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Tremors in white rhinoceros (*Ceratotherium simum*) during chemical immobilisation

By

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A dissertation in partial fulfilment of the requirements of the degree Masters in Veterinary Science in the Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria

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Animal Ethics Committee

PROJECT TITLE	**Tremors in the white rhinoceros *(Ceratotherium simum simum)* during chemical immobilization**
PROJECT NUMBER	**V087-13**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Ms. S de Lange**

STUDENT NUMBER (where applicable)	**280 310 84**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**White rhinoceros *(Ceratotherium simum simum)***	
NUMBER OF ANIMALS	**25**	
Approval period to use animals for research/testing purposes		**February 2014-December 2014**
SUPERVISOR	**Dr. L Meyer**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 25 November 2013
CHAIRMAN: UP Animal Ethics Committee	Signature

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Tremors in white rhinoceros (*Ceratotherium simum*) during chemical immobilisation

SS de Lange; A Fuller; A Haw & LCR Meyer

Summary

White rhinoceros (*Ceratotherium simum*) are susceptible to developing muscle tremors during chemical immobilisation induced by potent opioid receptor agonists. Whether these tremors result directly from the actions of the opioids or from other physiological changes associated with immobilisation is unknown. A pilot study on 8 boma-managed chemically immobilised rhinoceros was conducted using different supportive interventions for the animal's cardiorespiratory systems to test whether these interventions had an effect on tremors during chemical immobilisation. The pilot study revealed that butorphanol, a partial opioid agonist/antagonist, combined with nasotracheal oxygen insufflation, compared to the control, was the only intervention that decreased the observed tremor intensity and adequately stabilized the rhinoceros cardiorespiratory system in the immobilised rhinoceros. With this knowledge and using the same drug protocol (etorphine and azaperone and hyaluronidase) and supportive interventions (butorphanol and nasotracheal oxygen insufflation), a field study was conducted to quantify tremors, both objectively and subjectively, and record various physiological responses of 14 rhinoceros during a 25 minute chemical immobilisation period. Butorphanol was injected intravenously 6 minutes after the rhinoceros became laterally recumbent. Tracheal oxygen insufflation was also administered from this time. Occurrence (intensity) of tremors was assessed every minute throughout the 25 minute immobilisation period, both subjectively by human observation, and objectively by accelerometer data loggers placed on the front leg. Arterial blood pH, oxygen and carbon dioxide levels, electrolytes and plasma catecholamine concentrations were measured at 5 minute time points. The tremor intensity was highest (5 minutes – 28 counts/min) just after the animals became recumbent, but decreased (3 counts/min) after butorphanol and nasotracheal oxygen insufflation was administered. Tremor intensity was correlated with the mean pH, arterial partial pressure of oxygen, serum

potassium and median plasma adrenaline concentration. High tremor intensity occurred when plasma adrenaline concentrations were elevated and when hypoxaemia and acidaemia were at their worst. Hypoxaemia and acidaemia, both physiological stressors, were correlated with the increased plasma adrenaline concentrations. These correlations indicate that changes in blood oxygenation and pH could be the driving force behind the changes in the tremor intensity. Butorphanol and nasotracheal oxygen insufflation corrected the hypoxia and acidaemia and reduced tremor intensity. Therefore, tremor intensity could possibly indicate the severity of the pathophysiological effects of the capture drugs on a rhinoceros cardiorespiratory system.

Chapter 1

Introduction

Since the 1960's, rhinoceros have been chemically immobilised for relocation as part of conservation management plans (Kock *et al.* 1995). Initially, the black rhinoceros (*Diceros bicornis*) was the primary focus of these conservation efforts (Kock *et al.* 1990a; Kock *et al.* 1990b). However, with the recent rapid increase in poaching, intensive conservation management plans are needed for all rhinoceros species, including the white rhinoceros (*Ceratotherium simum*). Currently, rhinoceros are regularly chemically immobilised for management practices such as relocation, data collection for research, de-horning, as well as other veterinary interventions (Kock *et al*. 1995; Wenger *et al*. 2007). Chemical immobilisation thus is essential for these conservation efforts. However, the immobilisation process in itself may place a rhinoceros at risk as a result of physiological derangements related to the stress of the capture event and drug-induced side-effects (Kock *et al*. 1990a, 1990b; Heard *et al*. 1992; Kock 1992).

Muscle tremor is one particular opioid-induced side-effect that occurs commonly in immobilised white rhinoceros (Kock *et al*. 1995; Moreira 2010). Although tremors are well recognised in rhinoceros, there is a lack of knowledge as to the mechanisms and how best to minimise these immobilisation-induced tremors. This study was an opportunistic investigational study in white rhinoceros that were being chemically immobilised to determine the effects of different drug interventions used to improve cardiorespiratory function during immobilisation. In boma-managed rhinoceros (the initial part of this study) the characteristics of tremors during immobilisation were determined by subjective visual observations. In field-captured rhinoceros (the second part of this study) an objective method of measuring tremors, by using activity loggers, was assessed alongside subjective visual observations. In both boma and field studies, measured physiological variables were used to determine a potential cause of these tremors.

The aims of this investigational study were:

1. To determine the possible mechanisms that may lead to muscle tremors in immobilised white rhinoceros.
2. To establish a reliable method of measuring the tremor intensities objectively.
3. To determine whether butorphanol, a partial opioid agonist/antagonist, and nasotracheal oxygen insufflation affect tremor intensity.

Chapter 2

Literature review

During field immobilisation of wild animals, a short induction period is essential in order to minimize the time during which the animal can injure itself (Bush *et al.*1980; Bush & de Vos 1987; Morkel 1994; Raath 1994; Radcliffe & Morkel 2008). A quick induction also decreases complications associated with excessive stress and exertion such as tachycardia, hypertension, hyperthermia and metabolic acidosis (Morkel 1994). In rhinoceros, a quick induction is achieved by administering a potent opioid that causes a reversible catatonic immobilisation. Opioids such as etorphine and thiafentanil are regularly used to chemically immobilise both captive and free-living animals (Portas 2004; Foggin *et al.* 2012). The negative effects of these opioids, including muscle tremors, cardiovascular alterations and respiratory depression (with subsequent hypoxia and hypercapnia), have been well documented and may result in life-threatening complications (Radcliff *et al*. 2000; Atkinson *et al.* 2002; Portas 2004; Fahlman 2008; Moreira 2010). Of these effects, muscle tremors have been studied the least and very little is known about their cause, and their possible effects on a rhinoceros.

2.1 Defining tremors

A tremor is a “quivering movement that cannot be controlled” (Hawker & Waite 2007). Trembling (tremors), shivering, shaking and quaking are all terms that are used to describe the same basic movement of involuntary rapid repetitive muscle contraction and relaxation (Carithers 1995). Some specialists combine the use of the words tremble (shiver, quake and quiver) and tremor. Tremble has been used to describe a higher frequency of the contraction of skeletal muscles (induced by fear, fatigue or temperature regulation) (Carithers 1995), whereas tremors are an uncontrolled contraction and relaxation of antagonistic muscles. There are various types of tremors, which have been investigated in humans and animals, which differ in frequency as well as in their distribution in the body. Some tremors are defined based on the body’s support against

gravity and whether there are concurrent voluntary or involuntary movements (postural and rest tremors). Other types of tremors are defined when specific tasks are performed (task-specific tremors), or when tremors occur during voluntary movements (kinetic tremors). Just as there are many types of tremors or trembles, so too are there many different causes of these tremors. Some tremors occur as a result of a non-infectious disease (Wilsons, Parkinson's and neuropathic disease) or a pathophysiological condition (anxiety or metabolic disorders like acidosis), while others occur due to an infection or trauma. Low blood calcium (hypocalcaemia), low blood glucose (hypoglycaemia) and high blood potassium (hyperkalaemia) are specific metabolic derangements that are known to cause trembling or tremors (Carithers 1995). Some tremors may also be toxin- or drug-induced (Carithers 1995; Findley 1996; Dalvi & Premkumar 2011).

2.2 Opioid-induced muscle tremors

Etorphine, a μ-, δ- and κ-opioid receptor agonist, is a drug that possibly induces muscle tremors and muscle rigidity in various species (Haigh 1990; Portas 2004; Mentaberre *et al*. 2010; Moreira 2010), including rhinoceros (Bush *et al*. 2004). Indeed, muscle tremors in immobilised rhinoceros have been found to increase in severity with additional doses of etorphine (Heard *et al.* 1992; Kock *et al.* 1995)

When animals are chemically immobilised it is very seldom that opioids are used as the sole agent for this purpose. Usually a tranquilizer or sedative is added to the opioid to aid in the immobilising process (Burroughs *et al.* 2012a). A tranquilizer, e.g. azaperone, is most commonly used in combination with etorphine to immobilise rhinoceros. Azaperone is believed to reduce the induction time in rhinoceros and, to some extent, it can decrease the vascular hypertension induced by etorphine (Moreira 2010). Azaperone belongs to the chemical class butyrophenones, which affect the body through dopaminergic and peripheral adrenergic blockade, causing both tranquilization and alterations in motor function. Some of the most common side-effects of this class are severe hypotension, ataxia, hypertonia and tremors (Mentaberre *et al.* 2010).

