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# **Pain-Induced Distress and Its Alleviation Using Butorphanol After Ovariohysterectomy Of Bitches**

**A thesis presented in partial fulfilment of the requirements  
for the degree of Doctor of Philosophy  
in Veterinary Clinical Science at  
Massey University**

**Steven M. Fox, MS, DVM, MBA  
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# **Pain-Induced Distress and Its Alleviation Using Butorphanol After Ovariohysterectomy Of Bitches**

**Volume I**

**Text**

**(Volume II contains the Tables, Figures and Appendices)**

**On the strength of one link in the cable,  
Dependeth the might of the chain.  
Who knows when thou may'st be tested?  
So live that thou bearest the strain!**

**Reef Points, 1964-65  
United States Naval Academy**

## Abstract

Ovariohysterectomy is the most frequently performed surgical procedure in companion animal veterinary practice. It is regarded by many as being quite benign; however, questioning of that premise prompted this investigation. There were no satisfactory data available to determine how benign or noxious this procedure might be, yet this query is of considerable clinical importance. There exists the further vagary of assessment for pain-relief measures, whether associated with surgery or injury. Great value would lie in establishing a routinely available criterion for pain assessment. The present work was therefore undertaken to examine this area of clinical relevance and to establish a model for further study of postsurgical pain-induced distress and its alleviation.

Most previous studies in this area had omitted satisfactory control or baseline animals in that the anaesthetic or analgesic treatments were rarely, if ever, applied to animals that were not also subjected to surgery. Accordingly, the following nine treatments were made: Control, Anaesthesia, Analgesia, Analgesia plus Anaesthesia, Anaesthesia plus Analgesia at intubation, Anaesthesia plus Analgesia at extubation, Anaesthesia plus Surgery, Analgesia plus Anaesthesia plus Surgery, and Anaesthesia plus Surgery plus Analgesia. These were designed so that, with the inclusion of surgery, all the major variations in treatment were independently examined.

The parameters used to evaluate the extent of pain-induced distress were changes in plasma cortisol concentration and behaviour. Cortisol is a well established physiological parameter of distress, and behaviour is a cue used by most clinicians. Butorphanol was selected as the analgesic of choice in this investigation based upon its wide use, margin of safety, across-species efficacy, versatility in route of administration, long shelf-life, competitive pricing, and freedom from the requirement for documented use by controlling authorities.

The following conclusions were drawn from the cortisol data. Psychogenic stimuli in conscious control bitches were responsible for a transient rise in cortisol concentrations not seen in anaesthetised dogs which were unconscious. Butorphanol elicited a large cortisol response,

attributable to dysphoria, which was again prevented by anaesthetic administration. As judged by cortisol response there was no apparent benefit of preoperative butorphanol administered intravenously 30 minutes prior to or at the time of anaesthetic induction. However, there was an earlier decline in cortisol concentration when butorphanol was given at extubation and this was interpreted to reflect an earlier decrease in postoperative pain-induced distress.

The study commenced with 166 behavioural parameters (interactive and noninteractive) from which it was found that 76 occurred at insufficient frequencies as to be valuable as indices of postoperative pain-induced distress. Among the discriminating behaviours, noninteractive parameters characteristic of the nonanalgesic surgery group were drawing the rear limbs up into a pike position, lip licking, cage circling, incision licking, vomition, and 'look back' (flank gazing), while the only characteristic interactive behaviour was an extended neck. Vocalisation was associated with the dysphoria of analgesia rather than pain-induced distress.

The major contributions of this research were: (1) establishing ovariohysterectomy as a model of pain-induced distress to examine the benefit of various pain-control strategies, (2) elimination of a number of commonly seen behaviours and identification of useful behaviours for identifying pain-induced distress, (3) clarification of the responses to control and 'base-comparison' treatments with regard to both cortisol and behavioural responses, (4) identification of specific pain-induced behaviours, (5) derivation of a mathematical function representing a numerical expression for the clinical intuition of the subjective impression of pain experience in dogs, and (6) identifying behaviours that can be used by the clinician to indicate the presence or absence of pain-induced distress following ovariohysterectomy. Results suggest that the ovariohysterectomy is associated with sufficient pain-induced distress to warrant the associated use of analgesia.

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*Pain-Induced Distress  
and Its Alleviation  
Using Butorphanol After  
Ovariohysterectomy Of Bitches*



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## Introduction

*"A man is ethical only when life, as such, is sacred to him, that of plants and animals as well as that of his fellow man, and when he devotes himself helpfully to all life that is in need of help."*

**-Albert Schweitzer**

This thesis deals with the contemporary and sentient topic of animal welfare and well-being. Specifically, it explores features associated with the surgical procedure of canine ovariohysterectomy.

Ovariohysterectomy is the most frequently performed surgical procedure in companion animal veterinary practice, and is most often considered to be a 'routine' operation. As such this operation is often accepted as a benign procedure and is performed without the administration of associated pain relieving drugs (analgesic).

With the mounting interest in animal welfare, the lay public, the veterinary profession, and the research community are now critically questioning the historical acceptance of an ovariohysterectomy as a benign procedure. The majority of a companion animal veterinarian's clientele are women, who believe on anthromorphic grounds that ovariohysterectomy performed on a bitch would be no less comfortable than the same operation that many women have directly or indirectly experienced. Additionally, veterinarians, having experienced the need to increase the depth of anaesthesia during manipulations of the visceral mesentery during an ovariohysterectomy, have only recently begun to interpret what this means in terms of intraoperative well-being for the patient. These considerations, as well as others, all raise the question, "How benign is an ovariohysterectomy in the bitch?"

The latter was the underpinning question of the present investigation. Further, is ovariohysterectomy actually 'painful'? In order to investigate these questions, one or more parameters were needed so that an answer could be expressed and proposed. By identifying and validating a

parameter which could be quantified, assessment of the degree of discomfort associated with ovariohysterectomy might then become possible.

Given the preinvestigative anthropomorphic hypothesis that ovariohysterectomy is a painful procedure, the ethical pursuit would be to relieve that pain. This phase of the investigation used the previously established parameter(s) to assess any reduction of discomfort by an analgesic. Further, the timing of analgesic administration was considered to be a significant factor. Reports from human patients (Katz et al., 1992; Woolf and Chong, 1993) suggest that presurgical administration of analgesia is more efficacious than postsurgical administration, and this hypothesis was held at the commencement of the study.

Plasma cortisol concentration is widely considered to be a most reliable physiological parameter of distress. Although this parameter has its critics (Rushen, 1986; Moberg, 1987; Sackman, 1991), within its interpretative restrictions cortisol has been validated as a reliable index in a large number of animal species including humans (Herd, 1966; Domzal et al., 1983; Chastain et al., 1986; Ley et al., 1991). Major objectives of this study included assessing whether plasma cortisol was as an accurate index of pain-induced distress in the bitch undergoing ovariohysterectomy, determine limitations for such use, and evaluate cortisol's efficacy for determining the degree of distress associated with ovariohysterectomy.

Plasma cortisol is a cumbersome tool for the timely assessment of surgically associated distress to the practicing veterinarian in a clinical setting, because the assay requires sophisticated instrumentation and two to three days for processing. Veterinarians, animal health technicians, and veterinary nurses interact with animals almost constantly, and they are thus keen observers of animal behaviour. Therefore, behaviour may better serve the veterinarian as a parameter for assessing distress. Were cortisol established as a valid parameter for appraising pain-induced distress in the first phase of the study, specific behaviours or categories of behaviours might also represent canine expressions of pain-induced distress, which could be verified by cortisol data.

It was presumed that dogs may show different behavioural responses to stimuli, depending on whether they are interacting with humans or are in relative isolation. This was based on the awareness that pet dogs can be conditioned to give a particular response to their owners. Recognising a difference was considered to be important for two reasons: firstly, a bitch's noninteractive response to a procedure known to be painful in the human may best identify the animal's 'true' uninhibited behavioural expression. Secondly, the veterinarian is most interested in an animal's postsurgical status, not in an isolated environment, but as it responds to his or her clinical inspection. Responses of a bitch over a given period of time may be quite different depending on whether the recovering animal is being examined or is left undisturbed and observed from afar.

This study of perioperative pain-induced distress was conducted in order to assess whether plasma cortisol and behaviours could be used for laboratory and clinical assessments. These parameters might then provide insights for subsequent alleviation of distress following routine abdominal surgery, thereby enhancing animal welfare and further advancing our ethical responsibility to those animals entrusted to our care.

### **Animal Welfare**

As elucidated by Campbell (1987) the human-animal bond is deeply rooted in history and the evolution of European culture. Ancient myths frequently portray animals as prominent symbols, and a relationship of man to animals has existed for centuries. Tales of animal ancestry or marriage between beast and maiden have been passed from generation to generation, denoting man's early respect for animals. Yet this respect faltered in the evolution of modern man with the development of agriculture and domestication, as animals became man's commodities (Chapman, 1992).

The industrial and technological revolutions changed man's regard for animals. With the technological revolution came man's apathetic attitude toward animal suffering, an attitude attributable to what Rollin (1987) refers to as the *common sense of science*. This prevailing attitude had foundation in the belief that, in and of itself, science has nothing to do with values in general or ethics in particular. Accordingly, the common sense of science saw ethics as a subjective matter of taste and personal

preference; as matters of emotion and not reason. Complimenting this lack of responsibility for balancing science and ethics came the psychology of *behaviourism*, introduced in 1913 by J.B. Watson. In its pure form, behaviourism denied the knowability and reality of consciousness in human beings or animals. So, for most of the century, although ordinary common sense did not deny animal pain and suffering, little attention was given to them (Rollin, 1987).

Only during the past few decades has a growing portion of the public demanded far greater accountability from the scientific community for animal use (Singer, 1975; Rowan and Rollin, 1983). This demand has now spread to any personnel entrusted with animal rearing and care. Demands have gained substance in the passage of laws, guidelines, and policies that have begun to acknowledge the rights of animals and diminish the absolute domain of man over his animals.

Rollin (1991) maintains that animals' rights and their welfare are an extension of the 1960's mind set, where concern for the rights of minorities and women was seen not as a new idea for discussion, but rather as the maturation of ethical principles already taken for granted in society within our moral and legal system.

Within the animal welfare movement have come three principal areas of focus: kindness to animals, cruelty to animals, and love for animals. Each focus has its own baggage of emotion, ethics, politics, and economic commitment.

Initially, welfare of only pet animals received attention, but now includes that of laboratory animals and production animals. As concern for more species has occurred the breadth and depth has increased. Although Fraser (1990) would differentiate the terms 'welfare' and 'well-being' with the latter reflecting an endogenous state, both refer to good states of being and their synonymous usage herein is adopted to avoid ambiguity.

"When applied to animals, including humans, the term 'welfare' (or 'well-being') usually denotes an absence of 'suffering' or an absence of what might be argued are major components of suffering, ie., anxiety, fear, pain and distress" (Mellor and Reid, 1994). Unfortunately, discussion of

anxiety, stress, suffering and pain is confused by misunderstandings in the use of these terms. As a starting point the *International Association for the Study of Pain* has drawn up a definition of terms relevant to pain experience and as guidance for the research community (Merskey, 1979). These dynamic, working definitions (quoted below) are guidelines to help recognise anxiety, stress, suffering and pain in animals and to facilitate a reasoned discussion of these phenomena (Panel report on the colloquium on recognition and alleviation of animal pain and distress, 1987).

*"Anxiety and Fear may have the same evolutionary benefit as pain.*

Anxiety can be defined as an emotional state involving increased arousal and alertness prompted by an unknown danger that may be present in the immediate environment. Fear can be defined similarly, except that fear would refer to an experienced or known danger in the immediate environment. Thus, anxiety appears to be a generalised, unfocused response to the unknown, and fear is a focused response to a known object or previous experience. A dog may tremble in a veterinarian's examination room during the first visit because of anxiety about what will happen. On the second visit, the dog may whine or try to escape from fear of a remembered event."

*"Stress can be defined as the effect of physical, physiological, or emotional factors (stressors) that induce an alteration in the animal's homeostasis or adaptive state. The adaptive response acts to return the animal to a base-line behavioural and physiological state. The response to stress often involves changes in the neuroendocrinologic function, in the autonomic nervous system, and in the mental state of the animal, as well as in its behaviour. Stress and subsequent responses may be categorised in three ways. Neutral stress is not in itself harmful to an animal and evokes responses that neither improve nor threaten the animal's well-being. Eustress involves environmental alterations that in themselves are not harmful to the animal but initiate responses that may, in turn, have potentially beneficial effects. Distress is a state in which the animal is unable to adapt to an altered environment or to altered internal stimuli."*



*"Suffering* is a colloquial term that is not defined in most medical dictionaries. It may be recognised as a highly unpleasant emotional response usually associated with pain and distress. Suffering can occur without pain, and although it might seem counter-intuitive, pain can occur without suffering. Modulation of suffering may involve removing or changing some aspect of the internal or external environment or giving the animal the opportunity to avoid, escape, or control some aspect of that environment."

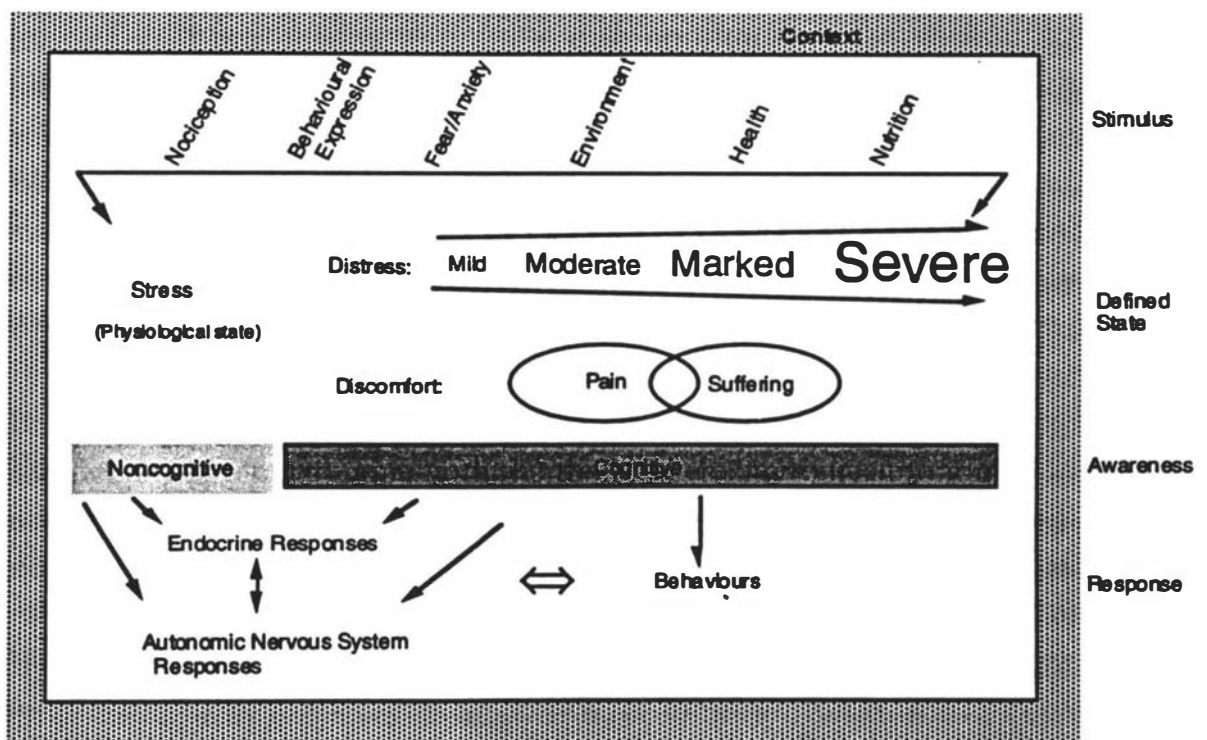
*"Pain* in human beings and in other animals is understood to have evolutionary survival value. Pain is a perception that depends on activation of a discrete set of receptors (nociceptors) by noxious stimuli. Further processing in neural pathways enables the noxious stimuli to be perceived as pain. Pain perception varies according to site, duration, and intensity of the stimulus and can be modified by previous experience, emotional states, and perhaps innate individual differences. Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Seemingly, the pain-detection threshold is uniform across species lines, whereas the pain-tolerance threshold may be more species-specific and subject to modification."

Animal husbandrymen commonly use the term 'stress' to indicate an environmental condition that is adverse to the well-being of an animal (Stott, 1981). This stress may be nutritional, climatic, social, or physiological. The biologist refers to various reactions undertaken by organisms to restore physiological integrity as 'biological stress', where stress often denotes the distorting influence or the distortion itself (Stott, 1981). In most contexts the word 'stress' has a largely negative connotation, indicating an undesirable state of affairs. Unfortunately, it is often assumed that because the same word has been used, then measures of adrenocortical activity are relevant to all meanings of the word stress. Lee (1965) presented a working definition of stress that is often used by physiologists: "stress denotes the magnitude of forces external to the bodily system which tend to displace that system from its resting or ground state." The appeal of this representation comes from the accent on forces that bring about changes in the bodily system, whether favourable

or adverse, rather than putting the inflection on unfavourable ambient conditions.

Reviewing pituitary-adrenal system responses in humans, Mason (1975) has suggested that different events produced the same physiological responses because they evoked the same emotional response. Dantzer and Mormède (1983) argue that it is the subjective experience of the animal that is responsible for the physiological response, and further, that these subjective experiences must be unpleasant or distressing in order to evoke the response. In contrast, there are many reasons for believing that 'unpleasantness' from an experimental technique or husbandry procedure cannot be determined by measuring plasma corticosteroid levels of animals (Rushen, 1986).

An animal confronted with a distressful situation relies on three major biological systems to cope with the distress: behaviour, autonomic nervous system, and neuroendocrine system (Moberg, 1987). If the behavioural response is insufficient to alleviate the distress or if the stimulus is of such magnitude, then the animal alters its biological function through the autonomic and neuroendocrine systems.



The above illustration may help to elucidate terminology used in this thesis. A clear distinction is made between stress and distress, where **distress** is not recognised as a vaguely defined upper limit of stress, but rather to encompass the resultant of all stimuli on the conscious animal. **Stress** is reserved to identify the physiological state of an animal; not a stimulus. The state of stress may be characterised by changes in such indices as blood pressure, heart rate and endocrine levels. In contrast, distress infers a state of consciousness, and even more embracing, includes the cognitive features of learned experience and perhaps judgement (if one believes dogs have this capacity).

Many stimuli are known to influence the dynamics of animal distress, which may progress in severity through stages of discomfort, pain, and suffering. Nociception, fear/anxiety, and environment are among those stimuli presented in the above illustration which were given particular attention in the present investigation. Cognitive processing of stimuli that contribute to the level of distress in an animal subsequently gives rise to changes in the autonomic nervous system, endocrine system, and behavioural traits. Further, a number of feed-back loops in response to distress are recognised. Although most behaviours would accompany only the conscious (cognitive) animal, the noncognitive (unconscious) animal may express behavioural changes that do not require the conscious state, eg. the animal may tremble resultant from the unconscious physiological response to hypothermia of anaesthesia. Additionally, endocrine and nervous system reactions to distress may influence behaviours, eg. salivation. These synergistic influences between endocrine and nervous system responses and behaviours are represented in the illustration by those lines with arrows at either end.

For a meaningful interpretation of the above definitions, one must accept the over-riding consideration of context. Distress, as it is explored in this study, focuses on noxious or unpleasant stimuli. Of course, distress is not confined to only such offensive stimuli. A feeling of euphoria or maximal well-being may also involve distress, as it is herein defined, accompanied by both endocrine and autonomic nervous system and behavioural responses; however, this study focused on the adverse end of the distress spectrum.

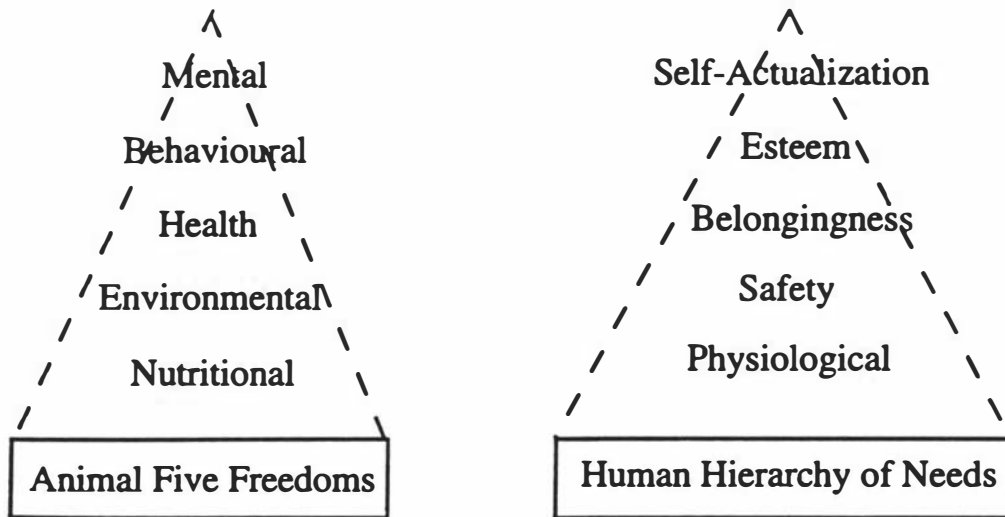
Given the recognition that distress results in a change in physiological indices and behaviours, the possibility exists that deviations in these parameters may be quantified, thereby providing indices of the magnitude of distress. Plasma cortisol is a physiological parameter responsive to many different stimuli of distress which is consistently seen in many different species of animals. This suggests that plasma cortisol may hold potential as a particularly good index of distress.

In the rush to fill society's pressing desire to obtain objective data concerning animal welfare issues there is potential for oversimplified interpretations of expedient types of measures. For instance, assessment of the activity of the pituitary-adrenal axis, often through measurement of plasma levels of corticosteroids, as an index of pain or welfare can be misunderstood or misrepresented as measuring pain or welfare. However, as depicted, both cortisol and behaviour do not measure pain or welfare, but represent distress responses to various stimuli. It is only through the careful examination of the effects of multiple stimuli acting within any situation that the impact of a single stimulus can be recognised.

The term **pain** infers consciousness, as pain and suffering do not exist in the unconscious subject. Although the term is used sparingly within this chapter, it is more fully developed in subsequent chapters. At this point, 'pain-induced distress' is coined to represent expression of the combination of: noxious nociceptive input, the cognitive responses to that nociception and associated contextual stimuli.

Returning to the issue of animal welfare, perhaps the broadest guidance on animal welfare is found within the concept of the 'five freedoms' formulated by the Farm Animal Welfare Council of the United Kingdom (Farm Animal Welfare Council press release, 1992). The five freedoms recognise that animals have nutritional, environmental, health, behavioural and mental needs. Through such a scheme a more comprehensive definition of the actual state of welfare of an animal becomes possible by reference to the extent of compromise of these needs (Mellor and Reid, 1994). Conceptually the animals' five freedoms can be seen as a pyramid, rising from animals' most basic needs in a manner similar to one of the most widely accepted theories of human motivation, the *Hierarchy of*

*Needs Theory* developed in the early 1940's by the psychologist Abraham Maslow (1943).



The infliction of 'pain' to animals is one of the most emotional aspects of animal welfare concern. Although animals have been used extensively as research subjects for studies of the mechanisms of pain, little research has been conducted with the specific goal of improving clinical management of pain in domestic species. Various procedures and diseases in livestock probably produce varying degrees of acute pain that may last for hours and days. These include mulesing of sheep, castration and tail docking in lambs and other animals, footrot in sheep, laminitis in horses, and slaughter of animals. Because of economic and logistic considerations, many of these animals do not always receive analgesics, yet most would acknowledge that they undoubtedly experience pain (AVMA Animal Welfare Forum, 1994).

There are several economic reasons why it is impractical to use potent analgesic agents in livestock. Available opioids and other systemic analgesics are said to be too dangerous for farmers to use safely. In the current economic climate, veterinary surgeons are not likely to attend the tail docking or castration of lambs. Caudal epidural block with local anaesthetic or infiltration to produce ring nerve blocks are possible, but some (Bonica, 1992) believe that the stress associated with firm physical restraint to enable such procedures must be weighed against the benefit to the animal. This issue is presently under investigation as a matter of animal welfare concern at Massey University, New Zealand.

Hansen and Hardie (1993) suggest that no one has questioned the adequacy of analgesic treatment as it is practised today. Most accounts of veterinary clinical experience with the recognition of pain in companion animal species appear to be made on the basis of anecdotal observations of presumed signs of pain. Few clinical reports provide recommendations for evaluation of pain on the basis of observation of behaviour. Treatment of experimentally induced pain in dogs and cats have been evaluated in several studies, but there have been few attempts to objectively evaluate the application of analgesics in a clinical setting.

Numerous reports in human beings (Marks and Sachar, 1973; Cohen, 1980; Melzack et al., 1987; Oden, 1989) and one in cats and dogs (Hansen and Hardie, 1993) indicate that postsurgical analgesia is frequently inadequate, and that many patients experience significant discomfort. The reasons for inadequate treatment are complex and include deficiencies in knowledge and skills, inappropriate attitudes, lack of objective criteria for assessment of postsurgical pain, great inter-species variability regarding pharmacokinetics and pharmacodynamics of opioids and other systemic analgesics (as well as local anaesthetics and regional anaesthesia), concern that postsurgical analgesia may have serious undesirable effects, and institutional barriers (Oden, 1989; Bonica, 1992). This study was conducted to further clarify several of these areas.

The initial objectives of this study were to answer the following questions:

- \* *What are the restrictions for using changes in plasma cortisol concentration as an index of pain-induced distress after ovariohysterectomy?*
- \* *What behavioural responses are distress-specific after ovariohysterectomy?*
- \* *What impact does the timing of butorphanol analgesia have on postsurgical pain-induced distress as assessed by cortisol and behaviour?*

- \* *Are interactive and non-interactive behavioural responses of the bitch to man different in the presence of pain-induced distress from ovariohysterectomy?*

### **Protocol**

Nine treatments were made. The nine treatments incorporated various combinations of anaesthesia, surgery and analgesia so that characteristic effects of each could be identified independently. Within these treatments the time for administration of analgesic was also varied so as to appraise presurgical versus postsurgical treatment effects. Blood sampling was performed at regular intervals for radioimmunoassay (RIA) of plasma cortisol concentrations (see Appendix A for RIA details). All bitches were videotaped for a total of six hours, and the video tapes were subsequently reviewed in detail to assess behaviours.

The descriptive behaviours (defined within Appendix B) were decided upon after an extensive literature review and discussions with colleagues holding anaesthesia specialty qualifications. For ease of management similar behaviours were grouped into subcategories. Noninteractive and interactive (palpation) behaviours were assessed using different behavioural characteristics, and it is recognised that the intervention of palpations conducted at the time of blood sampling may have had an influence on the noninteractive results.

Selection of butorphanol as the analgesic of choice in this study was based on its broad attributes. Butorphanol has been recognised as the most widely used opioid agonist-antagonist in the world.<sup>1</sup> It has a wide margin of safety, is efficacious in a diverse range of animal species, and can be administered by various routes. Butorphanol has a long shelf-life, is competitively priced, and most importantly, does not require documentation of use by drug controlling authorities (see Appendix C for further butorphanol details).

Most veterinarians perform their ovariohysterectomy surgeries in the morning and discharge their patients the same afternoon. The advertised duration of butorphanol action (one to four hours) well suits this protocol,

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<sup>1</sup> Professor Donald Sawyer. Central Veterinary Conference. Kansas City, 1994.

where the lingering effects of the analgesic do not mask a clinical assessment by the practitioner at the time of patient discharge.

Butorphanol is not presently registered for commercial trade in Australia or New Zealand, countries in which veterinarians seek its introduction. It was anticipated that this study might support dossier efficacy data for registration of butorphanol in those countries.

As the study proceeded several issues arose which merited further research and literature review:

### **Cortisol**

Is cortisol a valid parameter by which to assess pain-induced distress for ovariohysterectomy in the bitch, and if so, how useful is this index?

### **Opioids**

How might the pharmacodynamics of the agonist-antagonist butorphanol explain cortisol and behavioural responses resulting from its administration in the bitch?

### **Anaesthesia**

How might the pharmacodynamics of halothane anaesthesia influence cortisol and behavioural responses in the bitch?

### **Pain and Behaviour**

What is pain, and do bitches undergoing an ovariohysterectomy feel pain? If the prestudy hypothesis that they do feel pain is correct, then how can this be verified? Does the bitch display characteristic behaviours to pain of an ovariohysterectomy?

Additional information is now provided, and more details will be presented in later chapters.

### **Cortisol**

*Is cortisol a valid parameter by which to assess pain-induced distress for ovariohysterectomy in the bitch, and if so, how useful is this index?*



Cortisol (hydrocortisone) is the primary glucocorticoid secreted by the canine adrenal cortex (Reigh et al., 1950; Farrell, 1954; Farrell and Lamus, 1955). It is released in response to stimulation of adrenocortical cells by adrenocorticotrophic hormone (ACTH), a protein produced by the anterior portion of the pituitary gland (adenohypophysis) (Ganong et al., 1974). The ACTH release is modulated by hypothalamic secretion of a small peptide, corticotropin-releasing factor (CRF). Various stimuli increase, and circulating peripheral glucocorticoids decrease, ACTH release (Bassett and Hinks, 1969; Ganong et al., 1974).

The hypothalamic-pituitary-adrenal axis is a highly dynamic mechanism which initiates a complex cascade of effects that is highly responsive to an animal's environmental changes. Plasma cortisol is an index that has consistently been used in both physiological and clinical investigations to monitor activity of this axis. However, the acceptance of plasma cortisol as a parameter by which to assess the impact of surgical pain-induced distress on an animal is complicated by procedures associated with surgery i.e. anaesthesia and analgesia. Given that the hypothalamic-pituitary-adrenal axis is sensitive to the pharmacodynamics of both anaesthetics and analgesics, it is imperative that such influences be investigated and accounted for independently, utilising the latest available understanding in order to subsequently identify the distinct effects of surgery.

Parenthetically it is worth noting that the same caveat holds for behaviours as it does for cortisol regarding the peripherals to surgery, ie. the influences of anaesthesia and analgesia must be recognised before the behavioural expressions of pain-induced distress can be identified.

In human beings plasma cortisol becomes elevated during both the surgical and the postsurgical periods. Cortisol elevations are related to rises in ACTH (Cooper and Nelson, 1962) and are correlated with the severity of the surgical procedure and its duration (Sandberg et al., 1954; Madsen et al., 1976). Graded cortisol responses to surgical procedures in the dog are reported as similar to those in humans (Schmidt and Booker, 1982; Frank et al., 1992; Matthews et al., 1992). Thus it seems likely that with appropriate consideration, cortisol may be useful as an index of pain-induced distress in the dog.

## Opioids

*How might the pharmacodynamics of the agonist-antagonist butorphanol explain cortisol and behavioural responses resulting from its administration in the bitch?*

The word *Opiate* once referred to drugs of opium origin (Jaffe and Martin, 1992). After the development of synthetics with morphine-like actions, the word *opioid* was coined to refer in a generic sense to all drugs, natural and synthetic, with morphine-like actions. With usage *opioid* has also come to refer to antagonists of morphine-like drugs as well as to receptors that combine with such agents. The latter usage has not been adopted for this thesis.

Since evidence of multiple opioid receptors was first noted by Martin and colleagues (1976), understanding of opioid pharmacology has progressed steadily. The first two opioid receptors ( $\mu$  for morphine and  $\kappa$  for ketocyclazocine) mediate analgesia, which is neurologically generalised for the  $\mu$  agonist but considered spinal for the  $\kappa$  agonist. It is believed that no cross tolerance exists between the two (Itzhak, 1988). Mu receptors are subdivided into  $\mu_1$  and  $\mu_2$ . Opioid peptides including morphine have similar affinities for  $\mu_1$ , while morphine has the greatest affinity for  $\mu_2$  (Itzhak, 1988). Mu receptors are mainly responsible for supraspinal analgesia, euphoria, respiratory depression and physical dependency.

Kappa receptors have three subdivisions, although there is debate about whether  $\kappa_2$  and  $\epsilon$  (of which little is known) are the same receptor (Pleuvry, 1993). Kappa receptors are mainly responsible for spinal analgesia, miosis and sedation. The third receptor,  $\sigma$ , which has two subdivisions, does not mediate analgesia and has subsequently been associated with the psychotomimetic activity of opioids. Sigma receptors are mainly responsible for dysphoria, hallucinations, respiratory stimulation and various vasomotor effects. However, since naloxone is inactive at  $\sigma$  receptors (Walker et al., 1990), they are no longer classified as opioid receptors.

Delta receptors exist as two subdivisions and there is evidence that analgesia occurs through activation of  $\delta$  receptors (Heyman et al., 1988).

There is also some evidence that  $\mu$  and  $\delta$  receptors may exist in two forms, complexed together and non-complexed (Heyman et al., 1989). Both  $\mu$  and  $\delta$  receptors appear to be associated with the opening of potassium channels, while  $\kappa$  receptors are associated with the closure of calcium channels (Dickinson, 1991) (N channels of neurones (Gross and Macdonald, 1987)).

Opioids produce central effects by dynamically binding to specific receptors in the encephalon and spinal cord. Once opioids have interacted with the receptor site, either stimulation or depression of different neuronal populations is initiated. Butorphanol is considered to be a strong agonist-antagonist synthetic opioid with moderate kappa and sigma receptor activity (Benedetti and Butler, 1990). Kappa effects include spinal analgesia, miosis and sedation and sigma effects include dysphoria or psychotomimetic properties (Jaffe and Martin, 1992). Side effects of butorphanol in man include nausea, sweating, headache, vertigo, floating or pleasant sensations, dizziness, lethargy and confusion (Caruso et al., 1979).

The most important pharmacological property of opioid drugs is analgesia. It is now apparent that opioids exert this effect supraspinally, at the level of the brain stem, spinally by inhibiting nociceptive input, and peripherally when pain is produced by inflammation or a sympathetic pain syndrome (Pleuvry, 1993). Although benzomorphan relatives of pentazocine, such as butorphanol, interact quite selectively with  $\kappa$  receptors (Pfeiffer et al., 1986), the independent supraspinal analgesic activity of  $\kappa$  receptor activation is uncertain (Leighton et al., 1988; Millan, 1990; Dickinson, 1991).

## **Anaesthesia**

*How might the pharmacodynamics of halothane anaesthesia influence cortisol and behavioural responses in the bitch?*

The mechanism(s) of action for general anaesthetics is (are), as yet, unknown. Urban (1993) suggests that anaesthetics may interfere with the process of neurone signal modulation in a variety of fashions:

- 1) Anaesthetics may prevent a neurone from firing by enhancing inhibitory neuronal effects, or by inhibition of excitatory neurone effects.
- 2) Anaesthetics may modify an incoming excitatory signal that prevents a neurone from firing by altering either or both the firing rate or producing a temporal shift in the incoming signal.
- 3) Temporal shifts in impulse conduction could result in a normally inhibitory signal becoming ineffective, thus removing inhibition, and
  - i. interference with signal integration could occur (a network of neurones may respond to the integration of anaesthesia induced responses from its individual member neurones),
  - ii. partial inhibition may lead to complete inhibition in the next higher level of integration, or
  - iii. it may result in the removal of inhibition.

While the molecular basis of anaesthesia for most of the anaesthetic agents is unknown, it is a commonly held view that anaesthetic drugs have a greater effect on synaptic mechanisms in the central nervous system (CNS) than on the propagation of action potentials along axons (Griffiths, 1993). In particular, Group A anaesthetics (gaseous and volatile anaesthetics) have two sites of synaptic transfer in sensory pathways that are susceptible to the action of anaesthetics: the ventrobasal thalamus and the cortical layer V (Angel, 1993).

### **Pain and Behaviour**

*What is pain, and do bitches undergoing an ovariohysterectomy feel pain? If the prestudy hypothesis that they do feel pain is correct, then how can this be verified? Does the bitch display characteristic behaviours to pain of an ovariohysterectomy?*

Probably the most frequently used approach to determine whether animals are suffering or not is to make a comparison of their status with that of ourselves. The difficulty of trying to conclude whether an animal is suffering by comparison with ourselves is that there are no clear behavioural markers for suffering in any animal species. Animals have evolved a variety of species-specific behavioural patterns that suit them for

certain natural environments. Unless we understand a good deal about animal behaviour to interpret vocalisations, facial expressions, movement and postural changes, we could mistakenly assume that certain behavioural signs are evidence of underlying suffering. For example: what sounds like a (distress responsive) cry to us, simply because it resembles that of a baby, may represent a simple exchange of signals between two canids. Additionally, a wide variety of attention-getting behaviours has been described in dogs, ranging from chasing shadows, biting in the air, and lameness to medical signs such as coughing or diarrhoea (Hart, 1979). In many ways, using an animal's own behaviour to indicate its freedom from distress might be more reliable than attempting to evaluate its welfare by comparing its reactions to ours or even trying to establish a link between its physiological responses and its welfare.

