Universidad de León

Facultad de Veterinaria

Tesis Doctoral

USE OF CEREBRAL STATE INDEX AS A TOOL TO EVALUATE THE DEPTH OF ANESTHESIA USING PREDICTED PROPOFOL PLASMA CONCENTRATIONS.

EL USO DEL "INDICE DE ESTADO CEREBRAL" COMO HERRAMIENTA PARA EVALUAR LA PROFUNDIDAD ANESTESICA EN PERROS ANESTESIADOS CON PROPOFOL

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Abstract

In the last 20-30 years, advances in computing technology allowed the use of complex statistical modelling techniques for electroencephalogram (EEG) processing in order to generate a numerical scale of anesthetic depth assessment that could be easily interpreted by anaesthesiologists in humans. The investigation is focused on the development of monitors based on the EEG, once it is known that anesthetic drugs affect both cerebral physiology and EEG patterns. Similarly the EEG could be considered a promising physiological signal to assess the depth of anesthesia in dogs, since there are changes in the EEG patterns during increasing hypnotic concentrations. However until now none of the current monitors developed for humans seems to have the capability to detect accurately the variation in drug concentrations and in clinical endpoints.

The main objective of this work is to evaluate the performance of the human Cerebral State Index (CSI) during anesthesia with propofol in dogs. This monitor uses EEG as the input variable, which is analysed with fuzzy logic to process data. The fuzzy logic analysis is a problem-solving control system methodology that incorporates a simple, ruled-based" IF X and Y then Z approach for solving a control problem, rather than attempting to model a system mathematically. Thus, this mathematical algorithm used in this equipment seems to be, among other developed hypnotic monitors used in humans, the one that may yield better performance in quantifying depth of anesthesia in dogs.

The monitor also analyses the Burst suppression ratio (BSr) which is defined as the percentage of time in 30-s window where the amplitude of the electroencephalographic signal was less than 3.5μV. These EEG periods characterize the deepest levels of hypnosis.

All studies were performed at Porto Veterinary Hospital. All dogs came from an adoption program established between local animal shelters and the Porto Hospital. The studies were prepared with healthy dogs undergoing scheduled routine surgical procedures.

The study was performed in three phases aiming at evaluating the performance of the CSM monitor during anesthesia with propofol.

In all phases the Beth's pharmacokinetic model for propofol was incorporated in Ruggloop® software, which was used to drive the propofol infusion. This was done according to the desired propofol plasma target. The software also provided the estimated propofol plasma concentration achieved in the blood. The EEG was collected by three electrodes placed in specific positions in the head thereafter EEG signal is converted by the Cerebral State Monitor into the CSI.

In the first phase (chapter 3.1), the performance of the CSM monitor was evaluated after the administration of a propofol bolus dose of 6mg/kg. The cerebral electrical changes induced by increasing propofol concentrations in the dogs were detected by the cerebral state index (CSI), displayed by the CSM. The next step to evaluate the CSI is to find the accuracy of the monitor when correlated with clinical endpoints.

In the second phase (chapter 3.2), it was introduced the clinical end points, ocular reflexes position of the eyeball to evaluate the performance of the CSM monitor in dogs during induction of anesthesia with propofol. Five anesthetic planes, classified from A to E, were proposed based ocular on reflexes and position of the eyeball. During induction of anesthesia at $200mlh^{-1}$ it was observed a high correlation between the estimated propofol plasma concentrations (PropCp) and the clinical endpoints observed. However the performance of CSM was not consistent with the clinical observations on the different planes of depth of anesthesia. This results pointed CSM potential use limitation during routine induction of veterinary anesthesia.

In the third phase of the study (Chapter 3.3), the CSI and the previously identified anesthetic planes were studied during different steady-state propofol plasma concentrations, for 5 minutes each. It was observed a good correlation between PropCp and the anesthetic planes. Despite of a better correlation between CSI, PropCp and anesthetic planes than in the studies above, the CSI showed several limitations because it was not able to clearly distinguish among the anesthetic planes. Nevertheless the CSI was able to detect very deep planes of anesthesia when BSR occurs. These findings could compromise the use of CSI in dogs.

In conclusion the CSM showed important clinical limitations during induction and in the maintenance of anesthesia during routine anesthetic procedures with propofol in

dogs. Nevertheless, it seems that CSM is able to detect deeper levels of anesthesia when burst suppression occurs. The burst suppression rate was consistent in deep planes of anesthesia, which may be of relevance when considering the development of future Fuzzy logic based EEG monitors.

Resumen.

En los últimos 20-30 años, los avances en la tecnología informática permiten la utilización de complejas técnicas de modelización estadística del electroencefalograma (EEG) con el fin de generar una escala numérica "índice" para evaluar la profundidad anestésica y ayudar a los médicos anestesistas humanos.

Nuestro objetivo en esta memoria de tesis doctoral es el desarrollo de un índice basado en los patrones del electroencefalograma producidos por alteraciones en el fisiología cerebral cuando el animal esta anestesiado, y relacionarlos con la profundidad anestésica. Es decir la utilización del encefalograma para evaluar la profundidad anestésica, ya que hasta ahora ninguno de los signos clínicos que utilizamos puede, de forma cuantitativa, relacionarse con la profundidad anestésica.

En este trabajo realizamos la anestesia general con propofol en perros sanos a los que se les somete a una cirugía de orquitectomía, y que provienen de sociedades protectoras que tienen convenios con el Hospital Veterinario de Oporto (Portugal).

Se emplea el Electroencefalograma como variable de entrada, que es analizada con lógica difusa para procesar los datos. El algoritmo matemático que utiliza este equipo, parece ser que es el más adecuado para intentar obtener los índices que nos midan la profundidad anestésica.

En todos los estudios del trabajo de tesis doctoral, se incorporó el modelo farmacocinético del propofol desarrollado por Beths, T y col. 2001, mediante el Ruggloop software, que nos proporciona la estimación de la concentración plasmática de propofol en sangre.

Con el fin de evaluar el rendimiento del "Cerebral State Monitor", aparato que detecta el efecto de los anestésicos sobre el cerebro, recogiendo y procesando la actividad eléctrica cerebral, y convirtiendo esta señal eléctrica, a través de algoritmos matemáticos, en un índice reconocible, se realizó el estudio en tres fases:

1ª fase.- Se administró un bolo de propofol de 6 mg. /Kgpv. Los cambios de la actividad eléctrica cerebral fueron cuantificados mediante el cálculo del índice de estado cerebral. (Articulo "Brain monitoring in dogs using the cerebral state index Turing the induction of anesthesia via target-controlled infusión of propofol").

2ª fase.- Se administro propofol en infusión constante de 200 ml/hora en concentración del 1%. En este trabajo se introdujeron los signos clínicos, reflejos oculares, posición del globo ocular, etc. con el fin de observar la correlación entre las

concentraciones plasmáticas estimadas de propofol y los signos clínicos observados durante la inducción de la anestesia. Sin embargo el rendimiento del "Cerebral State Monitor", no se corresponde con las observaciones clínicas en los diferentes planos de profundidad anestésica. (Articulo "Correlation between clinical signs of depth of anesthesia and cerebral state index responses in dogs during induction of anesthesia with propofol").

3ª fase.- Se evalúa el "Índice de Estado cerebral" (CSI) medido por el "Cerebral State Monitor" (CSM) en perros anestesiados con diferentes concentraciones de propofol. Se demostró que a pesar de que hay una mayor correlación entre el CSI y los planos de profundidad anestésica, no siempre se podían distinguir unos planos anestésicos de otros. (Articulo "Correlation between clinical signs of depth of anesthesia and cerebral state index responses in dog with different target-controlled infusions of propofol").

En conclusión la utilización de monitores de profundidad anestésica puede guiarnos en la dosificación de anestésicos, evitando la sobre dosificación y reduciendo costes y efectos secundarios, pero el CSM mostró limitaciones clínicas importantes durante la evaluación de la inducción y mantenimiento de la anestesia en procedimientos rutinarios de anestesia en perros, aunque si permite detectar alertas en planos profundos de anestesia.

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Glossary

-	Absent
+	Present
AEP	Auditory-evoked potential
ANFIS	Adaptative Neuro Fuzzy Inference System
Awareness	When a patient has inadequate anesthesia and was consciousness
BIS	Bispectral index
BS	Burst Suppresion
BSr	Burst Suppresion ratio
Ce	Effect-site concentration
CNS	Central nervous system
CO2	Carbon dioxide
Cp	Plasma concentration
CR	Corneal reflex
CSI	Cerebral State index
CSM	Cerebral State Monitor
DoA	Depth of anesthesia
E0	Minimum effect
EC	Eye centred
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyography
EP	Evoked potential
ERV	Eyeball rotated ventromedially
FFT	Fast Fourier Transform
HR	Heart rate
Hz	Hertz
IM	Intramuscular
k10	Elimination parameter of central compartment
k12	Distribution rate from central to the second compartment
k13	Distribution rate between central and third compartment
k1e	Distribution rate between central and effect compartment

k21	Distribution rate between second and central compartment
k31	Distribution rate between third and central compartment
ke0	Elimination parameter of effect compartment
MAC	Minimum alveolar concentration
MAP	Mean arterial blood pressure
MF	Median frequency
MLAEP	Midlatency auditory evoked potential
MOAAS	Modified observer´s assessment of alertness and sedation
N	Negative
NaCl	Sodium cloride
P	Positive
PK	Prediction probability
PR	Palpebral reflex
PropCp	Estimated propofol plasma concentration
QEEG	Quantitative electroencephalogram variables
Re	Response entropy
S	Seconds
SE	State entropy
SEF	Spectral edge frequency
SQI	Signal quality index
Stat mode	Starting a continuous measurement
TCI	Target controlled infusions
TIVA	Total Intravenous Anesthesia
α	Alpha
β	Beta

Chapter 1

Introduction

The complexity in the evaluation of the depth of anesthesia during clinical practice, the different techniques applied in human medicine and the controversy about the reliability in veterinary anesthesia thwarts the development and routine use of monitors of depth of anesthesia. In an attempt to facilitate the understanding of the role played by monitors of depth of anesthesia, this first chapter addresses the main issues regarding the use of that tool in common veterinary practice. During this discussion special attention is devoted to the dog the target specie from this thesis.

1.1-Depth of anesthesia: What have we been monitoring?

1.1.1-History

The dogs had a great value in development of intravenous anesthesia. In 1657, Sir Christopher Wren the famous architect, carried out the first experiments at Duc de Bordeux`s house. Wren ligated the veins of a large dog, made an opening on the side of the ligature toward the heart and introduced the syringe in this opening. The syringe was made off animal bladder, to which a quill had been attached, and filled with a opium solution in one case and *crocus metallorum* in another. This experiment revealed that opium administered intravenously *"soon stupefied*" (probably a deep sedation) but did not kill the dog. Sir Wren was certainly unconscious of his anesthetic result from administering opium. On the contrary, when the *crocus metallorum* was administered, it induced the vomiting and caused death. (First Lieut Keys 1942) (Reference Librarian, Mayo clinic, Rochester, Minnesota, reviewed by War Manuscript Board) .

The classical concept of anesthesia described by Pinsker (Pinsker 1986) incorporated immobility (absence of movement or skeletal muscle tone), unconsciousness and the attenuation of stress response (heart rate and blood pressure) to painful stimulus, that reflects analgesia are the main three components of anesthesia.

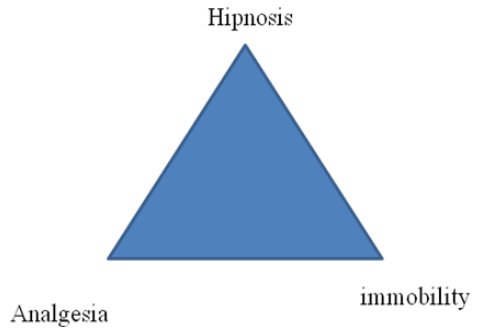


Figure 1 -Represents the classical components of anesthesia

Pry-Roberts (Prys-Roberts 1987) defined anesthesia as a state in which an individual is incapable to perceive or recall a noxious stimulus as a result of drug unconsciousness. In veterinary patients the objective of anesthesia is to promote reversible unconsciousness, amnesia, analgesia and immobility with minimal risk to the animal (Haskins 1996). When dogs are anesthetized, unconsciousness is expected. But does it always occur? Veterinary anesthesia undoubtedly induces unresponsiveness and amnesia. Nevertheless, the extent to which it causes unconsciousness is harder to establish. The present knowledge of mechanisms that produce unconsciousness is very limited in humans, and even more limited in animals.

In order to better understand consciousness in humans, it was separated from memory, and memory itself was separated in two forms: implicit and explicit. Consciousness is defined as the capability to execute simple motor tasks in response to a verbal command; explicit memory is the capability to recall events or words; implicit memory represents part of the subconscious and normally is related with changes in performance or behaviour (Rampil 2001). When applying these concepts to veterinary anesthesia, it is easy to see that veterinarians do not face an easy task. As owners sometimes report changes in the behaviour of their animal after the surgical procedures, this could lead us to thinking that these changes could be related to a surgical awareness. Nevertheless there are several factors that can be associated with these behavioural changes, since the environment and medical procedures are traumatic experiences, in different grades, for the patients. . In fact, anaesthesia can´t be detached from surgery and its implications. Prior to this, there is one main question that is worth addressing:

1.1.2-How deep are our veterinary patients anesthetized?

That question still goes on puzzling as time goes by because the concept of deep anaesthesia is dimensionless. Some reports, on human medical literature refer to the difficulty that teachers have to explain the students how deep anesthetized the patient is. "*The experienced teacher, noting that patient appears to be clinically satisfactory, often says the patient is in the second plane and walks away before he is asked to explain how he arrived at this decision*"(Jacoby, Hamelberg et al. 1960) .

Since the anesthesia was first introduced during the last century, multiple suggestions were introduced in order to characterize the depth of anesthesia, and consequently the amount of the drug that should be administered to the patient.

The depth of anesthesia has been evaluated based on clinical changes in blood pressure, heart rate, pulse rate, ocular signs, muscular tonus and respiration (Cullen, Eger et al. 1972). Interestingly enough, back in 1960, , experienced anesthesiologists claimed that the most important sign to observe in anesthetized patients is respiration, as many anaesthesiologists controlled the amount of drugs given in accordance to changes in ventilation (Jacoby, Hamelberg et al. 1960).

Despite of the possibility of using the ventilation to access the depth of anesthesia in veterinary, because most of the patients are anesthetized without neuromuscular blocking agents, the breathing rate may also be a sensitive indicator of more underlying problems. There are a lot of physiologic events like hypoxemia, hyperthermia, hypotension, atelectasis and pain that can affect the breathing rate (Haskins 1996).

The references in veterinary literature (Haskins 1992; Lumb and Jones 1996; Hall, Clarke et al. 2001) related to depth of anesthesia are normally extrapolated from human anesthesia. Guedel, in 1937 (Guedel 1937) described the classical signals of ether anesthesia for humans, which have been applied to animals in a first effort to characterized the depth of anesthesia (Soma 1971; Hall, Clarke et al. 2001). This evaluation of depth of anesthesia was based on observation of ocular reflexes, muscle tone, and respiration. Haskins described a variety of signs that can be helpful to evaluate the depth of anesthesia in dogs (Table 1) (Haskins 1992).

Table 1 - General guidelines for judging anesthetic depth.

Signs	Light	Medium	Deep
Spontaneous movement	**Maybe/unlike**	**No**	**No**
Reflex movement	**Yes/maybe**	**No**	**No**
Hemodynamic responses	**Yes**	**Maybe/no**	**No**
Vaporizer settings	**Low**	**1.1 to 1.5 MAC**	**Hight**
Muscle tone	**High/good**	**Moderate**	**None**
Palpebral reflex	**Yes**	**No**	**No**
Eyeball position	**Central**	**Ventromedial**	**Central**
Light reflex	**Yes**	**Maybe**	**No**
Moist córneas	**Yes**	**Yes**	**No**
Pupil aperture	**Medium**	**Smaller**	**Dilated**
Shivering	**Maybe**	**No**	**No**

Opinions in Small Animal Anesthesia in Veterinary Clinics of North America. Adapted from Haskings, S.C. (1992).

Burge and colleagues (1943), reported spontaneous movements in dogs anaesthetised with ether, which can be associated to a light or inadequate anesthesia. Nevertheless, these observations were reported repeatedly in the same dog. Under the same conditions, the same authors observed in different dogs that by decreasing the depth of anesthesia, the negative potential of the brain´s motor cortex would increase the probability of spontaneous movements (Burge 1943). Nevertheless, in humans' studies the high percentage of spontaneous movement is not correlated with a post-operatively recall (Stolzy, Couture et al. 1987). There are numerous reasons that could promote movement during surgery, including not just light anesthesia, but insufficient muscle relaxation, patient respiratory effort, cardiac function (ballisticcardiographic forces), mechanical ventilation and direct electrocautery stimulation (Adragna 1986).

Haskings (Haskins 1992) described that a gross spontaneous movement, or a reflex movement are reliable signs of light anesthesia.However, Antognini and Schwartz demonstrated that with volatile anesthetics the concentration to produce amnesia and unconsciousness is around 25-40% of the needs to inhibit purposeful movement (Antognini and Schwartz 1993).Similar findings were observed for propofol action at the lumbar dorsal spinal cord (Antognini, Wang et al. 2000). Thus, it is reasonable to suggest that purposeful movement by itself cannot be used as an indicator of level of depth of anesthesia, as anesthesia is a result of simultaneous actions at different sites in central nervous system.

The evaluation of depth of anesthesia in veterinary practice also depends on the autonomic responses to the noxious stimulus. The minimum alveolar concentration

(MAC) was first described by Merkel and Eger (Merkel and Eger 1963), and is by definition the steady minimum alveolar concentration of anesthetic required to prevent purposeful movement in 50% of animals population, in response to a selected noxious stimulation (Merkel and Eger 1963). Therefore, and as exposed in the last paragraph regarding the percentage of hypnotic to produce unconsciousness, using MAC as a single method to evaluate the depth of anesthesia would probably yield in excessive anesthetic administration, because its definition was based on purposeful movement. If one wants to abolish the movement the patient must be maintained under excessive levels of anesthesia (Whelan and Flecknell 1992).

The degree of muscle relaxation was also frequently used to monitor the depth of anesthesia. In 1937, Hendersoh and colleagues provided a simple description of muscular tonus under ether anesthesia. According to those authors, in early stages of anesthesia the higher nervous centres are depressed, but the lower motor centres are not inhibited, leading to an increase in the muscular tonus; on the other hand, in deep stages of anesthesia, the lower motor centres are also depressed, causing a diminished nerve discharge, leading to muscle relaxation and absence of muscular tonus (Hendersoh 1937).

During routine anesthetic procedures, the muscular tonus in veterinary patients is estimated by the resistance to open the mouth or eyelids. This clinical evaluation may become very subjective if we attend to the fact muscular tonus is influenced by the size of the patients (larger dogs have more tonus), and in some dogs it is almost impossible to detect the presence of mandibular tonus due, for example, to their very small size. Thus, in an ideal situation, veterinarians should be able to quantify the muscular tonus for each individual patient, but even then, muscular tonus would not be an objective measurement of the depth of anesthesia (Haskins 1992).

Ocular reflexes and eyeball position are two of the most used signs to access the depth of anesthesia in veterinary patients. In 1966 Guedel and colleagues reported that the eyeball is one of the most important signs in anesthesia at that time.

As long as the eyeball is oscillating or is in an eccentric position though stationary, there is no danger that too much ether has been given. Aside from extraneous circumstances, such as positional asphyxia, haemorrhage, or shock, if the eyeball is moving or is stationary but eccentric, the patient is safe and in a good condition(Guedel 1966).

In veterinary anesthesia the eyeball movements are similar with thiopental, propofol, halothane and isoflurane. When increasing from light to moderate anesthesia, the eyeball rotates ventromedially or ventrolaterally, returning to a central position in deeper levels of anesthesia (Hall, Clarke et al. 2001). However, there are some animals in which the eyeball position never changes (Haskins 1992), this is a very important fact to bear when one only uses this method to access anesthetic depth. Although the pupil size is also observed when analysing the eyeball position, there are many variations between individuals, and consequently it is not a reliable sign itself that can provide accurately information regarding the level of anesthetic depth (Haskins 1992).

The palpebral reflex, defined by the partial or complete closure of the eyelids, is a commonly useful tool to help monitoring the depth of anesthesia. Its occurrence means that the patient is under a light plan of anaesthesia. If palpebral reflex is absent or depressed, the patient could be in a medium to a deep level of anesthesia (Hall, Clarke et al. 2001). The corneal reflex may also be used to help determining the depth of anesthesia. A corneal reflex is the eyelid response obtained to a careful and gentle touch in the cornea, and should not cause any corneal damage. This reflex causes some controversy regarding its usefulness in accessing the depth of anesthesia. Some veterinary anaesthesiologists refer that corneal reflex is not a sign of deep anesthesia (Hall, Clarke et al. 2001), while others report that this reflex should always be present, unless the patient is too deep anaesthetised or dead. The absence of moist cornea may also be considered as a sign of an excessive depth of anesthesia (Haskins 1992).

As hypnotic drugs also depress the autonomic nervous system, it is expected that the heart rate and blood pressure decrease, when the level of anesthetic depth increases (Flaishon, Windsor et al. 1997). Nevertheless, these autonomic signs can be influenced by several physiological events, such as noxious stimulation or hypo/hyperperfusion situations. Combinations of analgesic and hypnotic drugs, such as propofol and opioids, can also by themselves cause hemodynamic depression unrelated to the depth of anesthesia (Hug 1990). Thus, although these variables are very important for monitoring and maintaining the patient's health condition under general anesthesia, they cannot be considered by themselves indicators of the level of depth of anesthesia.

According to the previous paragraphs, in order to have a safe and reliable monitoring of the depth of anesthesia, veterinarians should not rely on any particular sign, but use as much information as possible from the visual interaction with the patient, and from hemodynamic monitors. There is one study that produced a visual analogue scale (VAS)

for pigs (Martin-Cancho, Lima et al. 2003) based on the clinical experience of the researchers, and on the information suggested in Lumb & Jones Veterinary Anesthesia (Lumb and Jones 1996) (Table 2).

Table 2-Variables used to access depth of anesthesia in pigs (Martin-Cancho, Lima et al. 2003).

Variables	Anesthetic planes				
	1	2	3	4	5
Conscious	**Yes**	**No**	**No**	**No**	**No**
Breathing Rate	**Normal**	**Irregular and Increase**	**Moderately decrease**	**Moderately decrease**	**Slow and irregular**
Types of breathing	**Thoracic and abdominal**	**Thoracic and abdominal**	**Thoracic and abdominal**	**Thoracic but predominantly abdominal**	**Abdominal**
Pupil	**Normal**	**Dilated**	**Normal**	**Moderately dilated**	**Dilated**
Eyeball Position	**Normal**	**Variable**	**Fixed ventromedially**	**Normal**	**Normal**
Heart Rate	**Normal**	**Increased**	**Moderately decrease**	**Moderately decrease**	**Decreased**
Arterial blood pressure	**Normal**	**Increased**	**Normal**	**Decreased**	**Decreased**
Palpebral reflex	**Weak response**	**Strong response**	**Weak response**	**Not detected**	**Not detected**
Corneal reflex	**Weak response**	**Strong response**	**Weak response or not detected**	**Weak response or not detected**	**Not detected**
Pedal reflex	**Strong response**	**Strong response**	**Weak response**	**Not detected**	**Not detected**
Muscular tone	**Yes**	**Yes**	**Decreased**	**Decreased**	**Not detected**
Nocioceptive response	**Strong response**	**Strong response**	**Weak response**	**Not detected**	**Not detected**

Bispectral index, spectral edge frequency 95%, and median frequency recorded for various concentrations of isoflurane and sevoflurane in pigs. Adapted from Martin-Cancho and colleagues (Martin-Cancho, Lima et al. 2003).

1.2- Why do we need monitors to assess the depth of anesthesia?

The depth of anesthesia is usually determined by the response measured to a certain stimulus, and by the amount of drug required in the effect site to blunt the responsiveness (Stanski 2000). But this evaluation depends on the subjective assessment of the reflex physiological response (heart rate and blood pressure) to the ongoing procedure. Additionally, and as the anesthetic drugs have different physiologic responses, it is extremely important to have a detailed knowledge of mechanism of action of different drugs used in anesthesia, in order to more accurately interpret the clinical signs (Haskins 1992).

The major goal in veterinary and human anesthesia is to quantify the depth of anesthesia and, at the same time, to evaluate the level of unconsciousness that cannot be measured objectively. An increased accuracy of the process of measuring the depth of anesthesia would be a huge step towards the standardization of anesthetic practice in institutional animal care, in clinical practice and in research aiming at guarantying an adequate anesthetic protocol and less variation between individuals.

Some reflex response may be masked by the use of neuromuscular blocking agents, despite the fact that these are not currently used in veterinary practice, its use may increase as surgical techniques evolve and require more accurate anesthetic procedures. The introduction of devices that can monitor the depth of anesthesia in paralyzed animals reduces the chance of intraoperative awareness, or of overdosing the patient (Martin-Cancho, Carrasco-Jimenez et al. 2004).

In humans, the occurrence of awareness during surgery is of great concern because this very traumatic experience may lead to a completely change of personality, making living a nightmare for these patients (Bruhn, Myles et al. 2006). In veterinary anesthesia, the occurrence and the consequences of awareness are difficult to establish (Haskins 1996), but it does not mean that is not frequent.

Despite of the remarkable improvements in the assessment of the cardiovascular function during anesthesia, the direct determination of the effect of the anesthetic agent(s) on the central nervous system has remained a challenge (Moerman, Bonke et al. 1993). There are studies in humans that refer the awareness of the patient when the hemodynamic parameters remain stable (Flaishon, Windsor et al. 1997).

Wetzel and colleagues demonstrated significant hemodynamic responses to surgical stimuli in patients with undoubtedly criteria of brain death (Wetzel, Setzer et al. 1985). The same principles are applied to veterinary anesthesia (Hall, Clarke et al. 2001),
but the veterinary anesthetists have few alternatives to monitor the depth of anesthesia besides monitoring the autonomic parameters. The cardiovascular signs of anesthesia appear to be a balance between sympathetic stimulation and the direct depressant effects of anesthesia. It is difficult to evaluate the depth of anesthesia based on the autonomic activity, as some agents like CO_2, can influence such activity (Cullen, Eger et al. 1972). It is possible that unnecessary anesthetic overdose may occur during veterinary anesthesia, when only monitoring hemodynamic parameters (Whelan and Flecknell 1992). The development of a depth of anesthesia monitor has faced the problems related with the fact that there is no single clinical measurement that includes all anesthetic drugs used in current general anesthesia, because different drugs have different pharmacologic actions, and sometimes different physiological responses. This makes very difficult to determine the potency of a certain drug using just one monitoring parameter (Kissin 2000). For example, the use of opioids like fentanyl can alter the heart rate, mean arterial pressure and catecolamine response during isoflurane and desflurane anesthesia (Hilgenberg 1981; Daniel, Weiskopf et al. 1998). If we consider that balanced anesthesia uses the combination of different drugs, with different effects, to achieve a more stable and safe level of anesthesia, monitoring these anesthetic procedures require additional clinical information, where brain activity is one of the most important parameters. Many anesthesiologists confess that sometimes they have difficulties in administrating more or less of a particular drug, as balanced anesthesia is a more difficult technique to learn, despite being associated with several advantages (Jacoby, Hamelberg et al. 1960).

The brain electrical activity has an important relevance to assess the depth of anesthesia. The EEG is a non invasive indicator of the cerebral function during general anesthesia, as it represents cortical activity based on the sum of postsynaptic activity. New computer analysis can process the EEG to give us valuable information about the level of unconsciousness.

Currently, the veterinary anesthesia uses a multimodal anesthesia monitoring approach. Hemodynamic and respiratory parameters and muscle routinely controlled during anesthesia. However, the level of consciousness remains a challenge, the veterinary anesthesiologists do not have a reliable tool to monitor the “brain state”.

1.3- What is the origin of the EEG?

1.3.1- The electrical activity in the brain.

The EEG is by definition a recording of a spontaneous electrical activity of the cerebral cortex, and it represents the net summation of electric currents produced by cerebral neurons (Rampil 1998). The large pyramid of cells arranged sides by side in the fifth layer of the brain are responsible for the neuronal source of the EEG. The long straight apical dendrites, and neighboring dendrites have allowed the creation of dipoles in the neurons that cause currents to flow, which will have a greater effect on the surface electrodes (Rampil 1998; Bennett, Voss et al. 2009). The EEG had no regular, repetitive patterns in shape of the EEG waveform, and changes randomly over time (Rampil 1998).

In veterinary anesthesia, the EEG raised increased interest in the nineteenth decade, despite of the large ongoing research in animals for human purposes that boasted since the 19th century. In 1875 Caton described the presence of electrical current in the brain using a galvanometer in rabbits and monkeys (Caton 1875). Hans Berger of Jena, the "father of electroencephalography", recorded the electrical activity of the brain using electrodes in the head surface (Berger 1933). Nevertheless, the researchers have only studied in 1937 the relationship between anesthesia and the EEG, when Gibbs and Lennox hypothesized that the electroencephalogram is sensitive to anesthetic agents (Gibbs, Gibbs et al. 1937). In the same year, Loomis and colleagues described systematic changes of the EEG during human sleep, and were the first to describe five anesthetic stages, A–E, to distinguish different EEG patterns (Loomis, Harvey et al. 1937) (Fig 2).

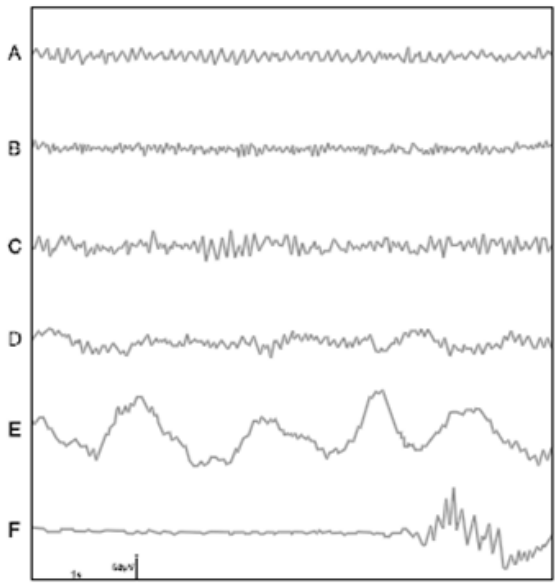


Figure 2-EEG patterns

(A) Represents an "awake" EEG with a high frequency and low amplitude of the waves. The application of anesthetics, such as volatile anesthetics, barbiturates, etomidate and propofol induces comparable and dose-dependent changes in the raw EEG signals according to the following pattern. In low doses, anesthetics lead to a short-term high frequency beta rhythm (B). With increasing anesthetic effect, a slowing of EEG frequency is observed, increasing theta and then delta activity, with a simultaneous increase in amplitude (C to E). A further increase in anesthetic dose results in an increasing inhibition of the electric activity until a flat EEG (suppression) is registered that is merely disturbed by short-term activity bursts (F); this is called a 'burst suppression pattern'.

In veterinary anesthesia, the first reports associating the EEG with anesthetic depth were introduced by Klem and Mallo in 1966 (Klemm and Mallo 1966). The EEG is considered a possible measurement to assess the depth of anesthesia in dogs because there are changes in the EEG patterns during anesthesia (Bergamasco, Accatino et al. 2003), that reflect electrical activity related to physiological changes in cerebral metabolism (Artru 1986) and in cerebral blood flow (Kuramoto, Oshita et al. 1979; Dong, Bledsoe et al. 1983). Anesthetic drugs affect both cerebral physiology and EEG patterns.

The electroencephalographic signal is described by using three basic parameters:

1. *Amplitude* is the size, or voltage of the recorded signal and ranges from 5 μV to 500 mV.
2. *Frequency* is the number of times per second the signal oscillates or crosses the zero voltage line (Stanski 2000). It is very interesting that the fluctuations are in a relatively narrow range from 0.5 Hz to 50 Hz, with most of the frequencies below 30 Hz (Holliday and Colette 1999).
3. *Time* is the duration of the sampling of the signal. The set of sequential samples is named an epoch, and the collection of all possible epochs from an EEG is entitle ensemble (Rampil 1998). The 2 seconds epochs have been recommended for monitoring the patients' EEG during surgical procedures. The advantage of averaging the epochs is the reduction of the power spectrum variance. In contrast the averaging could obliterate the characteristics of the EEG pattern (Levy, Shapiro et al. 1980). Contamination of the signal could be introduced by artifacts frequencies produced by the abrupt transitions at the epochs' end (Rampil 1998). Otto has been studying in dogs the effects of the different averaging on the EEG analysis in dogs and conclude that quantification EEG analysis in response to noxious stimulation are significantly depending of the number of epochs subjected to averaging. The average of 20 to 30 second period could the best for detecting changes in EEG data when an intense noxious stimulus is applied (Otto 2007).

From the clinical point of view, fortunately the brain has the tendency to limit the variation in the wave patterns observed in the normal wake EEG when the anesthetic agents are administered (Martin, Faulconer et al. 1959). Traditionally, frequency bands are categorized in *delta(δ)*(0-4Hz), *theta(θ)*(4-8Hz), *alpha(α)* (8-13Hz) and *beta(β)* (13-30Hz) (Rampil 1998). The high frequencies α and β waves predominate during the waking state, and the slower δ and θ waves predominate during sleep or under anesthesia (Bennett, Voss et al. 2009). Higher cortical activity normally promotes the desynchronization of the EEG, and the neurons are active more independently, creating the conscious behavior in humans. On the other hand the anesthetic agents promote the synchronization of the cortical activity (Rampil 1998). Desynchronization has been

described in laboratory animals when a noxious stimulus was applied (Antognini, Carstens et al. 2000). One of the most interesting challenge in the analysis of EEG is the effect of the noxious stimulus on EEG monitoring during surgery (Otto 2008). Noxious stimuli can cause four types of changes in the EEG:

- Desynchronization with observation of fast rhythms (20- 60Hz),
- Spindles 6-10Hz and
- Bursts of slow waves 1-3Hz.
- Synchronization of the EEG

Nevertheless in response to a noxious stimulation, it can occur a slowing of the EEG wave referred as synchronization (Otto 2008), also observed in cats by Kaad and colleagues (Kaada, Thomas et al. 1967). Miyauchi and colleagues described that sciatic stimulation in dogs has different effects on EEG, depending on the concentration of penthotal anesthesia. A distinct threshold concentration of thiopental seems to block the response to noxious stimuli during anesthesia in animals (Miyauchi, Sakabe et al. 1985). To simplify, any changes in EEG waveform due to a noxious stimulus should be carefully evaluated, as it may indicate a perception of pain (Otto 2008).

1.3.2-Artifacts

Artifacts entail some and have to be taken in account when the EEG is analyzed. There are different sources of artifacts:

1.3.2.1 Artifacts from outside the head

Artifacts from outside the head may include noise from electrical equipment, power line artifacts (which are the most important source of exogenous electrical artifact), external pacemakers, electrocardiogram, diathermy surgical movement of the patient and hammering or drilling (Rampil 1998; Bennett, Voss et al. 2009).

1.3.2.2-Artifacts from the head outside the brain

The artifact from the head but outside the brain is mainly the electromyography (EMG). The frequency of EMG ranges from 10-300Hz. It is known that EMG cannot be filtered out completely, and for this reason could influence the analysis of the EEG (Ribeiro, Ferreira et al. 2009). Despite the artifact effect of the EMG, the usefulness of this parameter as additional information about the anesthetic state of the patient is

controversial (Bennett, Voss et al. 2009). Campagnol and colleagues studies the effect in dogs of a noxious produced by electrical stimulation. It was observed that after a stimulation the BIS increased significantly in all treatments, but it must be taken in account that they founded a high correlation between BIS and EMG, which lead to interpret this results cautiously because the changes in BIS may not be related to awareness but to EMG response.(Campagnol, Teixeira Neto et al. 2007)

1.3.2.3- Artifact within the brain

The artifacts with origin in the brain are not well described in veterinary anesthesia because it is very difficult to establish the alteration in EEG due to artifact that are in the brain, like: low amplitude of EEG sometimes provoked by analgesic or volatile agents, seizure activity and post-ictal state which can occur due abnormal physiology, cerebral pathology or drugs, variable δ activity during stable anesthesia unrelated with surgical stimulation. Paradoxal δ activity occurs in one third of the human patients, noxious stimulus may cause an increase in slow δ waves .Finally the cerebral pathology and systemic problems, namely, hypovolemia, hypoglycemia, and cardiac arrest could slow the EEG waves (Bennett, Voss et al. 2009).

1.4 What are the principles to analyze EEG?

The EEG is a microvolt-range signal, thus the quality and consistency of the electrode connected to the skin is essential to obtain a the reliable interpretation of the signal (Rampil 1998).

In 1959 Martin and Faulconner exposed the difficulty in recording the EEG in the surgery room, because the great amount of amplification necessary created an enormous amount of artifacts (Martin, Faulconer et al. 1959; Levy, Shapiro et al. 1980). Nevertheless, the major problem was to perform a quantitative analysis of the EEG. The first EEG evaluations were visual, requiring highly qualified personnel for the interpretation of the EEG. But, even so, the highest average correlation, regarding conclusion from EEG interpretation, between seven experienced EEG readers was 56 percent (Berezowskyj, McEwen et al. 1976).

In the 80s , the advance in electronic technology brought about a renewed interest in the analysis of intraoperative EEG. The large amount of the raw EEG could be analyzed due to a newer computer techniques (microprocessor) enable to extract the EEG into a condensed, descriptive format called processed EEG (Edmonds and Markku 1985; Stanski 2000).

One of the earliest statistical analysis used for EEG was the period analysis. This method quantifies the number of times the EEG line crosses the zero and can be performed manually (Levy, Shapiro et al. 1980). Nevertheless, the obvious limitations of this analysis led to the use of other more accurate and computerized methods.

1.4.1-Power Spectrum Analysis

The Power Spectrum analysis consists of the conversion of continuous EEG waveform into a finite resolution numbers obtained in samples sequentially analyzed. The samples represent a finite interval time called an epoch (Rampil 1998). The analysis of the epoch is performed by using the Fast Fourier Transform (FFT), a complex mathematic technique. The FFT decompose the EEG epoch in sine and cosine waves of different amplitude at a certain frequency range. The sum of these sinusoidal waves represents the original waveform. The use of power analysis as the measurement of the amplitude is a convention reflecting the origins of Fourrier analysis in radio engineering (Levy, Shapiro et al. 1980). The power spectrum analysis is then calculated squaring the

amplitude of the individual frequency component (Fig.3.2) and represents the relative contribution of each frequency.

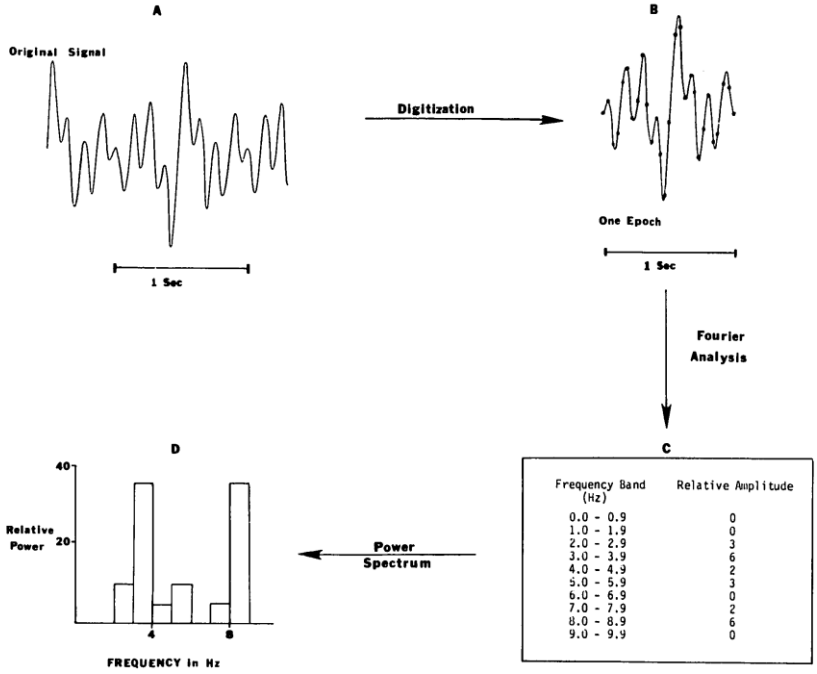


Figure 3-Schematic representation of the process of power spectrum analysis.

The original continuous waveform (A). Digital form of the EEG samples (B). Fourrier analysis into a microprocessor (C). Histogram of the power spectrum (D). Adapted from (Levy, Shapiro et al. 1980).

The Power spectrum analysis takes into account frequency and amplitude information of the EEG waves but the phase angle is not analysed. The phase angle(θ) represents the time offset of the sinus wave relative to time zero (Sigl and Chamoun 1994). The phase related to the start of the epoch is obtained by Fourrier analysis (Rampil 1998). Nevertheless the physiological meaning of the phase angles is unclear. In general, the awake brain has multiple signal pacemakers, working independently with little synchronization. In the "sleep brain" fewer independent signal generators are active (Rampil 1998).

1.4.1.1 Spectral edge frequency

The spectral edge frequency (SEF) is a statistical analysis of the EEG derived from power spectrum that represents the frequency below which 95% (SEF95%) of the power spectrum is located. Some authors use the SEF80% (Long, Shah et al. 1989).

1.4.1.2 Median frequency

Median frequency (MF) is the frequency below which 50% of the total EEG power spectrum is located (Schwender, Daunderer et al. 1998).

1.4.1.3 Relative power

Relative power of four frequency bands represents the percentage distribution of total power of each frequency band (Otto, Voight et al. 1996). The power band ratios, relative fractional θ power, relative fractional α power, and relative fractional β power are all related to the δ power (Long, Shah et al. 1989).

All of this measurements derived from the power spectrum analysis are designed by quantitative electroencephalogram variables (QEEG) (Otto 2008).

The performance of EEG derived indexes when used by monitors of depth of anesthesia are sometimes compromised due to the different pharmacological and physiological characteristics of the anesthetic drugs, and its effects on the EEG patterns (Rampil 1998). Studies performed in dogs by Itamoto and colleagues demonstrated that when administering 20µg of medetomidine alone, or combined with midazolam, or with midazolam and butorfanol, the EEG index values obtained are similar which means that QEEG should be carefully interpreted to assess the depth of anesthesia when using balanced anesthesia (Itamoto, Taura et al. 2002). Nevertheless these QEEG variables detected changes in the EEG caused by the anesthetic drugs, but the incapability of finding EEG calibration behavioral endpoints is the major issue behind the QEEG poor performance.

1.4.2-Bispectral analysis

The Bispectral analysis is a processing technique that quantifies nonlinear and linear changes in the generation of EEG.

The Bispectral analysis quantifies the relation between two primary frequencies, f1 and f2, and the modulation of the components of these frequencies f1+f2, detecting phase–coupling within the signal, and the variation as a response to the external stimulus. In the absence of phase coupling the bispectrum tends to zero; in the presence of phase coupling the bispectrum tends to non zero (Sigl and Chamoun 1994). The bispectrum is not just a reflex of phase coupling, as it is influenced by the amplitude of the EEG signal and by the degree of phase coupling. Bicoherence is used to incorporate these two variables. A high bicoherence value at (f1, f2) indicates that there is a phase coupling within the triplet of frequencies f1, f2, and f1 + f2. Strong phase coupling means that the sinusoidal components at f1 and f2 may have probably the same generator of the neuronal activity (Rampil 1998) .

1.4.3-Burst Suppression Quantification

In deep planes of anesthesia the EEG may develop an atypical pattern of activity. This pattern is called burst suppression , which is quantified by the burst suppression ratio (BSr) The BSR is characterized by alternating periods of normal to high voltage activity with low voltage or isoelectric EEG wave. The isoelectric EEG periods should be longer than 0.50 sec., and the waves' voltages should not exceed ±5.0 mV to be assumed as BS pattern. The time in a suppressed state is measured, and the BSr is the fraction of the epoch length where the EEG is suppressed.

The monitors to evaluate the depth of anesthesia incorporate more than one method of EEG analysis in an attempt to override the limitations of each EEG parameter itself.

1.5-How can the EEG signal be correctly collected?

Although the EEG is a microvolt-range signal, most of the artifacts influencing the EEG will present a millivolt magnitude. This makes the quality and consistency of the electrode to skin connection essential to the reliable interpretation of the signal.

The quantification of impedance is important to evaluate the quality of the electrical contact between the EEG electrode and the skin. In a simplistic definition, the impedance means the "resistance" or not to the electric current flow. Low impedance occurs when you have low resistances to the electrical current, which means a good contact between the skin and the electrode, allowing an increase in the quality of the EEG signal. High impedance values could interfere with the reliability of the monitoring results (Seitsonen, Yli-Hankala et al. 2000; Thogersen and Ording 2000; Anderson, Sartipy et al. 2007).

The impedances values during EEG monitoring should stay below 10 kΩ (Seitsonen, Yli-Hankala et al. 2000). For the example, the BIS monitor needs impedance values lower than 7,5 kΩ to startup (Greene, Benson et al. 2002). On the other hand, the Cerebral State Monitor needs impedance values lower than 5 kΩ to startup.

The skin electrodes used for neurological diagnosis usually have a multipolar assemble, but this complex method is impracticable for monitoring brain activity during routine anesthetic procedures. The bipolar assemble is the best way to collect EEG during anesthesia. Fortunately, in humans the anesthetic agents induce significant changes in the frontal cortex activity which allow the use of the forehead to stick the EEG skin electrodes to quantify the anesthetic effects on the brain electric activity (Bennett, Voss et al. 2009). In *bipolar* assembles, derivations are formed by two electrodes that are acknowledged to be located over active cerebral tissue. It is recommended that the electrodes form chains oriented longitudinally and/or transversely over the calvaria (Holliday and Colette 1999).

There are different electrodes assembles described in dogs: frontal-occipital (Campagnol, Teixeira Neto et al. 2007; Ribeiro, Ferreira et al. 2009) (Fig. 4), bifrontal (Carrasco-Jimenez, Martin Cancho et al. 2004) (Fig. 5), and frontal-temporal (Campagnol, Teixeira Neto et al. 2007) (Fig. 6). Bergamasco and colleagues described the anterior areas of the brain with predominance and prevalence of slowing frequency bands in propofol anesthetized dogs (Bergamasco, Accatino et al. 2003).

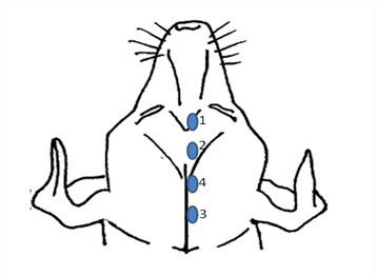


Figure 4-Represents the frontal-occipital position of electrodes.

All the electrodes are in the midline. Electrode 1 is placed 1 cm dorsal to an imaginary line connecting the medial canthi of the eyes, and electrode 3 was placed on the occipital crest. Because of the design of the sensor, electrodes 2 (ground), and 4 (reference) were placed at 2-cm distance from each other (Campagnol, Teixeira Neto et al. 2007).