Sedatives that are commonly used during capture include drugs belonging to the chemical classes of α_2-adrenergic receptor agonists and the benzodiazepines. Drugs belonging to both classes induce muscle relaxation (Burroughs *et al*. 2012b). Although α_2-adrenergic receptor agonists have been shown to reduce muscle tension in chemically immobilised rhinoceros (Wenger *et al.* 2007), they are not commonly used in this species as they exacerbate respiratory depression and hinder gas exchange in the lungs (Burroughs *et al*. 2012b). The benzodiazepines; diazepam and midazolam, have been used successfully to treat muscle tension that is accompanied by jerking and twitching in rhinoceros (Burroughs *et al.* 2012b). However, when midazolam was combined with etorphine, at the same dose described by Burroughts *et al*. (2012b), to immobilise rhinoceros, obvious muscle tremors occurred and only decreased after butorphanol was administered (Moreira 2010). It has also been observed by field practitioners that muscle tremors decrease when butorphanol is administered to chemically immobilised rhinoceros (Burroughs *et al*. 2012b; M. Hofmeyr, 2013, personal communication).

In contrast, the administration of 0.075 mg/kg butorphanol to horses, 5 minutes after they were sedated with xylazine, caused an increase in trembling of their lips and head (Lascurain *et al.* 2006). Butorphanol, which appears to act as a partial agonist/antagonist at μ-opioid receptors as well as being an agonist at κ-opioid receptors (Radcliffe *et al*. 2000; Prado *et al.* 2008; Knych *et al.* 2012), is often administered during chemical immobilisation in order to reduce etorphine-induced cardiorespiratory depression (Portas 2004; Miller *et al.*2013). Whether butorphanol reduces or exacerbates muscle tremors still needs to be clarified.

The effects of opioids on muscle tremors, whether direct or indirect, still needs to be determined. Opioids cause catatonic immobilisation through indirect effects in the central nervous system, where they influence the concentration of neurotransmitters, particularly dopamine, in central neurons involved in motor control (Kania 1985). Whether these effects result in tremors is unknown. The possible direct effects of opioids on muscle tremors have been investigated to a greater extent than the indirect

effects. Opioids interact directly with muscle cells, particularly those in the heart and gastrointestinal system. Fentanyl has been found to decrease the sensitivity of myofilaments to calcium, as well as decrease the intracellular calcium concentration (Kanaya *et al.* 1998). In contrast, morphine at higher doses reportedly increases the intracellular calcium concentration, but also to decreases myofilament calcium sensitivity (Kanaya *et al.* 1998) and potentially act to protect the cells. Previous studies reported that morphine protected cardiac cells from ischaemic injury (Liang & Gross 1999; Zhang *et al.* 2008).

When the neuronal-structured opioid receptors in the gastrointestinal tract are stimulated, different effector pathways are activated. One of these pathways results in a decrease in the voltage-gated calcium channels (Sternini *et al*. 2004), which results in a decrease in peristaltic movement. Under normal circumstances, peristaltic movement occurs as a result of an influx of calcium through the channels into the smooth muscle cells, while calcium is also released from intracellular storage (Huizinga & Lammers 2009). These channels occur not only in the smooth muscle cells of the gastrointestinal tract, but also in cardiac and skeletal muscles, where they have a direct impact on the intensity and frequency of contractions (Catterall 2011). Opioid receptors are also found on skeletal muscles (Kurz & Sessler 2003); however the distribution of different opioid-receptor subtypes varies among cell types, layers and regions, and even among different species (Ventura *et al*. 1992; Storr *et al.* 2000; Evans *et al*. 2001; Kurz & Sessler 2003; Sternini *et al.* 2004; Lin *et al*. 2008; Holzer 2009). It may be reasonable to speculate that, when opioids are administered to rhinoceros, skeletal muscles may tremor because of the direct activation of opioid receptors, which influence the contractile mechanisms of skeletal muscle cells.

2.3 Changes in physiological variables that can potentially induce tremors

Immobilising drugs might not be the only cause of muscle tremors during chemical immobilisation to capture rhinoceros. Especially in free-ranging rhinoceros, capture results in a “fight-or-flight” response, which triggers the release of catecholamines

(adrenaline, noradrenaline and dopamine) into the bloodstream (Moreira 2010; Fitzgerald 2012; Meltzer & Kock 2012). Catecholamines play a vital role in controlling the biochemical and physiological responses during exercise (Williams *et al*. 2002) and stress. Adrenaline and noradrenaline are released via activation of the sympathetic nervous system and exert their effect on the organs that have adrenoreceptors (Fitzgerald 2012). Adrenaline has been found to have a greater effect on the α-adrenoreceptors, while noradrenaline has a greater effect on the β-adrenoreceptors (Owen 1986). Increased circulating catecholamines cause muscle tremors (Anderson & Aitken 1977). The release of catecholamines into the bloodstream can also result in a chain-reaction which may alter other physiological variables. Previous studies in anaesthetised dogs reported an increase in either adrenaline or noradrenaline that resulted in an increase in oxygen consumption and glucose uptake in the upper jejunum (Grayson & Oyebola 1983); when both adrenaline and noradrenaline were administered the blood glucose concentration increased and remained elevated for a longer period than the increased oxygen consumption. However, when noradrenaline was administered on its own, the glucose concentrations were lower and oxygen consumption remained elevated for a longer period of time. Apart from oxygen consumption and glucose uptake, catecholamines also have an effect on plasma potassium levels. Administration of adrenaline and noradrenaline can result in a change in the plasma potassium concentration (hypokalaemia or hyperkalaemia), depending on the dose, the species and the route of administration (Moratinos & Reverte 1993). Potassium is one of the most abundant cations found in the body. The balance of intra- and extracellular potassium can be influenced by several factors, including a change in the pH (Moratinos & Reverte 1993). Hydrogen ion concentration is closely related to changes in plasma potassium concentration and catecholamines have also been found to have an effect on the blood pH (Jeffers 1986; Primmett *et al.* 1986). Moreover, it is widely accepted that catecholamines prompt an increase in heart rate (Taylor & Meeran 1973) with adrenaline stimulating a greater increase than noradrenaline (Lees & Taverno 1970).

Many of the physiological alterations induced by catecholamines can indirectly induce or influence muscle tremors. The direct stimulation of β_2-adrenergic receptors can cause sweating and muscle tremors in horses (Yovich *et al*. 1984). β-blockers have been used to control tremors in chemically immobilised captive bears (Bush *et al*. 1980), and also in anaesthetised humans (Frishman 2003; Arbaizar *et al*. 2008). Therefore it is plausible that a capture-induced release of catecholamines might cause tremors as a result of β_2-receptor activation.

The effect of changing catecholamine concentrations on blood pH may also indirectly influence muscle tremors. Blood pH is also affected by a range of other variables, including potassium (K^+) (Moratinos & Reverte 1993) and lactate (Sahlin *et al*. 1976; Snow & Mackenzie 1977). Several studies on the relationship between the pH and lactate have been done in both humans (Sahlin *et al.* 1976) and animals, such as horses (Snow & Mackenzie 1977). In both cases, it has been reported that the blood pH decreased during exercise and then returned to normal levels a short period after the exercise. For example, in horses (Snow & McKenzie 1977) it took 15 minutes for the pH to return to normal after the exercise was completed. Not surprisingly, it was observed that during intensive rowing exercise in humans, plasma potassium and arterial blood lactate concentration increased, while arterial pH decreased (Atanasovska *et al.* 2014).

As mentioned, hyperkalaemia can induce tremors and it may be plausible that acidosis and hyperlactaemia may also play a role. It has been previously speculated that muscle tremors in rhinoceros during chemical immobilisation might be linked to acidosis, hypercapnia and hypoxaemia arising from opioid-induced respiratory depression (Kock & Garnier 1993). Hypoxia, which is a known side effect of opioids (Radcliff *et al.* 2000; Atkinson *et al*. 2002; Portas 2004; Fahlman 2008; Moreira 2010), stimulates peripheral chemoreceptors, which results in an increase in ventilation and redistribution of blood flow in the body and lungs, as well as an increase in arterial blood pressure. The chemoreceptor response can also be enhanced by additional factors such as acidosis, hypercapnia, systemic hypotension and catecholamines (Heistad & Abboud 1980). It has been proposed that noradrenaline might be released as a result of hypoxaemia,

which then stimulates chemoreceptor activation. In contrast, dopamine decreases chemoreceptor activation in humans and has been shown to decrease the response to hypoventilation (Heistad & Abboud 1980).

2.4 Potential consequences of muscle tremors

Tremors and shivering increase metabolic heat (and possibly body temperature), heart rate (which consequently may result in an increase in blood flow) and oxygen consumption (Diaz & Becker 2010; Sessler 2011). During capture and immobilisation rhinoceros often become hyperthermic, hypoxic and hypertensive, and therefore tremors may exacerbate these capture-induced side-effects (Diaz & Becker 2010). Apart from the possible physiological consequences of tremors in rhinoceros, high tremor intensities may also make it difficult to collect samples and work with the immobilised rhinoceros.