Intense anxiety and fear are an integral part of the distress response/experience. They greatly enhance hypothalamic responses through cortical stimulation. Several authors (Corman et al., 1958; Janis, 1958; Chapman and Cox, 1977; Volicer, 1978) have noted in humans that presurgical anxiety markedly influences anxiety within the postsurgical period, particularly in patients with inadequate psychological preparation. Prior to an operation many human patients develop anxiety, apprehension and fear; feelings which may be carried into the postsurgical period and cause distress and arousal.

In humans admission to the hospital produces anxiety and distress which correlate highly with the incidence and intensity of postsurgical suffering (Janis, 1958; Volicer, 1978). Volicer (1978) showed that human patients scoring high in 'hospital stress' presurgically had more pain and morbidity postsurgically and less improvement after discharge than did patients who had a lower score.

Chapman (1978) has pointed out that the postsurgical human patient's state of anxiety is composed of, or caused by, three basic determinants: fear or fright, uncertainty, and helplessness. Fright is a reaction to the recognition of suffering as soon as the effects of anaesthesia disappear. The suffering seems worse than expected, and the patient feels frantic and threatened, especially if this is the first such experience for the patient. Uncertainty, the second component, has a serious impact on anxiety,

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especially in those who do not know what to expect after the operation. The unfamiliar surroundings in recovery accentuate the anxiety and the patient remains hypervigilant and anxious, which in turn decreases the discomfort threshold and increases the perception of discomfort, thus creating a vicious cycle. This is further enhanced by helplessness. The patient is often placed in a fixed position and, because movement exacerbates the discomfort, feels unable to cope. Although these observations are made regarding the human patient, they prompt speculation for similarities in the bitch. Of course, it is not possible to convince an animal that the discomfort which occurs subsequent to surgery is to be expected and that it will only be temporary. Accordingly, anxiety may be a major contributor to postsurgical restlessness. Animals recovering from surgery may well be surprised and confused because they hurt: sedation is often appropriate as it dissipates the related anxiety.

### Statistical Analyses

Rather extensive statistical analyses were performed on data within each chapter of the following study, especially the behaviour data. An introductory section to statistics is presented here to provide a foundation to which specifics are added within each chapter.

"In the modern sense of the word, statistics is concerned with the development and application of methods and techniques for collecting, analysing, and interpreting quantitative data in such a way that the reliability of conclusions based on the data may be evaluated objectively by means of probability statements "(Shott, 1991). As long as generalisations are not made regarding calculated measurements, observed descriptions are the limit of data presentation. However, with implementation of statistical methods inductive generalisations take the form of statistical inferences, giving greater credibility to interpretations.

Statistical methods based on specific distributional assumptions such as normality are called parametric statistics. When parametric statistical assumptions hold, parametric methods of analysis are most appropriate because they are more powerful, ie., they have a better chance of rejecting the null hypothesis when it is false (Haycock et al., 1992). In some cases data that do not meet parametric assumptions can be transformed (eg., by

logarithms, square roots, or reciprocals) into data that satisfy these assumptions, thereby justifying the use of parametric techniques.

The need for techniques that apply more broadly than within the restrictions of parametrics has led to the development of nonparametric methods. These do not require that the underlying populations have any particular probability distribution, and some even apply to nonnumerical data. "In place of parameters such as means and variances and their estimators, these methods use ranks and other measures of relative magnitude; hence the term 'nonparametric'" (Shott, 1991). If violation of parametric assumptions cannot be corrected by transformation, or the untransformed data are preferred, then nonparametric statistical procedures should be implemented.

The point of multivariate analysis is to consider several variables simultaneously, each one being considered equally important at the start of the analysis. The importance of measured parameter relatedness is expressed by Kendall (1957), "The variates are dependent among themselves so that we cannot split off one or more from the others and consider it by itself. The variates must be considered together". Further, the problem addressed with the multivariate analysis technique of discriminant function analysis is the separation of two or more treatments given measurements for these treatments on several variables.

Several extensive data sets were collected from experiments reported in this thesis. With the provision that underlying distributional and model assumptions were satisfied, some of the data (eg., cortisol measurements) could be analysed using parametric procedures. On the other hand, some of the data (eg., behavioural data) were more appropriately analysed by nonparametric procedures, especially when specific hypotheses were being explored. Much of the behavioural data were analysed on an exploratory basis using semi-exploratory parametric models. Additional details are presented in the appropriate chapters.

The statistical techniques applied in this thesis involved mainly:

1. analysis of variance, which included repeated measures analysis and subsequent 'post hoc' analyses such as Scheffé's test, Fischer's protected least significant distance (PLSD) test, and simple student's t-tests;

2. principal components, canonical discriminant analysis, and related multivariate statistical techniques, and
3. correlation and regression analyses.

Although each of these techniques will be described where appropriate in the text, it is fitting at this time to present an overview and explanation of what they aim to achieve.

### *Analysis of Variance (ANOVA)*

A one-way analysis of variance model attempts to determine if all the treatments have the same mean or 'centre', and the technique has been developed for both parametric and non-parametric models. For this thesis the emphasis is on ANOVA for parametric (continuous) variables. An analysis of variance studies the effect of independent variables on a continuous dependent variable when the independent variables are usually nominal (eg., treatments) rather than continuous. Analysis of variance determines the significance of the effects in a model by calculating how much of the variability in the dependent variable can be explained by the effect in question.

A one-way analysis of variance attempts to identify if all groups have the same mean. For a formal application of the ANOVA it is assumed that random samples from normally distributed populations have the same underlying variance despite different means. In practice, the normality assumption is not critical, and the assumption of equal variance can also be violated, provided the groups are of similar size. The assumption of random sampling is very important.

Given the assumption of the same underlying variance, the variability is estimated with a generalised pooled estimate of variance. The ANOVA then computes the means of the groups and compares their variance to the pooled variance. The comparison statistic follows an F distribution. Larger F-values (ie., smaller p-values) propose that the means differ more than expected based on the underlying sample-to-sample variability inherent among measurements within groups. On the other hand, smaller F-values (ie., larger p-values) support the hypothesis that the means are not discernibly different.



If F-values are found to be small (ie., there is no treatment difference) then the statistical analysis is usually terminated at this stage. If, however, the ANOVA shows significant differences among treatments, then further 'post hoc' analyses or multiple comparisons are usually implemented to determine which treatments are different. There are many of these 'post hoc' tests from which to choose. Those tests used in the analysis of data from the present studies were: the Scheffé's F test and the Fischer's protected least significance distance test for simultaneous comparisons, and the Dunnet's test for the difference between a treatment and a control. The Student's t-test was used to contrast two treatments at different stages of analyses.

A special type of ANOVA involves the analysis of measurements made on the same variable (eg., cortisol measurements) at several points in time. These are called 'repeated measures' analysis of variance. There are several analyses available for such data and relevant methods used in this thesis are presented when appropriate.

***Principle components, canonical discriminant analysis, and related multivariate analysis techniques***

Several multivariate statistical techniques are potentially applicable to the data sets generated within this thesis. Two techniques were mainly used: principal components analysis, and multivariate analysis of variance, which leads to an exploratory use of canonical discriminant analysis.

Principal components analysis is a technique for reducing the dimensionality of a data set. For example: 15 variables may be measured on each individual in a given treatment. Since these measurements (variables) are taken on the same individual, it is conceivable that they would be correlated, and that some particular variables may be highly correlated. If two variables are highly correlated then one can be predicted from the other. In turn, for certain statistical analyses, one of the variables is effectively redundant and could be eliminated. This reduces the dimensionality of the data set. Principle components does not reduce the dimensionality by 'dropping' some variables; instead, it replaces the original number of variables by a smaller number of derived variables, each of which is a (linear) mathematical combination of the

original variables. It is the analyst's hope that only a few of these 'new' or 'derived' variables will contain almost all of the information in the original data set. Thereafter, in further analysis of the data set, one may choose to work with only these fewer 'new' variables, thereby reducing the dimensionality of the problem.

A given property of these 'new' variables is their construction as orthogonal (or independent) to one another. This means that each 'new' variable holds information about the data in a 'dimension' which is independent of the others. Inspection of the mathematical equations generating the new variables occasionally suggests 'descriptive' terms for these new variables. For example: original measurements on weights and lengths of an object may create a new variable depicting the 'size' of the object. It is worthy of note, however, that it is not always possible to reduce the dimensionality of some data sets. This is especially true if the original variables have few correlations among themselves, suggesting that they are already representing independent dimensions of the data set. Thus, it is not possible to find a smaller set of derived variables to account for a large proportion of the total variance (or information) in the original variables.

Principal components analysis was applied to the data on behaviours as part of an exploratory data analysis procedure. Further details are given in Chapters 4 and 5.

Another multivariate technique used on the behaviour data is the canonical discriminant analysis. Like principal component analysis, this model is also effectively a dimension-reduction technique. The fine difference is that canonical discriminant analysis is applied to data for several treatments and the procedure incorporates the information that there are several classes or treatments in the data set. The goal of the procedure is to produce new (derived) variables, which are combinations of the original variables, such that these new variables best summarise or highlight the differences among the treatments. Given two or more treatments with measurements of several quantitative variables on each within-treatment object, canonical discriminant analysis derives a new set of variables from mathematical functions of the original variables whose new values distinguish, or separate, with maximal ability, among members

of the given treatments. Thus, instead of using the multiple original variables to study or highlight treatment differences, a lower, and perhaps more manageable, number of new variable that contain almost all the information about the differences among the treatments are used.

As will be seen in Chapters 4 and 5, a large number of behaviour variables were recorded for each bitch in various studies. The technique of canonical discriminant analysis was used to investigate if only a few derived variables from the many original behaviour variables could be used to summarise the treatment differences.

### ***Linear Regression***

The technique of linear regression is usually used to determine if a variable (Y) is linearly related to another variable or constant (X), in which case the linear regression of Y on X is considered. A simple extension allows for the possibility of several X variables. For the following studies, linear regression was used to investigate the linear relationship between plasma cortisol concentration measurements and the frequencies of some behaviours. Most of this endeavour is semi-descriptive in order to gather information on potentially 'predictive' behaviours.

### **Thesis Format**

The classical approach to a PhD project is sequential investigations that build upon a common theme. However, because of the time required to collect and process plasma cortisol samples, the complexity of behavioural data processing, and my continual commitment to the veterinary hospital as a clinician, this approach was not feasible. Instead, an omnibus scheme was pursued. Considerable time (18 months) and detail was given to the design of this research so that treatments required to consider specific questions were not omitted.

The study is documented in the ensuing chapters. Firstly, a pilot study was conducted (Fox et al., 1994) (see published study: Appendix D) using six bitches in four treatments: Control, Anaesthesia, Analgesia, and Anaesthesia plus Surgery. Data from animals included within this pilot

study were also included in the parent study which this thesis reports. The pilot study publication reported only cortisol parameters and not behaviours. The published pilot study reports an additional ACTH challenge trial conducted in parallel with the distress and pain-induced distress cortisol assessments. The ACTH stimulation trial was conducted to confirm normal endocrine responses from trial bitches and to assess response times. Please consult the enclosed publication (Appendix D), for additional details, as they are not duplicated in this thesis.

The thesis is separated into six major chapters:

1. Background and introduction,
2. Cortisol results for nonsurgical treatments,
3. Cortisol results for surgical treatments,
4. Behaviour results for nonsurgical treatments,
5. Behaviour results for surgical treatments, and
6. General discussion.

The contents of the different sections were arranged to help the reader assimilate the large data set, ie. both cortisol and behavioural data from nine treatments. Although results from nonsurgery treatments precede the chapter for results from surgery treatments, a collective interpretation of these two groups is made within the chapter on surgical treatments for both the cortisol and the behaviour data.

Chapter 2 addresses the effects of anaesthesia and analgesia in different combinations on plasma cortisol concentrations. This chapter supports the credibility of plasma cortisol as an index of distress, elucidates treatments that serve as base comparisons for surgical treatments discussed in Chapter 3, and identifies some limitations on the use of cortisol as a parameter for measuring distress.

Utilising the discriminating base groups established in Chapter 2, data from the treatments with ovariohysterectomy surgery in Chapter 3 add credibility to cortisol as a parameter for evaluating associated pain-induced distress. Chapter 3 also contains analysis of the efficacy for presurgical versus postsurgical butorphanol administration. Within this analysis a limitation in protocol design involving the timing and route of presurgical analgesic administration is identified and discussed.

Having established the usefulness of cortisol as a parameter for assessing distress in Chapters 2 and pain-induced distress in Chapter 3, the effects on behaviour of anaesthesia and analgesia in different combinations are evaluated in Chapter 4. In a like manner to earlier chapters, Chapter 4 establishes behavioural 'bases' for assessing behavioural responses to the surgery treatments presented in Chapter 5. In both Chapters 4 and 5, noninteractive behaviour is presented first followed by interactive (palpation) behaviours.

Approximately 166 behaviours were evaluated in total. Each of these individual behaviours was specifically defined in an effort to avoid ambiguity. Detailed definitions of these behaviours are provided in Appendix B. In the interests of brevity these detailed definitions are not duplicated within Chapters 4 and 5 in the belief that the descriptive terms are sufficient for clarity. To further accommodate the reader most illustrations, graphs and tables are contained in Volume 2, so that text and supporting data in Figures and Tables can be consulted with greater ease.

In summary, three major objectives have been set. Firstly is the validation of cortisol as a index for assessing pain-induced distress for ovariohysterectomy. Secondly is the explanation of the influence of anaesthesia and analgesia on both the plasma cortisol and behaviours associated with ovariohysterectomy. Thirdly is an attempt to use cortisol as a link to support the use of behaviours in assessing distress from ovariohysterectomy in the bitch.

Publications referencing this thesis work are:

1. Research in Veterinary Science, 1994, **57**, 110-118,
2. Proceedings 226: Animal Pain and its Control. 1994, Adelaide, Australia; Post Graduate Committee in Veterinary Science, University of Sydney,
3. Proceedings: New Zealand Physiological Society. 1994, Palmerston North, and
4. Proceedings: Central States (Veterinary) Conference. 1994, Kansas City, Kansas, U.S.A.

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## Chapter 2

### Effects of different combinations of anaesthesia and analgesia on plasma cortisol concentrations in bitches

#### Chapter Summary

Plasma cortisol responses were assessed in response to six treatments: Control, Anaesthesia, Analgesia, Analgesia followed by Anaesthesia, Anaesthesia followed by Analgesia at intubation and Anaesthesia followed by Analgesia at extubation. An 80 minute sustained rise in cortisol concentration within the Control group was attributed to the moderate distress associated with experiencing a novel environment. The more pronounced and more protracted rise in cortisol concentration seen in the Analgesia group was ascribed to the dysphoric state of bitches under the influence of the sigma receptor agonist-antagonist butorphanol. The treatment of halothane anaesthesia alone resulted in no change of plasma cortisol concentration.

Cortisol concentrations rose for a period of 120 minutes in the group given butorphanol; however, cortisol concentrations fell when halothane anaesthesia was given 40 minutes after butorphanol administration. When analgesic was given after anaesthesia was induced or while the animal was still under the strong influence of anaesthesia (immediately after extubation), there was no immediate rise in cortisol. Halothane anaesthesia appeared to suppress the cortisol expression of butorphanol analgesia for a period of up to 60 minutes. Plasma cortisol concentrations in all groups had returned to pretreatment values by 24 hours.

It is concluded that plasma cortisol responses to the six treatments are reflections of the state of the bitches' consciousness. Whereas plasma cortisol concentrations fall under the influence of anaesthesia in the absence of noxious nociceptor stimulation, when the bitch is conscious the cognitive psychological distress experienced determines the extent of the pituitary-adrenal axis response.

## Introduction

The responses of the hypothalamic-pituitary-adrenal axis to environmental alterations (Vial et al., 1979; Chastain et al., 1986; Frank et al., 1992; Knol et al., 1992; Vincent and Michell, 1992), anaesthesia (Church et al., 1994), and surgery (Campbell and Watts, 1973; Schmidt and Booker, 1982; Matthews et al., 1992; Church et al., 1994) have been studied infrequently in the dog. Cortisol is a commonly used parameter for measuring these responses in both humans and animals (Herd, 1966; Domzal et al., 1983; Chastain et al., 1986; Ley et al., 1991). Cortisol has specifically been used as an index of physiological distress, and marked rises in cortisol concentrations have been associated with a variety of surgical procedures conducted under anaesthesia in humans (Hashimoto and Migita, 1979), dogs (Frank et al., 1992), sheep (Pearson and Mellor, 1975) and goats (Pearson and Mellor, 1975). Such cortisol rises have been shown to occur during and after the administration of most anaesthetic agents, and are further increased with surgery (Matthews et al., 1992). Surgically induced increases in cortisol reportedly relate to the severity of the operative trauma, and are much greater during intra-abdominal surgery than during body surface procedures in humans, horses and dogs (Clarke, 1970; Schmidt and Booker, 1982; Taylor, 1985).

These observations suggested that changes in plasma cortisol concentrations would be a useful index of surgical pain-induced distress in 'spayed' bitches. However, before cortisol could be utilised in this manner for the present study, it was first necessary to define the cortisol responses in bitches to a range of 'base' treatments not involving surgery. Thus, by defining the cortisol responses of bitches to the administration of various combinations of anaesthesia and analgesia without surgery, cortisol responses to similar analgesia and anaesthesia plus surgery could be elucidated. The present study was thus conducted to evaluate plasma cortisol response to various combinations of halothane-in-oxygen anaesthesia and butorphanol analgesia.

## Materials and Methods

### *Animals*

Sixteen New Zealand cross-bred working bitches with an average age of 20 months were used in addition to bitches used in the pilot study (Appendix D). Each bitch was fasted overnight before undergoing one to three of the six treatments noted below. The interval between treatments in bitches which received more than one treatment was approximately eight weeks. The treatments were:

- \* (1) deliberate Control (11 bitches),
  - 6 bitches were the same as in the pilot study,
- \* (2) Anaesthesia (11 bitches),
  - 6 bitches were the same as in the pilot study,
- \* (3) Analgesia (11 bitches),
  - 6 bitches were the same as in the pilot study,
- \* (4) Analgesia plus Anaesthesia (10 bitches),
- \* (5) Anaesthesia plus Immediate Analgesia (at intubation) (10 bitches),  
and
- \* (6) Anaesthesia plus Analgesia (at extubation) (10 bitches).

All treatments were conducted in parallel over the entire period of data collection so as to minimise any influence of seasonal effect. The allocation of bitches to treatments and the order of treatments was random.

Physical examination revealed that the bitches were healthy. Ethical constraints restricted bitches receiving these treatments to non-pet animals, which were housed in an indoor-outdoor rural facility<sup>2</sup> and brought to Massey University Veterinary Hospital for 48 hours to undergo each treatment, after which they were returned to the rural facility.

All bitches were admitted to the hospital during the afternoon of day one and were placed in an indoor cage (840 cm x 942 cm x 790 cm) within a restricted access, but not isolated, ward. One of the treatments

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<sup>2</sup> Massey University Animal Health Services Centre

was commenced at approximately 10:00 AM on day two, and the bitches were returned to the rural facility after noon on day three. This sequence was followed for all treatments.

### *Treatments*

**Deliberate Control (Con):** The bitch was moved from the ward to the anaesthesia induction room, a cephalic vein catheter was placed, and a blood sample was taken. The bitch was gently restrained on a table in the presence of low activity (traffic of three to four persons) for approximately 53 minutes, and was then returned to the ward. Throughout the following five hour period of observation, blood sampling was performed at the regular intervals indicated in Table 2.1. Blood collection was via the catheter until it became blocked or was dislodged by the animal, after which samples were taken by cephalic venipuncture (22 gauge needles) in the contralateral limb.

**Anaesthesia (Ans):** After arrival in the induction room and cephalic vein catheterisation, without preanaesthetic medication, anaesthesia was induced with barbiturate (2.5% sodium thiopentone, approximately 20-25 mg/kg) to a depth allowing intubation, after which a blood sample was taken through the cephalic vein catheter (Table 2.1). Surgical plane anaesthesia was then maintained with halothane in oxygen at a Fluotec setting of 2 for about 47 minutes.

**Analgesia (Anl):** After arrival in the induction room and blood sampling via a cephalic vein catheter, 0.4 mg/kg of butorphanol (Torbugesic<sup>3</sup>, Fort Dodge Laboratories, Fort Dodge, Iowa) was administered intravenously (bolus). The bitch was thereafter gently restrained on the table in the presence of low activity for approximately 51 minutes before being returned to the ward. Blood samples were taken as indicated in Table 2.1.

**Analgesia plus Anaesthesia (Anl/Ans):** Thirty minutes prior to presentation in the induction room 0.4 mg/kg of butorphanol was administered intravenously via a cephalic vein catheter. The bitch was

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<sup>3</sup> Torbugesic: Lot # 454127

then allowed to rest in the ward cage until movement to the induction room and subsequent handling as with the Anaesthesia group. Throughout the following five hour period of observation blood sampling was performed at regular intervals (Table 2.1).

Anaesthesia plus Immediate Analgesia (Ans/I Anl): The procedure was the same as that in the Anaesthesia group, except that 0.4 mg/kg of butorphanol was administered intravenously immediately following intubation within the anaesthesia induction room (Table 2.1).

Anaesthesia plus Analgesia (Ans/Anl): The bitches were treated in the same way as the Anaesthesia group, except that they received 0.4 mg/kg of butorphanol intravenously at the time of extubation, after the bitch was returned to the ward cage (Table 2.1).

Zero minutes (Table 2.1) marked the beginning of each treatment.

- The Analgesia group received butorphanol at 34 minutes.
- The Analgesia plus Anaesthesia group received butorphanol at 0 minutes.
- The Anaesthesia plus Immediate Analgesia group received butorphanol at 40 minutes.
- The Anaesthesia plus Analgesia group received butorphanol at 91 minutes.

Commencing with the 121 minute blood sample (30 minutes after extubation), the abdomen was gently palpated immediately prior to each blood collection. Palpation was part of the protocol for a video-taped behaviour study (Chapters 4 and 5) conducted in parallel with this cortisol study. The interval between the beginning of palpation and the end of the blood sampling was approximately two to three minutes.

### ***Cortisol Assays***

Blood samples (5 mls) were collected in lithium heparinised vacutainers, centrifuged and the plasma stored at -20 °C until required. Total plasma cortisol concentrations were determined by radio-immunoassay (Appendix A), the lowest detectable concentration being 0.22 ng/ml and



the intra-assay and inter-assay coefficients of variation being 16% and 22%, respectively for plasma standards of 1, 16 and 19 ng/ml.

### *Statistical Analyses*

Scheffé's F test was used to differentiate the plasma cortisol responses to each of the treatments. Selection of this test was based upon its robustness to assumption violations and its conservatism across treatment comparisons. T-test analysis was used to examine return of plasma cortisol concentrations after each treatment to pretreatment concentrations. Although some formal statistical test results are reported, the emphasis is on describing and comparing the patterns of change in cortisol concentrations. Experimental design excluded repeated measurements analysis.

In order to give a single measure of both the magnitude and the duration of increases in plasma cortisol concentrations after a treatment, the integrated cortisol responses were calculated. The integrated response is defined as the area between a horizontal line drawn through the pretreatment concentration and the cortisol curve during the period when the concentrations were greater than the pretreatment value (Mellor and Murray, 1989).

### **Results**

No clinical complications were detected at any stage of the study and there were no consistently high or low responders with respect to plasma cortisol concentrations. The pretreatment concentrations (mean  $\pm$  SEM) on day two (Figures 2.1-2.4) were:  $17 \pm 3.4$ ,  $17 \pm 2.2$ ,  $23 \pm 4.4$ ,  $26 \pm 4.7$ ,  $29 \pm 5.1$ , and  $25 \pm 3.6$  ng/ml for the Control (n=11), Anaesthesia (11), Analgesia (11), Analgesia plus Anaesthesia (10), Anaesthesia plus Immediate Analgesia (10), and Anaesthesia plus Analgesia (10) groups, respectively.

In Control bitches there was no significant change in plasma cortisol concentrations between the pretreatment sample (0 minutes) and the first sample taken in the induction room (34 minutes). Plasma cortisol

concentrations rose significantly after 34 minutes until the time that the bitches were returned to their ward cage, after which values returned to pretreatment levels where they remained for the duration of the observation period (Figure 2.1).

Plasma cortisol concentrations for the Anaesthesia group did not change significantly throughout the course of the sampling period (Figure 2.2).

Plasma cortisol concentrations for the Analgesia alone group showed a pattern similar to that of the Controls until 90 minutes: an insignificant rise before 34 minutes, but a marked rise between 34 and 90 minutes. Cortisol concentrations reached a peak at 121 minutes. Thereafter, the mean concentration began to fall, reaching a significantly ( $P<0.05$ ) lower value at 180 minutes than at the peak. In contrast to the Control bitches which showed a significant ( $P<0.05$ ) fall in cortisol concentrations in the sample taken after they returned to their cages, the Analgesia group did not show a significant fall until 90 minutes after returning to their cages. Peak plasma concentrations were observed at approximately 85 minutes after butorphanol administration and the concentrations did not return to pretreatment levels until approximately four hours (235 minutes) after the butorphanol administration. Thereafter plasma concentrations for the Analgesia group were similar to those of the Control group (Figure 2.3).

The Analgesia plus Anaesthesia group showed an immediate rise in cortisol concentrations after analgesic administration at 0 minutes. However, after the administration of anaesthetic, concentrations fell to values which were numerically above the pretreatment values, but they were not significantly different from these pretreatment values (Figure 2.4). Additionally, there was a rise in concentration between 181 and 391 minutes, but this too was not significant.

In the Anaesthesia plus Immediate Analgesia group plasma cortisol concentrations fell between zero and 34 minutes, when the animals were introduced to the anaesthesia induction room. The concentrations continued to fall after the administration of butorphanol, at 40 minutes, until 30 minutes after extubation. Then the cortisol concentrations rose above pretreatment values where they remained for the rest of the

observation period. They reached peak values at 271 minutes (approximately 160 minutes after extubation and 231 minutes after butorphanol administration)(Figure 2.4).

In the Anaesthesia plus Analgesia group cortisol concentrations were lower after anaesthesia than they were before anaesthesia. Plasma concentrations began to rise 60 minutes after extubation and continued to rise, reaching a peak at approximately three hours (160 minutes) after extubation. At 391 minutes (five hours after extubation) cortisol concentrations began to fall (Figure 2.4).

After extubation there was no difference in plasma cortisol concentrations between the Anaesthesia plus Immediate Analgesia and the Anaesthesia plus Analgesia groups until 151 minutes (Figure 2.4). After 151 minutes the plasma cortisol concentrations of the Anaesthesia plus Analgesia group rose to peak at a significantly ( $P<0.05$ ) higher level than concentrations of the Anaesthesia plus Immediate Analgesia group. The integrated cortisol response from 151 to 391 minutes was significantly different ( $P<0.05$ ) between these two groups:  $59.4\pm21.43$  for Anaesthesia plus Immediate Analgesia and  $163.2\pm36.29$  for Anaesthesia plus Analgesia (Figure 2.5). Likewise, compared to the Anaesthesia plus Analgesia group the plasma cortisol concentrations between 151 and 391 minutes in the Analgesia plus Anaesthesia group were significantly lower ( $P<0.05$ ) and the corresponding integrated cortisol response was also lower ( $P<0.05$ ). In all groups plasma cortisol concentrations had returned to pretreatment values at 24 hours.

#### Summarising original results:

1. Whereas cortisol concentrations rose in the Control and the Analgesia groups, the Anaesthesia bitches showed no change in plasma cortisol concentrations. Cortisol concentrations returned to pretreatment values by 151 minutes in the Control group and 271 minutes in the Analgesia group.
2. In the three groups given both analgesic and anaesthetic, anaesthesia effected a fall in plasma cortisol concentration that was sustained for a period of no less than 30 minutes after extubation. After 151 minutes (one hour after extubation) subsequent elevations in cortisol concentrations were sustained

at a higher level in the group which received butorphanol at extubation. In the group given analgesic, subsequent anaesthetic administration induced a fall in cortisol concentration that was not seen when anaesthesia was not given. When analgesic was given after anaesthesia was induced or while the animal was still under the strong influence of anaesthesia (Anaesthesia plus Analgesia group) there was no immediate rise in cortisol.

## Discussion

The work described in this chapter demonstrated a responsiveness of plasma cortisol concentrations to several different treatments, and established various 'base' data for the interpretation of results from additional surgical treatments to be discussed in Chapter 3.

On this basis it is suggested that the major influences of halothane anaesthetic and butorphanol analgesic on plasma cortisol concentrations were:

- firstly, compared to Controls, because anaesthetised bitches were not conscious of surrounding novel experiences, halothane-in-oxygen anaesthesia effected a decrease in plasma cortisol concentration;
- secondly, elevated cortisol concentrations from the administration of the agonist-antagonist synthetic opioid butorphanol, in the conscious bitch, are consistent with that of a drug exhibiting sigma receptor activity and associated dysphoria;
- thirdly, the influence of butorphanol on plasma cortisol concentrations is suppressed by halothane anaesthesia for a period of up to 60 minutes after extubation;
- and finally, the duration of effect of butorphanol on plasma cortisol concentrations is related to its biological clearance: marked elevations in cortisol concentrations were seen for up to four to five hours, but returned to pretreatment values by 24 hours.

Plasma corticosteroid concentrations are most frequently used as a measure of distress (Moberg, 1987). Behavioural, autonomic nervous or neuroendocrinologic responses can be observed simultaneously with

elevations of plasma cortisol concentrations within one minute of stimulation (Knol et al., 1992), and basal plasma cortisol values have been shown to be significantly lower for dogs tested in their home environment compared to dogs tested in an unfamiliar veterinary hospital (Vial et al., 1979).

The rise in plasma cortisol concentration for Control bitches during their stay in the anaesthetic induction room (34-90 minutes) may therefore be attributed to the conscious 'novel experiences' of unfamiliar surroundings and low traffic flow of unfamiliar personnel. This is supported by two observations:

- (1) the plasma cortisol concentrations returned to pretreatment values once the Control bitches were returned to their familiar ward cage (Figure 2.1), and
- (2) at the stage when cortisol concentrations in the Control group rose, those of the Anaesthesia group which were unconscious and therefore emotionally unresponsive, remained unchanged (Figure 2.2).

Further, there is a large body of evidence (Mason, 1968; Hennessy and Levine, 1979) showing that animals of many species respond with a rise in glucocorticoid levels when placed in a novel environment.

Increases in plasma concentrations of glucocorticoids are considered to be an integral component of the general hormonal response to anaesthesia in man (Taylor, 1987; Napolitano and Chernow, 1988). However, it appears that short-term (<1 hour) general anaesthesia in dogs, with the techniques used in this study, caused either no change (Figure 2.2) or a significant decrease in plasma cortisol concentration (Figure 2.4). The findings of Kruse-Elliott and others (1987) conflict with these observations. They reported increased cortisol concentrations in dogs in which anaesthesia was induced with thiopental sodium and maintained with halothane and oxygen, but in their study it is not clear if surgery had commenced at the time blood was sampled for cortisol assay.

The effects of general anaesthesia on human adrenocortical function remain controversial. Halothane significantly increased plasma cortisol levels before surgery in two studies (Oyama et al., 1968; Murakawa et

al., 1987) while a third study conducted by Frieling and Brandt (1985) suggested halothane-oxygen anaesthesia depressed adrenocortical function. Freiling and Brandt state that a review of the literature showed that findings contrary to theirs may be due to concomitant use of N<sub>2</sub>O or the distress of intubation.

The plasma cortisol response to anaesthesia seen here in the bitch is markedly different to that in the horse. Gaseous anaesthesia in the horse induces an increase in plasma cortisol concentration (Taylor, 1989). Of all species the horse is especially prone to a variety of complications during anaesthesia such as hypercarbia, hypoxaemia, hypotension and relative hypovolaemia that could be responsible for producing responses in cortisol (Muir, 1990).

Reasons for the rise in plasma cortisol concentrations prior to 121 minutes in the Analgesia group (Figure 2.3), a rise greater than the peak seen in the Control group, are not clear. There are four likely explanations:

- 1) The plasma cortisol concentrations were elevated in response to direct hormonal effect(s) from butorphanol administration. There may be a direct stimulatory effect on the adrenal cortex or hypothalamus, unrelated to potentially unpleasant side effects of butorphanol, although there is apparently no specific information in the literature on this point and it is highly unlikely since opioids act in the hypothalamus to inhibit the release of both corticotropin-releasing factor (CRF) and adrenocorticotropin (ACTH)(Jaffe and Martin 1992).
- 2) The cortisol concentrations were elevated as an indirect consequence of neuro-pharmacological effects of butorphanol on opioid receptor sites. Although the indirect effect of the agonist-opioid morphine is suppression of cortisol (Jaffe and Martin, 1992), responses to the agonist-antagonist butorphanol have not apparently been documented.
- 3) The rise in plasma cortisol concentration might be due to reflex responses to increased carbon dioxide or to hypotension, similar to secondary consequences seen during morphine-induced ventilatory depression and/or vasodilation (Caruso et al., 1979). However,

hypotension is not association with butorphanol administration in the dog.<sup>4</sup>

- 4) The rise in plasma cortisol concentration may be a hypophyseal-adrenal axis response to the psychogenic consequences (dysphoria) of butorphanol administration. Support for this explanation is provided by the observation that butorphanol is a sigma receptor agonist associated with dysphoria in humans (Caruso et al., 1979). Accordingly, an induced dysphoria may give rise to an anxiety-like state as described for humans by Chapman (1978). This is considered to be the most likely explanation, as described below.

The decline in plasma cortisol concentration after 121 minutes in the Analgesia group suggests that any effects of butorphanol on systemic cortisol concentration were dissipating. Predicted intravenous pharmacokinetics using a one compartment model (Pfeffer et al., 1980) reveal that the concentration of butorphanol at 121 minutes would have been approximately 29 mg/l, nearly half the predicted zero time concentration of 53 mg/l. Although clinical analgesic effects of butorphanol are reported to be immediate following its intravenous administration (Jaffe and Martin, 1992), plasma cortisol responses peaked at 121 minutes, approximately 1.25 biological half-lives of butorphanol after its administration; the cortisol concentrations returned to pretreatment values after 211 minutes; about 2.2 butorphanol biological half-lives. It is evident that the patterns of change in the plasma concentrations of butorphanol and cortisol would have been different. In contrast to the immediate rise in plasma concentration of butorphanol after intravenous administration and thereafter an exponential decay (assuming a one compartment model), the plasma cortisol concentration rose to a peak reached at 121 minutes and then declined slowly over the next two to four hours.

Results from this investigation suggest that butorphanol, acting via sigma receptors, induces a dysphoric state through the limbic system of the CNS. Thereafter the CNS activates feed-back mechanisms, including activations of the sympathetic nervous system, which generate multiple sources for stimulation of cortisol release (Figure 2.6).

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<sup>4</sup> Personal communication. Professor Donald C. Sawyer, DVM, PhD: Michigan State University, USA.

Anaesthesia appears to block this cascade at the level of the limbic system.

The Analgesia plus Anaesthesia group showed a significant rise in cortisol concentration from the time of analgesic administration within the ward cage to the time of blood sampling on arrival in the induction room (Figure 2.4); a rise very similar (34 ng/ml vs 32.2 ng/ml) to that seen in the Analgesia group immediately following butorphanol administration (Figure 2.3). This rise between 0-34 minutes could reflect environmental stimuli from the change of location; however, since the Control bitches showed no significant rise in plasma concentrations having experienced the same environmental changes moving to the induction room, this rise in plasma concentration may be attributed to the influence of butorphanol.

Based upon observations from the Analgesia alone group (Figure 2.3), the cortisol concentrations of the Analgesia plus Anaesthesia group (Figure 2.4) would have been expected to continue to rise beyond the 40 minute point if anaesthesia had no effect. However, once consciousness was lost due to anaesthesia in the Analgesia plus Anaesthesia group, cortisol concentrations returned to pretreatment values.

In the absence of an influence from anaesthesia on plasma cortisol concentration, both the Anaesthesia plus Immediate Analgesia and the Anaesthesia plus Analgesia groups should have shown an immediate rise in cortisol concentrations after butorphanol administration. This was not the case, and a marked influence of anaesthesia on cortisol concentration is demonstrated in the suppression of such a rise. This influence of anaesthesia appeared to prevent any rise of cortisol in both the Anaesthesia plus Immediate Analgesia and the Anaesthesia plus Analgesia groups until 60 minutes after extubation (Figure 2.4). Despite the fact that the predicted butorphanol concentrations would have been much lower in the former than in the latter group, neither group showed a rise in cortisol concentration that was above the value at the time of extubation until 60 minutes after extubation (at 151 minutes).



For all three treatments involving the combinations of anaesthesia and analgesia (Figure 2.4) the plasma cortisol concentrations 30 minutes after extubation were significantly ( $P < 0.05$ ) lower than the concentrations immediately following intubation. This uniform observation using the index cortisol supports the previously cited findings of Frieling and Brandt (1985) that halothane-oxygen anaesthesia depressed adrenocortical function, and additionally suggests that the effect of anaesthesia is sufficient to 'override' the butorphanol effect on plasma cortisol concentration. Apparently, this overriding effect of anaesthesia is dissipated by 60 minutes after extubation (151 minutes) as plasma cortisol concentrations then began to rise.