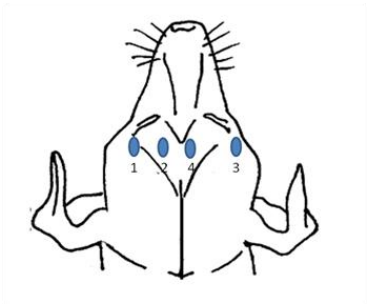


Figure 5-Represents the bifrontal position of electrodes

Electrodes 1 and 3 were placed 1 cm caudal to the lateral canthus of each eye. Electrode 4 (reference) was placed on the midline, and electrode 2 (ground) was placed between electrodes 1 and 4 (Campagnol, Teixeira Neto et al. 2007).

Figure 6-Represents the frontal-temporal position of electrodes.

The electrode 1 was placed in the midline on the rostral third portion of an imaginary line connecting the zygomatic process of the frontal bone, and the caudal portion of the frontal crest. Electrodes 2 (ground) and 4 (reference) were each placed at an angle of 15^{o} to 30^{o} to the transverse plane. As a result of this placement, electrode 2 remained dorsal to the eyelid, whereas electrode 4 was placed caudodorsal to the lateral canthus of the left eye. Electrode 3 was placed on the zygomatic process, cranial to the base of the left ear (Campagnol, Teixeira Neto et al. 2007).

1.5.1-Types of electrodes:

The most used brain monitor is the BIS monitor, and most of the studies performed in dogs uses the BIS Quatro Sensor (Aspect Medical Systems, Newton, Mass). In order to use this sensor in dogs, it is necessary to shave the dogs' head, which is a strong limitation for veterinary practice. Green and colleagues compare the performance between patch electrode (BIS sensor, Aspect medical systems Inc) and subdermal electrodes using a modified cable of ECG using three 29 gauge platinum needles. They concluded that both types of electrodes had similar skin impedance, and the results obtained are not statistically significant. The EEG clamp electrodes are also used because they are easy to apply during routine clinical use, and because they reduce the cost of EEG monitoring (Seitsonen, Yli-Hankala et al. 2000; Akavipat, Dumrongbul et al. 2006). Hemmerling and Harvey refer that the use of ECG c electrodes could replace the patch electrodes of the BIS monitor after a good preparation of the head in humans (Hemmerling and Harvey 2002). The same results were observed for the CSI monitor where the electrocardiogram (ECG) electrodes maintained the same accuracy (Anderson, Sartipy et al. 2007). In conclusion, to record the EEG it can be used small needle electrodes placed in the subcutaneous tissues over the calvaria, small metal disk electrodes applied to the skin surface, or small alligator clamps attached to the skin after dulling the teeth of the clamp, whenever impedance values are respected.

1.6-How to validate the monitor?

The ideal monitor to assess the depth of anesthesia should correlate brain electric activity and anesthetic drug concentration under general anesthesia, reflecting the clinical state of the patient, regardless of the anesthetic drug used. Two concepts must be taken in account when looking for a good monitor for depth of anesthesia: validity and reliability.

Validity is a measurement of accuracy and is difficult to quantify since there is no gold standard for anesthetic depth. Validity represents the agreement between the monitor and another reference instrument (Bruhn, Myles et al. 2006).

Reliability is a measurement of uniformity values obtained between repeated measures. Reliability can be evaluated by testing and retesting the reliability, in this case concurrent or repetitive measurements of depth of anesthesia will be comparable at a stable anesthetic state with clinical variables for the example (Bruhn, Myles et al. 2006).

1.6.1-Characteristics and qualities of the ideal monitor to measure the depth of anesthesia.

1.6.1.1- Analysis of the EEG as tool to assess the anesthetic depth.

To use monitors to evaluate the depth of anesthesia we must assume that the EEG represents a cluster of pos-synaptic potentials that can be accurately and reliably correlated with different anesthetic states (Stanski 2000). It is still unclear which is the optimal EEG processing technique for brain monitor, and that is why it exists depth of anesthesia monitors based on spontaneous potentials, evoked potentials or in multiple modalities. The monitors that combine spontaneous EEG indices with evoked potentials show the best potential, and are currently under development (Schneider, Hollweck et al. 2005). An important consideration in the evaluation of clinical monitors is the distinction between the desired signal, and the artifacts on the EEG and on EMG (Edmonds and Markku 1985).

1.6.1.2- Correlation between processed EEG and cerebral drug effect.

Cerebral drug effect represents the amount of drug on the effect site (brain). Anesthetic dose-response curves are essential to validate and characterize the utility of the brain monitor. It is expected that gradually increasing anesthetic concentrations will be reflected directly on the EEG index (fig. 7).

In 1977, Stullken and colleagues described that, in dogs, the cerebral oxygen consumption dose response curves at MAC of halothane, isoflurane, and enflurane less than 1 are nonlinear. This fact was attributed to a rapidly decrease of cerebral oxygen consumption when the dogs pass from the awake to anesthetized state (Stullken, Milde et al. 1977). Thus, although it is easy to think that the EEG dose-response curve should be exclusively linear for anesthetic agents (Palanca, Mashour et al. 2009), the dose response curve that better described the anesthetic index with the anesthetic concentration is typically sigmoidal. Beyond the loss of responsiveness there is a plateau in the dose response across a wide range of clinically relevant anesthetic concentrations (Walling and Hicks 2006; Palanca, Mashour et al. 2009). This has some implications in the use of brain monitors to titrate the anesthetic drugs because if the loss of responsiveness occurs near the plateau, it will be difficult for the monitor to correlate with anesthetic depth (fig. 7). In veterinary patients, the main reference used to evaluate the brain monitors is the correlation of the EEG derived indexes with MAC, and multiples of MAC values (Greene, Benson et al. 2002; Carrasco-Jimenez, Martin Cancho et al. 2004; Campagnol, Teixeira Neto et al. 2007).

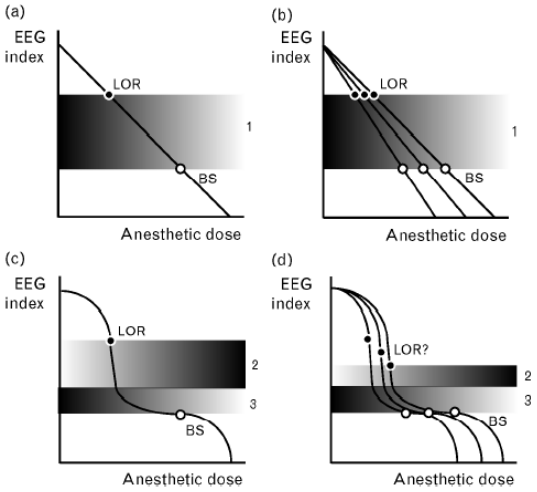


Figure 7-Represents the different dose-response curves for anesthetic concentration and EEG index (Palanca, Mashour et al. 2009).

a) Linear dose response.
b) Represents the individual variability in linear dose response curves
c) Dose-response curves typically sigmoidal
d) Represents the individual variability in sigmoidal dose response curves.
The linear dose response is ideal to obtain the correlation between anesthetics concentrations and clinical endpoints. The dose response curve that better described the anesthetic index with the anesthetic concentration of the drugs is typically sigmoidal
The most informative range of the EEG monitor lies in region 2, where small changes in anesthetic dose lead to large changes in EEG index. Region 3 represents a range in which the EEG monitor provides little information across a wide range in anesthetic concentration. The targeting of this range can lead to excessive anesthetic administration.
Individual variability in the EEG indices corresponding to LOR can complicate anesthetic dosing by reducing the informative range of EEG in region 2.
LOR, loss of responsiveness; pEEG, processed EEG; BS, burst suppression.
Adapted from Palanca, Mashour et all 2009.

1.6.1.3-Correlation between electroencephalographic indexes and clinical endpoints.

It is imperative that depth of anesthesia monitors have the ability to distinguish different states of anesthesia, sedation, and unconsciousness. In humans, sedation scales (Modified Observer´s Assessment of Alertness and Sedation, MOAAS) have been as markers of cerebral activity and drug concentration (Jensen, Litvan et al. 2006). In veterinary anesthesia the methods commonly used to analyze the performance of the monitors of depth of anesthesia are the ocular reflexes, eyeball position, mandibular tonus, movement, and response to a noxious stimulus (Greene, Benson et al. 2002; Carrasco-Jimenez, Martin Cancho et al. 2004; Campagnol, Teixeira Neto et al. 2007).

The burst suppression is another reasonable endpoint. This type of cerebral electrical activity is not observed in the natural sleep, and it is observed in depth levels of anesthesia, presumably incompatible with memory formation.

In conclusion, monitors to assess depth of anesthesia should show graded responses to altering depth of anesthesia at all levels, from light sedation to deep surgical anesthesia. The variety of combinations of anesthetics should not affect the accuracy of the monitor, and the monitor should respond to surgical stimulus when anesthesia is inadequate. The variables in the monitor display should be easily quantified, readily interpreted, and be unaffected by routine operating theatre interference, such as electrocautery.

1.7-What monitors had been studied for monitoring depth of anesthesia?

The monitor to assess the depth of anesthesia can be divided in two categories. The quantitative electroencephalographic monitors, who analyze and quantify the variables obtained from spontaneous electrical activity from the brain and a second type of monitor, which analyze the EEG after stimulation, the Auditory Evoked Potentials (AEP) monitors. Recently there are under investigation monitors with multiple modalities, these monitors combine the analysis of spontaneous EEG with metrics based on evoked potentials (John and Prichep 2005; Horn, Pilge et al. 2009)

1.7.1- Quantitative electroencephalographic monitors

1.7.1.1- Bispectral index.

The bispectral index® (BIS®) monitor (Aspect Medical, Newton, MA, USA) was the first technology approved by US Food and drugs administration (October 1996) for depth of anesthesia monitoring.

BIS is a dimensionless number scale ranging from 0 to 100. These values correspond to complete electrical silence to awake respectively (Johansen and Sebel 2000). The BIS is a complex parameter that integrates a various EEG descriptors into a single variable (John and Prichep 2005). The first step on the BIS construction starts with the exclusion of the artifacts from high- and low-frequency. The artifacts detected are removed from the epoch and repaired by interpolation. The epoch artifact free is used to calculate the amount of burst suppression, another concept was introduced in the analysis of burst suppression called QUAZI and was designed to detect the burst suppression in the wandering baseline voltage (Rampil 1998; Johansen and Sebel 2000).

At the same time the fast fourier transformation, power spectrum and the bispectrum of the epoch are calculated. The values obtained are then computed to Beta ratio and Synchfastslow.

The Beta ratio is the subparameter based on the power of two frequency bands and is calculated by: $\log(P_{30\text{-}47}Hz)/ (P_{11\text{-}20}Hz)$. SynchFastSlow is a subparameter derived from the bispectrum analysis (Fig 8).

An important concept in the development of the BIS is the fact that these subparameters were adjusted based on prospective collected database of anesthetized volunteers to evaluate the clinical sedative endpoints and hypnotic drugs (Glass, Bloom et al. 1997).

The BIS is the most studied depth of anesthesia monitor in veterinary anesthesia, there are studies in different kind of species, cats, dogs, pigs, horses, goats and rabbits (Antognini, Wang et al. 2000; Greene, Benson et al. 2002; Haga and Dolvik 2002; March and Muir 2003; Martin-Cancho, Carrasco-Jimenez et al. 2004; Martin-Cancho, Lima et al. 2006). All of this studies were performed with inhalant anesthetics and the effects of MAC and MAC multiples on BIS were evaluated. In all of these species the reference values for surgical anesthesia were not founded.

Green and colleges studied the relationship between BIS multiples of sevoflurane MAC (0.8, 1, 1.5 and 2) with concomitant administration of neuromuscular blocking agents in the intention of diminish the effect of EMG on values of BIS, since is known that EMG is the principal source of the artifacts. It was observed a decrease in BIS values when the multiples MAC values for sevoflurane increase. Nevertheless this reduction was not significant different between all MAC multiples detected by BIS (Greene, Benson et al. 2002). Another study with different concentration of isoflurane, in absence of noxious stimulus showed poor correlation with BIS, which implies that BIS could not reflect the changes on depth of anesthesia (Campagnol, Teixeira Neto et al. 2007).

In BIS monitor the presence of burst suppression could lead to a false increase of BIS values. This is due to the fact that this pattern is recognized by the monitor as high frequency related with a more awake brain (March and Muir 2005).

The results obtained in different studies for sevoflurane and isoflurane at deep levels of anesthesia reveal that the burst suppression was observed more commonly in dogs anesthetized with sevoflurane at 2MAC multiple (Greene, Benson et al. 2002; Greene, Tranquilli et al. 2003). This could reflex a slight difference between activation and suppression of the EEG for inhalant anesthetic agents. BIS correlated with end tidal sevoflurane better than with isoflurane. Clinical use of BIS monitoring in dogs may require evaluation of specie anesthetic drug combinations (Green, Benson et al. 2002).

The effects of propofol on BIS in dogs are not well studied, there are just one study using three infusion rates of propofol and. forty and fifty minutes after the beginning of the infusions there a greater decrease in BIS values for infusion $0{,}8 mg kg^{-1} h^{-1}$ when compared with infusions at 0,2 and $0{,}4 mg kg^{-1} h^{-1}$ (Lopes, Nunes et al. 2008). One of the limitations of this study is the administration of the bolus before the start of the infusions which can interfere in the consequent analysis of the BIS at different infusion rates during the first thirty minutes.

There are a lack of studies in dogs that evaluate the relationship between clinical end-points and EEG derivate monitors Carrasco-Jimenez and colleagues evaluate the swallowing and palpebral reflexes during recovery of the surgery, the BIS increased significantly at the resumption of these reflexes (Carrasco-Jimenez, Martin Cancho et al. 2004).

The measure of the anesthetic effect on the brain should be independent of the anesthetic agent used and accuracy of the method should detect easily the inadequate anesthetic plane.

Clinical veterinary anesthesia increases the use of a multi-agent approach with the simultaneous use of various sedatives and analgesics in addition to an inhalation anesthetic. Because multiple drugs are often used, it is not always clear what the effect of a specific drug is on the BIS.

The association of medetomidine with isoflurane was related with decrease in BIS values when compared with use of isoflurane alone. (Greene, Tranquilli et al. 2003). The opioids are currently used in association with general anesthetics and it is known that they reduce the dose of volatile anesthetics and propofol used (Vuyk 1997).

A study performed with standard doses of morphine associated to a constant concentration of isoflurane showed that the administration of morphine did not affect significantly the values of BIS (Henao-Guerrero, McMurphy et al. 2009).

Ueyama and colleagues had determined the effects of perzinfotel, fentanyl and the association fentanyl-perzinfotel on MAC of isoflurane and consequently in BIS values in dogs. It was founded that BIS increased as MAC of isoflurane decreased when dogs were treated with fentanyl, perzinfotel, or both. n (Ueyama, Lerche et al. 2009). This study did not reflect the effect of fentanyl or the perzinfotel on BIS since the values of isoflurane concentration were not constant. Similar conclusions were observed in the study evaluating the effect on MAC isoflurane from the infusion of morphine, lidocaine, ketamine. Their combination reduced isoflurane MAC in all dogs and consequently BIS increased as MAC of isoflurane decreased in all dogs, independent of the drug infusion administered (Muir , Wiese et al. 2003).

Nociceptive stimulus could induce EEG changes that may be indicative of arousal from anesthesia (Bimar and Bellville 1977). In a variety of species, increase in the EEG median frequency following noxious stimulation may be suggestive of cortical perception of pain during anesthesia (corticocerebral arousal) (Murrell, Johnson et al. 2003; Otto and Mally 2003).

Intra-operative BIS monitoring in veterinary medicine could be used to minimize conscious arousal. BIS values in the clinical practice may not predict the depth anesthesia for every patient as it must be considered the interindividual variability in drug response.

The change in BIS after a noxious stimulus provides a direct measure of an anesthetic or pre-anesthetic drug's efficacy in suppressing nociceptive transmission and arousal from the hypnotic state (March and Muir 2005). Recently the BIS was used to monitor the depth of anesthesia in puppies using multiples of sevoflurane MAC, it was observed the capacity of BIS, at lower level of anesthesia, to respond to a noxious stimulus, however the BIS cannot distinguish accurately between different MAC concentrations (Morgaz, Granados et al. 2009). The BIS was also studied in cats were it was observed that BIS during isoflurane anesthesia could detect corticocerebral responsiveness to somatic or visceral noxious stimulus (March and Muir 2003). Leigh and colleagues studied the effects on BIS by different MAC multiples of sevoflurane and isoflurane, it was observed in both studies that the BIS values recorded were consistently low, which means that the endpoints used for humans could not be applied to cats (Lamont, Greene et al. 2004; Lamont, Greene et al. 2005). The BIS was also studied in pigs, rabbits and horses with poor results (Haga and Dolvik 2002; Martin-Cancho, Carrasco-Jimenez et al. 2004; Martin-Cancho, Lima et al. 2006). In goats the studies performed showed that BIS can be used to monitor the depth of anesthesia, nevertheless more studies should be performed (Antognini, Wang et al. 2000).

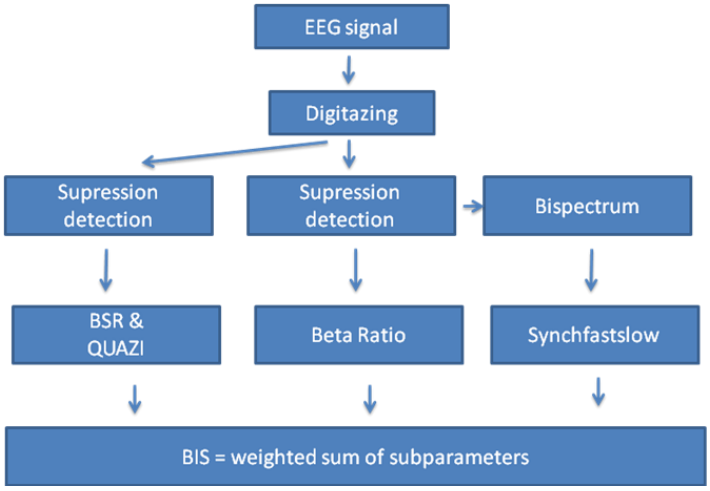


Figure 8-Represents the flow chart for the calculation of BIS,adapted from (Rampil 1998).

1.7.1.2- Spectral entropy

Neuronal systems have a nonlinear behavior like the EEG waveforms and are considered not a sum of sine waves but a chaotic pattern (Pritchard and Duke 1992)

The truer understanding of the brain changes that result in a chaotic dynamics should be analyzed by nonlinear models (Walling and Hicks 2006). Entropy is a concept that analyzes the "amount of order" in the EEG (Vanluchene, Vereecke et al. 2004).

Shannon and Weaver were the first to describe entropy in 1949 (Shannon 1948) and in 1984 John and Shore applied it to a power spectrum. Entropy describes the irregularity, complexity and unpredictable characteristics of the signal (Viertio-Oja, Maja et al. 2004).

Various entropy algorithms have been developed in the past two decades to characterize the signal of EEG. It could be computed a time domain called the approximate entropy or Shannon entropy and in frequency domain called spectral entropy.

The first step of the algorithms used in Datex-Ohmeda Entropy TM Module (Datex-Ohmeda Helsinki, Finland) is the caption of the spectrum applying the FFT, thereafter a called Shannon entropy is applied to get the spectral entropy. The principal advantage of this approach is the independence of absolute scales such as amplitude and frequency of the signal (Vakkuri, Yli-Hankala et al. 2004; Viertio-Oja, Maja et al. 2004).

For optimal response to minimized the time delay of the monitor a time window for each particular frequency is individually chosen, for example, at a frequency of 0.5Hz, a time window of 30 s would be required to obtain 15 full cycles of the 0.5Hz variation. For a frequency of 50Hz, the same number of full cycles could be obtained with only 0.3 s of data. This leads to a concept called time-frequency balanced spectral entropy (Vakkuri, Yli-Hankala et al. 2004; Viertio-Oja, Maja et al. 2004).

In the entropy module, the shortest time window is used for the frequency range between 32Hz and 47Hz. The longest time window is used only for frequencies below 2Hz. For frequencies between 2Hz and 32Hz, window lengths between these two extremes are used. The very short window of less than 2s for the range of frequencies from 32Hz to 47Hz ensures that the entropy value rises readily at arousal. In particular, it provides for immediate indication of EMG activation (Viertio-Oja, Maja et al. 2004).

The module measured the biosignal collected from the skin of the forehead and the side of the head, it contains both EEG and EMG of the frontal muscle. The EMG signal has a wide noise-like spectrum with frequencies higher than 30 Hz, the EEG signal

components put on display frequencies lower than 30 Hz during anesthesia (Viertio-Oja, Maja et al. 2004). It is important to refer that in humans the facial muscles are less sensitive to the effects of neuromuscular blocking agents than the other muscles. Neuromuscular blocking inhibit spontaneous EMG activity, they do not totally abolish the ability of facial muscles to react to noxious stimuli when used in clinically practical amounts (Paloheimo 1990).

The M/Entropy S/5 monitor shows two parameters that are calculated from two different frequency ranges, namely State entropy (SE) and Response entropy (RE). SE is computed with a frequencie between 0,8Hz and 32 Hz which represents the EEG and a small amount of EMG, RE is computed with frequencies between 0,8Hz and 47Hz,

These ranges of frequencies include the EMG-dominant and EEG-dominant part of the spectrum.

Because electrical activity exceeding 32Hz originates mainly in the EMG and only for a small part in the EEG, the EMG activity is calculated the by difference of the RE and SE values. The short calculation delay between 32 and 47Hz is useful in practice because it immediately reveals the EMG activation of the frontal muscle related to inadequate anesthesia, the imminence of regaining consciousness and strong nociception. If there is no measurable activity in the patient's frontal muscle (i.e. 'adequate' anesthesia), there is no activity over 32Hz in the biosignal, and the RE and SE values are identical (Vakkuri, Yli-Hankala et al. 2004; Viertio-Oja, Maja et al. 2004). The monitor shows the values of BSR, nevertheless did not affect the calculation of SE and Re.

The a-dimensional values of SE vary between 0 and 91. RE values oscillate from 0 to 100 .The RE–SE difference is used to measure EMG and some authors consider that a RE–SE difference >10 indicates the presence of nociception (Takamatsu, Ozaki et al. 2006). With an awake subject, EEG is highly irregular, and the amount of entropy is high. With increasing depth of anesthesia, EEG turns towards more regular patterns, decreasing entropy (Fig 9 and 10).

There is just one study in dogs that evaluate the spectral entropy to measure the anesthetic depth and antinociception (Morgaz, Granados et al. 2010). The main reason for the use of this monitor is the fact that the analysis of the EEG being independent of the frequency or amplitude of the signal. For the authors this is a potential advantage for its use in veterinary medicine because it may allow for EEG analysis and anesthetic depth monitoring in all species Morgaz and colleges concluded that the M/Entropy S/5

can distinguish between awake and unconscious state because the monitor could predict the movement of the animals at 0,75 sevoflurane MAC. It was found a paradoxical pattern at 1,75 MAC which leads to the elevation of spectral index when compared to 1,5 MAC, that makes the monitor unable to detect different planes of the anesthetic depth in sevoflurane anesthetized Beagles (Morgaz, Granados et al. 2010).

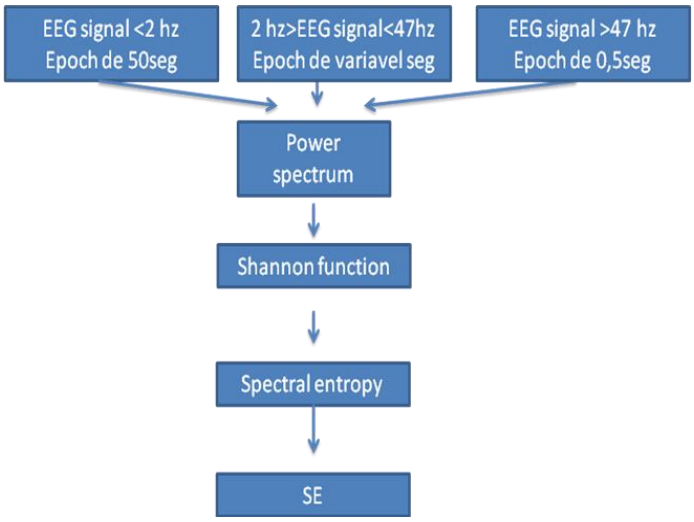


Figure 9-Represents the flow chart for the calculation of Spectral entropy**.**

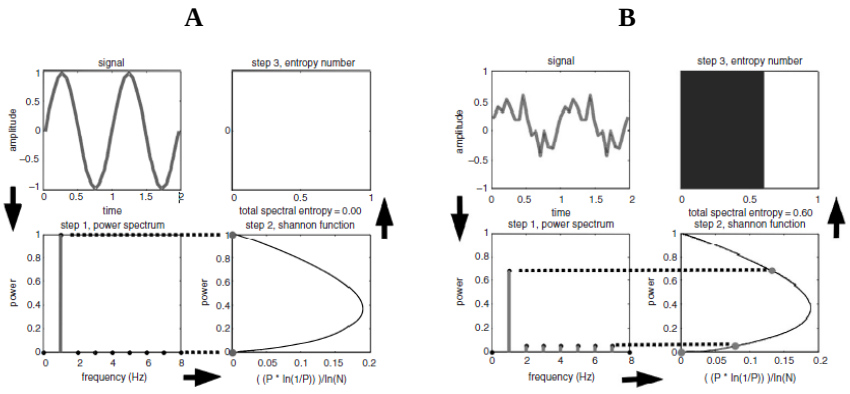


Figure 10-Description of the Entropy algorithm as applied in the Datex-Ohmeda S/5 Entropy Module

A) The perfect sine wave includes only one nonzero spectral component, which is normalized to 1 in the normalization step (2). In the Shannon mapping, both values 1 and 0 contribute to a value of 0, thus corresponding to entropy=0.

B) Some amount of white noise is superimposed on top of the sine wave. After normalization, the spectrum includes one high component corresponding to the frequency of the sine wave, and 6 smaller nonzero components. In the Shannon mapping, both types of components contribute nonzero values to the entropy of the signal, corresponding to a total entropy=0.12* 6 * 0.08=0.60.

Adapted from (Viertio-Oja, Maja et al. 2004).

1.7.1.3-Narcotrend index

The Narcotrend an EEG monitor designed to measure the depth of anesthesia and developed at the Medical School, University of Hannover, Germany.

The principle of this monitor is the establishment of a database of EEG patterns which are classified based on the principles first described by Loomis and colleges in 1937, where during the sleep there are systematic changes classified in five stages from A to E (Loomis, Harvey et al. 1937). More recently Shultz and colleges create a substages A, $B_{0–2}$, $C_{0–2}$, $D_{0–2}$, $E_{0,1}$ and $F_{0,1}$ (Schultz, Schultz et al. 2000) in an attempt to characterized and classified electroencephalographic patterns observed during anesthesia. There to simplify the interpretation a Narcotrend index was developed as an indication for the depth of anesthetic level; from 100 to 90=awake, from 89 to 80=sedation, from 79 to 65=lightly anesthetized, from 64 to 37=general anesthesia, from 36 to 13=deeply hypnotized and from 12 to 1=increased burst suppression until EEG silence (Kreuer and Wilhelm 2006).

In the development of Narcotrend monitor a database, was incorporated with the typical examples of EEG from the stages and substages obtained during anesthesia with thiopental–enflurane or propofol. More than 1000 artifact-free epochs with a length of 20s were visually classified to form the basis for the development of automatic classification algorithms (Kreuer and Wilhelm 2006).

For the classification a Fourier transformation is applied and a numerous quantitative features from the time and the frequency domain were extracted, power spectrum of the four frequency power bands (δ; 0.5–3.5 Hz, θ; 3.5–7.5 Hz, α; 7.5–12.5 Hz and β; 12.5–45 Hz), MF (50% quantile), the SEF (95% quantile) total absolute power (amplitude), entropy measures and autoregressive parameters. (Kreuer, Biedler et al. 2003). The extracted parameters were statistically analyzed to identify a subset of electroencephalographic parameters to discriminate more precisily the different visually determined electroencephalographic sub stages. This “memory” created in the monitor could be called the background of the monitor. When a new patient is monitored extensive algorithms for artifact detection are applied, thereafter, the electroencephalographic parameters that are relevant for suppression detection and that contribute to the discriminant functions are calculated, and the epoch is classified into one of the sub stages. A sufficient similarity of the epoch to one of the typical

electroencephalographic stages is required for a classification to be made (Fig 11) (Kreuer, Biedler et al. 2003; Kreuer and Wilhelm 2006) .

A study on beagles, the narcotend was used to detect burst suppression induced with high doses of hypnotics (pentobarbital or etomidate) and/or seizures development caused by compounds, known by induced seizures in animal (der Linde, Van Deuren et al. 2010). Van der Linde and colleges concluded that it is possible to reliably calculate the burst suppression ratio, spiking and seizure activity induced by drugs based on a one lead EEG signal using the Narcotrend module. It is important to refer that during anesthesia with fentanyl/etomidate protocol the Narcotrend index calculated in six dogs was between 64 and 38 units (general anesthesia) and stable over 3h. All of this dogs were under the effect of neuromuscular blocking agents (der Linde, Van Deuren et al. 2010).

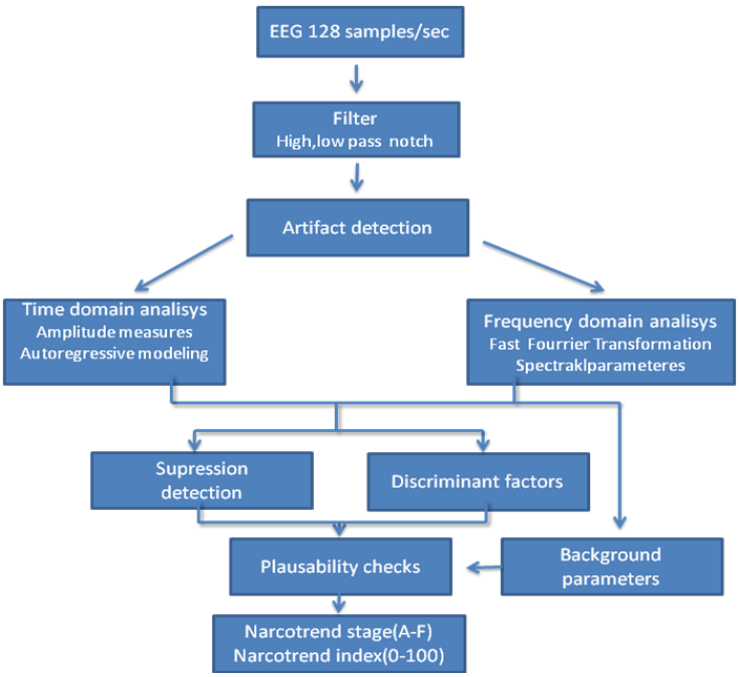


Figure 11-Represents the flow chart for the calculation of Narcotrend stage or index, adapted from Narcotrend monitor (Kreuer and Wilhelm 2006).

1.7.1.4-Cerebral State Index

The CSI is based on the analysis of the frequency content of the EEG signal. These are used to define two energy ratios called alpha (α) and beta (β). Both α and β show a shift in the energy content from the higher to the lower frequencies with deeper anesthesia. The relationship between these shifts is also analyzed by CSM as a separate parameter (β-α). The monitor, who works on-line, also evaluates the amount of instantaneous burst suppression (BS) in each thirty-second period that quantifies the amount of "silent" or "flat line "EEG. These EEG periods characterize the deepest levels of hypnosis. The four parameters are then used as inputs to a fuzzy logic classifier system that calculates the CSI. Fuzzy reasoning permits the implementation of very complex processes and can also be successfully applied to high non-linear processes, where it can simplify modeling considerably (Jensen, Litvan et al. 2006). The four parameters mentioned above are used as inputs for this fuzzy Adaptative Neuro Fuzzy Inference System (ANFIS), which governs the rules that govern relations between the input parameters using a least mean squares approach.

This approach (ANFIS) establishes the relationship between variables based on a no restricted mathematical structure; the data is analyzed with no prior assumption of the mathematical relationship between variables. The advantage of using ANFIS is that it is a data-driven approach that does not assume an underlying mathematical model governing the relationship between the anesthetic drugs and the response –effect (Gambus, Jensen et al. 2010). There are just one study with CSM monitor made by Bollen and Saxtorph, during sedative procedures using medetomidine, it observed an increase in the CSI values with concurrent decrease in BSR as a response to a noxious stimulation (Bollen and Saxtorph 2006).

1.7.2-Evoked Potential monitors.

These type of monitors have different approaches to assess electrical brain activity. The analysis is based on evoked responses, series of oscillations in the brain electrical activity, derived from the EEG response to a different stimulus: auditory, somatosensory, nociceptive and visual (Thornton and Sharpe 1998).

1.7.2.1-Auditory evoked potential monitors.

The auditory evoked potential (AEP) consists of characteristic peaks and troughs, that represents the auditory pathway from cochlea to cerebral cortex (Fig.1.12) (Thornton and Sharpe 1998). This series of waves are caracterized by their latency, amplitude and polarity. In AEP studies the peaks are labelled according to polarity (P=positive and N=negative) followed by a number or letter. The latency of each peak in the evoked potential (EP) waveshape reflects the time required for the neuronal encoded information about the stimulus to be transmitted to successive structures in the sensory pathway (John and Prichep 2005).

The waves produced by auditory stimulus have three different origins. Brainstem waves are represented by Roman numeral (I-V) but the analysis is not useful during humans anesthesia because with IV agents the waves are not afected (Thornton, Konieczko et al. 1989), nevertheless it is extensively studied in rats (Shaw 1988), but unfortunatly it was not found any reports in dogs during anesthesia.

The early cortical or midle latency waves were investigated during anesthesia.It was observed alterations in the shape of the waves when differents concentrations of the great majoraty of hipnotic agents were used (Thornton and Sharpe 1998). For this reason midlatency components of the AEP (MLAEP), in the range of 20–100 ms after the auditory stimulus have received special attention as a possible monitor for depth of anesthesia. With general anesthesia, MLAEP peak latencies increase and peak amplitudes decrease (Horn, Pilge et al. 2009). This waves are generated in the geniculate and primary auditory cortex and called No,Po,Na,Pa and Nb (fig. 1.12).

Late cortical waves are not evaluated because they are completely abolish with sedative agents (Thornton and Sharpe 1998).

AEP is the response of the brain to click stimuli through the hearing nerve AEP is a very weak electrical signal wrapped in the EEG background activity. The repetition of sound inputs and the use of computer averaging and filtering techniques allow the AEP to be distinguished from EEG background noise. The signal is time-locked to the stimulus and most of the noise occurs randomly, allowing the noise to be averaged out with averaging of repeated response (Thornton and Sharpe 1998). One of the most important features of depth of anesthesia monitors is the time needed to extract the AEP during surgery. In attempt to use MAEP during anesthesia different mathematical approaches were used to reduce the complexity of AEP and permit the creation of AEP indexes (Doi, Gajraj et al. 1997; Mantzaridis and Kenny 1997; Thornton and Sharpe 1998). The AEP indexes have been proposed as single numerical variable based on the morphology of the curves and is calculated from the difference of the amplitude of successive segments of the curve. This numerical variable is determined by a moving time average of 3 seconds intervals (Doi, Gajraj et al. 1997).

Código de campo alterado

Recent advances in computation had permit the development of an objective method for analysis of the MLAEP resulted in autoregressive model with an exogenous input (ARX model). ARX model is used to analyze these amplitudes and latencies to derive a dimensionless number between 0 and 100 known as the A-line ARX-Index (AAI Index – monitor, Fig. 13) (Jensen, Lindholm et al. 1996; Vereecke, Vasquez et al. 2005).

The MLAEP has been studied in dogs with conflicting results. Murrell and colleagues evaluate MLAEP in awake dogs and after two concentration of sevoflurane, it was observed that sevoflurane cause alterations in the waveforms with an increase of the latency in the peaks P0, Na and Pa, however there was no differences between low and high concentration of sevoflurane (Murrell, de Groot et al. 2005).

Interesting studies performed by Van Oostrom and collegues where they use the MLAEP and somatosensory evoked potentials to distinguish the sedatives effects from analgesic effects of dexmededtomidine at different constant rate infusions, concluding that sedative effects was obtained at lower doses than analgesia effect (van Oostrom, Doornenbal et al. 2011).

The MLAEP index was applied in a closed loop anesthetic system in dogs to control the delivery of propofol. During this study it showed a 89.2% accuracy rate for classifying anesthesia depth (Huang, Lu et al. 1999).

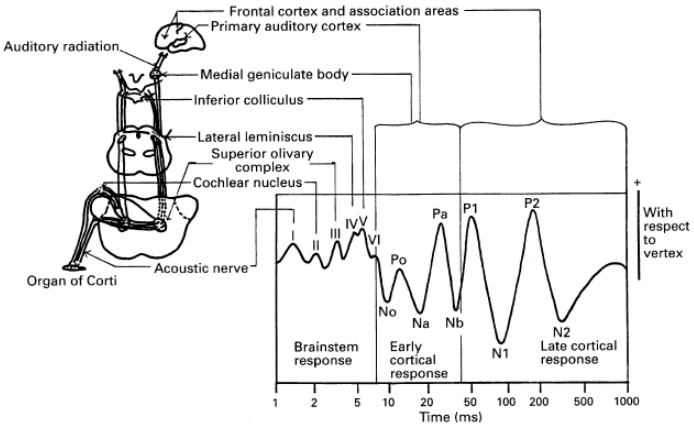


Figure 12-The schematic representation of an auditory evoked response.

The diagram describes the nomenclature of response and shows its anatomical relationship with auditory neuroaxi (Adapted (Thornton and Sharpe 1998)

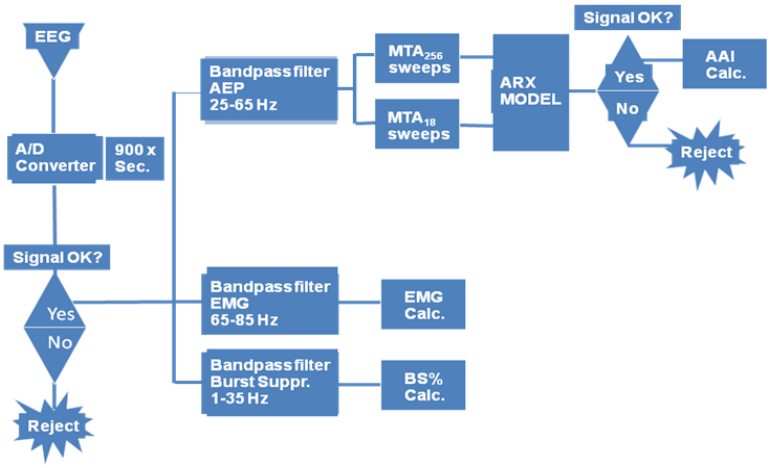


Figure 13-Represents the flow chart for the calculation AAI index.

Adapted from Clinical Department Alaris Medical Systems International 2002

1.7.3- Comparison between monitors

The most studied monitor in veterinary anesthesia is the BIS, followed with by the less researched MLAEP. The cerebral state index was the less studied, however the algorithm of analysis used by this monitor can give us promising results. Nevertheless, until now all studied monitors have significant limitations when intermediate planes of anesthesia are evaluated.

The monitor of depth of anesthesia should show graded responses to changing depth of anesthesia at all levels from light sedation to deep surgical anesthesia. However, so far this was not observed in dogs. The indicator should be easily quantified, readily interpreted, and unaffected by routine operating procedures. The monitor specificity should be independent of anesthetic technique and should respond to surgical stimulus when anesthesia is inadequate.

It is difficult to compare the performance of depth of anesthesia monitors and their correlations with anesthetic drugs and anesthetic planes. This is due to variability between patient and study conditions with are not standard.

The progress of technology and new short-acting anesthetic agents has allowed the development of total intravenous anesthesia (TIVA). This technique is generally used in human medicine as a result of improved for the intravenous pharmacokinetic studies.

There are many advantages of TIVA over volatile anesthetic agents of, in particular, hemodinamica stability, and reduction of pollution in the operating room, soft recovery and great variety of protocols for the administration of drugs that can be used with TIVA. There are several manual regimes that have been described for the dogs. However, the intermittent administration of the bolus of a drug can lead à side effects that alternate periods of deep anesthesia with surface anesthesia with possibility of wake up the patient. The administration by continuous infusions allow, achieving more stable concentrations in the plasma reducing the total dose of the drug.

1.8-Target controlled infusions

Progress in computing technology gave rise to the development of target controlled infusions (TCI) devices. In anesthesia, TCI uses real time pharmacokinetic models to calculate drugs, bolus doses and infusion rates allowing drugs to be delivery in order to achieve an estimated plasma concentration. After the propofol intravenous administration to a central compartment (the blood), it is rapidly distributed to a second compartment (composed mainly by the muscular tissues) and slowly distributed to a third compartment that corresponds mainly to the body fat tissue storage. Thus, TCI for propofol uses three compartmental pharmacokinetic/pharmacodynamic mathematic models that takes into account the drug distribution, and estimates the propofol plasma and cerebral concentrations according to a given propofol infusion dose (Marsh, White et al. 1991; Schnider, Minto et al. 1999).

The development of a three-compartment pharmacokinetic model for propofol for dogs (Beths, Glen et al. 2001) allowed the use of TCI in veterinary anesthesia practice.

This model may be incorporated in a computer software, allowing the selection of a desired target drug concentration set by anesthesiologists. In veterinary anesthesia, TCI is just applied to plasma concentration, predicted by the pharmacokinetic model. The software calculates the infusion rate at fixed intervals that is required to achieve and maintain the pre-selected target concentration.

The few previous veterinary studies about TCI at induction and maintenance of anesthesia (Beths, Glen et al. 2001; Musk, Pang et al. 2005; Auckburally, Pawson et al. 2008; Beier, Aguiar et al. 2009) using Beths and colleagues' pharmacokinetic model (Beths, Glen et al. 2001) reported that the TCI is an appropriate technique for induction of anesthesia in healthy dogs, and can better titrate the delivery rate to a desire clinical effect, while minimizing side effects (Musk, Pang et al. 2005). The TCI allows changing the depth of anesthesia more quickly and effectively (Beths, Glen et al. 2001), and could be a useful tool to anesthetize dogs.

1.8.1-Three compartment pharmacokinetic model .

In many drugs that the pharmacokinetics is better explained by the use of multicompartment models (Sheiner, Stanski et al. 1979). The three-compartment model is the most suitable for describing the pharmacokinetics of propofol (Fig.14)(Tackley, Lewis et al. 1989; Schnider, Minto et al. 1998)

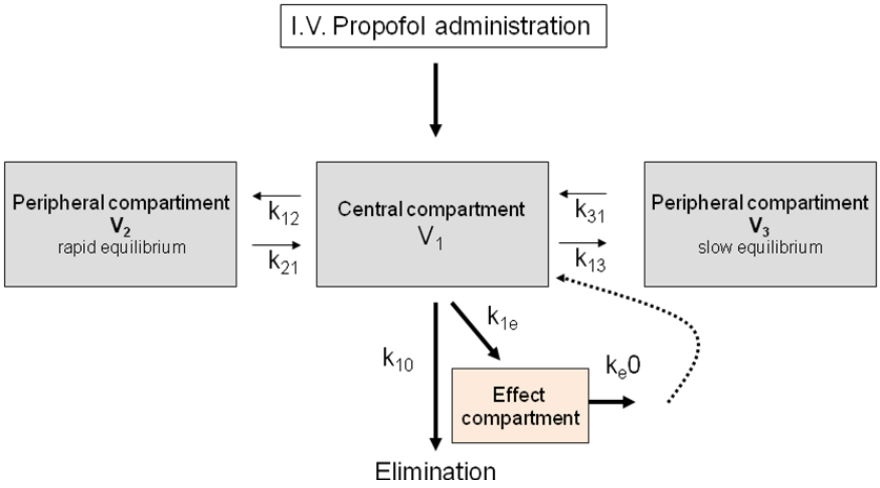


Figure 14-Representation of three-compartment model of propofol.

The drug is administered into a central compartment, from which it is eliminated. From the central compartment, drug is distributed into a rapidly equilibrating second peripheral compartment (generally compose by splanchnic and muscle tissue store) and more slowly into a third compartment (fat store). Compartmental models assume

that the drug added to the central compartment is instantaneously and completely mixed within the arterial circulation. The time course that describes the exchange drugs to achieve the equilibrium between central compartment and two peripheral compartments are obtained by intercompartimental rate constants ($K_{12,}K_{21,}$ ...). This pharmacokinetics model must encompass the elimination of the drug described by K_{10} rate constant and/or biotransformation of the drug.

It would be useful to increase the use of kinetic-driven drug delivery and/or drug displays systems, identifying the propofol kinetic parameter set that best characterizes propofol plasmatic concentration (Cp) across a variety of dosing conditions. In addition, it would also be useful to develop a better understanding of the predictive accuracy of compartmental models compared with physiologically based models across different dosing conditions.

The first model described for propofol and applied to veterinary anesthesia was the Beth`s pharmacokinetic model for propofol in dogs (Table 3) (Fig 14).

Beth`s Pharmacokinetic model for the TCI infusion of propofol in dogs incorporated in the Rugloop II software	
V_1(ml/Kg)	780
Cl_{TB}	(54.6)*
K_{10}(minute)	0,07
K_{12}(minute)	0.0365
K_{21}(minute)	0,0312
K_{13}(minute)	0,0049
K_{31}(minute)	0,0011

.

Table 3-Beth`s Pharmacokinetic model for propofol.

* Clearance is calculated from V_1 and K_{10} , but is not required for the TCI. V_1-Volume of central compartment, CL_{TB} Total body clearance, K_{10} Elimination constant rate , K_{12} K_{21} K_{13} K_{31}, Rate constants for transfers between compartments V_1 ,$V_{2,}$ V_3.

Aims and objectives of the thesis

The president of the American Animal Hospital Association declared that we are witnessing an evolution in the veterinary profession (Brady and Palmeri 2007). The core of veterinary services grew at a rate of 10% during the last decade, which is mainly due to medical advances (Brady and Palmeri 2007).

The anthropomorphization of pets led the pet owners to want the same best-in-class care for their pets, as for themselves. Forexample, in USA in 2003, owners spent $1,32 billion in the surgery for Cranial Cruciate Ligament Rupture. Anesthesia is a field of medicine that "per se" does not promote the cure of the patient, but allows the development of increasingly complex surgical and medical procedures.

A large study performed in UK to evaluate the quality of sedation anesthesia, enrolled 117 veterinary practices including 98036 dogs and 79178 cats. Results showed a percentage of death of 0,17% for dogs, and 0,24% for cats within 48 hours after the procedure. In healthy dogs and cats the results were 0,05% and 0,11% respectively. On the other hand in sick patients the values were seriously higher: in dogs the risks were 1,33% and in cats 1,40% (Brodbelt, Blissitt et al. 2008).