2.5 Measurement of muscle tremors

In immobilised wildlife, the relationship among tremors, capture-drugs, hypoxaemia, catecholamine release and various other physiological factors has been especially difficult to evaluate as current measurements of tremor intensity are subjective, rather than objective. Indeed, an objective method of measuring the intensity of muscle tremors is needed to be able to investigate whether tremors have any clinical significance. There are some difficulties with measuring tremor intensity by subjective visual scoring methods. These difficulties may include the differences in the subjectivity of the person observing the rhinoceros and the variability of tremor intensity at different sites on the rhinoceros body that the person may be observing. Some of the objective methods used for humans, such as measuring the amount of water spilled from a cup, are easy to use and cheap, but are not practical in rhinoceros. Other methods, such as accelerometry and electromyography (EMG), are more expensive and require some expertise (Kugelberg 1947; Bain 1998). Electromyography has been used to differentiate between varieties of muscular disorders in humans (Kugelberg 1974). The

use of electromyography in the field is problematic, as the measurement devices were built for the human hospital setting and are not designed for field use. The devices are also very expensive and heavy, require a large power supply and are not easily portable (Williams *et al*. 2008). Lighter and more portable electromyographs however, are being developed and have been used previously in birds (Lesku *et al.* 2011). Currently, a popular method of measuring muscle activity is using activity data loggers, such as Actical or Sigma-Delta activity loggers. These activity loggers have different sensitivity levels. The Actical® logger (Mini-Mitter Co., Inc., Bend, OR) has a uniaxial or omnidirectional accelerometer that is most sensitive in a single plane (Lascelles *et al.* 2008). This specific device was originally designed for humans (Heil 2006; Paul *et al.* 2007; Lascelles *et al*. 2008) but it and other similar accelerometer loggers have been used with success in numerous animal studies to measure activity levels (Mitchell *et al.* 1997; Lascelles *et al.* 2008; Papailiou *et al.* 2008; Culp *et al.* 2009; Hetem *et al.* 2010; Hetem *et al*. 2012; McFarland *et al.* 2013). The Sigma-Delta logger has also been used to measure activity patterns in animals and it appears to be more sensitive as it records activity across three-planes and is equally sensitive across all of them (McFarland *et al.* 2013). Having a specific method of measuring tremors, such as using an accelerometer, can be useful when attempting to understand the changes in tremor intensity that are visually observed (Elble *et al.* 2006). Accelerometers have been used to measure tremor amplitude in humans that suffer from hand tremors (Elble *et al.* 2006). However, uniaxial accelerometers are unable to record the complexity of the three-dimensional movements that occur in muscle tremors (Elble *et al.* 2006). Nevertheless, as far as we know, measuring the intensity of muscle tremors using activity loggers in rhinoceros during chemical immobilisation has not previously been attempted. By establishing a better technique to measure tremor intensity and by investigating the relationship among tremors and physiological variables, we aim to provide a better understanding of the tremor intensity in rhinoceros.

Chapter 3

Materials and Methods

3.1 Subjective measurement of tremor intensity in white rhinoceros immobilised in a boma environment

3.1.1 Experimental design

Eight sub-adult white rhinoceros (*Ceratotherium simum*) that were housed in purpose-built bomas at Skukuza in the Kruger National Park, South Africa (S24°59.696' E031°35.217) were used in an opportunistic investigational study to determine the efficacy of different supportive interventions in reducing opioid-induced respiratory depression. Once these rhinoceros were chemically immobilised, the characteristics of the tremors which they developed, and how the tremors changed after each intervention, was determined by subjective visual observations of the tremor intensity. The rhinoceros were chemically immobilised four times every 2 weeks, so that each individual received each intervention (see 3.1.2 & Table 3.2) in a random order. The supportive interventions were administered 6 minutes after the rhinoceros became laterally recumbent and data was recorded during the full 20 minute immobilisation period. Visual observations of the tremors that occurred during the immobilisations were done by one person every 5 minutes, using a pre-determined scoring system (see 3.1.4 & Table 3.3). For practical reasons the observer was not blinded to which of the interventions were administered to the rhinoceros. Arterial blood gases were measured and recorded at the same 5 minute time points during the immobilisation period for comparison with tremor intensity measures.

3.1.2 Chemical immobilisation and handling

The rhinoceros were darted with a mixture of etorphine hydrochloride (M99, Novartis, Kempton Park, South African, 9.8 mg/ml), azaperone (Stressnil, Janssen

Pharmaceutical Ltd, Halfway House, South Africa, 10 mg/ml) and hyalase (hyaluronidase - lyophilized hyalase, Kyron Laboratories, Midrand, South Africa) by remote injection using a CO_2-powered dart gun (Dan-Inject, South Africa). The dose used to induce the rhinoceros was determined by using a scale of an approximate weight-to-dose ratio (Table 3.1). This scale was developed and is used by the Veterinary Wildlife Services (VWS) at Skukuza for rhinoceros capture. The veterinarian darted the rhinoceros from a walkway on top of the bomas. Once the chemical immobilising drugs had taken effect, resulting in standing immobilisation, a blindfold was placed over the rhinoceros's eyes. The rhinoceros was guided, with the aid of a rope, into lateral recumbency, which occurred within 15 minutes after being darted. A catheter was inserted into the medial auricular artery, on the inner aspect of the pinna, for sampling arterial blood. Blood samples (1 ml) were taken at 5 minute intervals starting 5 minutes after the rhinoceros was recumbent until the end of the immobilisation at 20 minutes (Fig. 3.1). Six minutes after recumbency, each rhinoceros received one of the four supportive interventions which included a control, intravenous butorphanol, continuous nasotracheal oxygen insufflation, and a combination of intravenous butorphanol and continuous nasotracheal oxygen insufflation (Table 3.2). Oxygen was administered using an equine 'stomach' tube (9.5 mm o.d. x 213 cm, Kyron Laboratories, Midrand, South Africa), which was inserted into the nasal cavity and then guided ventrally into the tracheal region (Bush *et al*. 2004). Catheters and tubing were removed after 20 minutes. The rhinoceros was stimulated to stand and guided into a weighing-crate with the aid of ropes and a prodder. Once weighed (Nagata Electronic Crane Scale (HB/HC – 33)), the rhinoceros received the opioid antagonist, naltrexone, intravenously into an auricular vein (50 mg/ml, Kyron Laboratories, Midrand, South Africa) at a dose of 20 times the given etorphine dose (Table 3.1). Naltrexone completely antagonized the effects of etorphine and reversed the immobilisation. The rhinoceros was released back into the boma as soon as the reversal agent had taken full effect.

Table 3.1 Doses for chemical immobilisation and antagonisation, based on estimated body weights, of rhinoceros

Weight (kg)	**M99** (~10 mg/ml) (~0.002 mg/kg)		**Azaperone** (40 mg/ml) (~0.03 mg/kg)		**Butorphanol** (20 mg/ml) (15 x M99 mg)		**Naltrexone** (50 mg/ml) (20 x M99 mg)	
	mg	**ml**	**mg**	**ml**	**mg**	**ml**	**mg**	**Ml**
500-750	1.5	0.15	22.5	0.56	22.5	1.125	30	0.6
750-1000	2.0	0.2	30.0	0.75	30.0	1.5	40	0.8
1000-1250	2.5	0.25	37.5	0.94	37.5	1.875	50	1.0
1250-1500	3.0	0.3	45.0	1.125	45.0	2.25	60	1.2
1500-1750	3.5	0.35	52.5	1.31	52.5	2.625	70	1.4
1750-2000	4.0	0.4	60.0	1.5	60.0	3	80	1.6

Table 3.2 Supportive interventions administered during chemical immobilisation of rhinoceros (IV = intravenous)

	Control (Saline)	**Butorphanol**	**Butorphanol and oxygen insufflation**	**Oxygen insufflation**
Administration	IV	IV	IV (butorphanol) and nasotracheally (oxygen)	nasotracheally
Dosage	2 ml	15 mg/mg etorphine		30 L/min

3.1.3 Measurement of arterial blood gases

The auricular artery was catheterized using a 22G x 25.4mm IV catheter (Nipro Safelet Cath, Nipro Corporation, New Jersey, United States of America). At 5, 10, 15 and 20 minutes (time 0 = start of lateral recumbency) 1ml of blood was drawn into pre-heparinized syringes for blood gas analyses. A portable pre-calibrated blood gas analyser with pre-calibrated blood gas cassettes (Roche OPTI CCA Analyzer + OPTI cassette B, Kat Medical, Johannesburg, South Africa) was used for the arterial blood analyses.