The antagonistic effect of halothane-in-oxygen anaesthesia on plasma cortisol concentration responses to butorphanol, which apparently lasts for approximately 60 minutes after the withdrawal of the volatile gas anaesthesia may be (conjecturally) explained in several ways:

- 1) A dominance phenomenon of the anaesthetic could result from agonist concentration at the site(s) of pharmacological receptors. Over-abundance of active anaesthetic moieties may take command of subsequent physiological responses. Further, these responses may not dissipate until moiety concentrations fall to a given level from expiration of the volatile anaesthetic gases.
- 2) Anaesthetic gases may transiently change the conformation of pharmacological receptors (such as  $\sigma$  receptors) rendering them ineffective to butorphanol for a given period of time. Only after these opioid receptors return to their original conformation could they then respond in the anticipated manner. There are no apparent studies in the literature to illuminate this proposal.
- 3) A third explanation is also possible: the anaesthetic and analgesic may together form a complex that acts uniquely at the receptor(s). This complex may give rise to the observed fall in cortisol concentration, a response that was not seen with administration of anaesthesia alone (Figure 2.2). However, this possibility is refuted by recognition that in the Anaesthesia plus Analgesia group butorphanol was given as halothane anaesthesia had dissipated. The design of this study did not allow further exploration of this hypothesis.

- 4) If the rise in plasma cortisol concentration caused by butorphanol were predominantly psychogenic, the loss of consciousness due to anaesthesia would be expected to dissipate this psychogenic response, and it would be expected to rise as consciousness is recovered.

This fourth explanation of cause-and-effect of consciousness on plasma cortisol concentration is favoured for both its simplicity and conformance to what is known regarding the central nervous system's response of 'fight or flight' as described by Selye (1936). If a spiked rise in plasma cortisol concentration is seen in response to acute psychological distress, it is logical to infer an absence of cortisol response in the void of psychological challenge.

It is highly unlikely that thiopentone contributed to the post-extubation lingering effect of anaesthesia on cortisol concentrations. Since the short duration of thiopentone-induced anaesthesia is due to the rapidly declining levels of the drug in the brain, which can be attributed to redistribution, the biological half-life (initial phase of disposition:  $14.9 \pm 3.3$  minutes) would be expected to approximate the duration of anaesthesia (Brandon and Baggot, 1981). At the time of extubation approximately four biological half-lives of thiopentone would have passed.

Clinically, the recovery from gaseous anaesthesia is rapid, depending upon such factors as solubility of the anaesthetic used and fat depots in the patient. Using nuclear magnetic resonance techniques, Wyrwicz and others (1983) have found that substantial amounts of halothane, or a breakdown product of halothane, remains in the brain of rabbits for as long as 98 hours after the administration of 1 percent halothane for a duration of 30 minutes. Halothane metabolites most likely dominated these results, as autoradiographic studies indicate that 90 percent of the halothane introduced into the gray matter of the brain of monkeys by a brief anaesthetising exposure disappeared from the brain within 20 minutes after exposure (Cohen et al., 1972). Results from the present study show that although bitches may show early behavioural signs of recovery from halothane anaesthesia, as is documented in later chapters,

halothane suppression of a butorphanol-induced rise in plasma cortisol remains for at least 30 minutes after exposure.

Saidman and Eger (1964) have shown that after opioid administration, less inhalant agent is required to maintain anaesthesia- a so called 'anaesthetic-sparing' effect. This reduction is measured as a lowering of the minimum alveolar concentration (MAC) for that inhalant (ie., MAC-sparing effect). In dogs, the agonists morphine and fentanyl have an enflurane-sparing effect (Murphy and Hug, 1982a; Murphy and Hug, 1982b).

The period over which anaesthesia was administered in this study was not supervised by trained anaesthesia personnel. Consequently, a consistent plane of anaesthesia for all bitches could not be ensured. Routinely, a surgical plane of anaesthesia was established and anaesthetic administration was thereafter unaltered until the end of the anaesthetic period. The argument may be proposed that in this study butorphanol administration to a bitch under the influence of halothane anaesthesia may have been MAC-sparing, and should have dictated lowering the dose of halothane. Without doing so, an excessive amount of volatile gas anaesthetic would have been inspired by the patient. Since blood pressure, body temperature, respiration rate, heart rate and end tidal anaesthetic concentrations were not measured, this proposal cannot be clarified. However, in contrast to the enflurane-sparing effect of the mu-agonists morphine and fentanyl, one study (Quandt et al., 1994) has shown that butorphanol does not reduce the minimum alveolar concentration of halothane in dogs. Further, the simultaneous rise of plasma cortisol concentrations in the Anaesthesia plus Immediate Analgesia and Anaesthesia plus Analgesia groups at 121 minutes (Figure 2.4) would suggest that in these two groups, at least, anaesthesia was consistent.

Cortisol responses to the three treatments involving both analgesic and anaesthetic may best be discussed by considering observations before and after 151 minutes. Between 0 and 151 minutes the Analgesia plus Anaesthesia cortisol curve of Figure 2.4 stands alone, while the curves of the Anaesthesia plus Immediate Analgesia and the Anaesthesia plus Analgesia groups appear to be quite similar. After 151 minutes the

integrated cortisol response for the Anaesthesia plus Analgesia group was greater than that for the Anaesthesia plus Immediate Analgesia group. This could be attributed to the greater predicted systemic concentrations of butorphanol at that time in the former group as well as the greater 'contact time' of butorphanol and the anaesthetic. If the difference in plasma cortisol concentration between the two groups at time 181 minutes was a consequence of different systemic butorphanol concentrations, then this difference would have to be attributable to a difference of no more than 9-10 mg/l between the two groups, as predicted by IV pharmacokinetics (Pfeffer et al., 1980). There are no data in the literature by which to make comparisons of systemic butorphanol concentrations and plasma cortisol concentrations.

Reportedly, the action of butorphanol is immediate after IV administration (Jaffe and Martin, 1992) and the mean serum half-life is 1.62 hours in dogs (Pfeffer et al., 1980). Sedation is reported to decrease after 30 minutes and become absent after 60 minutes (Trim, 1983). The data from the present study demonstrated that elevations in cortisol concentration were far more prolonged (Figure 2.5) than the cited clinical observation of sedation.

Cortisol responses to the Control treatment of this study were predictable, considering its use as an index in response to environmental changes. Among several insights provided by the Control group is the observation that the interactive palpation immediately preceding blood sampling, apparently, had minimal influence on plasma cortisol concentrations. In contrast to the Controls, cortisol responses in bitches undergoing the Analgesia and the Anaesthesia treatments were unexpected. Explanation of these responses are open for interpretation because little is known regarding the action of anaesthetics and analgesics on the central nervous system, and therefore, on cortisol response. However, investigation and proposed explanations of the plasma cortisol responses to the treatments of this study were necessary before the responses to treatments involving surgery and perioperative analgesia could be explored.

Cortisol rises due to the treatments of this study probably result from cognitive phenomena, in agreement with the beliefs of Mason (1975),

and Dantzer and Mormède (1981, 1983) that the amount of psychological distress that an animal experiences determines the extent of the pituitary-adrenal axis response. It will remain unknown if the experiences were unpleasant. In the chapter to follow, bitches experienced nociceptor inputs while the animal was in a state of unconsciousness from anaesthesia. Within this newly imposed condition of surgery the response of cortisol to nociceptor barrage was observed, unaffected by a cognitive component until such time as the animal regained consciousness. After regaining consciousness the collective effects of both the nociceptor inputs and cognitive recognition on cortisol response were measured.

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### **Chapter 3**

#### **Effects of presurgical and postsurgical analgesia on plasma cortisol concentrations in ovariohysterectomised bitches**

##### **Chapter Summary**

Plasma cortisol responses were assessed in response to four treatments: Control, Anaesthesia plus Surgery, Analgesia plus Anaesthesia plus Surgery and Anaesthesia plus Surgery plus Analgesia. A marked rise in plasma cortisol concentration was associated with surgery in all groups; a rise which was sustained above pretreatment values for greater than five hours after surgery. Giving butorphanol 30 minutes prior to surgery had no effect on the surgically-induced rise in plasma cortisol concentration and no effect on the postsurgical plasma cortisol concentration. In contrast, administration of butorphanol at extubation did reduce plasma cortisol concentrations in the postsurgical period.

Differences in plasma cortisol concentration ascribed to the response from different treatments were interpreted as reflecting the levels of pain-induced distress experienced by the respective groups. Lower postsurgical cortisol concentrations in the Anaesthesia plus Surgery plus Analgesia group were attributed to the analgesic properties of butorphanol. Prior to commencement of this study it was anticipated that the preemptive analgesia group (Analgesia plus Anaesthesia plus Surgery) would show a major difference from the Anaesthesia plus Surgery group in cortisol response to surgery; however, this was not the case. It is suggested that this unexpected response is due, at least in part, to administration of the butorphanol outside its most efficacious use, ie. it was given too early before surgery by the route of intravenous administration.

Three distinct phases with different stimuli for cortisol response were identified for all bitches undergoing surgery. First was the period preceding the induction of anaesthesia, where bitches were exposed to changing environmental influences and associated novel experiences including butorphanol induced dysphoria. Second was the period of anaesthesia-induced unconsciousness and associated surgical-induced

noxious nociceptor stimulation. Finally, there was the postsurgical period of regained consciousness during which the bitch was responding to a combination of stimuli: surgically-induced noxious stimuli, cognitive or 'emotional' responses to that 'percieved' pain, and any other novel experiences. Plasma cortisol concentrations observed in this study reflected the different distressful stimuli experienced by bitches during these different periods.

## Introduction

Advances in laboratory methodology have led to the development of catecholamine assays to demonstrate conclusively that pain and specific surgical events in humans induce transient catecholamine increases (Hoar et al., 1980; Taborsky et al., 1982; Davies et al., 1984) and it is well recognised that noxious stimuli such as skin incision elicit an adrenergic response characterised by increased plasma concentrations of noradrenaline and adrenaline (Hoar et al., 1980; Stanley et al., 1980). This response can be prevented or decreased by increased anaesthetic depth or administration of analgesic agents (Hoar et al., 1980; Stanley et al., 1980; Flacke et al., 1983). With an appreciation that the methodology of cortisol assays is less demanding than that of catecholamines, the following study was conducted in an attempt to characterise pain-induced distress by plasma cortisol responses from ovariohysterectomy surgery in the bitch.

A number of hypothalamic-pituitary hormones, including corticotropin-releasing factor, vasopressin, oxytocin and brain opioid peptides are involved in the distress response. An enhanced sympathetic and depressed parasympathetic activity develops in distress responses, resulting in increased circulating amounts of adrenaline, noradrenaline, and enkaphalin peptides. Sympathetic activity also results in increased amounts of circulating vasopressin and substance P (Breazile, 1987).

The reaction of the hypothalamic-pituitary-adrenal axis (HPA) to environmental alterations (Vial et al., 1979; Chastain et al., 1986; Frank et al., 1992; Knol et al., 1992; Vincent and Michell, 1992;), anaesthesia (Church et al., 1994), and surgery (Campbell and Watts, 1973; Schmidt and Booker, 1982; Matthews et al., 1992; Church et al., 1994) has been

studied infrequently in the dog. However, cortisol is a commonly used index of distress in both humans and animals and is frequently assayed in evaluation of the activity of the HPA axis (Herd, 1966; Domzal et al., 1983; Chastain et al., 1986; Ley et al., 1991). Marked rises in cortisol concentrations of plasma have been associated with a variety of surgical procedures conducted under anaesthesia in humans (Hashimoto and Migita, 1979), dogs (Frank et al., 1992), sheep (Pearson and Mellor, 1975) and goats (Pearson and Mellor, 1975). A cortisol rise is associated with the administration of most anaesthetic agents (although this premise is contrary to those findings reported in Chapter 2), and is further increased with surgery (Matthews et al., 1992). Surgically induced increases in cortisol have been related to the severity of the operative trauma, being much greater during intra-abdominal surgery than during body surface procedures in humans, horses and dogs (Clarke, 1970; Schmidt and Booker, 1982; Taylor, 1985).

With the recognition of cortisol as an effective index of pain-induced distress in humans, and having established plasma cortisol responses to combinations of analgesia and anaesthesia (Chapter 2), the following study was performed to investigate the effects on plasma cortisol of pre- and postsurgical analgesic administration in bitches undergoing ovariohysterectomy. However, before proceeding to describe the study it is necessary to present additional background material in order to provide more details about the context of this work.

### *Neuronal Transmission and Anaesthesia*

The details of synaptic transmission within the CNS have generally been identified (Bonica, 1990a). The principal events may be summarised as follows: a nerve cell receives information by way of synaptic contacts all over the dendrites and cell body. Impulses travelling in the presynaptic fibres invade the terminal branches to depolarise the nerve endings. This depolarisation leads to the opening of the voltage-gated calcium channels and an influx of calcium into the terminal. The subsequent increase in intracellular calcium is the trigger for secretion of transmitter substance from the nerve terminal. The released transmitter diffuses across the synaptic cleft and binds to specific receptor sites on the postsynaptic membrane. Activation of the receptors results in a change in the

permeability of the membrane to particular ions, which in turn leads to a change in membrane potential and to excitation or inhibition of the next neurone of the network, depending on the nature of the synapse considered. Many CNS transmitters activate second messenger systems (adenylate cyclase-cAMP and inositol polyphosphate-[Ca<sup>2+</sup>]).

Neurotransmitter systems have been subdivided according to their classification into (1) amino acids (aspartate, glutamate, gamma-aminobutyrate (GABA) and glycine), (2) other classical transmitters ((catecholamines, dopamine, noradrenaline and adrenaline, 5-hydroxytryptamine (5-HT)) and acetylcholine) and (3) neuropeptide systems (McMahon and Nicholls, 1991). In view of their wide distribution and suggested roles in the CNS, the excitatory amino acid transmitter systems constitute an important potential target for anaesthetic action. GABA is the major inhibitory neurotransmitter in the CNS (Horton, 1989). By acting on both pre- and postsynaptic receptors, GABA causes an increase in chloride ion conductance which results in hyperpolarisation of the nerve membrane. 5-HT has been implicated as an important transmitter in the feeding, sleep, mood, behaviour and cardiovascular control pathways and acetylcholine is believed to play an important role in the control of consciousness (Althaus et al., 1985). Unfortunately, present knowledge of the effects of particular anaesthetic agents on discrete presynaptic activities is for each agent dependent on only one or two studies. Often these have been carried out using differing experimental systems making the comparison of results equivocal.

Anaesthesia may be the net result of a combination of a large number of nonspecific toxicological effects on CNS function that any particular agent exerts. If so, the 'physiological mechanism' of anaesthesia may well be different for any given agent (Griffiths and Norman, 1993). Nevertheless, influx of extracellular calcium is essential for the coupling of electrical excitation to neurotransmitter release, and release of different neurotransmitters may rely on different phases of calcium transport (Cohen and Van der Kloff, 1985). Documented effects of halothane on specific neurotransmitter release is summarised in Table 3.1.

### *Obtunding Distress*

Noxious stimuli such as those associated with surgery elicit a cascade of physiological responses (Figure 3.1), and resultant pain-induced distress may evoke harmful responses that interfere with well-being and comfort, and are capable of inducing overt pathological changes (e.g. severe postsurgical distress can result in a catabolic state leading to death). Many responses to noxious stimuli originate in the central nervous system utilising a number of neurological and neuroendocrine mechanisms, as illustrated in Figure 3.1, and in addition, some responses are elicited by somatic cells interacting with the nervous system and may even be elicited without neural involvement (Breazile, 1987). Selective modulation of these mechanisms holds potential for controlling pain-induced distress and thereby animal welfare.

Figure 3.2 depicts a simplified anatomic scheme representing interactions associated with pain-induced distress. The thalamus serves as an entry for essentially all sensory nervous signals (excepting olfactory) originating from the transmitter (T) cell to the cerebrum. It is the primary relay from peripheral receptors to the cerebral cortex. Associated with the thalamus are collateral fibres connecting the limbic and reticular systems. The reticular system integrates the experience or cognitive component of a distress response, while the limbic system is associated with the emotional component (Breazile, 1987). Collateral fibres from the thalamus are also integrated with the locus coeruleus and the periaqueductal gray nucleus, both of which influence descending anti-nociceptive pathways.

The limbic system of the brain is central to the organisation of most distress responses (Breazile, 1987) (Figure 3.2). The hypothalamus serves as an integral part of the limbic system, providing its principal outflow through influence on hypophyseal endocrine and autonomic neural control mechanisms. As such the hypothalamus represents a brain centre in which the regulation of several 'distress' hormones is co-ordinated with the organised activity of the autonomic nervous system (Figure 3.1) in eliciting a wide variety of animal behaviours (Chapters 4 and 5).

In laboratory animals and humans, responses to noxious stimuli of surgery are mediated by neural impulses, including those arising from

injury at the surgical site and acting via the hypothalamus. The response can be abolished either by regional anaesthesia of the relevant area or by suppression of the hypothalamus and cerebral cortex with injectable anaesthetics (Morishima et al., 1980; Anand et al., 1987; Sanhoury et al., 1991) (Figure 3.3).

A number of sites have potential for modulation of pain-induced distress as depicted in the simplified illustration of Figure 3.3. Local anaesthetics block the peripheral nerve or peripheral receptor, thereby defusing the cascade of events initiated by a noxious stimulus. The periaqueductal gray nucleus has been shown to contain a large component of opioid receptors, and opioids are effective in blocking transmission from the limbic system to the cerebral cortex. This latter blockade dampens the emotional component of distress making the pain-induced distress more tolerable. The locus coeruleus contains a high concentration of opioid and noradrenergic receptors so that a state of sedation and some analgesia may be induced in the animal with  $\alpha_2$ -agonists. The periaqueductal gray nucleus is also responsive to  $\alpha_2$ -agonists. Tranquillisers are most effective in dampening cerebral cortex input from the reticular system, while dissociogenics induce a cataleptoid state with immobilisation but not relaxation, and yield incomplete analgesia.

Surgical stimulation in humans results in increased plasma noradrenaline, and it is suggested that analgesic agents that suppress its release do so via decreased perception of pain (Hargreaves et al., 1986). In dogs, plasma catecholamine concentrations increase during laparotomy similar to the response observed in humans (Taborsky et al., 1982), and analgesics for humans are often administered to dogs and cats, either on the basis of scientific evidence or, more often, with the view that if it works in humans it will be effective in the dog or cat.

Although the mechanisms of many analgesic drugs are becoming clarified, the molecular basis for the action of most anaesthetic agents remains unknown. Yet, it is a commonly held view that general anaesthetic drugs have a more pronounced effect on synaptic mechanisms in the central nervous system (CNS) than on the propagation of electrical signals along axons (Larrabee and Posternak, 1952). A more germane issue is whether or not anaesthetic agents act generally, producing a range of metabolic

alterations which together result in anaesthesia or at, as yet, unidentified specific sites in neuronal mechanisms (Franks and Lieb, 1984).

### *The Nociceptive Pathway*

The nociceptive pathway is a complex afferent maze that gives rise to many considerations in elucidating its dynamics.

"In operations involving the abdominal or thoracic viscera (of humans), the total pain experience is produced by input from three sites of injury: the skin, the deep somatic structures, and the involved viscus or viscera. *The cutaneous component*, which results both from liberation of algogenic substances and from damaged cutaneous nerves, is characterised by a sharp stimulating quality, is localised, and is often accompanied by a burning sensation. *The deep somatic component*, also resulting both from liberation of algogenic substances and from the consequent lowering of nociceptive threshold, as well as from the damaged nerve axons in the fascia, muscle, pleura, or peritoneum, produces a diffuse aching discomfort that is felt either locally, in an area of reference, or both. *The visceral component* of pain results from the pathophysiology inherent in the surgical disease, and also from surgical trauma of the viscus that often cause persistent nociceptive input. The pain is characterised by a dull, aching, diffuse quality, and is felt locally, in an area of reference in the abdominal wall or chest, or both (Bonica, 1990b)."

Peripheral tissue injury provokes two kinds of modification in the responsiveness of the nervous system: *peripheral sensitisation*, a reduction in the threshold of nociceptor afferent peripheral terminals, and *central sensitisation*, an activity-dependent increase in the excitability of spinal neurones, where spinal cord neurons are triggered by and their activity outlasts nociceptive afferent inputs ( Woolf, 1983; Woolf and Chong, 1993). Exactly what quantity and what specific types of input are required to initiate central sensitisation, the precise time course of changes, and whether more input will produce longer lasting effects have not been adequately studied. However, brief periods of nociceptor input can produce central hypersensitivity changes that alter responses to subsequent



inputs, lasting between 10 to 200 times the duration of the initiating stimulus (Woolf and Chong, 1993). This facilitation appears to be mediated in part by the local release of glutamate which, acting through an N-methyl-D-aspartic acid (NMDA) receptor, produces long-term changes in neuronal excitability (Haley et al., 1990). While *windup* (a term coined to describe exaggerated responsiveness from both peripheral and central sensitisation to noxious stimuli) requires the NMDA site for its initiation, the NMDA receptor is not required for its sustenance (Abram and Yaksh, 1993). Together, peripheral and central sensitisation contribute to a state of post-injury stimulus hypersensitivity found postsurgically, a state which manifests as an increase in the response to noxious stimuli and a decrease in the stimulus threshold, both at the site of injury and in the surrounding uninjured tissue.

### *Pre-emptive Analgesia*

Promise exists for the prevention of nociceptive afferent hypersensitisation by the use of *pre-emptive analgesia*. By administering pre- and/or intra-operative analgesics the barrage of surgically induced afferent activity can be impeded. Three classes of analgesic drugs have been used in this role: local anaesthetics, opioids, and non-steroidal anti-inflammatory drugs (NSAIDs). Preventing peripheral sensitisation has been assumed to be the major action of NSAIDs in all species. This prevention relies on inhibition of prostaglandin production by blocking the enzyme cyclooxygenase (Dahl and Kellet, 1991). Experimental observations have shown that low doses of morphine prevent central sensitisation and that high doses are required to suppress central sensitisation once it is already present (Woolf and Wall, 1986), and a number of trials have compared the presurgical administration of opioids (not butorphanol) with nonopiate sedatives (Woolf and Chong, 1993). Pre-emptive opioid administration in humans has resulted in a reduction in total dose of postsurgical analgesia, increase in the time to demand for analgesia, and decrease in number of patients requesting postsurgical analgesia (George et al., 1974; Kiss and Killan, 1992).

Opioids administered either systemically or spinally before a noxious stimulus have been shown to block C-fibre evoked sensitisation (Woolf

and Wall 1986; Dickenson and Sullivan, 1987; Dickinson, 1991). Some of the electrophysiological studies of C-fibre-induced spinal sensitisation have been performed in rats under a surgical plane of anaesthesia using the volatile agent halothane (Dickenson and Sullivan, 1987; Schouenborg and Dickenson, 1988). These observations (as well as other evidence) suggest that, in contrast to opioids or NMDA antagonists (ketamine), spinal facilitation (in the rat) is not diminished by many inhalation anaesthetics such as halothane (Abram and Yaksh, 1993).

### *Visceral Nociception*

Using the colorectal distention model it has been determined that both cutaneous and visceral nociceptive transmission in the spinal cord are subject to descending inhibitory modulation by activation of components of the endogenous pain control system, and no significant differences were detected between the modulation of cutaneous or visceral nociceptive transmission from these sites in the brain stem (Gebhart, 1992). Yet, pharmacological studies have shown that spinal visceral nociceptive transmission is significantly more sensitive to modulation by the drug morphine than is spinal cutaneous nociceptive transmission (Ness and Gebhart, 1989). Therefore, distinctly different visceral and cutaneous inputs to the spinal cord exhibit significantly different sensitivities to the commonly used  $\mu$ -opioid morphine. Similar investigations have not been made with the  $\kappa$ -opioid butorphanol.

Using the colorectal distention model in rats, Gebhart (1992) has observed that  $\mu$ -opioid receptor agonists were both more potent and more efficacious for reducing visceromotor responses than were either  $\delta$ - or  $\kappa$ -opioid receptor agonists. In contrast, 0.4 mg/kg of butorphanol produced significant relief for 30 minutes in the horse from experimentally induced pain created by a light source and from visceral induced pain by a caecal balloon for 90 minutes (Kalpravidh et al., 1984). The relatively short duration of visceral analgesia in the dog (45-60 minutes) provided by butorphanol (Harvey, 1994; Sawyer, 1994) is in contrast to the much longer duration of 169-350 minutes reported in cats (Sawyer and Rech, 1987). This could be the result of more rapid metabolism in the dog or

because of a possible species difference in the response pattern of  $\kappa$ -opioid receptors.

Ovariohysterectomy is the most commonly performed surgical procedure in small animal practice (Pearson, 1970; Dorn and Swist, 1977; Sawyer, 1988). Consequently, this operation is considered to be routine and innocuous by many veterinarians, and the procedure is often performed without the associated use of analgesics. Notwithstanding, human operations that involve the abdominal wall are usually followed by severe pain in 5 to 15% of patients and moderate pain in 30 to 50% (Bonica, 1990c). In humans the mean duration of lingering moderate to severe pain after operations such as amputations, prostatic surgery, anorectal surgery and major skin grafts is 2 days (Bonica, 1990c).

Hansen (1990) suggests that ovariohysterectomy in the bitch is less benign than routinely accepted and is, in fact, associated with distressful consequences. Reduction of stress associated with canine ovariohysterectomy could therefore hold potential for important progress in animal welfare.

Having investigated the cortisol responses to combinations of analgesic and anaesthetic from the base treatments in Chapter 2, the present study was undertaken to evaluate the pain-induced distress response of plasma cortisol to ovariohysterectomy. This chapter describes changes in plasma cortisol concentrations in healthy bitches undergoing ovariohysterectomy. Responses of plasma cortisol concentrations to different surgical treatments further establish cortisol as a useful physiological parameter for the assessment of pain-induced distress. Additionally, the impact of timing on the intravenous administration of butorphanol (presurgical and postsurgical analgesia) on surgical pain-induced distress is described.

## Materials and Methods

### Animals

Thirty-three bitches of various breeds and an average age of 19 months were used for three surgical treatments. Twenty-one of the bitches were presented to the veterinary hospital for routine ovariohysterectomy by their owners who signed a consent form for the study, while the others were sourced from the Massey University Animal Health Services Centre. Each bitch was fasted overnight before undergoing a treatment described below. The treatments were:

- \* (1) deliberate Control (11 bitches),
  - 6 bitches were the same as in the pilot study,
- \* (2) Anaesthesia plus Surgery (12 bitches),
  - 6 bitches were the same as in the pilot study,
- \* (3) Analgesia plus Anaesthesia plus Surgery (10 bitches), and
- \* (4) Anaesthesia plus Surgery plus Analgesia (10 bitches).

Within the limitations of the research design, bitches were assigned to treatments randomly and Control bitches also underwent one of the surgical treatments, but with an interval of no less than eight weeks between the two. Patient data are presented below:

<b>Bitches</b>		
<b>Breed</b>	<b>Number</b>	<b>Mean Age (Mo)</b>
Labrador X	5	8.2
Huntaway X	4	30.3
NZ Border Collie	7	12.3
Terrier	2	17.5
Spaniel	2	24.3
German Shepherd	4	25.5
Wiemaraner	1	24
Yellow Lab	2	22
Ridgeback	1	14
Doberman	1	7
Great Dane	1	14
Newfoundland	1	37
Toy Poodle	1	7
Corgi	1	25
<b>Total Group</b>	<b>33</b>	<b>19</b>

All bitches were admitted to the hospital during the afternoon of day one and were placed in an indoor cage (840 cm x 942 cm x 790 cm) within a restricted access, but not isolated, ward. Physical examination revealed that the bitches were healthy. One of the treatments was commenced at approximately 10:00 AM on day two, and the bitches were discharged after noon on day three. This sequence was followed for all treatments.

### *Treatments*

Deliberate Control (Con): The bitch was moved from the ward to the anaesthesia induction room, a cephalic vein catheter was placed, and a blood sample was taken. The bitch was gently restrained on a table in the presence of low activity (traffic of three to four persons) for approximately 53 minutes, and was then returned to the ward. Throughout the following five hour period of observation, blood sampling was performed at the regular intervals indicated in Table 3.2. Blood collection was via the catheter until it became blocked or was dislodged by the animal, after which occasions samples were taken by cephalic venipuncture (22 gauge needles) in the contralateral limb. These were the same animals as the Controls in Chapter 2. They were studied contemporaneously with the other treatments reported in both this Chapter and Chapter 2.

Anaesthesia plus Surgery (Ans/Sx): After arrival in the induction room and cephalic vein catheterisation, without preanaesthetic medication, anaesthesia was induced with barbiturate (2.5% sodium thiopentone, approximately 20-25 mg/kg) to a depth allowing intubation, after which a blood sample was taken through the cephalic catheter. Following preparation for surgery which included hair clipping and antiseptic cleansing of the skin, and movement to the surgical theatre, a three-clamp ovariohysterectomy (Fingland, 1990) was performed. The abdominal wall was closed with no greater than appositional tension using a simple interrupted pattern of absorbable sutures<sup>5</sup>. The skin incision was closed with monofilament absorbable sutures in an intradermal pattern<sup>1</sup>.

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<sup>5</sup> Maxon. Davis & Geck, Auckland, New Zealand.

Anaesthetic administration for this group lasted approximately 55 minutes (Table 3.2).

Analgesia plus Anaesthesia plus Surgery (Anl/Ans/Sx): Thirty minutes prior to presentation in the induction room 0.4 mg/kg of butorphanol (Torbugesic<sup>6</sup>, Fort Dodge Laboratories, Fort Dodge, Iowa) was administered intravenously. The bitch was then allowed to rest in the ward cage until movement to the induction room and subsequent handling as with the Anaesthesia plus Surgery group (Table 3.2).

Anaesthesia plus Surgery plus Analgesia (Ans/Sx/Anl): The bitches were treated in the same way as the Anaesthesia plus Surgery group, except that 0.4 mg/kg of butorphanol was administered intravenously at the time of extubation after the bitch was returned to the ward cage (Table 3.2).

Zero minutes (Table 3.2) marked the beginning of each treatment.

- ° The Analgesia plus Anaesthesia plus Surgery group received butorphanol at 0 minutes.
- ° The Anaesthesia plus Surgery plus Analgesia group received butorphanol at 91 minutes.

The 60 minute and 63 minute blood samples (Table 3.2) were taken approximately one to two minutes after skin incision and visceral manipulation, respectively.

Commencing with the 121 minute blood sample (30 minutes after extubation), the abdomen was gently palpated immediately prior to each blood collection. Palpation was part of the protocol for a video-taped behaviour study conducted in parallel with this cortisol study (Chapters 4 and 5). The interval between the beginning of palpation and the end of the blood sampling was approximately two to three minutes.

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<sup>6</sup> Torbugesic: Lot # 454127

### *Cortisol Assays*

Blood samples (5 ml) were collected in lithium heparinised vacutainers, centrifuged and the plasma stored at -20°C until required. Total plasma cortisol concentrations were determined by radio-immunoassay (Appendix A), the lowest detectable concentration being 0.22 ng/ml and the intra-assay and inter-assay coefficients of variation being 16% and 22%, respectively for plasma standards of 1, 16 and 19 ng/ml.

### *Statistical Analyses*

Scheffé's F test was used to differentiate the plasma cortisol responses to each of the treatments. Selection of this test was based upon its robustness to assumption violations and its conservatism across treatment comparisons. T-test analysis was used to examine return of plasma cortisol concentrations after each treatment to pretreatment concentrations. Although some formal statistical test results are reported, the emphasis is on describing and comparing the patterns of change in cortisol concentrations. Experimental design excluded repeated measurements analysis.

In order to give a single measure of both the magnitude and the duration of increases in plasma cortisol concentrations after a treatment, the integrated cortisol responses were calculated. The integrated response is defined as the area between a horizontal line drawn through the pretreatment concentration and the cortisol curve during the period when the concentrations were greater than the pretreatment value (Mellor and Murray, 1989).

### **Results**

No clinical complications were detected at any stage of the study and there were no consistently high or low responders with respect to plasma cortisol concentrations. The pretreatment concentrations (Mean  $\pm$  SEM) on day two were:  $17 \pm 3.4$ ,  $26 \pm 3.6$ ,  $31 \pm 6.5$ , and  $34 \pm 5.1$  ng/ml for the Control (n=11), Anaesthesia plus Surgery (12), Analgesia plus Anaesthesia

plus Surgery (10), and Anaesthesia plus Surgery plus Analgesia (10) groups, respectively.

As reported in Chapter 2, in Control bitches there was no significant change in plasma cortisol concentrations between the pretreatment sample (0 minutes) and the first sample taken in the induction room (34 minutes). Plasma cortisol concentrations rose significantly after 34 minutes until the time that the bitches were returned to their ward cage, after which values returned to pretreatment levels for the rest of the observation period (Figure 3.4).

In Anaesthesia plus Surgery bitches plasma cortisol concentrations remained unchanged from pretreatment values until the skin incision was made at 60 minutes. Plasma concentrations rose significantly ( $P<0.05$ ) over the course of the laparotomy and continued to rise, reaching a peak 90 minutes after extubation (181 minutes). Plasma concentrations did not change significantly thereafter for the duration of the observation period (Figure 3.5), although they had returned to pretreatment values by 24 hours.

After the administration of butorphanol at zero minutes (arrow in Figure 3.5), plasma cortisol concentrations for the Analgesia plus Anaesthesia plus Surgery group rose significantly ( $P<0.05$ ) until the induction of anaesthesia. Plasma concentrations rose significantly ( $P<0.05$ ) over the course of the laparotomy and continued to rise significantly for sixty minutes after extubation. Peaking at 151 minutes, plasma cortisol concentrations did not fall significantly until 391 minutes. Plasma concentrations did not return to pretreatment values during the observation period (Table 3.2), but had returned by 24 hours.

Plasma cortisol concentrations fell significantly ( $P<0.05$ ) between zero minutes and the induction of anaesthesia for the Anaesthesia plus Surgery plus Analgesia group. Plasma concentrations rose significantly ( $P<0.05$ ) over the course of the laparotomy and continued to rise for sixty minutes after extubation (Figure 3.5). Peaking at 151 minutes, plasma cortisol concentrations did not fall significantly until 211 minutes ( $P<0.05$ ). Plasma concentrations continued to fall until 271 minutes, where they remained for the rest of the observation period. Plasma concentrations



had returned to pretreatment values by 24 hours: Controls ( $19 \pm 4.3$  ng/ml), Anaesthesia/Surgery ( $30 \pm 6.6$ ), Analgesia/Anaesthesia/Surgery ( $33 \pm 4$ ), and Anaesthesia/Surgery/Analgesia ( $37 \pm 3.9$ ).

Integrated cortisol responses ( $\text{ng ml}^{-1} \times \text{hours}$ ) for the three surgery groups were similar between zero and 151 minutes: Anaesthesia plus Surgery, ( $69.17 \pm 29.9$ ); Analgesia plus Anaesthesia plus Surgery, ( $89.33 \pm 35.2$ ); Anaesthesia plus Surgery plus Analgesia, ( $67.83 \pm 34.5$ ). After 151 minutes the response was similar for the Anaesthesia plus Surgery ( $215.45 \pm 46.6$ ) and the Analgesia plus Anaesthesia plus Surgery ( $209.07 \pm 29.1$ ) groups, but was significantly lower ( $P < 0.05$ ) for the Anaesthesia plus Surgery plus Analgesia ( $122.52 \pm 17.2$ ) group.

## Discussion

Plasma corticosteroid concentrations are frequently used as a measure of distress (Moberg, 1987). Having examined the interpretation of cortisol responses to combinations of butorphanol analgesic and halothane anaesthetic in Chapter 2, it is now possible to examine the response of plasma cortisol concentrations to combinations of anaesthesia, surgery and analgesia.

As seen in Chapter 2, the administration of anaesthetic prevented a rise in cortisol that was observed in conscious bitches. Whereas there were no significant cortisol changes in the Anaesthesia bitches (Chapter 2), here the anaesthetised bitches showed a significant rise in cortisol concentration after the surgical incision was made.

Major new observations in this phase of the study involved this cortisol rise, as follows:

- The immediate and marked rise in plasma cortisol concentration that occurred in response to surgical stimulation was attributed to sensory input, including nociceptor stimuli, of the operative procedure while the bitch was unconscious. Once consciousness returned and effects of the anaesthesia wore off, the sustained elevation of plasma cortisol concentration was due to the combined effects of residual noxious stimuli and contextual cognitive responses.

- Giving butorphanol 30 minutes prior to surgery had no effect on the surgically induced rise of plasma cortisol concentration and no effect on the postsurgical plasma cortisol concentration.
- Administration of butorphanol at extubation could obviously have no effect on the surgically-induced rise in plasma cortisol concentration, but it was associated with reduced plasma cortisol concentration in the postsurgical period. This reduction was interpreted as reflecting lower levels of pain-induced distress.
- Plasma cortisol concentration returned to pretreatment values within 24 hours, at which point residual pain-induced distress was interpreted as being minimal.