In veterinary anesthesia the standard monitoring measures are heart rate, noninvasive blood pressure and arterial oxygen saturation. The monitoring of the depth of anesthesia is based only in clinical signs. Nevertheless, the human EEG based depth of anesthesia monitor, CSM, seems to have some advantages in its applicability in dogs. Additionally, in recent years there was a progressive transition from the use of volatile agents to the use of intravenous hypnotics in human anesthesia. Total Intravenous anesthesia (TIVA) is nowadays well established in human anesthesia, being a common practice and, in many cases, the technique of choice. However, the developments of TIVA in humans were not yet followed in Veterinary anesthesia.

The lack of studies in dogs that evaluate the relationship between clinical end-points and EEG based depth of anesthesia brought the need to study the correlation between depth of anesthesia and an objective visual scale that correlates with EEG depression and different propofol plasmatic concentrations in dogs under general anesthesia using propofol.

For the reasons explained above the overall purposes of this thesis are the improvement of the quality of anesthesia, testing the electroencephalogram responses during propofol

based general anesthesia, and studying clinical responses during propofol based general anesthesia in dogs by:

1) Analyzing the potential advantage of the CSM and its algorithm
2) Developing an objective visual clinical scale for correlating with different degrees of EEG depression under TIVA/TCI with propofol
3) Correlating CSM parameters with clinical depth of anesthesia
4) Correlating CSM with propofol expected effects

Objetivos de la memoria de tesis doctoral (espanhol)

Como declaro el Presidente de la "American Animal Hospital Association", estamos asistiendo a una evolución importante de la Profesión Veterinaria, debido principalmente a los avances médicos, lo que nos ha llevado entre otras causas a un aumento de mas del 10% en los servicios veterinarios (Brady; Palmeri 2007).

La antropomorfizacion, utilizando el término en el sentido de que se atribuyen cualidades humanas a las mascotas, hace que sus dueños quieran que los cuidados de salud que se presten a sus mascotas sean tan buenos como los que reciban ellos mismos.

En el caso de la anestesia, campo de la medicina que permite el desarrollo de procedimientos quirúrgicos y médicos cada vez más complejos, se demanda que cada vez sea más segura y eficiente.

En un estudio multicéntrico realizado en el Reino Unido cuya finalidad era evaluar la calidad de la anestesia y sedación y en el que participaron 117 centros veterinarios entre los resultados obtenidos (98.036 perros y 79.178 gatos) podemos reseñar

> El porcentaje de mortalidad en animales anestesiados fue del 0,1 % en perros y 0,24 % para los gatos, considerando el periodo de tiempo, desde el comienzo de la anestesia hasta 48 horas después de finalizar el procedimiento quirúrgico.
>
> El porcentaje de mortalidad en perros gatos sanos anestesiados fue de 0,05 y 0,11 % respectivamente.
>
> El porcentaje en pacientes enfermos anestesiados los valores fueron mas altos 1,33 % en perros y 1,40 % en gatos (Brodbelt, Blissitt 2008)

En anestesia veterinaria la monitorización estándar es ritmo cardiaco, presión arterial no invasiva, y la saturación arterial de oxígeno, como indicadores básicos.

El control de la profundidad de la anestesia se basa solo en signos clínicos.

En anestesia humana, en los últimos tiempos está incrementándose la utilización de la anestesia total intravenosa (TIVA) sobre la anestesia inhalatoria. Esta tendencia no es tan marcada en veterinaria como en humana, pero cada vez se va imponiendo más.

Por eso en el presente trabajo quisimos comprobar si la medición de la profundidad anestésica se podía cuantificar mediante el uso del EEG con ayuda del "Cerebral State

Monitor" (CSM) para obtener un Índice de Estado cerebral (CSI) que estuviese relacionado con los planos de anestesia.

La falta de estudios de este tema en perros nos llevó a realizar el presente estudio con el fin de mejorar la calidad de la anestesia, testar las respuestas del electroencefalograma durante la anestesia general con propofol, y establecer la relación de las respuestas clínicas durante la anestesia intravenosa con propofol y el índice de estado cerebral.

Chapter 2

2-Material and General Methodology

All data analyzed in this thesis was obtained during routine orchiectomy procedures at Porto Veterinary Hospital. All dogs came from an adoption program between local animal societies and the Porto Veterinary Hospital.

A written consent was obtained from the institutions which have participated in the project.

2.1- Patients and hemodynamic monitoring

Dogs undergoing scheduled routine orquiectomy were studied. All dogs were considered healthy based on its clinical history, clinical examination and packed cell volume, total protein, blood urea and an ASA score of one. No medications were administered prior to the study.

A cannula was inserted in the cephalic vein for drug and fluid administration. A three way stopcock was used to connect the intravenous catheter to the propofol and sodium chloride 0.9% delivery lines. A Braun infusion pump (Braun, Melsungen, Germany) was used for the administration of sodium chloride at a constant infusion rate of 10 ml kg hr^{-1} during the entire study period.

A S/5 Datex monitor (Datex-Ohmeda; Helsinki, Finland) was used for monitoring the hemodynamic parameters. The blood pressure was measured non-invasively in the cranial metatarsal region of the left hindlimb. Cuff width was choosing to be around 40% of circumference of the limb. Three measurements were obtained and used to calculate the medium value made immediately after the preceding one (Datex S/5 "STAT" mode). A lead II electrode ECG was monitored.

Anesthesia was induced with propofol 1% (Fresenius Kabi; Bad Homburg, Germany) using a syringe pump (Asena GH, Alaris Medical Systems) programmed to allow a maximum infusion rate of 600 ml h^{-1}. Rugloop II® software (Demed Engineering, Temse, Belgium) was used to drive the propofol syringe pump and store all hemodynamic and electroencephalographic data from the respective monitors, every 5 seconds, and also to estimate the propofol plasma concentrations using Beths' pharmacokinetic model (Beths, Glen et al. 2001). Data was then extracted from Rugloop II R using the Labgrab software (Demed Engineering, Temse,Belgium), and

exported to an *Excel* file. The anesthesia setup (devices) used for data collection is showed in figure 15

In the Rugloop Software the pharmacokinetic model for propofol is not included for dogs. It was necessary to open the program and create a file were it was inserted the Beth´s pharmacokinetic model. When we start the study with a dog it was required to introduce the variables in this file related to the weight of the animal studied.

The Rugloop software enables the control of the syringe pum with four modes for propofol administration , bolus, constant rate infusion, and TCI(plasma or effect site concentration) (Fig 16).

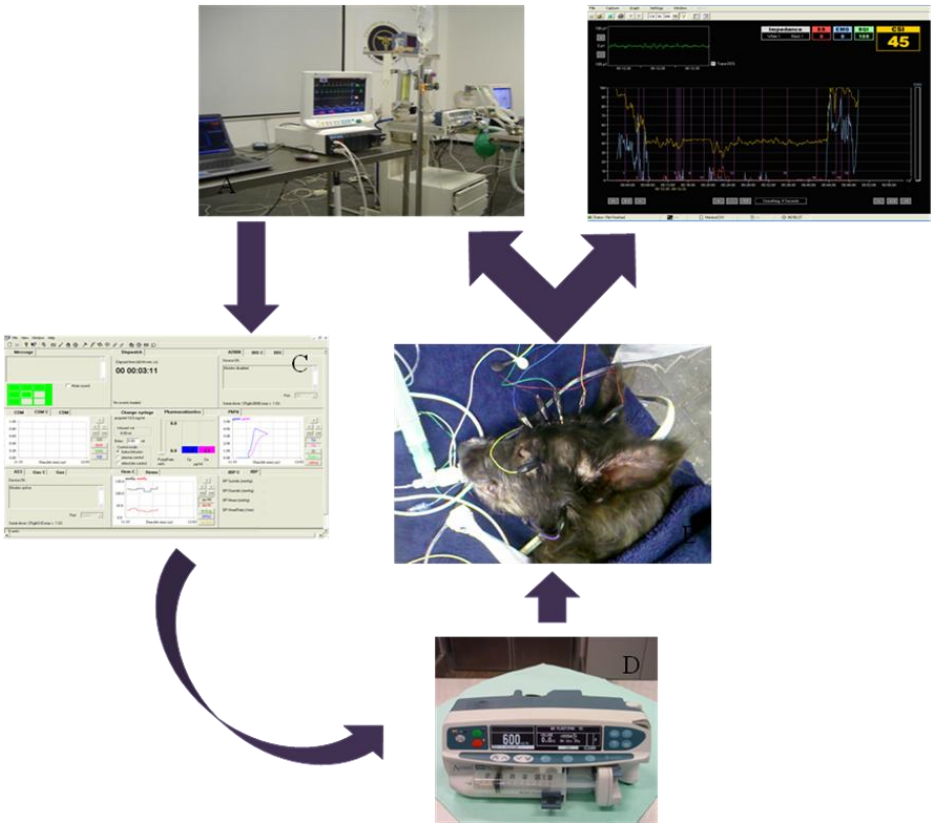


Figure 15-Anesthesia Setup:

The dog anesthesia monitoring (E) is performed by a Datex monitor(A) and CSI monitor(B). Thereafter the RugLoop II(C) software connected to the monitors recorded all data and facilitates the control of the Asena syringe pump(D) by the operator.

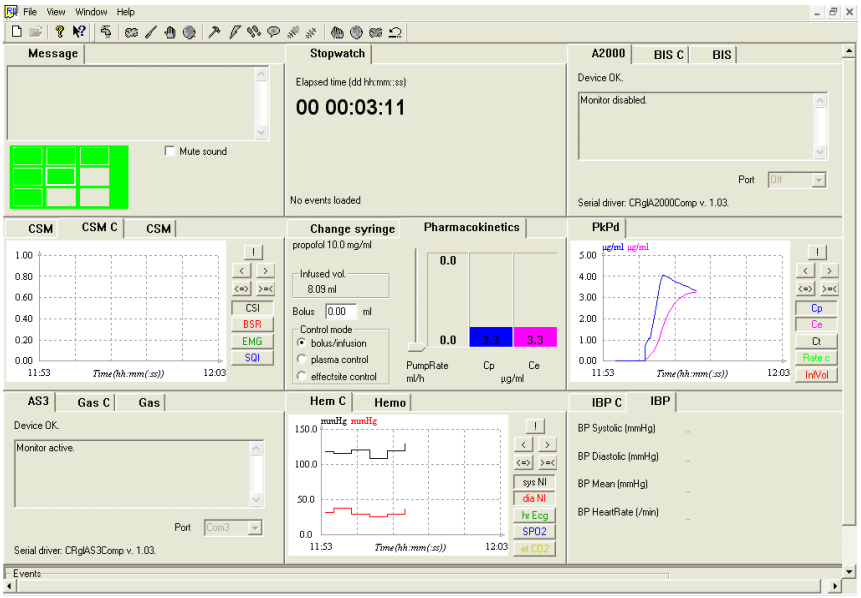


Figure 16-Rugloop II Software.

2.2-Cerebral state index monitoring.

The Cerebral State Index (CSI) is a commercial index for monitoring the depth of anesthesia, provided by the Cerebral State Monitor (CSM, figure 17) using multi parametric EEG processing techniques previously explained (Fig 17).

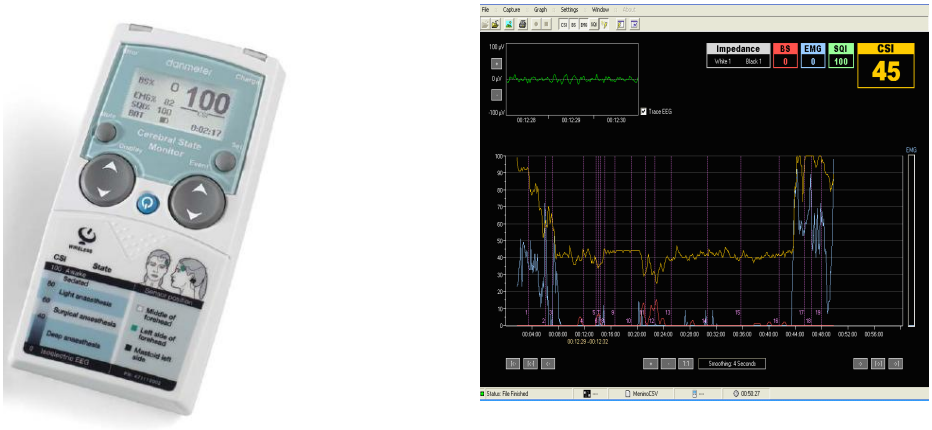


Figure 17-a) CSI monitor (image from CSI user manual). b) Displayed software in the personal computer.

The CSI and frontal EMG activity data were collected every five seconds from a Cerebral State Monitor (CSM) (Danmeter, DK-5000 Odense C, Denmark). A modified ECG cable with clamp electrodes was connected to the CSM cable. The use of ECG clamp electrodes facilitates the clinical use and reduces the cost of EEG monitor (Seitsonen, Yli-Hankala et al. 2000; Akavipat, Dumrongbul et al. 2006) Before the application of clamp electrodes the skin was cleaned with alcohol with 5 minutes before the data collection to allow drying (Fig.18).

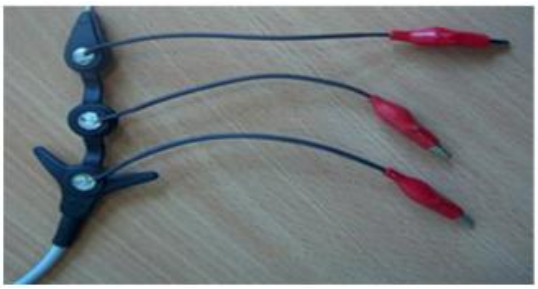


Figure 18-Alligator clamps adapted to collect dog's cerebral data.

The Bipolar EEG waveform used was derived from the signal recorded by two different sensor positions of the electrodes using three clamp electrodes. The first position was placed according to the manufacturer's instructions for human CSI monitoring: in the middle of the forehead/frontal bone (white), to the left side of the forehead near the lateral eye socket, and on the temporal mastoid process on the left side of the head (Black). The EEG waveform was derived from the signal recorded between the frontal (white) and mastoid (black) electrodes (Fig 19).

A B

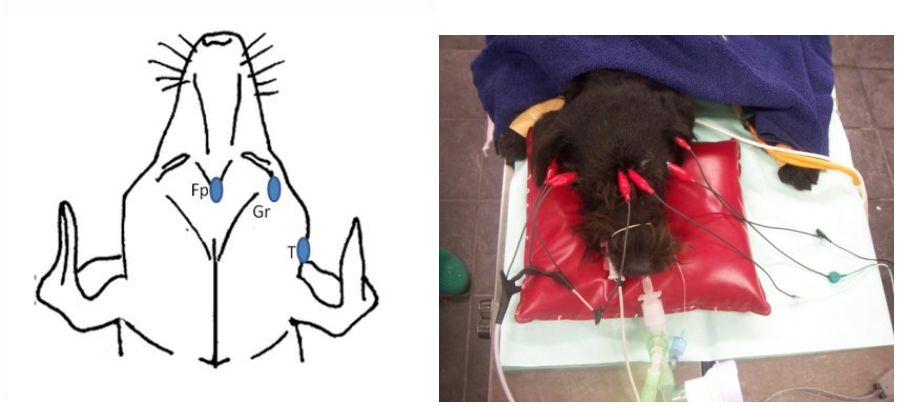


Figure 19-Representation of the electrodes placement.

A) Dorsal view of canine cranium showing the placement of the EEG recording electrodes. Fp (frontopolar electrode), Gr (ground reference electrode) and temporal(T);
B). Dorsal view of the canine cranium showing the placement of electrodes during a study comparing BIS monitor and CSM monitor. CSI electrodes were Placed in left side

The second position, taking into account the craniums anatomy (Fig 20), and the EEG waveform was derived from the signal recorded between the frontopolar electrode (positive) represented by the white color, and the occipital electrode (negative) represented by the black color, according to Pelegrino and colleagues' studies (Pellegrino and Sica 2004). The ground or the reference electrode was placed in the parietal position (Fig.21 A and B).

A B

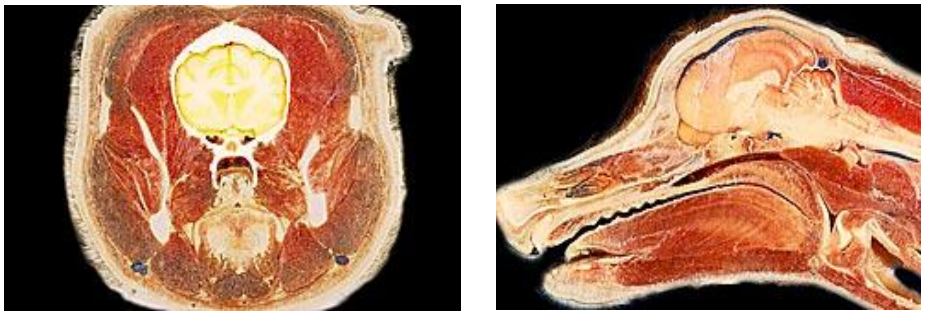


Figure 20-Canine head and neck anatomy.

A) Canine head transverse section . B) Canine head and neck sagital section. Extracted from canine planar anatomy website of University of Minnesota College of Veterinary Medicine.

A B

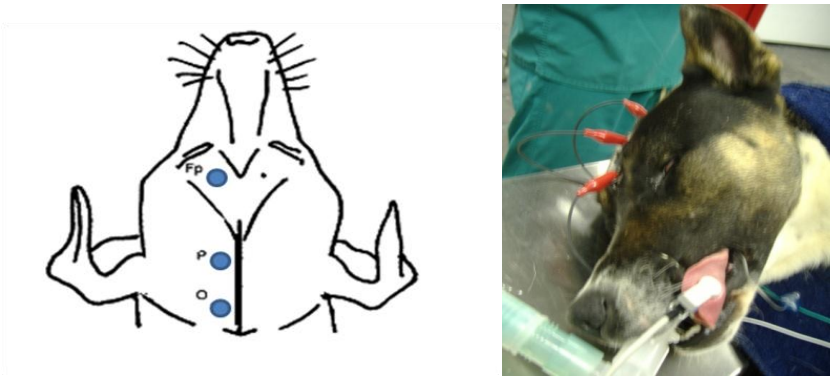


Figure 21-Representation of the electrodes placement.

A). Dorsal view of canine cranium showing the placement of the EEG recording electrodes. Fp (frontopolar electrode), P (parietal electrode) and O (occipital electrode);
B. Lateral view of the left side of the canine cranium showing the placement of electrodes.

The CSM display the EEG signal continuously. The sensitivity of EEG was ±400µV and the CSM monitor collected 2000 samples sec^{-1} (14bits equivalent), with an every second update. To calculate the CSI, frequencies outside the 6-42 Hz range were filtered out. The CSI is based on the combination of four subparameteres of the electroencephalographic signal (β ratio, α ratio, β-α ratio and BS. The first three are depicted from the spectral analysis and the fourth is the burst BSr calculated by the monitor. These sub-parameters are used to define two energy ratios called alpha (α) and beta (β). Both α and β show a shift in the energy content from the highest to the lowest frequencies within deeper anesthesia

These subparameteres are calculated by the following approach (Formula 1)

$$\beta\text{ratio} = \log \frac{E_{30-42.5Hz}}{E_{11-21Hz}}$$

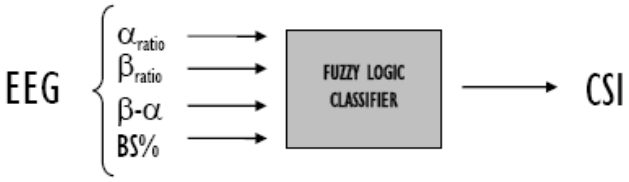
$$\alpha\text{ratio} = \log \frac{E_{30-42.5Hz}}{E_{6-12Hz}}$$

$$(\beta - \alpha)\text{ratio} = \log \frac{E_{6-12Hz}}{E_{11-21Hz}}$$

Formula 1: The energy of EEG is evaluated in specific frequency bands used to define two energy ratios called alpha (α) and beta (β). Both show a shift in the energy content from higher to lower frequencies during anesthesia. The relationship between these quantities is also analysed as a separate parameter (α-β) (Danmeter 2004).

Burst suppression is defined as the percentage of time in 30-s window where the amplitude of the electroencephalographic signal was less than 3.5µV. These EEG periods characterize the deepest levels of hypnosis.

These parameters are then used as inputs to a fuzzy logic classifier to calculate cerebral state index (Jensen, Litvan et al. 2006) Scheme 1 .



Scheme CSI subparameters.
These subparameters are then used as inputs to a fuzzy logic inference system that calculates the CSI. Fuzzy logic is a problem-solving control system methodology that incorporates a simple, rule based "IF X AND Y THEN Z" approach to a solving control problem rather than attempting to model a system mathematically.

The four parameters mentioned above are used as inputs for this fuzzy Adaptative Neuro Fuzzy Inference System (ANFIS), which governs the rules that govern relations between the input parameters using a least mean squares approach.

However, by combining the parameters, a higher correlation coefficient can be reached. An intuitive explanation to this fact is that the ANFIS system, shown in figure 22, automatically uses the best parameter, meaning that when one fails, another might still be a good correlate. The burst suppression parameter indicates deep anesthesia; in this case, the weight on the spectral parameters will be inferior because they are not good correlates during deep anesthesia with burst suppression due to the nonstationary nature of the electroencephalogram in this situation. The structure of the ANFIS systems ensures that each linguistic term is represented by only one fuzzy set.

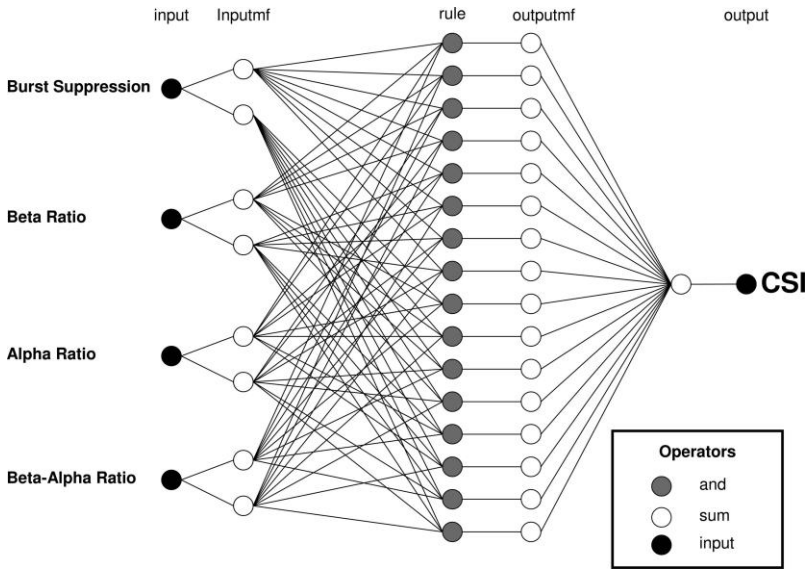


Figure 22-Representative scheme of ANFIS structure.

Legend: CSI - Cerebral State Index; inputmf - membership function of input; outputmf - membership function of output (adapted from Jensen et al., 1996)

The CSI is a unit-less scale from 0 to 100 (Table.4), where 0 indicates a flat EEG and 100 indicates EEG activity corresponding to the awake state. The adequate anesthesia is designed to range 40 and 60. The table present approximate values based on the mean values of the patient behavior.

CSI	Clinical sate
90-100	Awake
80-90	Drowsy
60-80	Light anesthesia or sedation
40-60	Surgical anesthesia
10-40	Deep anesthesia normally with BSr
0-10	Close to coma (BSr larger than 75)

Table 4-CSI description

Signal quality index (SQI) is collected at a constant rate. The SQI is calculated using the quality of the acquired EEG signal and on the signal artefacts during the previous one minute period. The electrode-to-skin impedance is included in the SQI calculation. The artifact rejection algorithm ensures that the incoming EEG is not contaminated by external noise (<2µVp-p, <0.4µV RMS, 1 – 250 Hz). When excessive noise is detected, the signal quality index is also diminished, thus reflecting the disturbance. The SQI is displayed numerically as percentage units (0-100%, 100% equals to the best signal quality). Electrode-to-skin impedances at 1 kΩ result in a SQI of 100. If the impedance of the white or black sensors exceeds 1kΩ, the SQI will gradually decrease. If the sensor impedance is >5kΩ the CSI, BS and EMG will be blank ("- -" displayed). In our study, impedances were kept low (within 1 kΩ and 3 kΩ) by using a special wax (Elefix, paste for EEG, Nihon Kohden Corporation, Japan) placed between the electrodes and the skin.

High levels of facial muscular or EMG activity can interfere with the CSI under certain circumstances. The monitor incorporates an EMG filter that removes most of the potential interfering EMG activity. The EMG bar shows the energy of the EMG level in the 75–85 Hz frequency band (0–100 logarithmic).

The EEG data collection ended before the movement of the patient to the appropriate positional and the beginning of the surgery.

2.3. Anesthetic clinical end points based on ocular reflexes.

The assumption proposed in this work was that the ocular reflexes could describe the anesthetic stage of the dog. The palpebral and corneal reflexes were tested three times with a humidified swab in serum to prevent lesions in cornea. This procedure was made always in the right eye. The reflexes were considered negatives if there were no response to these three stimulations. The palpebral reflex was tested gently at the lateral canthus of the eye, and the corneal reflex was elicited by a smooth pressure on the cornea. The patient was also observed for eyeball position.

Palpebral reflex (PR) and corneal reflex (CR) were classified as present (+) or absent (-); the position of the eyeball was described as eyeball rotated ventromedially (ERV) if the pupil was looking towards the middle eye corner, or centred (EC) if the pupil was centred between the superior and inferior eyelid. The tests were performed by the same anesthesiologst for all of the dogs and the observed responses allowed the preposition of Table 5

Anesthetic planes	Ocular reflexes
A	**PR+/EC/CR+**
B	**PR+/ERV/CR+**
C	**PR-/ERV/CR+**
D	**PR-/EC/CR+**
E	**PR-/EC/CR-**

Table 5-Dog's anesthetic clinical end points (anesthetic planes).

The ocular reflexes are monitored as: palpebral reflex (PR), eyeball centered in the eye (EC), corneal reflex (CR) and eyeball rotated ventrally (ERV). In the presence of the reflex, it is symbolized as +, in its absence, it is symbolized as −.

2.4 Anesthetic Protocol

Data was collected according to three different protocols.

2.4.1 Anesthetic protocol A

Fifteen mixed-breed dogs, ten of which were female, aged 3.4±2.4 years and weighing 22.5±10.5kg were analyzed.

All dogs were premedicated with 0.5 mg kg^{-1} morphine sulphate (Morphine injection ;Martindale Pharmaceuticals) and with 0.03 mg kg^{-1} acepromazine IM (Vetoquinol®; Univete), thirty minutes prior to the beginning of the induction of anesthesia.

Anesthesia was induced using a 6 mg kg^{-1} bolus dose of propofol 1% (Fresenius Kabi®, Bad Homburg, Germany) via a syringe pump (Asena GH, Alaris Medical Systems) programmed to allow a maximum infusion rate of 600 ml h^{-1}. A propofol bolus dose of 6 mg kg^{-1} was decided upon previous clinical observations where the CSI reached minimum values around 50 seconds after propofol administration (Ferreira, Ribeiro et al. 2006). RugLoop II® software (developed by Tom DeSmet (Demed Engineering, Temse, Belgium) and Michel Struys (Ghent University, Gent, Belgium)), running on a personal computer, was used to drive the propofol syringe pump.

Propofol infusion was ceased after the bolus administration. At this point the CSI was expected to have attained its minimum value. Then, with a decrease in PropCp a resulting increase in the CSI values was expected. In order to avoid a total recovery from anesthesia, the study methodology limited the recovery to a CSI value of 70. Once the CSI reached 70, the study ended; the depth of anesthesia was increased and the planned surgical procedures were carried out.

During the entire study dogs were allowed to breathe spontaneously via a facial mask administering 100% of oxygen. If apnea were to occurred, the study would be interrupted, the dogs would be intubated, mechanically ventilated and subsequently discarded from the final data analysis. No stimuli is applied at any time.

2.4.1 Anesthetic protocol B

Fourteen mixed breed dogs aged 2.6±0.9 years, including 8 females, weighing 25.3±5.28 kg were analysed. Dogs were premedicated with 0.05 $mgkg^{-1}$ acepromazine IM (Vetoquinol, Univete, Lure,France) thirty minutes prior to the beginning of the induction of anesthesia. Anesthesia was divided in the study and surgical periods: the study period had not no external interference and was divided in induction and

maintenance; the surgical period was characterized by external stimulus and other drug administration. During induction of anesthesia, dogs were allowed to breathe spontaneously via a facial mask where 100% oxygen was provided.

2.4.1.1 First study

In the first phase of the study period, propofol was administered at a constant infusion rate of 200 mlh^{-1}.the dogs were placed in sternal recumbence.

The first reflexes were tested when the dogs changed to a lateral recumbence without a cervical tonus after the beginning of the propofol infusion.

The palpebral and corneal reflexes were tested every 30 seconds with a swab moistened with serum. The eyeball position was also observed at each test. The tests were performed by the same operator in all dogs

The study was stopped if apnea occurred (no breathe for more than 15 seconds), or hypotension (mean arterial blood pressure below 60 mmHg) occurred, or until loss of corneal reflex. At this time point, dogs were intubated, the propofol infusion was stopped and the propofol plasma concentration was allowed to decrease to 3 μg ml−1. After intubation, dogs were manually ventilated using a closed circuit to maintain an end-tidal CO2 of 35 mmHg level measured by the Datex S/5 monitor.

2.4.1.1 Second study

In the second phase of the study, propofol was administered by TCI using seven different constant steps of propofol plasma concentrations: 3 μgml^{-1}, 4 $\mu g\ ml^{-1}$, 5 $\mu g\ ml^{-1}$, 6$\mu g\ ml^{-1}$, 7 $\mu g\ ml^{-1}$, 9 $\mu g\ ml^{-1}$ and 11 μg ml−1. At each level the propofol plasma concentration was maintained unaltered for 5 minutes and the dogs' reflexes evaluated by the same anesthesiologist. During this phase, the palpebral and corneal reflexes were tested. Each reflex was tested three times and was considered negative if there was no response in all three times. The study would be interrupted if hypotension (mean arterial pressure below 60 mmHg) occurred, or if the corneal reflex was lost. Dogs would be discarded from data analysis in the respective propofol plasma concentration level if hypotension occurred. Hemodynamic variables were monitored during the entire study. No stimuli were applied besides testing of the reflexes.

2.4 Statistics

Heart rate and mean arterial pressure values were collected before the administration of the propofol infusion, those values were then compared to those observed at maximum PropCp using analysis of variance.

The results obtained from testing the reflexes were analyzed and grouped according to the propofol concentrations and, consequently, with anesthetic depth. A letter representing a different plane of anesthesia was given to each group of reflexes. Data was tested for normal distribution using the Shapiro Wilk test.

The Spearman Rank correlation analysis was used to compare CSI and PropCp, CSI and anesthetic planes, and anesthetic planes and PropCp. The Spearman Rank correlation was used to compare anesthetic planes with CSI and EMG.

The analysis of the Electroencephalogram parameters change in the last study, in which the relationship between the anesthetic planes and different steps of PropCp were explored, based on an appropriate measure of performance to identify indicators for anesthetic depth, the prediction probability (PK). Ideally, anesthetic depth indicator values should correlate perfectly with anesthetic depth along a lighter to a deeper anesthesia progression on a continuum way. The mathematical basis of PK was described by Smith *et* al (Smith, Dutton et al. 1996). A PK value of 1 means that the values of the predicting variable, *e.g.*, anesthetic depth indicator correctly predicts the value of the observed anesthetic depth. A PK value of 0.5 means that the values of the indicator predict no better than a 50–50 chance.

Heart rate, mean arterial pressure and CSM data (CSI, BS, SQI and EMG) were compared to baseline values (twenty minutes after the administration of acepromazine, since its difficulty to apply the electrodes without sedation) using the Wilcoxon or paired sample T tests.

The time delays for the CSI calculation range from 53 to 55 (Pilge, Zanner et al. 2006). In our analysis, a delay of 50 seconds in CSI data was taken into account when analyzing CSI data. Statistical analysis was performed using SPSS v.13.0 for Windows.

Capítulo 2

2-Material y Metodología General

Los datos analizados en esta memoria se obtuvieron cuando los perros a los que se anestesiaba fueron sometidos a una intervención quirúrgica de orquitectomía.

Los animales provenían de Sociedades Protectoras que tenían convenios con el Hospital Veterinario de Oporto (Portugal). Siguiendo el protocolo de forma estándar y rutinaria se obtuvo el consentimiento por escrito, tanto para la anestesia y cirugía, como para someterlos a los protocolos de este trabajo.

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2.1- Pacientes y estudio hemodinámico

En el estudio solo se incluyeron perros sanos, evaluándose su estado de salud mediante la historia clínica, el examen clínico, y análisis clínicos (hematocrito, proteínas totales, urea, etc.).

Todos ellos fueron clasificados en el grupo de "Estado Físico I" según la clasificación ASA.

Ninguno de los animales estaba recibiendo ningún fármaco antes del estudio.

Se administraron los fármacos a través de la vena cefálica. Se utilizo una válvula de tres vías conectada al catéter (una vía para la administración del propofol, y otra para la administración de suero salino (NaCl al 1%) mediante una bomba de infusión de marca "Braun" para la administración del suero a un flujo constante de 10 ml.Kg/h (10 ml. por Kg. de peso vivo/hora), durante todo el tiempo del estudio.

La monitorización de los parámetros hemodinámicos se llevo a cabo mediante un monitor "Datex S/5". Se registró el electrocardiograma en la derivación II.

La presión arterial se midió de modo no invasivo.

Se indujo la anestesia con propofol al 1% (Fresenius Kabi; Bad Homburg, Alemania) utilizando una bomba de jeringa (Asena GH, Alaris Medical Systems) programada para permitir una velocidad de infusión máxima de 600 ml h^{-1}.

El Software de Rugloop II ® (Demed Engineering, Temse, Bélgica) fue utilizado para guardar los datos del propofol y almacenar todos los datos hemodinámicos y electroencefalográficos de los respectivos monitores, cada 5 segundos

y también para estimar la concentración plasmática de propofol utilizando el modelo farmacocinético de Beths (Beths, Glen et al. 2001).

Los datos del Rugloop II R fueron tratados con el software Labgrab (Demed Engineering, Temse, Bélgica) y exportados a un archivo de Excel.

En el Rugloop Software no esta incluido, cuando se compra, el modelo farmacocinético del propofol en perros. Fue necesario modificar su programación y crear un archivo donde se insertó la farmacocinética de Beth´s.

Al comenzar el estudio de cada perro introducimos las variables relacionadas con el peso del animal estudiado.

El sistema de anestesia utilizado para la recogida de datos se muestra en la figura.15

El software Rugloop permite controlar la jeringa infusora con diferentes formas de administración del propofol: bolus, tasa de infusión constante y TCI (Fig. 16).

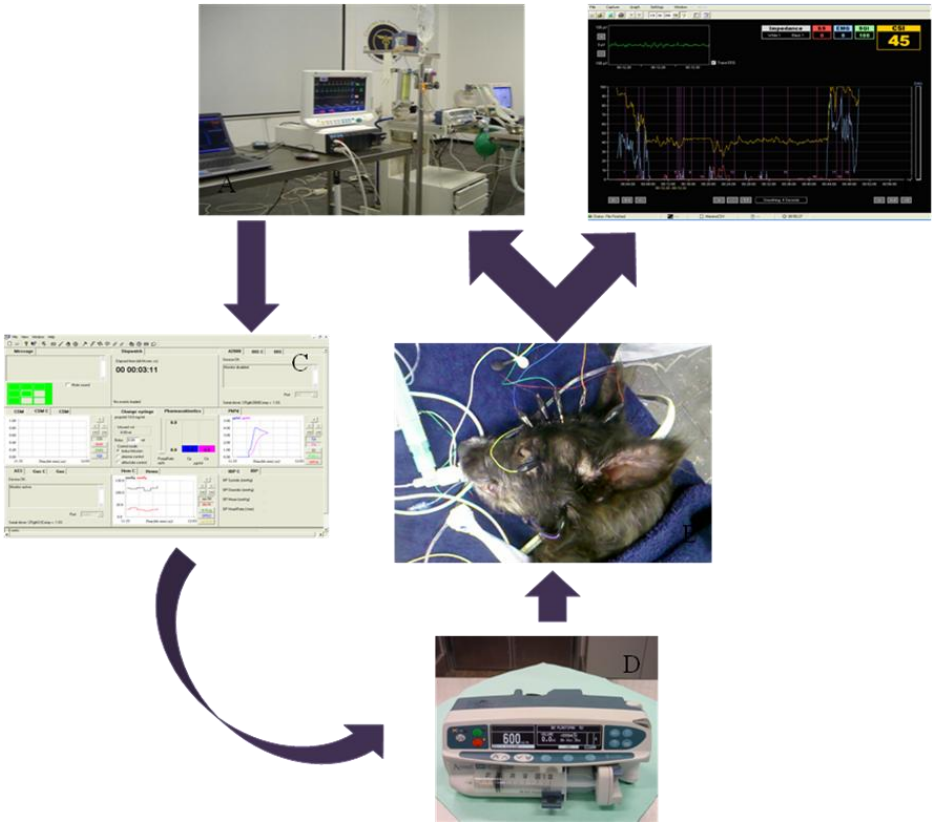


Figure 15: Anesthesia Setup: The dog anesthesia monitoring (E) is performed by a Datex monitor(A) and CSI monitor(B). Thereafter the RugLoop II(C) software connected to the monitors recorded all data and permit to the operator control the Asena syringe pump(D).

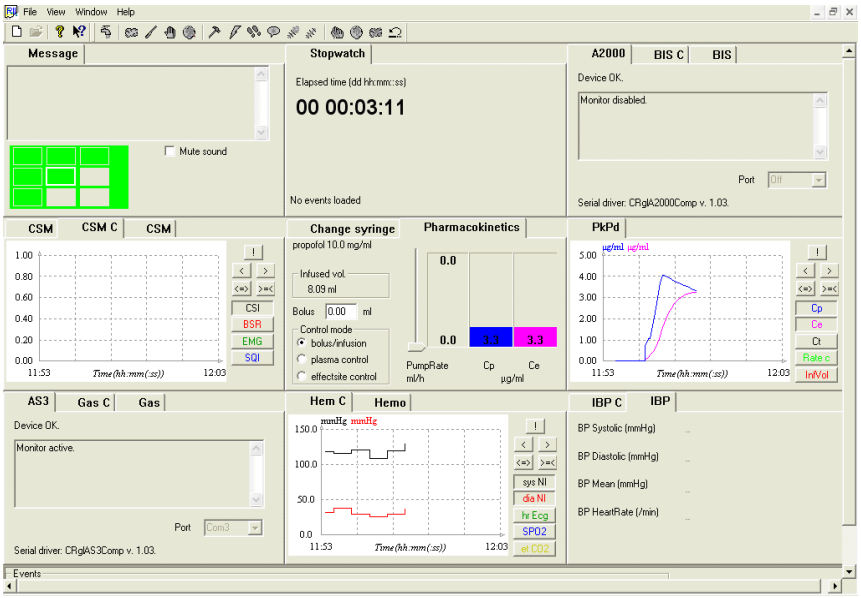


Fig 16 – Rugloop II Software.

2.2- Monitorización del “Cerebral state index” (CSI).

El índice de estado Cerebral (CSI) es un índice con el que se quiere evaluar la profundidad de la anestesia y que es calculado por el “Cerebral State Monitor” (CSM, figura 17) utilizando técnicas de procesamiento del EEG.

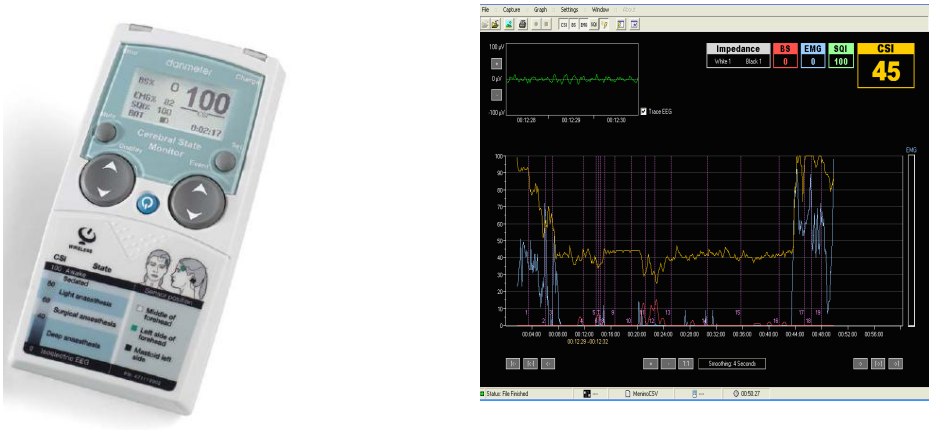


Figure 17: a) CSI monitor (image from CSI user manual). b) Display software in the personal computer.

El CSI y la actividad electromiográfica frontal (EMG) son datos recogidos cada cinco segundos por el Cerebral State Monitor (CSM) (Danmeter, DK-5000 Odense C, Dinamarca).

Un cable modificado de ECG con pinzas de cocodrilo o aligator se conectaron al cable CSM. El uso de esos electrodos de pinza facilita el uso clínico y reduce el coste del monitor EEG (Seitsonen, Yli-Hankala et al 2000; Akavipat, Dumrongbul et al. 2006).

Antes de la aplicación de los electrodos de pinza la piel se limpia con alcohol 5 minutos antes de la recogida de los datos para permitir su secado (Fig. 18).

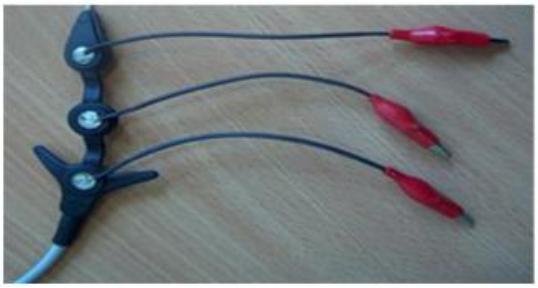


Fig .18 Alligator clamps adapted to collect dog's cerebral data

La onda bipolar del EEG en nuestro estudio se deriva de la señal grabada por dos posiciones diferentes de los electrodos utilizando tres electrodos en pinza.

La primera posición donde se colocada un electrodo se hace de acuerdo con las instrucciones del fabricante para la monitorización en humanos de la CSI: en el medio del hueso frontal (blanco), en el lado izquierdo de la frente cerca del borde lateral del ojo y en el proceso mastoides temporal del lado izquierdo de la cabeza (negro). La forma de onda de EEG se deriva de la señal registrada entre el electrodo blanco y el negro (Fig 19A e B).

A B

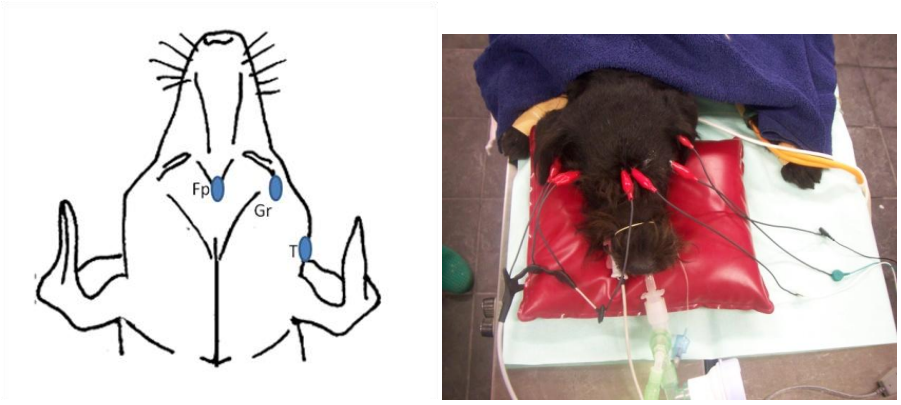


Fig 19A) Dorsal view of canine cranium showing the placement of the EEG recording electrodes. Fp (frontopolar electrode), Gr (ground reference electrode) and temporal(T);
B). Dorsal view of the canine cranium showing the placement of electrodes during a study comparing BIS monitor and CSM monitor. CSI electrodes were Placed in left side

La segunda posición, teniendo en cuenta la anatomía de cráneo (Fig. 20) y la onda de EEG se deriva de la señal registrada entre el electrodo frontopolar (positivo) representada por el color blanco y el electrodo occipital (negativo) representada por el color negro, según estudios de Pelegrino y col. (Pellegrino y Sica 2004). El electrodo de referencia se situó en la posición parietal (Fig.21 A y B).

A B

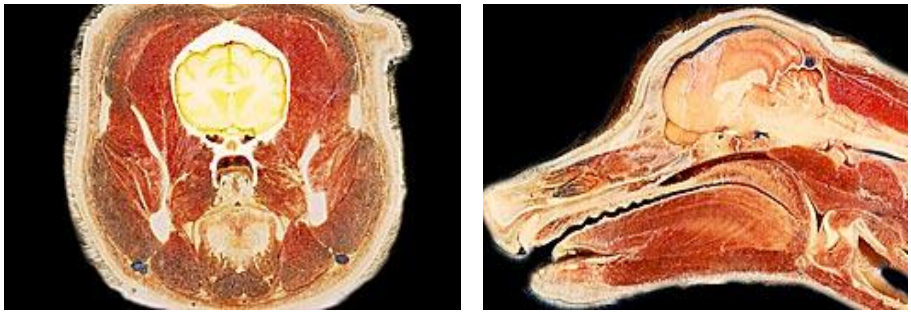


Fig 20 Extracted from canine planar anatomy website of University of Minnesota College of Veterinary Medicine. A) Canine head transverse section . B) Canine head and neck sagital section

A B

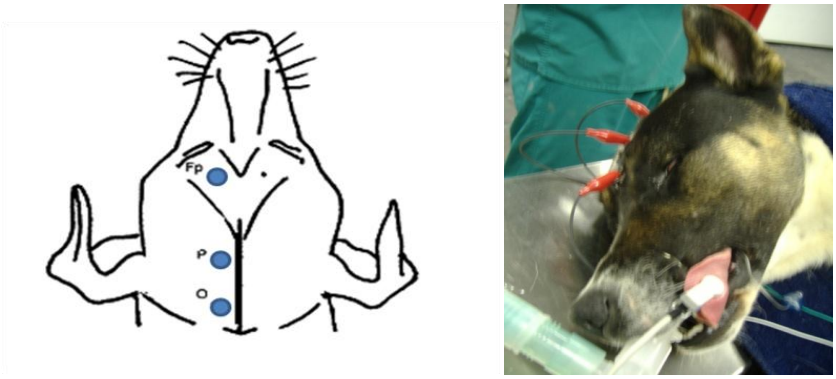


Fig 21 A). Dorsal view of canine cranium showing the placement of the EEG recording electrodes. Fp (frontopolar electrode), P (parietal electrode) and O (occipital electrode);
B. Lateral view of the left side of the canine cranium showing the placement of electrodes.

En el "Cerebral State Monitor" (CSM) se recoge la señal de EEG continuamente. La sensibilidad de EEG fue ±400μV y el monitor CSM recogió 2000 muestras por segundo (14bits equivalente), con una actualización cada segundo.