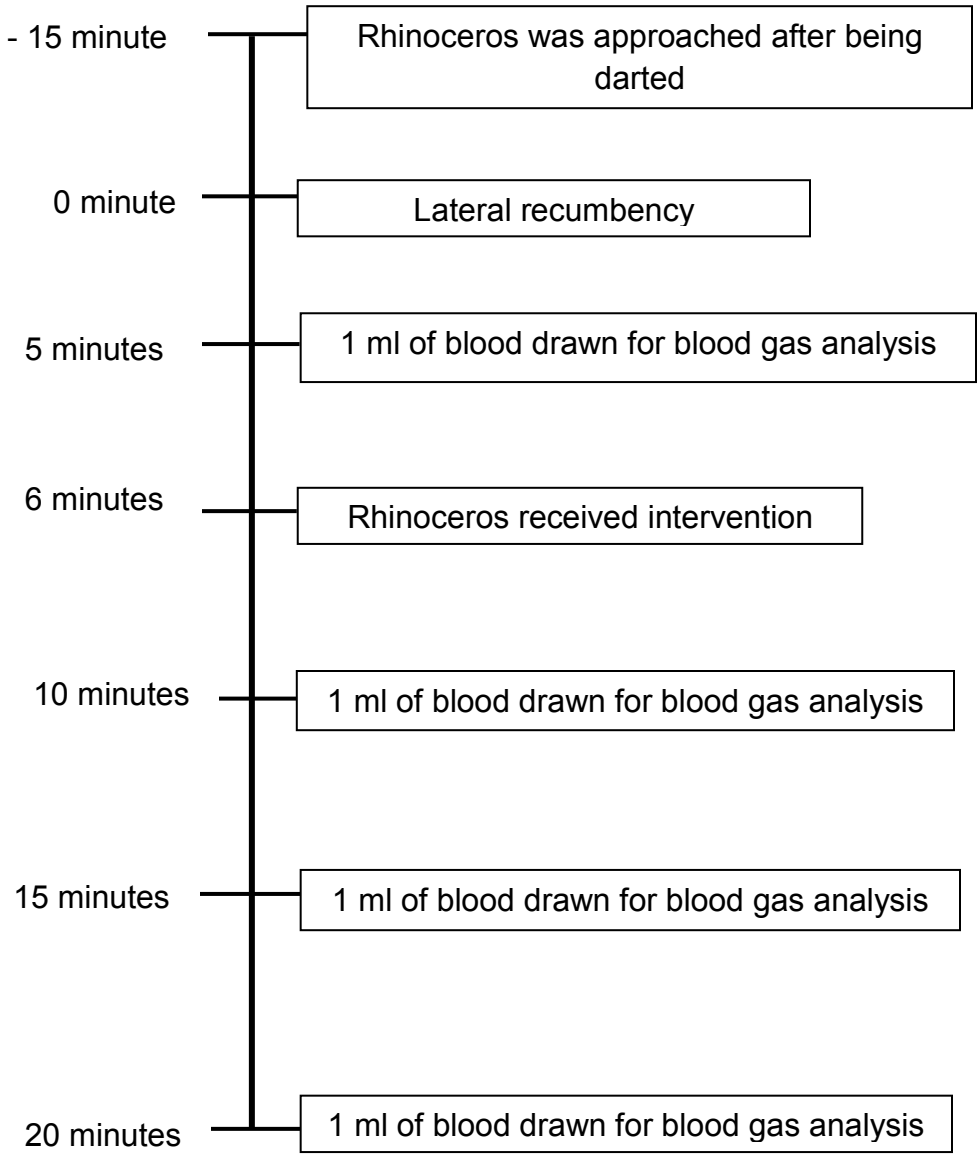

Figure 3.1 Time points at which arterial blood samples were drawn while the rhinoceros were in lateral recumbency.

3.1.4 Subjective measurements and recordings of tremor intensity

The intensity of the tremors during lateral recumbency were recorded every 5 minutes (at the same time points at which blood samples and clinical variables were recorded). The muscle tremor intensity was subjectively assessed by one observer and using a predetermined scoring scale (Table 3.3).

Table 3.3 Scoring system used to rank tremor intensity in the rhinoceros.

Observation	
No visible tremors	0
Slight tremors - Tremors resulting in leg and foot movement only	1
Mild tremors - Tremors resulting in shoulder and chest movement with leg and foot movement	2
Moderate tremors - Tremors resulting in gross shoulder, chest, leg and foot movement	3
Severe tremors - Tremors resulting in whole body and head movement	4

3.1.5 Data analysis

The sample size from the boma-study was too small to allow for normality testing. Therefore, based on an excepted data distribution of each variable in a normal population, the data was treated as parametric or nonparametric. A repeated measure non-parametric one-way analysis of variance (ANOVA) with Dunn's multiple comparisons test was used to determine significant differences in tremor intensity over time for each of the interventions when compared to the control intervention and also among the four interventions at the various time points. For the arterial partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) and blood pH, a repeated measure of one-way analysis of variance (ANOVA) with Dunnett's multiple comparison was used to determine significant differences between the interventions and the control intervention. To determine the relationship between the median tremor intensity (score) with the mean PaO_2, $PaCO_2$ and mean blood pH across the interventions, the Spearman's rank-

order correlation was used. GraphPad® Prism (version 6.02) was used to analyse the data, with the level of statistical significance set at $p < 0.05$.

3.1.6 Ethical clearance

This project was approved by the University of Pretoria's Animal Ethics Committee (approval number V087-13; Addendum A), and by the University of the Witwatersrand's Animal Ethics Screening Committee (clearance certificate 2012/23/04; Addendum B).

3.2 Objective and subjective measurement of tremor intensity in free-living white rhinoceros

3.2.1 Experimental design

To determine the possible causes of tremors and evaluate the association between physiological variables and the tremor intensity during chemical immobilisation under field conditions, 14 rhinoceros were immobilised in the field using a combination of etorphine, azaperone and hyaluronidase at the same doses used in the boma study (see 3.1). This study took place in the southern section of the Kruger National Park (S24°59.696' E031°35.217). Over the course of 9 days, all 14 rhinoceros were darted in the early morning, during the coolest time of the day. The 14 rhinoceros were sub-adult males that had been selected randomly in the field, and ranged in ages from 3 to 8 years. After the rhinoceros were chemically immobilised and had been recumbent for 6 minutes, butorphanol was injected intravenously and nasotracheal oxygen insufflation started. The nasotracheal oxygen insufflation then continued throughout the immobilisation period. To determine an objective measurement of tremor intensity in rhinoceros, activity loggers were attached to a leg. In addition, an individual observer scored tremor intensity subjectively, as per Table 3.3. The tremors were measured using both visual observations (subjectively) and activity loggers (objectively); at 1 minute time points throughout the immobilisation period (see 3.2.5). Arterial blood variables were measured and recorded while the rhinoceros were chemically

immobilised. The measured blood variables included blood pH, blood glucose, plasma catecholamines (noradrenaline, adrenaline and dopamine), blood electrolytes, and arterial partial pressure of oxygen (PaO_2) and carbon dioxide (PaO_2). These variables were used to establish the possible mechanisms associated with muscle tremors by correlating them with tremor intensity.

3.2.2 Rhinoceros capture

Each rhinoceros was immobilised with the same mixture of chemical immobilising drugs and a dart-gun, just as the rhinoceros in the boma (see 3.2.2). The doses used to dart the rhinoceros were calculated based on an estimate of the body mass of the rhinoceros, using the same scale that was used to determine the doses for the rhinoceros that were kept in the bomas (Table 3.1). Each rhinoceros was darted from a helicopter. Once the rhinoceros displayed initial effects of the capture drugs, the helicopter pilot guided the rhinoceros towards a road or open area where a ground-crew could reach the rhinoceros. As soon as the rhinoceros started stumbling and had limited co-ordination, the Veterinary Wildlife Services (VWS) staff approached the rhinoceros and a blindfold was thrown over the eyes, followed by a rope that was tied around its head, just behind the second horn. The rhinoceros was then pushed down into sternal recumbency, before being manoeuvred into left lateral recumbency. A 22G X 25.4 mm IV catheter (Jelco®, Smiths Medical, Kempton Park, South Africa) was inserted into the medial auricular artery of the right ear, while an 18G x 25.4 mm IV catheter (Jelco®, Smiths Medical, Kempton Park, South Africa) was inserted into an auricular vein on the lateral aspect of the left ear. While the rhinoceros were recumbent, 1ml and 10 ml blood samples were drawn at 1 minute, 5 minute, 10 minute, 15 minute and 20 minute time points (see 3.2.3). After 6 minutes of lateral recumbency, the rhinoceros received butorphanol (Kyron Laboratories, Midrand, South Africa, 20 mg/ml), as well as oxygen delivered at a flow rate of 30 L/min via nasotracheal intubation. The tube was inserted into the nostril and then guided ventrally into the trachea, in the same way as in the rhinoceros in the boma. The dose of butorphanol for each rhinoceros was calculated by taking the dose of etorphine that was in the dart and multiplying it by 15 (Table 3.1).