The rise in plasma cortisol concentration for the Control bitches during their stay in the anaesthetic induction room (34-90 minutes, Figure 3.4) is attributed to the conscious 'novel experiences' of unfamiliar surroundings and low traffic flow of unfamiliar personnel (Chapter 2). This is supported by the observation that the plasma cortisol concentrations returned to pretreatment values once the Control bitches were returned to their familiar ward cage.

In human beings surgical distress results in elevated plasma cortisol concentrations. These cortisol elevations are related to rises in ACTH (Cooper and Nelson, 1962) and correlate with the severity of the surgical procedure and its duration (Sandberg et al., 1954; Madsen et al., 1976). Schmidt and Booker (1982) suggest that graded cortisol responses to surgical procedures in the dog (n=6) are similar to those in humans, while a more recent report from Church and others (1994) shows that the type of surgical procedure (thoracotomy, orthopaedic or laparotomy) does not influence the magnitude of this response in dogs (n=25). Supporting data from the Schmidt and Booker study are weak considering that only one blood sample (at four hours) was taken between the presurgical and the 24-hour sample in that report.

The immediate rise of plasma cortisol concentration with the commencement of surgery in the Anaesthesia plus Surgery group for this study is consistent with the rise seen by Church and others (1994) during canine laparotomies, although the minimum period of general anaesthesia in that study was four hours. Knol and others (1992) have reported that

plasma corticosteroid levels can rise within one minute of exposure to stimuli, with resultant behavioural, autonomic nervous or neuroendocrinologic responses. In this study significant rises in plasma cortisol levels were seen from the blood sample taken approximately one to two minutes after visceral manipulation. The marked elevations in plasma cortisol concentrations which were maintained for nearly two hours after extubation in the Anaesthesia plus Surgery bitches are most likely due to sensory mechanisms, including nociceptor input arising from the skin incision and/or manipulations of the abdominal viscera. After extubation, the low plasma concentrations of cortisol in the Anaesthesia group (Chapter 2) suggest that the early period of recovery from anaesthesia itself was not distressful, and therefore would not have contributed significantly to the increases in cortisol concentrations in the surgery groups during the same period.

Three distinct phases are recognised for all bitches undergoing surgery in this study. First was the period leading to anaesthetic induction, in which bitches were exposed to changing environmental influences and associated novel experiences. In the case of the presurgical analgesic group, this period also involved those conscious responses accompanying the dysphoric effects of butorphanol. Second was the period of anaesthesia-induced unconsciousness where the principal noxious stimulus was surgically-induced nociceptor stimulation. This period was followed by a transition to consciousness with the bitches' associated cognitive responses that add to those arising from the surgically induced noxious stimuli. Once the bitch regained consciousness she was now in a third phase, responding to a combination of stimuli: surgically-induced noxious stimuli, cognitive or 'emotional' response to that 'perceived' pain, and any other novel experiences. Accordingly, the resultant cortisol response was an aggregate of different afferent signals as they affected the whole animal within its contextual environment.

Halothane anaesthesia does not cause plasma cortisol concentration to increase (and may cause them to decrease, Chapter 2). The nociceptive events of the Anaesthesia plus Surgery group are apparently sufficient to overwhelm such a halothane effect during the period of unconsciousness induced by anaesthesia. The nociceptive inputs from surgery plus any additional cortisol responsive stimuli (i.e. cognitive features: anxiety, fear,

or memorable associations) affiliated with the immediate postsurgical period apparently persist for more than five hours after extubation (Figure 3.5). The mechanisms underlying these effects are unknown, but give rise to speculation.

The early rise in plasma cortisol concentration following butorphanol administration in the Analgesia plus Anaesthesia plus Surgery group is attributed to the dysphoria or psychotomimetic effects of this sigma receptor opioid (Benedetti and Butler, 1990) (Chapter 2). In humans, butorphanol side effects include nausea, sweating, headache, vertigo, floating or pleasant sensations, dizziness, lethargy and confusion (Caruso et al., 1979). Since halothane anaesthesia suppresses plasma cortisol concentrations, despite the systemic presence of butorphanol (Chapter 2, Figure 2.4, Analgesia/Anaesthesia), then the rise in plasma cortisol concentration over the anaesthesia period in the Analgesia plus Anaesthesia plus Surgery group must be ascribed to the effects of surgery itself, as with the Anaesthesia plus Surgery group.

Differences in plasma cortisol concentrations after 151 minutes between the Anaesthesia plus Surgery plus Analgesia group and the other two surgery groups suggest that the postsurgical administration of butorphanol was more effective in suppressing cortisol concentrations, as well as postsurgical pain-induced distress, than was its presurgical administration. Two possible explanations for this observation are proposed, the first of which is favoured.

- 1) The systemic concentration of butorphanol at 151 minutes would have been greater for the postsurgically than for the presurgically administered butorphanol group (33 mg/l vs 19 mg/l, by predicted IV pharmacokinetics (Pfeffer et al., 1980)). If it is assumed that butorphanol's effects are concentration dependant, the relative systemic concentrations of butorphanol may result in altered plasma cortisol concentrations, where the greater levels of butorphanol suppressed pain-induced distress, and therefore, yielded a lower cortisol value.
- 2) The pharmacological activity of halothane anaesthesia may have irreversibly altered the antinociceptive effects of the presurgically administered butorphanol in the Analgesia plus Anaesthesia plus Surgery group. Such an irreversible transformation, as might occur

with conformational changes in the butorphanol moiety or the butorphanol receptor, might leave the butorphanol ineffective as an analgesic once changed by halothane or its metabolites.

The lower cortisol concentrations seen in the Anaesthesia/Surgery/Analgesia group after surgery are attributed to both the analgesic and sedative properties of butorphanol. Recognition was made earlier that bitches recovering from surgery express the combined response to a variety of stimuli, namely the surgically-stimulated noxious stimuli, cognitive response to the 'perceived' pain and other novel experiences. From reported studies in humans (Lippmann et al., 1977), it is assumed that postsurgical bitches received the same analgesic relief from pain.

Opportunity exists to influence postsurgical pain-induced distress by using known analgesic treatments, and by changing the time or duration of administration (Woolf and Chong, 1993). Findings that sensory signals generated by surgically damaged tissue trigger a prolonged state of CNS excitability have encouraged investigations testing whether presurgical medications can pre-empt postsurgical pain by preventing the establishment of central sensitisation (Katz et al., 1992; Woolf and Chong, 1993). The underlying principle herein is that therapeutic intervention is made in advance of the pain rather than in reaction to it. This was the premise for comparing pre- to post-operative analgesic effects in the present study. In our study, however, the analgesic effect of butorphanol may have dissipated by the time of anaesthetic extubation at 91 minutes. In an intravenous administration study using the colorectal distention model Houghton and others (1991) reported the longest mean duration of antinociception occurring with the 0.4 mg/kg dose of butorphanol was  $38 \pm 9$  minutes, although one of eight dogs demonstrated a 75 minute duration of antinociception at this dose.

Butorphanol given intravenously and 30 minutes before surgical stimulation was not effective in decreasing postsurgical pain-induced distress as determined by plasma cortisol concentration. Intravenous administration would have resulted in a rapid body clearance. By dosing 30 minutes before surgery, systemic concentrations of butorphanol at the time of return of consciousness after surgery were probably too low to

produce an effect. Intravenous administration of butorphanol for this study was selected as the route of choice to minimise the variability of bioavailability. Among those parameters that influence bioavailability are the site of injection and fat depots at that site, and by administering butorphanol intravenously the variability associated with subcutaneous or intramuscular injection was eliminated. The time for presurgical administration (30 minutes before anaesthetic induction) conformed to the most common time for administration of presurgical drugs in clinical practice. Approximately two years after the commencement of this study Houghton and others (1991) and Sawyer and others (1991) published findings that the analgesic properties of butorphanol in the dog lasted approximately 40 minutes. In retrospect, giving butorphanol at the above time and by the above route did not allow the drug to be efficacious. One of two alternative protocols would have been more informative: either administer butorphanol (at the same dose) subcutaneously or intramuscularly 30 minutes before anaesthetic induction, or administer the butorphanol intravenously 5-10 minutes before making the surgical incision. Alternatively, butorphanol could be administered at 30 minute intervals.

In assessing the pain-induced distress associated with ovariohysterectomy, most practitioners focus on the visceral component of the procedure, as bitches frequently exhibit a clinical response to visceral manipulation despite being under general anaesthesia. The cutaneous component of the surgery is often under-emphasised. Tissue damage causes the release of endogenous chemicals (algogenic substances) including serotonin, histamine, prostaglandins, bradykinin, substance P,  $H^+$ ,  $K^+$ , and many others (Bonica, 1990b). These products bypass the normal sensory nervous system and are absorbed directly by the hypothalamus, that part of the brain that controls the pituitary and thereby much of the endocrine system (Wall, 1990). In addition to direct excitatory action on the membrane of nociceptors, these agents may have an indirect excitatory action by altering the local microcirculation (Bonica, 1990b). Although the precise mechanism by which endogenous chemicals participate in peripheral transduction of nociceptive stimuli into nociceptive impulses is not known, prostaglandins are recognised to facilitate the pain-induced distress evoked by chemical and physical stimuli by sensitisation of nociceptors while being ineffective in evoking distress themselves (Yaksh

and Hammond, 1982). For example, bradykinin activates nociceptors and the action of bradykinin on nociceptors is potentiated by prostaglandins present in the injured tissue compartment. Prostaglandins E<sub>2</sub> and F<sub>2</sub> $\alpha$  have little algogenic effect when administered intra-arterially in the dog, but they potentiate the action of intra-arterial bradykinin, resulting in marked "pseudoaffective" and autonomic responses by the animal (Ferreira et al., 1973). Local injection of prostaglandins also greatly accentuates the response to pressure in the inflamed paw of the rat and the inflamed knee joint of the dog (Yaksh and Noueihed, 1985).

The role of  $\kappa$  receptor activation in the production of analgesia is controversial. While drugs with  $\kappa$  agonist activity produce analgesia at the spinal level, many possess activity at other receptors, both opioid and non-opioid, which could explain their action (Pleuvry, 1993).

Powerful analgesic effects in attenuating the discomfort from inflammation may be obtained with opioids acting at peripheral receptors. Inflammatory mediators, such as prostaglandin E<sub>2</sub>, activate adenylyl cyclase via a stimulatory G protein causing nociceptor sensitisation. Activation of  $\mu$  receptors switches off this process via inhibitor G proteins and thus prevents nociceptor sensitisation (Levine and Taiwo, 1989). Prevention of prostaglandin E<sub>2</sub> hyperalgesia is not a property shared by  $\delta$  and  $\kappa$  agonists, but they can prevent bradykinin-induced hyperalgesia. Bradykinin induces the release of nociceptor sensitising agents, including prostanoids, from postganglionic sympathetic nerves, thus it is thought that  $\delta$  and  $\kappa$  receptors mediating peripheral analgesia are situated on the sympathetic nerves and prevent the release of mediators, which may themselves initiate noxious responses (Taiwo and Levine, 1991). Using tail pinch as a method of studying analgesia, evidence has been presented that  $\kappa$  opioid agonists, but not morphine, activate noradrenergic and serotonergic pathways in mice (Kunihara et al., 1992).

Where examined, the following rank order of prostaglandins is made regarding their sensitising effects: PGE<sub>1</sub>>PGE<sub>2</sub>>PGF<sub>2a</sub>≥PGF<sub>2b</sub>>PS>PGA<sub>1</sub>=PGB<sub>2</sub>=PGI<sub>2</sub> (Ferreira et al., 1973; Yaksh and Hammond, 1982). In damaged human skin there is a marked elevation of prostanoid levels, which is blocked by cyclo-oxygenase inhibitors (Winkelmann, 1978).

To exclude the cutaneous nociceptive input to plasma cortisol concentration from the influence of prostaglandins in our study, non-steroidal anti-inflammatory drugs (cyclo-oxygenase inhibitors) could have been administered presurgically. Alternatively, pre-emptive spinal anaesthesia could have been administered. A rise in plasma cortisol concentration under the suppression of spinal nociceptive pathways from spinal anaesthesia would strongly suggest a marked influence from the general class eicosanoids, of which prostaglandins are a subclass.

For the ovariohysterectomised bitch both cutaneous and visceral nociceptive inputs could be considerable, and may collectively or independently override the analgesic effect of presurgical butorphanol. The two nociceptive inputs (cutaneous and visceral) could be synergistic through spinal reflex self-excitation. That is, cutaneous and visceral primary afferents could be converging on viscerosomatic spinothalamic tract neurons (Bonica, 1990b). Stimulation of the somatomotor neurons in the anterior horn may be occurring, thereby producing reflex skeletal muscle spasm, which acts as positive feedback to create and sustain an agonistic circle of excitation (Bonica, 1990b).

It is informative to assess area under the cortisol curve values after extubation (91-391 minutes) in the following treatments:

Treatment	Integrated Cortisol Response (ng ml <sup>-1</sup> x min)
Controls (Figure 2.1)	1,920
Analgesia alone (Figure 2.3)	7,430
Anaesthesia plus Analgesia (Figure 2.4)	8,050
Anaesthesia plus Surgery (Figure 3.5)	19,060
Anaesthesia plus Surgery plus Analgesia (Figure 3.5)	12,600

Using the integrated cortisol response as an index for scaling distress, the above table suggests the environmental stimuli experienced by the Controls represent a mild distress. Compared to Controls, the Analgesia alone group showed a much higher value of integrated cortisol response which is



attributed to the dysphoric side effects of butorphanol. Insignificantly different is the slightly greater response in bitches of the Anaesthesia plus Analgesia treatment. While each of these three treatments involved the cognitive features of distress from varying stimuli, they did not experience the noxious stimuli of surgery. In the Anaesthesia plus Surgery treatment, where the additional stimulus of nociception was present, the response was significantly ( $P<0.05$ ) greater than those seen in the other groups. However, in the presence of postsurgical butorphanol analgesia, this latter rise was significantly ( $P<0.05$ ) reduced, although the value was still significantly ( $P<0.05$ ) greater than in treatments not experiencing noxious surgical stimuli. These data support the use of cortisol as an index for both distress and pain-induced distress, and defend the proposal that butorphanol contributes to the suppression of pain-induced distress in bitches following ovariohysterectomy.

Treatments in this study document the short-term duration of pain-induced distress following ovariohysterectomy as assessed by plasma cortisol, suggesting that significant pain-induced distress is present for a duration of more than five hours after surgery in the absence of analgesia. It remains unknown how long this pain-induced distress persists, although results suggest this distress is maintained no longer than 24 hours.

In summary the results from the integrated cortisol response show that pain-induced distress from 'routine' ovariohysterectomy is significantly suppressed by the administration of postsurgical butorphanol. Unfortunately, the pre-study hypothesis that pre-emptive butorphanol would be more effective than postsurgical butorphanol in suppressing pain-induced distress could not be illustrated because of design restrictions identified late within the conduct of the study.

Presently, data from this research supports the use of cortisol as an index for measuring the intensity and duration of pain-induced distress. Use of cortisol to determine the efficacy of butorphanol analgesic has been based on only one treatment, although that evidence is persuasive. However, the clinical practitioner needs immediate guidelines for the administration of analgesics, and thus a cortisol index is unsuitable. In Chapters 4 and 5 behaviours of those same animals utilised to investigate cortisol responses

are examined, but in the search for a more timely index of pain-induced distress.

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## Chapter 4

### Effects of different combinations of halothane anaesthesia and butorphanol analgesia on behaviour in bitches

#### Chapter Summary

Noninteractive and interactive behaviours were investigated for differences in effect from six treatments: Control, Anaesthesia, Analgesia, Analgesia followed by Anaesthesia, Anaesthesia followed by Analgesia at intubation and Anaesthesia followed by Analgesia at extubation. The noninteractive hourly behaviours which characterised the Control group were high frequencies of normal speed position change and head lifts and low frequencies of stretching and grooming. The Anaesthesia group was characterised by lip licking and cage sniffing, while the Analgesia group demonstrated no unique noninteractive hourly behaviours. Groups treated with combinations of anaesthetic and analgesic all showed vocalisation.

While noninteractive behaviours are valuable in recognising the animals' responses in the absence of humans, interactive behaviours are of greatest interest to the animal handler who often palpates the animal for an indication of behavioural response. Control bitches most often maintained a standing position throughout the palpation event, maintained a level head position with ears back and tail low, looked ahead and showed a high frequency of arched back, lip licking and tail wagging. In contrast, the Anaesthesia group most often commenced the palpation in a sitting position, but ended in a variety of positions and frequently showed lip licking. Whining was common in the Analgesia group. The Anaesthesia plus Analgesia and the Anaesthesia plus Immediate Analgesia groups both demonstrated vocalisation for five hours after the treatment. The Anaesthesia plus Analgesia group seldom changed from a lateral recumbency position during the palpation period, showed sleepy or closed eyes, commonly stretched and frequently moaned or groaned.

From the behavioural results of this study it was concluded that the rise in plasma cortisol concentrations documented in Chapter 2 that were observed within the groups administered analgesic were probably due to distress associated with an inability to express a state of restlessness. The sedative effects of butorphanol were apparently sufficient to prevent these bitches from relieving their distress through active movement.

The statistical technique of discriminant canonical analysis was very useful for identifying specific behaviours distinct to different groups and for providing a single figure (the discriminant function,  $Z$ ) to qualitatively characterise each bitch within a group.

## Introduction

Livingston (1994) states, "Perhaps the simplest form of neurologic evaluation with regard to pain is that of simple observation. The behavioural responses to pain can usually be detected in most species with a little training." Difficulty arises in the scientific acceptance of this proposal because there are no unequivocal standards for judging distress (or 'pain') from animal behaviours.

Excluding the dogmatic opponents to the idea, contemporary ethics dictate that higher developed animals such as the dog (*Canis domestica*) do feel pain! Anatomically and physiologically dogs have similar neurological receptors to humans, and dogs are frequently used as substitute models for investigating human pain. Dogs produce many behaviours similar to what we as humans would expect when pain occurred under similar circumstances, and their responses are abated with the same analgesics that abate apparently similar pain in humans. Given the voluminous literature of support in the disciplines of physiology, ethics and animal well-being, developed animals experiencing circumstances that induce what we humans call pain, most likely experience similar pain in response to similar noxious stimuli.

It is necessary to present additional background material before proceeding with a description of the first of two behavioural studies

which were conducted as part of the present work. This material is presented to help guide the reader to a greater appreciation for the context of the work that follows.

### *Behaviour as a Parameter of Distress*

The response of a conscious subject to distress is not limited to alterations of plasma hormone concentrations (and other physiological variables), but may also involve appropriate behavioural alterations. Hormonal and behavioural responses can be intimately related in distressful situations (Dantzer and Mormede, 1983), and often the first indication that an animal may be distressed is a change in behaviour away from normal (Blackshaw and Allan, 1985).

Environmental events may trigger a wide range of physiological changes. Two such changes are the emergency reaction and the general adaptation syndrome. The emergency reaction, as described by Cannon (1935), relates to the activation of the sympathetic nervous system and the adrenal medulla. This reaction is a short latency response involving hormonal factors, such as catecholamines, that enable the subject to quickly mobilise its resources for the behavioural responses of fight or flight. The general adaptation syndrome was initially described by Selye (1936) and is characterised by the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland, which in turn, activates corticosteroid release from the adrenal cortex. Corticosteroids serve many functions some of which are to increase the magnitude and duration of metabolic activity initiated by short-lived catecholamines.

Hormonal reactions and related behavioural responses can assume at least three different interactions (Leshner, 1978): (1) a given emotional state can trigger and co-ordinate endocrine and behavioural responses, (2) hormones can affect behaviour, and (3) endocrine status can be modified if the subject engages in specific behaviours.

It is often possible to identify psychological distress in animals by combining analyses of behavioural changes with physiological measures such as changes in cortisol secretion or heart rate (Moberg, 1985; Line, 1987; Broom, 1988). Changes in such physiological variables often

correlate with escape attempts, exploratory conduct, and immobility or defecation when animals are subjected to experimental distress stimuli, such as handling or placement in unfamiliar surroundings (Archer, 1973; Archer, 1979).

The work of Flecknell's group (1994) in laboratory animals as well as Mellor and others (1989; 1991) in farm animals has led to the ability to recognise different levels of clinical distress in these animals and to the proposal of scoring systems. The work of Mellor's group on lambs subjected to castration and taildocking showed correlations between abnormal behaviour scores and cortisol levels in young lambs<sup>7</sup>; the work concentrated mainly on postural responses, as these could be recorded at a distance with minimal interference from the observer. Similar correlations between cortisol and behaviour have been demonstrated in the cat (Carlstead et al., 1993).

Behaviour studies have become an important means of recognising when the welfare of an animal may be compromised; however, the difficulty with behavioural indices arises in assessing what behaviour actually gives an accurate measure of welfare. Often the first indication that an animal may be distressed is a change in behaviour away from normal (Blackshaw and Allan, 1985). Buchenauer (1981) suggests that all behavioural patterns that differ from normal for a species can be considered as disturbed behaviour, yet it is difficult to identify to what extent modification in behaviour is within the 'normal' range. In a most general sense, abnormal behaviour might be defined as "a behavioural pattern shown by a minority of the animal population that is not due to any obvious disease state, that is not adaptive in an evolutionary sense and that is not maintained by a concurrent conditioning reinforcer or caused by identifiable events in early experience" (Hart and Miller, 1985).

Individual differences in behaviour may be examined from two perspectives (Manteca and Deag, 1993). Firstly, from the more qualitative perspective of temperament, which differs between individuals (Lawrence et al., 1991), or alternatively from a more quantitative perspective using physiological parameters. The latter

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<sup>7</sup> Unpublished data.

often determines the former and involves such measurements as frequency, duration and patterns of specific behaviours (Mendl and Harcourt, 1988).

### *Pain and Behaviours*

A major goal of better defining pain-associated behaviours in animals is to detect pain when they experience it. Pain is likely to be present in situations where two or more of the following are applicable (Headley, 1993): 1) actual or potential tissue damage, 2) changes in the individual's normal behaviour, and 3) return to the individual's normal behaviour following analgesic therapy. Outward behaviours considered to be expressions of pain have a large impact on the practice of veterinary medicine. These behaviours may be the result of physical impairment by a painful disorder, they may be strategies employed by the animal to reduce pain, or they may be protective, in order to prevent initiation or exacerbation of discomfort (Turk and Flor, 1987).

Subjective evaluation of pain intensity by the veterinary clinician is often based on only the severity of discomfort that is likely to be elicited from a given noxious stimulus, based on human pain perception standards. Yet, it must be appreciated that evolution has resulted in masking the expression of pain in some animals, as those feral animals signalling pain, weakness or distress may become prey (Wright et al., 1985). Behaviour profiles of dogs indicate that some behavioural traits discriminate between breeds better than others (Hart and Miller, 1985), which may in fact be an evolutionary phenomenon considering that dogs have evolved under the influence of a variety of uses by humans. For instance, stoicism is characteristic in several breeds (Crane, 1987).

Nociceptive pathways in the human are generally considered more complex, sensitive and responsive than in other animals, because humans have made greater evolutionary neurological advances than animals. With this in mind, anthropomorphism may have its physiological limitations when comparing distress responses due to abdominal surgery between ovariohysterectomy in bitches and hysterectomy in women.

*Guidelines for Pain Assessment*

Soma (1985) proposed the following behavioural signs of acute pain:

<i>Sign</i>	<i>Modifier</i>
Sweating	In the Horse
Guarding	Attempting to protect, move away, or bite
Crying	Movement or palpitation
Mutilation	Licking, biting, scratching, shaking
Restlessness	Pacing, lying down and getting up, shifting weight
Recumbency	Unusual length of time

The Association of Veterinary Teachers and Research Workers has proposed the following list of factors<sup>8</sup> as worthy of attention in the assessment of pain:

- History of the animal & environment
- Details of the animal
- Physiological measurements
- Activity
- Gait
- Reaction to handling
- Response to analgesics
- Clinical examination
- "Mental" status
- Posture
- Facial expression
- Vocalisation

At least three types of response to pain can be recognised<sup>2</sup>:

- 1) "Responses designed to modify the animal's conscious behaviour: to enable it to learn and thus avoid repetition of the situation leading to the pain being felt. Pain makes the animal feel 'bad' and it attempts to avoid the situation. Such manifestations may be seen as an animal resists entry to a veterinary clinic, assuming the animal has made the association with an unpleasant experience."
- 2) "Responses, often automatic, which are designed to protect the animal or parts of the animal. A withdrawal reflex may be regarded as a minimal response and may be exaggerated to the

<sup>8</sup> Guidelines for the recognition and assessment of pain in animals. Universities Federation for Animal Welfare, 1989.



point of running away. This may be seen as escape behaviour or pawing at a restraining cage door."

- 3) "Responses which are designed to convey the presence of the experience to others of the same species or other species which may help to alleviate the situation, eg., pain specific vocalisation, facial expression, and posture."

<i>Response</i>	<i>Conscious/Automatic</i>
Modification of behaviour	Conscious
Protective response	Conscious or automatic
Response designed to minimise pain	May be automatic
Response designed to convey experience to others	Conscious or automatic

*The Assessment of Pain by Behaviours*

In human medicine pain assessment is an area of increasing investigation. Chambers and Price (1967) have proposed a modified pain rating scale with the following specific categories for evaluating human patients' acute pain: attention seeking, anxiety, verbalisation, skeletal muscle response (restlessness, tenseness, grimaces, and frowns), perspiration, sounds (cries, moans, groans, sighs and grunts), and nausea. Correlations between evaluator scores and the patients' verbal statement of pain intensity range from  $r=0.66$  to  $r=0.87$  ( $r$ = Pearson's correlation).

In a study (Mateo and Krenzischek, 1992) using four categories from the Chambers and Price model (restlessness, tense muscles, frowning and grimacing, and patient sounds) correlations between patients' verbal descriptions and behaviour within the first postsurgical hour were: frowning and grimacing  $r=0.69$ ; patient sounds  $r=0.59$ ; muscle tension  $r=0.53$ ; and restlessness  $r=0.48$ .

Pain in newborn humans is considered to be different from that in adult humans; it is perhaps more similar to pain in animals, with the behavioural dimension assuming primary importance (crying, fussiness, grimacing) and physiological dimensions (increased heart and

respiratory rates, profuse perspiration) being manifested as well (Pigeon et al., 1989). Crying, limb movement, agitated state, tachycardia, and voiding behaviours appear to be the behavioural signs most often cueing nurses' perceptions of pain in the neonatal intensive care unit (Pigeon et al., 1989). When evaluating these criteria there was good inter-rater agreement in the coding of nurses' responses, however there was less discrimination of the behaviours on the basis of intensity of pain (Pigeon et al., 1989). Another study of 109 nurses who worked in units where newborns were hospitalised identified fussiness, restlessness, crying and grimacing as salient signs 96.2%, 93.7%, 79% and 79% of the time, respectively (Jones, 1989). In still another study (Bradshaw and Zeanah, 1986) of 99 paediatric nurses, an average of 4.5 criteria were used to evaluate pain, and the most experienced nurses used a broader range of the criteria than the least experienced. In decreasing order behaviours used were: body language, oral expression, physiological measures, affective signs (withdrawal, anxiety, irritability and depression) and verbal requests.

"Pain-induced vocalisation has been studied in a number of mammalian species, and such studies appear to offer a useful model for understanding pain-related behaviour in prelingual children" (Levine and Gordon, 1982). Spectrographic analysis of vocalisation in infants has confirmed the general impression that cries of distress, pain and hunger are distinguishable from each other (Levine and Gordon, 1982). A characteristic of human verbal expression of pain is that it contains a mixture of private suffering and public display. It is reasonable to assume that such features of vocalisation also occur in other social animals.

Pain-induced vocalisation is assumed to have evolved as a means by which the young of a species can influence parental behaviour (Ferreira et al., 1973; Yaksh and Hammond, 1982; Holt, 1986). The screaming cry induced by noxious stimuli expresses a high degree of excitement, which is almost always transferred to other animals (Winter et al., 1966; Randolph and Mason, 1969). It has an immediate and compelling effect on the mother, causing her to approach, retrieve and render aid and comfort to her infant (King, 1963; Randolph and Mason, 1969).

### *Background for This Phase of the Study*

Although plasma cortisol is an established parameter for assessing animal distress, at present it cannot be used clinically by practitioners as a prompt for analgesic administration because of the time taken to complete the assay. A more immediate and timely index would be behavioural expressions. At the beginning of this study it was proposed that behaviour may be substituted for the use of cortisol assay in assessing pain-induced distress. If this substitution could be validated, then further, preemptive analgesia could be compared in efficacy to postsurgical analgesia using behaviour as a parameter for assessment.

Several caveats were considered at the commencement of this phase of the study. Firstly, it was assumed that dogs probably tolerate distress up to a given magnitude beyond which they express behavioural modification. Therefore, behaviour may not be an accurate index for scaling distress, and any given behaviour may simply be present or not present under certain circumstances. Secondly, animal behaviour by itself cannot serve as an indicator of distress without association to some established distress index. Accordingly, it was held that the established index of cortisol, as elucidated in Chapters 2 and 3, might serve as that link between behaviour and distress.

In an attempt to identify an animal's response to surgery one must first recognise behaviours associated with procedures conducted with the surgery itself, ie. anaesthesia and any associated perioperative analgesia. As there is no such information in the veterinary literature, that phase of the study reported in this chapter was conducted with normal canine bitches with the intent of identifying such associated behaviours. In a manner similar to that described in Chapter 2, treatments in this chapter served as base treatments for comparison with actual surgery treatments, which are later presented in Chapter 5.

This chapter deals with treatments from which 'normal' patterns and trends of behaviour were identified. A major objective was to use behaviours to gain further insights into the cause of plasma cortisol elevations in conscious bitches seen earlier and to explore the consideration that butorphanol may induce distress. In Chapter 5, behavioural responses to treatments from this chapter are compared

with those to treatments involving surgery and perioperative analgesia, to identify the behavioural effects of both surgery and analgesia.

### ***Interactive vs Noninteractive Behaviour Classifications***

An initial assumption in the present study was that bitches (pets in particular) may express different behaviours, depending on the presence or absence of humans. This supposition was based on the premise that bitches may have become conditioned to specific behaviours in expectation of a given response from their handler. Further, distinguishing between behaviours which occur in the presence or the absence of humans is important for clinical application. While the research scientist may be interested in the bitches' behaviour when humans are not present, in contrast, the clinical practitioner is acutely interested in behavioural responses the bitch shows to his/her physical manipulations. Therefore the study was designed to accomodate both interests.

All behaviours discussed in this chapter and Chapter 5 are explicitly defined in Appendix B. In the interests of brevity and with the belief that the behavioural terms are sufficiently descriptive, definitions of the terms are not duplicated in the following chapters. Within the interactive and non-interactive categories subclasses for identifying similar behavioural expressions were grouped. Subclasses are described in the materials and methods section. Finally, at periodic intervals within the text of both this chapter and Chapter 5 timely summaries are presented in the interest of orderliness and reader comprehension.

## **Materials and Methods**

### ***Animals***

Sixteen New Zealand cross-bred working bitches with an average age of 20 months were used in addition to those from the pilot study (Appendix D) for no more than three treatments each. Each bitch was fasted overnight before undergoing one to three of the six treatments below. The interval between treatments in bitches that received more than one was approximately eight weeks. The treatments were:

- \* (1) deliberate Control (11 bitches),
  - 6 bitches were the same as in the pilot study,
- \* (2) Anaesthesia alone (11 bitches),
  - 6 bitches were the same as in the pilot study,
- \* (3) Analgesia alone (11 bitches),
  - 6 bitches were the same as in the pilot study,
- \* (4) Analgesia followed by Anaesthesia (10 bitches),
- \* (5) Anaesthesia followed by Analgesia at intubation (10 bitches), and
- \* (6) Anaesthesia followed by Analgesia at extubation (10 bitches).

All treatments were conducted in parallel over the entire period of data collection so as to minimise any influence of seasonal effect. The allocation of bitches to treatments and the order of treatments were random.

Physical examination revealed that the bitches were healthy. They were housed in an indoor-outdoor rural facility and brought to Massey University Veterinary Hospital for 48 hours to undergo each treatment, after which they were returned to the rural facility.

All bitches were admitted to the hospital during the afternoon of day one and were placed into an indoor cage (840 cm x 942 cm x 790 cm) within a restricted access, but not isolated, ward. One of the treatments was commenced at approximately 10:00 AM on day two, and the bitches were returned to the rural facility after noon on day three. This sequence was followed for all treatments.

### ***Treatments***

**Deliberate Control (Con):** The bitch was moved from the ward to the anaesthesia induction room, a cephalic vein catheter was placed, and a blood sample was taken. The bitch was gently restrained on a table in the presence of low activity (traffic of three to four persons) for approximately 53 minutes, and was then returned to the ward. Throughout the following five hour period of observation, blood sampling was performed at the regular intervals indicated in Table 4.1. Blood collection was via the catheter until it became blocked or was dislodged by the animal, after which samples were taken by cephalic

venipuncture (22 gauge needles) in the contralateral limb. These were the same animals as the Controls in Chapters 2 and 3.

**Anaesthesia (Ans):** After arrival in the induction room and cephalic vein catheterisation, without preanaesthetic medication, anaesthesia was induced with barbiturate (2.5% sodium thiopentone, approximately 20-25 mg/kg) to a depth allowing intubation, after which a blood sample was taken through the cephalic vein catheter (Table 4.1). Surgical plane anaesthesia was then maintained with halothane in oxygen at a Fluotec setting of 2 for about 47 minutes (Table 4.1).

**Analgesia (Anl):** After arrival in the induction room and blood sampling via a cephalic vein catheter, 0.4 mg/kg of butorphanol (Torbugesic<sup>9</sup>, Fort Dodge Laboratories, Fort Dodge, Iowa) was administered intravenously. The bitch was thereafter gently restrained on the table in the presence of low activity for approximately 51 minutes and was then returned to the ward. Blood samples were taken as indicated in Table 4.1

**Analgesia then Anaesthesia (Anl/Ans):** Thirty minutes prior to presentation in the induction room 0.4 mg/kg of butorphanol was administered intravenously by venipuncture. The bitch was then allowed to rest in the ward cage until movement to the induction room and subsequent handling as with the Anaesthesia group (Table 4.1).

**Anaesthesia then Immediate Analgesia (Ans/I Anl):** The procedure was the same as that in the Anaesthesia group, except that 0.4 mg/kg of butorphanol was administered intravenously immediately following intubation within the anaesthesia induction room (Table 4.1).

**Anaesthesia then Analgesia (Ans/Anl):** The bitches were treated in the same way as the Anaesthesia group, except that they received 0.4 mg/kg of butorphanol intravenously at the time of extubation, after the bitch was returned to the ward cage (Table 4.1).

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<sup>9</sup> Torbugesic: Lot # 454127

Treatment Abbreviations		
Con	⇔	Control
Ans	⇔	Anaesthesia
Anl	⇔	Analgesia
Anl/Ans	⇔	Analgesia plus Anaesthesia
Ans/ I Anl	⇔	Anaesthesia plus Immediate Analgesia
Ans/Anl	⇔	Anaesthesia plus Analgesia

- The Analgesia group received butorphanol at 34 minutes.
- The Analgesia plus Anaesthesia group received butorphanol at 0 minutes.
- The Anaesthesia plus Immediate Analgesia group received butorphanol at 40 minutes.
- The Anaesthesia plus Analgesia group received butorphanol at 91 minutes.

Commencing with the 121 minute blood sample (30 minutes after extubation), the abdomen was gently palpated immediately prior to each blood collection.

***Behavioural Recording***

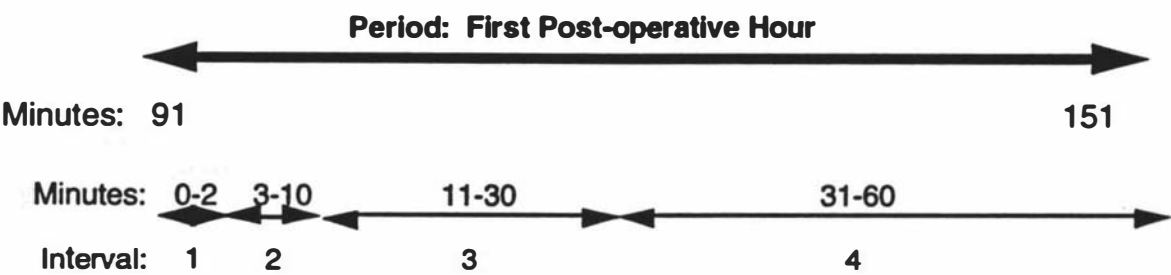
All behaviours were video-taped<sup>10</sup> with the bitch confined in the cage, which was illuminated with a fluorescent light affixed to the cage ceiling. The cage contained no bedding or bowls, and the door was constructed of vertical metal bars. The camera was mounted on a tripod approximately four meters in front of the cage adjacent to an additional light source directed into the cage. Isolating the cage and camera within the restricted ward area was an opaque screen suspended from the walls. Bitches were video taped for five continuous hours following their return to the ward cage, and this filming provided the data for behavioural scoring. Interactive behaviours were scored immediately prior to blood sampling commencing at 121 minutes (Table 4.1). At the time of blood sampling the investigator approached the cage through the isolating screen, pausing approximately two seconds

<sup>10</sup> Panasonic MS 1, S-VHS video camera. Viewing was performed on a Panasonic FS100, S-VHS video cassette recorder with jog/shuttle control.

before opening the cage door. The bitch was greeted with a "hello", (animal's name) and patted gently on the head three times. The ventral abdomen was gently squeezed three times with a single open hand, palm up. The investigator then withdrew, closed the cage door and exited the screened area. The investigator then re-entered the screened area to take the blood sample. Only three persons performed palpations, and the same investigator did not always serve as palpator for a given animal treatment.