Para calcular el índice (CSI) se eliminaron las frecuencias que estaban fuera del rango de 6-42 Hz. En el cálculo del CSI se utiliza la combinación de cuarto subparámetros de la señal electroencefalográfica. La primera de ellas se deriva del análisis espectral y la cuarta es el ratio de la supresión (BSR %) calculada por el monitor. Estos subparámetros se utilizan para definir dos ratios de energía llamados alfa (α) y beta (β). α y β muestran cambios de energía que se obtienen desde las frecuencias mayores hasta las menores con una anestesia profunda.

Estos subparámetros se calculan con las siguientes ecuaciones (Fórmula 1)

$$\beta\text{ratio} = \log \frac{E_{30-42.5Hz}}{E_{11-21Hz}}$$

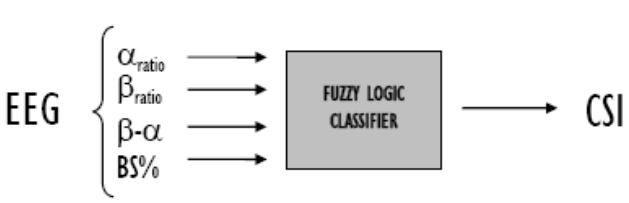
$$\alpha\text{ratio} = \log \frac{E_{30-42.5Hz}}{E_{6-12Hz}}$$

$$(\beta - \alpha)\text{ratio} = \log \frac{E_{6-12Hz}}{E_{11-21Hz}}$$

Formula 1: The energy of EEG is evaluated in specific frequency bands. These are used to define two energy ratios called alpha (α) and beta (β). Both of these show a shift in energy content from higher to lower frequencies during anesthesia. The relationship between these quantities is also analysed as a separate parameter (α-β).Adapted from Danmeter manual (Danmeter 2004).

La Salva-Supresión (BSR %)se define como el porcentaje de tiempo en una ventana de 30-s donde la amplitud de la señal electroencefalográfica es inferior a 3.5μV. Estos períodos de EEG son los que caracterizan los niveles más profundos de hipnosis.

Estos parámetros se usan como entradas, para un clasificador de lógica difusa, que calcula el índice de estado cerebral (Jensen, Litvan et al 2006) esquema 1



Scheme 1. These subparameters are then used as inputs to a fuzzy logic inference system that calculates the CSI. Fuzzy logic is a problem-solving control system methodology that incorporates a simple, rule based "IF X AND Y THEN Z" approach to a solving control problem rather than attempting to model a system mathematically.

Los cuatro parámetros mencionados arriba, se utilizan como entradas para esta lógica difusa Adaptative Neuro Fuzzy Inference System (ANFIS), que regula las normas que rigen las relaciones entre los parámetros de entrada(Fig 22).

.

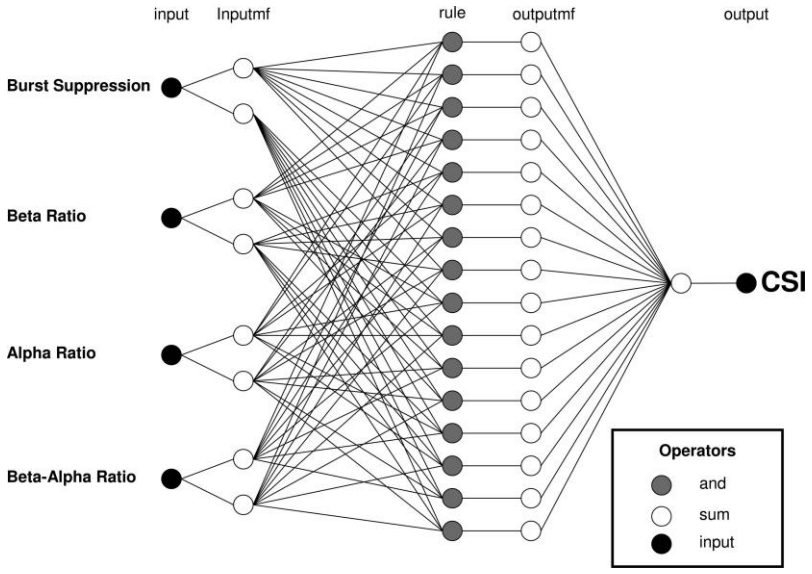


Figure 22 - Representative scheme of ANFIS structure.

Legend: CSI - Cerebral State Index; inputmf - membership function of input; outputmf - membership function of output (adapted from Jensen et al., 1996)

El CSI tiene una escala de 0 a 100 (tabla. 2.1), donde 0 indica un EEG plano y 100 indica la actividad del EEG correspondiente al estado despierto.
La anestesia adecuada en las cirugías ordinarias está diseñada para que el valor del Índice este comprendido entre 40 y 60.
Todos los valores de la tabla son valores aproximados, basados en los valores medios del comportamiento del paciente.

CSI	Estado
90-100	Despierto
80-90	Soñoliento
60-80	Ligera anestesio o sedación
40-60	Anestesia quirúrgica
10-40	Anestesia profunda normalmente con BSr
0-10	Cerca del coma (BSr mayor que 75)

Table 4 CSI description

El índice de calidad de señal (SQI) se recoge constantemente. Se calcula utilizando la calidad de la señal del EEG adquirida pero teniendo en cuenta los artefactos de la señal durante el minuto anterior y la impedancia del electrodo hasta la piel. El algoritmo de rechazo del artefacto asegura que el EEG no está contaminado por ruido externo (< 2µVp-p, < 0.4µV RMS, 1 - 250 Hz).
El SQI se mide como unidades porcentuales (0-100%, siendo el 100% la mejor calidad de señal).
Una impedancia del electrodo hasta la piel de 1 kΩ resulta en un SQI de 100.
Si la impedancia de los sensores blanco o negro supera 1kΩ, el SQI disminuirá gradualmente.
Si la impedancia del sensor es > 5kΩ los valores del índice y la Electromiografía dejaran de valorarse.
En nuestro estudio, las impedancias se mantuvieron bajas (dentro de 1 kΩ y kΩ 3) utilizando una cera especial (Elefix, pasta para EEG, Nihon Kohden Corporation, Japón) entre los electrodos y la piel.

La actividad electromiográfica (EMG) elevada, de la musculatura facial puede interferir en el índice CSI, pero el monitor incorpora un filtro que elimina la mayor parte de las interferencias.

2.3. Evaluación de la profundidad anestésica basada en la utilización de los reflejos oculares.

Una de las hipótesis que hicimos en nuestro trabajo es que los reflejos oculares podían relacionarse con el plano de anestesia.

Las pruebas fueron realizadas por el mismo anestesista en todos los perros.

Los reflejos palpebrales y cornéales se probaron tres veces con un hisopo humidificado en suero para evitar lesiones en la córnea: Este procedimiento se hizo siempre en el ojo derecho. El reflejo palpebral (PR) fue probado suavemente en el borde lateral del ojo, y el reflejo corneal (CR) fue suscitado por una suave presión sobre la córnea. Los reflejos palpebral (PR) y corneal(CR) fueron clasificados como presente (+) o ausente (-).

También se observó la posición del globo ocular del paciente:

Globo ocular girado ventromedial (ERV) si la pupila estaba mirando hacia la esquina del ojo,

Centrado (CE) si la pupila se centró entre el párpado superior e inferior.

Las respuestas observadas se recogen en la siguiente tabla 5

Planos anestésicos	Reflejos oculares
A	**PR+/EC/CR+**
B	**PR+/ERV/CR+**
C	**PR-/ERV/CR+**
D	**PR-/EC/CR+**
E	**PR-/EC/CR-**

Table 5- Dog's anesthetic clinical end points (anesthetic planes).
The ocular reflexes are monitored as: palpebral reflex (PR), eyeball centred in the eye (EC), corneal reflex (CR) and eyeball rotated ventrally (ERV). In the presence of the reflex, it is symbolized as +, in its absence, it is symbolized as −.

2.4 Protocolo anestésico

Empleamos tres protocolos diferentes.

2.4.1 Protocolo Anestésico A

Se analizaron quince perros sin raza, diez de los cuales fueron hembras, con una edad de 3.4±2.4 años y un peso 22.5±10.5 kg.

Todos los perros fueron medicados previamente con 0,5 mg $Kg.^{-1}$ de sulfato de morfina (Morphine injection; Martindale Pharmaceuticals) y con 0,03 mg $Kg.^{-1}$ de acepromazina IM (Vetoquinol ®; Univete), treinta minutos antes del comienzo de la inducción de la anestesia.

La anestesia fue inducida mediante una dosis 6 mg $Kg.^{-1}$ de propofol 1% (Fresenius Kabi ®, Bad Homburg, Alemania).La cantidad de propofol administrada se eligio basándonos en anteriores observaciones clínicas (Ferreira, Ribeiro et al 2006).

La administración de propofol se hizo utilizando una bomba de jeringa (Asena GH, Alaris Medical Systems) programada para permitir una velocidad de infusión máxima de 600 ml h^{-1} La infusión de propofol cesó después de la administración del bolo, momento en el que se debía tener un valor mínimo del CSI. Según pasaba el tiempo debía producirse una disminución de PropCp por lo que teníamos un aumento en los valores del CSI. El estudio se terminaba cuando el índice alcanzaba un valor de 70.

Durante el estudio todos los perros respiraban espontáneamente a través de una máscara facial a traves de la qual se administraba oxígeno al 100%. Si en algún caso se producía apnea, el estudio se interrumpía y se descartaba ese animal para incluirlo en el estudio.

Para poder realizar los procedimientos quirúrgicos planificados se aumentaba la cantidad de propofol administrada, ya que los animales estaban próximos a despertarse, pero estos valores no se contabilizaban en el estudio.

2.4.1 Protocolo Anestesico B

Se estudiaron catorce perros sin raza, con 2.6±0.9 años de edad, incluyendo 8 hembras, con un peso de 25.3±5.28 kg.

Los perros fueron medicados con 0,05 $mgkg^{-1}$ de acepromazina IM (Vetoquinol, Univete, Lure, Francia), treinta minutos antes del comienzo de la inducción de la anestesia.

Durante la inducción de la anestesia, los perros podían respirar espontáneamente a través de una máscara facial y se les suministraba oxígeno al 100%.

La anestesia se realizo en dos estudios o protocolos:

2.4.1.1 Primer estudio

En la primera fase del período de estudio, se administró propofol en una infusión constante de 200 mlh^{-1} .

Los perros fueron colocados en decúbito esternal, y se evaluaron los primeros reflejos cuando los perros se cambiaron a una posición de decúbito lateral, cuando yo no había tono cervical

Los reflejos palpebrales y cornéales se observaron cada 30 segundos, anotándose también la posición del globo ocular. Las observaciones fueron realizadas por el mismo anestesista en todos los perros.

El estudio fue detenido en caso de apnea (el perro no respira durante más de 15 segundos), o hipotensión (presión arterial menor de 60 mm.Hg), o hasta la pérdida del reflejo corneal.

2.4.1.1Segundo studio

En la segunda fase, se administró propofol en diferentes concentraciones mediante el TCI (Target controlled infusions),con concentraciones de propofol en plasma de: 3 µg/ml., 4 µg/ml, 5 µg/ml., 6µg/ ml., 7 µg/ml., 9 µg/ ml. y 11 µg/ ml.

En cada nivel, la concentración plasmática de propofol se mantuvo inalterada durante 5 minutos.

Los reflejos de los perros, palpebrales y cornéales, fueron evaluados por el mismo anestesiólogo. Cada reflejo fue probado en tres ocasiones y fue considerado negativo si no hubo respuesta las tres veces.

El estudio se interrumpió si se produjo hipotensión (presión arterial menor de 60 mm Hg), o si el reflejo corneal se pierde. Los datos de los perros con hipotensión fueron descartados.

Las variables hemodinámicas fueron controladas durante todo el estudio.

2.4 Estadisticas

El ritmo cardíaco y presión arterial se midieron antes del inicio de la infusión de propofol y comparados con los observados en PropCp mediante análisis de la varianza.

Los resultados obtenidos de las pruebas de los reflejos fueron analizados y agrupados, en función de las concentraciones de propofol y, consecuentemente, con profundidad anestésica. Los datos fueron analizados mediante la prueba Shapiro Wilk.

El análisis de correlación de Spearman Rank se utilizo para comparar: CSI con PropCp; CSI con planos anestésicos y planos de anestésicos con PropCp. La correlación de rango de Spearman fue utilizada también para comparar planos anestésicos con CSI (Índice de estado cerebral) y con EMG (electromiografía).

En el último estudio, la relación entre los planos de anestésicos y diferentes medidas de PropCp, se basó en una medida apropiada de rendimiento para identificar indicadores de profundidad anestésica, la probabilidad de predicción (PK) (Smith, Dutton et al. 1996)

Un valor de PK de 1 significa que los valores de la variable de predicción, por ejemplo, el indicador de profundidad anestésica predice correctamente el valor de la profundidad anestésica observado. Un valor de PK de 0,5 significa que los valores del indicador solo predicen una probabilidad de 50–50.

La frecuencia cardíaca, presión arterial media y los datos obtenidos con el "Cerebral State Monitor" (CSI, BS, SQI y EMG) se compararon con los valores de referencia (obtenidos veinte minutos después de la administración de acepromazina, que se necesitaba para poder aplicar los electrodos), mediante las pruebas de t de muestras pareadas o Wilcoxon.

El análisis estadístico se realizó mediante SPSS v. 13.0 para Windows.

Chaper 3

Results

3.1 Section One

Brain monitoring in dogs using the cerebral state index during the induction of anesthesia via target-controlled infusion of propofol.

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3.1.1Abstract

The aim of this study was to evaluate the correlation between the Cerebral State Index (CSI) and the estimated propofol plasma concentrations in dogs during induction of anesthesia.

Fifteen healthy dogs undergoing scheduled routine surgical procedures were enrolled in this study. Target Controlled Infusion (TCI) software, based on the pharmacokinetic model for propofol, was used to control the syringe pump and to estimate plasma propofol concentrations (PropCp) and the CSI values every five seconds. Three electrodes placed in the centre of the forehead, on the left side of the forehead and on the left mastoid were used to collect the electroencephalographic (EEG) signal converted by the Cerebral State Monitor into the CSI.
The cerebral electrical changes induced by increasing propofol concentrations appear to be detected by CSI monitoring in dogs. The negative correlation between CSI and

PropCp demonstrates that the CSI could be used to assess electrical brain activity in dogs during the induction of anesthesia with propofol.

3.1.2-Introduction

Anaesthesiology has improved notably thanks to developments in monitoring cardiovascular parameters during general anesthesia. Anaesthesiologists are now able to routinely control physiological parameters such as blood pressure, heart rate and respiratory rate. However, the direct determination of the effects of anesthetic and sedative agents on the Central Nervous System remains a challenge (Flaishon R 1997).

Target Controlled Infusion (TCI) is an anesthetic delivery system now widely used in human anesthesia (Guarracino, Lapolla et al. 2005). In veterinary medicine, some reports have been published using TCI in dogs during anesthesia induction and maintenance (Beths, Glen et al. 2001; Musk, Pang et al. 2005), which state that TCI is an appropriate technique for use in dogs. TCI can improve the delivery rate of a desired clinical effect while minimising side effects (Musk, Pang et al. 2005) and allows a more effective and easy control of the depth of anesthesia (Beths, Glen et al. 2001).

In human medicine, a variety of analytical concepts have been proposed to quantify changes in the EEG during general anesthesia. The monitor most widely used for this purpose is the bispectral index (BIS), which is largely based on the bispectrum analysis of the EEG (Stanski 2000). On the other hand, the Cerebral State Monitor (CSM) was recently introduced as an intra-operative monitor of anesthetic depth (Zhong, Guo et al. 2005). The Cerebral State Index (CSI) provided by CSM monitors is based on a combination of four sub parameters of the electroencephalographic signal. Three of these derive from spectral analysis of the EEG, and the fourth is the burst suppression (BS) calculated by the CSM (Jensen, Litvan et al. 2006). The CSI is a numerical scale between “0” and “100”, where “0” represents an isoelectric EEG line and “100” represents the EEG electrical activity of a fully awake individual. CSI is based on the analysis of the EEG frequency using a fuzzy logic classifier, whereas BS is the percentage of isoelectrical EEG signal during each 30-second period (Danmeter 2004). The ideal anesthetic level for humans was established to be between 40 and 60 (Jensen, Litvan et al. 2006).

In veterinary medicine, BIS monitoring has been proposed as a way to assess depth of anesthesia in dogs (Muir , Wiese et al. 2003), cats (March and Muir 2003) and pigs (Martin-Cancho, Lima et al. 2006). However its applicability is not clear when it comes to variation between species and drugs. Furthermore the correlation between EEG depression and the BIS index is unclear (March and Muir 2005). In animals, increased

concentrations of anesthetic agents showed a reduction in BIS values, but it was not possible to establish a BIS interval where it is safe to maintain anesthesia (Lamont, Greene et al. 2005; March and Muir 2005)

The CSM may be a useful monitor to access depth of anesthesia in veterinary medicine. This monitor provides a similar brain monitoring function to that of the BIS. The CSM's small size and low cost when compared to other brain monitors makes it an appealing instrument for use by veterinary professionals in anesthetic procedures. The CSM monitor has not been studied to the same extent as the BIS. In veterinary anesthesia, the CSM monitor has only been studied in dogs by Bollen and Saxtorph, during sedative procedures using medetomidine (Bollen and Saxtorph 2006).

To our knowledge, no studies have been published involving the use of CSI monitoring on dogs during anesthesia. The CSM algorithm used to estimate CSI is different from that used by the BIS monitor. The CSM uses a fuzzy logic analysis tool which is not specifically adjusted to use on humans. On the contrary, the BIS' calculations use data from 5000 human anesthetic procedures stored in a database (Johansen and Sebel 2000). Therefore, it is possible that CSI monitoring could yield better results than BIS when monitoring brain activity in dogs during anesthetic procedures. The objective of this study is to analyse the correlation between CSI and propofol-estimated plasma concentrations during induction of anesthesia in dogs.

3.1.3-Material and Methods

3.1.3.1-Patients and hemodynamic monitoring

After Research Committee approval and informed consent, fifteen healthy dogs undergoing scheduled routine surgical procedures were enrolled in this study. All dogs were premedicated with 0.5 mg kg^{-1} morphine sulphate (Morphine injection ;Martindale Pharmaceuticals) and with 0.03 mg kg^{-1} acepromazine IM (Vetoquinol®; Univete), thirty minutes prior to the beginning of the induction of anesthesia.

A peripheral catheter was inserted in the cephalic vein for drug and fluid administration. A three-way stopcock was used to connect the intravenous catheter to the propofol and to the sodium chloride 0.9% delivery lines. A Braun infusion pump (Braun, Melsungen, Germany) was used to administrate sodium chloride 0.9 % at a constant infusion rate of 10 ml kg^{-1} hr^{-1} throughout the study period.

A S/5 Datex monitor (Datex-Ohmeda, Helsinki, Finland) was used to collect the hemodynamic data. The non-invasive blood pressure was measured using an automated oscillometric method on the posterior left limb using repeated measurements: each measurement was carried out immediately after the preceding one (Datex S/5 "STAT" mode) which corresponds to a continuous mode for collecting blood pressure measurements throughout the study. Heart rate was monitored using three ECG electrodes placed in accordance with Academy of Veterinary Cardiology Committee specifications.

3.1.3.2-Cerebral state index monitoring

The CSI and frontal electromyographic activity (EMG) data were collected from a Cerebral State Monitor (CSM) (Danmeter, DK-5000 Odense C, Denmark) using three clamp electrodes placed according to the manufacturer's instructions for human CSI monitoring: in the middle of the forehead/frontal bone (white), to the left side of the forehead near the lateral eye socket, and on the temporal mastoid process on the left side of the head (Black). The EEG waveform was derived from the signal recorded between the frontal (white) and mastoid (black) electrodes. The frequency content was between 2-35 Hz and the CSM monitor collects 2000 samples sec^{-1} (14bits equivalent), updating every second and filtering between 6 and 42 Hz. (Danmeter 2004). Impedances were kept at a low level (within 1 kΩ and 3 kΩ) by placing wax (Elefix, paste for EEG. Nihon Kohden Corporation)between the electrodes and the skin.

The CSI is based on analysis of the frequency content of the EEG signal. The EEG energy is characterised into specific frequency bands. These are used to define two energy ratios, namely alpha (α) and beta (β). There is a shift exhibited in the energy content of both α and β, from a relatively high to a relatively low frequency level, when the animal is in a deeper state of anesthesia. The relationship between these shifts is also analysed by CSM as a separate parameter (β-α). The monitor, that works online, also evaluates the amount of instantaneous BS during each thirty-second period of the EEG in order to quantify the amount of time the EEG is "silent" or "flat-line". These EEG patterns typify the very deepest levels of hypnosis. The four parameters are inputted into a fuzzy-logic classifier system that calculates the CSI. Fuzzy reasoning enables very complex processes to be carried out and can also be successfully applied to high non-linear processes, where it can considerably simplify modelling (Jensen, Litvan et al. 2006).

The signal quality index (SQI) was collected at a constant rate. The SQI is calculated based on the quality of the EEG signal obtained and the signal artefacts recorded during the preceding one-minute period. The electrode-to-skin impedance is included in the SQI calculation. The SQI is displayed numerically as percentage units (0-100%, 100% equals optimum signal quality).Electrode-to-skin impedances at 1 kΩ result in a SQI of 100. If the impedance of the white or black sensors exceeds 1kΩ, the SQI will gradually decrease. The artefact rejection algorithm ensures that the incoming EEG is not contaminated by external noise (<2μVp-p, <0.4μV RMS, 1 – 250 Hz). When excessive noise is detected, the signal quality index is also diminished, thus reflecting the disturbance (Danmeter 2004).

Electromyographic (EMG) activity from head muscles was also recorded by the CSM every 5 seconds.

3.1.3.3-Anesthetic protocol

Anesthesia was induced using a 6 mg kg^{-1} bolus dose of propofol 1% (Fresenius Kabi®, Bad Homburg, Germany) via a syringe pump (Asena GH, Alaris Medical Systems) programmed to allow a maximum infusion rate of 600 ml h^{-1}. A propofol bolus dose of 6 mg kg^{-1} was decided upon previous clinical observations where the CSI reached minimum values around 50 seconds after propofol administration (Ferreira, Ribeiro et al. 2006). RugLoop II® software (developed by Tom DeSmet (Demed Engineering, Temse, Belgium) and Michel Struys (Ghent University, Gent, Belgium)), running on a

personal computer, was used to drive the propofol syringe pump. The pharmacokinetic model for propofol from Beths and colleagues (Beths, Glen et al. 2001) was incorporated in the RugLoop II® software and was used to estimate the propofol plasma concentrations (PropCp). RugLoop II® was also used to store all pharmacokinetic, hemodynamic and electroencephalographic data at five-second intervals.

Propofol infusion was ceased after the bolus administration. At this point the CSI was expected to have attained its minimum value. Then, with a decrease in PropCp a resulting increase in the CSI values was expected. In order to avoid a total recovery from the anesthesia, the study methodology limited the recovery to a CSI value of 70. Once the CSI reached 70, the study ended; the depth of anesthesia was increased and the planned surgical procedures were carried out.

During the entire study period dogs were allowed to breathe spontaneously via a facial mask administering 100% oxygen. If apnea were to occur, the study would be interrupted, the dogs intubated and mechanically ventilated and subsequently discarded from the final data analysis. No stimuli were applied at any time.

3.1.3.4-Statistical analysis.

Data was tested for normal distribution and for homogeneity of variance using the Shapiro Wilk and the Levene tests respectively. Heart rate and mean arterial pressure values were collected before propofol infusion was begun and compared to those observed at maximum PropCp using analysis of variance. The CSI and PropCp data, collected at five-second intervals after the start of propofol infusion, were compared using Spearman Rank correlation analysis. A delay of 50 seconds in CSI data was taken into account when analyzing CSI data (Pilge, Zanner et al. 2006). Statistical analysis was performed using SPSS v.13.0 for Windows. Data are expressed in mean±sd; $P<0.05$ was considered statistically significant.

3.1.4-Results

Fifteen mixed-breed dogs, ten of which were female, aged 3.4±2.4 years and weighing 22.5±10.5kg were analysed. Prior to the induction of anesthesia, CSI was 90.7± 4, heart rate was 103.6±20.3 bpm and mean arterial pressure was 83.1± 14.9 mmHg.

During the induction of anesthesia, the maximum PropCp occurred 46.3±45.9 seconds after propofol infusion was started and was 7.18±0.39 µg ml^{-1}, which corresponded to a CSI value of 67±12, with SQI and EMG values of 78.0±12.4 % and 32.9±30.8 % respectively. At this point, heart rate was 122±33.3 bpm and mean arterial pressure was 78.4±19.4 mmHg. There was a 13% decrease in mean arterial pressure ($P<0.05$) and a 17% increase in heart rate ($P<0.05$) between awake values and those values recorded at maximum PropCp (table 6).

The minimum CSI values observed were 52.3±9.6 and occurred with a PropCp of 5.2±0.96 µg ml^{-1}, 1.8±1.5 minutes after the maximum PropCp value was attained; SQI and EMG values were 83±12.1% and 30.7±26.5% respectively. At this point in time, heart rate was 117.4±31.9 bpm and mean arterial pressure was 73.3±16.2 mmHg (table 7).

At the end of the study, CSI was 70.3±1.2 with SQI and EMG values of 76.8±13.5% and 31.5±14% respectively; PropCp was 3.83±1.3µg ml^{-1}, heart rate was 110.5±28 bpm and mean arterial pressure was 74.3±14 mmHg.

A significant negative correlation was observed between CSI and PropCp (correlation coefficient of -0.579; $P<0.01$) (figure 23).

Table 6-Values obtained at maximum concentration of propofol from each individual dog.

	Weight(Kg)	HR(bpm)	MAP(mmhg)	PropCp(máx)µg/ml	CSI	EMG(%)
D1	21	117	55	7.15	67	0
D2	12	98	85	7.35	55	48
D3	5.7	121	93	7.51	56	80
D4	35	123	77	6.74	83	20
D5	29	156	51	6.76	44	0
D6	6,7	78	64	7.34	63	52
D7	21	128	94	6.61	61	77
D8	17	65	72	7.19	57	0
D9	10	203	65	7.47	78	82
D10	28	158	62	7.02	70	6
D11	45	103	63	6.58	68	0
D12	6.3	129	97	7.52	66	53
D13	25	143	66	7.09	93	38
D14	25	102	117	7.02	77	28
D15	18	109	70	7.78	71	10

P=Patient, HR = heart rate, MAP=Mean arterial pressure, PropCP= Predicted Propofol Plasma Concentration, maximum, CSI=Cerebral State index, EMG=Electromyography.

Table 7-Values obtained from each individual at minimum CSI value

	HR(bpm)	MAP(mmhg)	PropCp µg/ml	CSI	EMG
D1	109	50	5.96	50	0
D2	94	77	5.79	47	57
D3	102	84	5.02	45	39
D4	112	75	3.54	56	0
D5	150	51	5.83	38	0
D6	84	56	5.36	54	43
D7	128	81	4.20	51	24
D8	76	67	4.90	52	0
D9	191	61	6.69	70	87
D10	158	62	5.85	63	10
D11	101	63	6.09	68	0
D12	123	97	6.50	49	47
D13	110	81	4.76	38	26
D14	88	77	4.75	55	27
D15	130	104	3.63	55	0

P=Patient, MAP=Mean arterial pressure, PropCP= Predicted Propofol Plasma Concentration, CSI=Cerebral State index, EMG=Electromyography.

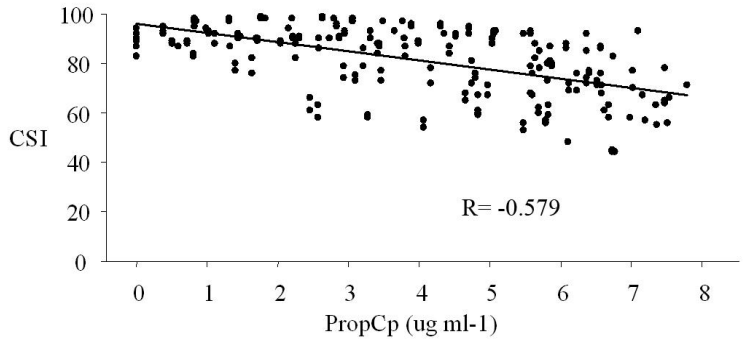

Figure 23-Correlation between CSI and propofol estimated plasma concentrations (PropCp) from the beginning of the study until maximum achieved estimated propofol concentration.

3.1.5-Discussion

The present study analysed the correlation between estimated PropCp concentrations and their effects on CSI monitoring in dogs at different points during induction of anesthesia: at maximum PropCp, at minimum CSI values and when the CSI value reached 70 during the washout phase after the propofol bolus.

There are no universally accepted standard electrode locations for EEG recording in dogs considered to be ideal for electrode placing during clinical research or clinical practice (Pellegrino and Sica 2004). Therefore, the location chosen by us to place the electrodes was the same as that referred to by Grenne, Benson and colleagues(Greene, Benson et al. 2002) and described by Pellegrino and Sica as suitable for EEG recording (Pellegrino and Sica 2004).

Prior to induction of anesthesia, CSI values were 90.7±4 in the dogs used in our study. In humans, CSI values between 90-100 represent the electrical brain activity of an awake individual while CSI values between 80 to 90 indicate a low level of sedation (Danmeter 2004). The effects of pre-medication with acepromazine and morphine (Pascoe 2000) may explain the CSI values observed in dogs at the outset of our study.

Nolan and Reid suggested that tracheal intubation should not be performed when the average propofol blood concentration is below 5.4 μg ml^{-1} (Nolan and Reid.J. 1993). Based on previous clinical observations, we decided to administer a 6mg kg^{-1} propofol bolus to induce anesthesia. This dose raised PropCp to a maximum of 7.18 μg ml^{-1}on average. Thus, the maximum PropCp attained in our study is clinically acceptable for anesthesia induction and tracheal intubation.

Our results showed that increasing the concentration of propofol during induction of anesthesia in dogs resulted in a progressive decrease in CSI values. However, a large inter-individual variability in CSI responses was also observed (table 1 and table 2). The differences in time taken to attain the maximum PropCp and CSI response may be justified by the large weight variability among the dogs used and by the maximum infusion rate of 600 ml h^{-1} permitted by the syringe pump during bolus administration. The plasma drug concentrations cannot in themselves predict the time span or magnitude of drug effect (Holford and Sheiner 1981). When the plasma drug concentrations are increased, a time lag occurs until an equilibrium between plasma and effect site drug concentration is reached (Jacobs 1995). The time needed to transfer the drug to the effect site (the brain in the case of hypnotics) depends on the concentration

gradient, and an increased gradient requires less time to induce anesthesia (Musk, Pang et al. 2005).

Beths' pharmacokinetic model (Beths, Glen et al. 2001) was developed using dogs with a wide range of ages and weights. In humans, pharmacokinetic and pharmacodynamic differences are described among children, elderly patients and adults (Kazama, Ikeda et al. 1999; Hermán R. Muñoz 2004). A parallel could be inferred for veterinary medicine where significant pharmacokinetic differences exist for propofol use across different breeds, ages and weights. This could prove limiting when trying to apply the pharmacokinetic model. Nevertheless, there are studies showing that TCI using this pharmacokinetic model in dogs allowed for better propofol titration with improved control of anesthetic depth (Beths, Glen et al. 2001) and fewer unwanted side effects (Musk, Pang et al. 2005) when compared to manual infusion.

EMG values higher than 30% were observed in seven dogs at maximum PropCp and in five dogs at minimum CSI values. EMG activity is the most significant source of EEG artefact. Variations in EMG activity are associated with rapid changes in BIS values in dogs (Greene, Benson et al. 2002). Despite the fact that the CSI algorithm also incorporates an EMG filter that removes most potentially interfering EMG activity, the EMG still has a significant influence on the CSI value (Jensen, Litvan et al. 2006). However, there are no published studies that quantify the total influence of EMG on CSI. Low EMG values are ideal when monitoring brain activity. One way of achieving this would be the administration of myorelaxant drugs. However, as our study was performed during induction of anesthesia it would be unethical and unsafe to administer myorelaxant drugs prior to tracheal intubation.

Another important parameter to take into account when using CSI monitoring is the SQI, which represents the quality of the EEG signal received. SQI calculation is based on a series of artefacts that occur during each one-minute period. The SQI is also influenced by the level of impedance of the electrodes on the skin. Impedance of the black and white sensors above a level of 3kΩ will negatively influence SQI and thus affect CSI monitoring (Danmeter 2004). Throughout the entire study period, impedance was kept low (within 1 kΩ and 3 kΩ) with the use of wax placed between electrodes and the skin, thus allowing us to obtain average SQI values of around 80% over the course of the study period.

The CSI could be a satisfactory alternative to BIS for monitoring depth of anesthesia in humans (Hoymork, Hval et al. 2007). The CSI demonstrates more variability in the

baseline when compared with BIS. However, both have a high probability of predicting loss of consciousness, loss of reflexes and correlate well with the Modified Observer's Assessment of Alertness/Sedation Scale (MOAAS) (Zhong, Guo et al. 2005). There are few animal studies reporting on monitoring depth of anesthesia using BIS technology. The BIS monitor appears to have certain limitations when used in animals, especially when burst suppression is observed after the administration of large doses of anesthetics (March and Muir 2005). In these studies, the hypnotic agents were essentially halogenated and the results showed BIS' usefulness to be limited when monitoring the degree of CNS depression (Greene, Benson et al. 2004; Martin-Cancho, Lima et al. 2006). Increased concentrations of anesthetic agents decreased BIS but it could not accurately estimate a safe BIS interval for anaesthetising veterinary patients (Lamont, Greene et al. 2005; March and Muir 2005). In pigs for example, BIS seems to interpret burst suppression in the EEG as an indication that the animal is waking up, showing high BIS values even during a steady state anesthesia (Greene, Benson et al. 2004).

The PropCp decreased after cessation of the propofol bolus according to the estimates performed by Beths' pharmacokinetic model (Beths, Glen et al. 2001). This was reflected by a progressive increase in CSI values, indicating a lighter depth of anesthesia. Although this correlation has already been shown in humans (Zhong, Guo et al. 2005), this is the first time that it has been reported in dogs.

Propofol decreases the arterial blood pressure by centrally depressing sympathetic neural output, which results in a decrease in systemic vascular resistance (Clayes, Gepts et al. 1988). Episodes of hypotension (mean arterial pressure <60mmHg) were observed in only two dogs in our study for a very brief period of time when PropCp reached its maximum concentration. Other than these two cases, heart rate and mean arterial pressure were always within normal physiological limits. Thus, a possible effect of systemic hemodynamics on cerebral hemodynamics and on resulting CSI values appears unlikely.

In conclusion, cerebral electrical changes induced by increasing and decreasing propofol concentrations appear to be detected by CSI monitoring of dogs during induction of anesthesia. The negative correlation between CSI and PropCp indicates that the CSI could be used to assess electrical brain activity in dogs during induction of anesthesia using propofol and suggests that it could potentially be used for monitoring the depth of anesthesia during general anesthetic procedures. However it must be underlined that CSM is a brain monitor not yet validated for use in veterinary anesthesia

and, this must therefore be taken into account. Nevertheless, this study shows that CSI monitoring could be a potentially useful tool for accessing the depth of anesthesia in dogs.

3.2-Section Two

Correlation between clinical signs of depth of anesthesia and cerebral state index responses in dogs during induction of anesthesia with propofol.

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3.2.1-Absctract

The Cerebral State Index (CSI) is used for monitoring EEG and depth of anesthesia. The objective of this study was to analyse the correlation between ocular reflexes, CSI and estimated propofol plasma concentrations (PropCP) in dogs during induction of anesthesia with propofol.

Fourteen dogs were premedicated with acepromazine 0.05mg kg-1 IM. Anesthesia was induced with a 200 ml h-1 propofol 1% constant infusion rate until loss of corneal reflex using RugLoop II software with Beths' pharmacokinetic model to estimate PropCp.

Palpebral reflex (PR) and the corneal reflex (CR) were tested every 30s and classified as present (+) or absent (-), and eyeball position was registered as rotated ventromedialy (ERV) or centred (EC).

Heart rate (HR), mean arterial pressure (MAP) and CSI values were analyzed from baseline before the beginning of propofol infusion (T0) until loss of CR; CSI and PropCp, CSI and anesthetic planes, and PropCp and anesthetic planes were compared using correlation analysis.

PropCp reached 7.65±2.1 ug ml-1 at the end of the study. CSI values at T0 were 89.2±3.8. Based on the observation of ocular reflexes and eyeball position, it was possible to define five anesthetic planes: A (superficial) to E (deep), being A

(PR+/CR+/EC), B (PR+/ERV/CR+), C (PR-/ERV/CR+), D (PR-/EC/CR+) and E (PR-/EC/CR-). There was a significant correlation between PropCp and the anesthetic planes (R=0,861; P<0.01). No significant correlation was observed between CSI and the anesthetic planes or between CSI and PropCp. MAP decreased significantly from T0 until loss of corneal reflex (from 98±14 mmHg to 82±12 mmHg); HR did not change significantly (from 101±30 bpm to 113±16 bpm).

The CSI monitoring was not consistent with the clinical observations observed in the different stages of depth anesthesia. This could limit the use of CSI for monitoring depth of anesthesia with propofol.

3.2.2-Introduction

The concept of depth anesthesia is dimensionless. Without any objective monitoring, the subjective descriptions `too light´, `too deep´ or `enough´ are not helpful for a careful assessment of the adequate plane of anesthesia (Pomfrett 1999). The observation of physical signs, such as somatic muscle tone, respiratory patterns and ocular signs allowed assigning to a given stage of anesthesia (Heiko 2004). So far, only ether was used to define the clinical stages according to anesthetic depth (Bhargava, Setlur et al. 2004) The development of a brain monitor that can be used in dogs is a real necessity to monitor depth of anesthesia more objectively. The electroencephalogram (EEG) provides a direct measurement of the functional state of the brain which makes the monitoring of increasing anesthetic depth and the detection of unnecessary depth of anesthesia possible(Lunn, Rosen et al. 1987). Different EEG patterns such as decreasing EEG frequency and increasing wave amplitude can be observed with increasing anesthetic concentrations. These and other changes can be described and quantified statistically (Miller, Sleig et al. 2004), and algorithms can be introduced in EEG monitoring devices and used to access the depth of anesthesia.

The Cerebral State Monitor (CSM), an EEG monitor, was recently introduced for monitoring the depth of anesthesia in humans, proving to be a useful tool. (Jensen, Litvan et al. 2006; Hoymork, Hval et al. 2007)This monitor has not yet been validated in dogs but there is a possibility that the Cerebral State Index (CSI) displayed by the CSM could be used for EEG monitoring in dogs (Ribeiro, Ferreira et al. 2007).

The objective of this study is to analyse the correlation between depth of anesthesia by monitoring ocular reflexes, and CSI and estimated propofol plasma concentrations in dogs during induction of anesthesia with propofol.

3.2.3-Methods

3.2.3.1-Patients and hemodynamic monitoring

Fourteen healthy dogs undergoing scheduled routine surgical procedures were enrolled in this study. All dogs were premedicated with 0.05 mg kg^{-1} Acepromazine IM (Calmivet®, Vetoquinol, France), thirty minutes prior to the beginning of the induction of anesthesia.

A cannula was inserted in the cephalic vein for drug and fluid administration. A three way stopcock was used to connect the intravenous catheter to the propofol and sodium chloride 0.9% delivery lines. A Braun infusion pump (Braun, Melsungen, Germany) was used for the administration of sodium chloride at a constant infusion rate of 10 ml kg^{-1} hr^{-1} during the entire study period.

An S/5 Datex monitor (Datex-Ohmeda; Helsinki, Finland) was used for monitoring the hemodynamic parameters. The blood pressure was measured non-invasively in the anterior carpus of the left hind leg using repeated measurements: each measurement was made immediately after the preceding one (Datex S/5 "STAT" mode). Heart rate was monitored by three ECG electrodes placed according to Academy of Veterinary Cardiology Committee.

3.2.3.2-Cerebral state index monitoring

The CSI and frontal electromyographic activity (EMG) data were collected every five seconds from a Cerebral State Monitor (CSM) (Danmeter, DK-5000 Odense C, Denmark) using three clamp, ECG electrodes (Seitsonen, Yli-Hankala et al. 2000), placed in standard position in the midline of the head. The EEG waveform in our study was derived from the signal recorded between the frontopolar electrode (positive)represented by the white color and the occipital electrode (negative) represented by the black color, according to Pelegrino and colleagues' studies (Pellegrino and Sica 2004). The ground or the reference electrode was placed in the parietal position. The frequency of the waveform was between 2-35 Hz and CSM monitor collected 2000 samples sec^{-1} (14bits equivalent), with an update every one second. To calculate the CSI, frequencies outside the 6-42 Hz range were filtered out (Danmeter 2004). Impedances were kept low (within 1 kΩ and 3 kΩ) by using a special wax (Elefix, paste for EEG, Nihon Kohden Corporation) placed between the electrodes and the skin.

The CSI is calculated using four sub-parameters of the electroencephalogram: β ratio, α ratio, β ratio - α ratio, and burst suppression. These sub-parameters are used to define two energy ratios called alpha (α) and beta (β). Both α and β show a shift in the energy content from the higher to the lower frequencies with deeper anesthesia. This information can be used to achieve an index which varies from 0 to 100 (Jensen, Litvan et al. 2006).

The monitor also evaluates the amount of instantaneous burst suppression (BS) in each thirty-second period that quantifies the amount of isoelectric waves in the EEG. These EEG periods characterise the deepest levels of hypnosis.

Signal quality index (SQI) was collected at a constant rate. The SQI is calculated using the quality of the acquired EEG signal and on the signal artefacts during the previous one minute period. The electrode-to-skin impedance is included in the SQI calculation. The SQI is displayed numerically as percentage units (0-100%, 100% equals best signal quality). Electrode-to-skin impedances at 1 kΩ result in a SQI of 100. If the impedance of the white or black sensors exceeds 1kΩ, the SQI will gradually decrease (Danmeter 2004). When excessive noise is detected, the SQI is also diminished reflecting the disturbance (Danmeter 2004). If the sensor impedance is >5kΩ the CSI, BS and EMG will be blank ("- -" displayed).

3.2.3.3-Anesthetic protocol

RugLoop II® software (developed by Tom DeSmet (Demed Engineering, Temse, Belgium) and Michel Struys (Ghent University, Gent, Belgium)) running on a personal computer was used to drive the propofol syringe pump. The pharmacokinetic model for propofol from Beths and colleagues (Beths, Glen et al. 2001) was incorporated in the RugLoop II® software and was used to estimate the propofol plasma concentrations (PropCp). RugLoop II® was also used to store the pharmacokinetic, hemodynamic and electroencephalographic data every five seconds.

Anesthesia was induced using a constant infusion rate of 200 ml h^{-1} of propofol 1% (Fresenius Kabi®; Bad Homburg, Germany). Throughout this period, the animals were breathing 100% oxygen through a face mask. The end point of the propofol administration was the moment when the dogs lost their corneal reflex. However if the dogs became apnoeic (did not breathe for more than 15 seconds) or hypotensive (meaning their arterial blood pressure dropped below 60 mmHg) the propofol

administration was stopped . After the end of the study the dog was intubated and prepared for the scheduled surgery.

No stimuli were applied at any time to the dogs other than testing reflexes.

3.2.3.4-Clinical observation of the depth of anesthesia

Before the induction of anesthesia and after premedication, the dogs were placed in sternal recumbency. The first reflexes were tested when the dogs changed to a lateral recumbence without a cervical tonus after the beginning of the propofol infusion.

The palpebral and corneal reflexes were tested every 30 seconds with a swab moistened with serum. The palpebral reflex was tested gently at the lateral canthus of the eye, and corneal reflex was elicited by a smooth pressure on the cornea. Each reflex was tested three times and considered negative if there was no response to the three taps with the swab. The eyeball position was also observed at each test. Thus, palpebral reflex (PR) and corneal reflex (CR) were classified as present (+) or absent (-); the position of the eyeball was described as eyeball rotated ventormedially (ERV) if the pupil was looking towards the middle eye corner, or centred (EC) if the pupil was centred between the superior and inferior palpebral. The tests were performed by the same operator for all of the dogs

3.2.3.5-Data and statistical analysis

The results obtained from testing the reflexes were analyzed and grouped according to the propofol concentrations and, consequently, with anesthetic depth. A letter representing a different plane of anesthesia was given to each group of reflexes. Data was tested for normal distribution using the Shapiro Wilk test. Heart rate, mean arterial pressure and CSI values were analyzed from baseline values before the beginning of propofol infusion (T0) until the loss of corneal reflex using the Wilcoxon. The Spearman Rank correlation analysis was used to compare CSI and PropCp, CSI and anesthetic planes, and anesthetic planes and PropCp. A delay of 50 seconds in CSI data was taken into account when analyzing CSI data (Pilge, Zanner et al. 2006). Statistical analysis was performed using SPSS v.13.0 for Windows. Data was expressed in mean±sd; $|R|>0.5$ and $P<0.05$ was considered statistically significant.

3.2.4-Results

Fourteen mixed breed dogs aged 2.6±0.9 years, including 8 females, weighing 25.3±5.28 kg were analysed. The results from prospectively analyzing the ocular reflexes indicated five levels of anesthetic depth (A to E) (table 8). CSI decreased from baseline to plane E (table 9) but no significant correlation was observed between CSI and these anesthetic planes (R=0.26; Fig 3.2.4.1), or between CSI and PropCp) (R=0.478). Nevertheless, a strong correlation was observed between PropCp and the anesthetic planes (R=0.887; $P<0.01$).

In plane E, all dogs lost their palpebral and corneal reflex, and were intubated with laryngeal reflex absent. At this time point lower EMG values associated with lower CSI values were observed. Four dogs showed high EMG values in this plane associated with high CSI values.

Mean arterial blood pressure decreased by 16 % from the baseline to plane E ($P<0.05$). Heart rate did not change significantly during the study (Table 9).

Table 8-Anesthetic planes identified based on increasing estimated propofol plasma concentrations in all dogs. Anesthetic planes are represented from A to E, according to anesthetic depth based on the ocular reflexes monitored: Palpebral reflex (PR), eyeball centred in the eye (EC), corneal reflex (CR) and eyeball rotated ventrally (ERV).

Anesthetic planes	Ocular reflexes
A	PR+/EC/CR+
B	PR+/ERV/CR+
C	PR-/ERV/CR
D	PR-/EC/CR+
E	PR-/EC/CR-

A-Presence of palpebral reflex, eyeball centred and corneal reflex positive; B- Presence of palpebral reflex, eyeball rotated ventrally and corneal reflex positive; C- Absence palpebral reflex, eyeball rotated ventrally and corneal reflex positive; D- Absence palpebral reflex, eyeball centred and corneal reflex positive ;E- Absence palpebral reflex, eyeball centred, absence of corneal reflex.

Table 9-Hemodynamic, electroencephalographic and propofol data observed during baseline and defined anesthetic planes A to E in this study.