After 25 minutes of being in lateral recumbency, the catheters were removed. To ensure that the rhinoceros was not re-darted from the helicopter, heavy-duty silver tape was taped around the right back foot and around the horn as temporary markers. The rhinoceros was then stimulated to walk into a crate, where it was weighed (Nagata Electronic Crane Scale (HB/HC – 33)). Thereafter, the rhinoceros received naltrexone (IV), an opioid antagonist, at a dose 20 times that of the etorphine used (Table 3.1), to reverse the immobilisation. The rhinoceros was then released back into the field.

3.2.3 Blood samples

At 5 minute time points, starting from the moment the rhinoceros was in lateral recumbency, 1ml of blood was drawn from the right auricular artery into pre-heparinised syringes for blood gas and electrolyte analysis. An Epoc^{TM} BGEM blood analysis system (Kyron Laboratories, Midrand, South Africa) was used for arterial blood analyses. It determined the blood pH, calcium (Ca^{+}), glucose, chloride (Cl^{-}), sodium (Na^{++}), potassium (K^{+}) and lactate concentrations, and also the arterial partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$). Within the first minute of placing the rhinoceros into lateral recumbency, a 10ml blood sample was collected through the catheter in the left ear. The blood was placed in two 5ml EDTA (ethylenediaminetetraacetic acid) tubes (BD Vacutainer®, Johannesburg, South Africa). Thereafter, at 5 minute time points during the immobilisation, 10ml of blood was drawn and placed in EDTA tubes. These blood samples were used to determine the catecholamine concentrations for each rhinoceros at the various time points after being placed into recumbency. After 25 minutes, 10ml of blood was drawn from a vein in the front lower left leg and placed in EDTA tubes (Fig. 3.2).

All the EDTA tubes were placed directly onto ice and then, within 10 minutes after collection in the field, centrifuged, at 2500 rpm for 10 minutes. The plasma was pipetted and placed in cryotubes (Greiner Bio-One, Frickenhausen, Germany), and then snap-frozen in liquid nitrogen in the field. These samples were then stored in a -80°C freezer at the VWS facilities for two months. They were transported on dry ice to a laboratory at

the University of the North West to determine catecholamine (noradrenaline, adrenaline and dopamine) concentrations using high-performance liquid chromatography (HPLC; see 3.2.4).

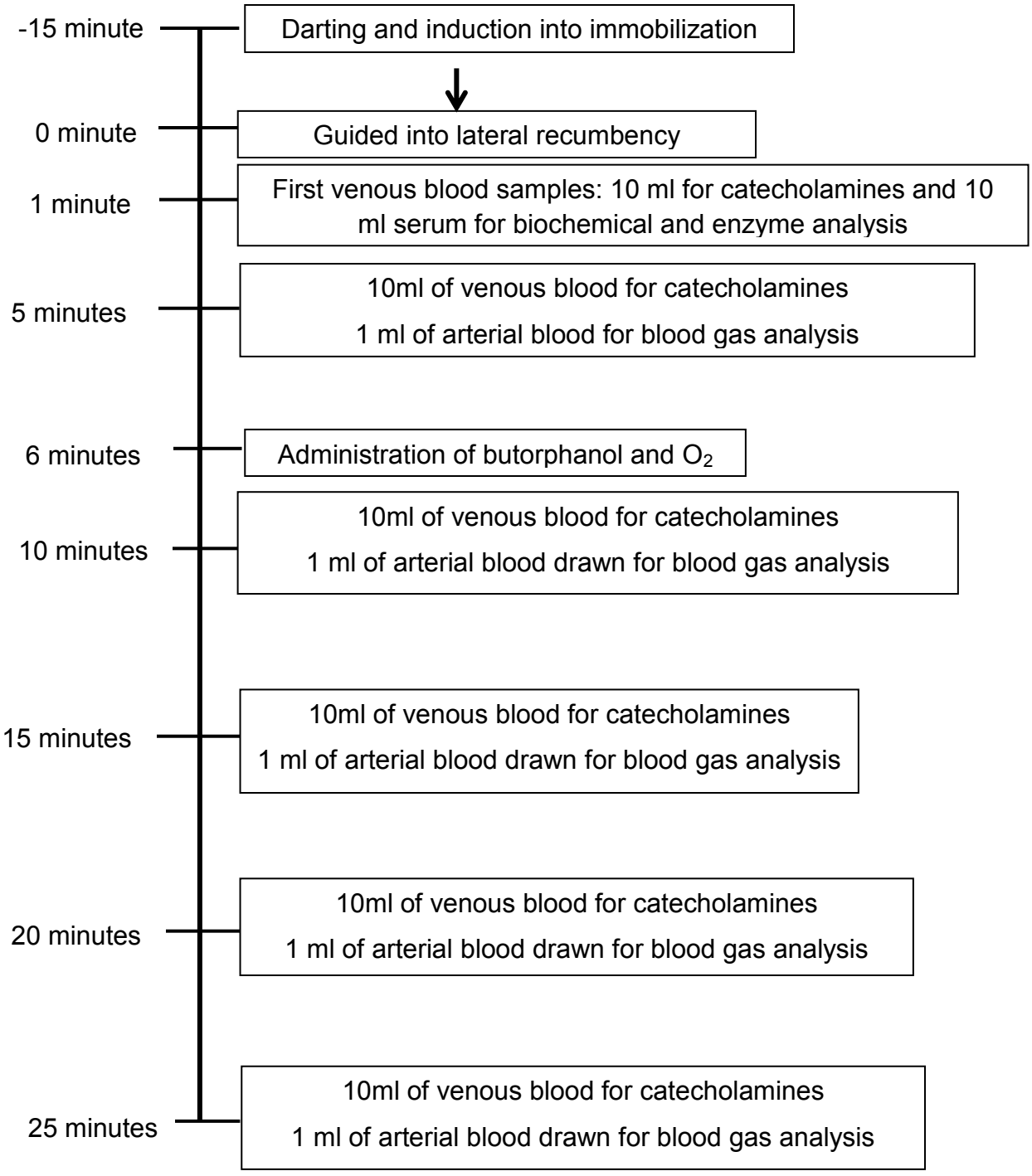

Figure 3.2 Time points of blood sampling while the rhinoceros were in lateral recumbency

3.2.4 Determination of catecholamine concentrations by High-Performance Liquid Chromatography (HPLC)

The HPLC method used for determining adrenaline, noradrenaline and dopamine concentrations in blood plasma was a modification of the methods used in previous studies (De Villiers *et al*. 1987; Coetzee 2006). The test was done at the School of Pharmacy of the Faculty of Health Science, University of the North West, in Potchefstroom, South Africa. Cryotubes containing the frozen plasma samples were stored in a -80°C freezer until the day of analysis. On the day of analysis, the cryotubes with the frozen plasma samples were placed on ice so as to thaw slowly. An empty 1.5ml Eppendorf tube received approximately 50 mg of acid-washed alumina (type WA – 4, acid) then 900 µl of plasma, 500 µl Tris buffer and 20 µl of internal standard, at a concentration of 7.5 µg/ml. The Tris buffer allowed the catecholamines to bind to the alumina. Once all the solutions had been placed in the eppendorf tube, the mixture was mixed vigorously for 30 minutes. The mixture was then centrifuged at room temperature for 10 to 15 minutes at 14 000 rpm. The supernatant that formed at the top of the eppendorf tube was removed with a micropipette and discarded. The alumina that remained in the eppendorf tube was rinsed off twice with 1ml of double-distilled water and the resulting supernatant was removed. To decrease the pH of the alumina to 2, 200 µl of 0.1M perchloric acid solution was added. The mixture was then placed on ice for 30 minutes to allow for the catecholamines to slowly desorb from the alumina due to a change in pH. After 30 minutes, the mixture was then vortexed (Vortex mixer VM – 300), and centrifuged for 5 minutes. The entire acidic supernatant sample was placed in a sample tray of an Agilent 1200 Series, equipped with an isocratic pump, an autosampler and an ESA Coulochem III Electrochemical detector with a coulometric flow and Chromeleon® Chromatography Management System version 6.8. Of the sample, 20 µl were programmed to be injected, at a flow rate of 1.00 ml/min, into the column (Synergi 4 µ Hydro-RP, 250 x 4.6 mm, 4 µ, 80 Å pores, Phenomenex, Torrance, CA) and a guard column (SecurityGuard™, HPLC Guard Cartridge System, with SecurityGuard Cartridges, C18, 4.0 x 3.0 mm, Phenomenex, Torrance, CA) for analysis. Using the same method, standard curves were created using an internal standard

(I.STD) of isoprenaline, which consisted of 1mg of DL-Isoproterenol hydrochloride dissolved in 10ml of solution A. The peak area of each sample was recorded on a spread-sheet in nanograms per millilitre (ng/ml), in the form of the standard curve straight-line equation (F. Viljoen, personal communication 2014).

3.2.5 Measurement of tremors

Two methods of measuring the tremor intensity were used. The first method was visual observations to score tremor intensity subjectively. The same pre-determined scoring system was used as when the rhinoceros were immobilised in the bomas (see Table 3.3). The second method used activity loggers, or accelerometers, to measure the tremor intensity.