The video tape recording was assessed as follows: for the first hour after extubation 51 behaviours were evaluated (Figure 4.1) during four intervals: minutes 0-2, 3-10, 11-30 and 31-60. These behaviours are referred to as **minute behaviours**. At the commencement of this study it was anticipated that many behaviours might occur rapidly as soon as the bitch was extubated. By dividing the first hour after extubation into four intervals as presented below, it was believed that such activity might be captured in the best manner.

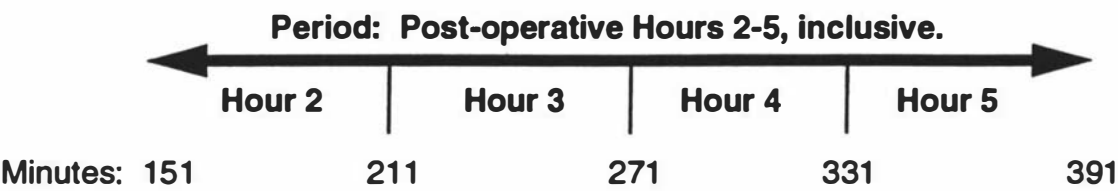
*Minute Behaviour:*



For the next four hours (hours 2-5, inclusive, after extubation) 48 behaviours were evaluated (Figure 4.2) in frequency by hourly increments. These behaviours are referred to as **noninteractive hourly behaviours**.



*Noninteractive Hourly Behaviour:*



The entire postsurgical period thereby consisted of the combined minute and noninteractive hourly behaviour periods.



Sixty-seven behaviours were evaluated during each palpation period (approximately three minutes in duration) commencing at 121 minutes (Figure 4.3). These behaviours are referred to as **interactive palpation behaviours** and were obtained at the time of blood sampling for plasma cortisol (Table 4.1).

*Interactive Palpation Behaviour:*



The minute, hourly and interactive palpation behaviours are noted as follows:

**Noninteractive Minute Behaviours:**

Each observed behaviour is listed below within a group which includes similar types of behaviours. Behaviours within each of these groups are listed in columns read down, then to the right ( $\downarrow \Rightarrow$ ).

**Stationary positions**

lateral	hang stand
sternal curl	normal sit
sternal other	hang sit
normal stand	

**Position changes**

number of changes	torso weight shifts
-------------------	---------------------

**Modifiers**

ataxia	increased thoracic limb
slow speed	weight bearing
normal speed	

**Motion**

walking

**Modifiers**

normal speed	first stand
slow speed	

**Activities**

slow motion cage circling		quiet alert
normal speed cage circling		head lifts
pacing	thrashing	head nodding
drawing legs up	stretching	door biting
cage licking	yawning	door pawing
cage sniffing	incision licking	grooming
cage digging	lip licking	trembling
manipulations	urination	paddling
vomition	salivation	
look back	defaecation	

**Breathing**

pant	normal
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**Vocalisation**

whine	bark
groan/moan	howl

Noninteractive Hourly Behaviours:

Each observed behaviour is listed below within a group which includes similar types of behaviours. Behaviours within each of these groups are listed in columns read down, then to the right ( $\Downarrow \Rightarrow$ ).

**Stationary major behaviours**

lateral rest or sleep	sternal curl	sit other
lateral awake	sit alert	normal stand
sternal awake	hang sit	hang stand

**Position change**

slow motion position change  
normal speed position change  
torso weight shifts  
thoracic limb weight shifts  
draws legs up  
stretching

**Movement behaviours**

attention seeking	normal speed cage circle
escape behaviour	slow speed cage circling

**Short behaviours**

head lifts	look back	cage licking
head nodding	pacing	cage digging
ataxia	yawning	door pawing
thrashing	trembling	door biting
normal breathing	salivation	whine
panting	urination	groan/moan
lip licking	vomition	bark
incision licking	defaecation	howl
grooming	cage sniffing	manipulation

## Interactive Palpation Behaviours:

Each observed behaviour is listed below within a group which includes similar types of behaviours. Behaviours within each of these groups are listed in columns read down, then to the right ( $\Downarrow \Rightarrow$ ).

**Starting positions**

lateral	sit	
sternal	stand	

**Position changes**

yes	no	
-----	----	--

**End position**

lateral	sit	
sternal	stand	

**Head position**

high	lowered	sway
high alert	hang	scooping
level	rest on surface	tilt
sudden head lift		

**Ear position**

forward alert	neutral	back
alternating	flat to sides	

**Eye position**

wide-eyed	eyebrow lift	wary
stare ahead	frantic searching	glance/avert
watch	sleepy or lidded	closed

**Tail position**

high	fast wag/curl	tuck
high arch	slow wag/curl	low
level	no wag/curl	on surface

**Vocalisation**

whine	bark
groan/moan	yelp/scream

**Orientation**

stare ahead	deliberate avert	sharp belly
tester	hide	slow belly

**Breathing**

normal	catch breath	pant
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**Other behaviours**

lip licking	drawing legs up	retreat
rigid stance	stretching	restrained
extended neck	lip lift	escape
arched back	bite/snap	

Minute and noninteractive hourly behaviours were recorded from the video tape as the number of total occurrences during the designated interval, ie. 0-2, 3-10, 11-30 and 31-60 minutes and hour 2, 3, 4 and 5. Interactive palpation behaviours were recorded as a single occurrence during a single palpation event.

### *Analysis and Presentation of Results*

The results of minute behaviours (the first hour after extubation) are presented for the purpose of description only. The minute behaviour data set was excluded from statistical analysis because of the highly variable state of consciousness when bitches were extubated. In an attempt to keep the same elapsed time at extubation (approximately 91 minutes) for all treatments, some bitches were prematurely extubated while others were extubated as they were coughing when regaining consciousness. Accordingly, this first hour after extubation (91-151 minutes) showed an inherently wide range of variability, much of which reflected the depth of anaesthesia and subsequent stage of consciousness at extubation.

A six-stage process was followed in both this chapter and Chapter 5 for identification of behaviours which could be used to differentiate between treatments. The first phase of this process dealt with the identification of behavioural frequencies and the second phase dealt with major differences between groups. A description of this process follows. Although processing of the noninteractive hourly behaviours is used for the description, the same process was implemented for the interactive palpation behaviours. Whereas notable outcomes from this process are cited in the results section additional data manipulations leading to these outcomes are contained in Appendix E. This approach has been taken for the convenience of the reader so that insights into the derivation of significant results are provided, but not at the expense of reader distraction from excessive detail.

### *Identification of behavioural frequencies:*

1. From the raw scores (Figure F2: Appendix F) of behavioural frequency recorded for each bitch individually, an average

- occurrence of every behaviour within each interval of observation (eg, hour 2, 3, 4 and 5) was obtained for each group.
2. Behavioural frequencies for each of the four hours were then averaged to obtain a mean occurrence of each behaviour per group (eg, Figure 4.4).
  3. Behaviours occurring with an average frequency of  $<2$ /hour were considered rare. Behaviours occurring with an average frequency of  $\geq 2$ /hour were classified as: low (2-6/hour), medium (7-10/hour) or high ( $>10$ /hour).
  4. Rare behaviours ( $<2$ /hour) were excluded from subsequent analysis because their low frequency of occurrence did not contribute to identifying differences due to treatment effect, and simply served as 'statistical clutter'.

To this point in the analysis, behaviours of note had been identified, but there was still the question of whether the noted behaviours actually allowed responses to treatment to be distinguished by behaviour, and if so what relative importance did each behaviour play in making the distinction.

#### *Major Differences Between Groups*

5. Canonical discriminate analysis was then performed to rank specific behaviours by their influence on making group distinctions. The analysis also provided a graphic display of group distinction by treatment effect. A statistical analysis system (SAS) computer program was used to perform the canonical discriminant function analysis, screening for behavioural differences (Manly, 1986). Two features of this technique are noteworthy. Firstly, the canonical analysis is used as a tool for the reduction of variables thereby making the data set more manageable. Secondly, canonical discriminant function coefficients statistically confirmed the negligible contribution toward treatment differentiation made by those behaviours recognised with rare occurrence.
6. Data from step 5 were then collated with data from step 2 to characterise the frequency of specific behaviours as they were ranked by importance for differentiating the groups.

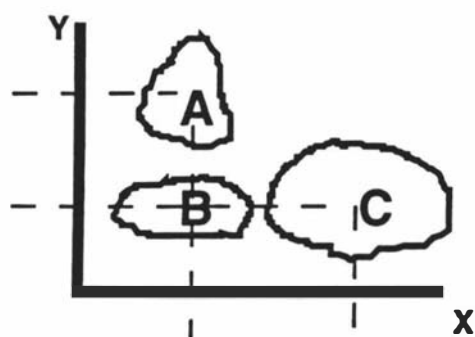
Results of both the hourly and palpation data are presented firstly by identification of major behaviours seen in all groups. After describing those major behavioural features of each group, the behaviours are identified which allowed groups to be differentiated by treatment effect.

Data from the Anaesthesia, Analgesia/Anaesthesia and Anaesthesia/Analgesia groups within this chapter will again be utilised in Chapter 5 in order to identify the different effects on behaviour from treatments involving surgery .

### *Canonical Discriminant Analysis*

With the assumption that the reader may not be familiar with the statistical technique of canonical discriminant analysis, the following section is presented to provide a greater comprehension (Cooley and Lohnes, 1971; Manly, 1986).

Understanding of canonical discriminate function analysis, which was used for the examination of behaviours in this thesis, begins with an appreciation that two-dimensional data which when plotted on a bivariate axis can be represented mathematically as a vector. Firstly, for this technique a statistical analysis system (SAS) computer program generates the 'best' linear combination of variables by which to describe a treatment. These linear combinations are referred to as **canonical discriminant functions (Z)**, and consist of contributing variables (V) with weighting coefficients (c); ie.,  $Z_1 = c_1 V_1 + c_2 V_2 + \dots$  etc.. By this approach it may be possible to determine several linear combinations for separating different treatments. Each function (Z) gives the maximum possible F ratio ( $\text{Mean Square}_{\text{Between Groups}} / \text{Mean Square}_{\text{Within Groups}}$ ) on a one-way analysis of variance for the differentiation of variation within and between groups. When more than one function is used to account for the treatment variability, then each additional function maximises the F ratio on a one-way analysis of variance subject to the condition that there is no correlation between the respective functions. Finding the coefficients of the canonical discriminant functions, which indicate the weighting of each variable for its discriminating value, is an eigenvalue mathematical problem.



Plotting the discriminant functions ( $Z_1$  on the X axis and  $Z_2$  on the Y axis) in this study allowed visualisation of treatment differences where behavioural results in each group were found to be in distinct and separate areas of the chart. In the illustrative example,

treatment C is easily differentiated from treatments A and B on the X axis, however A and B are indistinguishable on this axis because of similar treatment means. In contrast, reference to the treatment means on the Y axis is required to differentiate between treatments A and B, but this axis is ineffective in distinguishing between treatments B and C. Of course, it is difficult to visualise or to diagram functions representing more than three dimensions (3D). Common treatments clustered into a pattern that is well separated from other treatment clusters suggest that the treatment in question can be differentiated by the variables considered, ie, in the illustration, treatments A, B, and C are differentiated by the variables generating the plot as seen by their independent patterns. In contrast, overlapping patterns are less differentiated.

Canonical discriminant function analysis was applied to the behavioural data in Chapters 4 and 5. When graphically plotted, separation of one treatment group from another indicates that the treatments are well differentiated based upon the variables (behaviours) used to generate the canonical functions. Coefficients of the canonical functions can be considered as weightings of each respective variable as they reflect discrimination of treatment effects.

The statistical analysis system program generates several different coefficients that can be selected as weighting for the discriminant function variables. Different coefficients are generated in conformance to varying corollaries of mathematical models. Accordingly, which coefficient best explains the data is a contentious issue among statisticians. Some would suggest using the *pooled within-class standardised canonical coefficients* (SCC) while others would use the *pooled within canonical structure correlations* (CS). The former



weights the variable by considering the collective influence of all other variables, while the latter relies on a correlation of the individual behaviour to the canonical discriminant function, ignoring all the other variables. In Chapters 4 and 5 both of these coefficients have been employed, from which a composite has been obtained. This composite coefficient for each behaviour variable has then been used to weight the respective behaviour for determining its relative merit (rank) of importance in differentiating treatment effect.

There follows an illustration of the canonical discriminant analysis technique used to explore noninteractive hourly behavioural data from the nonsurgery treatments. All results presented hereafter in both Chapters 4 and 5 that were obtained from this technique were derived in a similar manner, therefore their independent derivations are not presented in the chapter text, but specific steps in obtaining textual data may be found in Appendices E (for Chapter 4) and G (for Chapter 5).

Figure 4.5<sup>11</sup> is a plot of the noninteractive hourly behaviours from the nonsurgical treatments for two canonical discriminant functions which are represented on the X and Y axes. In this analysis  $Z_1$  captures 78% of the differences between groups and  $Z_2$  captures 11.5% of the between-group differences (SAS calculation). Together they capture a comprehensive proportion of the between-group differences (approximately 90%). Greater treatment separation occurs with the horizontal axis values of  $Z_1$  than with  $Z_2$  on the vertical axis, and the two functions graphed together demonstrate group differences by identifiably separate (although overlapping) areas.

For analysis of group differences, canonical 1 (X axis) is more effective in separating the Analgesia, Control and Anaesthesia treatments, which have class means of -0.58, 1.49, and 3.73 respectively, from the Anaesthesia/Analgesia, Analgesia/Anaesthesia, and Anaesthesia/Immediate Analgesia treatments with class means of -1.68, -1.66, and -1.45, respectively. Similarly, on the Y-axis (canonical 2) the Anaesthesia/Immediate Analgesia treatment is more easily differentiated from the Anaesthesia/Analgesia, Analgesia/Anaesthesia, or Analgesia

<sup>11</sup> Figure 4.5a is a 2 dimensional (2D) plot of canonical 1 and canonical 2 data. Figure 4.4b is a 3 dimensional (3D) plot including canonical 3, which represents approximately 7% of the between-group differences for this data set.

treatments where the class means are 1.42, -0.19, -0.25 and -0.83, respectively. Neither canonical axis well differentiates the Analgesia/Anaesthesia treatment from the Anaesthesia/Analgesia treatment.

Separation of treatment patterns in Figure 4.5 suggests the degree of group differentiation that can be made by treatment effect on the behaviour variables. Each point on the chart represents a group bitch plotted against its  $Z_1$  and  $Z_2$  values. The SCC and CS coefficients developed within the multivariate analysis technique provide a scheme by which each behaviour may be weighted for its importance in differentiating the groups by treatment effect. The relative importance of each behaviour is judged by its absolute value; the positive or negative prefix simply denotes a vector orientation. Since either the SCC or the CS prefix may be used, these coefficients are tabled with their associated behaviour in descending order for the purpose of comparison. Table 4.2 shows these computer-generated (SAS program) coefficients for noninteractive hourly behaviours identified as most relevant for the differentiation of nonsurgical treatments (canonical 1).

The same data are provided in Table 4.3, but the behaviours are ordered (middle column) from the highest to lowest as determined by both the SCC and CS coefficients. The behaviours in Table 4.3 provide an order of importance for identifying major differences between groups, but by two different criteria: SCC and CS coefficient weighting. For example, 'draws legs up' is first under the order of SCC coefficient weighting, but eleventh under the CS coefficient weighting. Therefore the two criteria are now collated, providing input from each criterion. By giving each behaviour in Table 4.3 the number of points equal to its SCC and its CS order and then ranking the behaviours from the lowest to the highest total points, 'draws legs up' now ranks as tied for third with 'slow motion position change' in importance for differentiating treatments as based on total points. Table 4.4 shows the resultant ranking.

To this point, results from the canonical 1 analysis have suggested that the Analgesia, Control and Anaesthesia groups can be differentiated by behaviours, but that the Anaesthesia/Analgesia, Analgesia/Anaesthesia

and Anaesthesia/ Immediate Analgesia groups appear too much alike to be differentiated (Figure 4.5), ie. their group means are very similar on the X axis. Employment of the canonical 2 analysis (Y axis) allows differentiation of the Anaesthesia/Immediate Analgesia group from the Anaesthesia/Analgesia and Analgesia/Anaesthesia groups, the latter two remaining virtually indistinguishable by the noninteractive hourly behaviours, ie. group means for the Anaesthesia/Analgesia and Analgesia/Anaesthesia are too similar on either axis for differentiation by treatment effect.

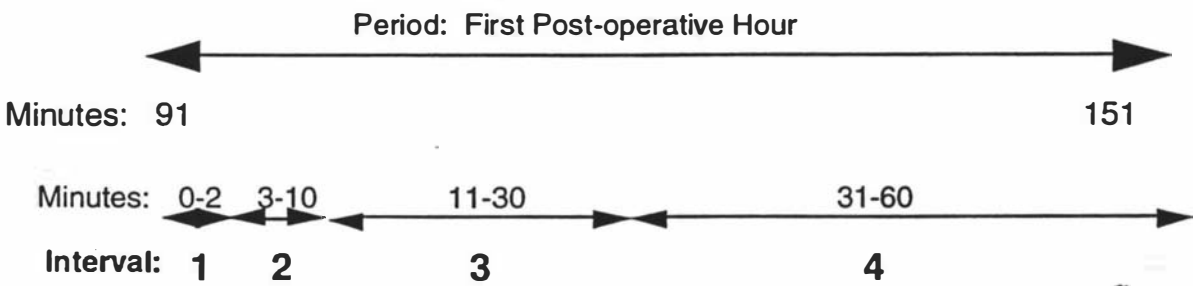
Pursuing the same process as for the canonical 1 analysis, data for the canonical 2 analysis of noninteractive hourly behaviours is presented in Tables 4.5 through 4.7. Based on the canonical 2 combination of SCC and CS coefficient order from Tables 4.5 and 4.6, the ranking of noninteractive hourly behaviours noted in Table 4.7 best differentiates the Anaesthesia/Immediate Analgesia group from the 1) Anaesthesia/Analgesia, Analgesia/Anaesthesia, and 2) Analgesia groups.

The canonical 2 analysis suggests that 'panting' serves first importance as the noninteractive hourly behaviour for differentiating the Anaesthesia/Immediate Analgesia group from the Anaesthesia/Analgesia and Analgesia/Anaesthesia groups, which themselves remain undifferentiated because they 'look' the same under the restraints of the canonical 1 and canonical 2 analyses.

Results

**Minute Behaviours**

Minute behaviours were summed over the four intervals comprising the first hour after 'extubation'.



Summation of minute behaviours over the four intervals comprising the first hour after extubation (91-151 minutes) for each group is presented in Figure 4.6. Totals for each separate interval (one to four) within this hour (91-151 minutes) are displayed in Figures 4.7 and 4.8<sup>12</sup> (3 Dimension and Hi-Lo charts).

Minute behaviours that were expressed rarely (<2 total occurrences over the first postsurgical hour) in all groups were:

Sternal curl	Door biting
Normal sit	Door pawing
Hang sit	Incision licking
Normal stand	Yawning
Hang stand	Trembling
Slow speed position change	Manipulation behaviours
Increased thoracic limb weight bearing	Urination
Walking	Defaecation
Normal speed motion	Vomition
Slow speed motion	Salivation
Increased thoracic limb weight bearing	Paddling
Motion ataxia	Look back
Slow motion cage circling	Pant
Quite alert	Whine
Drawing legs up	Bark
Cage digging	Groan/moan
Cage licking	Howl
	Bandage chew
	IV licking

Those minute behaviours of more notable occurrence in all groups are summarised in Table 4.8. Again, these frequencies represent a summation of the four intervals comprising the first hour after 'extubation'.

<sup>12</sup> For this and subsequent figures that involve both a 3Dimension and a Hi-Lo chart, the 3Dimension chart is presented for an appreciation of the comparative trends among groups while the Hi-Lo chart is presented for detail of actual frequency values. Hi-Lo simply refers to the highest and lowest values represented on the X axis irrespective of group.

## Description of Minute Behaviours for Each Group

### Control

In the Control group, the period that corresponded to the first hour after extubation in other groups was characterised by *low frequencies* of awake, sternal other, torso weight shift, lip licking, normal speed cage circling, cage sniffing and grooming, *medium frequency* in normal speed position change, and *high frequency* of head lifts and number of position changes. Controls showed the highest frequency of head lifts of any of the treatments (Table 4.9)(Figure 6). Behaviours characteristically not seen in this group, but displayed in other groups were: hang stand, positional ataxia, thrashing, and head nodding.

### Anaesthesia

The Anaesthesia group was characterised by *low frequencies* in lateral position, number of position changes, thrashing, head nodding, stretching, cage sniffing, grooming and pacing, *medium frequency* in torso weight shifts and head lifts, and *high frequency* in normal speed position change, position ataxia, lip licking, and normal speed cage circling. This group showed the highest occurrence of normal speed position change of any of the treatments (Table 4.9)(Figures 4.7 and 4.8).

### Analgesia

The butorphanol treatment was characterised by a *low frequency* of awake, sternal other, torso weight shift, normal speed position changes, position ataxia and normal speed cage circling. *Medium frequency* of head lifts was seen, and no behaviours were seen with high frequency. This group showed the highest occurrence of the sternal curl position and salivation (Table 4.9)(Figures 4.7 and 4.8).

### Analgesia plus Anaesthesia

Head lifts were seen in *high frequency* for this group, while normal speed cage circling was seen with *medium frequency*, and asleep, lateral, normal speed position changes and positional ataxia were seen with *low frequency*. This group showed the second highest frequency of head lifts and normal speed cage circling (Table 4.9)(Figures 4.7 and 4.8).

**Anaesthesia plus Immediate Analgesia**

This treatment resulted in *low frequency* of positional ataxia, asleep, lateral position, and stretching, *medium frequency* of normal speed position change and head lifts. No behaviours were seen with high frequency in this group. Among all the treatments this group showed the highest frequency of asleep and lateral position (Table 4.9)(Figures 4.7 and 4.8).

**Anaesthesia plus Analgesia**

This group was characterised mainly by a *medium frequency* of head lifts. *Low frequencies* included asleep, lateral position, normal speed position changes, positional ataxia, and lip licking. No behaviours were seen with high frequency in this group. Among all the treatments head lifts were third in frequency within this group (Table 4.9)(Figures 4.5 and 4.6).

A summary of the minute behavioural data for the nonsurgical groups is presented in Table 4.9.

**Noninteractive Hourly Behaviours**

For the noninteractive hourly behaviours (two to five hours, inclusive, post-extubation) frequencies were ranked as rare (<2), low (2-6), medium (7-10) and high (>10).

Noninteractive hourly behaviours which were rare in all groups (averaging <2 occurrences/hour) and would therefore have been of little use to discriminate between groups, were identified (Figure 4.5) and discarded from the analyses. Figure 4.5 is a graph showing the low, medium and high values of each noninteractive hourly behaviour for all groups. Ordinate values are averages of the behavioural frequency for the second through fifth hours, inclusive, after extubation. Rare noninteractive hourly behaviours included:

- |                       |                       |
|-----------------------|-----------------------|
| Lateral rest or sleep | Sternal rest or sleep |
| Lateral awake         | Sternal awake         |
| Sternal curl          | Sit alert             |

Sit other (lazy)	Incision licking
Hang sit	Groan/moan
Hang stand	Howl
Normal stand	Pacing
Slow motion position change	Ataxia
Draws legs up	Trembling
Attention seeking	Manipulation behaviours
Slow speed cage circling	Vomition
Thrashing	Salivation
Yawning	Urination
Cage licking	Defaecation
Cage digging	Look back
Door biting	IV licking
Door pawing	Bandage chew
Head nodding	

Those noninteractive hourly behaviours of more notable occurrence for the nonsurgical groups are presented in Table E1 (Appendix E).

**Description of NonInteractive Hourly Behaviours for Each Group**

**Controls**

Controls were characterised by *high frequencies* of: normal speed position change, and head lifts. Behaviours seen in low frequency were torso weight shift, stretching and grooming. Behaviours characteristically not seen in this group, but displayed in other groups were: hang stand, slow motion position change, and whining (Table 4.10)(Figures 4.9 and 4.10).

**Anaesthesia**

*High frequency* behaviours were: normal speed position change, and head lifts. Normal speed cage circling was seen with *medium frequency*. *Low frequency* behaviours were: torso weight shift, lip licking, cage sniffing, and grooming. Of all groups, this group showed the highest frequency of grooming (Table 4.10)(Figures 4.9 and 4.10).

**Analgesia**

After this treatment bitches showed only low and medium frequency behaviours. Torso weight shift was characteristically seen with *low*

*frequency*, while normal speed position change and head lifts were seen with *medium frequency* (Table 4.10)(Figures 4.9 and 4.10).

### **Analgesia plus Anaesthesia**

This treatment resulted in no high frequency behaviours. *Medium frequency* behaviours were normal speed position change, normal speed cage circling and head lifts. *Low frequency* behaviours were thoracic limb weight shifts, stretching and whining (Table 4.10)(Figures 4.9 and 4.10).

### **Anaesthesia plus Immediate Analgesia**

This treatment caused *low frequency* of stretching, escape behaviours, whining, panting and barking, *medium frequency* of normal speed position changes, thoracic limb weight shifts and head lifts, and *high frequency* of normal speed cage circling. Of all groups, this group showed the highest frequency of panting and vocalisation-- whining, barking and howling (Table 4.10)(Figures 4.9 and 4.10).

### **Anaesthesia plus Analgesia**

Behaviours of *low frequency* were thoracic limb weight shifts, lip licking and whining. Head lifts were seen with *medium frequency* and normal speed cage circling was seen with *high frequency*. Of all groups, this group showed the highest frequency of normal speed cage circling (Table 4.10)(Figures 4.9 and 4.10).

A summary of the noninteractive hourly behaviours per treatment is presented in Table 4.10.

### **Discriminating Behaviours In Each Group**

Those behaviours which allowed the responses to different treatments to be discriminated were determined by the canonical multivariate analysis. This, together with the frequency of each noninteractive hourly behaviour within each group (Figures 4.9 & 4.10), contributes to the creation of Table 4.11, showing the behavioural frequencies unique to the Control, Anaesthesia, and Analgesia groups compared to the other groups (Analgesia/Anaesthesia, Anaesthesia/Analgesia, and Anaesthesia/Immediate Analgesia) by canonical 1 discrimination.



*Within the constraints of the canonical 1 analysis, Table 4.11 is best utilised to note distinction between the Control, Anaesthesia and Analgesia groups.*

The Control, Anaesthesia and Analgesia groups are best differentiated by the canonical 1 analysis (Figure 4.5). The Canonical 2 analysis is further employed for differentiation of the Anaesthesia/Immediate Analgesia group (Table 4.12); however, even the additional implementation of canonical 2 analysis still fails to differentiate the Analgesia/Anaesthesia and Anaesthesia/Analgesia groups: they continue to appear similar.

*Within the constraints of the canonical 2 analysis, Table 4.12 is best utilised to note distinction between the Anaesthesia/Immediate Analgesia group and the other nonsurgical groups.*

The frequencies of some noninteractive hourly behaviours showed trends (Figure F2, Appendix F) during the period from the second to the fifth hour after 'extubation'. These trends are presented in Table 4.13.

### ***Interactive Palpation Behaviours***

Those observations presented above were based upon non-interactive behaviours, as the bitches were somewhat isolated within a screened area for the purpose of video taping. For insights into the bitches' 'interactive' behaviours, responses were assessed during each period of palpation which preceded blood sampling for cortisol assays.

Interactive palpation behaviours were assessed in a manner similar to the minute and noninteractive hourly behaviours. Unlike the noninteractive hourly behaviours, none of these behaviours was occurring more than once during a given palpation procedure i.e., a behaviour was either present or absent during a palpation. The frequency distribution of these behaviours (Figure 4.11) was such that qualitatively there was little point in making three divisions as with the

noninteractive hourly behaviours, therefore frequencies were grouped as high ( $>0.5$ ), low ( $0.3-0.5$ ) or rare ( $\leq 0.3$ ).

Rare interactive palpation behaviours were:

<b>Position change</b>	<b>Level</b>
Yes	High
<b>End Position</b>	<b>Tuck</b>
Sit	High arch
<b>Head Position</b>	No wag/curl
High	Slow wag/curl
Hang	Fast wag/curl
High alert	<b>Vocalisations</b>
Scooping	Whine
Tilt	Bark
Sway	Moan/groan
Sudden head lift	Yelp/scream
<b>Ear Position</b>	<b>Orientation</b>
Forward alert	Sharp belly
Alternating	Slow belly
Flat to sides	Deliberate avert
<b>Eye Position</b>	Hide
Glance/avert	<b>Breathing</b>
Wary	Pant
Eyebrow lift	Catch breath
Wide-eyed	<b>Other Behaviours</b>
Sleepy/lidded	Lip licking
Closed	Rigid stance
Frantic searching	Extended neck
<b>Tail Position</b>	Retreat
Escape	
Restrained	
Stretching	
Drawing legs up	
Lip lift	
Bite/snap	

Those interactive palpation behaviours of notable occurrence for all the nonsurgical groups are presented in Table E2. The interactive palpation behaviours from each palpation period within each treatment is summarised in Table 4.14. Additional presurgical (pre-op) and 24 hours observations are presented in Table 4.15. The presurgical data were obtained from a one-hour video recording on the day before commencing a treatment, immediately after the bitch was admitted to the hospital. The 24 hour data were obtained the day following the treatment, on the day the bitch was discharged from the hospital.

#### **Description of Interactive Palpation Behaviours for Each Group**

**Control** bitches were characterised by accepting palpation in a standing position and not changing this position after palpation. They characteristically held their head level, ears back, tail low, and stared ahead during the palpation. They did not vocalise, their breathing was normal, and they oriented their attention in staring ahead. During palpation the Control bitches frequently arched their back (Table 4.16)(Figures 4.12-4.16).

Several contrasting features characterised different treatments (Table 4.16)(Figures 4.12-4.16). **Anaesthesia** bitches accepted the palpation in a sitting position, changed positions over the course of the palpation and ended the exercise in a variety of positions. An arched back and lip licking were behaviours common to this group. In the **Analgesia** group bitches vocalised by whining for the first hour after being returned to their ward cage. An arched back was commonly seen in this group. Bitches in the **Analgesia plus Anaesthesia** group showed inconsistent starting positions and usually maintained this position after the palpation. Stretching and lip licking were commonly seen in this group. **Anaesthesia plus Immediate Analgesia** bitches routinely commenced the palpation in sternal recumbency and then assumed the lateral position at the termination of palpation. This treatment resulted in vocalisation of groaning/moaning between 1.5 and five hours, and after two hours this group changed their orientation from staring ahead to the tester. After two hours this group also showed panting. The **Anaesthesia plus Analgesia** group seldom changed from their lateral

recumbent position during palpation. Eye position was sleepy or closed until 1.5 hours into this treatment, after which time stare ahead was the common position. This group vocalised with moaning/groaning after two hours, and stretching was a commonly demonstrated behaviour.

#### **Discriminating Behaviours in Each Group**

Interactive hourly behaviours for the nonsurgical groups are presented in the canonical plot of Figure 4.17. In this analysis Z<sub>1</sub> captures 39% of the differences between groups and Z<sub>2</sub> captures 27% of the between-group differences (SAS calculation). (Z<sub>3</sub> seen in the 3 dimension graphic captures 17%)

The frequency of occurrence for ranked interactive palpation behaviours by canonical 1 analysis noting major differences between groups is seen in Table 4.17 (Figures 4.12-4.16 and Tables 4.14 & 4.16).

*Within the constraints of the canonical 1 analysis, Table 4.17 best highlights major differences of all groups except Controls, which is best differentiated by the canonical 2 analysis.*

Behavioural frequencies ranked by canonical 2 analysis which allow the major differences of the Control group to be compared with the other groups is seen in Table 4.18 (Figures 4.12-4.16 and Tables 4.14 & 4.16).

*Within the constraints of the canonical 2 analysis Table 4.18 best highlights differences in the Control group. The canonical 2 analysis poorly discriminates groups other than the Controls (Figure 4.17).*

The frequencies of some interactive palpation behaviours were observed to exhibit trends (Figure F3) during the period over which palpations were performed (121-391 minutes). These trends are presented in Table 4.19.

Average times from extubation to first stand were:

Anaesthesia,  $33 \pm 10.6$  minutes;  
 Analgesia plus Anaesthesia,  $77 \pm 17.6$  minutes;  
 Anaesthesia plus Immediate Analgesia,  $106 \pm 18.2$  minutes; and  
 Anaesthesia plus Analgesia,  $70 \pm 8.1$  minutes.

Behaviours, as they are presented above, have been utilised to identify differences in treatment effects, ie, the emphasis has been placed on treatments. Behaviour analysis with the emphasis on specific behaviours and how these behaviours changed in response to specific treatments is presented in Appendix F.

## **Discussion**

Both noninteractive and interactive behaviours were assessed in this study:

### **Noninteractive Hourly Behaviours Characterising Treatments**

#### *Control*

- High frequency of normal speed position change and head lifts
- Low frequency of stretching and grooming
- No hang stand, slow motion position change or whining

#### *Anaesthesia*

- Lip licking and cage sniffing

#### *Analgesia*

- No unique noninteractive hourly behaviours

#### *Combination: Anaesthesia and Analgesia*

- Vocalisation

### **Interactive Palpation Behaviours Characterising Treatments**

#### *Control*

- Standing position throughout the palpation event
- Level head position
- Ears back
- Tail low
- Looked ahead

- Highest frequency of arched back, lip licking and tail wagging

#### *Anaesthesia*

- Commenced sitting, but ended in a variety of positions
- Lip licking was common

#### *Analgesia*

- Whining, but with decreasing frequency over time

#### *Anaesthesia plus Analgesia and Anaesthesia plus*

##### *Immediate Analgesia*

- Vocalisation throughout the 5 hours after extubation

#### *Anaesthesia plus Analgesia*

- Seldom changed position from lateral recumbency
- Sleepy or closed eye position
- Stretching was common
- Moan/groan was common

Additional novel findings in this study were:

1. Behaviours which allowed the responses to different treatments to be discriminated could be determined by the canonical multivariant analysis statistical technique. Coefficients of variables generated by this technique may be used to weight respective variables for their merit in differentiating treatment effects.
2. Control bitches were easily differentiated from other groups using both noninteractive behaviours (with data from the canonical 1 analysis) and interactive behaviours (with data from the canonical 2 analysis).
3. Using noninteractive behaviours Control, Anaesthesia and Analgesia treatments were more easily differentiated than treatments involving combinations of analgesia and anaesthesia. Within treatments involving both analgesic and anaesthetic, the Anaesthesia/Analgesia and the Analgesia/Anaesthesia treatments were virtually indistinguishable by the behavioural indices assessed.

Observations on the effects of anaesthetics and analgesics in animals deal with physiological parameters such as cardiovascular response, respiratory alterations and body temperature changes (Trim, 1983; Muir and Robertson, 1985; Tyner et al., 1989; Greene et al., 1990; Sawyer et al., 1991). It is rare for behavioural responses also to be documented. This is most likely attributable to the lack of core behaviours by which to judge normality. Results of this study suggest that normal behaviours can be defined and treatment effects can be identified in behavioural alterations, yet considerable development is needed in this area before subtle treatment effects can be recognised.

Comparing results from this behavioural study with results from the plasma cortisol study in Chapter 2 gives rise to speculation on interactive correlations. Data from this behavioural study suggests that a high frequency of activities which may be construed to represent restlessness (such as normal speed position changes, normal speed cage circling, positional ataxia and torso weight shifts) are associated with anaesthesia (Tables 4.9 and 4.10). In comparison, anaesthesia was associated with low levels of plasma cortisol, as presented in Chapter 2 (Figure 2.2). On the other hand, all the groups treated with analgesic in this study generally showed lower frequencies of the above listed behaviours considered representative of restlessness, but were associated with higher levels of plasma cortisol than were the Anaesthesia group (Figure 2.5). It may well be that the Anaesthesia group was able to express their anxious state by the activity of motion, whereas those bitches under the influence of sedation from the butorphanol were restricted in their ability to express this anxiety which, in turn, was distressful and was manifest as elevation in plasma cortisol concentrations.

During the course of this study a clinical impression was gained that bitches recovering from anaesthesia were unsettled until such time as they achieved postural control. This was most apparent in the Anaesthesia group. Of additional note is the time to first stand which was significantly less ( $P < 0.05$ ) for the Anaesthesia group than for the groups treated with both analgesic and anaesthetic. Obviously, the sedation effect of the butorphanol delayed the time to first stand in these

latter groups, a time over which it is proposed that they were restricted from relieving their distress from restlessness.