	N	PropCp (μg/ml)	CSI	EMG (%)	BS (%)	SQI (%)	HR (bpm)	MAP (mmhg)
Baseline	14	0.00	89.2 ± 3.8	94.0 ± 11.5	0.0	63.7 ± 21.0	101±30	98±14
Anesthetic planes								
A	14	3.03±1.01	91.9 ± 4.9	93.7 ± 9.2	0.0	68.9 ± 20.3	110±18	95±15
B	10	4.29±1.66	92.8 ± 7.9	84.5 ± 27.6	0.0	71.3 ± 23.6	101±21	99±12
C	12	5.54±1.34	83.3 ± 24.8	66.0 ± 36.8	44±0[1]	70.1 ± 25.4	103±20	91±12
D	5	6.72±2.15	71.2 ± 31.2	40.4 ± 25.7	46±0[1]	85.0 ± 12.7	117±7	88±11
E			60.4 ±		7.5			
	14	7.65±2.16	25.6[3]	25.1 ± 35.0[4]	±15.8	80.7 ± 17.2	113±16	82±12[2]

N- Number of dogs in which were observed the respective anesthetic plane. PropCp- Estimated Propofol Plasma Concentration ; CSI- Cerebral State Index ; EMG-Electromyography; BS- Burst suppression; SQI- Signal Quality Index ; HR- heart rate; MAP- Mean arterial pressure.

[1]Only one dog started to show burst suppression on plane C, which then followed to plane D and E, five dogs showed burst suppression only in plane E.

CSI, HR and MAP data from all anesthetic planes were compared to the baseline values: [2]P<0.05 [3]P<0.01.

[4] Four dogs in plane E have high values of EMG (>30%)

Figure 24-Correlation between CSI and anesthetic planes.

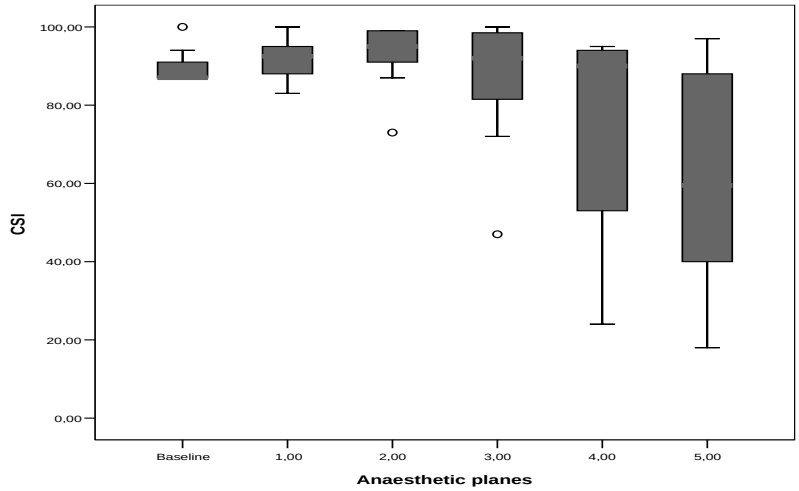

Figure 24 - Relationship between baseline infusion (T0) and anesthetic planes A, B, C, D and E and CSI during the study period. "o" represents de outliers. Data presented as a box plot; 25th and 75th percentile are the boxes' borders; whiskers are the lowest and highest values for the 5th and 95th percentiles, respectively.

3.2.5-Discussion

This study analysed the effect of propofol on CSI, ocular reflexes, heart rate and mean arterial blood pressure during the induction of anesthesia with propofol in dogs. Five different anesthetic planes based on the analysis of ocular reflexes were identified in dogs under propofol anesthesia. In fact PropCp showed a strong correlation with these anesthetic planes and so, different PropCp intervals are proposed in order to achieve a desired depth of anesthesia. On the other hand, CSI decreased with increasing propofol concentrations but there was no correlation between CSI and the depth of anesthesia provided by the anesthetic planes in this study. Thus, CSI does not seem to provide trustworthy clinical information for monitoring the depth of anesthesia in dogs during the induction of anesthesia with propofol.

The hemodynamic parameters monitored in this study provided very little information about the depth of anesthesia when using propofol as hypnotic agent. In fact only mean arterial pressure decreased by about 16% from baseline to plane E, which is of limited clinical use when monitoring depth of anesthesia.

The development of a three-compartment pharmacokinetic model for propofol for dogs (Beths, Glen et al. 2001) allowed the use of TCI in veterinary anesthesia practice. The concentrations obtained with the propofol constant rate infusion of 200 ml h^{-1} were based on Beths pharmacokinetic model (Beths, Glen et al. 2001). Although in our study no blood analyses were performed to measure the real propofol concentration in the blood, there are studies showing that TCI using this pharmacokinetic model in dogs allows a better propofol titration with improved control of anesthetic depth (Beths, Glen et al. 2001) and less undesired side effects (Musk, Pang et al. 2005) when compared to manual infusion.

The effects of propofol on the EEG are well described in humans (Sleigh, Steyn-Ross et al. 2001). The administration of low doses of propofol increases the amplitude and the EEG alpha wave rhythm, followed by a shift to EEG gamma and theta frequency. Higher doses produce burst suppression and decrease in the EEG amplitude (Jensen, Litvan et al. 2006). A good monitor of depth of anesthesia should accurately distinguish these EEG changes and correlate them with different anesthetic depth. The most studied monitor in veterinary science is the bispectral index monitor BIS (March and Muir 2005). This monitor seems to have some limitations when used in animals, especially when burst suppression is observed after the administration of large anesthetic doses

(March and Muir 2005). In these studies, the hypnotic agents were essentially halogenated and the results showed limited BIS usefulness for monitoring the degree of Central Nervous System (CNS) depression (Greene, Benson et al. 2004; Martin-Cancho, Lima et al. 2006). Increased concentrations of anesthetic agents decreased BIS but could not accurately estimate the BIS interval where it is safe to anaesthetise veterinary patients (Lamont, Greene et al. 2005; March and Muir 2005). For example, in pigs BIS seems to interpret the burst suppression in the EEG as an indicator of awakening, showing high BIS values even during a steady state of anesthesia (Greene, Benson et al. 2004).

In humans, CSI monitoring has recently been introduced to monitor the hypnotic state of patients during anesthetic procedures, but demonstrates more variability in the baseline when compared with BIS. However, both monitors have a high probability of predicting loss of consciousness, loss of reflexes and good correlations with the Modified Observer's Assessment of Alertness/Sedation Scale (MOAAS) (Zhong, Guo et al. 2005). Jensen and colleagues concluded that the CSI could be a reasonable alternative to the BIS monitor. In a previous study, our group proposed the CSI as a possible satisfactory alternative to BIS for monitoring depth of anesthesia in dogs (Ribeiro, Ferreira et al. 2007). This study showed that the cerebral electrical changes induced by a 6 mg kg^{-1} bolus dose of propofol was detected by the CSI monitoring of dogs during induction of anesthesia. The negative correlation between CSI and PropCp suggested that the CSI could be used to assess electrical brain activity in dogs. However, results from this study indicate that when anesthesia is induced using a propofol constant infusion rate, CSI correlation is lower than the previous study (Ribeiro, Ferreira et al. 2007). A possible reason for such differences may be related to the time needed to transfer the drug to the effect site. The concentration at the brain in the case of hypnotics depends on the concentration gradient (Musk, Pang et al. 2005). When a bolus of 6 mg kg^{-1} of propofol is administered, the time lag needed for the propofol plasma and effect-site concentrations to reach the equilibrium is small. Thus, the CSI rapidly reflects the propofol concentrations in the brain by showing lower CSI values. In a constant rate of propofol infusion, the propofol plasma concentration increases more slowly. Thus, the gradient of concentration of propofol between the plasma and the effect-site is lower, and the time to transfer the drug to the effect site is longer. Furthermore, this study showed a higher standard deviation at the maximum estimated propofol plasma concentration (7.65±2.16 µg ml^{-1}) when compared with the

bolus study (7.18±0.39 µg ml^{-1}). This may be explained by the fact that in this study propofol infusion was stopped based on clinical assessments.

According to the CSI manufacturer, CSI values in humans higher than 90 indicate an "awake" brain, between 80 to 70 indicate deep sedation, between 60 and 40 indicate general anesthesia and below 40 indicate excessive anesthesia (Danmeter 2004). As we can see in table 2, the CSI values are far from correlating with these intervals. Furthermore CSI had no correlation with propofol-estimated plasma concentrations and no correlation with the clinical signs of the anesthetic planes referred in this study.

It is known that EMG activity is the most significant source of EEG artefact. Despite the fact that the CSI algorithm also incorporates an EMG filter that removes most of the potential interfering EMG activity, the EMG still has a significant influence on the CSI value (Jensen, Litvan et al. 2006). The influence of EMG in dogs may be particularly important due to the amount of muscles in the head compared with humans. In an attempt to diminish the influence from EMG produced by temporal muscles electrodes were placed in the mid-line. The influence of EMG on the CSI values was a constant observation in our study and it seems to be the main reason for the high standard deviation observed in the CSI values during the anesthetic planes C, D and E (Table2).

Isoelectric EEG periods interrupted by brief periods of high amplitude EEG activity indicates a non-specific reduction in cerebral metabolic activity known as burst suppression (Rampil 1998). Onset of burst suppression has historically been associated with a surgical plane of anesthesia (stage III, planes 2 and 3) (March and Muir 2005). In our study, the BS was observed in three out of the four dogs that had values of CSI below 40 and happened more consistently in plane E of anesthesia where all dogs lost their corneal reflex.

Another important parameter to take into account when using CSI monitoring is the SQI. The SQI represents the quality of the received EEG signal. The calculation of SQI is based on a series of artefacts that occur during each one-minute period. The SQI is also influenced by the impedance of the electrodes in the skin. Impedances of the black and white electrodes superior to 3kΩ will negatively influence SQI and thus affect CSI monitoring (Danmeter 2004). During the entire study period, impedances were kept low (within 1 kΩ and 3 kΩ) by using wax placed between electrodes and the skin.

Propofol decreases the arterial blood pressure by central depression of sympathetic neural output, which results in a decrease in systemic vascular resistance and potential hypotension (Clayes, Gepts et al. 1988). Studies in sheep show that rapid injection of

propofol for the induction of anesthesia produces more effects on cardiovascular systems than a slower infusion rate (Zheng, Upton et al. 1998). In our study, no hypotensive episodes were observed and mean arterial blood pressure showed an average maximum decrease of 16% from "awake" values to the deepest anesthetic stage observed (Plane E). Heart rate was also within physiologic normal range during the entire study. The slow rate of propofol infusion (a constant infusion of 200 ml min^{-1}) and the dogs' weight of over 20 kg may help to explain the hemodynamic stability.

In conclusion, this study proposes five anesthetic planes based on objective eyeball position and reflex observations during induction of anesthesia with propofol. The performance of CSM was not consistent with the clinical observations on the different planes of depth of anesthesia. This may limit its use during the induction of veterinary routine anesthesia. The good hemodynamic stability provided by some hypnotic agents even at higher concentrations, as seems to occur with propofol, provides little clinical information for monitoring depth of anesthesia and strengthens the need for an EEG based monitor for veterinary use. Until then, it seems that clinical reflexes still provide useful information that cannot be minimized. Nevertheless, these reflexes are of limited clinical value when using myorelaxant drugs during general anesthesia.

3.3-Section Three

Correlation between clinical signs of depth of anesthesia and cerebral state index responses in dogs with different target-controlled infusions of propofol.

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3.3.1-Abstract

Objective To evaluate if the Cerebral State Index (CSI), measured by a Cerebral State Monitor (CSM), can predict depth of anesthesia as assessed clinically, or estimated propofol plasma concentrations.

Study design Prospective clinical study.

Animals Fourteen mixed breed dogs, weiging 24.5 ± 4.7 kg, scheduled to undergo neutering procedures.

Methods Dogs were premedicated with 0.05 mg kg^{-1} acepromazine intramuscularly. The CSM and cardiovascular monitoring equipment were attached. Anesthesia was induced with propofol using a target controlled infusion (TCI) to varying plasma propofol targets (PropCp). Following endotracheal intubation the dogs were ventilated with oxygen. Anesthetic maintenance was with propofol by TCI. A PropCp of 3 µg dL^{-1} was set initially, then PropCps were increased in 1 µg dl^{-1} steps to 7, 9 and then 11 µg dL^{-1}. Each PropCp was held constant for a 5 minute period, at the end of which depth of anesthesia was classified using a previously evaluated scale of 'planes' based on palpebral and corneal reflexs and eye position. Cerebral state index (CSI), burst suppression (BSR) and electromyogram were measured at these time points. The

Prediction Probability (PK) of these variables, or of the PropCp in predicting depth of anesthesia was calculated.

Results The PKs for predicting anesthetic planes were 0.74, 0.91, 0.76 and 0.78 for CSI, BSR, EMG and PropCp, respectively. The PKs for PropCp to predict CSI, BSR and EMG were 0.65, 0.71 and 0.65, respectively.

Conclusion and clinical relevance The Cerebral State Monitor was able to detect very deep planes of anesthesia when BSR occurs, but was not able to distinguish between the intermediate anesthetic planes likely to be used in clinical anesthesia.

3.3.2-Introduction

It is difficult to assess the depth of anesthesia during balanced anesthesia using both anesthetic and neuromuscular blocking agents, and even in humans, hemodynamic changes do not always detect intraoperative awareness (Rampil 2001). The electroencephalogram (EEG) provides a direct measure of the functional state of the cerebral cortex which makes it possible to monitor increasing anesthetic depth and detect if anesthesia is unnecessarily deep (Lunn, Rosen et al. 1987). Different EEG patterns can be observed with increasing anesthetic concentrations.

In humans (Kiyama and Takeda 1997; Gajraj, Doi et al. 1998) and in animals (Antunes et al. 2003; Otto 2008) the analysis of the EEG with methods based on power spectrum analysis showed no correlation with depth of anesthesia. However, in humans the Bispectral Index (BIS) is used very successfully to provide a scale of hypnosis. In veterinary medicine, the efficacy of BIS monitoring in assessing the depth of anesthesia in dogs (Muir , Wiese et al. 2003), cats (March and Muir 2003) and pigs (Martin-Cancho, Lima et al. 2006) has been studied, but in animals the correlation between EEG depression and the BIS index is unclear, especially when burst suppression is observed after the administration of large anesthetic doses (March and Muir 2005). This may reflect the fact that "BIS' calculations use data collected from 5000 human EEG studies collected during anesthetic procedures (Johansen and Sebel 2000) which makes its applicability difficult between species.

The Cerebral State Monitor (CSM) is a fuzzy logic based analysis from the EEG monitor and has been used for monitoring the depth of anesthesia in humans (Jensen, Litvan et al. 2006; Hoymork, Hval et al. 2007). The main advantage of this method is that because the relationship between the EEG and the clinical state cannot be easily modeled by a mathematical function, the fuzzy logic analysis has the potential to offer a better alternative to establish this relationship (Jensen, Litvan et al. 2006). In a previous study carried out in our laboratory (Ribeiro, Ferreira et al. 2008) an intravenous (IV) propofol bolus of 6 mg kg^{-1} resulted in a progressive decrease in Cerebral State Index (CSI) values displayed by the CSM, and a slight negative correlation between the plasma target concentration (PropCp) and CSI was observed. This suggested the possibility that CSM could be useful in monitoring depth of anesthesia in dogs. However, in a subsequent study in which propofol was given by a continuous infusion of 200 mL $hour^{-1}$ to induce anesthesia to varying clinical depths, the CSI was not

consistent with clinical observations of the depth of anesthesia reached during these infusions (Ribeiro, Ferreira et al. 2009). The objective of this current study is to evaluate CSM correlation with different estimated propofol steady state plasma concentrations and clinical anesthetic planes in dogs during maintenance of anesthesia.

3.3.3-Methods

All procedures were approved by a local ethics committee, and fully informed consent for data collection, for altering the planes of anesthesia and for enrolment in research was given by the owners.

3.3.3.1-Dogs and hemodynamic monitoring

We previously reported the CSM performance during induction of anesthesia with propofol in 14 dogs (Ribeiro, Ferreira et al. 2009). This current report is of the continuation of anesthesia with propofol in these subjects. All dogs were scheduled for a routine neutering procedure and were considered healthy based on clinical history, clinical examination and blood analysis (packed cell volume, total protein and blood urea).

All dogs were premedicated with 0.05 mg kg^{-1} acepromazine IM (Calmivet®, Vetoquinol, France) 30 minutes prior to the beginning of the induction of anesthesia. A cannula (18 gauge) was inserted in the cephalic vein for drug and fluid administration. A three way stopcock was used to connect the IV catheter to the propofol and sodium chloride 0.9% delivery lines. Sodium chloride at a constant infusion rate of 10 mL kg^{-1} $hour^{-1}$ was administered during the entire study period via an infusion pump.

A S/5 Datex monitor (Datex-Ohmeda, Finland) was used for monitoring the hemodynamic parameters. Blood pressure was measured non-invasively in the cranial metatarsal region of the left hindlimb, and mean arterial pressures are reported. Cuff width was chosen to be around 40% of circumference of the limb. Three measurements were obtained and used to calculate the average value made immediately after the preceding one (Datex S/5 "STAT" mode). A lead II electrode ECG was monitored.

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3.3.3.2-Cerebral state index monitoring.

The CSM used was the same as that employed in the previous studies (Ribeiro, Ferreira et al. 2008). The electrodes were already in position for the induction of anesthesia (Ribeiro, Ferreira et al. 2009) and were left in place. The bipolar EEG waveform in our study was derived from the signal recorded between the frontopolar electrode (positive),

and the occipital electrode (negative), as described by (Pellegrino and Sica 2004). The ground or the reference electrode was placed in the parietal position (Fig.25).

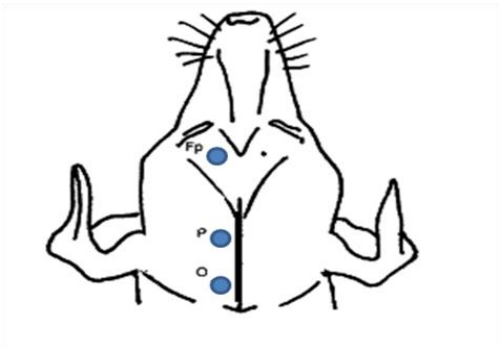

Figure 25-Electrodes placement for EEG recording

Dorsal view of canine cranium showing the placement of the EEG recording electrodes.
Fp (frontopolar electrode), P (parietal electrode) and O (occipital electrode);

A modified ECG cable with clamp electrodes, connected to the CSM cable, was used to provide these electrodes. Before the application of clamp electrodes the skin was cleaned with alcohol 5 minutes before the data collection to allow drying.

The CSM displays the EEG signal continuously, and also four parameters derived from EEG analysis, CSI, BS, EMG and SQI. Details of how these parameters are derived has been described fully in our previous work (Ribeiro, Ferreira et al. 2009). CSI is based on a combination of four sub-parameters of the electroencephalographic signal: β ratio, α ratio, β-α ratio and burst suppression (BS). The CSI is displayed in a scale from 0 to 100 that represents the different depths of anesthesia. The BS is defined as the percentage of time in a 30-second window where the amplitude of the electroencephalographic signal was less than 3.5μV. These EEG periods characterise the deepest levels of hypnosis. The SQI is calculated using the quality of the acquired

EEG signal and on the signal artefacts during the previous 1-minute period. The electrode-to-skin impedance is included in the SQI calculation. High levels of facial muscular or electromyographic (EMG) activity can interfere with the CSI under certain circumstances. The monitor incorporates an EMG filter that removes most of the potential interfering EMG activity. The EMG bar shows the energy of the EMG level in the 75–85 Hz frequency band (0–100 logarithmic scale).

The EEG data collection ended before the patient was moved to the appropriate position for the commencement of surgery.

3.3.3.3-Anesthetic protocol and clinical observations

RugLoop II software (developed by Tom DeSmet (Demed Engineering, Belgium) and Michel Struys (Ghent University, Belgium)) running on a personal computer was used to drive the propofol syringe pump. The pharmacokinetic model incorporated in the RugLoop II software and used to estimate the propofol plasma concentrations was that of Beths et al. (Beths, Glen et al. 2001). RugLoop II was also used to store the pharmacokinetic, hemodynamic and electroencephalographic data every 5 seconds.

Thirty minutes after premedication, anesthesia was induced with propofol 1% (Fresenius Kabi; Germany) using a constant rate of infusion (CRI) of 200 mL $hour^{-1}$ until various planes of anesthesia were achieved. CSI was measured during this induction as previously described (Ribeiro, Ferreira et al. 2009). Once this previous study was completed, the trachea was intubated and the dogs were mechanically ventilated with 100% oxygen in order to maintain the end-tidal CO_2 between 35-45mmHg (4.6-6 kPa). All dogs were positioned in left lateral recumbency during the entire study period. The propofol target control infusion (TCI) was then initialized by switching the infusion mode in the RugloopII from CRI to PropCp. An initial plasma target of 3 µg dL^{-1} was set. It required (mean ± SD) 9.5 ± 2.4 minutes for the initial high plasma concentrations of propofol achieved during induction of anesthesia to decrease. During this period the Rugloop II software control stopped the infusion and when the estimated correct value was reached, the pump was automatically re-started and the plasma target of 3 µg dL^{-1} was maintained for 5 minutes. At the end of this 5-minute period, reflex testing to assess anesthetic depth commenced.

Reflex testing was as described in our previous study (Ribeiro, Ferreira et al. 2009). The palpebral and corneal reflexes of the right eye were tested three times with a swab damped with serum. The reflexes were considered negative if there were no response to these three stimulations. The palpebral reflex was tested gently at the lateral canthus of the eye, and the corneal reflex was elicited by a smooth pressure on the cornea. The eyeball position was observed. The ocular reflexes were grouped in anesthetic 'planes' of depth, A to E as described previously (Ribeiro, Ferreira et al. 2009) and in table 10.
Once the reflexes had been tested, the PropCps were increased in 1µg dL^{-1} steps every 5 minutes to 7µg dL^{-1}, and then increased to 9 µg dL^{-1}, then to 11µg dL^{-1} . These PropCps were each maintained for 5 minutes after each step increase. The same procedures were applied for evaluating the reflexes at the end of each 5 minute step. If mean arterial blood pressure decreased below 60 mmHg, the propofol administration was stopped.
After the end of the study a TCI of 3µg dL^{-1} was set and a bolus of fentanyl 5 µg kg^{-1} was administered before surgery commenced. At the end of the surgery carprofen 4 mg kg^{-1} SC and a single dose of morphine 0.4 mg kg^{-1} SC were administered.

3.3.3.4-Data and statistical analysis.

The efficacy of electroencephalogram parameters to predict depth of anesthesia was evaluated using prediction probability (PK). The mathematical basis of PK was as described by Smith *et* al (Smith, Dutton et al. 1996). A PK value of 1 means that the values of the predicting variable, *e.g.*, anesthetic depth indicator, correctly predicts the value of the observed anesthetic depth. A PK value of 0.5 means that the values of the indicator predict no better than a 50–50 chance. The PK calculation was performed using the program PKMACRO.
Data for heart rate, mean arterial pressure and CSM data (CSI, BS, SQI and EMG) are described as means ± SD for each anesthetic plane. Data showed as a box plot: 25th and 75th percentile are the boxes' borders; whiskers are the lowest and highest values for the 5th and 95th percentiles, respectively. The time delays for the CSI calculation ranged from 53 to 55 seconds (Pilge, Zanner et al. 2006). In our analysis, a delay of 50 seconds in CSI data was taken into account when analyzing CSI data.

3.3.4-Results

The subjects were 14 dogs, of mixed breeds, 8 females and 6 males, aged 2.6 ± 1 years, and weight 24.5 ± 4.67 kg. One dog was excluded from data analysis because it showed an anomalous EEG flat line throughout the whole study period.

Two dogs required initial PropCp doses of 5 and 6 µg dL^{-1} respectively to maintain anesthesia, as the target 3 µg dL^{-1} was insufficient. Depth of anesthesia in one dog did not reach anesthetic plane E even at 11 µg dL^{-1}.

The plane of anesthesia (as defined by Table 10) increased with administration of increasing PropCp, but the five separate planes were not observed in all dogs. In most such cases depth of anesthesia increased, missing a plane (eg. from C to E). However, in one dog the plane decreased from D to C despite an increased PropCp.

The hemodynamic and electroencephalographic data and its relationship with the identified anesthetic planes are shown in Table 11. The results demonstrate that CSI distinguished plane C and E from the baseline (awake). Only four dogs passed through plane D which impaired the analysis. EMG decreased with the depth of anesthesia, and at plane E was minimal.

The PK for CSM parameters related with anesthetic planes and PropCp are described in Table 12. The prediction probability (PK) for CSM parameters to be related with anesthetic planes were at intermediate values in the region of 0.75. Thus the monitor did not always predict increases or decreases in the depth of anesthesia. The lowest probability observed was between CSI and PropCp; the PK between PropCp and anesthetic planes was higher (PK=0.78). The highest value for PK was for BSR (PK =0.91) related to anesthetic planes. When BSR was analyzed with PropCp the PK decrease to a value of 0.71. At deep levels of anesthesia the CSI was sensitive to burst suppression, 12 dogs reached the plane E and eight of them showed burst suppression (BS) the percentage BS during plane E was on average 11%. Burst suppression was also observed in planes C and D in two dogs with low values, less than 3%.

Figure 2 demonstrates the relationship between the CSI and clinically assessed anesthetic planes. The CSI showed a tendency to decrease with increasing propofol concentrations and increased anesthetic depth. However it did not differentiate the intermediate anesthetic planes. Although a great difference was observed in plane E - the deepest levels of anesthesia, considerable overlap between CSI values obtained at the different anesthetic planes was observed.

Table 10-Anesthetic planes identified with increasing PropCp (target propofol plasma concentrations) in 13 dogs, in which anesthesia was first induced, then maintained by propofol CRI.

Anesthetic planes	Ocular reflexes
A	PR+/EC/CR+
B	PR+/ERV/CR+
C	PR-/ERV/CR+
D	PR-/EC/CR+
E	PR-/EC/CR-

The anesthetic planes were allocated a letter A to E, according to the anesthetic depth based on the presence (+) or absence (-) of ocular reflexes and the position of the eye: palpebral reflex (PR), corneal reflex (CR) eyeball centred in the eye (EC) and eyeball rotated ventrally (ERV).

Table 11-Hemodynamic and electroencephalographic data observed before induction of anesthesia (baseline) and when the dogs were at anesthetic planes A to E.

	CSI	EMG (%)	BS (%)	SQI (%)	HR(bpm)	MAP(mmHg)
Baseline	89 ± 3	94 ± 11	0.0	63 ± 21	101 ± 30	98±14
Anesthetic planes						
A	88 ± 13	68 ± 27	0.0	87 ± 8	99 ± 23	91 ± 23
B	79 ± 17	52 ± 31	0.0	78 ± 11	88 ± 19	84 ± 15
C	57 ± 16	20 ± 30	0.1 ± 0.4	82 ± 14	87 ± 20	83 ± 13
D	59 ± 18	24 ± 22	0.8 ± 1.7	78 ± 17	98 ± 25	88 ±15
E	42 ± 14	6 ± 16	11.2 ± 18.8	88 ± 10	94 ± 17	79 ± 17

CSI (Cerebral state index), EMG (Electromyography), BS (Burst suppression), SQI (Signal quality index), HR (Heart rate) and MAP (Mean arterial pressure). All data is mean ± SD

Table 12-Prediction probability (PK) for observed anesthetic planes and for target propofol plasma concentrations (PropCp)

	PK for anesthetic planes (see	**PK for PropCp**

Table 1)		
CSI	**0.74**	**0.65**
BSR	**0.91**	**0.71**
EMG	**0.76**	**0.65**
PropCp	**0.78**	

CSI (Cerebral state index), BSR (Burst suppression ratio) and EMG (Electromyography) .

Figure 26

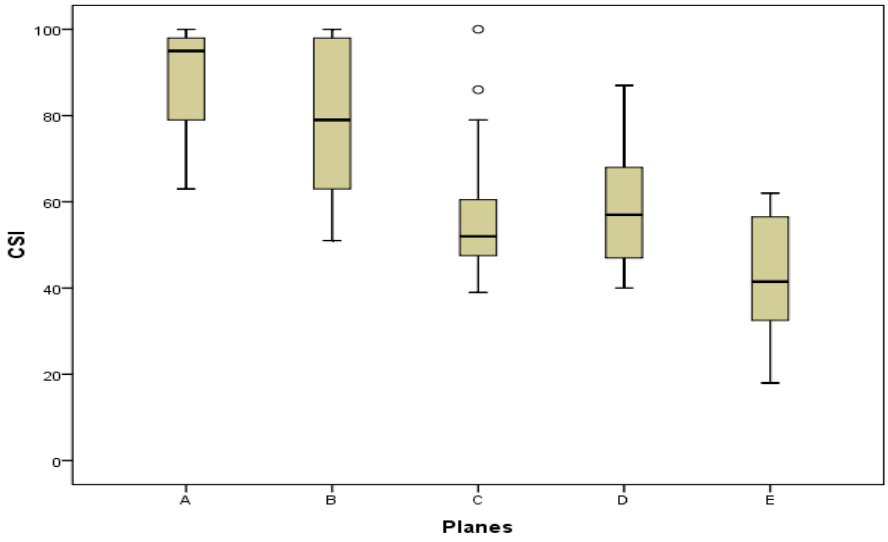

Figure 26-Relationship between anesthetics planes A, B, C, D and E, and CSI.

"o" represents the outliers. Data are presented as a box plot: 25th and 75th percentile are the boxes' borders; whiskers are the values for the 5th and 95th percentiles. Overlap between CSI values obtained in different anesthetic planes is observed.

3.3.5-Discussion

The CSM calculates the CSI and uses four subparameters derived from the time domain analysis (burst ratio) and frequency domain analysis (α-ratio,β-ratio, β-ratio - α-ratio) of the EEG. To deliver an index these subparameters are used as inputs to a fuzzy logic analysis. Fuzzy logic is a problem-solving control system methodology that incorporates a simple, ruled-based "If X and Y then Z" approach to solving a control problem rather than attempting to model a system mathematically (Jensen, Litvan et al. 2006).

In humans the CSM monitor has been studied using the Modified Observer's Assessment of Alertness and Sedation Scale (OAA/S) (Jensen, Litvan et al. 2006). However, in veterinary practice there is no sedation scale accepted universally, and studies are difficult to perform in animals using loss of consciousness or loss of righting reflexs as assessment points without interfering with the collection of the EEG signal. In our previous study we identified a scale of anesthetic depth based on ocular reflexes (Ribeiro, Ferreira et al. 2009). In this earlier study in which propofol was infused at a constant rate to induce anesthesia and then to increase depth to the planes of anesthesia identified in the scale, results with the CSM monitor were disappointing as, although there was some correlation between CSM and estimated propofol plasma concentrations, the CSM did not predict anesthetic planes accurately. As it is possible that this failure was because a steady state propofol concentration had not been reached at the effector cells, the objective of this current continuation study was to re-evaluate the CSM performance, this time during maintenance of anesthesia with propofol with different target concentrations. A monitor of depth anesthesia can only be called accurate if it (1) provides an accurate correlation with cerebral drug concentration, (2) correlates with the clinical state of the patients, and (3) informs the clinician when excessive levels of anesthesia are present (Jensen, Litvan et al. 2006). In our study, these three aspects were analyzed.

The first condition for CSM was not met as the PK value was 0.65 which means that the monitor did not always predict increments or decrements in the amount of propofol. The analysis was carried out 5 minutes after propofol was anticipated to have reached the selected plasma target concentration. At the end of each propofol stage, it was assumed that the PropCp was similar to the propofol concentration at the effect site This

assumption was based on the half-life for the propofol plasma-effect-site equilibration being around 2.3 minutes for humans (Kazama, Ikeda et al. 1999). Musk et al in studies with TCI allowed three minutes for the equilibration between blood and drug site action(Musk, Pang et al. 2005).

A potential reason for the poor correlation between CSI and PropCp in this study compared with the previous work (Ribeiro, Ferreira et al. 2009) is that a different method of statistical analysis was employed. A further reason could be the application of Beths' pharmacokinetic model (Beths et al. 2001). The model is derived from a population sample of dogs, with its inevitable variations between real and target plasma concentrations of propofol. In humans a 20-30% variation between real and target propofol plasma concentration can be acceptable clinically for TCI use (Coetzee, Glen et al. 1995; Fechner, Albrecht et al. 1998). However, the PropCp based on Beths' pharmacokinetic model could be even more variable as a result of the great breed variability in the dog population. It important to refer that in the Beth`s model the performance of the TCI was evaluated with the administration of an opioid and in present study the acepromazine alone was used for premedication(Beths, Glen et al. 2001).

In our study no analyses were performed to measure the real propofol concentration in the blood.

Regarding the second condition, the present study showed that the sequential increase of the anesthetic depth from plane A to E was followed by a decrease in CSI values, but the CSI did not distinguish accurately between the anesthetic planes B, C and D as the CSI values were overlapped. Although CSI seems to provide useful information at the deepest levels of anesthesia when burst suppression occurs; its clinical usefulness was limited because it could not provide reliable clinical information during intermediate levels of anesthesia.

EMG activity is the most significant source of EEG artefact (Jensen, Litvan et al. 2006). The influence of EMG in dogs may be particularly important due to the amount of muscles in the head compared with humans. The influence of EMG on the CSI values was a constant observation in our study and could be another reason for the low values obtain for PK.

The CSM appeared to detect the excessive levels of anesthesia that were reached at plane E. Some studies in humans also suggest that the CSI has a better performance than BIS in detecting deep levels of anesthesia adequately (Jensen, Litvan et al. 2006).

Burst suppression refers to isoelectric periods interrupted by brief intervals of high amplitude EEG activity. An isoelectric EEG pattern is continuous, uninterrupted by burst activity. This pattern is developed during deep anesthesia (Rampil 1998; Greene, Benson et al. 2002). In dogs, an increase in suppression ratio could lead to paradoxical increases of BIS values (BIS>60) (March and Muir 2005). Nevertheless, this was not observed in our study, in which burst suppression was observed frequently during plane E at the time of the lowest CSI values. During burst suppression, the α and β ratio are no longer monotonously decreasing as a function of anesthetic depth and therefore they cannot be used in the calculation of the final index (Jensen, Litvan et al. 2006).

The hemodynamic parameters were monitored in this study to ensure that the dogs were not harmed by the increasing depth of anesthesia. However, they provided very little information regarding depth of anesthesia. It is known that propofol decreases the arterial blood pressure by central depression of sympathetic neural output, which results in a decrease in systemic vascular resistance and potential hypotension (Clayes, Gepts et al. 1988). The velocity of the propofol infusion is also a very important factor influencing hemodynamic effects (Brás, Bressan et al. 2009). In our study, dogs showed a surprisingly stable hemodynamic profile, the maximum decrease of MAP being 24% from baseline in plane E. This hemodynamic stability when propofol is infused may limit the usefulness of hemodynamic data for monitoring depth of anesthesia and strengthen the need for a EEG based monitor for veterinary use, but in this current study no painful stimuli were administered so the cardiovascular response to surgery was not investigated.

In conclusion, this study evaluates the use of the CSM in detecting depth of anesthesia with propofol as scaled in five previously identified anesthetic planes (Ribeiro, Ferreira et al. 2009). Although there is some prediction probability between CSI values and the anesthetic planes, the CSI showed clinically important limitations as it was not able to distinguish between anesthetic planes B, C, and D (ie those depths likely to be used in clinical practice), as CSI values overlapped. Although CSI was able to detect deeper levels of anesthesia when burst suppression occurs, it would be hoped that such deep levels would not normally be reached. However, the burst suppression was a consistent finding in deep planes of anesthesia, and this fact could be relevant for the use of Fuzzy logic analysis in future monitors.

Chapter 4

4.1-Global summary of results

In general the results from this thesis point for limited capacity of the CSM monitor to detect accurately different anesthetic planes representing clinical signs observed and different concentrations of propofol.

Although CSI seems to be able to detect very deep levels of anesthesia when burst suppression occurs, it would be expected that such levels would not normally be reached under general anesthesia.

Anesthestic planes observed during the studies seems to be a useful tool in the future to assess the performance of brain EEG based monitors

In particular the results of these studies represent different ways to evaluate the performance of the CSM monitor under different protocols for propofol administration. Furthermore, in the latter two studies the monitor was also evaluated based on clinical endpoints using ocular reflexes based on the developed visual clinical scale. Specific results from this work may be divided in the following headings:

4.1.1-CSI response to predicted plasma propofol concentration infused

In the first study during the induction of anesthesia with a propofol bolus of $6mgKg^{-1}$ a significant negative correlation was observed between CSI and PropCp (correlation coefficient of -0.57; *P<0.01)*. In the second study when a propofol $200ml^{-1}$infusion was used to achieved the anesthetic planes desired, no correlation was observed between CSI and PropCp (R=0,47).In the third study, during maintenance of anesthesia with different target controlled infusions of propofol, the PK between CSI and PropCp was 0,65. All these results represent a poor correlation between the CSI and the estimated drug concentration.

4.1.2- CSI relation with clinical end points

The results obtained from the ocular reflexes analyses indicated five possible clinical levels of anesthetic depth (A to E). These anesthetic planes were essential for developing the first visual objective clinical scale to monitor depth of anesthesia in dogs under general anesthesia with propofol, and to evaluate the CSM monitor performance in the subsequent studies.

In the second study, during induction of anesthesia with propofol 200ml/h, no significant correlation were observed between CSI and anesthetic planes despite the CSI decreases from baseline to plane E. Nevertheless, it was observed a strong correlation, or prediction probability between anesthetic planes of the clinical scale and PropCp (R=0.887; *P<0.01*).

In the third study, the PK between PropCp and anesthetic planes was high (PK=0,78). Prediction probability showed intermediate values near 0,75 between CSM parameters and anesthetic planes. This means that the monitor did not always predict increments or decrements in the level of anesthesia.

4.1.3-CSM monitor performance evaluation at deep levels of anesthesia.

The detection of the deep levels of anesthesia and burst suppression is one of the biggest virtues of the CSM monitor when applied to dogs.

In the first study only three dogs present burst suppression, related with the lower values of CSI. In the second study, in plane E, all dogs lost their palpebral and corneal reflex, and were intubated with laryngeal reflex absent. At this time point lower EMG values associated with lower CSI values were observed. With the exception of one dog that have burst suppression in plane C and D, the BSR was only observed in plane E.

In the third study the highest value observed for PK was between BSR (PK =0,91) and anesthetic planes. When BSR was related to PropCp, the PK decreased to 0,71. At deep levels of anesthesia the CSI was sensitive to burst suppression: twelve dogs reached the plane E, and CSM detected the excessive depth of anesthesia in eight of them, by revealing an average of 11% of burst suppression (BS). Burst suppression was also observed in Planes C and D in two dogs with BS values less than 3%.

The influence of the EMG in the CSI values was a constant finding during all studies, and it seems to be the main reason for the high standard deviation observed in the CSI values during the anesthetic planes C, D and E.

In the first study EMG values higher than 30% were observed in seven dogs at maximum PropCp, and in five dogs at minimum CSI values.

In the third study was interesting to verify that EMG decreases with the increment in depth of anesthesia, and showed a high correlation with CSI (R=0,80).

Hemodynamic variables were not a concern and did not influence the studies of anesthetic depth. In the first study there was a 13% decrease in mean arterial pressure (*P<0.05*) and a 17% increase in heart rate (*P<0.05*) between awake values and those

values recorded at maximum PropCp. In the second study mean arterial blood pressure decreased by 16 % from the baseline to plane E ($P<0.05$). Heart rate did not change significantly during the study. In the third study mean arterial blood pressure and heart rate did not change significantly, with the exception for MAP in plane C were the difference was significantly ($P<0.05$) from the baseline. Nevertheless, mean arterial blood pressure did not decrease below the minimum threshold value of 60 mmHg in any study, which could have compromised the cerebral autoregulation and influence the results

Capítulo 4

4.1.Resumen global de los resultados

Los resultados obtenidos por nosotros muestran que la capacidad del monitor CSM es muy limitada cuando queremos determinar con precisión diferentes planos anestésicos y que además nos los relacione con los signos clínicos observados cuando utilizamos diferentes concentraciones de propofol en la anestesia de perros.

Aunque el CSI se muestra efectivo en el plano más profundo de la anestesia (plano E), cuando se produce la BS, este plano anestésico no se alcanza normalmente en las anestesias que hacemos en la clínica diaria.

Los resultados concretos de esta memoria podemos agruparlos en los apartados siguientes:

4.1.1- Respuesta del CSI a la concentración plasmática previsible de propofol.

En el primer estudio durante la inducción de la anestesia con un bolo de propofol de $6mgKg^{-1}$ se observó una correlación negativa significativa entre CSI y PropCp (coeficiente de correlación de-0.57; $P < 0,01$).

En el segundo estudio cuando se utilizo una infusión de propofol de 200 ml^{-1} para alcanzar los planos anestésicos deseados, no se observó ninguna correlación entre CSI y PropCp ($R = 0, 47$).

En el tercer estudio, durante el mantenimiento de la anestesia con infusiones de distintas concentraciones de propofol, el PK fue 0,65 cuando se estudio la relación del CSI con PropCp.

Todos estos resultados representan una baja correlación entre la CSI y la concentración estimada de propofol.

4.1.2- Relación de CSI con los reflexos oculares observados en los perros.

Lo primero fue establecer una escala visual de los reflejos oculares para relacionarlos con los cinco planos de profundidad anestésica (A, B, C, D, E), cuando anestesiábamos a perros con propofol.

En el segundo estudio, durante la inducción de la anestesia con propofol 200 ml/h, no se observó ninguna correlación significativa entre CSI y planos anestésicos a pesar de las disminuciones CSI desde el plano A hasta al plano E.

Sin embargo, se observó una fuerte correlación, o probabilidad de predicción entre planos anestésicos de la escala clínica y la PropCp ($R = 0.887$; $P < 0,01$).

En el tercer estudio, la PK entre PropCp y los planos de anestésicos fue alta (PK = 0, 78).

El uso de PK mostró valores cercanos al 0,75 cuando estudiamos la relación entre CSI con planos de anestésicos. Esto significa que el monitor no siempre es capaz de indicarnos incrementos o disminuciones del nivel de anestesia.

4.1.3- Evaluación de desempeño de monitor CSM en la anestesia profunda.

La detección de niveles de anestesia y la BSR es una de las mayores virtudes del monitor CSM cuando se aplica a los perros.

En el primer estudio sólo tres perros presentan BSR, relacionada con los valores inferiores de CSI.

En el segundo estudio, en el plano E, todos los perros pierden su reflejo palpebral y corneal y fueron intubados con reflejo laríngeo ausente. En esta fase los valores EMG más bajos están asociados con los valores más bajos de CSI.

Con la excepción de un perro que presentaba BSR en los planos C y D, el BSR sólo se observó en el plano E en los demás perros.

En el tercer estudio el valor más alto observado de PK (PK = 0,91) fue para BSR relacionados con los planos anestésicos. Cuando BSR se analizó relacionadolo con la PropCp, el PK disminuyó a 0,71.

En niveles de anestesia profundos el CSI es sensible a la BSR. Doce perros alcanzaron el plano E, y el CSM (Cerebral State Monitor) detecto la excesiva profundidad de

anestesia en ocho de ellos, presentando un promedio del 11% BS. La BS también se observó en los planos C y D en dos perros con valores inferiores a 3%.

La influencia del EMG en los valores de CSI fue una constante en todos los estudios, asi el primer estudio se observaron valores de EMG superiores al 30 % en siete perros en PropCp máximo y en cinco perros en valores mínimos de CSI.

En el tercer estudio fue interesante comprobar que el EMG disminuye con el incremento de la profundidad de la anestesia y mostro una alta correlación con el CSI (R = 0, 80).

Las variables hemodinámicos no eran objeto prioritario de estudio en estos trabajos y los resultados obtenidos confirman que las variables hemodinámicas no estaban relacionadas con la profundidad anestésica.

En el primer estudio, hubo una disminución de 13% en la presión arterial media ($P < 0,05$) y un aumento de 17% en la frecuencia cardiaca ($P < 0,05$) cuando se compraban los valores medidos en los animales despiertos y los valores registrados en PropCp máxima.

En el segundo estudio la presión arterial media disminuía un 16% cuando se comparaban los valores obtenidos cuando el animal estaba sin anestesia con los obtenidos cuando el animal estaba en el plano E ($P < 0,05$). La frecuencia cardíaca no cambió significativamente durante el estudio.

En la tercer estudio la presión arterial y ritmo cardíaco no cambiaron significativamente en los diferentes planos de anestesia., con la excepción de la presión arterial media en el plano de anestesia C, con una diferencia fue significativa ($P < 0,05$) respecto al valor medido al comienzo de la inducción anestésica. Sin embargo, cuando la presión arterial cayo por debajo de 60 mmHg , ese animal se elimino del estudio.

Chaper 5

5.1 General discussion

In current veterinary anesthesia one of the "Holy Grail" is to find a monitor to assess the depth of anesthesia. The deleterious effects of the awareness in companion animal are difficult to establish (Haskins 1996), but these events could modify the behavior and the quality of life for animals and it is an important welfare matter. The evaluation of the depth of anesthesia based on clinical observation, ocular reflexes, movement, mandibular tonus is completely useless when neuromuscular blocking agents are being used. Thus, the accurate assessment of the depth of anesthesia will be a major contribute to the standardization of animal care, in clinical practice and in research aiming at achieving an adequate anesthetic protocol.

At the beginning of the time frame of this thesis the CSM had recently been introduced as an intra-operative monitor of anesthetic depth (Zhong, Guo et al. 2005). The basic CSM analysis uses the collected EEG as input variables to a fuzzy logic analysis. This methodological approach seems to be the best way to quantify the depth of anesthesia in dogs with a previously developed human monitor because this monitor establishes the relationship between variables based on a non-restricted mathematical structure. The data is analyzed with no prior assumption of the mathematical relationship which means that the monitor does not assume an underlying mathematical model governing the relationship between the anesthetic drugs and the response effect (Gambus, Jensen et al. 2010). Another reason to use this monitor was the fact that this was a novel small handheld monitor that derives the EEG from three forehead electrodes, but with a connector that makes it possible to use ordinary ECG electrodes with snap connectors. This allows an easy application and it is also an inexpensive way of using electrodes in clinical practice (Seitsonen, Yli-Hankala et al. 2000).

The efficacy of depth of anesthesia monitoring devices are not the same for the quantification of sedation, measuring the effect site concentration of anesthetics and detection the level of consciousness (Bruhn, Myles et al. 2006). These variables require different calibration points and scales that should be analyzed based on a graded dose of anesthetics.

In order to evaluate the applicability of the CSM monitor to veterinary anesthesia, some requirements have to be present: a) accurate correlation with cerebral

drug concentration, b) accurate correlation with the clinical state of the patients, and c) to inform the clinician when excessive levels of anesthesia are present (Jensen, Litvan et al. 2006). In the present thesis all of these aspects were analyzed.