The data loggers contain accelerometers, which record activity continuously at pre-set time points. Two types of activity loggers were used to measure muscle tremors at 1 minute points. One was an Actical (Mini-Mitter Co., Inc., Bend, OR; Lascelles *et al.* 2008), which contains an omnidirectional accelerometer. The second was a Sigma-Delta logger (Mlog_AT1, Sigma-Delta Technologies, Wembly, Australia), which has a triaxial accelerometer. The time interval over which data are recorded and averaged is known as an epoch, thus both logger types were set at an epoch of 1 minute.

To reduce the possibility of losing data if one of the loggers happened to fail, each logger was duplicated using a back-up logger on each rhinoceros at each site. Two Actical and two Sigma-Delta loggers were placed next to one another to make one logger pack. By placing them next to one another they were kept in a standard position throughout the study. The loggers were then bound together using heavy duty tape in order to protect them from damage (Fig. 3.3 & Fig. 3.4). A total of two packs were placed on each rhinoceros. Therefore, a total of 8 loggers were placed on the rhinoceros. One pack of encased loggers was secured to the shoulder (Fig. 3.4 A) and a second pack was placed just above the foot of the rhinoceros (Fig. 3.4 B). The logger packs were kept in place using rubber-bands. After the loggers had been placed and

secured on the leg, a rope was bound around the foot of this leg and held fast to try to prevent large kicking movements. The reason for this was to decrease the possibility of any large limb movements being recorded, instead of the actual tremors. All the loggers functioned normally and therefore the data from only four of the loggers were used for analysis.

To decide which logger to use as an objective measurement for measuring tremor intensity, the data from all the activity loggers were compared to each other. Between the Actical and the Sigma-Delta activity loggers, the logger that recorded the finer tremor intensity was chosen for further analysis (see 4.3.4). The visual data (tremor scores) and the logger activity data were correlated to determine whether the logger data and visual data were comparable. Tremor intensity data were also used to determine if there were any relationships between the clinical data and the blood variables that were collected while the rhinoceros were immobilised (see 4.5).

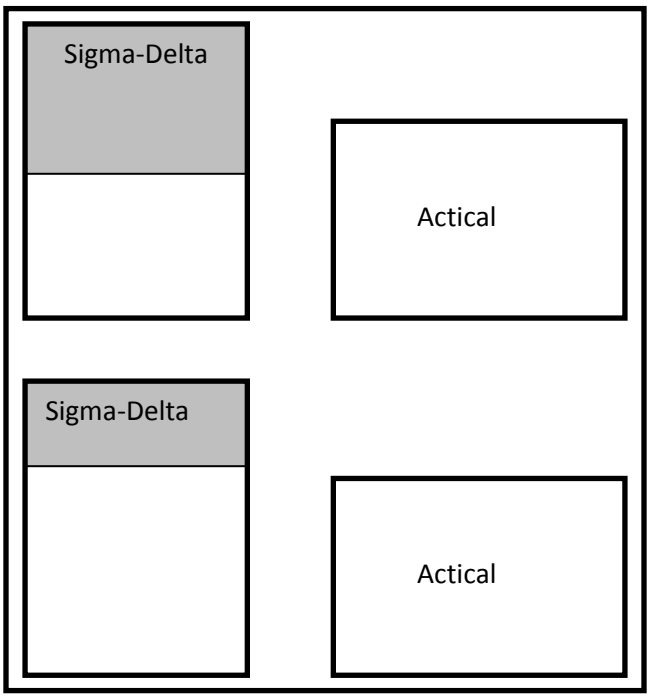

Figure 3.3 A “pack”, which contained two Actical and two Sigma-Delta activity loggers

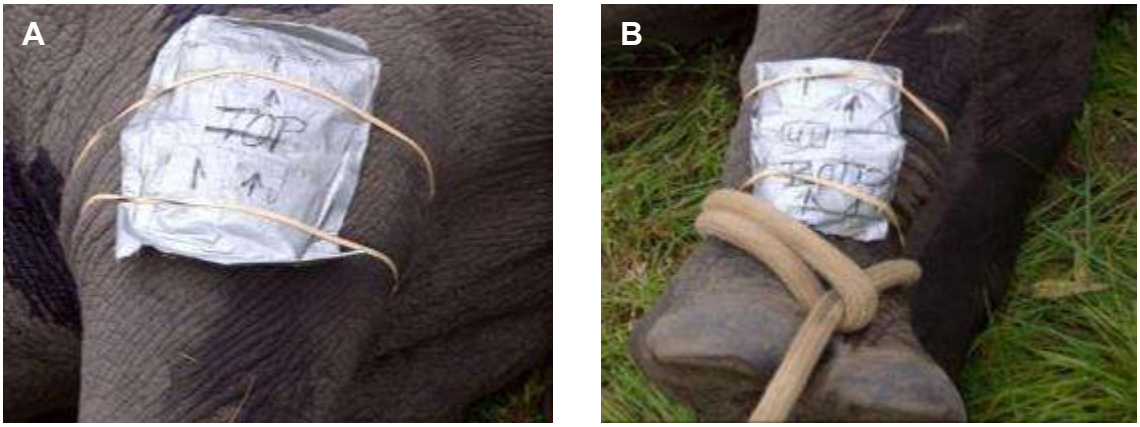

Figure 3.4 Upper part of the right leg of the rhinoceros with the upper pack (A) and the lower part of the right leg with the lower pack (B), which contained two Actical and two Sigma-Delta loggers. The packs were held in place with rubber bands.

3.2.6 Data analysis

The relationship between tremor intensity and the different physiological variables were modeled using a linear and generalized linear mixed model regression (R v3.0.2, R: A Language and Environment for Statistical Computing; R Core Team; R Foundation for Statistical Computing; Vienna; Austria; 2014). However, model fit was poor and therefore simple univariate non-linear (Spearman's rank-order) correlations were used to determine the relationship between tremor intensities and physiological variables.

A Spearman's rank-order correlation was used to determine the relationship between the activity loggers and the visual tremor intensity scores. For this analysis, the median observed tremor intensity (score) that was observed for all 14 rhinoceros at each minute was correlated with the median tremor intensity (counts/minute) that was recorded by the logger. When data from two activity loggers were compared, they were converted into percentages of the maximum value that was recorded for that specific logger during the study. However, to make the logger data linear, thereby allowing for a parametric (Pearson's product-moment) correlation when it was analysed with the measured variables, the data were transformed to the base of 10 (log). Similarly adrenaline, noradrenaline and lactate concentrations were also transformed in the same manner, thereby enabling Pearson's product-moment correlations to be used. Out of the three known catecholamines, namely adrenaline, noradrenaline and dopamine, only adrenaline and noradrenaline were detected by the HPLC method; the dopamine concentrations were too low in the plasma samples to be recorded by this method. Because catecholamines are not only released in response to a psychological stress, but also can be released in response to physiological stress, the relationship between mean catecholamines and mean physiological variables (PaO_2, $PaCO_2$, electrolytes, glucose, pH and lactate) in the 14 rhinoceros over the immobilization were assessed using Pearson's product-moment correlation

Actual drug doses were correlated, using Spearman's rank-order correlation, to determine if there was any relationship with the original tremor intensity count. The dose

per kilogram (mg/kg) of etorphine and azaperone was correlated with the tremor intensity at the 1 and 5 minute time points only. The actual amount of the butorphanol, was also calculated (mg/kg) and correlated with tremor intensity for the time points at 10, 15 and 20 minutes. In addition, the total distance that the rhinoceros ran before and after being darted was correlated with tremor intensity that was recorded at the first minute that the rhinoceros were placed in recumbency, using the Spearman's rank-order correlation.

A paired, parametric one-way analysis of variance (ANOVA), followed by a Tukey's multiple comparison post-hoc test, was used to determine how blood pH, glucose, calcium (Ca^{+}), glucose, chlorine (Cl^{-}), sodium (Na^{++}) and potassium (K^{+}) concentrations, arterial partial pressure of oxygen (PaO_2) and arterial partial pressure of carbon dioxide ($PaCO_2$) data changed over time, and whether there were any differences among the data before butorphanol and nasotracheal oxygen insufflation were administered. To determine how the lactate, catecholamines and tremor intensity (both the observed tremor intensity and the tremor intensity recorded by the loggers) changed over time, a Friedman's test, followed by a Dunn's multiple comparison post-hoc test, was used. Data analysis was performed using GraphPad® Prism (version 6.02), with the level of statistical significance set at $p < 0.05$.

3.2.7 Ethical clearance

Approval to perform the study was granted by the Animal Ethics Committee (approval number V087-13; Addendum A) at the Faculty of Veterinary Science, University of Pretoria, SANParks' Scientific committee (approval number HAWA1042; Addendum C) and the Animal Ethics Screening Committee from the University of the Witwatersrand (clearance certificate 2012/23/04; Addendum B). Additional Threatened or Protected Species (TOP's) permits were applied for to move the frozen plasma samples across province borders.