The somewhat isolated confinement of the bitches in this study may have been a contributing factor in the low occurrence of vocalisation (barking and whining) within the Control group. This gives rise to several points regarding vocalisation. Studies by Adams and Johnson (1994) showed that urban dogs have a sleep/wake cycle (during the night) averaging 21 minutes, so they effectively wake 3 times per hour. Their observations of sleeping dogs suggested a possible decrease in response to external stimuli during active sleep, similar to rapid eye movement (REM) sleep in humans. The same authors later showed that dogs were more likely to bark or become more alert in response to barking than to other auditory stimuli (Adams and Johnson, 1994). Accordingly, individual bitches in this study might have been influenced by the occasional boisterous dog housed elsewhere in the hospital. Barking is proposed as a juvenile characteristic which is promoted during domestication, as wild adult wolves do not bark (Coppinger and Feinstein, 1991). Being part of a social group could be an evolutionary mechanism that affords greater survival for dogs. Barking in pairs or groups may be synergistic by allowing dogs to gain increased security within their social group (Coppinger and Feinstein, 1991).

Historically, veterinarians and animal handlers have inferred a 'painful' state from animals vocalising after extubation from surgery. In this study, which did not include any surgical treatments and therefore no recognised source of pain-induced distress, all of the groups except the Control and the Anaesthesia bitches showed vocalisation as an interactive behaviour after extubation, ie. every treatment in which the analgesic was administered was characterised by vocalisation. Such vocalisation may well be a behavioural expression of the bitch's analgesic induced dysphoria.

Within the Anaesthesia treatment ataxia and slow motion position change are behaviours expected to be of high frequency, both attributable to recovery of the central nervous system from effects of gaseous anaesthesia. As bitches in the present study regained consciousness and became more alert over time, they regained normal



speed of position change. For reasons previously cited (latter part of materials and methods), return to consciousness was variable, even within treatments.

Two neurotransmitters which may influence behavioural responses resulting from the administration of the general anaesthesia, halothane, and the synthetic opioid, butorphanol used in this study, are 5-hydroxytryptamine (5-HT) and acetylcholine. While the molecular basis of the action of most anaesthetic agents is unknown, it is often assumed that general anaesthetic drugs have a more pronounced effect on synaptic mechanisms in the central nervous system than on the propagation of electrical signals along axons (Larrabee and Posternak, 1952). Further, it is unknown whether anaesthesia results from neuronal mechanisms at specific sites or from a range of metabolic alterations with lesser specificity. The diversity of different transmitter substances and complex interplay between systems of transmission give rise to many permutations for potential sites of action. Behavioural responses to the anaesthetic and analgesic administered in this study could well be an expression of pharmacodynamic effects on these and other neurotransmitters. Neurotransmitter systems have been subdivided into three classes: amino acids, so-called "classical transmitters", and neuropeptide systems (McMahon and Nicholls, 1991). Among the classical transmitters is 5-HT. Although a precise functional role for 5-HT has as yet to be fully established, it has been implicated as an important transmitter in the feeding, sleep, mood, behaviour and cardiovascular control pathways (Althaus et al., 1985). Another classical neurotransmitter gaining recognition for its importance in the control of consciousness is acetylcholine. In a study on acetylcholine transmission, the release of acetylcholine from the surface of rabbit brain was shown to decrease during barbiturate anaesthesia and increase after a period of feeding and walking, indicating a role for acetylcholine in the maintenance of consciousness (Collier and Mitchell, 1967).

Post extubation lip licking was characteristically seen in dogs recovering from the Anaesthesia treatment (Table 4.11), yet this behaviour has not apparently been reported in the literature. Lip licking cannot ostensibly be attributed to salivation because salivation and lip licking were not correlated in this study, and, if acetylcholine

decreases as a result of anaesthesia (Collier and Mitchell, 1967), salivation would also be expected to decrease since the parasympathetic stimulus for salivation is principally under cholinergic control.

Cage sniffing was ranked high (#1) as a discriminator for the Anaesthesia treatment by canonical 1 analysis of noninteractive hourly behaviours. Cage sniffing was frequently seen when a conscious bitch was first introduced to her new cage after admission to the hospital, but ceased after a short period of time. In contrast, bitches in the Anaesthesia group continued this behaviour for an extended period. There are at least two possible explanations for this phenomenon. Firstly, a pharmacological effect of the anaesthetic may have 'blunted' the functional integrity of the olfactory system anywhere from cranial nerve I to the cerebral cortex, causing the bitch to 'work harder' for recognition of its environment. Secondly, the anaesthetic may interfere with the recall or recollection of the bitches' environment causing the bitch to continuously re-acquaint itself with its surroundings.

Intense anxiety and fear are an integral part of the distress response/experience. Any behavioural expression of anxiety in this study may have been altered by the administration of butorphanol: initially by the strong influence of dysphoria and later by the strong influence of sedation. Several authors (Corman et al., 1958; Janis, 1958; Chapman and Cox, 1977; Volicer, 1978) have noted that presurgical and postsurgical anxiety in humans dramatically influence the postsurgical period, particularly in patients with inadequate psychological preparation. Prior to an operation many human patients develop apprehension and fear, feelings that are carried into the postsurgical period causing anxiety and arousal. A similar phenomenon may well have occurred in the bitches in this study. Distressful psychological stimuli unique to captive environments may result from an animal's inability to perform active behaviours directed to a predicted outcome (Dantzer, 1989). For example, an animal may be restricted in contrast to a more permissive environment where it can find a hiding place or escape from a threat. Correlations between distress and environmental restrictions are well documented in both the rat (Irwin et al., 1989) and the cat (Carlstead and Brown, 1993). In general, threats perceived as inescapable or uncontrollable elicit a

'conservation-withdrawal' response strategy, resulting in an animal reacting passively, usually submitting or withdrawing (Archer, 1979; Henry, 1982). Increased adrenocortical and vagal activity, decreased gonadal steroid secretion, and elevated blood pressure may also be observed under such restraints (Moberg, 1985). Although these latter physiological parameters were not assessed in this study, bitches under the influence of butorphanol may be recognised to have exhibited this conservation-withdrawal type of profile. However, the strong pharmacological influence of analgesic sedation probably influenced an expression of this anticipated behaviour.

Although physiological responses to the administration of opioids in dogs are well documented, behavioural responses are not. A report of the opioid effects on social behaviour of kennel dogs was published in 1983 by Panksepp and others (Panksepp et al., 1983). In their experiment, 'kennel-dogs' were generally more obedient following treatment with a low dose of morphine (0.25 mg/kg, subcutaneous) and more uncontrollable following naloxone treatment (0.25 mg/kg, subcutaneous). The authors concluded that morphine may have simulated a state resembling that of being re-united with their original homes, thereby permitting the animal to be more responsive to humans, while opioid blockage may have further intensified a state of social isolation, thereby making the animal less capable of deriving comfort from human interaction. Further, Davis (1983) observed that in well-socialised dogs, naloxone amplified solicitous canine behaviour patterns, namely tail-wagging and face licking, while morphine reduced such behaviours.

Agonist opioids tend to elicit dose dependent responses, while partial agonist opioids (such as butorphanol) show ceiling effects (Jaffe and Martin, 1992). This differentiation, together with an appreciation that morphine and butorphanol act through different receptors (Chapter 1), makes behavioural responses to morphine a poor model for behavioural responses to butorphanol. Notwithstanding, Domer and Josselson (1964) observed that ten to twenty seconds after the first intravenous administration of morphine into a conscious dog, a behavioural response occurred which they termed "sham rage". The response was characterised by violent struggling, yelping and snapping. After this

sham rage, the duration of which depended on the dose of morphine used, the characteristic effects of narcosis, salivation and miosis were produced (Domer and Josselson, 1964). Urination and defaecation were also observed with the highest doses used (5 mg/kg) and occasionally a second episode of sham rage occurred after the initial response had subsided. The findings of Fennessy and Ortiz (1968) agree with Domer and Josselson's hypothesis that sham rage produced in the dog by morphine was due to a release of histamine in the brain. In 1966 Holobut fluorimetrically assayed the plasma of dogs for adrenaline and noradrenaline, and found that small doses of histamine preferentially released adrenaline, whereas higher doses released both adrenaline and noradrenaline. A response similar to sham rage is predictably absent with the intravenous administration of butorphanol since the agonist-antagonist butorphanol acts at a different opioid receptor.

Representative interactive palpation behaviours for each treatment are presented in Table 4.16. Anaesthesia and/or analgesia altered the starting and ending position from that of Controls. Standing was seen on both occasions in only the Control group. This is most likely a result of the sedative effect from the halothane or butorphanol in the other groups. A similar explanation is proposed for the change of head position, where all groups are different from the Controls. Most different from the 'level position' of the Controls was 'rest on surface', which was characteristic of the two groups experiencing the highest systemic concentrations of butorphanol at the time of observation.

In conclusion, quantitative physiological responses to distress have been investigated for quite some time and our understanding of how drugs influence the central nervous system has advanced dramatically over the past decade. Linking both the behavioural and physiological responses to distress could further enable us to use both our present drugs as well as newly developed drugs more responsibly and more creatively.

Within this chapter both noninteractive and interactive behaviours resulting from nonsurgical treatments have been explored. The assignment of *rare*, *low*, *medium*, and *high* categories for behavioural frequencies was somewhat arbitrarily based upon the total distribution within all treatments. Multivariate analysis has been used to verify

differences among treatments and to weight behaviours as they influence major differences between groups. Having established reference data on behavioural responses to nonsurgical treatments it is now possible to search for pain-induced distress behaviours associated with the surgical procedure of ovariectomy. Thereafter the effect of presurgical and postsurgical analgesia using butorphanol can be assessed.

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## Chapter 5

### Effects of surgery as well as presurgical and postsurgical butorphanol analgesia on behaviour in ovariohysterectomised bitches

#### Chapter Summary

Both noninteractive and interactive behaviours were investigated for differences in effect from four treatments: Controls, Anaesthesia plus Surgery, Analgesia plus Anaesthesia plus Surgery and Anaesthesia plus Surgery plus Analgesia. Results support the presumption that noteworthy pain-induced distress accompanies 'routine' ovariohysterectomy in the bitch, and minor behavioural changes can be recognised which accompany this pain-induced distress. Noninteractive behaviours associated with surgery were a decrease in normal speed cage circling and an increase in frequency of drawing legs up, stretching, and torso weight shift. Drawing legs up (as in a pike position) is proposed as the most easily recognised pain-induced distress behaviour for animal care attendants, as the other discriminating noninteractive behaviours are more subtle and require a pre-treatment control to allow assessment. The infrequent noninteractive behaviours of looking back at the flank area, vomition and incision licking were also considered to be behavioural expressions of pain-induced distress from ovariohysterectomy. A general demeanour of restlessness characterised surgery bitches who were not given analgesic and these bitches characteristically maintained a sternal position when palpated.

During the postsurgical period those bitches receiving analgesic moved less frequently than those not receiving analgesic, and they moved with greater ease when they did so. Agreeing with previous findings, bitches receiving analgesia in this study were more vocal than those that did not. Such vocalisation was attributed to the dysphoria induced by butorphanol, which limits the use of the traditional behaviour of vocalisation as an index of postsurgical pain.

Control bitches in this study were characterised by accepting the palpation exercise in a standing position and not changing this position after

palpation. They characteristically held their head level, ears back, tail low, and stared ahead during the palpation. They did not vocalise, their breathing was normal, and they oriented their attention in staring ahead. During palpation they arched their back. Major deviations from these characteristics, given a similar environment, should be considered abnormal.

There were no characteristic behaviours recognised as unique to the presurgical analgesic group. This was attributed to the combination of timing and route of butorphanol administration for this treatment. Giving intravenous butorphanol 30 minutes prior to anaesthesia induction was concluded to be too early for influencing any postsurgical behaviours by its analgesic effects.

Results from this study show that characteristic behaviours can be associated with both the plasma cortisol concentration rise resulting from the pain-induced distress of ovariohysterectomy and the cortisol fall accompanying the administration of butorphanol. Some of the behaviours related to the butorphanol are ascribed to the drug's analgesic properties while other behaviours are attributed to the sedative attributes of the drug.

Canine ovariohysterectomies are frequently performed in companion animal veterinary practice and veterinarians have historically contended that bitches 'tolerate' the procedure fairly well, even in the absence of analgesic. From this study it is concluded that routine ovariohysterectomy is accompanied by sufficient pain-induced distress as to warrant the concurrent administration of butorphanol analgesic, which was seen to reduce this pain-induced distress. Characteristic behaviours associated with the pain-induced distress of this well-tolerated 'routine' procedure have been identified, and it is proposed that more pronounced and easily recognised behaviours would be associated with more severe surgical trauma; making the behavioural response to analgesics, in turn, more pronounced.

## Introduction

Part of the declaration from all members of the Royal College of Veterinary Surgeons (UK) and the American Veterinary Medical Association is a commitment to the welfare of animals. This implies an endeavour to increase knowledge and understanding of animal pain in order to reduce it to the minimum consistent with the goals of society.

Most support for the belief that animals experience pain is based on similarities between nociceptive reflexes and reactions in man and animals. As Thomas Lewis (1942) states, "We have no knowledge of pain beyond that derived from human experience; yet we may judge of its presence in animals by bodily reactions that human experience has brought us to recognise as its frequent accompaniment or by use of stimuli that similar experience tells us should be painful."

Scientists investigating analgesic drugs often quantify the occurrence and latency of simple behaviours as well as more complex responses that animals show to a normally painful stimulus. In most cases the investigator infers pain or analgesia from behavioural changes. Yet one cannot quantify animal pain directly.

"Pain is far from being an emotionally neutral experience; it is almost always accompanied by emotional disturbance and distress" (Shott, 1991). "In animals, emotional pain is defined as an unpleasant perception in response to external or internal stimuli that results in a state similar to anxiety or frustration" (Spinelli and Markowitz, 1987). Beecher (1969) contends that the pain experience involves not only a sensory signal but an emotional reaction to that signal as well, maintaining that a meaningful study of pain (in humans, at least) cannot be conducted in exclusion of concomitant emotional reactions.

Probably the most frequently used approach to determine whether animals are suffering from distress or not is to make a comparison of their status with that of ourselves. Presently we know very little about the species-typical behavioural patterns that have evolved in animals, and most often we try to explain animal behaviours by the same cause and effect which we know from human behaviour; as if animals were under-developed humans. While humans have developed intellectually over time many animals have

also advanced with evolution, but in areas of more sophisticated vocalisation and behavioural communications. This is becoming more apparent as we learn more about the wildlife around us. In many ways, using an animal's own behaviour to indicate its freedom from distress is more reliable than attempting to evaluate its welfare by comparing its reactions to ours or even trying to establish a link between its physiological responses and its welfare.

The recognition of pain in animals is inherently difficult and there are many factors to be considered. While pain is a sensation, the perception of pain in advanced vertebrates is considered to be physiologically similar to that in humans (Haskins, 1991). If a noxious stimulus were considered to be painful to a human, it may well be considered painful to any other developed animal. Accordingly, studies indicate that the pain threshold, or level at which a noxious stimulus results in the perception of pain, is very similar regardless of the species tested (Haskins, 1991).

Those entrusted with the alleviation of pain-induced distress in animals should strive to anticipate painful situations, recognise signs of pain-induced distress, objectively assess the degree of suffering, and then choose an appropriate protocol for relief. Insights into the first premise, anticipation of a painful stimulus, have historically been guided by schemes such as the following (Werner and Taboada, 1994):

### Classification of Pain Severity Associated with Surgical Procedures

#### Severe

Procedures involving the:  
 Eyes and periorbital area  
 Shoulder musculature and humerus  
 Cervical vertebrae  
 Amputation  
 Sternal thoracotomy

#### Moderate

Procedures involving the :  
 Head and face  
 Thoracic and lumbar vertebrae  
 Anorectal area  
 Lateral thoracotomy  
 Celiotomy  
 Orthopaedic procedures

#### Mild

Minor superficial procedures

Clinical assessment of the patient should involve critical observation and interpretation of nocifensive behaviours, which are behavioural responses evoked by a noxious stimulus that is presumed to be associated with pain. Animal handlers recognising nocifensive clues, should then render appropriate treatment including analgesia. As Crane (1987) states, "No apologies are needed for the use of analgesics in the patient with discomfort or pain on the basis of anthropomorphic considerations of the veterinarian. To provide such therapy is consistent with the Veterinarian's Oath."

In evaluating an animal for physical pain one must rely more on the art of clinical evaluation than on 'pure' science (Spinelli and Markowitz, 1987). If a procedure has a high probability of inducing pain, and there is a radical change in an animal's behaviour after the procedure, it is most probable that pain-induced distress is the cause of that change. A presumptive diagnosis of pain-induced distress can be made when clinical signs of distress are alleviated by the use of an appropriate analgesic or by the use of a local anaesthetic (Spinelli and Markowitz, 1987).



"Within the higher central nervous system, arousal of the hypothalamic-pituitary axis during fear or anxiety probably sensitises the patient to the nociceptive afferent barrage" (Chapman and Turner, 1990). LeResche (1982) has confirmed a human facial expression unique to acute pain, and in human clinical practice it is the behaviour of the patient in pain, more than any physiological indicator, that defines the severity of the pain problem for the staff (Chapman and Turner, 1990).

Canine behaviours that veterinarians have historically attributed to pain or discomfort include many spontaneous and interactive behaviours with a broad spectrum of features. The ideal clinical situation would provide opportunity for observation of each patient's 'normal' behaviour in the hospital prior to performing surgery or some other procedure perceived as painful, and then compare this 'normal' behaviour with its behaviour following the onset of pain-induced distress. This is seldom possible, and clinicians rarely evaluate patient behaviour before distress is induced. Nevertheless, a number of behaviours observed in hospitalised dogs are *presumed* to represent responses to pain, yet validity, specificity and sensitivity of these behaviours to degrees of pain-induced distress have not been verified.

Most accounts of veterinary clinical experience with the recognition of pain appear to be made on the basis of anecdotal observations of signs of pain-induced distress, and few clinical reports provide recommendations for evaluation of distress on the basis of rigorously observed behaviour (Hansen and Hardie, 1993). Having identified the behavioural responses to combinations of analgesia and anaesthesia from the 'base' treatments of Chapter 4, the phase of the study described in this chapter was conducted with the intention of (1) elucidating behaviours associated with an ovariohysterectomy, and (2) identifying changes in those behaviours resulting from pre- or postsurgical administration of butorphanol.

Effects of pain-induced distress from surgery cannot be identified without reference to the base groups of Chapter 4; therefore, details from the Anaesthesia, Analgesia plus Anaesthesia, and Anaesthesia plus Analgesia groups are once again presented so that conclusions can be drawn. Such conclusions are of central importance to the clinician, whose postsurgical

care and treatment decisions are most often initiated by changes in patient behaviour

## Materials and Methods

### *Animals*

*Three treatments from Chapter 4 are referenced as comparative bases:*

- 1) Anaesthesia*
- 2) Analgesia plus Anaesthesia*
- 3) Anaesthesia plus Analgesia*

*The Anaesthesia group has served as a comparative base to the Anaesthesia/Surgery group in the distinction of responses to surgery, while the Analgesia/Anaesthesia and Anaesthesia/Analgesia groups have served as comparative bases to the Anaesthesia/Surgery group in the distinction of responses to butorphanol analgesia.*

Thirty-three bitches of various breeds and an average age of 19 months were used for three surgical treatments in this study. Twenty-one of the bitches were presented to the veterinary hospital for routine ovariohysterectomy by their owners who signed consent forms for the study, while the others were from the Massey University Animal Health Services Centre. Each bitch was fasted overnight before undergoing a treatment described below. The treatments were:

- \* (1) deliberate Control (11 bitches),
  - 6 bitches were the same as in the pilot study (Appendix D),
- \* (2) Anaesthesia plus Surgery (12 bitches),
  - 6 bitches were the same as in the pilot study (Appendix D),
- \* (3) Analgesia plus Anaesthesia plus Surgery (10 bitches), and
- \* (4) Anaesthesia plus Surgery plus Analgesia (10 bitches).

All treatments were conducted in parallel over the entire period of data collection so as to minimise any influence of seasonal effect. The allocation of bitches to treatments and the order of treatments were random.

Patient data are as follows:

Bitches		
Breed	Number	Mean Age (Mo)
Labrador X	5	8.2
Huntaway X	4	30.3
NZ Border Collie	7	12.3
Terrier	2	17.5
Spaniel	2	24.3
German Shepherd	4	25.5
Wiemaraner	1	24
Yellow Lab	2	22
Ridgeback	1	14
Doberman	1	7
Great Dane	1	14
Newfoundland	1	37
Toy Poodle	1	7
Corgi	1	25
Total	33	19

All bitches were admitted to the hospital during the afternoon of day one and were placed into an indoor cage (840 cm x 942 cm x 790 cm) within a restricted access, but not isolated, ward. Physical examination revealed that the bitches were healthy. One of the treatments was commenced at approximately 10:00 AM on day two, and the bitches were discharged after noon on day three. This sequence was followed for all treatments.

Deliberate Control (Con): The bitch was moved from the ward to the anaesthesia induction room, a cephalic vein catheter was placed, and a blood sample was taken. The bitch was gently restrained on a table in the presence of low activity (traffic of three to four persons) for approximately 53 minutes, and was then returned to the ward. Throughout the following five hour period of observation, blood sampling was performed at regular intervals as indicated in Table 5.1. Blood collection was via the catheter until it became blocked or was dislodged by the animal, after which samples were taken by cephalic venipuncture (22

gauge needles) in the contralateral limb. Bitches were video taped<sup>13</sup> for five continuous hours following their return to the ward cage (Table 5.1). These were the same animals as the Controls in Chapters 2, 3, and 4. They were studied contemporaneously with the other treatments reported in this Chapter.

Anaesthesia plus Surgery (Ans/Sx): After arrival in the induction room and cephalic vein catheterisation, without preanaesthetic medication, anaesthesia was induced with barbiturate (2.5% sodium thiopentone, approximately 20-25 mg/kg) to a depth allowing intubation, after which a blood sample was taken through the cephalic catheter. Following routine preparation for surgery and movement to surgical theatre, a three-clamp ovariohysterectomy (Fingland, 1990) was performed. The abdominal wall was closed with no greater than appositional tension using a simple interrupted pattern of absorbable suture<sup>14</sup>. The skin incision was closed with monofilament absorbable sutures in an intradermal pattern<sup>14</sup>. Anaesthetic administration for this group lasted approximately 55 minutes. Bitches were video taped as for the Control group (Table 5.1).

Analgesia plus Anaesthesia plus Surgery (Anl/Ans/Sx): Thirty minutes prior to presentation in the induction room 0.4 mg/kg of butorphanol (Torbugesic<sup>15</sup>, Fort Dodge Laboratories, Fort Dodge, Iowa) was administered intravenously. The bitch was then allowed to rest in the ward cage until movement to the induction room and subsequent handling as with the anaesthesia plus surgery group. Bitches were video taped as for the Control group (Table 5.1).

Anaesthesia plus Surgery plus Analgesia (Ans/Sx/Anl): The bitches were treated in the same way as the Anaesthesia plus Surgery group, except 0.4 mg/kg of butorphanol was administered intravenously at the time of extubation after the bitch was returned to the ward cage. Bitches were video taped as for the Control group (Table 5.1).

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<sup>13</sup> Panasonic MS 1, S-VHS video camera. Viewing was performed on a Panasonic FS 100, S-VHS video cassette recorder with jog/shuttle control.

<sup>14</sup> Maxon. Davis & Geck, Auckland, New Zealand.

<sup>15</sup> Torbugesic: Lot # 454127

Treatment Abbreviations		
<hr/>		
Con	⇔	Control
Ans/Sx	⇔	Anaesthesia/Surgery
Anl/Ans/Sx	⇔	Analgesia/Anaesthesia/Surgery
Ans/Sx/Anl	⇔	Anaesthesia/Surgery/Analgesia

The Analgesia plus Anaesthesia plus Surgery group received butorphanol at zero minutes while the Anaesthesia plus Surgery plus Analgesia group received butorphanol at 91 minutes (Table 5.1). Commencing with the 121 minute blood sample (30 minutes after extubation), the abdomen was gently palpated immediately prior to each blood collection. The period from the beginning of palpation to the end of blood sampling was rarely greater than 3 minutes.

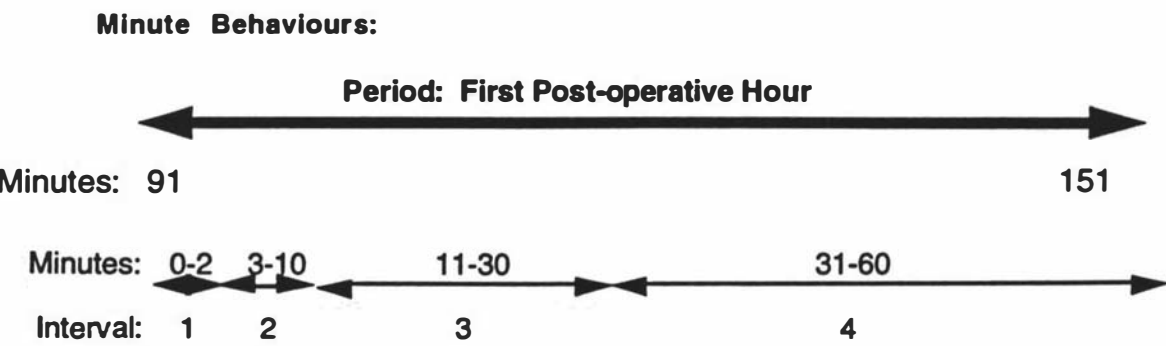
*Behavioural Recording*

All behaviours were video-taped with the bitch contained in the indoor cage. The cage was illuminated with a fluorescent light affixed to the cage ceiling. It contained no bedding, no bowls, and the cage door was constructed of vertical metal bars. The camera was mounted on a tripod approximately four meters in front of the cage adjacent to an additional light source directed into the cage. Isolating the cage and camera within the restricted ward area was an opaque screen suspended from the walls. Bitches were video taped for five continuous hours following their return to the ward cage.

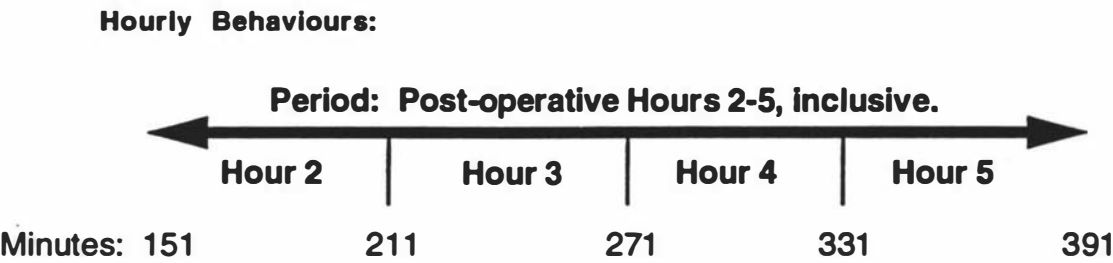
Continuous filming provided non-interactive behavioural scoring over the intervals noted. Interactive behaviours were scored during the short periods of blood sampling. At the time of blood sampling the investigator approached the cage through the isolating screen, pausing approximately two seconds before opening the cage door. The bitch was greeted with a "hello", (animal's name) and patted gently on the head three times. The ventral abdomen was gently squeezed three times with a single open hand, palm up. The investigator then withdrew, closed the cage door and exited the screened area. The investigator then re-entered the screened area to take the blood sample. Only three persons performed all palpations, and

the same investigator did not always serve as palpator for a given bitch treatment.

The video tape recording was assessed as follows: for the first hour after extubation 51 behaviours were evaluated (Figure 5.1) during four intervals: minutes 0-2, 3-10, 11-30 and 31-60. These behaviours are referred to as **minute behaviours**. At the commencement of this study it was anticipated that many behaviours might be occurring rapidly as soon as the bitch was extubated. By dividing the first hour after extubation into four intervals as presented below, it was believed that such activity might be captured in the best manner.



For the next four hours (hours two to five, inclusive, post-extubation) 48 behaviours were evaluated (Figure 5.2) in frequency by hourly increments. These behaviours are referred to as **hourly behaviours**.



The entire postsurgical period thereby consisted of the combined minute and hourly behaviour periods.



Sixty-seven behaviours were evaluated during each palpation period (approximately three minutes in duration) commencing at 121 minutes (Figure 5.3). These behaviours are referred to as **palpation behaviours** and were obtained at the time of blood sampling for plasma cortisol (Table 5.1).

**Palpation Behaviours:**



The minute, hourly and palpation behaviours are noted as follows:

Noninteractive Minute Behaviours:

Each observed behaviour is listed below within a group which includes similar types of behaviours. Behaviours within each of these groups are listed in columns read down, then to the right (↓⇒).

**Stationary positions**

lateral	hang stand
sternal curl	normal sit
sternal other	hang sit
normal stand	

**Position changes**

number of changes	torso weight shifts
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**Modifiers**

ataxia	increased thoracic limb
slow speed	weight bearing
normal speed	

**Motion**

walking

**Modifiers**

normal speed	first stand
slow speed	

**Activities**

slow motion cage circling		defaecation
normal speed cage circling		quiet alert
pacing	thrashing	head lifts
drawing legs up	stretching	head nodding
cage licking	yawning	door biting
cage sniffing	incision licking	door pawing
cage digging	lip licking	grooming
manipulations	urination	trembling
vomition	salivation	paddling
look back		

**Breathing**

pant	normal
------	--------

**Vocalisation**

whine	bark
groan/moan	howl



## Noninteractive Hourly Behaviours:

Each observed behaviour is listed below within a group which includes similar types of behaviours. Behaviours within each of these groups are listed in columns read down, then to the right ( $\Downarrow \Rightarrow$ ).

### Stationary major behaviours

lateral rest or sleep	sternal curl	sit other
lateral awake	sit alert	normal stand
sternal awake	hang sit	hang stand

### Position change

slow motion position change  
 normal speed position change  
 torso weight shifts  
 thoracic limb weight shifts  
 draws legs up  
 stretching

### Movement behaviours

attention seeking	normal speed cage circle
escape behaviour	slow speed cage circling

### Short behaviours

head lifts	look back	cage licking
head nodding	pacing	cage digging
ataxia	yawning	door pawing
thrashing	trembling	door biting
normal breathing	salivation	whine
panting	urination	groan/moan
lip licking	vomition	bark
incision licking	defaecation	howl
grooming	cage sniffing	manipulation

Interactive Palpation Behaviours:

Each observed behaviour is listed below within a group which includes similar types of behaviours. Behaviours within each of these groups are listed in columns read down, then to the right (↓⇒).

<b>Starting positions</b>		
lateral	sit	
sternal	stand	
<b>Position changes</b>		
yes	no	
<b>End position</b>		
lateral	sit	
sternal	stand	
<b>Head position</b>		
high	lowered	sway
high alert	hang	scooping
level	rest on surface	tilt
sudden head lift		
<b>Ear position</b>		
forward alert	neutral	back
alternating	flat to sides	
<b>Eye position</b>		
wide-eyed	eyebrow lift	wary
stare ahead	frantic searching	glance/avert
watch	sleepy or lidded	closed
<b>Tail position</b>		
high	fast wag/curl	tuck
high arch	slow wag/curl	low
level	no wag/curl	on surface
<b>Vocalisation</b>		
whine	bark	
groan/moan	yelp/scream	
<b>Orientation</b>		
stare ahead	deliberate avert	sharp belly
tester	hide	slow belly
<b>Breathing</b>		
normal	catch breath	pant
<b>Other behaviours</b>		
lip licking	drawing legs up	retreat
rigid stance	stretching	restrained
extended neck	lip lift	escape
arched back	bite/snap	

Obviously, several of the noninteractive and interactive behaviours occur in a composite fashion, ie., a bitch in lateral recumbency will likely have its tail on the surface and a standing bitch will not have its tail on the surface.

Minute and noninteractive hourly behaviours were recorded from the video tape as the number of total occurrences during the designated interval, ie. 0-2, 3-10, 11-30 and 31-60 minutes and hour 2, 3, 4 and 5. Interactive palpation behaviours were recorded as a single occurrence during a single palpation event.

### *Analysis and Presentation of Results*

The results of minute behaviours (the first hour after extubation) are presented for the purpose of description only. The minute behaviour data set was excluded from statistical analysis because the bitches were at a highly variable state of consciousness when extubated. In an attempt to keep the same elapsed time at extubation (approximately 91 minutes) for all treatments, some bitches were prematurely extubated while others were extubated as they were coughing when regaining consciousness. Accordingly, this first hour after extubation (91-151 minutes) showed an inherently wide range of variability, much of which reflected the depth of anaesthesia and subsequent stage of consciousness at extubation.

A six-stage process was followed as in Chapter 4 for identification of behaviours which could be used to differentiate between treatments. The first phase of this process dealt with the identification of behavioural frequencies and the second phase dealt with major differences between groups. A description of this process follows. Although processing of the noninteractive hourly behaviours is cited in the description, the same process was implemented for the interactive palpation behaviours. Whereas notable outcomes from this process are presented in the results section, additional data manipulations leading to these outcomes are contained in Appendix G. This approach has been taken for the convenience of the reader so that insights into the derivation of significant results are provided, but not at the expense of reader distraction from excessive detail.

### *Identification of behavioural frequencies:*

1. From the raw scores (Figure F5: Appendix F) of behavioural frequency recorded for each bitch individually, an average occurrence of every behaviour within each interval of observation (eg, hour 2, 3, 4 and 5) was obtained for each group.
2. Behavioural frequencies for each of the four hours were then averaged to obtain a mean occurrence of each behaviour per group (eg, Figure 5.4).
3. Behaviours occurring with an average frequency of  $<2$ /hour were considered rare. Behaviours occurring with an average frequency of  $\geq 2$ /hour were classified as: low (2-6/hour), medium (7-10/hour) or high ( $>10$ /hour).
4. Rare behaviours ( $<2$ /hour) were excluded from subsequent analysis because their low frequency of occurrence did not contribute to identifying differences due to treatment effect, and simply served as 'statistical clutter'.

To this point in the analysis, behaviours of note had been identified, but there was still the question of whether the noted behaviours actually allowed responses to treatment to be distinguished by behaviour, and if so what relative importance did each behaviour play in making the distinctions.

### *Major Differences Between Groups*

5. Canonical discriminate analysis was then performed to rank specific behaviours by their influence on making group distinctions. The analysis also provided a graphic display of group distinction by treatment effect. A statistical analysis system (SAS) computer program was used to perform the canonical discriminant function analysis, screening for behavioural differences (Manly, 1986). Two features of this technique are noteworthy. Firstly, the canonical analysis is used as a tool for the reduction of variables thereby making the data set more manageable. Secondly, canonical discriminant function coefficients statistically confirmed the negligible contribution toward treatment differentiation made by those behaviours recognised with rare occurrence.

6. Data from step 5 were then collated with data from step 2 to characterise the frequency of specific behaviours as they were ranked in importance for differentiating the groups.

Results are presented within two major sections: 1) identification of characteristic *behaviours associated with surgery*, and 2) identification of characteristic *behaviours associated with butorphanol analgesia*. Data from the Anaesthesia, Analgesia/Anaesthesia and Anaesthesia/Analgesia groups of Chapter 4 were utilised to discern the effects of surgery on behaviour. As subsections, results of both the noninteractive hourly and interactive palpation data are presented firstly by identification of major behaviours seen in each group. After describing the major behavioural features of each group, behaviours are identified that allow groups to be differentiated. The presentation format is:

1. *Behaviours associated with surgery* that allowed treatment characterisation:

Comparative treatments:

- A. Anaesthesia, Anaesthesia/Surgery, Control
- B. Analgesia/Anaesthesia,  
Analgesia/Anaesthesia/Surgery, Control
- C. Anaesthesia/Analgesia,  
Anaesthesia/Surgery/Analgesia, Control

I. Hourly Behaviours

II. Palpation Behaviours

2. *Behaviours associated with butorphanol analgesia* that allowed treatment characterisation:

Comparative Treatments:

- A. Control
- B. Anaesthesia/Surgery
- C. Analgesia/Anaesthesia/Surgery
- D. Anaesthesia/Surgery/Analgesia

I. Minute Behaviours

- Description for each treatment

II. Hourly Behaviours

- Description of behaviours in each group
- Discriminating behaviours in each group

III. Palpation Behaviours

- Description of behaviours in each group
- Discriminating behaviours in each group

## Results

Process for deriving the results:

1. Simple observation of the frequency of particular behaviours allowed 76 of the 99 original behaviours to be discarded because they occurred too rarely to be of value in distinguishing different responses to the treatments.
2. Further observation of the remaining interactive and noninteractive behaviours produced low, medium and/or high quantitative rankings of frequency and general descriptions of the responses to different treatments.
3. In order to provide precision and statistical validity to those features observed for effectively distinguishing behavioural responses to different treatments, discriminant canonical analysis was extremely useful to:
  - a) identify specific behaviours distinctive of different treatments \*
  - b) give a single figure (the discriminant function, Z) that would quantitatively characterise each bitch within a group. The Z function may thereby be considered as a value similar to the qualitative impression presented from overall responses by the bitch to the observer, ie., that general 'feel' about an animal's status that is proclaimed by animal handlers. \*

### *Characteristic Behaviours Associated with Surgery*

The distinctive responses to surgery in the surgery treatments were compared to those in their relevant 'base' groups:

Surgery Treatment	Base Treatment for Comparison
Anaesthesia/Surgery	Control Anaesthesia
Analgesia/Anaesthesia/Surgery	Control Analgesia/Anaesthesia
Anaesthesia/Surgery/Analgesia	Control Anaesthesia/Analgesia

The ranking of noninteractive hourly behaviours that generally differentiate the effects of any of the surgery treatments are (Table 5.6):

1. Draws legs up

2. Normal speed position change

3. Normal speed cage circle

Stretching

Slow motion position change

4. Lip licking

5. Panting

Cage sniffing

6. Head lifts

7. Hang stand

8. Grooming

9. Ataxia

Torso weight shift

10. Whine

Thoracic limb weight shift

11. Bark

1. All bitches undergoing ovariohysterectomy showed the characteristic noninteractive behaviour of 'draws legs up', a behaviour which was not seen in nonsurgery bitches. This behaviour was seen with a lower frequency in the groups receiving analgesic.