The effect of the propofol in the brain is expected to gradually augment with increasing drug concentrations, which should be directly reflected on the EEG index. However, in all of our studies this was not exactly observed. The highest value had a predicted probability of 0,65 for the relation between CSI values and the PropCp during the maintenance of anesthesia with different PropCp. It must be taken into account that PK value of 1.0 means an exact prediction, while a PK of 0.5 is no better than a 50:50 accuracy. Thus, the PK value of 0,65 is considered a low value for prediction probability in this case.

A potential reason for the poor correlation between CSI and PropCp in our studies could be the application of the Beth´s model. This model is depicted from a dogs' population sample, with its inevitable variations between real plasma concentrations of propofol and plasma target concentration (PropCp). In humans it was observed that 20-30% variations between propofol plasma concentrations and PropCp could be clinically acceptable for TCI use (Coetzee, Glen et al. 1995; Fechner, Albrecht et al. 1998). Although one of the main limitations during this study could be the lack of quantification of the real propofol concentration in the blood, the PropCp used were estimated by a reliable pharmacokinetic model used in several international scientific publications.

The PropCp had better correlation and predicted probability with clinical endpoints, rather than with CSI which could be a safe way of monitoring depth of anesthesia while there are no reliable depth of anesthesia monitors for veterinary use..

In the discussion of our results, we must take into account that when a human monitor is used to evaluate the dogs EEG patterns the accuracy could be diminished. For example, when the BIS monitor was applied to monitoring the depth of anesthesia in pigs, it seems to interpret burst suppression in the EEG as an indication that the animal is waking up, showing high BIS values (Greene, Benson et al. 2004). Another factor that may be related to this poor correlation is the EMG, which is the most significant source of EEG artifact. Variations in EMG activity are associated with rapid changes in BIS values in dogs (Greene, Benson et al. 2002). Despite the fact that the CSI algorithm also incorporates an EMG filter that removes most potentially interfering EMG activity, the EMG still has a significant influence on the CSI algorithm (Jensen,

Litvan et al. 2006). The influence of EMG in dogs may be particularly important due to the amount of muscles in the head compared to humans. The influence of EMG on the CSI values was a constant observation in our study and it seems to be the main reason for the high standard deviation observed in the CSI values in the second study. In the third study it was observed a PK of 0,8 between EMG and CSI.

When the performance of the CSM monitor was evaluated during induction of anesthesia, and based on the clinical state of the patient it was not observed a significant correlation between CSI and anesthetic planes. However, when dogs were anaesthetized with TCI, a sequentially increase in the anesthetic depth from plane A to E followed by a decrease in CSI values was observed. The PK obtained was 0,74 which means that CSI cannot distinguish accurately the anesthetic levels B, C and D because CSI values were overlapped at different anesthetic planes. The main reason for the differences between both studies may be related with the influence of EMG on the CSI values, because during induction of anesthesia at 200ml/h higher EMG values were observed in planes C, D and E, than in the third study where TCI was used.

It is fundamental for the clinician to know when excessive levels of anesthesia are present, and burst suppression plays an important role in this context. Burst suppression refers to isoelectric periods interrupted by brief intervals of high amplitude EEG activity. This pattern is developed during deep anesthetic levels (Rampil 1998; Greene, Benson et al. 2002). The burst suppression was frequently observed during plane E in both studies, and was associated with the lowest CSI values when the majority of the dogs lost their corneal reflex. In fact, it is important to highlight the 0.91 PK value observed between BSR and anesthetic planes.

The hemodynamic parameters monitored in this study provided very little information regarding depth of anesthesia when using propofol. It is known that propofol decreases the arterial blood pressure by central depression of sympathetic neural output, which results in a decrease in systemic vascular resistance and potential hypotension (Clayes, Gepts et al. 1988). The velocity of the propofol infusion is also a very important factor that influences the propofol hemodynamic effects (Brás, Bressan et al. 2009). In our studies, dogs showed stable hemodynamic profile The good hemodynamic stability provided by propofol limits the usefulness of hemodynamic data for monitoring depth of anesthesia, and strengthens the need for an EEG based monitor for veterinary use.

Capítulo 5

5.1 Discusión general

En anestesia veterinaria el encontrar un monitor que nos mida la profundidad anestésica de forma eficiente y fiable se ha convertido en la búsqueda del "Santo Grial".

Es imposible la evaluación de la profundidad de la anestésica basada en la observación clínica, reflejos oculares, movimiento ocular, tono mandibular, etc., cuando se utilizan bloqueantes neuromusculares.

Por lo tanto, la evaluación de objetiva de la profundidad anestésica será una gran ayuda en la normalización del cuidado de los animales, en la práctica clínica y en la investigación para garantizar el adecuado protocolo anestésico.

Al comienzo de esta tesis el CSM se había introducido recientemente como un monitor intraoperatorio de profundidad anestésica (Zhong, Guo et al 2005).

El análisis básico del CSM utiliza las variaciones del electroencefalograma durante la anestesia, que después son analizadas según de la lógica difusa. Este enfoque metodológico parece ser la mejor manera de cuantificar la profundidad de la anestesia en perros, utilizando un monitor humano previamente desarrollado, porque el monitor establece la relación entre las variables basándose en una estructura matemática no restringida. (Gambus, Jensen et al 2010).

Otra razón por la que elegimos este monitor fue que es pequeño, que se transporta con facilidad, y que solo utiliza tres electrodos en la obtención del EEG, siendo además posible el utilizar electrodos de ECG normales, de fácil aplicación y baratos (Seitsonen, Yli-Hankala et al 2000).

La eficacia de los dispositivos que monitorizan la profundidad de la anestesia, no es la misma para la cuantificación de sedación,que para medir la concentración del fármaco en el "effect site" de los anestésicos y para detección del nivel de conciencia (Bruhn, Myles et al 2006). Cada una de estas variables requiere puntos de calibración diferentes.

Para evaluar la aplicabilidad del monitor CSM en anestesia veterinaria, los valores obtenidos del Índice deben cumplir algunas premisas:

a. Exacta correlación del Índice con la concentración del fármaco en el cerebro,

b. Exacta correlación del Índice con el estado clínico de los pacientes.
c. El Índice obtenido debe indicar al clínico cuando existen niveles excesivos de anestesia, o cuando aumenta o disminuye la profundidad de la anestesia. (Jensen, Litvan et al. 2006).

En la presente memoria presentada para obtener el grado de doctor se analizaron todos estos aspectos.

Esperábamos que el efecto de propofol en el cerebro aumentara gradualmente con el aumento de las concentraciones del fármaco, y que por otro lado, estos cambios se debierían reflejarse directamente en el índice obtenido a partir de los cambios del EEG. Sin embargo, en todos nuestros estudios esto no se ha observó exactamente. El valor más alto tenía un PK de 0,65 para la relación entre los valores de la CSI y la PropCp durante el mantenimiento de la anestesia con diferentes PropCp. Debe tenerse en cuenta que el valor PK 1,0 significa una predicción exacta siempre, mientras que un PK de 0.5 no es mejor que una precisión de 50:50. Por lo tanto, el valor de PK de 0,65 se considera un valor bajo de probabilidad de predicción.

Una razón posible para la baja correlación entre CSI y PropCp en nuestros estudios podría ser la aplicación del modelo Beth´s. Este modelo se obtuvo con muestras de una población de perros, pero tenía inevitables variaciones entre las concentraciones en plasma real de propofol y la concentración plasmática de destino (PropCp).

En los seres humanos se observó que variaciones de un 20-30% entre las concentraciones en plasma propofol y PropCp eran clínicamente aceptables para uso TCI (Coetzee, Glen et al. 1995; Fechner, Albrecht et al. 1998).

Sin embargo nosotros seguimos pensando que una de las principales limitaciones de este estudio pudo ser la falta de cuantificación de la concentración de propofol real en la sangre, y que tendríamos que profundizar en la farmacocinética del propofol (Beths, T y col. 2001,

También debemos tener cuenta que cuando se utiliza un monitor para evaluar los patrones EEG en perros, que esta calibrado para usarse en el hombre, la precisión puede disminuir.

La PropCp tuvo una mejor correlación y un PK mas alto con los signos clínicos de la escala basada en los reflejos oculares, que con el CSI, por lo que consideramos que esta evaluación es una manera mas segura de controlar la profundidad de la anestesia mientras no encontremos un monitor fiable que sirva para medir la profundidad

anestésica para uso veterinario. Por ejemplo, cuando el monitor BIS se aplicó a la evaluación de la profundidad de la anestesia en cerdos, los resultados suministrados parecían interpretar el BS en el EEG como una indicación de que el animal se está despertando, mostrando valores elevados de índice biespectral (BIS). (Greene, Benson et al. 2004). Otro factor que puede estar relacionado con esta baja correlación es el EMG, que es la fuente más importante de artefactos de EEG, Las variaciones en la actividad de EMG se asocian con rápidos cambios en los valores de BIS en perros (Greene, Benson et al. 2002). A pesar de que el algoritmo CSI incorpora un filtro de EMG que elimina la mayoría de la actividad EMG, todavía puede interferir, y tener una influencia significativa en el algoritmo del CSI (Jensen, Litvan et al. 2006).

La influencia de EMG en perros puede ser particularmente importante debido a la cantidad de músculos que hay en la cabeza del perro en comparación con los seres humanos. La influencia de EMG en los valores del índice CSI fue una observación constante en nuestro estudio. En el tercer trabajo, se observó un PK de 0,8 entre EMG y CSI.

En el siguiente estudio se administro propofol en infusión constante de 200 ml/hora en concentración del 1%. En este trabajo se introdujeron los signos clínicos, reflejos oculares, posición del globo ocular, etc. con el fin de observar la correlación entre las concentraciones plasmáticas estimadas de propofol y los signos clínicos observados durante la inducción de la anestesia. Sin embargo el rendimiento del "Cerebral State Monitor", no se corresponde con las observaciones clínicas en los diferentes planos de profundidad anestésica.

Durante el mantenimiento da la anestesia con TCI (Target controlled infusions) en que se aumento secuencialmente el PropCp se observó un incremento de la profundidad anestésica desde el plano A al plano E, seguido por una disminución en los valores del Índice de Estado Cerebral (CSI). El PK obtenido fue de 0,74 lo significa que el CSI no puede distinguir con precisión entre los planos anestésicos, B, C y D, porque los valores del índice se superponen en diferentes planos anestésicos.

La principal razón de las diferencias entre ambos estudios puede estar relacionada con la influencia de EMG en los valores del CSI, porque durante la inducción de la anestesia com propofol a una velocidad de 200 ml/h se observaron valores EMG mayores en los planos, C, D y E, que en el tercer estudio donde se utilizó el TCI

Es fundamental para el clínico saber cuando esta en planos profundos de la anestesia y en ellos la BS (Burst supresión) desempeña un papel importante.

La BS se refiere a períodos isoeléctrico interrumpidos por breves intervalos de actividad del EEG de gran amplitud. Este patrón se desarrolla durante los niveles de anestesia profunda (Rampil 1998; Greene, Benson et al. 2002).La BS se observó con frecuencia durante el plano E en ambos estudios y se asoció con los valores más bajos del Índice (CSI), que era cuando la mayoría de los perros perdió su reflejo corneal. De hecho, es importante destacar el valor de PK 0.91 observado entre BSR y los planos anestésicos.

Los parámetros hemodinámicos analizados en esta memoria proporcionan muy poca información en cuanto a la profundidad de la anestesia conseguida con la administración de propofol. Se sabe que el propofol disminuye la presión arterial de la sangre porque produce depresión simpática central, que da como resultado una disminución en la resistencia vascular sistémica y una potencial hipotensión (Clayes, Gepts et al. 1988). La velocidad de la infusión de propofol también es un factor importante que influye en los efectos hemodinámicos producidos por el propofol (Brás, Bressan et al 2009).

En nuestros estudios, los perros mostraban un perfil hemodinámico estable.La buena estabilidad hemodinámica observada durante los estudios realizados por nosotros en la anestesia con propofol en perros limita la utilidad de los datos hemodinámicos para controlar la profundidad de la anestesia y refuerza la necesidad de desarrollar un monitor basado en las modificaciones del EEG para uso veterinario.

Chapter 6

6- General conclusion.

In human medicine, monitors of depth of anesthesia are currently used and commercially available. Such monitors accurately detect the variation in terms of concentrations of drugs and clinical endpoints.

During the development of this thesis, one of the main problems dealt with was the impossibility of achieving reliable clinical endpoints to measure the depth of anesthesia. In an attempt to overcome this problem it was developed an objective visual clinical scale to evaluate the depth of anesthesia based on ocular reflexes. The results obtained showed that this scale could be a useful tool to assess depth of anesthesia in dogs.

The main conclusions from the investigational studies in this thesis are:

1. The establishment of a visual objective clinical scale based on the assessment of ocular reflexes, for monitoring the depth of anesthesia in dogs;
2. The ECG electrodes could be used to collect the EEG signal since low skin-electrode impedances indicating reliable skin-electrode contact with skin, were obtained after carefully preparation with both abrasion paste and alcohol;
3. Cerebral electrical changes induced by increasing and decreasing propofol concentrations in dogs appear to be detected by CSI monitoring during induction of anesthesia with propofol;

4. During induction of anesthesia the performance of CSM was not consistent with the clinical observations on the different planes of depth of anesthesia. This may limit its use during the induction of veterinary routine anesthesia;

5. The CSI values and the anesthetic planes proposed showed a good prediction probability when TCI at very different anesthetic targets was used. However, the CSI revealed important clinical limitations because it was not able to distinguish between intermediate planes of anesthesia;

6. The CSM is able to detect deeper levels of anesthesia when burst suppression is present;

7. The EMG is an important variable to take in account since the performance of the CSM monitor could be greatly affected by this type of electrical activity;

8. TCI showed to be a user-friendly technique, making it easier to change the anesthetic planes desired by the anesthesiologist. Its use will probably increase in general veterinary anesthesia practice, since it is a good methodology to perform TIVA;

9. Hemodynamics provides few clinical information for monitoring the depth of anesthesia, because good hemodynamic stability was observed with propofol even at very high concentrations.

6.1- Future research

During this researh it became clear that the approach should be the development of a monitor of depth of anesthesia just to be used in veterinary patients. The optimal monitor of depth of anesthesia will be one that integrates multivariables that are displayed in the monitor, and interpreted according to the anesthesia moments. Monitors that combine both spontaneous and evoked cerebral electrophysiologic signals are now under investigation for humans (Horn, Pilge et al. 2009). Probably the EMG could be used as a part of the signal, where the EMG's usefulness is related to the immediate activation of the frontal muscle, which in turn is related to inadequate anesthesia, imminence of regaining consciousness and strong nocioception (Viertio-Oja, Maja et al. 2004). In the future, the equipment to measures cerebral drug effect will be considered as an integral part of the anesthetic monitoring set up. This will allow the anesthesiologists to be capable of differentiating and quantifying the various anesthetic drug effects.

Capítulo 6

6- Conclusiones.

1.- La buena estabilidad hemodinámica proporcionada por el propofol, incluso en altas concentraciones, proporciona poca información clínica para controlar la profundidad anestésica.

2.- El establecimiento de una escala clínica objetiva visual para evaluar la profundidad de la anestesia basada en los reflejos oculares puede ser una herramienta útil para conocer la profundidad anestésica en perros, ya que no encontramos otros signos clínicos más fiables y mejores para medir la profundidad anestésica.

3.- Los electrodos utilizados en la realización de un electrocardiograma se pueden utilizar para recoger la señal del electroencefalograma desde valores bajos de las impedancias piel-electrodo, pero hay que tener especial cuidado en conseguir un buen contacto, y haber preparado antes la piel con limpieza y alcohol.

4.- Cambios eléctricos cerebrales inducidos por la variación de concentraciones del propofol en la etapa de inducción de la anestesia se detectan en el Índice de Estado cerebral (CSI) medido por el "Cerebral State Monitor" (CSM).

5.- Sin embargo el rendimiento del CSM no mostraba relaciones constantes y fiables con las observaciones clínicas en los diferentes planos de profundidad anestésica, lo que limitaría su uso al periodo de inducción anestésica en anestesias de rutina.

6.- Los valores del Índice de Estado cerebral (CSI) medido por el "Cerebral State Monitor" (CSM) muestran una alta probabilidad de relación (PK) con los planos anestésicos obtenidos, cuando suministramos diferentes concentraciones de propofol con el TCI (Target controlled infusions), pero no era capaz de distinguir los planos intermedios de anestesia.

7.- El "Cerebral State Monitor" (CSM) es capaz de detectar y funcionar con planos profundos de anestesia cuando existe BS (Burst supresión).

8.- La electromiografía es una técnica que hay que tener en cuenta porque la eficacia del monitor CSM puede verse afectada por este tipo de actividad eléctrica.

9.- En nuestra experiencia el TCI es una técnica fácil de usar, pero todavía tiene que mejorar y avanzar para conseguir mejores monitores para medir los diferentes planos de profundidad anestésica, y que su desarrollo se adapte para pacientes veterinarios.

6.1-Futuras investigaciones

Durante nuestras investigaciones quedó claro que el enfoque futuro debe ser el desarrollo de un monitor de profundidad anestésica diseñado sólo para pacientes veterinarios.

Monitores que combinan señales de electrofisiología cerebral espontánea y potenciales evocados están ahora desarrollándose para los seres humanos (Cuerno, Pilge et al. 2009).

El monitor óptimo para medir la profundidad de la anestesia será uno que integre multivariables que se muestren en el monitor y que se puedan interpretar y relacionar con los diferentes planos de anestesia.

Probablemente el EMG podría utilizarse como parte de la señal, porque como hemos visto el EMG está relacionado con la activación del músculo frontal, que a su vez está relacionada con la anestesia inadecuada, y la inminencia de recuperar la conciencia y fuerte nociception (Viertio-Oja, Maja et al 2004).

En el futuro, estos monitores que nos cuantifican el efecto de las drogas en el cerebro se considerarán como parte integrante del control de la anestesia. Esto permitirá a los anestesiólogos ser capaces de diferenciar y cuantificar los efectos de las drogas anestésicas.

References:

Adragna, M. G. (1986). "An unusual cause of patient movement during anesthesia." anesthesiology **64**(1): 89-90.

Akavipat, P., K. Dumrongbul, et al. (2006). "Can electrocardiogram electrodes replace bispectral index electrodes for monitoring depth of anesthesia?" Journal of The Medical Association of Thailand **89**(1): 51-55.

Anderson, R. E., U. Sartipy, et al. (2007). "Use of conventional ECG electrodes for depth of anaesthesia monitoring using the cerebral state index: a clinical study in day surgery." Brithish Journal of Anaesthesia **98**(5): 645-648.

Antognini, J. F., E. Carstens, et al. (2000). "Isoflurane depresses electroencephalographic and medial thalamic responses to noxious stimulation via an indirect spinal action." Anesthesia Analgesia **91**(5): 1282-1288.

Antognini, J. F. and K. Schwartz (1993). "Exaggerated anesthetic requirements in the preferentially anesthetized brain." Anesthesiology **79**(6): 1244-1249.

Antognini, J. F., X. W. Wang, et al. (2000). "Isoflurane anaesthetic depth in goats monitored using the bispectral index of the electroencephalogram." Veterinary research communications **24(6)**: 361-370.

Antognini, J. F., X. W. Wang, et al. (2000). "Propofol directly depresses lumbar dorsal horn neuronal responses to noxious stimulation in goats." Canadian Journal Anesthesia **47**(3): 273-279.

Artru, A. A. (1986). "Cerebral metabolism and EEG during combination of hypocapnia and isoflurane-induced hypotension in dogs." anesthesiology **65**(6): 602-608.

Auckburally, A., P. Pawson, et al. (2008). "A comparison of induction of anaesthesia using a target-controlled infusion device in dogs with propofol or a propofol and alfentanil admixture" Veterinary Anaesthesia and Analgesia, **35**: 319-325.

Beier, S. L., A. J. d. A. Aguiar, et al. (2009). "Effect of remifentanil on requirements for propofol administered by use of a target-controlled infusion system for maintaining anesthesia in dogs." AJVR **70(6)**: 703-709.

Bennett, C., L. J. Voss, et al. (2009). "Practical use of the raw electroencephalogram waveform during general anesthesia: the art and science." Anesthesia Analgesia **109**(2): 539-550.

Berezowskyj, J. L., J. A. McEwen, et al. (1976). "A study of anaesthesia depth by power spectral analysis of the electroencephalogram (EEG)." Canadian Anesthesia Society Journal **23**(1): 1-8.

Bergamasco, L., A. Accatino, et al. (2003). "Quantitative electroencephalographic findings in beagles anaesthetized with propofol." Veterinary Journal **166**(1): 58-66.

Berger, H. (1933). "Uber das Elektrenkephalogramm des Menschen." Arch. Psychiat **101**: 452.

Beths, T., J. B. Glen, et al. (2001). "Evaluation and optimisation of a target-controlled infusion system for administering propofol to dogs as part of a total intravenous anaesthetic technique during dental surgery." Vet Rec **148**(7): 198-203.

Bhargava, A. K., R. Setlur, et al. (2004). "Correlation of Bispectral Index and Guedel's Stages of Ether Anesthesia." Anesthesia Analgesia **98**(1): 132-134.

Bimar, J. and J. W. Bellville (1977). "Arousal reactions during anesthesia in man." anesthesiology **47**(5): 449-454.

Bollen, P. J. A. and H. Saxtorph (2006). "Cerebral state monitoring in Beagle dogs sedated with medetomidine." Veterinary Anaesthesia and Analgesia **33**: 237-240.

Brady, D. and C. Palmeri (2007). The Pet Economy. Bloomberg businessweek online.

Brás, S., N. Bressan, et al. (2009). "A step towards effect-site target-controlled infusion with propofol in dogs: a k(e0) for propofol." Journal Veterinary Pharmacology Therapeutics **32**(2): 182-188.

Brodbelt, D. C., K. J. Blissitt, et al. (2008). "The risk of death: the confidential enquiry into perioperative small animal fatalities." Vet Anaesth Analg **35**(5): 365-373.

Bruhn, J., P. S. Myles, et al. (2006). "Depth of anaesthesia monitoring: what's available, what's validated and what's next?" Brithish Journal Anaesthesia **97**(1): 85-94.

Burge, W. E. (1943). "Mode of Action of Anesthetics in Abolishing Voluntary Muscular Movement." Anesthesia and Analgesia November-December: 341-344.

Campagnol, D., F. J. Teixeira Neto, et al. (2007). "Effects of epidural administration of dexmedetomidine on the minimum alveolar concentration of isoflurane in dogs." Am J Vet Res **68**(12): 1308-1318.

Campagnol, D., F. J. Teixeira Neto, et al. (2007). "Use of bispectral index to monitor depth of anesthesia in isoflurane-anesthetized dogs." American Journal of Veterinary Research **68**(12): 1300-1307.

Carrasco-Jimenez, M. S., M. F. Martin Cancho, et al. (2004). "Relationships between a proprietary index, bispectral index, and hemodynamic variables as a means for evaluating depth of anesthesia in dogs anesthetized with sevoflurane." Am J Vet Res **65**(8): 1128-1135.

Caton, R. (1875). "Electrical Currents of the Brain." The Journal of Nervous and Mental Disease **2**(4): 610.

Clayes, M. A., E. Gepts, et al. (1988). "Haemodynamic changes during anesthesia induced and mantained with propofol " British Journal Anaesthesia **60**: 3-9.

Coetzee, J. F., J. B. Glen, et al. (1995). "Pharmacokinetic model selection for target controlled infusions of propofol. Assessment of three parameter sets." anesthesiology **82**(6): 1328-1345.

Cullen, D. J., E. I. Eger, 2nd, et al. (1972). "Clinical signs of anesthesia." anesthesiology **36**(1): 21-36.

Daniel, M., R. B. Weiskopf, et al. (1998). "Fentanyl augments the blockade of the sympathetic response to incision (MAC-BAR) produced by desflurane and isoflurane: desflurane and isoflurane MAC-BAR without and with fentanyl." anesthesiology **88**(1): 43-49.

Danmeter (2004). Cerebral State Monitor User Manual. UK.

der Linde, H. J., B. Van Deuren, et al. (2010). "EEG in the FEAB model: Measurement of electroencephalographical burst suppression and seizure liability in safety pharmacology." Journal of Pharmacological and Toxicological Methods.

Doi, M., R. J. Gajraj, et al. (1997). "Relationship between calculated blood concentration of propofol and electrophysiological variables during emergence from anaesthesia: comparison of bispectral index, spectral edge frequency, median frequency and auditory evoked potential index." Brithish Journal Anaesthesia **78**(2): 180-184.

Dong, W. K., S. W. Bledsoe, et al. (1983). "Profound arterial hypotension in dogs: brain electrical activity and organ integrity." anesthesiology **58**(1): 61-71.

Edmonds, H. L. and P. Markku (1985). "Computerized monitoring of the EMG and EEG during anesthesia. An evaluation of the anesthesia and brain activity

monitor (ABM®)." Journal of Clinical Monitoring and Computing **1(4)**: 201-210.
Fechner, J., S. Albrecht, et al. (1998). "[Predictability and precision of "target-controlled infusion" (TCI) of propofol with the "Disoprifusor TCI" system]." Anaesthesist **47**(8): 663-668.
Ferreira, D., L. N. Ribeiro, C.S., et al. (2006). Brain monitoring with cerebral state index during induction of anesthesia with propofol target controlled infusion in dogs. IX world congress of veterinary anesthesia,12-16 sptember 2006 santos-SP.Brasil, SP.Brasil.
First Lieut Keys, T. E. (1942). Development of anesthesia. Rochester, Minnesota, Reference Librarian, Mayo clinic, on leave
Flaishon R, W. A., Sebel PS (1997). "Recover of consciousness after thiopental or propofol. Bispectral index and isolated forearm technique.
." Anesthesiology **86**: 613 -619
Flaishon, R., A. Windsor, et al. (1997). "Recover of consciousness after thiopental or propofol. Bispectral index and isolated forearm technique." Anesthesiology **86**: 613 -619
Gajraj, R. J., M. Doi, et al. (1998). "Analysis of the EEG bispectrum, auditory evoked potentials and the EEG power spectrum during repeated transitions from consciousness to unconsciousness." Brithish Journal Anaesthesia **80**(1): 46-52.
Gambus, P. L., E. W. Jensen, et al. (2010). "Modeling the Effect of Propofol and Remifentanil Combinations for Sedation-Analgesia in Endoscopic Procedures Using an Adaptive Neuro Fuzzy Inference System (ANFIS)." Anesth Analg.
Gibbs, F. A., E. L. Gibbs, et al. (1937). "Effect of electro-encephalogram of certain drugs which influence the nervous activity." Archives Internal Medicine **60**: 154.
Glass, P. S., M. Bloom, et al. (1997). "Bispectral analysis measures sedation and memory effects of propofol, midazolam, isoflurane, and alfentanil in healthy volunteers." anesthesiology **86**(4): 836-847.
Green, S. A., G. J. Benson, et al. (2002). Bispectral index in dogs anesthetized with isoflurane: comparison with sevoflurane. Proceedings of the American College of Veterinary Anesthesiologists 26th Annual Meeting, 2001New Orleans, Veterinary Anaesthesia and Analgesia 2002.
Greene, S. A., G. J. Benson, et al. (2002). "Relationship of Canine Bispectral Index to Multiples of Sevoflurane Minimal Alveolar Concentration, Using Patch or Subdermal Electrode" Comparative medicine **Vol 52, No 5** Pages 424-428.
Greene, S. A., G. J. Benson, et al. (2004). "Effect of Isoflurane, Atracurium, Fentanyl, and Noxious Stimulation on Bispectral Index in Pigs" Comparative medicine **Vol 54, No 4**.
Greene, S. A., W. J. Tranquilli, et al. (2003). "Effect of medetomidine administration on bispectral index measurements in dogs during anesthesia with isoflurane." American Journal Veterinary Research **64**(3): 316-320.
Guarracino, F., F. Lapolla, et al. (2005). "Target Controled Infusion:TCI." Minerva Anestesiologica **71**(6): 335-337.
Guedel, A. E. (1937). Inhalational Anesthesia: A fundamental guide. New York, Macmillan.
Guedel, A. E. (1966). "Third Stage Ether Anesthesia: A Sub-Classification Regarding the Significance of the Position and Movements of the Eyeball." Survey of Anesthesiology. **10(5)**: 515-521.

Haga, H. A. and N. I. Dolvik (2002). "Evaluation of the bispectral index as an indicator of degree of central nervous system depression in isoflurane-anesthetized horses." Am J Vet Res **63**(3): 438-442.

Hall, L. W., K. W. Clarke, et al. (2001). Patient monitoring and clinical measurement. Veterinary Anesthesia. L. W. Hall, K. W. Clarke and Trim.C.M., Saunders,W.D. .

Hall, L. W., K. W. Clarke, et al. (2001). Veterinary Anesthesia, Saunders,W.D. .

Haskins, S. C. (1992). General guidelines for judging anesthetic depth Opinions in Small Animal Anesthesia. V. C. o. N. America., W.B.Saunders Company **22**.

Haskins, S. C. (1996). Monitoring anesthetized patient. In lumb and Jones Veterinary Anesthesia W. V. Lumb and E. W. Jones, Baltimore,Lippincott Williams and Wilkins**:** 408-423.

Heiko, R. (2004). Depht of anesthesia-Monitoring and concepts European Society of Anaesthesiologists

Lisbon.

Hemmerling, T. M. and P. Harvey (2002). "Electrocardiographic electrodes provide the same results as expensive special sensors in the routine monitoring of anesthetic depth." Anesthesia Analgesia **94**(2): 369-371, table of contents.

Henao-Guerrero, P. N., R. McMurphy, et al. (2009). "Effect of morphine on the bispectral index during isoflurane anesthesia in dogs." Veterinary Anaesthesia Analgesia **36**(2): 133-143.

Hendersoh, Y. (1937). "Muscle Tone and Anesthesia." Anesthesia and Analgesia(January-February): 43-46.

Hermán R. Muñoz, M. D., M.Sc.,Luis I. Cortinez, M.D.,Mauricio E. Ibacache, M.D., Fernando R. Altermatt, M.D. (2004). "Estimation of the Plasma Effect Site Equilibration Rate Constant (Ke0) of propofol in children Using the Time to Peak Effect." Anesthesiology **101**: 1269-1274.

Hilgenberg, J. C. (1981). "Intraoperative awareness during high-dose fentanyl--oxygen anesthesia." anesthesiology **54**(4): 341-343.

Holford, N. H. G. and L. B. Sheiner (1981). "Understanding the dose-effect relationship: Clinical application of pharmacokinetic-pharmacodynamic models." Clinical Pharmacokinetics **6**: 429-445.

Holliday, T. A. and W. Colette (1999). "Clinical Electroencephalography in Dogs " Veterinary Neurology & Neurosurgery Journal **1**(1).

Horn, B., S. Pilge, et al. (2009). "A combination of electroencephalogram and auditory evoked potentials separates different levels of anesthesia in volunteers." Anesthesia Analgesia **108**(5): 1512-1521.

Hoymork, S. C., K. Hval, et al. (2007). "Can the Cerebral State Monitor replace the Bispectral Index in monitoring hypnotic effect during propofol/remifentanil anaesthesia?

." Acta Anaesthesiologica Scandinavica

51: 210-216.

Huang, J. W., Y. Y. Lu, et al. (1999). "Depth of anesthesia estimation and control." IEEE Trans Biomed Eng **46**(1): 71-81.

Hug, C. C., Jr. (1990). "Does opioid "anesthesia" exist?" anesthesiology **73**(1): 1-4.

Itamoto, K., Y. Taura, et al. (2002). "Quantitative electroencephalography of medetomidine, medetomidine-midazolam and medetomidine-midazolam-butorphanol in dogs." J Vet Med A Physiol Pathol Clin Med **49**(4): 169-172.

Jacobs, J. R. (1995). "Infusion rate control algorithms for pharmacokinetic model-driven drug infusion devices." Int Anesthesiol Clin **33**: 65-82

Jacoby, J., W. Hamelberg, et al. (1960). "Control of anesthetic depth during balaced anesthesia." Anesthesia Analgesia **39**: 275-282.
Jensen, E. W., P. Lindholm, et al. (1996). "Autoregressive modeling with exogenous input of middle-latency auditory-evoked potentials to measure rapid changes in depth of anesthesia." Methods Inf Med **35**(3): 256-260.
Jensen, E. W., H. Litvan, et al. (2006). "Cerebral State Index during Propofol Anesthesia: A Comparison with the Bispectral Index and the A-Line ARX Index. ." Anesthesiology **105(1)**: 28-36.
Johansen, J. and P. Sebel (2000). "Development and clinical application of electroencephalographic bispectrum monitoring." Anesthesiology **93**: 1336-1344.
Johansen, J. W. and P. S. Sebel (2000). "Development and clinical application of electroencephalographic bispectrum monitoring." Anesthesiology **93**(5): 1336-1344.
John, E. R. and L. S. Prichep (2005). "The anesthetic cascade: a theory of how anesthesia suppresses consciousness." anesthesiology **102**(2): 447-471.
Kaada, B. R., F. Thomas, et al. (1967). "EEG synchronization induced by high frequency midbrain reticular stimulation in anesthetized cats." Electroencephalogr Clin Neurophysiol **22**(3): 220-230.
Kazama, T., K. M. K. Ikeda, et al. (1999). "Comparison of the Effect-site keO s of Propofol for Blood Pressure and EEG Bispectral Index in Elderly and Younger Patients." Anesthesiology **90(6)**: pp 1517-1527.
Kissin, I. (2000). "Depth of anesthesia and bispectral index monitoring." Anesthesia Analgesia **90**(5): 1114-1117.
Kiyama, S. and J. Takeda (1997). "Effect of extradural analgesia on the paradoxical arousal response of the electroencephalogram." British Journal Anaesthesia **79**(6): 750-753.
Klemm, W. R. and G. L. Mallo (1966). "Clinical electroencephalography in anesthetized small animals." Journal American Veterinary Medical Association **148**(9): 1038-1042.
Kreuer, S., A. Biedler, et al. (2003). "Narcotrend monitoring allows faster emergence and a reduction of drug consumption in propofol-remifentanil anesthesia." anesthesiology **99**(1): 34-41.
Kreuer, S. and W. Wilhelm (2006). "The Narcotrend monitor." Best Pract Res Clin Anaesthesiol **20**(1): 111-119.
Kuramoto, T., S. Oshita, et al. (1979). "Modification of the Relationship between Cerebral Metabolism, Blood Flow, and Electroencephalogram by Stimulation during Anesthesia in the Dog." anesthesiology **51**(3): 211-217.
Lamont, L. A., S. A. Greene, et al. (2004). "Relationship of bispectral index to minimum alveolar concentration multiples of sevoflurane in cats." Am J Vet Res **65**(1): 93-98.
Lamont, L. A., S. A. Greene, et al. (2005). "Relationship of Feline Bispectral Index to Multiples of Isoflurane Minimum Alveolar Concentration." Comparative medicine **Vol 55, No 3**
Pages 269-274.
Levy, W. J., H. M. Shapiro, et al. (1980). "Automated EEG processing for intraoperative monitoring: a comparison of techniques." anesthesiology **53**(3): 223-236.

Long, C. W., N. K. Shah, et al. (1989). "A comparison of EEG determinants of near-awakening from isoflurane and fentanyl anesthesia. Spectral edge, median power frequency, and delta ratio." Anesthesia Analgesia **69**(2): 169-173.

Loomis, A. L., E. N. Harvey, et al. (1937). "Cerebral states during sleep, as studied by human brain potentials." Journal of Experimental Psychology **21**(2): 127-144.

Lopes, P. C., N. Nunes, et al. (2008). "Bispectral index in dogs at three intravenous infusion rates of propofol." Veterinary Anaesthesia Analgesia **35**(3): 228-231.

Lumb, W. V. and E. W. Jones (1996). In lumb and Jones Veterinary Anesthesia Baltimore,Lippincott Williams and Wilkins.

Lunn, J., N. Rosen, et al. (1987). Consciousness,Awareness,Pain and General Anesthesia. London:Butterworths.

Mantzaridis, H. and G. N. Kenny (1997). "Auditory evoked potential index: a quantitative measure of changes in auditory evoked potentials during general anaesthesia." Anaesthesia **52**(11): 1030-1036.

March, P. A. and W. W. Muir (2003). "Use of the bispectral index as a monitor of anesthetic depth in cats anesthetized with isoflurane." American Journal of Veterinary Research **64**(12): 1534-1541.

March, P. A. and W. W. Muir (2005). "Bispectral analysis of the electroencephalogram: a review of its development and use in anesthesia." Veterinary Anaesthesia and Analgesia **32**(5): 241-255.

March, P. A. and W. W. Muir (2005). "Bispectral analysis of the electroencephalogram:a review of its developement and use in anesthesia." Veterinary Anaesthesia and Analgesia(32): 241-255.

March, P. A. and W. W. Muir, 3rd (2003). "Minimum alveolar concentration measures of central nervous system activation in cats anesthetized with isoflurane." American Journal of Veterinary Research **64**(12): 1528-1533.

March, P. A. and W. W. Muir, 3rd (2003). "Use of the bispectral index as a monitor of anesthetic depth in cats anesthetized with isoflurane." American Journal of Veterinary Research **64**(12): 1534-1541.

Marsh, B., M. White, et al. (1991). "Pharmacokinetic model driven infusion of propofol in children." British Journal Anaesthesia **67**(1): 41-48.

Martin-Cancho, M. F., M. S. Carrasco-Jimenez, et al. (2004). "Assessment of the relationship of bispectral index values, hemodynamic changes, and recovery times associated with sevoflurane or propofol anesthesia in pigs." American Journal of Veterinary Research **65**(4): 409-416.

Martin-Cancho, M. F., J. R. Lima, et al. (2006). "Relationship of bispectral index values, haemodynamic changes and recovery times during sevoflurane or propofol anaesthesia in rabbits." Laboratory Animals **40**(1): 28-42.

Martin-Cancho, M. F., J. R. Lima, et al. (2003). "Bispectral index, spectral edge frequency 95%, and median frequency recorded for various concentrations of isoflurane and sevoflurane in pigs." American Journal of Veterinary Research **64**(7): 866-873.

Martin-Cancho, M. F., J. R. Lima, et al. (2006). "Bispectral index, spectral edge frequency 95% and median frequency recorded at varying desflurane concentrations in pigs " Research in Veterinary Science **81**(3): Pages 373-381.

Martin-Cancho, M. F., J. R. Lima, et al. (2006). "Relationship of bispectral index values,haemodynamic changes and recovery times during sevoflurane anesthesia in rabbits " Laboratory animals **40**: 28-42.

Martin, J. T., A. Faulconer, Jr., et al. (1959). "Electroencephalography in anesthesiology." anesthesiology **20**(3): 359-376.

Merkel, G. and E. I. Eger, 2nd (1963). "A comparative study of halothane and halopropane anesthesia including method for determining equipotency." anesthesiology **24**: 346-357.

Miller, A., J. W. Sleig, et al. (2004). "Does bispectral analysis of the electroencephalogram add anything but complexity." British Journal of Anesthesia **92(1)**: 8-13.

Miyauchi, Y., T. Sakabe, et al. (1985). "Responses of EEG, cerebral oxygen consumption and blood flow to peripheral nerve stimulation during thiopentone anaesthesia in the dog." Canadian Anesthesia Society Journal **32**(5): 491-498.

Moerman, N., B. Bonke, et al. (1993). "Awareness and recall during general anesthesia. Facts and feelings." anesthesiology **79**(3): 454-464.

Morgaz, J., M. D. Granados, et al. (2010). "Evaluation of spectral entropy to measure anaesthetic depth and antinociception in sevoflurane-anaesthetised Beagle dogs." Veterinary Journal.

Morgaz, J., M. M. Granados, et al. (2009). "Relationship of bispectral index to hemodynamic variables and alveolar concentration multiples of sevoflurane in puppies." Research Veterinary Science **86**(3): 508-513.

Muir , W. W., A. J. Wiese, et al. (2003). "Effects of morphine, lidocaine, ketamine, and morphine-lidocaine-ketamine drug combination on minimum alveolar concentration in dogs anesthetized with isoflurane." American Journal of Veterinary Research **64**(9): 1155-1160.

Murrell, J. C., H. N. de Groot, et al. (2005). "Investigation of changes in the middle latency auditory evoked potential during anesthesia with sevoflurane in dogs." American Journal Veterinary Research **66**(7): 1156-1161.

Murrell, J. C., C. B. Johnson, et al. (2003). "Changes in the EEG during castration in horses and ponies anaesthetized with halothane." Veterinary Anaesthesia Analgesia **30**(3): 138-146.

Musk, G. C., D. S. J. Pang, et al. (2005). "Target-controlled infusion of propofol in dogs - evaluation of four targets for induction of anaesthesia." Veterinary Record. **157**(24): 766-.

Nolan, A. and Reid.J. (1993). "Pharmakokinetics of propofol Administered by infusionin dogs undergoing surgery." British Journal of Anesthesia **70**: 546-551.

Otto, K. A. (2007). "Effects of averaging data series on the electroencephalographic response to noxious visceral stimulation in isoflurane-anaesthetized dogs." Research Veterinary Science **83**(3): 385-393.

Otto, K. A. (2008). "EEG power spectrum analysis for monitoring depth of anaesthesia during experimental surgery." Lab Anim **42**(1): 45-61.

Otto, K. A. and P. Mally (2003). "Noxious stimulation during orthopaedic surgery results in EEG 'arousal' or 'paradoxical arousal' reaction in isoflurane-anaesthetised sheep." Research Veterinary Science **75**(2): 103-112.

Otto, K. A., S. Voight, et al. (1996). "Differences in quantitated electroencephalographic variables during surgical stimulation of horses anesthetized with isoflurane." Vet Surg **25**(3): 249-255.

Palanca, B. J., G. A. Mashour, et al. (2009). "Processed electroencephalogram in depth of anesthesia monitoring." Curr Opin Anaesthesiol **22**(5): 553-559.

Paloheimo, M. (1990). "Quantitative surface electromyography (qEMG): applications in anaesthesiology and critical care." Acta Anaesthesiol Scand Suppl **93**: 1-83.

Pascoe, P. J. (2000). "Opioid analgesics." Veterinary Clinics of North America **30(4)**.

Pellegrino, F. C. and R. E. P. Sica (2004). "Canine electroencephalographic recording technique:

findings in normal and epileptic dogs." Clinical Neurophysiology **115**: 477–487.
Pilge, S., R. Zanner, et al. (2006). "Time Delay of Index Calculation: Analysis of Cerebral State, Bispectral, and Narcotrend Indices
[Laboratory Investigations]
" Anesthesiology
Volume 104(3)
pp 488-494

Pinsker, M. C. (1986). "Anesthesia: a pragmatic construct." Anesthesia Analgesia **65**(7): 819-820.
Pomfrett, C. J. D. (1999). "Heart rate variability, BIS and 'depth of anaesthesia'." British Journal Anaesthesia. **82**(5): 659-662.
Pritchard, W. S. and D. W. Duke (1992). "Measuring chaos in the brain: a tutorial review of nonlinear dynamical EEG analysis." Int J Neurosci **67**(1-4): 31-80.
Prys-Roberts, C. (1987). "Anaesthesia: a practical or impractical construct?" British Journal Anaesthesia **59**(11): 1341-1345.
Rampil, I. J. (1998). "A Primer for EEG Signal Processing in Anesthesia " Anesthesiology **84(2)**: 980-1002.
Rampil, I. J. (2001). "Monitoring depth of anesthesia." Curr Opin Anaesthesiol **14**(6): 649-653.
Ribeiro, L. M., D. A. Ferreira, et al. (2009). "Correlation between clinical signs of depth of anaesthesia and cerebral state index responses in dogs during induction of anaesthesia with propofol." Res Vet Sci.
Ribeiro, L. M., D. A. Ferreira, et al. (2009). "Correlation between clinical signs of depth of anaesthesia and cerebral state index responses in dogs during induction of anaesthesia with propofol." Res Vet Sci **87**(2): 287-291.
Ribeiro, L. M., D. A. Ferreira, et al. (2007). "Brain monitoring in dogs using the cerebral state index during the induction of anaesthesia via target-controlled infusion of propofol." Research in Veterinary Science **Article in press**.
Ribeiro, L. M., D. A. Ferreira, et al. (2008). "Brain monitoring in dogs using the cerebral state index during the induction of anaesthesia via target-controlled infusion of propofol." Research in Veterinary Science **85(2)**: 227-232.
Schneider, G., R. Hollweck, et al. (2005). "Detection of consciousness by electroencephalogram and auditory evoked potentials." Anesthesiology **103**(5): 934-943.
Schnider, T. W., C. F. Minto, et al. (1998). "The influence of method of administration and covariates on the pharmacokinetics of propofol in adult volunteers." anesthesiology **88**(5): 1170-1182.
Schnider, T. W., C. F. Minto, et al. (1999). "The influence of age on propofol pharmacodynamics." anesthesiology **90**(6): 1502-1516.
Schultz, A., B. Schultz, et al. (2000). Sleeping stage based systems (Narcotrend),. New Aspects of High Technology in Medicine 2000. H. P. Bruck, F. Koeckerling, F. Bouchard and C. Schug-Pass. Bolognia, Monduzzi**:** 285–291.
Schwender, D., M. Daunderer, et al. (1998). "Power spectral analysis of the electroencephalogram during increasing end-expiratory concentrations of isoflurane, desflurane and sevoflurane." Anaesthesia **53**(4): 335-342.
Seitsonen, E., A. Yli-Hankala, et al. (2000). "Are electrocardiogram electrodes acceptable for electroencephalogram bispectral index monitoring?" Acta Anaesthesiologica Scandinavica **44**(10): 1266-1270.