Chapter 4

Results

4.1 The relationship between treatment interventions and tremor intensity during chemical immobilisation of white rhinoceros in the boma

4.1.1 Tremors during chemical immobilisation

Tremors were visually observed in all rhinoceros that were immobilised with etorphine and azaperone. The observed tremor intensities (subjective tremor intensity scores, see Table 3.3) were highest at 5 minutes into lateral recumbency (median tremor intensity score = 2.5 to 3) for all the rhinoceros (Fig. 4.1.1 A, B, C and D), with distinct tremors resulting in gross shoulder, chest, leg and foot movement. The tremor intensity decreased over the 20 minutes of lateral recumbency when no supportive intervention was administered to the rhinoceros (control, $p = 0.003$; $F_3 = 14.18$, Fig. 4.1.1 A). However there was no significant difference in the tremor intensity when the 10, 15 and 20 minute time points were each compared to the 5 minute time point (pre-intervention). From 10 minutes of lateral recumbency onwards, mild tremors were observed, resulting in shoulder and chest movement with leg and foot movements (median tremor intensity score = 2 to 2.5). Between individuals, there was substantial variability in the tremor intensity scores at the 15 and 20 minute time points, with some rhinoceros showing slight tremors that resulted in movement in the legs and foot only (median tremor score = 0.5) and others moderate tremors that resulted in gross shoulder, chest, leg and foot movement (median tremor intensity score = 3).

4.1.2 Effects of butorphanol and nasotracheal oxygen insufflation on tremor intensity during chemical immobilisation

When the partial opioid agonist/antagonist, butorphanol, was administered intravenously at 6 minutes peri-recumbency, the tremor intensity decreased significantly over the 20 minute immobilisation period ($p = 0.0003$, $F_3 = 18.5$, Fig. 4.1.1 B). Four minutes after

butorphanol was administered, namely at the 10 minute time interval, the tremor intensity had not changed significantly compared to the pre-intervention tremor scores at 5 minutes. However, at the 15 minute ($p = 0.01$, median tremor intensity score = 0.5) and 20 minute ($p = 0.003$, median tremor intensity score = 0) time points, the tremor intensity significantly decreased from moderate tremors that resulted in gross shoulder, chest, leg and foot movements at the 5 minute time point, to either no tremors or localised tremor movements in the foot at the 15 and 20 minute time points.

Butorphanol combined with nasotracheal oxygen insufflation led to a similar decrease in tremor intensity to that when butorphanol was used on its own. The tremor intensity of the rhinoceros decreased after the administration of butorphanol with nasotracheal oxygen insufflation ($p = 0.001$, $F_3 = 17.02$, Fig. 4.1.1 C). Similarly to butorphanol alone, no immediate change occurred in the tremor intensity following the intervention at the 10 minute time point (median tremor intensity score = 1), but there was a significant decrease in the tremor intensity at 15 minutes ($p = 0.01$, median tremor intensity score = 0) and 20 minutes ($p = 0.02$, median tremor intensity score = 0.5) compared to the 5 minute pre-intervention time point.

In contrast to the butorphanol alone, and butorphanol with nasotracheal oxygen insufflation, the nasotracheal oxygen insufflation on its own did not lead to a significant change in tremor intensity ($p = 0.06$, $F_3 = 7.44$, Fig. 4.1.1 D) during the immobilisation period. At the pre-intervention time point (5 minutes) the rhinoceros had moderate tremors (median tremor intensity score = 2 to 3). During the post-intervention periods (10, 15 & 20 minutes), the rhinoceros had moderate tremors (median tremor intensity score = 3) or mild tremors (median tremor intensity score = 2).

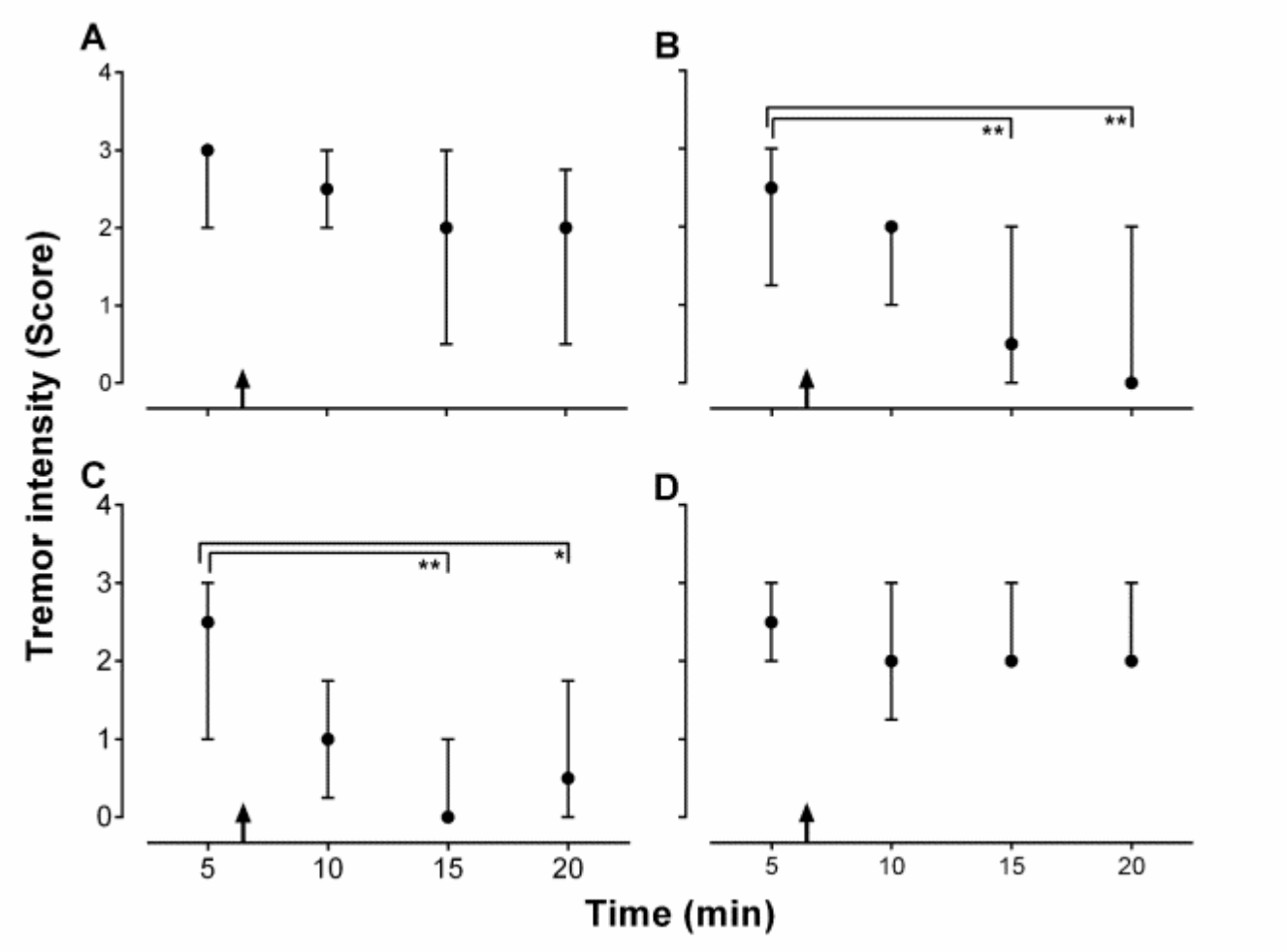

Figure 4.1.1 The median observed tremor intensity score with interquartile ranges for the 8 boma-managed rhinoceros while they were chemically immobilised with a mixture of etorphine and azaperone. Six minutes after they were placed into lateral recumbency, each rhinoceros received one of four interventions (arrow), on four separate occasions. The interventions included 2ml sterile water (intravenously) (control) (A), butorphanol (intravenously) only (B), the combination of butorphanol (intravenously) and nasotracheal oxygen insufflation (C), or nasotracheal oxygen insufflation only (D). The brackets indicate the significant differences between tremor intensities compared to the pre-intervention administration at 5 minutes (* $p < 0.05$; ** $p < 0.01$; Friedman's and *post-hoc* Dunn's tests).