2. Surgery resulted in a decrease in normal speed cage circling.

3. 'Stretching' and 'torso weight shift' were generally seen with greater frequency after a surgery treatment when compared to an associated base group.
4. None of the interactive palpation behaviours were consistently different between a surgery treatment and its base group (Table 5.12).
5. Commencement of the palpation exercise was typified by a sternal position in all of the surgery groups. This 'start: sternal' position was not a feature in two (Anaesthesia and Anaesthesia/Analgesia) of the base groups.
6. Maintaining a 'head: level' posture was characteristic of bitches in all of the base groups; however, this behaviour was seen in bitches from only one of the surgery treatments (Analgesia/Anaesthesia/Surgery).
7. The only group in which vocalisation (whine) was seen was the Anaesthesia/Surgery/Analgesia treatment.
8. For the interactive behaviour 'orient: stare ahead' surgery resulted in: an increased frequency relative to its base group Anaesthesia, an increased frequency relative to its base group Anaesthesia/Analgesia, and a decrease in frequency relative to its base group Analgesia/Anaesthesia.
9. A standing posture at the end of the palpation exercise was characteristic of the Control bitches as well as the Analgesia/Anaesthesia/Surgery bitches. In contrast, this behaviour was not seen in the Anaesthesia/Surgery or Anaesthesia/Surgery/Analgesia bitches.
10. A sternal posture at the end of the palpation period was seen in only the Anaesthesia/Surgery group.



### *Characteristic Behaviours Associated with Butorphanol Analgesic*

The ranking of noninteractive hourly behaviours that generally differentiate the effects of any of the analgesic treatments are (Table 5.18):

1. Slow motion position change
2. Draws legs up
3. Ataxia
4. Normal speed position change  
Torso weight shifts
5. Stretching
6. Normal speed cage circle
7. Head lifts  
Panting
8. Thoracic limb weight shift
9. Bark
10. Whine
11. Grooming
12. Cage sniffing  
Hang stand

1. Control bitches showed the following (normal) noninteractive behaviours under the conditions of this study: frequent normal speed position changes and head lifts; absence of both hang stand and groan/moan.
2. Noninteractive hourly behaviours characteristic of the nonanalgesic group (Anaesthesia/Surgery) were:
  - Drawing the rear legs up (toward a pike position)
  - Lip licking
  - Cage circling
  - Incision licking
  - Vomition

3. The noninteractive behaviours of vocalisation were significantly ( $P<0.01$ ) more frequent in the presurgical analgesic group (Analgesia/Anaesthesia/Surgery) than in the other groups.
4. The noninteractive posture of hang stand was significantly ( $P<0.01$ ) more frequent in the postsurgical analgesic group (Anaesthesia/Surgery/Analgesia) than in the other groups.
5. The Control group is well differentiated by interactive palpation behaviours. Control bitches accepted the palpation exercise in a standing position and did not change this position after palpation. They characteristically held their heads level, ears back, tails low, and stared ahead during the palpation. They did not vocalise, their breathing was normal, and they oriented their attention in staring ahead, and they arched their back.
6. There was no consistent change in frequencies of ranked interactive palpation behaviours between pre- and postsurgical analgesia groups by which to differentiate treatment effect (Tables 5.24 and 5.25).

### *Responses to Surgery*

This section contains data developed from the base treatments in Chapter 4 (Anaesthesia, Analgesia/Anaesthesia and Anaesthesia/Analgesia) as well as the surgical treatments (Anaesthesia/Surgery, Analgesia/Anaesthesia/Surgery and Anaesthesia/Surgery/Analgesia) to determine the treatment effect of surgery on both noninteractive hourly and interactive palpation behaviours. Minute behaviours are not presented in this section because they served no value in differentiating the groups by treatment effect. Minute behaviours are described in the later section entitled, 'responses from butorphanol analgesic'.

Treatment comparisons:

- A) Anaesthesia, Anaesthesia/Surgery, and Control
- B) Analgesia/Anaesthesia, Analgesia/Anaesthesia/Surgery, and Control
- C) Anaesthesia/Analgesia, Anaesthesia/Surgery/Analgesia, and Control

Hourly Behaviours

Simple observations of the frequency in occurrence of particular behaviours unique to each group are summarised in Table 5.2. The separation of Control, Anaesthesia and Anaesthesia/Surgery groups by canonical analysis is illustrated by Figure 5.5. In this analysis Z<sub>1</sub> captures 92% of the group differences and Z<sub>2</sub> captures 8% of the group differences. The Anaesthesia/Surgery group is well differentiated from the Anaesthesia group on the canonical 1 axis. The frequency of occurrence for ranked hourly behaviours discriminating between the Anaesthesia and the Anaesthesia/Surgery groups by canonical 1 analysis is presented in Table 5.3.

The separation of Control, Analgesia/Anaesthesia and Analgesia/Anaesthesia/Surgery groups is illustrated by Figure 5.6. In this analysis Z<sub>1</sub> captures 73% of the group differences and Z<sub>2</sub> captures 27% of the group differences. The Analgesia/Anaesthesia/Surgery group is best differentiated from the Analgesia/Anaesthesia group on the canonical 1 axis. The frequency of occurrence for ranked hourly behaviours discriminating between the Analgesia/Anaesthesia and the Analgesia/Anaesthesia/Surgery groups by canonical 1 analysis is presented in Table 5.4.

The separation of Control, Anaesthesia/Analgesia and Anaesthesia/Surgery/Analgesia groups is illustrated by Figure 5.7. In this analysis Z<sub>1</sub> captures 55% of the group differences and Z<sub>2</sub> captures 45% of the group differences. The Anaesthesia/Surgery/Analgesia group is best differentiated from the Anaesthesia/Analgesia group on the canonical 1 axis. The frequency of occurrence for ranked hourly behaviours discriminating between Anaesthesia/Analgesia and the Anaesthesia/Surgery/Analgesia groups by canonical 1 analysis is presented in Table 5.5.

A comparison of discriminating noninteractive hourly behaviours among all the groups is presented in Table 5.6 (a collation of Tables 5.3-5.5).

The behaviour **draws legs up** is seen in only the surgery groups. It is seen with *low frequency* in the groups given analgesic, yet it is seen with *higher frequency* in the surgery group not given the analgesic (Table 5.6).

Surgery caused a decrease in frequency of **normal speed cage circling** in all groups: Anaesthesia (medium)  $\Rightarrow$  Anaesthesia/Surgery (low); Analgesia/Anaesthesia (medium)  $\Rightarrow$  Analgesia/Anaesthesia/Surgery (low); Anaesthesia/Analgesia (high)  $\Rightarrow$  Anaesthesia/Surgery/Analgesia (low).

In two of the three grouped (surgery vs nonsurgery 'base') comparisons an increase in frequency of **stretching** was seen associated with the treatment effect of surgery. Whereas stretching was not a feature of pre- or postanaesthesia analgesia, stretching was seen with low frequency in both the pre- and postsurgical analgesia groups.

The same observation was made for **torso weight shift** as was made above for stretching.

Palpation Behaviours

‘Interactive’ behaviours were assessed during each period of palpation which preceded blood sampling for cortisol assays. This is in contrast to the noninteractive behaviours presented above which were obtained from video taping the bitches in a somewhat isolated area.

Interactive palpation behaviours were categorised as high ( $>0.5$ ), low ( $0.3$ ) or rare ( $\leq 0.3$ ) and the rare behaviours were discarded from further analysis. The frequency distribution of the palpation behaviours suggested that two categories was most appropriate. With the exception that the interactive palpation behaviours were separated into two categories while the noninteractive hourly behaviours were separated into three categories, analyses of these data were made in a similar manner.

Distinct interactive palpation behaviours for each group are summarised by behavioural category in Table 5.7. This table is the synthesis of Tables 4.16 and 5.8.

The separation of Control, Anaesthesia and Anaesthesia/Surgery groups is illustrated by Figure 5.8. In this analysis Z1 captures 92% of the group differences and Z2 captures 8% of the group differences. The Anaesthesia/Surgery group is best differentiated from the Anaesthesia group on the canonical 2 axis. The frequency of occurrence for ranked palpation behaviours discriminating between Control, Anaesthesia and Anaesthesia/Surgery groups by canonical 2 analysis is presented in Table 5.9.

The separation of Control, Anaesthesia/Analgesia and Anaesthesia/Surgery/Analgesia groups is illustrated by Figure 5.10. In this analysis Z1 captures 89% of the group differences and Z2 captures 11% of the group differences. The Anaesthesia/Surgery/Analgesia group is best differentiated from the Anaesthesia/Analgesia group on the canonical 1 axis. The frequency of occurrence for ranked palpation behaviours discriminating between Control Anaesthesia/Analgesia and Anaesthesia/Surgery/Analgesia groups by canonical 1 analysis is presented in Table 5.10.

The separation of Control, Analgesia/Anaesthesia and Anaesthesia/Surgery/Analgesia groups is illustrated by Figure 5.11. In this analysis Z1 captures 97% of the group differences and Z2 captures 3% of the group differences. The Analgesia/Anaesthesia/Surgery group is best differentiated from the Analgesia/Anaesthesia group on the canonical 1 axis. The frequency of occurrence for ranked palpation behaviours discriminating between Control Analgesia/Anaesthesia and Anaesthesia/Surgery/Analgesia groups by canonical 1 analysis is presented in Table 5.11.

A comparison of discriminating interactive palpation behaviours among all the groups is presented in Table 5.12 (a collation of Tables 5.9-11).

**Start: stand.** Bitches in the Anaesthesia/Surgery, Anaesthesia/Analgesia and Anaesthesia/Surgery/Analgesia treatments did not show this behaviour, while bitches in the Anaesthesia, Analgesia/Anaesthesia and

Analgesia/Anaesthesia/Surgery groups showed this behaviour with low frequency.

**Start: sternal.** This behaviour was seen with low frequency in all the surgery groups. In contrast, it was not seen in the Control bitches and was seen with only low frequency in the Analgesia/Anaesthesia base treatment.

**Head: level.** Control bitches and bitches in the Anaesthesia/Analgesia, Analgesia/Anaesthesia and Analgesia/Anaesthesia/Surgery groups showed low frequency, while bitches in the Anaesthesia/Surgery and Anaesthesia/Surgery/Analgesia treatments did not exhibit this behaviour.

**Vocal: whine.** This behaviour was seen only in the Anaesthesia/Surgery/Analgesia group, where it was seen with low frequency

**Orient: stare ahead.** Control bitches showed this behaviour with low frequency. The postsurgical analgesia group (Anaesthesia/Surgery/Analgesia) showed this behaviour with low frequency, while the other two surgery groups (Anaesthesia/Surgery and Analgesia/Anaesthesia/Surgery) showed this behaviour with high frequency.

**End: sternal.** Only the Anaesthesia/Surgery group showed activity of this behaviour.

**End: stand.** This behaviour was commonly seen (high frequency) in the Control bitches, but was not seen in the post-anaesthesia analgesia group (Anaesthesia/Surgery/Analgesia) or in the Anaesthesia/Surgery group. Standing after palpation was seen with low frequency in the Anaesthesia and the Analgesia/Anaesthesia groups, and with high frequency in the Analgesia/Anaesthesia/Surgery group.

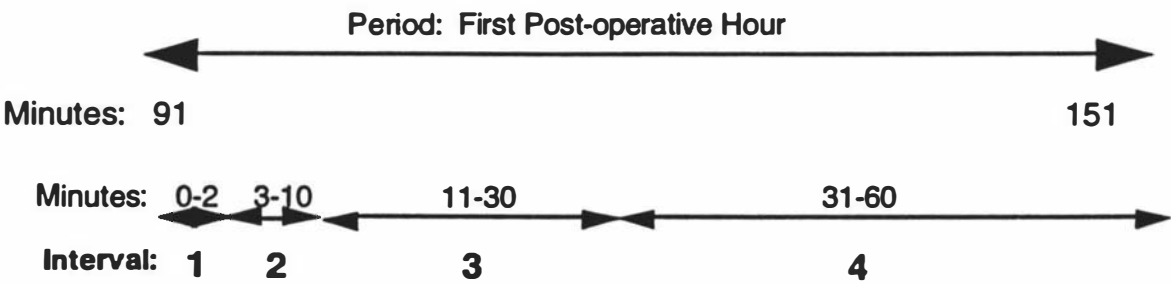
There were no consistent changes seen in ranked interactive palpation behaviours between groups undergoing surgery compared to their base groups serving as treatment 'controls' (Table 5.12).

Responses to Butorphanol Analgesic

- Treatment comparisons:
- A. Control
  - B. Anaesthesia/Surgery
  - C. Analgesia/Anaesthesia/Surgery
  - D. Anaesthesia/Surgery/Analgesia

Minute Behaviours

Minute behaviours were summed over the four intervals comprising the first hour after 'extubation'.



The summation of minute behaviours over the four intervals comprising the first hour after extubation (91-151 minutes) for each treatment is presented in Figure 5.12. Totals for each separate interval (one through four) within this hour (91-151 minutes) are displayed in Figures 5.13 through 5.15<sup>16</sup> (3 Dimension and Hi-Lo charts).

Minute behaviours that were rare in all groups (<2 total occurrences over the first postsurgical hour) and therefore discarded were:

<sup>16</sup> For this and subsequent figures that involve both a 3Dimension and a Hi-Lo chart, the 3Dimension chart is presented for an appreciation of the comparative trends among groups while the Hi-Lo chart is presented for detail of actual frequency values.

Sternal curl	Incision licking
Normal sit	Yawning
Hang sit	Pacing
Normal stand	Trembling
Hang stand	Manipulation behaviours
Slow motion cage circling	Urination
Quiet alert	Defaecation
Cage digging	Vomition
Cage licking	Salivation
Door biting	Bark
Door pawing	Howl

Those minute behaviours of more notable occurrence were tabulated across the treatments (Table 5.13).

#### *Descriptions of Non-interactive Minute Behaviours for Each Group*

##### **Controls**

In the Control group, the period that corresponded to the first hour after extubation in other groups was characterised by *low frequencies* of awake, sternal other, torso weight shift, lip licking, normal speed cage circling, cage sniffing and grooming, *medium frequency* in number of position changes, and *high frequency* of head lifts and number of position changes. Behaviours characteristically not seen in this group, but displayed in other groups were: hang stand, ataxia, thrashing, head nodding, and look back (Figures 5.12-15).

##### **Anaesthesia plus Surgery**

Groups given an ovariohysterectomy without the administration of analgesic were characterised by *high frequencies* of: number of position changes, lip licking, head lifts, drawing legs up, and whining. Among all groups this group showed the highest frequency of number of position changes. Behaviours seen with *low frequency* were lateral recumbency, torso weight shifts, slow speed position change, position ataxia, head nodding, normal speed cage circling, stretching, look back, and groan/moan (Figures 5.12-15).

##### **Analgesia plus Anaesthesia plus Surgery**

Behavioural responses in the presurgical analgesia group included *high frequencies* of number of position changes, head lifts, draws legs up,



whining, and groan/moan. *Medium frequency* behaviours included torso weight shift, positional ataxia and normal speed cage circling. *Low frequencies* of behaviours were seen in normal speed position changes, lip licking, thrashing, stretching and panting (Figures 5.12-15).

**Anaesthesia plus Surgery plus Analgesia**

Behaviours resulting from the treatment of postsurgical analgesic were characterised by *high frequencies* in normal speed position changes, head lifts, draws legs up, stretching, whining, and groan/moan; *medium frequencies* in number of position changes, positional ataxia and paddling; and *low frequencies* in thrashing, normal speed cage circling and panting. Among all groups these bitches showed the highest frequency of normal speed position changes, positional ataxia, walking, thrashing, stretching, paddling and whining (Figures 5.12-15).

Those two groups receiving analgesic (Analgesia/Anaesthesia/Surgery and Anaesthesia/Surgery/Analgesia) showed significantly ( $P<0.01$ ) more vocalisation (whine, and groan/moan) than the group that received no analgesic (Anaesthesia/Surgery). The minute behavioural data is summarised in Table 5.14.

<b>Hourly Behaviours</b>
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For the hourly behaviours (two through five hours, post-extubation) frequencies were ranked as rare (<2), low (2-6), medium (7-10) and high (>10).

*Description of Behaviours in Each Group*

Hourly behaviours which were rare in all groups (averaging <2 occurrences/hour) and would therefore have been of little use to discriminate between groups, were identified (Figure 5.4) and discarded from the analyses. Rare hourly behaviours were:

Lateral rest or sleep	Door biting
Sternal curl	Urination
Sternal rest or sleep	Defaecation
Lateral awake	Cage digging
Sternal awake	Door pawing
Sit alert	Head nodding
Sit other (lazy)	Incision licking
Hang sit	Bark
Normal stand	Howl
Attention seeking	Pacing
Slow speed cage circling	Trembling
Escape behaviour	Manipulation behaviours
Thrashing	Vomition
Yawning	Salivation
Cage licking	Look back
Cage sniffing	

These hourly behaviours were excluded from further analysis because they occurred so rarely within all treatments. Those remaining hourly behaviours of greater occurrence were compared across the groups and are presented in Table 5.15.

*Descriptions of Noninteractive Hourly Behaviours for Each Group*

**Controls**

Controls were characterised by *high frequencies* of: normal speed position change, and head lifts. Behaviours characteristically not seen in this group, but displayed in other groups were: hang stand, slow motion position change, slow speed cage circling, whining, ataxia, look back, salivation and pacing. These bitches showed the highest frequency

of head lifts and normal speed position changes, and were the only animals that chewed their IV catheter bandage (Figures 5.4, 5.16-17).

### **Anaesthesia plus Surgery**

Only the 'head lift' behaviour was seen with *high frequency* in this group. Among all groups, this group showed the highest frequency of slow motion position change, draws legs up, torso weight shift, normal speed cage circling, and lip licking (Figure 5.4). 'Draws legs up' was significantly ( $P<0.01$ ) greater in this group than in any other group. This was the only group that showed vomition and incision licking. Behaviours of *medium frequency* were: slow motion position change, normal speed position change, and draws legs up. Behaviours with *low frequency* were: thoracic limb weight shift, stretching, whining, grooming and ataxia. Although the behaviours torso weight shift, normal speed cage circling and lip licking were seen with low frequency, they were still highest in this group when compared to the other groups.

### **Analgesia plus Anaesthesia plus Surgery**

The presurgical analgesia group was characterised by *high frequency* of head lifts, and *medium frequency* of the normal speed position change behaviour. This group showed *low frequencies* of: slow motion position changes, torso weight shifts, draws legs up, stretching, normal speed cage circling, lip licking, whining, panting, and groan/moan. This group showed the highest frequency of panting, barking, groan/moan, and pacing. Barking, moan/groan, and panting were significantly ( $P<0.01$ ) greater in this group than in all other groups (Figures 5.4, 5.16-17).

### **Anaesthesia plus Surgery plus Analgesia**

These bitches showed the highest frequency of hang stand, thoracic limb weight shift, stretching, escape behaviour, whining, and door pawing among all groups (Figure 5.4). Door pawing was seen only within this group. The following behaviours were seen with *medium frequencies* in the postsurgical analgesia group: normal speed position change and head lifts. *Low frequencies* of the following behaviours were seen: hang stand, torso weight shift, thoracic limb weight shift, draws legs

up, stretching, normal speed cage circling, lip licking, and whine. Behaviours not seen in this group were: bark, pant, and yawn.

Distinguishing behaviours for each group are summarised as low, medium and high in Table 5.16.

The frequencies of some noninteractive hourly behaviours were observed to exhibit trends (Figure F5, Appendix F) during the period from the second to the fifth hour postsurgically. These trends are noted in Table 5.17.

#### *Discriminating Behaviours in Each Group*

Differentiation of the Control and surgical groups by noninteractive hourly behaviours is seen in the canonical analysis plot of Figure 5.18. In this analysis  $Z_1$  captures 60% of the group differences and  $Z_2$  captures 29% of the group differences (SAS calculation). Canonical 1 (X axis) is more relevant than canonical 2 in separating the Anaesthesia/Surgery group (with a class mean of 2.56) from the Control, Anaesthesia/Surgery/Analgesia and the Analgesia/Anaesthesia/Surgery groups (with class means of -1.45, -0.77 and -0.70, respectively). In comparison, the Control group is more easily differentiated from the Analgesia/Anaesthesia/Surgery and the Anaesthesia/Surgery/Analgesia groups on the Y-axis (Canonical 2) where the class means are -1.64, 1.08, and 1.11, respectively. The presurgical and postsurgical analgesic treatments are not well differentiated on either canonical axis.

*Within the constraints of the canonical 1 analysis, characterisation of the different surgery groups that received analgesic (Analgesia/Anaesthesia/Surgery and Anaesthesia/Surgery/Analgesia) and the group that did not (Anaesthesia/Surgery) is most easily achieved by use of Table 5.18. (Reference: Figure 5.18, canonical 1 vs. canonical 2 axis).*

*Within the constraints of the canonical 2 analysis, Table 5.19 is best for distinguishing Controls from the surgery groups.*

Palpation Behaviours
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### Description of Behaviours in Each Group

Rare palpation behaviours were:

#### Position Change

Yes

#### End Position

Lateral

Sit

#### Head Position

High

Hang

High alert

Scooping

Tilt

Sway

Sudden head lift

#### Ear Position

Forward alert

Alternating

Flat to sides

#### Eye Position

Glance/avert

Wary

Eyebrow lift

Wide-eyed

Sleepy or lidded

Closed

Frantic searching

#### Tail Position

Level

High

Tuck

High arch

No wag/curl

Slow wag/curl

Fast wag/curl

#### Vocalisations

Bark

Moan/groan

Yelp/scream

#### Orientation

Slow belly

Sharp belly

Deliberate avert

Hide

#### Breathing

Pant

Catch breath

#### Other Behaviours

Lip licking

Extended neck

Retreat

Escape

Restrained

Stretching

Drawing legs up

Lip lift

Bite/snap

Those interactive palpation behaviours retained for analysis are cited in Table 5.20. A summary of the interactive palpation behaviours for each palpation period within each group is presented in Table 5.21. Additional presurgical and 24 hours observations are presented in Table 5.22. The presurgical data were obtained on the day before commencement of the treatment and followed a one-hour video taping after the bitch was admitted to the hospital. The 24 hour data were obtained the day following commencement of a treatment, on the day the bitch was discharged from the hospital.

#### *Descriptions of Interactive Palpation Behaviours for Each Group*

The following descriptions are based upon the most representative (frequent) behaviour for each behavioural category as presented in Table 5.8; eg, start position, end position, head position, and others. These representative behaviours, in turn, were derived from those observed most frequently within each major category over the total number of palpation periods (Figure 5.9).

Control bitches were characterised by accepting the palpation exercise in a standing position and not changing this position after palpation. They characteristically held their head level, ears back, tail low, and stared ahead during the palpation. They did not vocalise, their breathing was normal, and they oriented their attention in staring ahead, and they arched their back (Table 5.9 and Figures 5.19-21).

Different palpation behaviours were seen within the various groups that contrasted to the Controls (Table 5.9 and Figures 5.19-21). With the Anaesthesia/Surgery treatment, bitches did not change from their initial sternal position over the course of the palpation and accordingly their tails were on the cage floor. These bitches' ears were more neutral than back, and their orientation was more on the tester than staring ahead. As with the Controls, these bitches showed an arched back, but they commonly showed lip licking, rigid stance when they were standing, and extended neck.

Analgesia/Anaesthesia/Surgery bitches maintained a standing position with their tails low throughout the palpation period. Their ears were more neutral than back, and whining was characteristic of this group. Retreat and escape behaviours were also characteristic of this group.

Bitches in the Anaesthesia/Surgery/Analgesia treatment usually accepted the palpation in lateral recumbency with their tail on the cage floor. As time progressed this starting position changed from lateral, to sternal, to stand. In contrast to the Controls, these bitches more often held their heads high rather than level. Vocalisation by whining was characteristic of this group. Additional behaviours commonly seen in this group were arched back (as in the Controls), retreat, and draws legs up.

The frequencies of some interactive palpation behaviours were observed (Figure F6, Appendix F) to exhibit trends over the duration of palpations from 121-391 minutes. These trends are listed in Table 5.23.

#### *Discriminating Behaviours in Each Group*

Separation of the groups by treatment effect is illustrated in the canonical discriminant function plot (Figure 5.22). In the canonical analysis Z<sub>1</sub> captures 74% of the group differences and Z<sub>2</sub> capture 14% of the group differences. For group distinction canonical 1 was more effective than canonical 2 for separating the Anaesthesia/Surgery/Analgesia, Analgesia/Anaesthesia/Surgery or Anaesthesia/Surgery and Control groups whose class means were 4.30, -0.78 & -0.79, and -2.30, respectively. The Analgesia/Anaesthesia/Surgery group (class mean: -1.91) was best differentiated from the other groups using the canonical 2 function: Control (0.79), Anaesthesia/Surgery (0.64) and Anaesthesia/Surgery/Analgesia (0.26).

The order of ranking behaviours for their effect in differentiating between groups is provided by the canonical analysis. Canonical multivariant analysis data, together with the frequency of each palpation behaviour within each treatment group (Figure 5.9), contribute to the creation of Table 5.24 which shows the frequencies of behavioural occurrences ranked for discrimination among treatments (canonical 1).

*Within the constraints of the canonical 1 analysis, Table 5.24 best distinguishes the Anaesthesia/Surgery/Analgesia group. Canonical 1 data are less discriminating for the Control group and are virtually ineffective in differentiating between the Anaesthesia/Surgery and the Analgesia/Anaesthesia/Surgery groups.*

Those interactive palpation behaviours most characteristic of the Analgesia/Anaesthesia/Surgery group are best identified using data from the canonical 2 analysis (Table 5.25).

*Within the constraints of the canonical 2 analysis, Table 5.25 best distinguishes the Analgesia/Anaesthesia/Surgery group. This data poorly discriminates the other groups (Control, Anaesthesia/Surgery and Anaesthesia/Surgery/Analgesia. Reference: Figure 5.22).*

Average times from extubation to first stand were:

Anaesthesia / Surgery,  $38 \pm 5.5$  minutes;

Analgesia / Anaesthesia / Surgery,  $29 \pm 7.1$  minutes; and

Anaesthesia / Surgery / Analgesia,  $66 \pm 24.9$  minutes.

## Discussion

Since animals do not communicate in our language, our perception of their pain is often deduced indirectly from behavioural patterns associated with injury or disease, or from nocifensive responses to acute noxious stimuli (Carstens, 1994). Carstens (1994) recognises nocifensive responses (mostly in laboratory animals) to acute noxious stimuli as: 1) segmental withdrawal reflexes (eg. rodent tail flick reflex; limb withdrawal), 2) more complex, unlearned organised responses (eg., vocalisation; jump, flinch), and 3) learned responses (avoidance; 'operantly' conditioned bar-press). Conclusions drawn from this study were based mostly on unlearned organised responses.

Results from this study support the belief that 'routine' ovariohysterectomy in the bitch results in pain-induced distress. Experience with coeliotomies in humans together with the historical observation that bitches respond to visceral manipulation during



ovariohysterectomy, even under a surgical plane of general anaesthesia, provide a strong indication that the procedure is painful. In addition, as seen in Chapter 3, ovariohysterectomy stimulated an immediate rise in plasma cortisol concentration which remained elevated if analgesia was not given than if it were given postsurgically. Also, distress from ovariohysterectomy was sufficient to modify bitches' postsurgical behaviours from behaviours of Controls. Additionally, this pain-induced distress persisted for at least five hours after the surgery, during which time plasma cortisol concentrations were significantly elevated above pretreatment values. During this same postsurgical period surgery groups demonstrated a characteristic decrease in frequency of the normal speed cage circling and increase in frequency of draws legs up, stretching and torso weight shift. High levels of plasma cortisol concentration in the Anaesthesia/Surgery group was associated with retention of the sternal posture over the palpation exercise in this group.

Drawing legs up (pike position) has potential for nursing care attendants as being the most easily recognised pain-induced distress behaviour following an ovariohysterectomy. This behaviour ranks first (Table 5.6) as a differentiating behaviour among those noninteractive behaviours collated from the surgery groups and their base treatments. Drawing legs up was seen only in the surgery bitches and was seen with greater frequency in the surgery group not receiving analgesic than in those surgery groups receiving analgesic. From this study the conclusion is made that drawing legs up is a pain-induced distress behaviour characteristic of focal or regional discomfort associated with the ovariohysterectomy. \*

In humans the parietal peritoneum is richly supplied with nerve endings derived from the intercostals and other spinal nerves and is sensitive to stretch as well as chemicals. Although stimulation of the ovaries gives rise to no sensations there is considerable sensation associated with traction on the parietal peritoneum and the ligaments of the uterus (Bonica, 1990a). Muscular rigidity or contraction is one of the most important clinical manifestations of deep somatic or visceral insult in humans (Bonica, 1990a). The muscular segments involved not only depend on the spinal segments supplying the deep somatic structure or viscera, but also on the intensity of the noxious stimuli. Additionally, intense and prolonged noxious stimuli not only influence the extent of the referred pain involved,

but also the extent and duration of associated muscle contraction. Correlations between the segments involved in the muscle spasm and the nerve supply is seen by the location of spasm in cholecystitis and appendicitis in humans: in cholecystitis the spasm is in the upper abdominal muscle, and in appendicitis it is in the lower abdominal muscle.

The behaviours of drawing legs up and arched back by bitches in this study are probably associated with reflex muscle contractions associated with pain-induced distress of ovariohysterectomy. In humans generalised peritoneum insult is associated with severe abdominal muscle spasms as well as vomiting, pain is markedly aggravated by motion and patients find pain relief by being on one side with their hips flexed (Bonica, 1990b).

Four behaviours, showing rare frequency ( $\leq 0.3$ ) in all surgery groups, had a significantly greater ( $P < 0.05$ ) frequency in the Anaesthesia/Surgery group than in any of the other surgery groups (Figure F5 and F6.

Appendix F):

Look back	(noninteractive behaviour)
Vomition	(noninteractive behaviour)
Incision licking	(noninteractive behaviour)
Extended neck	(interactive behaviour)

The frequency of both vomition and incision licking support the suspicion of visceral discomfort in the Anaesthesia/Surgery group (although incision licking may also be associated with somatic annoyance), and an extended neck is often seen as a clinical symptom of dogs with a 'guarded' abdomen<sup>17</sup> (as a feature of the 'sawhorse' posture). The 'look back' behaviour, sometimes called flank gazing, is considered pathognomonic for colic in horses (Blood et al., 1983) and would appear to signal similar visceral discomfort in the ovariohysterectomised bitch. In addition, the canine behaviour of placing the ventral abdomen on a cool surface, as was seen characteristically in the interactive behaviours of the Anaesthesia/Surgery group, is regarded as a sign of abdominal discomfort<sup>17</sup>.

The Anaesthesia/Surgery bitches showed a significantly greater ( $P < 0.05$ ) frequency of slow motion position change and slow motion cage circling

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<sup>17</sup> Personal communication: Dr. Grant Guilford BVSc, PhD, Diplomate ACVIM, FRCVSc; Massey University.

than any other surgery group (Figure F5). These behaviours suggest a state of restlessness in the Anaesthesia/Surgery group, and when considered with the above behaviours of regional discomfort, readily differentiate the surgery groups receiving analgesic from the surgery groups that did not. Surgery bitches that did not receive analgesic (and the associated sedation) were likely to be more anxious, nervous or fearful when awake, presumably slept less restfully, and therefore exhibited signs of exhaustion. The resultant manifestations were accentuated slowness in position change, lack of smooth transition from position to position and 'collapsing' into a position change.

In a study of human patients (Mateo and Krenzischek, 1992) using four categories from the Chambers and Price model (restlessness, tense muscles, frowning and grimacing, and patient sounds) correlation of patients' verbally described pain and behaviour within the first postsurgical hour was: frowning and grimacing,  $r=0.69$ , patient sounds,  $r=0.58$ , muscle tension,  $r=0.53$  and restlessness,  $r=0.48$  ( $r$ =Pearson correlation). These factors of human behaviour agree with the present study of bitches. Although facial expression was inappropriate as a behavioural parameter in this study, 'patient sounds' in the above human study could be accepted as similar to the canine behaviour of whine and groan/moan. However, the correlation of patient sounds from this study differ from that seen in human studies. Vocalisation in this study was seen from bitches given analgesic, the same finding as in Chapter 4. Human 'muscle tension' may be equivalent to muscle contraction leading to drawing legs up, which was characteristic of the nonanalgesic surgery groups. And lastly, restlessness in the human study can be similar to slow motion position change, torso weight shift, and thoracic limb weight shift which were seen within the surgery group not given analgesic.

Bitches that received analgesic moved less frequently during the postsurgical period than those bitches not receiving analgesic, and they moved with greater ease when they did so. Signs of focal or regional discomfort were infrequent. However, those bitches receiving analgesic showed greater vocalisation. Vocalisation may be related with the dysphoria of analgesia, an impression supported by the observation that these bitches often demonstrated the hang stand posture which is associated with the sedation of butorphanol analgesia. Additionally, the act of

vocalisation requires an active abdominal effort, which the Anaesthesia/Surgery bitches may have been reluctant to initiate.

Pain-induced-vocalisation appears to be at the top of a hierarchy of responses to noxious stimuli, and is reported to have a higher threshold than reflex withdrawal or escape behaviour (Carroll and Lim, 1960). In addition, it is abolished by lesions in the central nervous system at the level of the thalamus which do not affect more reflexive reactions to noxious stimuli (Carroll and Lim, 1960). Low doses of narcotics block sustained post-stimulus vocalisation, the presumed 'emotional' component of vocalisation, and "pain-induced vocalisation is the only pain-induced behaviour in animals that is blocked by narcotics in the range of doses used in humans with clinical pain" (Levine and Gordon, 1982). This last statement does not find support in the present canine study. Bitches receiving either pre- or postsurgical analgesic showed more vocalisation during the first hour after extubation than did ovariohysterectomised bitches receiving no analgesic. Additionally, vocalisation did not rank high (whine, 10 of 12; bark, 9 of 12) among discriminating noninteractive behaviours (canonical 1 analysis). The frequency of whining as a noninteractive behaviour was seen in the following order:

Anaesthesia/Immediate Analgesia (Chapter 4)

>Anaesthesia/Surgery/Analgesia >Anaesthesia/Analgesia (Chapter 4)

>Analgesia/Anaesthesia (Chapter 4) >Anaesthesia/Surgery >Analgesia

(Chapter 4) >Anaesthesia (Chapter 4) > Control. As an interactive

behaviour, vocalisation (the combined frequency of whining and groan/moan) was significantly ( $P<0.05$ ) greater for the Anaesthesia/Surgery/Analgesia group than for any other group in both this study and the study reported in Chapter 4.

The Analgesia/Anaesthesia/Surgery and the Anaesthesia/Surgery/Analgesia groups are virtually indistinguishable by noninteractive hourly behaviours (Figure 5.18). This similarity gives support to the argument that the analgesic actions of butorphanol are relatively short-lived. Two years after the initiation of this study, in a butorphanol study using the colorectal distention model, Houghton and others (1991) reported the longest mean duration of antinociception after a 0.4 mg/kg dose (intravenous) of butorphanol was  $38\pm9$  minutes, although one of eight dogs demonstrated a 75 minute duration of antinociception at that dose. Accordingly,

postsurgical differences in behaviour which were anticipated to reflect the suppression of windup (central sensitisation) from the preemptive administration of butorphanol in this study may have been lost due to the timing of administration (30 minutes before the induction of anaesthesia).

Assuming that the analgesic properties of butorphanol had probably dissipated before the onset of noxious stimuli in the Analgesia/Anaesthesia/Surgery group, it would be expected that the behavioural responses of this group would be very similar to those in the Anaesthesia/Surgery group without the effects of sedation on the former group. Accordingly, the behavioural manifestations of suppressing windup were never appreciated and differences in behaviour between the Anaesthesia/Surgery and the Analgesia/Anaesthesia/Surgery group were a consequence of sedation on the pain-induced distress of surgery. The lower frequencies of draws legs up and slow motion position change seen in the Analgesia/Anaesthesia/Surgery compared to the Anaesthesia/Surgery group (Table 5.6) are probably a result of sedation. Plasma cortisol concentrations in the postsurgical period for these two groups were similar (Figure 3.5), and support the proposal that although behaviours may be modified under the influence of sedation, pain-induced distress is not. This point emphasises a limitation of using behaviour as an index of distress.