Shannon, C. E. (1948). "A mathematical theory of communication " Bell System Techn Journal **27**: 379-423: 623 - 356.
Shaw, N. A. (1988). "The auditory evoked potential in the rat--a review." Prog Neurobiol **31**(1): 19-45.
Sheiner, L. B., D. R. Stanski, et al. (1979). "Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to d-tubocurarine." Clin Pharmacol Ther **25**(3): 358-371.
Sigl, J. C. and N. G. Chamoun (1994). "An introduction to bispectral analysis for the electroencephalogram." J Clin Monit **10**(6): 392-404.
Sleigh, J. W., D. A. Steyn-Ross, et al. (2001). "Comparison of changes in electroencephalographic measures during induction of general anaesthesia: influence of the gamma frequency band and electromyogram signal." British Journal Anaesthesia. **86**(1): 50-58.
Smith, W. D., R. C. Dutton, et al. (1996). "Measuring the performance of anesthetic depth indicators." Anesthesiology **84**(1): 38-51.
Soma, L. R. (1971). Depth of general anesthesia Textbook of veterinary anesthesia. L. R. Soma. Baltimore,Williams and Wilkins company**:** 178-187.
Stanski, D. (2000). Monitoring deph of anesthesia New York, Churchill Livingstone.
Stolzy, S. L., L. J. Couture, et al. (1987). "A study of spontaneous and purposeful movement as a predictor of postoperative recall during fentanyl/oxide/diazepam anesthesia." Anesthesiology **67** (3A): A376.
Stullken, E. H., Jr., J. H. Milde, et al. (1977). "The nonlinear responses of cerebral metabolism to low concentrations of halothane, enflurane, isoflurane, and thiopental." anesthesiology **46**(1): 28-34.
Tackley, R. M., G. T. Lewis, et al. (1989). "Computer controlled infusion of propofol." British Journal Anaesthesia **62**(1): 46-53.
Takamatsu, I., M. Ozaki, et al. (2006). "Entropy indices vs the bispectral index for estimating nociception during sevoflurane anaesthesia." British Journal Anaesthesia **96**(5): 620-626.
Thogersen, B. and H. Ording (2000). "Bispectral index monitoring: comparison of two types of electrode." Anaesthesia **55**(3): 242-246.
Thornton, C., K. M. Konieczko, et al. (1989). "Effect of propofol on the auditory evoked response and oesophageal contractility." British Journal Anaesthesia **63**(4): 411-417.
Thornton, C. and R. M. Sharpe (1998). "Evoked responses in anaesthesia." British Journal Anaesthesia **81**(5): 771-781.
Ueyama, Y., P. Lerche, et al. (2009). "Effects of intravenous administration of perzinfotel, fentanyl, and a combination of both drugs on the minimum alveolar concentration of isoflurane in dogs." American Journal Veterinary Research **70**(12): 1459-1464.
Vakkuri, A., A. Yli-Hankala, et al. (2004). "Time-frequency balanced spectral entropy as a measure of anesthetic drug effect in central nervous system during sevoflurane, propofol, and thiopental anesthesia." Acta Anaesthesiologica Scandinavica **48**(2): 145-153.
van Oostrom, H., A. Doornenbal, et al. (2011). "Neurophysiological assessment of the sedative and analgesic effects of a constant rate infusion of dexmedetomidine in the dog." Veterinary Journal.
Vanluchene, A. L., H. Vereecke, et al. (2004). "Spectral entropy as an electroencephalographic measure of anesthetic drug effect: a comparison with

bispectral index and processed midlatency auditory evoked response." anesthesiology **101**(1): 34-42.
Vereecke, H. E., P. M. Vasquez, et al. (2005). "New composite index based on midlatency auditory evoked potential and electroencephalographic parameters to optimize correlation with propofol effect site concentration: comparison with bispectral index and solitary used fast extracting auditory evoked potential index." anesthesiology **103**(3): 500-507.
Viertio-Oja, H., V. Maja, et al. (2004). "Description of the Entropy algorithm as applied in the Datex-Ohmeda S/5 Entropy Module." Acta Anaesthesiologica Scandinavica **48**(2): 154-161.
Vuyk, J. (1997). "Pharmacokinetic and pharmacodynamic interactions between opioids and propofol." J Clin Anesth **9**(6 Suppl): 23S-26S.
Walling, P. T. and K. N. Hicks (2006). "Nonlinear changes in brain dynamics during emergence from sevoflurane anesthesia: preliminary exploration using new software." anesthesiology **105**(5): 927-935.
Wetzel, R. C., N. Setzer, et al. (1985). "Hemodynamic responses in brain dead organ donor patients." Anesthesia Analgesia **64**(2): 125-128.
Whelan, G. and P. A. Flecknell (1992). "The assessment of depth of anaesthesia in animals and man." Laboratory Animal **26**(3): 153-162.
Zheng, D., R. N. Upton, et al. (1998). "The influence of the bolus injection rate of propofol on its cardiovascular effects and peak blood concentrations in sheep." Anesthesia Analgesia **86**(5): 1109-1115.
Zhong, T., Q. L. Guo, et al. (2005). "Comparative evaluation of the cerebral state index and the bispectral index during target-controlled infusion of propofol." British Journal Anaesthesia **95**(6): 798-802.

Appendix

The author declares (under the current law) that he has participated in the design,execution of experimental work necessary to obtain the presented results used in the manuscripts and thesis development. Under the goal of this PhD thesis, the manuscripts and short publications enumerated below were prepared by the author name stated as **Lénio Ribeiro.**

Articles in journals of international circulation with peer review:

2008-Ribeiro, L.M., Ferreira, D.A., Bressan, N., Nunes, C.A., Amorim, P., Antunes, L.M. Brain monitoring in dogs using the cerebral state index during the induction of anaesthesia via target-controlled infusion of propofol, Res Vet Sci 85(2): 227-232.

2009-Ribeiro, L.M., Ferreira, D.A., Brás, S. Castro, A. Nunes, C.A. Amorim, P.Antunes, L.M. Correlation between clinical signs of depth of anaesthesia and cerebral state index responses in dogs during induction of anaesthesia with propofol, Res Vet Sci 87(2): 287-291.

2009-Brás, S., Bressan, N., Ribeiro, L., Ferreira, D. A., Antunes, L., Nunes, C. S.
A steptowards effect-site target controlled infusion with propofol in dogs: a k_{e0} for propofol Journal of Veterinary Pharmacology and Therapeutics;, 32 (2), 182-188.

2011-Silva, A., Ribeiro L.M., Bressan, N., Oliveira, P., Ferreira, D.A., Antunes, L.M.
Dogs mean arterial pressure and heart rate responses during high propofol plasma concentrations estimated by a pharmacokinetic model.Res Vet Sci. Oct;91(2):278-80.

2012-Ribeiro, L.M., Ferreira, D.A., Brás, S., Castro,A., Gonzalo-Orden, J.M., Antunes, L.M. Correlation between clinical signs of depth of anaesthesia and cerebral state index responses in dogs with different target-controlled infusions of propofol,Vet Anaesth Analg 39(1): 21-28.

2012-Brás, S., Gouveia, S., Ribeiro, L., Ferreira, D.A., Antunes, L., Nunes, C. S.
Fuzzy Logic Model to describe anesthetic effect and muscular influence on EEG Cerebral State Index, Research in Veterinary Science.(Submitted)

Articles in scientific meeting proceedings

2007-Brás, S., Bressan, N., Ribeiro, L., Ferreira, D.A., Antunes, L., Nunes, C.S.
Nonlinear modeling of cerebral state index in dogs. Conf Proc IEEE Eng Med Biol Soc.; 537-40

2011-Brás, S., Ferreira, D. A., Antunes, L., Ribeiro, L., Nunes, C. S., Gouveia, S.
EMG contributes to improve Cerebral State Index modeling in dogs anesthesia, 33rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBS), Boston, USA, IEEE. 6593-6596

Abstracts in scientific meeting proceedings:

2006-Ferreira, D., Ribeiro, L.M., Nunes, C.S.; Bressan, N.; Amorim, P.; Alves, H.; Antunes, L.
Brain monitoring with cerebral state index during induction of anesthesia with propofol target controlled infusion in dogs. IX World Congress of Veterinary Anesthesia,12-16 September, Santos-SP. Brasil

2006-Ribeiro, L.M., Ferreira, D., Nunes, C.S.; Bressan, N.; Amorim, P.; Alves, H.; Antunes, L.
Target Controlled Infusion (TCI) – a useful tool to anesthetize dogs. IX World Congress of Veterinary Anesthesia, 12-16 September, Santos-SP.Brasil

2006-Ribeiro, L.M., Ferreira, D.,Nunes, C.S.; Bressan, N.; Amorim, P.; Alves, H.; Antunes, L.
Time to peak effect using the cerebral state index (CSI): determining the pharmacokinetics of propofol in dogs. IX World Congress of Veterinary Anesthesia,12-16 September, Santos-SP. Brasil.

2007-Brás, S. Bressan, N. Ribeiro, L. Ferreira, D. A. Antunes, L. Nunes C. S.Propofol Pharmacodynamic Effect On The Cerebral State Index In Dogs";First World Conference of Total Intravenous Anaesthesia, 27-29 September,Veneza, pp 95

2008-Ribeiro, L., Ferreira, D., Brás, S., Castro, A., Nunes, C.S., Amorim, P., Antunes L. M. Clinical and Cerebral State index responses in dogs to different estimated propofol plasma concentrations during induction of anesthesia. AVA spring meeting Bristol, UK.

2008-Mendes, M.C., Brás,S., Ribeiro, L., Antunes, L. M.
Evaluation of the bispectral index (BIS) and the cerebral state index (CSI) brain monitors in dogs during isoflurane anesthesia. AVA Autumn meeting Barcelona

2008-Mendes, M.C., Brás,S., Ribeiro, L., Antunes, L. M.
Preliminary evaluation of the bispectral index (BIS) and the cerebral state index (CSI) brain monitors during the recovering of dogs anaesthetised with isoflurane. AVA Autumn meeting Barcelona

2008-Brás, S., Ribeiro, L., Ferreira, D. A., Antunes, L., Amorim, P., Nunes, C. S.
Cerebral state index (CSI) values could be strongly contaminated by high EMG levels in dogs, Scientific Committee AVA Barcelona Meeting Poster; P-49

2009-Brás, S., Ribeiro, L., Ferreira, D. A., Antunes, L., Nunes, C. S.
Data Driven Identification of Propofol Pharmacokinetics in Dogs Versus Standard Model"; Second World Conference of Total Intravenous Anaesthesia, 23-25 Abril, Berlim,

Abstracts in journals of international circulation with peer review:

2007-Ribeiro, L., Ferreira D., Bressan, N., Amorim, P., Antunes, L.
Cerebral state index (CSI) monitoring in dogs during induction of anaesthesia with propofol. European Journal of Anaesthesiology, 24 (Sup. 39): 34

2007-Brás, S., Bressan, N., Ribeiro, L., Antunes, L., Nunes, C. S.
Pharmacokinetics and Pharmacodynamics of Propofol in Dog: KeO using the Cerebral State Index"; Anesthesiology, , 107: A827

2007-Nunes, C. S., Brás, S., Bressan, N., Ribeiro, L., Ferreira, D. A., Antunes, L. Amorim, P.
Time to Peak effect and pharmacokinetics of propofol using the Cerebral State Index in the dog"; Journal of Neurosurgical Anesthesia , ,19 (4): 341

2007-Nunes, C. S., Brás, S., Bressan, N., Ribeiro, L., Ferreira, D. A., Antunes, L., Amorim, P.
Pharmacokinetics and pharmacodynamics of propofol: differences between dogs and humans; Journal of Neurosurgical Anesthesia,19 (4): 337-338.

2008-Silva, A.M., Ribeiro, L., Bras, S., Ferreira, D. A., Antunes, L. M.
CSI response to incision in dogs. Veterinary Anaesth Analg 35(3) 19-21

2008-Brás, S., Ribeiro, L., Ferreira, D. A., Antunes, L., Nunes. C. S.
CSI response to high propofol concentrations is related to dog's weight, European Journal of Anaesthesiology 25 (Suppl 44): 9AP2

2008-Brás, S. Ribeiro, L. Ferreira, D. A. Antunes, L. Nunes; C. S. "A
Fuzzy Logic model for Cerebral State Index, during propofol anesthesia in dogs", Anesthesiology, 109: A1469

2008-Brás, S., Ribeiro, L., Ferreira, D. A., Antunes, L., Amorim, P., Nunes C. S.
Modeling the Effect of Propofol in Dogs Using Fuzzy Logic Theory
Journal of Neurosurgical Anesthesia20 (4): 311

2008-Brás, S., Ribeiro, L., Ferreira, D. A., Antunes, L., Amorim, P., Nunes, C. S., "The Cerebral State Index in Dogs Could Be Strongly Contaminated by the EMG"; Journal of Neurosurgical Anesthesia, 20 (4): 34

2008-Brás, S., Ribeiro, L., Ferreira, D. A., Antunes, L., Amorim, P., Nunes, C. S. Hemodynamic stability in Dogs Is Maintained with High Propofol Concentrations; Journal of Neurosurgical Anesthesia, 20 (4): 336

Original publications in this thesis

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Brain monitoring in dogs using the cerebral state index during the induction of anaesthesia via target-controlled infusion of propofol

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Abstract

The aim of this study was to evaluate the correlation between the cerebral state index (CSI) and the estimated propofol plasma concentrations in dogs during induction of anaesthesia.

Fifteen healthy dogs undergoing scheduled routine surgical procedures were enrolled in this study. Target controlled infusion (TCI) software, based on the pharmacokinetic model for propofol, was used to control the syringe pump and to estimate plasma propofol concentrations (PropCp) and the CSI values every five-seconds. Three electrodes placed in the centre of the forehead, on the left side of the forehead and on the left mastoid were used to collect the electroencephalographic (EEG) signal converted by the cerebral state monitor into the CSI.

The cerebral electrical changes induced by increasing propofol concentrations appear to be detected by CSI monitoring in dogs. The negative correlation between CSI and PropCp demonstrates that the CSI could be used to assess electrical brain activity in dogs during the induction of anaesthesia with propofol.

Keywords: CSI; Cerebral state index; TCI; Propofol; Brain monitoring

1. Introduction

Anaesthesiology has improved notably thanks to developments in monitoring cardiovascular parameters during general anaesthesia. Anaesthesiologists are now able to routinely control physiological parameters such as blood pressure, heart rate and respiratory rate. However, the direct determination of the effects of anaesthetic and sedative agents on the central nervous system remains a challenge (Flaishon and Sebel, 1997).

Target controlled infusion (TCI) is an anaesthetic delivery system now widely used in human anaesthesia (Guarracino et al., 2005). In veterinary medicine, some reports have been published using TCI in dogs during anaesthesia induction and maintenance (Beths et al., 2001; Musk et al., 2005), which state that TCI is an appropriate technique for use in dogs. TCI can improve the delivery rate of a desired clinical effect while minimising side effects (Musk et al., 2005) and allows a more effective and easy control of the depth of anaesthesia (Beths et al., 2001).

In human medicine, a variety of analytical concepts have been proposed to quantify changes in the EEG during general anaesthesia. The monitor most widely used for this purpose is the bispectral index (BIS), which is largely based on the bispectrum analysis of the EEG (Stanski, 2000). On the other hand, the cerebral state monitor (CSM) was recently introduced as an intra-operative monitor of anaesthetic depth (Zhong et al., 2005). The cerebral state index

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(CSI) provided by CSM monitors is based on a combination of four sub parameters of the electroencephalographic signal. Three of these derive from spectral analysis of the EEG, and the fourth is the burst suppression (BS) calculated by the CSM (Jensen et al., 2006). The CSI is a numerical scale between "0" and "100", where "0" represents an isoelectric EEG line and "100" represents the EEG electrical activity of a fully awake individual. CSI is based on the analysis of the EEG frequency using a fuzzy-logic classifier, whereas BS is the percentage of isoelectrical EEG signal during each 30 s period (Danmeter, 2004). The ideal anaesthetic level for humans was established to be between 40 and 60 (Jensen et al., 2006).

In veterinary medicine, BIS monitoring has been proposed as a way to assess depth of anaesthesia in dogs (Muir et al., 2003), cats (March and Muir, 2003) and pigs (Martin-Cancho et al., 2006). However, its applicability is not clear when it comes to variation between species and drugs. Furthermore, the correlation between EEG depression and the BIS index is unclear (March and Muir, 2005). In animals, increased concentrations of anaesthetic agents showed a reduction in BIS values, but it was not possible to establish a BIS interval where it is safe to maintain anaesthesia (Lamont et al., 2005; March and Muir, 2005).

The CSM may be a useful monitor to access depth of anaesthesia in veterinary medicine. This monitor provides a similar brain monitoring function to that of the BIS. The CSM's small size and low cost when compared to other brain monitors makes it an appealing instrument for use by veterinary professionals in anaesthetic procedures. The CSM monitor has not been studied to the same extent as the BIS. In veterinary anaesthesia, the CSM monitor has only been studied in dogs by Bollen and Saxtorph, during sedative procedures using medetomidine (Bollen and Saxtorph, 2006).

To our knowledge, no studies have been published involving the use of CSI monitoring on dogs during anaesthesia. The CSM algorithm used to estimate CSI is different from that used by the BIS monitor. The CSM uses a fuzzy logic analysis tool which is not specifically adjusted to use on humans. On the contrary, the BIS' calculations use data from 5000 human anaesthetic procedures stored in a database (Johansen and Sebel, 2000). Therefore, it is possible that CSI monitoring could yield better results than BIS when monitoring brain activity in dogs during anaesthetic procedures. The objective of this study is to analyse the correlation between CSI and propofol-estimated plasma concentrations during induction of anaesthesia in dogs.

2. Material and methods

2.1. Patients and haemodynamic monitoring

After research committee approval and informed consent, fifteen healthy dogs undergoing scheduled routine surgical procedures were enrolled in this study. All dogs were premedicated with 0.5 mg kg^{-1} morphine sulphate (Morphine injection; Martindale Pharmaceuticals) and with 0.03 mg kg^{-1} acepromazine IM (Vetoquinol®; Univete), thirty minutes prior to the beginning of the induction of anaesthesia.

A peripheral catheter was inserted in the cephalic vein for drug and fluid administration. A three-way stopcock was used to connect the intravenous catheter to the propofol and to the sodium chloride 0.9% delivery lines. A Braun infusion pump (Braun, Melsungen, Germany) was used to administrate sodium chloride 0.9% at a constant infusion rate of 10 ml kg^{-1} h^{-1} throughout the study period.

A S/5 Datex monitor (Datex-Ohmeda, Helsinki, Finland) was used to collect the haemodynamic data. The non-invasive blood pressure was measured using an automated oscillometric method on the posterior left limb using repeated measurements: each measurement was carried out immediately after the preceding one (Datex S/5 "STAT" mode) which corresponds to a continuous mode for collecting blood pressure measurements throughout the study. Heart rate was monitored using three ECG electrodes placed in accordance with Academy of Veterinary Cardiology Committee specifications.

2.2. Cerebral state index monitoring

The CSI and frontal electromyographic activity (EMG) data were collected from a cerebral state monitor (CSM) (Danmeter, DK-5000 Odense C, Denmark) using three clamp electrodes placed according to the manufacturer's instructions for human CSI monitoring: in the middle of the forehead/frontal bone (white), to the left side of the forehead near the lateral eye socket, and on the temporal mastoid process on the left side of the head (black). The EEG waveform was derived from the signal recorded between the frontal (white) and mastoid (black) electrodes. The frequency content was between 2 and 35 Hz and the CSM monitor collects 2000 samples s^{-1} (14 bits equivalent), updating every second and filtering between 6 and 42 Hz (Danmeter, 2004). Impedances were kept at a low level (within 1 kΩ and 3 kΩ) by placing wax (Elefix, paste for EEG. Nihon Kohden Corporation) between the electrodes and the skin.

The CSI is based on analysis of the frequency content of the EEG signal. The EEG energy is characterised into specific frequency bands. These are used to define two energy ratios, namely alpha (α) and beta (β). There is a shift exhibited in the energy content of both α and β, from a relatively high to a relatively low frequency level, when the animal is in a deeper state of anaesthesia. The relationship between these shifts is also analysed by CSM as a separate parameter ($\beta - \alpha$). The monitor, that works online, also evaluates the amount of instantaneous BS during each thirty-second period of the EEG in order to quantify the amount of time the EEG is "silent" or "flat-line". These EEG patterns typify the very deepest levels of hypnosis. The four parameters are inputted into a fuzzy-logic classifier system that calculates the CSI. Fuzzy reasoning enables very complex processes to be carried out and can also be successfully applied to high non-linear processes, where it can considerably simplify modelling (Jensen et al., 2006).

The signal quality index (SQI) was collected at a constant rate. The SQI is calculated based on the quality of the EEG signal obtained and the signal artefacts recorded during the preceding one-minute period. The electrode-to-skin impedance is included in the SQI calculation. The SQI is displayed numerically as percentage units (0–100%, 100% equals optimum signal quality). Electrode-to-skin impedances at 1 kΩ result in a SQI of 100. If the impedance of the white or black sensors exceeds 1 kΩ, the SQI will gradually decrease. The artefact rejection algorithm ensures that the incoming EEG is not contaminated by external noise (<2 μV_{p-p}, <0.4 μV RMS, 1–250 Hz). When excessive noise is detected, the signal quality index is also diminished, thus reflecting the disturbance (Danmeter, 2004).

Electromyographic (EMG) activity from head muscles was also recorded by the CSM every 5 s.

2.3. Anaesthetic protocol

Anaesthesia was induced using a 6 mg kg^{-1} bolus dose of propofol 1% (Fresenius Kabi®, Bad Homburg, Germany) via a syringe pump (Asena GH, Alaris Medical Systems) programmed to allow a maximum infusion rate of 600 ml h^{-1}. A propofol bolus dose of 6 mg kg^{-1} was decided upon previous clinical observations where the CSI reached minimum values around 50 s after propofol administration (Ferreira et al., 2006). RugLoop II® software (developed by Tom DeSmet (Demed Engineering, Temse, Belgium) and Michel Struys (Ghent University, Gent, Belgium)), running on a personal computer, was used to drive the propofol syringe pump. The pharmacokinetic model for propofol from Beths and colleagues (Beths et al., 2001) was incorporated in the RugLoop II® software and was used to estimate the propofol plasma concentrations (PropCp). RugLoop II® was also used to store all pharmacokinetic, haemodynamic and electroencephalographic data at five-second intervals.

Propofol infusion was ceased after the bolus administration. At this point the CSI was expected to have attained its minimum value. Then, with a decrease in PropCp a resulting increase in the CSI values was expected. In order to avoid a total recovery from the anaesthesia, the study methodology limited the recovery to a CSI value of 70. Once the CSI reached 70, the study ended; the depth of anaesthesia was increased and the planned surgical procedures were carried out.

During the entire study period dogs were allowed to breathe spontaneously via a facial mask administering 100% oxygen. If apnea were to occur, the study would be interrupted, the dogs intubated and mechanically ventilated and subsequently discarded from the final data analysis. No stimuli were applied at any time.

2.4. Statistical analysis

Data was tested for normal distribution and for homogeneity of variance using the Shapiro Wilk and the Levene tests, respectively. Heart rate and mean arterial pressure values were collected before propofol infusion was begun and compared to those observed at maximum PropCp using analysis of variance. The CSI and PropCp data, collected at five-second intervals after the start of propofol infusion, were compared using Spearman Rank correlation analysis. A delay of 50 s in CSI data was taken into account when analyzing CSI data (Pilge et al., 2006). Statistical analysis was performed using SPSS v.13.0 for Windows. Data are expressed in mean ± s.d.; $P < 0.05$ was considered statistically significant.

3. Results

Fifteen mixed-breed dogs, ten of which were female, aged 3.4 ± 2.4 years and weighing 22.5 ± 10.5 kg were analysed. Prior to the induction of anaesthesia, CSI was 90.7 ± 4, heart rate was 103.6 ± 20.3 bpm and mean arterial pressure was 83.1 ± 14.9 mm Hg.

During the induction of anaesthesia, the maximum PropCp occurred 46.3 ± 45.9 s after propofol infusion was started and was 7.18 ± 0.39 μg ml^{-1}, which corresponded to a CSI value of 67 ± 12, with SQI and EMG values of 78.0 ± 12.4% and 32.9 ± 30.8%, respectively. At this point, heart rate was 122 ± 33.3 bpm and mean arterial pressure was 78.4 ± 19.4 mm Hg. There was a 13% decrease in mean arterial pressure ($P < 0.05$) and a 17% increase in heart rate ($P < 0.05$) between awake values and those values recorded at maximum PropCp (Table 1).

The minimum CSI values observed were 52.3 ± 9.6 and occurred with a PropCp of 5.2 ± 0.96 μg ml^{-1}, 1.8 ± 1.5 min after the maximum PropCp value was attained; SQI and EMG values were 83 ± 12.1% and 30.7 ± 26.5%, respectively. At this point in time, heart rate was 117.4 ± 31.9 bpm and mean arterial pressure was 73.3 ± 16.2 mm Hg (Table 2).

At the end of the study, CSI was 70.3 ± 1.2 with SQI and EMG values of 76.8 ± 13.5% and 31.5 ± 14%, respectively; PropCp was 3.83 ± 1.3 μg ml^{-1}, heart rate was 110.5 ± 28 bpm and mean arterial pressure was 74.3 ± 14 mm Hg.

A significant negative correlation was observed between CSI and PropCp (correlation coefficient of −0.579; $P < 0.01$) (Fig. 1).

4. Discussion

The present study analysed the correlation between estimated PropCp concentrations and their effects on CSI monitoring in dogs at different points during induction of anaesthesia: at maximum PropCp, at minimum CSI values and when the CSI value reached 70 during the washout phase after the propofol bolus.

There are no universally accepted standard electrode locations for EEG recording in dogs considered to be ideal for electrode placing during clinical research or clinical

Table 1
Values from each individual dog at maximum propofol concentrations

Dogs	Weight (kg)	HR (bpm)	MAP (mm Hg)	Max PropCp (μg ml^{-1})	CSI	EMG (%)
P1	21	117	55	7.15	67	0
P2	12	98	85	7.35	55	48
P3	5.7	121	93	7.51	56	80
P4	35	123	77	6.74	83	20
P5	29	156	51	6.76	44	0
P6	6,7	78	64	7.34	63	52
P7	21	128	94	6.61	61	77
P8	17	65	72	7.19	57	0
P9	10	203	65	7.47	78	82
P10	28	158	62	7.02	70	6
P11	45	103	63	6.58	68	0
P12	6.3	129	97	7.52	66	53
P13	25	143	66	7.09	93	38
P14	25	102	117	7.02	77	28
P15	18	109	70	7.78	71	10

P: patient, HR: heart rate, MAP: mean arterial pressure, Max PropCp: maximum estimated propofol plasma concentrations, CSI: cerebral state index, EMG: Electromyography.

Table 2
Values from each individual dog at minimum cerebral sate index values

Dogs	HR (bpm)	MAP (mm Hg)	PropCp (μg ml^{-1})	CSI	EMG (%)
P1	109	50	5.96	50	0
P2	94	77	5.79	47	57
P3	102	84	5.02	45	39
P4	112	75	3.54	56	0
P5	150	51	5.83	38	0
P6	84	56	5.36	54	43
P7	128	81	4.20	51	24
P8	76	67	4.90	52	0
P9	191	61	6.69	70	87
P10	158	62	5.85	63	10
P11	101	63	6.09	68	0
P12	123	97	6.50	49	47
P13	110	81	4.76	38	26
P14	88	77	4.75	55	27
P15	130	104	3.63	55	0

P: patient, MAP: mean arterial pressure, PropCp: estimated propofol plasma concentrations, CSI: cerebral state index, EMG: Electromyography.

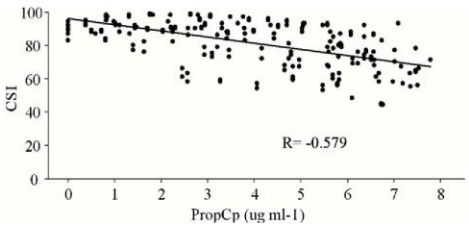

Fig. 1. Correlation between CSI and propofol-estimated plasma concentrations (PropCp) from the beginning of the study until maximum achieved estimated propofol concentration.

practice (Pellegrino and Sica, 2004). Therefore, the location chosen by us to place the electrodes was the same as that referred to by Grenne colleagues (Greene et al., 2002) and described by Pellegrino and Sica as suitable for EEG recording (Pellegrino and Sica, 2004).

Prior to induction of anaesthesia, CSI values were 90.7 ± 4 in the dogs used in our study. In humans, CSI values between 90 and 100 represent the electrical brain activity of an awake individual while CSI values between 80 and 90 indicate a low level of sedation (Danmeter, 2004). The effects of pre-medication with acepromazine and morphine (Pascoe, 2000) may explain the CSI values observed in dogs at the outset of our study.

Nolan and Reid suggested that tracheal intubation should not be performed when the average propofol blood concentration is below 5.4 μg ml^{-1}(Nolan and Reid, 1993). Based on previous clinical observations, we decided to administer a 6 mg kg $^{-1}$ propofol bolus to induce anaesthesia. This dose raised PropCp to a maximum of 7.18 μg ml^{-1} on average. Thus, the maximum PropCp attained in our study is clinically acceptable for anaesthesia induction and tracheal intubation.

Our results showed that increasing the concentration of propofol during induction of anaesthesia in dogs resulted

in a progressive decrease in CSI values. However, a large inter-individual variability in CSI responses was also observed (Tables 1 and 2). The differences in time taken to attain the maximum PropCp and CSI response may be justified by the large weight variability among the dogs used and by the maximum infusion rate of 600 ml h^{-1}permitted by the syringe pump during bolus administration. The plasma drug concentrations cannot in themselves predict the time span or magnitude of drug effect (Holford and Sheiner, 1981). When the plasma drug concentrations are increased, a time lag occurs until an equilibrium between plasma and effect site drug concentration is reached (Jacobs, 1995). The time needed to transfer the drug to the effect site (the brain in the case of hypnotics) depends on the concentration gradient, and an increased gradient requires less time to induce anaesthesia (Musk et al., 2005).

Beths' pharmacokinetic model (Beths et al., 2001) was developed using dogs with a wide range of ages and weights. In humans, pharmacokinetic and pharmacodynamic differences are described among children, elderly patients and adults (Kazama et al., 1999; Hermán et al., 2004). A parallel could be inferred for veterinary medicine where significant pharmacokinetic differences exist for propofol use across different breeds, ages and weights. This could prove limiting when trying to apply the pharmacokinetic model. Nevertheless, there are studies showing that TCI using this pharmacokinetic model in dogs allowed for better propofol titration with improved control of anaesthetic depth (Beths et al., 2001) and fewer unwanted side effects (Musk et al., 2005) when compared to manual infusion.

EMG values higher than 30% were observed in seven dogs at maximum PropCp and in five dogs at minimum CSI values. EMG activity is the most significant source of EEG artefact. Variations in EMG activity are associated with rapid changes in BIS values in dogs (Greene et al., 2002). Despite the fact that the CSI algorithm also incorporates an EMG filter that removes most potentially interfering EMG activity, the EMG still has a significant influence on the CSI value (Jensen et al., 2006). However, there are no published studies that quantify the total influence of EMG on CSI. Low EMG values are ideal when monitoring brain activity. One way of achieving this would be the administration of myorelaxant drugs. However, as our study was performed during induction of anaesthesia it would be unethical and unsafe to administer myorelaxant drugs prior to tracheal intubation.

Another important parameter to take into account when using CSI monitoring is the SQI, which represents the quality of the EEG signal received. SQI calculation is based on a series of artefacts that occur during each one-minute period. The SQI is also influenced by the level of impedance of the electrodes on the skin. Impedance of the black and white sensors above a level of 3 k will negatively influence SQI and thus affect CSI monitoring (Danmeter, 2004). Throughout the entire study period, impedance was kept low (within 1 kΩ and 3 kΩ) with the use of wax placed between electrodes and the skin, thus allowing us to obtain average SQI values of around 80% over the course of the study period.

The CSI could be a satisfactory alternative to BIS for monitoring depth of anaesthesia in humans (Hoymork et al., 2007). The CSI demonstrates more variability in the baseline when compared with BIS. However, both have a high probability of predicting loss of consciousness, loss of reflexes and correlate well with the modified observer's assessment of alertness/sedation scale (MOAAS) (Zhong et al., 2005). There are few animal studies reporting on monitoring depth of anaesthesia using BIS technology. The BIS monitor appears to have certain limitations when used in animals, especially when burst suppression is observed after the administration of large doses of anaesthetics (March and Muir, 2005). In these studies, the hypnotic agents were essentially halogenated and the results showed BIS' usefulness to be limited when monitoring the degree of CNS depression (Greene et al., 2004; Martin-Cancho et al., 2006). Increased concentrations of anaesthetic agents decreased BIS, but it could not accurately estimate a safe BIS interval for anaesthetising veterinary patients (Lamont et al., 2005; March and Muir, 2005). In pigs for example, BIS seems to interpret burst suppression in the EEG as an indication that the animal is waking up, showing high BIS values even during a steady state anaesthesia (Greene et al., 2004).

The PropCp decreased after cessation of the propofol bolus according to the estimates performed by Beths' pharmacokinetic model (Beths et al., 2001). This was reflected by a progressive increase in CSI values, indicating a lighter depth of anaesthesia. Although this correlation has already been shown in humans (Zhong et al., 2005), this is the first time that it has been reported in dogs.

Propofol decreases the arterial blood pressure by centrally depressing sympathetic neural output, which results in a decrease in systemic vascular resistance (Clayes et al., 1988). Episodes of hypotension (mean arterial pressure <60 mm Hg) were observed in only two dogs in our study for a very brief period of time when PropCp reached its maximum concentration. Other than these two cases, heart rate and mean arterial pressure were always within normal physiological limits. Thus, a possible effect of systemic haemodynamics on cerebral haemodynamics and on resulting CSI values appears unlikely.

In conclusion, cerebral electrical changes induced by increasing and decreasing propofol concentrations appear to be detected by CSI monitoring of dogs during induction of anaesthesia. The negative correlation between CSI and PropCp indicates that the CSI could be used to assess electrical brain activity in dogs during induction of anaesthesia using propofol and suggests that it could potentially be used for monitoring the depth of anaesthesia during general anaesthetic procedures. However, it must be underlined that CSM is a brain monitor not yet validated for use in veterinary anaesthesia and, this must therefore be taken into account. Nevertheless, this study shows that

CSI monitoring could be a potentially useful tool for accessing the depth of anaesthesia in dogs.

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References

Beths, T., Glen, J.B., et al., 2001. Evaluation and optimization of a target-controlled infusion system for administering propofol to dogs as part of a total intravenous anesthetic technique during dental surgery. Veterinary Records 148, 198–203.

Bollen, P.J.A., Saxtorph, H., 2006. Cerebral state monitoring in Beagle dogs sedated with medetomidine. Veterinary Anaesthesia and Analgesia 33, 237–240.

Clayes, M.A., Gepts, E., et al., 1988. Haemodynamic changes during anesthesia induced and maintained with propofol. British Journal of Anaesthesia 60, 3–9.

Danmeter, (2004). Cerebral state monitor user manual, UK.

Ferreira, D., L.N. Ribeiro, C.S., et al. (2006). Brain monitoring with cerebral state index during induction of anesthesia with propofol target controlled infusion in dogs. In: Proceedings of the IX World Congress of Veterinary Anesthesia, 12–16 September 2006, Santos-SP, Brasil.

Flaishon, R., Sebel, W.A.P.S., 1997. Recover of consciousness after thiopental or propofol. Anesthesiology 86, 613–619.

Greene, S.A., Benson, G.J., et al., 2002. Relationship of canine bispectral index to multiples of sevoflurane minimal alveolar concentration, using patch or subdermal electrode. Comparative Medicine 52 (5), 424–428.

Greene, S.A., Benson, G.J., et al., 2004. Effect of isoflurane, atracurium, fentanyl, and noxious stimulation on bispectral index in pigs. Comparative Medicine 54 (4).

Guarracino, F., Lapolla, F., et al., 2005. Target controled infusion: TCI. Minerva Anestesiologica 71 (6), 335–337.

Holford, N.H.G., Sheiner, L.B., 1981. Understanding the dose-effect relationship: clinical application of pharmacokinetic–pharmacodynamic models. Clinical Pharmacokinetics 6, 429–445.

Hoymork, S.C., Hval, K., et al., 2007. Can the cerebral state monitor replace the bispectral index in monitoring hypnotic effect during propofol/remifentanil anaesthesia? Acta Anaesthesiologica Scandinavica 51, 210–216.

Jacobs, J.R., 1995. Infusion rate control algorithms for pharmacokinetic model-driven drug infusion devices. International Anesthesiology Clinics 33, 65–82.

Jensen, E.W., Litvan, H., et al., 2006. Cerebral state index during propofol anesthesia: a comparison with the bispectral index and the A-line ARX index. Anesthesiology 105 (1), 28–36.

Johansen, J., Sebel, P., 2000. Development and clinical application of electroencephalographic bispectrum monitoring. Anesthesiology 93, 1336–1344.

Kazama, T., Ikeda, K.M.K., et al., 1999. Comparison of the effect-site KeO s of propofol for blood pressure and EEG bispectral index in elderly and younger patients. Anesthesiology 90 (6), 1517–1527.

Lamont, L.A., Greene, S.A., et al., 2005. Relationship of feline bispectral index to multiples of isoflurane minimum alveolar concentration. Comparative Medicine 55 (3), 269–274.

March, P.A., Muir, W.W., 2003. Use of the bispectral index as a monitor of anesthetic depth in cats anesthetized with isoflurane. American Journal of Veterinary Research 64 (12), 1534–1541.

March, P.A., Muir, W.W., 2005. Bispectral analysis of the electroencephalogram: a review of its development and use in anesthesia. Veterinary Anaesthesia and Analgesia 32 (5), 241–255.

Martin-Cancho, M.F., Lima, J.R., et al., 2006. Bispectral index, spectral edge frequency 95% and median frequency recorded at varying desflurane concentrations in pigs. Research in Veterinary Science 1 (3), 373–381.

Muir, W.W., Wiese, A.J., et al., 2003. Effects of morphine, lidocaine, ketamine, and morphine–lidocaine–ketamine drug combination on minimum alveolar concentration in dogs anesthetized with isoflurane. American Journal of Veterinary Research 64 (9), 1155–1160.

Muñoz, Hermán R., Luis, I., Cortinez, M.D., Mauricio, E., Ibacache, M.D., Fernando, R., Altermatt, M.D., 2004. Estimation of the plasma effect site equilibration rate constant (Ke0) of propofol in children using the time to peak effect. Anesthesiology 101, 1269–1274.

Musk, G.C., Pang, D.S.J., et al., 2005. Target-controlled infusion of propofol in dogs – evaluation of four targets for induction of anaesthesia. Veterinary Record 157 (24), 766.

Nolan, A., Reid, J., 1993. Pharmakokinetics of propofol administered by infusionin dogs undergoing surgery. British Journal of Anesthesia 70, 546–551.

Pascoe, P.J., 2000. Opioid analgesics. Veterinary Clinics of North America 30 (4).

Pellegrino, F.C., Sica, R.E.P., 2004. Canine electroencephalographic recording technique: findings in normal and epileptic dogs. Clinical Neurophysiology 115, 477–487.

Pilge, S., Zanner, R., et al., 2006. Time delay of index calculation: analysis of cerebral state, bispectral, and narcotrend indices [laboratory investigations]. Anesthesiology 104 (3), 488–494.

Stanski, D., 2000. Monitoring Depth of Anesthesia. Churchill Livingstone, New York.

Zhong, T., Guo, Q.L., et al., 2005. Comparative evaluation of the cerebral state index and the bispectral index during target-controlled infusion of propofol. British Journal of Anaesthesia 95 (6), 798–802.

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Correlation between clinical signs of depth of anaesthesia and cerebral state index responses in dogs during induction of anaesthesia with propofol

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ABSTRACT

The cerebral state index (CSI) is used for monitoring EEG and depth of anaesthesia. The objective of this study was to analyse the correlation between ocular reflexes, CSI and estimated propofol plasma concentrations (PropCP) in dogs during induction of anaesthesia with propofol.

Fourteen dogs were premedicated with acepromazine 0.05 mg kg^{-1} IM. Anaesthesia was induced with a 200 ml h^{-1} propofol 1% constant infusion rate until loss of corneal reflex using RugLoop II software with Beths' pharmacokinetic model to estimate PropCp.

Palpebral reflex (PR) and the corneal reflex (CR) were tested every 30 s and classified as present (+) or absent (−), and eyeball position was registered as rotated ventromedialy (ERV) or centred (EC).

Heart rate (HR), mean arterial pressure (MAP) and CSI values were analyzed from baseline before the beginning of propofol infusion (T0) until loss of CR; CSI and PropCp, CSI and anaesthetic planes, and PropCp and anaesthetic planes were compared using correlation analysis.

PropCp reached 7.65 ± 2.1 μg ml^{-1} at the end of the study. CSI values at T0 were 89.2 ± 3.8. Based on the observation of ocular reflexes and eyeball position, it was possible to define five anaesthetic planes: A (superficial) to E (deep), being A (PR+/CR+/EC), B (PR+/ERV/CR+), C (PR−/ERV/CR+), D (PR−/EC/CR+) and E (PR−/EC/CR−). There was a significant correlation between PropCp and the anaesthetic planes ($R = 0{,}861$; $P < 0.01$). No significant correlation was observed between CSI and the anaesthetic planes or between CSI and PropCp. MAP decreased significantly from T0 until loss of corneal reflex (from 98 ± 14 mmHg to 82 ± 12 mmHg); HR did not change significantly (from 101 ± 30 bpm to 113 ± 16 bpm).

The CSI monitoring was not consistent with the clinical observations observed in the different stages of depth anaesthesia. This could limit the use of CSI for monitoring depth of anaesthesia with propofol.

1. Introduction

The concept of depth anaesthesia is dimensionless. Without any objective monitoring, the subjective descriptions 'too light', 'too deep' or 'enough' are not helpful for a careful assessment of the adequate plane of anaesthesia (Pomfrett, 1999). The observation of physical signs, such as somatic muscle tone, respiratory patterns and ocular signs allowed assigning to a given stage of anaesthesia (Heiko, 2004). So far, only ether was used to define the clinical stages according to anaesthetic depth (Bhargava et al., 2004). The development of a brain monitor that can be used in dogs is a real necessity to monitor depth of anaesthesia more objectively. The electroencephalogram (EEG) provides a direct measurement of the functional state of the brain which makes the monitoring of increasing anaesthetic depth and the detection of unnecessary depth of anaesthesia possible (Lunn et al., 1987). Different EEG patterns such as decreasing EEG frequency and increasing wave amplitude can be observed with increasing anaesthetic concentrations. These and other changes can be described and quantified statistically (Miller et al., 2004), and algorithms can be introduced in EEG monitoring devices and used to access the depth of anaesthesia.

The cerebral state monitor (CSM), an EEG monitor, was recently introduced for monitoring the depth of anaesthesia in humans, proving to be a useful tool (Jensen et al., 2006). This monitor has not yet been validated in dogs but there is a possibility that the cerebral state index (CSI) displayed by the CSM could be used for EEG monitoring in dogs (Ribeiro et al., 2008).

The objective of this study is to analyse the correlation between depth of anaesthesia by monitoring ocular reflexes, and CSI and estimated propofol plasma concentrations in dogs during induction of anaesthesia with propofol.

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2. Methods

2.1. Patients and haemodynamic monitoring

Fourteen healthy dogs undergoing scheduled routine surgical procedures were enrolled in this study. All dogs were premedicated with 0.05 mg kg^{-1} Acepromazine IM (Calmivet®, Vetoquinol, France), thirty minutes prior to the beginning of the induction of anaesthesia.

A cannula was inserted in the cephalic vein for drug and fluid administration. A three way stopcock was used to connect the intravenous catheter to the propofol and sodium chloride 0.9% delivery lines. A Braun infusion pump (Braun, Melsungen, Germany) was used for the administration of sodium chloride at a constant infusion rate of 10 ml kg^{-1} hr^{-1} during the entire study period.

An S/5 Datex monitor (Datex-Ohmeda; Helsinki, Finland) was used for monitoring the haemodynamic parameters. The blood pressure was measured non-invasively in the anterior carpus of the left hind leg using repeated measurements: each measurement was made immediately after the preceding one (Datex S/5 "STAT" mode). Heart rate was monitored by three ECG electrodes placed according to Academy of Veterinary Cardiology Committee.

2.2. Cerebral state index monitoring

The CSI and frontal electromyographic activity (EMG) data were collected every five seconds from a cerebral state monitor (CSM) (Danmeter, DK-5000 Odense C, Denmark) using three clamp, ECG electrodes (Seitsonen et al., 2000), placed in standard position in the midline of the head. The EEG waveform in our study was derived from the signal recorded between the frontopolar electrode (positive) represented by the white color and the occipital electrode (negative) represented by the black color, according to Pelegrino and colleagues' studies (Pellegrino and Sica, 2004). The ground or the reference electrode was placed in the parietal position. The frequency of the waveform was between 2 and 35 Hz and CSM monitor collected 2000 samples s^{-1} (14 bits equivalent), with an update every one second. To calculate the CSI, frequencies outside the 6–42 Hz range were filtered out (Danmeter, 2004). Impedances were kept low (within 1 k and 3 k) by using a special wax (Elefix, paste for EEG, Nihon Kohden Corporation) placed between the electrodes and the skin.

The CSI is calculated using four sub-parameters of the electroencephalogram: β ratio, α ratio, β ratio–α ratio, and burst suppression. These sub-parameters are used to define two energy ratios called alpha (α) and beta (β). Both α and β show a shift in the energy content from the higher to the lower frequencies with deeper anaesthesia. This information can be used to achieve an index which varies from 0 to 100 (Jensen et al., 2006).

The monitor also evaluates the amount of instantaneous burst suppression (BS) in each thirty-second period that quantifies the amount of isoelectric waves in the EEG. These EEG periods characterise the deepest levels of hypnosis.

Signal quality index (SQI) was collected at a constant rate. The SQI is calculated using the quality of the acquired EEG signal and on the signal artefacts during the previous one minute period. The electrode-to-skin impedance is included in the SQI calculation. The SQI is displayed numerically as percentage units (0–100%, 100% equals best signal quality). Electrode-to-skin impedances at 1 kΩ result in a SQI of 100. If the impedance of the white or black sensors exceeds 1 kΩ, the SQI will gradually decrease (Danmeter, 2004). When excessive noise is detected, the SQI is also diminished reflecting the disturbance (Danmeter, 2004). If the sensor impedance is >5 kΩ the CSI, BS and EMG will be blank ("- -" displayed).

2.3. Anaesthetic protocol

RugLoop II® software (developed by Tom DeSmet (Demed Engineering, Temse, Belgium) and Michel Struys (Ghent University, Gent, Belgium)) running on a personal computer was used to drive the propofol syringe pump. The pharmacokinetic model for propofol from Beths and colleagues (Beths et al., 2001) was incorporated in the RugLoop II® software and was used to estimate the propofol plasma concentrations (PropCp). RugLoop II® was also used to store the pharmacokinetic, haemodynamic and electroencephalographic data every five seconds.

Anaesthesia was induced using a constant infusion rate of 200 ml h^{-1} of propofol 1% (Fresenius Kabi®; Bad Homburg, Germany). Throughout this period, the animals were breathing 100% oxygen through a face mask. The end point of the propofol administration was the moment when the dogs lost their corneal reflex. However if the dogs became apnoeic (did not breathe for more than 15 s) or hypotensive (meaning their arterial blood pressure dropped below 60 mmHg) the propofol administration was stopped. After the end of the study the dog was intubated and prepared for the scheduled surgery.

No stimuli were applied at any time to the dogs other than testing reflexes.

2.4. Clinical observation of the depth of anaesthesia

Before the induction of anaesthesia and after premedication, the dogs were placed in sternal recumbency. The first reflexes were tested when the dogs changed to a lateral recumbence without a cervical tonus after the beginning of the propofol infusion.

The palpebral and corneal reflexes were tested every 30 s with a swab moistened with serum. The palpebral reflex was tested gently at the lateral canthus of the eye, and corneal reflex was elicited by a smooth pressure on the cornea. Each reflex was tested three times and considered negative if there was no response to the three taps with the swab. The eyeball position was also observed at each test. Thus, palpebral reflex (PR) and corneal reflex (CR) were classified as present (+) or absent (−); the position of the eyeball was described as eyeball rotated ventormedially (ERV) if the pupil was looking towards the middle eye corner, or centred (EC) if the pupil was centred between the superior and inferior palpebral. The tests were performed by the same operator for all of the dogs.