Both noninteractive and interactive behaviours characterising the analgesic effects of butorphanol were quite subtle in contrast to the quantitative cortisol effects on pain-induced distress seen in Chapter 3 (Figure 3.5). Compared to the other surgery groups, bitches in the Anaesthesia/Surgery/Analgesia group showed a lower frequency of head lifts but a higher frequency of hang stand. The difference in head lift frequency can be attributed to a higher systemic level of sedation in these bitches, or it may be that these bitches were more comfortable and therefore less easily aroused. The Anaesthesia/Surgery/Analgesia bitches were probably more comfortable in the postsurgical period and therefore more apt to be upright, hence the standing posture, while the effect of sedation no doubt influenced the hanging head position. The two interactive behaviours of note in the postsurgical analgesic group were increased 'Vocal: whine' and decreased 'Orient: stare ahead'. Explanation for the decreased frequency of 'Orient: stare ahead' is similar

to that for the noninteractive behaviour of head lift: a higher systemic level of sedation in these bitches, or it may be that these bitches were more comfortable and therefore less 'intense'.

Lip licking and thrashing are two noninteractive behaviours worth further note. Lip licking ranked high among behaviours for identifying treatment differences (Table 5.18), and was seen in all surgery groups but not in the Controls; however, lip licking is apparently not recognised in the literature as a postsurgical phenomenon. The frequency of lip licking in the Anaesthesia/Surgery was significantly greater ( $P < 0.05$ ) than lip licking in either the presurgical or the postsurgical analgesia groups. This behaviour was not apparently correlated with salivation, which was also evaluated in the study. Lip licking could be associated with a dry mouth, a postsurgical anecdotal phenomenon in humans, but this hypothesis cannot be verified from this study as the bitches were deprived of water during the period of video taping. The Anaesthesia group reported in Chapter 4 showed a significantly greater ( $P < 0.05$ ) frequency of thrashing than the Anaesthesia/Surgery group in this study, and perhaps the discomfort associated with surgery may have been sufficient to discourage this distinct activity.

Plasma cortisol concentration is a numerical index of distress that easily lends itself to conventional statistical analysis for interpretation. On the other hand, the more complex index of behavioural expression is best analysed by less commonly used statistical methods. Canonical multivariant analysis is a statistical technique well suited for the analysis of behaviours. For this study canonical multivariant analysis graphically illustrates both the noninteractive and interactive difference in treatment effect between surgery bitches and their respective treatment base as controls, as well as between analgesic and nonanalgesic bitches undergoing ovariohysterectomy.

The contrasting separation of groups on the canonical plots of Figures 5.18 and 5.22 would suggest that interactive behaviours are a better index for differentiating groups by treatment effect than are noninteractive behaviours. Although this might be true, such a comparison cannot be used to infer that bitches respond differently in the presence of humans than if they were absent, an issue that was raised earlier. Since many of

the interactive behaviours were different from the noninteractive behaviours in this study, comparison of the data would be inappropriate for addressing this query. However, insight could be gained by repeating the protocol of noninteractive behavioural evaluation, but with one group in isolation and another similar treatment group in the presence of high human activity such as an intensive care facility.

To remind the reader, Control bitches in this study were characterised by accepting the palpation exercise in a standing position and not changing this position after palpation (Table 5.7). They characteristically held their head level, ears back, tail low, and stared ahead during the palpation. They did not vocalise, their breathing was normal, and they oriented their attention in staring ahead. During palpation the Control bitches arched their back. Major deviations from these characteristics, given a similar environment, should be considered abnormal.

Results of this study show that there is a notable difference in behaviours associated with the different treatments, and interpretive guidance has been proposed for identifying these behaviours. Measuring physiological parameters, such as adrenal corticosteroid hormones (Chapter 3), can further assist identification of behaviours associated with pain-induced distress, particularly under circumstances anticipated to involve noxious stimuli. Beyond this one can simply infer that if an animal is engaging in abnormal behaviour, it could be because of boredom or frustration or dementia, but it becomes a presumption as to whether the animal is experiencing pain. "Pain is best understood and managed when scientific data, behavioural data, and anthropomorphism are integrated and a rational approach is used" (Johnson, 1991).

The therapeutic approach to the use of analgesics under the above circumstances is eloquently stated by Lloyd Davis (1983): "One of the psychological curiosities of therapeutic decision-making is the withholding of analgesic drugs, because the clinician is not absolutely certain that the animal is experiencing pain. Yet the same individual will administer antibiotics without documenting the presence of a bacterial infection. Pain and suffering constitute the only situation in which I believe that, if in doubt, one should go ahead and treat."

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## Chapter 6

### General Discussion

The welfare of canine surgery patients prompted this study into the pain-induced distress associated with ovariohysterectomy in the bitch. A description of the term 'pain-induced distress' was made giving particular attention to the context in which interpretations are made, and considerations regarding the use of analgesics in association with ovariohysterectomy have been introduced. The initial objectives of this study were to answer the following questions:

- What are the restrictions for using changes in plasma cortisol concentration as an index of pain-induced distress after ovariohysterectomy?
- What behavioural responses are distress-specific after ovariohysterectomy?
- What impact does the timing of butorphanol analgesia have on postsurgical pain-induced distress as assessed by cortisol and behaviour?
- Are interactive and non-interactive behavioural responses of the bitch to man different in the presence of pain-induced distress caused by ovariohysterectomy?

A study with nine treatments was designed to assess plasma cortisol concentrations and both interactive and noninteractive behaviours. Findings are presented in Chapters 2 to 5, each of which begins with a chapter summary. The purpose of this chapter is to focus on and discuss the following issues:

- Classification of pain
- Cortisol as an indicator of distress
- Behaviour as an indicator of distress
- Association between cortisol and behaviours
- Study protocol: critical analysis
- Clinical application of research findings
- Scoring systems for pain
- Ovariohysterectomy: this research model
- Balanced analgesia
- Preemptive analgesia
- Major contributions of this research

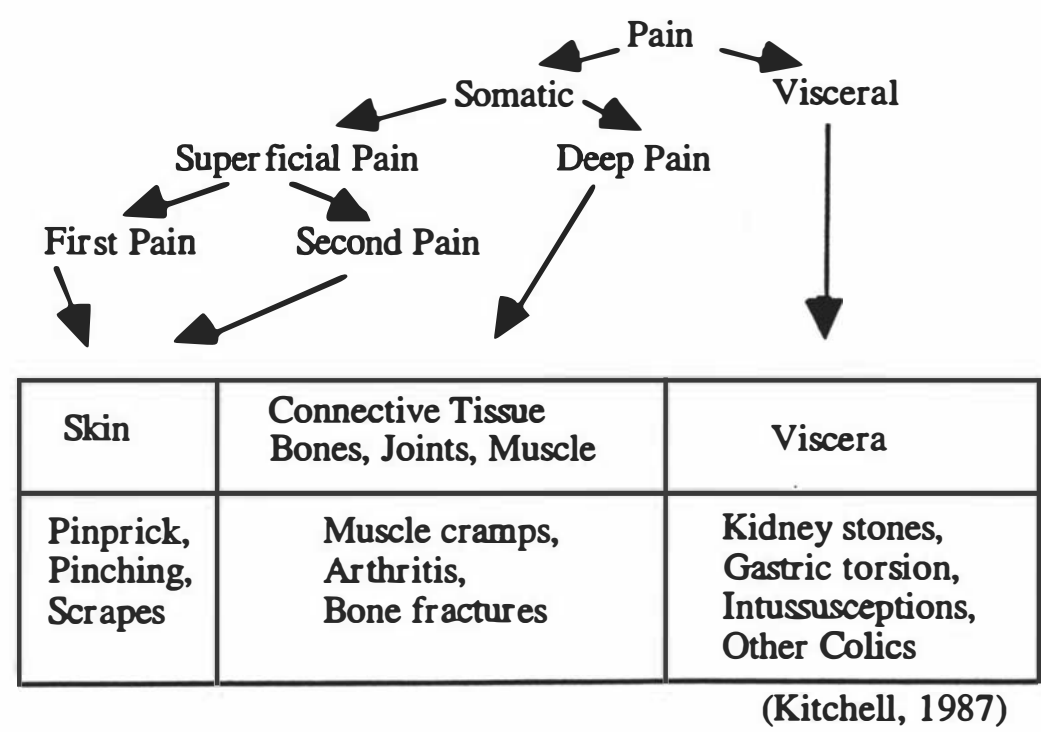
Classification of pain

Acute, Cancer and Chronic Pain

Pain may be divided into three categories based upon its onset and duration: acute pain, cancer pain, and chronic pain. *Acute pain* is more explicit and abrupt, and is associated with physical signs of autonomic nervous system activity. Acute pain is frequently alleviated by the use of analgesics and by the healing of injured tissue. *Cancer pain* usually presents with a well-defined onset and is often classified as acute-recurrent pain. Cancer pain is responsive to analgesics, but long-term control may lead to drug tolerance and addiction in humans. Pain is considered chronic when it persists for longer than six months (Kitchen et al., 1987). While *chronic pain* is the least understood of the three types of pain and is the most difficult type of pain to manage, acute pain can be the most devastating in its effects and the easiest to manage. Distress resulting from acute pain was the focus in this study.

Somatic and Visceral Pain

Qualities of acute pain are related to its site of origin (Kitchell, 1987).



Somatic pain is related to stimuli from skeletal muscle and skin while visceral pain is associated with tissues in the thoracic cavity and abdomen, and to some extent, bone. While stimuli that damage visceral tissue do not necessarily produce pain, stimuli associated with visceral pain include distention of hollow organs, ischaemia, inflammation, muscle spasm and mesenteric traction (Bonica, 1990a).

The *convergence-projection theory* of visceral sensation explains the typically diffuse and difficult localisation of visceral pain. Afferent inputs from the skin and viscera converge onto the same spinal dorsal horn neurones, which connect to the thalamus via the spinothalamic tract. In addition to somato-visceral convergence in the spinal cord, there is also viscerovisceral convergence: spinal neurons receiving convergent inputs from adjacent viscera. Visceral afferent fibres constitute 2-10% of all afferents entering the spinal cord, which is significantly out of proportion to the estimated 50-75% of neurons within the spinal cord that respond to visceral stimulation (Gebhart, 1994). The convergence-projection phenomenon is believed to be responsible for 'referred pain' where most visceral pain is felt in places other than the organ from which the sensation arises. This makes the origin of visceral pain difficult to identify in humans and understandably more so in animals.

In this study, pain-induced distress and changes in both plasma cortisol concentrations and behavioural responses associated with ovariectomy probably resulted from both somatic and visceral stimuli of nociception. Visceral pain is often associated with tenderness or increased sensitivity of the skin in the area of referral, a phenomenon called *hyperalgesia*. In recent years it has been documented experimentally (Ness and Gebhart, 1990) that cutaneous hyperalgesia develops after either deep somatic noxious stimulation or visceral stimulation and that the hyperalgesia differs in no significant way from that associated with experimental injury to the skin.

With this in mind, postsurgical palpation of the abdominal dermatome, as performed in this study, was likely to yield the most sensitive response to abdominal pain. Nonetheless behavioural responses to the interactive palpations were quite subtle. While the plasma cortisol response to ovariectomy was rather marked, this surgical procedure is

probably at the lower end of insults that elicit behavioural changes. The surgery was performed by a trained surgeon with considerable skill, who is disciplined in the principles of minimal surgical trauma. The amount of trauma associated with ovariohysterectomy, related to the skill of the individual surgeon could differ from surgeon to surgeon, and may influence postsurgical behavioural responses; but the design of the study did not allow exploration of this possibility.

### **Cortisol as an Indicator of Distress**

A summarising analysis of cortisol as an index of distress would include:

- Strengths:** Cortisol is an accepted index of distress seen in a variety of animal species.
- Weaknesses:** Cortisol levels may increase to adverse stimuli, but also to what may be considered innocuous or pleasant stimuli.  
Cortisol assays are tedious and time consuming.
- Opportunities:** Cortisol may serve as a standard by which other indices may be compared.
- Problems:** There is potential for over-interpretation or inappropriate interpretation of cortisol data.

Plasma cortisol is a commonly used index of distress in both humans and animals, and many investigators identify increased secretion of corticosteroids as proof for the presence of noxious stimuli. However, cortisol rises are also observed in situations considered innocuous, e.g., courtship, mating and active food consumption (Broom, 1988). Because cortisol responds to a variety of stimuli, both aversive and advantageous, some (Rushen, 1986) suggest that plasma cortisol is a poor index by which to assess animal suffering or distress. Difficulties in interpreting the meaning of cortisol responses to various stimuli often arise when the context of the circumstances is insufficiently defined. For example, cortisol levels in cattle accustomed to handling and bleeding differ from cattle which are not (Herd, 1989), cattle being herded show an affiliated rise in cortisol (Hattingh et al., 1980), and bulls loose-housed on slatted floors have cortisol levels greater than when they are housed on straw (Schlichting et al., 1983). It is important to realise that cortisol levels are difficult to interpret or evaluate when used to investigate the distress of a

situation on a single occasion, and failure to realise this often leads to cortisol being discredited as a valid index of distress. The design of this study with its consistent implementation of the nine treatments allowed confirmation of cortisol as a valid index for pain-induced distress with ovariectomy. Although physiological indices such as neuronal action potentials were not recorded within this study so as to confirm that the rise in cortisol during surgery in the unconscious bitch was attributable to the noxious stimuli of the surgery itself, this conclusion stands strongly on the collective findings from different treatments that exclude other explanations.

The adrenal cortex evolved in animals to assist their coping with various stimuli, and an adrenal cortical response is not necessarily evidence of the harmful effect of a stimulus or an indication that the animal is experiencing a loss of well-being. Before any biological end point can be used to validate distress, it must be established that a distress-induced change in that measurement is correlated with a meaningful change in the animal's well-being. Behavioural change offers additional insight, if not possible substitution, for appraisal of an animal's well-being.

In this study the response of plasma cortisol to the pain-induced distress from surgery of ovariectomy was quite marked, and the effect of butorphanol on this response was significant. However, elevations of plasma cortisol were also noted attributable to other factors which were not considered to compromise the animals' well-being. For the Analgesia alone group elevations of plasma cortisol were attributed to dysphoric side-effects of the butorphanol. Elevation of plasma cortisol in the groups receiving both analgesic and anaesthetic was suggested to result from the animals' inability to express restlessness due to the sedative effects of the butorphanol. Support for this proposal comes from the work cited by Dantzer (1989), who notes that when confronted with an aversive stimulant, the animal's degree of behavioural control is a major determinant of the behavioural and physiological impact of the stimulation.

### **Behaviour as an Indicator of Distress**

A summarising analysis of behaviour as an index of distress might look as follows:

- Strengths:** When validated for repeated reliability, behaviours offer a 'real time' index for assessing responses to distress.
- Weaknesses:** Behaviour as an index of distress is presently underdeveloped for interpretative use.  
Great variability exists in behavioural responses among species as well as individuals.
- Opportunities:** Animal handlers who become trained to identify behaviours specific for pain-induced distress as they are presently understood would likely further develop this index.
- Problems:** Behavioural responses to distress from one species can be unjustifiably attributed to another, or from one individual to another.

The value of behaviour as an index of distress has limitations similar to those for cortisol. It is not known if behaviours in animals respond linearly to pain-induced distress, and to the degree of noxious stimulus, or with an on-off behavioural response after reaching a given threshold of distress. The latter may confer advantages, e.g. it is counter-productive for animals of prey to change behaviour which would inhibit concealment or flight. In dogs there may be no social advantage within their pack from displaying pain-induced distress. Many behavioural responses in the pet *Canis* are likely learned responses directed to the pet's owner. A study might best select for learned behavioural responses by selecting pet bitches for all treatments, however in this study there was approximately equal representation of pet and non-pet bitches.

The ovariectomy model has merit as a base for comparing insult severity to pain-induced distress, because the procedure is usually uncomplicated, is performed with few variations from veterinarian to veterinarian (although level of surgical skill may differ), and is seldom performed under the influence of multiple interacting drug regimen, e.g., balanced anaesthesia together with balanced analgesia. Exploring the

proposal that behavioural changes are proportional to insult severity would require comparing data from a study such as this to results from other treatments involving procedures presumed to be more painful, e.g. thoracotomies and fractures. The latter could not be performed uniformly because techniques vary widely depending upon individual presentation of the case and many such patients are given a variety of therapeutic drugs, the interaction between which is seldom known. Even within our tightly controlled study it was difficult to specifically identify the influences on behaviour of anaesthesia or differentiate the sedation from the analgesic behavioural effects of butorphanol.

The conduct of this study identifies a very important point regarding clinical trials. Structured investigations of the more painful procedures mentioned above are never likely to be performed because of ethical constraints and prohibitive costs. The only acceptable opportunity to collect such data is through carefully designed prospective clinical trials. Accordingly, it is recommended that clinical research should be a major commitment of academic veterinarians and their institutions of employment.

#### **Association Between Cortisol and Behaviours**

Several studies of laboratory animals have established a relationship between circulating corticosteroid levels and frequency of particular classes of behavioural responses (Leshner, 1978). In the present study the restricted number of behavioural data points (4) made statistical analysis of associations between change in plasma cortisol concentrations and behavioural frequency impractical. An attempt to do so revealed no significant correlations, but on the basis of changes in cortisol concentrations that were significant and the change in frequency of particular behaviours that were significant, six classes of association have been identified (Tables 6.1 and 6.2): surgical treatments, 1) decreasing cortisol concentration / increasing behaviour frequency, 2) decreasing cortisol concentration / decreasing behaviour frequency, 3) high cortisol concentration / high behaviour frequency, 4) unchanging cortisol concentration / unchanging behaviour frequency; nonsurgical treatments, 5) corresponding cortisol concentration and behaviour frequency, and 6) inverse relationship between change in cortisol concentration and



frequency of behaviour. A more detailed description of associations between cortisol concentration and behavioural frequencies is provided in Appendix H.

### **Study Protocol: Critical Analysis**

#### ***Time of Preoperative Analgesia***

1. The presurgical analgesic butorphanol was administered 30 minutes prior to the induction of anaesthesia. In general veterinary practice this is the most common time for the administration of presurgical drugs, and was the reason for this choice in the study. As a premedication (preemptive analgesic), butorphanol would most frequently be given by intramuscular or subcutaneous injection in general practice, but in this study it was administered by intravenous injection to ensure consistent bioavailability. This combination of timing and route of administration may have been unfortunate, as the analgesic effect of butorphanol was recently reported to last approximately 38 minutes (a finding published two years after the commencement of this study). Accordingly, post-extubation observations of the pre-anaesthetic butorphanol group probably showed the residual sedative effects of the drug rather than its analgesic effects.

To reconcile this issue of analgesic route and timing, several additional combined butorphanol and surgical treatments would need to be investigated. One of the treatments would evaluate butorphanol administered by subcutaneous or intramuscular injection thirty minutes before anaesthetic induction. The second additional treatment would evaluate butorphanol (0.4 mg/kg) administered by intravenous injection, after the introduction of anaesthesia but just prior to entry into the surgical theatre, approximately 5-10 minutes before skin incision. Furthermore, doses of butorphanol greater than 0.4 mg/kg could be given at an earlier time, but calculated to provide the systemic concentration consistent with analgesia at the time of skin incision.

### *Other Parameters*

2. Monitoring more physiological parameters such as blood pressure and heart rate may have been of value. However, measurement of these has been reported in other studies and was not implemented here because it would have influenced behavioural observations. Remote monitoring of these parameters is possible, but resource constraint precluded it in this project.

Some (Stanley et al., 1980; Taborsky et al., 1982; Davies et al., 1984) contend that catecholamines, and even blood glucose, are as useful in evaluating distress as is plasma cortisol. No doubt recording of these additional data would be informative. However, plasma cortisol is the most widely accepted plasma index of distress and a large volume of literature contains reference to this index. Limitations of finance, personnel, and blood sample management dictated use of plasma cortisol concentration, which was considered to be the single most accepted index for assessment. A recent paper (Breslow et al., 1993) has reported the independent response of catecholamines and plasma cortisol to surgical distress in man, demonstrating that the two indices may not have correlated responses. Notwithstanding this, the additional considerations that sample handling for catecholamine analysis is more sophisticated than cortisol and that catecholamines are not as widely used as cortisol as an index of distress, catecholamine assays were not performed.

### *Separation of Visceral and Somatic Nociception*

3. The aggregate response of bitches undergoing surgery involved a major viscerosomatic nociception input, if dogs can be compared to humans (Bonica, 1990b). The primary somatic input came from the surgical wound and the primary visceral component largely from manipulation of the reproductive tract. These two components could be further separated experimentally by including an additional surgical treatment in which only a midline abdominal incision is made, so as to isolate the somatic component.

A less desirable alternative approach to this issue of viscerosomatic separation is addressed in Chapter 3. As cited in Chapter 3, the

administration of either nonsteroidal antiinflammatories or preemptive spinal anaesthesia is proposed to reduce somatic and visceral nociception, respectively. This approach is less desirable because it introduces the complexity of neurological chemophysiologic influences, of which much remains unknown; i.e., in addition to the well-documented peripheral mechanism, a central antinociceptive action of NSAIDs has been demonstrated in both animals (Attal et al., 1988) and humans (Willer et al., 1989).

Perhaps a more discriminating approach would be the use of local anaesthetic and opioid spinal anaesthesia. There is no convincing evidence that local anaesthetics have a significant interaction at opioid receptors, but Kosterlitz and Wallis (1964) reported that opioids may have a non-specific interaction with excitable membranes, producing a local anaesthetic effect.

#### *Blood Samples*

4. Initially, it was intended to place jugular catheters in all bitches for blood sampling. This was perceived to be the least invasive means for repeated blood sampling, but proved to be most impractical. The placement of jugular catheters was both irritating to the animals and frustrating to the investigator. After placement, catheters were secured with bandages, cyanoacrylate glue or a combination of the two. Invariably, the bitches had a low tolerance for the jugular vein catheters and quickly dislodged them.

The bitches were far more tolerant of cephalic vein catheters, which video taping revealed were most frequently ignored. However, the cephalic vein catheters frequently became occluded, necessitating their removal. Following removal, blood samples were obtained by venepuncture. Video taping further revealed that bitches very rarely gave attention to the site of venepuncture, from which it was concluded that repeated venepuncture is benign if properly performed. Knoll and others (1992) have reported that there is no difference between plasma cortisol values in samples taken by catheter or venepuncture in the laboratory dog.

### *Time of Extubation*

5. Greater utilisation of postsurgical behavioural data (e.g. minute behaviours) may have been achieved had the bitches been at a consistent stage of anaesthesia when extubated. The different depths of anaesthesia at the time of extubation caused difficulty in interpreting comparative data in the first hour after extubation. This situation resulted from the use of personnel with monitoring skills but minimum training in anaesthesia. Maintenance of anaesthesia by fully-trained anaesthesiologists may have provided bitches in a more consistent plane of anaesthesia at the time of extubation.

### *Duration of Sampling*

6. A recognised shortcoming of this study was failure to continue observing cortisol and behavioural changes until these parameters returned to pretreatment status. Unfortunately the resources for this investigation were too limited to allow this.

### *Value of Pilot Study*

7. A plasma cortisol pilot study consisting of four treatments was conducted prior to the parent study. This pilot study was most valuable for establishing parent study logistics and for the opportunity to resolve initial problem areas such as jugular catheterisation.

## **Clinical Application of Research Findings**

Three major points are made regarding the clinical application of findings from this study.

1. Routine ovariohysterectomy is accompanied by sufficient pain-induced distress to warrant the concurrent administration of analgesics, which reduces this pain-induced distress.
2. Few and somewhat subtle, noninteractive and interactive behavioural changes are associated with the pain-induced distress caused by ovariohysterectomy. Such changes are modified by the administration of analgesics.

3. Behavioural responses to pain-induced distress caused by ovariectomy accompany elevations in plasma cortisol concentrations. The cortisol rise itself supports the finding that ovariectomy is accompanied with significant pain-induced distress. This rise in plasma cortisol concentration is reduced by the administration of butorphanol.

### Scoring Systems for Pain

Because we live in an age of numbers, we tend to attribute respectability and reliability to phenomena which can be described in terms of numbers. In human beings pain analogue scales are used to quantitatively describe pain, and scoring systems have been devised in an attempt to identify pain intensity and guide the subsequent administration of appropriate drugs. Such systems include the McGill pain questionnaire, visual analogue scales, word descriptor responses (Chapman et al., 1985), the University of Alabama in Birmingham (UAB) pain behaviour scale (Richards et al., 1982) and pain induced vocalisation (PIV) (Levine and Gordon, 1982). These systems can be divided into three categories: 1) patient assessment based upon a linear scale from minimal to unbearable pain, 2) patient assessment based on the selection of a verbal term that best describes the intensity of their pain, and 3) patient assessment by some external observer. Although the categorisation based upon external observation is considered the least reliable, it is the only available method for recognising pain in infants and prelingual children. Veterinarians are confronted with the same difficulty in identifying pain in their patients as are those caring for these infants and children. Realistically, because pain is a complex, individually perceived interpretation, there will never be an accurate external measure of the presence or severity of animal pain. Nonetheless, this should not be a deterrent since such a pursuit ultimately advances our concern for the well-being of animals and enriches our standard of ethics.

Several criteria for the assessment of pain in animals have been advocated, from which scoring systems have been suggested (Sanford, 1992). Although the animal five freedom scheme (Farm Animal Welfare Council, 1992) is useful in identifying an animal's welfare, proposal of a similar scheme for the identification of animal pain is naive. The assessment of

animal pain is most complex considering variables such as species, breed, individuality, environment, and severity of insult. The establishment of standards will require investigations of large numbers of animals within rigidly controlled studies.

The number of animals utilised in this study was extremely small, and thereby restricted the ability to differentiate between the breeds of bitches observed. Additionally, data were analysed statistically, a process which has inherent compromises. The statistical analysis system (SAS) method of canonical multivariant analysis is well suited to the manipulation of these research data, but should not be interpreted as more than a 'best fit'. SAS generates considerable analytical results from input data and the 'most informative' set of results for interpretation remains a contentious issue among research statisticians.

The limitations of any scheme for scoring animal pain will include:

1. The assignment of scores to assessments does not confer objectivity.
2. The recognition of selected criteria will depend on the knowledge and experience of the observer.
3. Species differ enormously in their response to the same procedure, and any formal scoring scheme must allow for this variation.
4. Individual animals of the same species can show wide differences in their responses, as do people.

Although schemes for scoring animal pain have been proposed in the past and will, no doubt, be proposed in the future, they will be no more accurate than their size of data base, subjectivity of identification and weighting, and quality of data analysis. The desirability of a simple scoring system for animal pain will continue to prevail, principally from the need for guidance in pain relief as well as judgements to be made about research in animals. Its inherent caveat will be over-simplification and perceived invulnerability. Ultimately, successful implementation of an animal pain scoring system by a care provider will require complimentary husbandry skills and anthropomorphic compassion, guided by Professor Lloyd Davis' advice of 'when in doubt, administer analgesics'.

An apparent contradiction of results in the behavioural phase of this study was the distinct separation of treatments as seen in the canonical plots, yet

differences in specific behaviours for separate groups were much less clear. That is, although individual behaviours did not 'fall out' as clearly as the patterns of discriminant canonical analysis would suggest, the composite treatment effect, and therefore the qualitative impression of the behaviours, was quite distinct. This apparent dichotomy may be better understood by further considering the discriminant canonical analysis. As described earlier, a feature of the canonical discriminate analysis is to reduce the number of variables, and by so doing the original variables may be manipulated to appear in a different form. For instance, weight and height are examples of variables that might be considered in a more general form as size. Behaviours in this study may have been statistically manipulated in a similar manner such that interpretative results may not exactly correspond to the initial behaviours. Animal handlers claim that with experience they can recognise an animal in pain as well as differentiate between one that has had analgesic and one that has not. And yet they are often unable to clearly state the criteria on which they have based their judgement. This intuitive insight, which develops with experience, is quite important for the human paediatric nurse as well as the animal caretaker. This 'intuitive sense' may be similar to the Z value (discriminant function) of the canonical analysis. This 'intuitive Z value' may come with experience as an expression of the whole complex of displayed behavioural values. Ultimately, the Z value may fulfil our need for a number!

Although the subject of scoring animal pain is an interesting issue for scholarly debate, I believe a more pressing issue at present is the recognition of an animal suffering from pain-induced distress. Toward this end the following are proposed as postulates to support the presence of pain-induced distress in animals.

1. Within the context of the circumstances an anthropomorphic judgement is made that the insult would be painful. There must be no illicit anthropomorphism (Midgley, 1983), or projection onto animals of sophisticated human qualities that are clearly inappropriate.
2. A change in physiological response is associated with the insult. A recognised physiological index such as cortisol or catecholamines would fulfil this criterion.
3. A change in what is considered to be normal behaviour for the breed and species of animal in question is associated with the insult.

4. Administration of analgesic results in the post-insult physiological and behavioural changes returning toward those typical of the pre-insult status.
5. In the absence of analgesics, post-insult physiological and behavioural changes return to preinsult levels within an appropriate period of time.

### **Ovariohysterectomy: The Research Model**

Canine ovariohysterectomy is the most frequently performed surgical procedure in small animal veterinary practice (Pearson, 1970; Dorn and Swist, 1977; Sawyer, 1989), is performed with few variations, and is well suited as a model for the investigation of viscerosomatic nociception. Veterinary anaesthesiologists have long recognised the nocifensive aspect of ovariohysterectomy as patients routinely demonstrate marked responses at the time of operative manipulation of the ovarian pedicle.

Veterinarians have frequently questioned how much pain-induced distress results from ovariohysterectomy and whether this surgery ethically dictates the use of an analgesic. Historically, most concerns related to ovariohysterectomy in the postoperative period have been directed to anaesthetic rather than nocifensive issues, and the drugs administered have been selected for their sedative effect to facilitate anaesthesia recovery rather than for their analgesic attributes. This is apparently the first detailed study presenting supportive data that the 'routine' ovariohysterectomy in the bitch causes significant pain-induced distress.

The canine ovariohysterectomy model used for this study has potential for future development to:

- 1) evaluate different dosing regimens of butorphanol,
- 2) assess various administration routes of butorphanol,
- 3) judge different times of administration for butorphanol relative to the timing of surgery,
- 4) evaluate other analgesics,
- 5) evaluate different drug combinations,
- 6) serve as a standard for the weighting of responses from other surgical procedures as well as the standard for the conduct of the ovariohysterectomy procedure itself,



- 7) expand the reported data base with additional physiological parameters such as blood pressure, heart rate, capillary refill times, and the concentrations of catecholamines and glucose in the blood, and others,
- 8) compare the visceral nocifensive response of ovariectomy to other experimental models such as the colonic balloon, and
- 9) compare viscerosomatic responses of the dog to other animal species (i.e. cat).

### **Balanced Analgesia**

Although opioids produce their major effects on the central nervous system and the bowel, many behavioural studies examining peripheral antinociceptive effects of exogenous opioids have been reported (Stein, 1993). With one exception (Kayser et al., 1990) all studies reporting peripheral antinociceptive actions of opioids have used inflammation models. Inflammatory hyperalgesic conditions seem to be especially amenable to peripheral opioid antinociception. Depending on the particular circumstances,  $\mu$ ,  $\delta$ , and  $\kappa$  receptors can become active in peripheral tissue.

In addition to the use of parenteral opioids, non-steroidal anti-inflammatory drugs, and local anaesthetics, the use of spinal opioids to achieve analgesia has become a common method of treating pain. Intrathecal or epidural administration of opioids offers the advantage of prolonged analgesia (up to 24 hours) which is not associated with sympathetic blockage and hypotension, motor paralysis and hypothermia, as is the case with local anaesthetics. Further, a significantly enhanced antinociceptive effect has been obtained after injections of opioids and local anaesthetics in doses so low that alone they had little or no effect (Tejwani et al., 1992). Their relative concentration in the subarachnoid space appears to influence the agonistic receptivity of different opioid receptors. This synergism between opioids and local anaesthetics exists not only in somatic nociception models but also in visceral nociception models, where effects appear even more profound (Tejwani et al., 1992). Infiltration of the incision site with local anaesthetics/analgesics is more commonly being performed and techniques for epidural analgesia are becoming more popular in veterinary medicine.

For years veterinarians have been schooled on the efficacy of synergism of some antibiotic treatments. With similar appreciation they have come to accept the beneficial effects of 'balanced anaesthesia' and we are now at the threshold for accepting 'balanced analgesia'. With increasing knowledge of chemoreceptor activity, antinociceptive interactions and neuroanatomy, drugs of increasing selectivity will be developed. Until that time opportunity exists to make a substantial contribution to the management of postsurgical pain-induced distress by using known methods of analgesic treatment, but changing their timing or duration and route of administration.

### **Preemptive Analgesia**

Brief periods of nociceptor input can produce central hypersensitivity changes that alter responses to subsequent inputs; responses that last between 10 to 200 times the duration of the initiating stimulus (Woolf and Chong, 1993). The aim of preventing this central sensitisation while leaving physiological pain mechanisms intact has the theoretical advantage that the patient will not be totally analgesic, and thus allowing the ready recognition of postsurgical complications. Low doses of opioids (morphine) prevent central sensitisation while high doses are required to suppress central sensitisation once it is already present.

Prevention of pain before it occurs is proving to be a most successful form of pain control in both research studies and clinical practice. Presurgical application of local and regional analgesic techniques reduces both peripheral and central responses to pain, and is reported by some (Saidman and Eger, 1964) to reduce the concentration of inhalation analgesic needed during surgery. Opioid premedication, especially when combined with local infiltration or regional techniques, reduces the overall need for postsurgical analgesics (George et al., 1974; Kiss and Killan, 1992).

Presently, almost all analgesic agents produce sedation. This can present a complication for interpreting whether the behavioural response from an analgesic is due to its analgesic effect on pain-induced distress or is due to

effects of sedation; or, in contrast is the sedation of the analgesic drug contributing to the animal's distress by suppressing its normal response to certain stimuli. Moss (1992) states that there is an impressive amount of evidence to show that the pattern of hormonal response to (di)stress depends not on the physical characteristics of the noxious stimulus, but on the perceptual experience of the subject and the behaviour in which the subject engages. An illustration of the misinterpretation of analgesia based on behavioural observation comes from the early veterinary use of the drug ketamine. Ketamine effects in animals were presumed to be the same as those described in humans; immobilisation and analgesia. Many veterinarians, therefore, used ketamine as the sole agent under which to perform feline (and less frequently canine) ovariohysterectomies. The dissociative properties of ketamine were misinterpreted from the behavioural state of the animal to imply a state of analgesia, when in fact ketamine is a poor agent for visceral analgesia (Benson and Tranquilli, 1994). This example emphasises the importance of a clear understanding about the behavioural responses to a drug, an understanding that requires thorough investigation.

### **Major Contributions Of This Research**

The major findings and contributions of this study are:

1. Creation of a reliable canine ovariohysterectomy model for the study of perioperative analgesia.
2. Identification that ovariohysterectomy in the bitch is associated with significant distress as judged by the elevation of plasma cortisol concentration. Further, this distress is reduced by the postsurgical administration of intravenous butorphanol at 0.4 mg/kg.
3. After a preinduction rise in cortisol concentration, plasma cortisol levels fall during the unconscious period of surgical-plane (stage 3, plane 2) anaesthesia with halothane, and continue to fall until approximately 30-60 minutes after extubation.
4. Bitches recovering from ovariohysterectomy without the benefit of butorphanol can be identified by behavioural characteristics which are

- different from those exhibited by bitches receiving butorphanol, namely: drawing legs up into the pike position, looking back to the flank or incision area, licking the incision, vomiting, and extending the neck during postsurgical abdominal palpation.
- 5. Bitches treated with a combination of butorphanol and halothane anaesthesia but not ovariohysterectomy demonstrated different behavioural characteristics from other treatments, vocalisation being of particular note. Historically, vocalisations such as whining have been inferred to signal a painful state, yet this was not the finding of this study. Vocalisation of bitches in this study was attributed to the state of dysphoria induced by butorphanol.
- 6. Elevations in plasma cortisol concentration for bitches undergoing ovariohysterectomy, as well as those receiving perioperative butorphanol, returned to presurgical levels within 24 hours of the surgery.

It is proposed that it is possible to have a complex of significantly different behaviours in response to a treatment, but where differences are sufficiently subtle such that they can be difficult to individually describe and explain. It is further proposed that a quantitative measure of the distinction between behaviour sets in response to different treatments (i.e. the Z function of the canonical discriminant analysis used here) might represent the qualitative, subjective impression of the total demeanour an animal gives a technician.

Contrasting to the wide belief that ovariohysterectomy is a benign procedure, the present work supports the view that the pain-induced distress associated with ovariohysterectomy is sufficient to merit analgesic use. The critical clinical outcome of this research is the strong advice that analgesia should be provided in association with ovariohysterectomy. Further work is necessary, as indicated above, to clarify different analgesic strategies which might be more effective than those used here. The ovariohysterectomy model would be a good vehicle for testing analgesic strategies for ovariohysterectomy as well as more severe surgeries.

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