2.5. Data and statistical analysis

The results obtained from testing the reflexes were analyzed and grouped according to the propofol concentrations and, consequently, with anaesthetic depth. A letter representing a different plane of anaesthesia was given to each group of reflexes. Data was tested for normal distribution using the Shapiro Wilk test. Heart rate, mean arterial pressure and CSI values were analyzed from baseline values before the beginning of propofol infusion (T0) until the loss of corneal reflex using the Wilcoxon. The Spearman Rank correlation analysis was used to compare CSI and PropCp, CSI and anaesthetic planes, and anaesthetic planes and PropCp. A delay of 50 s in CSI data was taken into account when analyzing CSI data (Pilge et al., 2006). Statistical analysis was performed using SPSS v.13.0 for Windows. Data was expressed in mean ± sd; $|R| > 0.5$ and $P < 0.05$ was considered statistically significant.

3. Results

Fourteen mixed breed dogs aged 2.6 ± 0.9 years, including 8 females, weighing 25.3 ± 5.28 kg were analysed. The results from

prospectively analyzing the ocular reflexes indicated five levels of anaesthetic depth (A–E) (Table 1). CSI decreased from baseline to plane E (Table 2) but no significant correlation was observed between CSI and these anaesthetic planes ($R = 0.26$; Fig. 1), or between CSI and PropCp) ($R = 0.478$). Nevertheless, a strong correlation was observed between PropCp and the anaesthetic planes ($R = 0.887$; $P < 0.01$).

In plane E, all dogs lost their palpebral and corneal reflex, and were intubated with laryngeal reflex absent. At this time point lower EMG values associated with lower CSI values were observed. Four dogs showed high EMG values in this plane associated with high CSI values.

Mean arterial blood pressure decreased by 16% from the baseline to plane E ($P < 0.05$). Heart rate did not change significantly during the study (Table 2).

4. Discussion

This study analysed the effect of propofol on CSI, ocular reflexes, heart rate and mean arterial blood pressure during the induction of anaesthesia with propofol in dogs. Five different anaesthetic planes based on the analysis of ocular reflexes were identified in dogs under propofol anaesthesia. In fact PropCp showed a strong correlation with these anaesthetic planes and so, different PropCp intervals are proposed in order to achieve a desired depth of anaesthesia. On the other hand, CSI decreased with increasing propofol concentrations but there was no correlation between CSI and the depth of anaesthesia provided by the anaesthetic planes in this study. Thus, CSI does not seem to provide trustworthy clinical information for monitoring the depth of anaesthesia in dogs during the induction of anaesthesia with propofol.

The haemodynamic parameters monitored in this study provided very little information about the depth of anaesthesia when using propofol as hypnotic agent. In fact only mean arterial pressure decreased by about 16% from baseline to plane E, which is of limited clinical use when monitoring depth of anaesthesia.

The development of a three-compartment pharmacokinetic model for propofol for dogs (Beths et al., 2001) allowed the use of TCI in veterinary anaesthesia practice. The concentrations obtained with the propofol constant rate infusion of 200 ml h^{-1} were based on Beths pharmacokinetic model (Beths et al., 2001). Although in our study no blood analyses were performed to measure the real propofol concentration in the blood, there are studies showing that TCI using this pharmacokinetic model in dogs allows a better propofol titration with improved control of anaesthetic depth (Beths et al., 2001) and less undesired side effects (Musk et al., 2005) when compared to manual infusion.

The effects of propofol on the EEG are well described in humans (Sleigh et al., 2001). The administration of low doses of propofol increases the amplitude and the EEG alpha wave rhythm, followed by a shift to EEG gamma and theta frequency. Higher doses produce burst suppression and decrease in the EEG amplitude (Jensen et al., 2006). A good monitor of depth of anaesthesia should accurately distinguish these EEG changes and correlate them with different anaesthetic depth. The most studied monitor in veterinary science is the bispectral index monitor BIS (March and Muir, 2005). This monitor seems to have some limitations when used in animals, especially when burst suppression is observed after the administration of large anaesthetic doses (March and Muir, 2005). In these studies, the hypnotic agents were essentially halogenated and the results showed limited BIS usefulness for monitoring the degree of central nervous system (CNS) depression (Greene et al., 2004; Martin-Cancho et al., 2006). Increased concentrations of anaesthetic agents decreased BIS but could not accurately estimate the BIS interval where it is safe to anaesthetise veterinary patients (Lamont et al., 2005; March and Muir, 2005). For example, in pigs BIS seems to interpret the burst suppression in the EEG as an indicator of awakening, showing high BIS values even during a steady state of anaesthesia (Greene et al., 2004).

In humans, CSI monitoring has recently been introduced to monitor the hypnotic state of patients during anaesthetic procedures, but demonstrates more variability in the baseline when compared with BIS. However, both monitors have a high probability of predicting loss of consciousness, loss of reflexes and good correlations with the Modified Observer's Assessment of Alertness/Sedation Scale (MOAAS) (Zhong et al., 2005). Jensen and colleagues concluded that the CSI could be a reasonable alternative to the BIS monitor. In a previous study, our group proposed the CSI as a possible satisfactory alternative to BIS for monitoring depth of anaesthesia in dogs (Ribeiro et al., 2008). This study showed that the cerebral electrical changes induced by a 6 mg kg^{-1} bolus dose of propofol was detected by the CSI monitoring of dogs during induction of anaesthesia. The negative correlation between CSI and PropCp suggested that the CSI could be used to assess electrical brain activity in dogs. However, results from this study indicate that when anaesthesia is induced using a propofol constant infusion rate, CSI correlation is lower than the previous study (Ribeiro et al., 2008). A possible reason for such differences may be related to the time needed to transfer the drug to the effect site. The concentration at the brain in the case of hypnotics depends on the concentration gradient (Musk et al., 2005). When a bolus of 6 mg kg^{-1} of propofol is administered, the time lag needed for the propofol plasma and effect-site concentrations to reach the equilibrium is small. Thus, the CSI rapidly reflects the propofol concentrations in the brain by showing lower CSI values. In a constant rate of propofol infusion, the propofol plasma concentration increases more slowly. Thus, the gradient of concentration of propofol between the plasma and the effect-site is lower, and the time to transfer the drug to the effect site is longer. Furthermore, this study showed a higher standard deviation at the maximum estimated propofol plasma concentration (7.65 ± 2.16 μg ml^{-1}) when compared with the bolus study (7.18 ± 0.39 μg ml^{-1}). This may be explained by the fact that in this study propofol infusion was stopped based on clinical assessments.

According to the CSI manufacturer, CSI values in humans higher than 90 indicate an "awake" brain, between 80 and 70 indicate deep sedation, between 60 and 40 indicate general anaesthesia and below 40 indicate excessive anaesthesia (Danmeter, 2004). As we can see in Table 2, the CSI values are far from correlating with these intervals. Furthermore CSI had no correlation with propofol-estimated plasma concentrations and no correlation with the clinical signs of the anaesthetic planes referred in this study.

It is known that EMG activity is the most significant source of EEG artefact. Despite the fact that the CSI algorithm also incorporates an EMG filter that removes most of the potential interfering

Table 1
Anaesthetic planes identified based on increasing estimated propofol plasma concentrations in all dogs. Anaesthetic planes are represented from A to E, according to anaesthetic depth based on the ocular reflexes monitored: Palpebral reflex (PR), eyeball centred in the eye (EC), corneal reflex (CR) and eyeball rotated ventrally (ERV).

Anaesthetic planes	Ocular reflexes
A	PR+/EC/CR+
B	PR+/ERV/CR+
C	PR−/ERV/CR
D	PR−/EC/CR+
E	PR−/EC/CR−

A – presence of palpebral reflex, eyeball centred and corneal reflex positive; B – presence of palpebral reflex, eyeball rotated ventrally and corneal reflex positive; C – Absence palpebral reflex, eyeball rotated ventrally and corneal reflex positive; D – Absence palpebral reflex, eyeball centred and corneal reflex positive; E – Absence palpebral reflex, eyeball centred, absence of corneal reflex.

Table 2
Haemodynamic, electroencephalographic and propofol data observed during baseline and defined anaesthetic planes A–E in this study.

	N	PropCp (μg/ml)	CSI	EMG (%)	BS (%)	SQI (%)	HR (bpm)	MAP (mmhg)
Baseline	14	0.00	89.2 ± 3.8	94.0 ± 11.5	0.0	63.7 ± 21.0	101 ± 30	98±14
Anaesthetic planes								
A	14	3.03 ± 1.01	91.9 ± 4.9	93.7 ± 9.2	0.0	68.9 ± 20.3	110 ± 18	95±15
B	10	4.29 ± 1.66	92.8 ± 7.9	84.5 ± 27.6	0.0	71.3 ± 23.6	101 ± 21	99±12
C	12	5.54 ± 1.34	83.3 ± 24.8	66.0 ± 36.8	44 ± 0[a]	70.1 ± 25.4	103 ± 20	91±12
D	5	6.72 ± 2.15	71.2 ± 31.2	40.4 ± 25.7	46 ± 0[a]	85.0 ± 12.7	117 ± 7	88±11
E	14	7.65 ± 2.16	60.4 ± 25.6[c]	25.1 ± 35.0[d]	7.5 ± 15.8	80.7 ± 17.2	113 ± 16	82±12[b]

N – number of dogs in which were observed the respective anaesthetic plane; PropCp – estimated propofol plasma concentration; CSI – cerebral state index; EMG – electromyography; BS – burst suppression; SQI – signal quality index; HR – heart rate; MAP – mean arterial pressure.
[a] Only one dog started to show burst suppression on plane C, which then followed to plane D and E, five dogs showed burst suppression only in plane E.
[b] CSI, HR and MAP data from all anaesthetic planes were compared to the baseline values: $P < 0.05$.
[c] $P < 0.01$.
[d] Four dogs in plane E have high values of EMG (>30%).

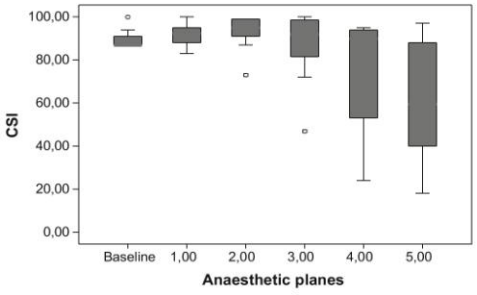

Fig. 1. Relationship between baseline infusion (T0) and anaesthetic planes A, B, C, D and E and CSI during the study period. "o" represents de outliers. Data presented as a box plot; 25th and 75th percentile are the boxes' borders; whiskers are the lowest and highest values for the 5th and 95th percentiles, respectively.

EMG activity, the EMG still has a significant influence on the CSI value (Jensen et al., 2006). The influence of EMG in dogs may be particularly important due to the amount of muscles in the head compared with humans. In an attempt to diminish the influence from EMG produced by temporal muscles electrodes were placed in the mid-line. The influence of EMG on the CSI values was a constant observation in our study and it seems to be the main reason for the high standard deviation observed in the CSI values during the anaesthetic planes C, D and E (Table 2).

Isoelectric EEG periods interrupted by brief periods of high amplitude EEG activity indicates a non-specific reduction in cerebral metabolic activity known as burst suppression (Rampil 1998). Onset of burst suppression has historically been associated with a surgical plane of anaesthesia (stage III, planes 2 and 3) (March and Muir, 2005). In our study, the BS was observed in three out of the four dogs that had values of CSI below 40 and happened more consistently in plane E of anaesthesia where all dogs lost their corneal reflex.

Another important parameter to take into account when using CSI monitoring is the SQI. The SQI represents the quality of the received EEG signal. The calculation of SQI is based on a series of artefacts that occur during each one-minute period. The SQI is also influenced by the impedance of the electrodes in the skin. Impedances of the black and white electrodes superior to 3 kΩ will negatively influence SQI and thus affect CSI monitoring (Danmeter, 2004). During the entire study period, impedances were kept low (within 1 kΩ and 3 kΩ) by using wax placed between electrodes and the skin.

Propofol decreases the arterial blood pressure by central depression of sympathetic neural output, which results in a decrease in systemic vascular resistance and potential hypotension (Clayes et al., 1988). Studies in sheep show that rapid injection of propofol for the induction of anaesthesia produces more effects on cardiovascular systems than a slower infusion rate (Zheng et al., 1998). In our study, no hypotensive episodes were observed and mean arterial blood pressure showed an average maximum decrease of 16% from "awake" values to the deepest anaesthetic stage observed (plane E). Heart rate was also within physiologic normal range during the entire study. The slow rate of propofol infusion (a constant infusion of 200 ml min^{-1}) and the dogs' weight of over 20 kg may help to explain the haemodynamic stability.

In conclusion, this study proposes five anaesthetic planes based on objective eyeball position and reflex observations during induction of anaesthesia with propofol. The performance of CSM was not consistent with the clinical observations on the different planes of depth of anaesthesia. This may limit its use during the induction of veterinary routine anaesthesia. The good haemodynamic stability provided by some hypnotic agents even at higher concentrations, as seems to occur with propofol, provides little clinical information for monitoring depth of anaesthesia and strengthens the need for an EEG based monitor for veterinary use. Until then, it seems that clinical reflexes still provide useful information that cannot be minimized. Nevertheless, these reflexes are of limited clinical value when using myorelaxant drugs during general anaesthesia.

References

Beths, T., Glen, J.B., et al., 2001. Evaluation and optimization of a target-controlled infusion system for administering propofol to dogs as part of a total intravenous anesthetic technique during dental surgery. Veterinary Records 148, 198–203.
Bhargava, A.K., Setlur, R., et al., 2004. Correlation of bispectral index and Guedel's stages of ether anesthesia. Anesthesia Analgesia 98 (1), 132–134.
Clayes, M.A., Gepts, E., et al., 1988. Haemodynamic changes during anesthesia induced and maintained with propofol. British Journal of Anesthesia 60, 3–9.
Danmeter, (2004). Cerebral State Monitor User Manual. UK.
Greene, S.A., Benson, G.J., et al., 2004. Effect of isoflurane, atracurium, fentanyl, and noxious stimulation on bispectral index in pigs. Comparative Medicine 54, 4.
Heiko, R., 2004. Depht of Anesthesia-Monitoring and Concepts. European Society of Anaesthesiologists, Lisbon.
Jensen, E.W., Litvan, H., et al., 2006. Cerebral state index during propofol anesthesia: a comparison with the bispectral index and the A-line ARX index. Anesthesiology 105 (1), 28–36.
Lamont, L.A., Greene, S.A., et al., 2005. Relationship of feline bispectral index to multiples of isoflurane minimum alveolar concentration. Comparative Medicine 55 (3), 269–274.
Lunn, J., Rosen, N., et al., 1987. Consciousness, Awareness, Pain and General Anesthesia. Butterworths, London.
March, P.A., Muir, W.W., 2005. Bispectral analysis of the electroencephalogram: a review of its development and use in anesthesia. Veterinary Anaesthesia and Analgesia 32, 241–255.

Martin-Cancho, M.F., Lima, J.R., et al., 2006. Bispectral index, spectral edge frequency 95% and median frequency recorded at varying desflurane concentrations in pigs. Research in Veterinary Science 81 (3), 373–381.

Miller, A., Sleig, J.W., et al., 2004. Does bispectral analysis of the electroencephalogram add anything but complexity. British Journal of Anesthesia 92 (1), 8–13.

Musk, G.C., Pang, D.S.J., et al., 2005. Target-controlled infusion of propofol in dogs - evaluation of four targets for induction of anaesthesia. Veterinary Records 157 (24), 766–770.

Pellegrino, F.C., Sica, R.E.P., 2004. Canine electroencephalographic recording technique: findings in normal and epileptic dogs. Clinical Neurophysiology 115, 477–487.

Pilge, S., Zanner, R., et al., 2006. Time delay of index calculation: analysis of cerebral state, bispectral, and narcotrend indices[laboratory investigations]. Anesthesiology 104 (3), 488–494.

Pomfrett, C.J.D., 1999. Heart rate variability, BIS and 'depth of anaesthesia'. British Journal of Anesthesia 82 (5), 659–662.

Rampil, I.J., 1998. A primer for EEG signal processing in anesthesia. Anesthesiology 84 (2), 980–1002.

Ribeiro, L.M., Ferreira, D.A., et al., 2008. Brain monitoring in dogs using the cerebral state index during the induction of anaesthesia via target-controlled infusion of propofol. Research in Veterinary Science 85 (2), 227–232.

Seitsonen, E., Yli-Hankala, A., et al., 2000. Are electrocardiogram electrodes acceptable for electroencephalogram bispectral index monitoring? Acta Anaesthesiologica Scandinavica 44 (10), 1266–1270.

Sleigh, J.W., Steyn-Ross, D.A., et al., 2001. Comparison of changes in electroencephalographic measures during induction of general anaesthesia: influence of the gamma frequency band and electromyogram signal. British Journal of Anesthesia 86 (1), 50–58.

Zheng, D., Upton, R.N., et al., 1998. The influence of the bolus injection rate of propofol on its cardiovascular effects and peak blood concentrations in sheep. Anesthesia Analgesia 86 (5), 1109–1115.

Zhong, T., Guo, Q.L., et al., 2005. Comparative evaluation of the cerebral state index and the bispectral index during target-controlled infusion of propofol. British Journal of Anesthesia 95 (6), 798–802.

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RESEARCH PAPER

Correlation between clinical signs of depth of anaesthesia and cerebral state index responses in dogs with different target-controlled infusions of propofol

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Abstract

Objective To evaluate if the cerebral state index (CSI), measured by a Cerebral State Monitor (CSM), can predict depth of anaesthesia as assessed clinically or by estimated propofol plasma concentrations.

Study design Prospective clinical study.

Animals Fourteen mixed breed dogs, weighing 24.5 ± 4.7 kg, scheduled to undergo neutering procedures.

Methods Dogs were premedicated with 0.05 mg kg^{-1} acepromazine intramuscularly. The CSM and cardiovascular monitoring equipment were attached. Anaesthesia was induced with propofol using a target controlled infusion (TCI) to varying plasma propofol targets (PropCp). Following endotracheal intubation the dogs were ventilated with oxygen. Anaesthetic maintenance was with propofol by TCI. A PropCp of 3 μg dL^{-1} was set initially, then PropCps were increased in 1 μg dL^{-1} steps to 7, 9 and then 11 μg dL^{-1}. Each PropCp was held constant for a 5 minute period, at the end of which depth of anaesthesia was classified using a previously evaluated scale of 'planes' based on palpebral and corneal reflexes and eye position. Cerebral state index (CSI), burst suppression (BSR) and electromyogram were measured at these time points. The prediction probability (PK) of these variables, or of the PropCp in predicting depth of anaesthesia was calculated.

Results The PKs for predicting anaesthetic planes were 0.74, 0.91, 0.76 and 0.78 for CSI, BSR, EMG and PropCp, respectively. The PKs for PropCp to predict CSI, BSR and EMG were 0.65, 0.71 and 0.65 respectively.

Conclusion and clinical relevance The Cerebral State Monitor was able to detect very deep planes of anaesthesia when BSR occurs, but was not able to distinguish between the intermediate anaesthetic planes likely to be used in clinical anaesthesia.

Keywords cerebral state index, depth anesthesia, electroencephalography, propofol.

Introduction

It is difficult to assess the depth of anaesthesia during balanced anaesthesia using both anaesthetic and neuromuscular blocking agents, and even in humans, haemodynamic changes do not always detect intraoperative awareness (Rampil 2001). The electroencephalogram (EEG) provides a direct measure of the functional state of the cerebral cortex which makes it possible to monitor increasing anaesthetic depth and detect if anaesthesia is

unnecessarily deep (Lunn & Rosen 1987). Different EEG patterns can be observed with increasing anaesthetic concentrations.

In humans (Kiyama & Takeda 1997; Gajraj et al. 1998) and in animals (Antunes et al. 2003; Otto 2008) the analysis of the EEG with methods based on power spectrum analysis showed no correlation with depth of anaesthesia. However, in humans the Bispectral Index (BIS) is used very successfully to provide a scale of hypnosis. In veterinary medicine, the efficacy of BIS monitoring in assessing the depth of anaesthesia in dogs (Muir et al. 2003), cats (March & Muir 2003) and pigs (Martin-Cancho et al. 2006) has been studied, but in animals the correlation between EEG depression and the BIS index is unclear, especially when burst suppression is observed after the administration of large anaesthetic doses (March & Muir 2005). This may reflect the fact that 'BIS' calculations use data collected from 5000 human EEG studies collected during anaesthetic procedures (Johansen & Sebel 2000) which makes its applicability difficult between species.

The Cerebral State Monitor (CSM) is a fuzzy logic based analysis from the EEG monitor and has been used for monitoring the depth of anaesthesia in humans (Jensen et al. 2006; Hoymork et al. 2007). The main advantage of this method is that because the relationship between the EEG and the clinical state cannot be easily modeled by a mathematical function, the fuzzy logic analysis has the potential to offer a better alternative to establish this relationship (Jensen et al. 2006). In a previous study carried out in our laboratory (Ribeiro et al. 2008) an intravenous (IV) propofol bolus of 6 mg kg^{-1} resulted in a progressive decrease in cerebral state index (CSI) values displayed by the CSM, and a slight negative correlation between the plasma target concentration (PropCp) and CSI was observed. This suggested the possibility that CSM could be useful in monitoring depth of anaesthesia in dogs. However, in a subsequent study in which propofol was given by a continuous infusion of 200 mL $hour^{-1}$ to induce anaesthesia to varying clinical depths, the CSI was not consistent with clinical observations of the depth of anaesthesia reached during these infusions (Ribeiro et al. 2009). The objective of this current study is to evaluate CSM correlation with different estimated propofol steady state plasma concentrations and clinical anaesthetic planes in dogs during maintenance of anaesthesia.

Methods

All procedures were approved by a local ethics committee, and fully informed consent for data collection, for altering the planes of anaesthesia and for enrolment in research was given by the owners.

Dogs and haemodynamic monitoring

We previously reported the CSM performance during induction of anaesthesia with propofol in 14 dogs (Ribeiro et al. 2009). This current report is of the continuation of anaesthesia with propofol in these subjects. All dogs were scheduled for a routine neutering procedure and were considered healthy based on clinical history, clinical examination and blood analysis (packed cell volume, total protein and blood urea).

All dogs were premedicated with 0.05 mg kg^{-1} acepromazine IM (Calmivet; Vetoquinol, France) 30 minutes prior to the beginning of the induction of anaesthesia. A cannula (18 gauge) was inserted in the cephalic vein for drug and fluid administration. A three way stopcock was used to connect the IV catheter to the propofol and sodium chloride 0.9% delivery lines. Sodium chloride at a constant infusion rate of 10 mL kg^{-1} $hour^{-1}$ was administered during the entire study period via an infusion pump.

A S/5 Datex monitor (Datex-Ohmeda, Finland) was used for monitoring the haemodynamic parameters. Blood pressure was measured non-invasively in the cranial metatarsal region of the left hindlimb, and mean arterial pressures are reported. Cuff width was chosen to be around 40% of circumference of the limb. Three measurements were obtained and used to calculate the average value (Datex S/5 'STAT' mode). A lead II electrode ECG was monitored.

Cerebral state index monitoring

The CSM used was the same as that employed in the previous studies (Ribeiro et al. 2008). The electrodes were already in position for the induction of anaesthesia (Ribeiro et al. 2009) and were left in place. The bipolar EEG waveform in our study was derived from the signal recorded between the frontopolar electrode (positive), and the occipital electrode (negative), as described by (Pellegrino & Sica 2004). The ground or the reference electrode was placed in the parietal position (Fig. 1). A

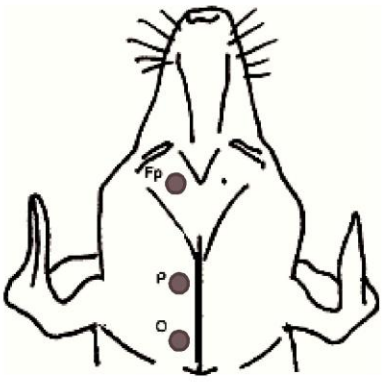

Figure 1 Adapted from (Pellegrino & Sica 2004). Electrodes placement for EEG recording. Dorsal view of canine cranium showing the placement of the EEG recording electrodes. Fp, frontal pole electrode; P, parietal electrode; O, occipital electrode.

modified ECG cable with clamp electrodes, connected to the CSM cable, was used to provide these electrodes. Before the application of clamp electrodes the skin was cleaned with alcohol 5 minutes before the data collection to allow drying.

The CSM displays the EEG signal continuously, and also four parameters derived from EEG analysis, CSI, BS, EMG and SQI. Details of how these parameters are derived has been described fully in our previous work (Ribeiro et al. 2009). CSI is based on a combination of four sub-parameters of the electroencephalographic signal: β ratio, α ratio, β-α ratio and burst suppression (BS). The CSI is displayed in a scale from 0 to 100 that represents the different depths of anaesthesia. The BS is defined as the percentage of time in a 30-second window where the amplitude of the electroencephalographic signal was <3.5 μV. These EEG periods characterise the deepest levels of hypnosis. The SQI is calculated using the quality of the acquired EEG signal and on the signal artefacts during the previous 1-minute period. The electrode-to-skin impedance is included in the SQI calculation. High levels of facial muscular or electromyographic (EMG) activity can interfere with the CSI under certain circumstances. The monitor incorporates an EMG filter that removes most of the potential interfering EMG activity. The EMG bar shows the energy of the EMG level in the 75–85 Hz frequency band (0–100 logarithmic scale). The EEG data collection ended before the patient was moved to the appropriate position for the commencement of surgery.

Anaesthetic protocol and clinical observations

RUGLOOP II software, (developed by Tom DeSmet (Demed Engineering, Belgium) and Michel Struys (Ghent University, Belgium)) running on a personal computer was used to drive the propofol syringe pump. The pharmacokinetic model incorporated in the RUGLOOP II software and used to estimate the propofol plasma concentrations was that of Beths et al. (2001). RUGLOOP II was also used to store the pharmacokinetic, haemodynamic and electroencephalographic data every 5 seconds.

Thirty minutes after premedication, anaesthesia was induced with propofol 1% (Fresenius Kabi, Germany) using a constant rate of infusion (CRI) of 200 mL hour^{-1} until various planes of anaesthesia were achieved. CSI was measured during this induction as previously described (Ribeiro et al. 2009). Once this previous study was completed, the trachea was intubated and the dogs were mechanically ventilated with 100% oxygen in order to maintain the end-tidal CO_2 between 35–45 mmHg (4.6–6 kPa). All dogs were positioned in left lateral recumbency during the entire study period. The propofol target control infusion (TCI) was then initialized by switching the infusion mode in the RugloopII from CRI to PropCp. An initial plasma target of 3 μg dL^{-1} was set. It required (mean ± SD) 9.5 ± 2.4 minutes for the initial high plasma concentrations of propofol achieved during induction of anaesthesia to decrease. During this period the RUGLOOP II software control stopped the infusion and when the estimated correct value was reached, the pump was automatically re-started and the plasma target of 3 μg dL^{-1} was maintained for 5 minutes. At the end of this 5-minute period, reflex testing to assess anaesthetic depth commenced.

Reflex testing was as described in our previous study (Ribeiro et al. 2009). The palpebral and corneal reflexes of the right eye were tested three times with a swab damped with serum. The reflexes were considered negative if there were no response to these three stimulations. The palpebral reflex was tested gently at the lateral canthus of the eye, and the corneal reflex was elicited by a smooth pressure on the cornea. The eyeball position was observed. The ocular reflexes were grouped in anaesthetic 'planes' of depth, A to E as described previously (Ribeiro et al. 2009) and in Table 1.

Table 1 Anaesthetic planes identified with increasing PropCp (target propofol plasma concentrations) in 13 dogs, in which anaesthesia was first induced, then maintained by propofol infusion.

Anaesthetic planes	Ocular reflexes
A	PR+/EC/CR+
B	PR+/ERV/CR+
C	PR–/ERV/CR+
D	PR–/EC/CR+
E	PR–/EC/CR–

The anaesthetic planes were allocated a letter A to E, according to the anaesthetic depth based on the presence (+) or absence (–) of ocular reflexes and the position of the eye: PR, palpebral reflex; CR, corneal reflex; EC, eyeball centred in the eye; ERV, eyeball rotated ventrally.

Once the reflexes had been tested, the PropCps were increased in 1 μg dL^{-1} steps every 5 minutes to 7 μg dL^{-1}, and then increased to 9 μg dL^{-1}, then to 11 μg dL^{-1}. These PropCps were each maintained for 5 minutes after each step increase. The same procedures were applied for evaluating the reflexes at the end of each 5 minute step. If mean arterial blood pressure decreased below 60 mmHg, the propofol administration was stopped.

After the end of the study a TCI of 3 μg dL^{-1} was set and a bolus of fentanyl 5 μg kg^{-1} was administered before surgery commenced. At the end of the surgery carprofen 4 mg kg^{-1} SC and a single dose of morphine 0.4 mg kg^{-1} SC were administered.

Data and statistical analysis

The efficacy of electroencephalogram parameters to predict depth of anaesthesia was evaluated using prediction probability (PK). The mathematical basis of PK was as described by Smith et al. (1996). A PK value of 1 means that the values of the predicting variable, e.g. anaesthetic depth indicator, correctly predicts the value of the observed anesthetic depth. A PK value of 0.5 means that the values of the indicator predict no better than a 50–50 chance. The PK calculation was performed using the program PKMACRO.

Data for heart rate, mean arterial pressure and CSM data (CSI, BS, SQI and EMG) are described as means ± SD for each anaesthetic plane. For data shown as a box plot: 25th and 75th percentile are the boxes' borders; whiskers are the lowest and highest values for the 5th and 95th percentiles, respectively. The time delays for the CSI calculation ranged from 53 to 55 seconds (Pilge et al. 2006). In our analysis, a delay of 50 seconds in CSI data was taken into account when analyzing CSI data.

Results

The subjects were 14 dogs, of mixed breeds, eight females and six males, aged 2.6 ± 1 years, and weight 24.5 ± 4.67 kg. One dog was excluded from data analysis because it showed an anomalous EEG flat line throughout the whole study period.

Two dogs required initial PropCp doses of 5 and 6 μg dL^{-1} respectively to maintain anaesthesia, as the target 3 μg dL^{-1} was insufficient. Depth of anaesthesia in one dog did not reach anaesthetic plane E even at 11 μg dL^{-1}.

The plane of anaesthesia (as defined by Table 1) increased with administration of increasing PropCp, but the five separate planes were not observed in all dogs. In most such cases depth of anaesthesia increased, missing a plane (e.g. from C to E). However, in one dog the plane decreased from D to C despite an increased PropCp.

The haemodynamic and electroencephalographic data and its relationship with the identified anaesthetic planes are shown in Table 2. The results demonstrate that CSI distinguished plane C and E from the baseline (awake). Only four dogs passed through plane D which impaired the analysis. EMG decreased with the depth of anaesthesia, and at plane E was minimal.

The PK for CSM parameters related with anaesthetic planes and PropCp are described in Table 3. The prediction probabilities (PK) for CSM parameters to be related with anaesthetic planes were at intermediate values in the region of 0.75. Thus the monitor did not always predict increases or decreases in the depth of anaesthesia. The lowest probability observed was between CSI and PropCp; the PK between PropCp and anaesthetic planes was higher (PK = 0.78). The highest value for PK was for BSR (PK = 0.91) related to anaesthetic planes. When BSR was analyzed with PropCp the PK decrease to a value of 0.71. At deep levels of anaesthesia the CSI was sensitive to burst suppression, 12 dogs reached the plane E and eight of them showed burst suppression (BS) the percentage BS during plane E was on average 11%. Burst suppression was also observed in planes C and D in two dogs with low values, <3%.

Table 2 Haemodynamic and electroencephalographic data observed before induction of anaesthesia (baseline) and when the dogs were at anaesthetic planes A to E (see Table 1)

	CSI	EMG (%)	BS (%)	SQI (%)	HR (beats minute^{-1})	MAP (mmHg)
Baseline	89 ± 3	94 ± 11	0.0	63 ± 21	101 ± 30	98 ± 14
Anaesthetic planes						
A	88 ± 13	68 ± 27	0.0	87 ± 8	99 ± 23	91 ± 23
B	79 ± 17	52 ± 31	0.0	78 ± 11	88 ± 19	84 ± 15
C	57 ± 16	20 ± 30	0.1 ± 0.4	82 ± 14	87 ± 20	83 ± 13
D	59 ± 18	24 ± 22	0.8 ± 1.7	78 ± 17	98 ± 25	88 ± 15
E	42 ± 14	6 ± 16	11.2 ± 18.8	88 ± 10	94 ± 17	79 ± 17

CSI, Cerebral state index; EMG, Electromyography; BS, Burst suppression; SQI, Signal quality index; HR, Heart rate; MAP, Mean arterial pressure.
All data are mean ± SD.

Table 3 Prediction probability (PK) for observed anaesthetic planes and for target propofol plasma concentrations (PropCp)

	PK for anaesthetic planes (see Table 1)	PK for PropCp
CSI	0.74	0.65
BSR	0.91	0.71
EMG	0.76	0.65
PropCp	0.78	

CSI, Cerebral state index; BSR, Burst suppression ratio; EMG, Electromyography.

Figure 2 demonstrates the relationship between the CSI and clinically assessed anaesthetic planes. The CSI showed a tendency to decrease with increasing propofol concentrations and increased anaesthetic depth. However it did not differentiate the intermediate anaesthetic planes. Although a great difference was observed in plane E – the deepest levels of anaesthesia, considerable overlap between CSI values obtained at the different anaesthetic planes was observed.

Discussion

The CSM calculates the CSI and uses four subparameters derived from the time domain analysis (burst ratio) and frequency domain analysis (α-ratio, β-ratio, β-ratio – α-ratio) of the EEG. To deliver an index, these subparameters are used as inputs to a fuzzy logic analysis. Fuzzy logic is a problem-solving control system methodology that incorporates a simple, ruled-based 'If *X* and *Y* then *Z*' approach to solving a control problem rather than attempting to model a system mathematically (Jensen et al. 2006).

In humans the CSM monitor has been studied using the Modified Observer's Assessment of Alertness and Sedation Scale (OAA/S) (Jensen et al. 2006). However, in veterinary practice there is no sedation scale accepted universally, and studies are difficult to perform in animals using loss of consciousness or loss of righting reflexes as assessment points without interfering with the collection of the EEG signal. In our previous study we identified a scale of anaesthetic depth based on ocular reflexes (Ribeiro et al. 2009). In this earlier study in which propofol was infused at a constant rate to induce anaesthesia and then to increase depth to the planes of anaesthesia identified in the scale, results with the CSM monitor were disappointing as, although there was some correlation between CSM and estimated propofol plasma concentrations, the CSM did not predict anaesthetic planes accurately. As it is possible that this failure was because a steady state propofol concentration had not been reached at the effector cells, the objective of this current continuation study was to re-evaluate the CSM performance, this time during maintenance of anaesthesia with propofol with different target concentrations. A monitor of depth anaesthesia can only be called accurate if it 1) provides an accurate correlation with cerebral drug concentration, 2) correlates with the clinical state of the patients, and 3) informs the clinician when excessive levels of anaesthesia are present (Jensen et al. 2006). In our study, these three aspects were analyzed.

The first condition for CSM was not met as the PK value was 0.65 which means that the monitor

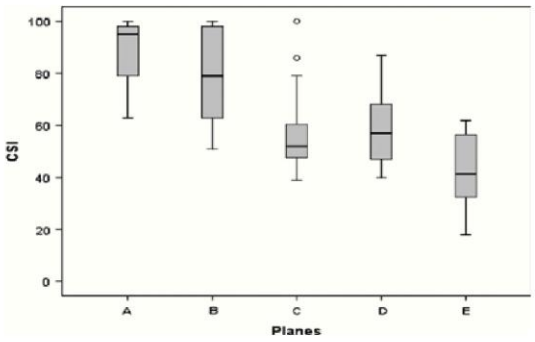

Figure 2 Relationship between anaesthetics planes A, B, C, D and E, and CSI; 'o' represents the outliers. Data are presented as a box plot: 25th and 75th percentile are the boxes' borders; whiskers are the values for the 5th and 95th percentiles. Overlap occurs between CSI values obtained in different anaesthetic plans.

did not always predict increments or decrements in the amount of propofol. The analysis was carried out 5 minutes after propofol was anticipated to have reached the selected plasma target concentration. At the end of each propofol stage, it was assumed that the PropCp was similar to the propofol concentration at the effect site This assumption was based on the half-life for the propofol plasma-effect-site equilibration being around 2.3 minutes for humans (Kazama et al. 1999). Musk et al. (2005) in studies with TCI allowed 3 minutes for the equilibration between blood and drug site action.

A potential reason for the poor correlation between CSI and PropCp in this study compared with the previous work (Ribeiro et al. 2009) is that a different method of statistical analysis was employed. A further reason could be the application of Beths' pharmacokinetic model (Beths et al. 2001). The model is derived from a population sample of dogs, with its inevitable variations between real and target plasma concentrations of propofol. In humans a 20–30% variation between real and target propofol plasma concentration can be acceptable clinically for TCI use (Coetzee et al. 1995; Fechner et al. 1998). However, the PropCp based on Beths' pharmacokinetic model could be even more variable as a result of the great breed variability in the dog population. It is important to note that in Beths' model the performance of the TCI was evaluated with the administration of an opioid and in the present study acepromazine alone was used for premedication (Beths et al. 2001).

In our study no analyses were performed to measure the real propofol concentration in the blood. Regarding the second condition, the present study showed that the sequential increase of the anaesthetic depth from plane A to E was followed by a decrease in CSI values, but the CSI did not distinguish accurately between the anaesthetic planes B, C and D as the CSI values were overlapped. Although CSI seems to provide useful information at the deepest levels of anaesthesia when burst suppression occurs; its clinical usefulness was limited because it could not provide reliable clinical information during intermediate levels of anaesthesia.

EMG activity is the most significant source of EEG artefact (Jensen et al. 2006). The influence of EMG in dogs may be particularly important due to the amount of muscles in the head compared with humans. The influence of EMG on the CSI values was a constant observation in our study and could be another reason for the low values obtain for PK.

The CSM appeared to detect the excessive levels of anaesthesia that were reached at plane E. Some studies in humans also suggest that the CSI has a better performance than BIS in detecting deep levels of anaesthesia adequately (Jensen et al. 2006). Burst suppression refers to isoelectric periods interrupted by brief intervals of high amplitude EEG activity. An isoelectric EEG pattern is continuous, uninterrupted by burst activity. This pattern is developed during deep anaesthesia (Rampil 1998; Greene et al. 2002). In dogs, an increase in suppression ratio could lead to paradoxical increases of BIS values (BIS > 60) (March & Muir 2005).

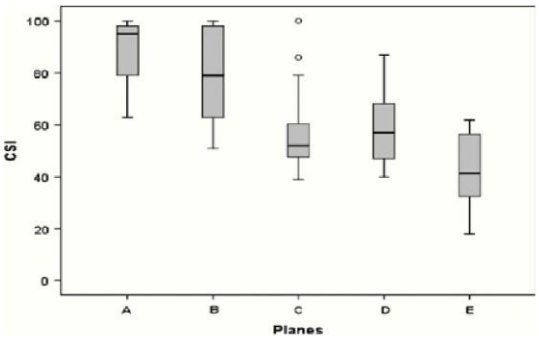

Figure 2 Relationship between anaesthetics planes A, B, C, D and E, and CSI; 'o' represents the outliers. Data are presented as a box plot: 25th and 75th percentile are the boxes' borders; whiskers are the values for the 5th and 95th percentiles. Overlap occurs between CSI values obtained in different anaesthetic plans.

did not always predict increments or decrements in the amount of propofol. The analysis was carried out 5 minutes after propofol was anticipated to have reached the selected plasma target concentration. At the end of each propofol stage, it was assumed that the PropCp was similar to the propofol concentration at the effect site This assumption was based on the half-life for the propofol plasma-effect-site equilibration being around 2.3 minutes for humans (Kazama et al. 1999). Musk et al. (2005) in studies with TCI allowed 3 minutes for the equilibration between blood and drug site action.

A potential reason for the poor correlation between CSI and PropCp in this study compared with the previous work (Ribeiro et al. 2009) is that a different method of statistical analysis was employed. A further reason could be the application of Beths' pharmacokinetic model (Beths et al. 2001). The model is derived from a population sample of dogs, with its inevitable variations between real and target plasma concentrations of propofol. In humans a 20–30% variation between real and target propofol plasma concentration can be acceptable clinically for TCI use (Coetzee et al. 1995; Fechner et al. 1998). However, the PropCp based on Beths' pharmacokinetic model could be even more variable as a result of the great breed variability in the dog population. It is important to note that in Beths' model the performance of the TCI was evaluated with the administration of an opioid and in the present study acepromazine alone was used for premedication (Beths et al. 2001).

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Nevertheless, this was not observed in our study, in which burst suppression was observed frequently during plane E at the time of the lowest CSI values. During burst suppression, the α and β ratio are no longer monotonously decreasing as a function of anaesthetic depth and therefore they cannot be used in the calculation of the final index (Jensen et al. 2006).

The haemodynamic parameters were monitored in this study to ensure that the dogs were not harmed by the increasing depth of anaesthesia. However, they provided very little information regarding depth of anaesthesia. It is known that propofol decreases the arterial blood pressure by central depression of sympathetic neural output, which results in a decrease in systemic vascular resistance and potential hypotension (Clayes et al. 1988). The velocity of the propofol infusion is also a very important factor influencing haemodynamic effects (Brás et al. 2009). In our study, dogs showed a surprisingly stable haemodynamic profile, the maximum decrease of MAP being 24% from baseline in plane E. This haemodynamic stability when propofol is infused may limit the usefulness of haemodynamic data for monitoring depth of anaesthesia and strengthen the need for an EEG based monitor for veterinary use, but in this current study no painful stimuli were administered so the cardiovascular response to surgery was not investigated.

In conclusion, this study evaluates the use of the CSM in detecting depth of anaesthesia with propofol as scaled in five previously identified anaesthetic planes (Ribeiro et al. 2009). Although there is some prediction probability between CSI values and the anaesthetic planes, the CSI showed clinically important limitations as it was not able to distinguish between anaesthetic planes B, C, and D (i.e. those depths likely to be used in clinical practice), as CSI values overlapped. Although CSI was able to detect deeper levels of anaesthesia when burst suppression occurs, it would be hoped that such deep levels would not normally be reached. However, the burst suppression was a consistent finding in deep planes of anaesthesia, and this fact could be relevant for the use of Fuzzy logic analysis in future monitors.

References

Beths T, Glen JB, Reid J et al. (2001) Evaluation and optimisation of a target-controlled infusion system for administering propofol to dogs as part of a total intravenous anaesthetic technique during dental surgery. Vet Rec 148, 198–203.

Brás S, Bressan N, Ribeiro L et al. (2009) A step towards effect-site target-controlled infusion with propofol in dogs: a k(e0) for propofol. J Vet Pharmacol Ther 32, 182–188.

Clayes MA, Gepts E, Camu F (1988) Haemodynamic changes during anesthesia induced and mantained with propofol. Br J Anaesth 60, 3–9.

Coetzee JF, Glen JB, Wium CA et al. (1995) Pharmacokinetic model selection for target controlled infusions of propofol. Assessment of three parameter sets. Anesthesiology 82, 1328–1345.

Fechner J, Albrecht S, Ihmsen H et al. (1998) Predictability and precision of "target-controlled infusion" (TCI) of propofol with the "Disoprifusor TCI" system. Anaesthesist 47, 663–668.

Gajraj RJ, Doi M, Mantzaridis H et al. (1998) Analysis of the EEG bispectrum, auditory evoked potentials and the EEG power spectrum during repeated transitions from consciousness to unconsciousness. Br J Anaesth 80, 46–52.

Greene SA, Benson GJ, Tranquilli WJ et al. (2002) Relationship of canine bispectral index to multiples of sevoflurane minimal alveolar concentration, using patch or subdermal electrode. Comp Med 52, 424–428.

Hoymork SC, Hval K, Jensen EW et al. (2007) Can the Cerebral State Monitor replace the Bispectral Index in monitoring hypnotic effect during propofol/remifentanil anaesthesia? Acta Anaesthesiol Scand 51, 210–216.

Jensen EW, Litvan H, Revuelta M et al. (2006) Cerebral state index during propofol anesthesia: a Comparison with the bispectral index and the A-line ARX index. Anesthesiology 105, 28–36.

Johansen J, Sebel P (2000) Development and clinical application of electroencephalographic bispectrum monitoring. Anesthesiology 93, 1336–1344.

Kazama T, Ikeda KMK, Kikura M et al. (1999) Comparison of the effect-site keO s of propofol for blood pressure and EEG bispectral index in elderly and younger patients. Anesthesiology 90, 1517–1527.

Kiyama S, Takeda J (1997) Effect of extradural analgesia on the paradoxical arousal response of the electroencephalogram. Br J Anaesth 79, 750–753.

Lunn J, Rosen N (1987) Consciousness, Awareness, Pain and General Anesthesia. Butterworths, London.

March PA, Muir WW (2003) Use of the bispectral index as a monitor of anesthetic depth in cats anesthetized with isoflurane. Am J Vet Res 64, 1534–1541.

March PA, Muir WW (2005) Bispectral analysis of the electroencephalogram: a review of its development and use in anesthesia. Vet Anaesth Analg 32, 241–255.

Martin-Cancho MF, Lima JR, Luis L et al. (2006) Bispectral index, spectral edge frequency 95% and median frequency recorded at varying desflurane concentrations in pigs. Res Vet Sci 81, 373–381.

Muir WW, Wiese AJ, March PA (2003) Effects of morphine, lidocaine, ketamine, and morphine-lidocaine-ketamine drug combination on minimum alveolar concentration in dogs anesthetized with isoflurane. Am J Vet Res 64, 1155–1160.

Musk GC, Pang DSJ, Beths T et al. (2005) Target-controlled infusion of propofol in dogs – evaluation of four targets for induction of anaesthesia. Vet Rec 157, 766–770.

Pellegrino FC, Sica REP (2004) Canine electroencephalographic recording technique: findings in normal and epileptic dogs. Clin Neurophysiol 115, 477–487.

Pilge S, Zanner R, Schneider G et al. (2006) Time delay of index calculation: analysis of cerebral state, bispectral, and narcotrend indices. Anesthesiology 104, 488–494.

Rampil IJ (1998) A primer for EEG signal processing in anesthesia. Anesthesiology 84, 980–1002.

Rampil IJ (2001) Monitoring depth of anesthesia. Curr Opin Anaesthesiol 14, 649–653.

Ribeiro LM, Ferreira DA, Bressan NM et al. (2008) Brain monitoring in dogs using the cerebral state index during the induction of anaesthesia via target-controlled infusion of propofol. Res Vet Sci 85, 227–232.

Ribeiro LM, Ferreira DA, Bras S et al. (2009) Correlation between clinical signs of depth of anaesthesia and cerebral state index responses in dogs during induction of anaesthesia with propofol. Res Vet Sci 87, 287–291.

Smith WD, Dutton RC, Smith NT (1996) Measuring the performance of anesthetic depth indicators. Anesthesiology 84, 38–51.

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