4.1.3 The relationship between tremor intensity and arterial blood gases during chemical immobilisation

When comparing tremor intensity across the trials (butorphanol only, butorphanol and nasotracheal oxygen insufflation and nasotracheal oxygen insufflation only), at 5 minutes, which was 1 minute before the interventions were administered, there was no significant difference in observed tremor intensity ($p = 0.28$, $F_3 = 3.84$, Fig. 4.1.2, Fig. 4.1.3, Fig. 4.1.4 A) compared to the control intervention (saline solution). At the 10 minute ($p = 0.012$, $F_3 = 10.50$, Fig. 4.1.2, Fig. 4.1.3, Fig. 4.1.4 B), 15 minute ($p = 0.0014$, $F_3 = 15.63$, Fig. 4.1.2, Fig. 4.1.3, Fig. 4.1.4 C) and 20 minute ($p = 0.0022$, $F_3 = 14.56$, Fig. 4.1.2, Fig. 4.1.3, Fig. 4.1.4 D) time points, there were differences among the interventions. The post-hoc test, however, revealed no difference between the control (saline solution) and treatment groups at the 15 and 20 minute time points. Therefore, when compared to the control, butorphanol administration did not reduce tremor intensity at 10 minute (Fig. 4.1.2, Fig. 4.1.3, Fig. 4.1.4 B), 15 minute (Fig 4.1.2, Fig. 4.1.3, Fig. 4.1.4 C) or 20 minute (Fig. 4.1.2, Fig. 4.1.3, Fig. 4.1.4 D) points. Butorphanol combined with nasotracheal oxygen insufflation did lead to a significant decrease in tremor intensity compared to the control group at the 10 minute time interval, 4 minutes after the combination intervention had been administered ($p = 0.02$, median tremor intensity score = 1). However, at the 15 minute (median tremor intensity score = 0) and 20 minute (median tremor intensity score = 0.5) time points, even though the tremor intensities appeared to decline when compared to the control, this difference was not statistically significant. When nasotracheal oxygen insufflation was administered on its own, the tremor intensity did not significantly change compared to the control at any of the time points (median tremor intensity score = 2 at all the time points, Fig. 4.1.2, Fig. 4.1.3, Fig. 4.1.4 B, C & D).

4.1.4 The relationship between tremor intensity, arterial blood gases and blood pH during chemical immobilisation

The arterial partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) and blood pH were also recorded at the same time points that the tremor intensities were recorded at. When comparing the data at 5 minutes from the treatment interventions (butorphanol only, butorphanol and nasotracheal oxygen insufflation and nasotracheal oxygen insufflation only) to the control (saline solution), there was no significant difference in PaO_2 ($p = 0.16$, $F_{3, 21} = 2.12$, Fig. 4.1.2 E), $PaCO_2$ ($p = 0.6$, $F_{3, 21} = 0.58$, Fig. 4.1.3 E) and pH ($p = 0.43$, $F_{3, 21} = 0.897$, Fig. 4.1.4 E).

Once the interventions (butorphanol only, butorphanol and nasotracheal oxygen insufflation and nasotracheal oxygen insufflation only) were administered, significant differences to the control (saline solution) were observed for the PaO_2 at the 10 minute ($p = 0.0002$, $F_{3, 21} = 37.37$, Fig. 4.1.2 F), 15 minute ($p = 0.0001$; $F_{3, 21} = 39.99$, Fig. 4.1.2 G) and 20 minute ($p = 0.001$, $F_{3, 21} = 27.63$, Fig. 4.1.2 H) time points. Butorphanol administration led to an increase in PaO_2, when compared to the control (5 minutes – mean PaO_2 = 27.13 mmHg; 10 minutes – mean PaO_2 = 26.63 mmHg; 15 minutes – mean PaO_2 = 29 mmHg & 20 minutes – mean PaO_2 = 31.25 mmHg), at all post-intervention time points(10 minutes - $p < 0.0001$, mean PaO_2 = 60.4 mmHg; 15 minutes - $p < 0.0001$, mean PaO_2 = 56.1 mmHg & 20 minutes - $p < 0.0001$, mean PaO_2 = 53.8 mmHg, Fig. 4.1.2 F, G & H). The rhinoceros however remained hypoxaemic (normal mean PaO_2 = 98.2 mmHg, range = 90.2 - 108.6 mmHg; Citino & Bush 2007) throughout the immobilisation period. Similarly, butorphanol combined with nasotracheal oxygen insufflation led to a significant increase in the PaO_2 at all the time points, when it was compared to the control (10 minutes - $p = 0.001$, mean PaO_2 = 98.7 mmHg; 15 minutes - $p = 0.0004$, mean PaO_2 = 136.3 mmHg & 20 minutes - $p = 0.001$, mean PaO_2 = 154.3 mmHg, Fig. 4.1.2 F, G & H). Indeed, this combination intervention of butorphanol and nasotracheal oxygen insufflation completely corrected the immobilisation-induced hypoxaemia. When nasotracheal oxygen insufflation was administered on its own compared to the control, the PaO_2 increased slightly at the 10 minute ($p = 0.02$, mean

PaO_2 = 37.4 mmHg, Fig. 4.1.2 F), 15 minute (p = 0.022, mean PaO_2 = 43.5 mmHg, Fig. 4.1.2 G) and 20 minute (p = 0.02, mean PaO_2 = 53.6 mmHg, Fig. 4.1.2 H) time points. However, even though the PaO_2 increased, the rhinoceros remained severely hypoxaemic.

A pattern emerged between the PaO_2 and the tremor intensity (score), in that when the hypoxia was corrected or at least partially corrected, the tremor intensity (score) seemed to decrease. This pattern was supported by a significant negative correlation between the mean PaO_2 and the median tremor intensity (score) for the interventions at the 5 minute ($p < 0.0001$, $r^2 = 0.6$, Fig. 4.1.2 I; Spearman rank-order correlation), 10 minute ($p < 0.0001$, $r^2 = 0.9$, Fig. 4.1.2 J; Spearman rank-order correlation) and 15 minute ($p < 0.0001$, $r^2 = 0.9$, Fig. 4.1.2 K; Spearman rank-order correlation) time points. There was no association at the 20 minute time point ($p = 0.167$, $r^2 = 0.9$, Fig. 4.1.2 L; Spearman rank-order correlation).

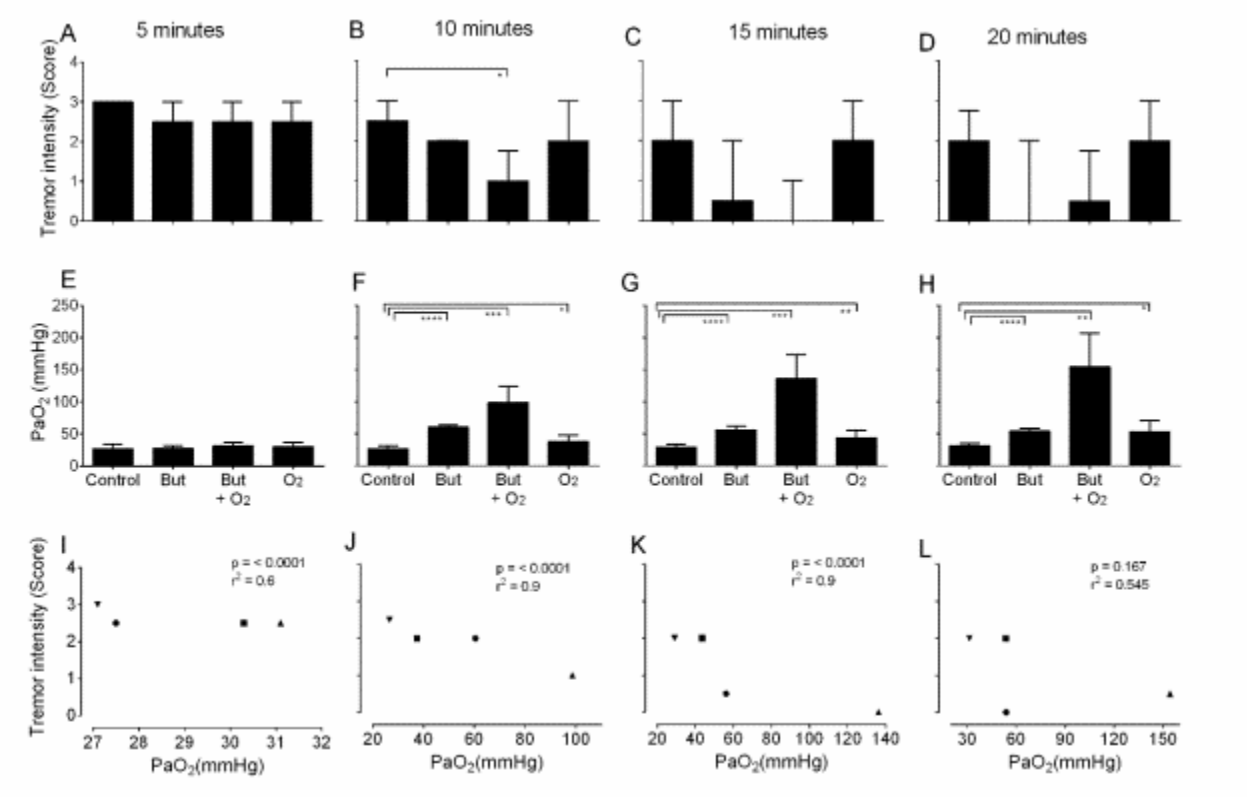

Figure 4.1.2 The median visual tremor intensity scores with interquartile ranges and the mean arterial partial pressure of oxygen (± standard deviation) (PaO_2) at the 5 minutes (left panel), 10 minutes (panel second from the left), 15 minutes (panel second from the right) and 20 minutes (right panel) in 8 white rhinoceros, which were chemically immobilised with etorphine and azaperone. On four separate occasions and at 6 minutes after being placed into lateral recumbency, the rhinoceros received either, sterile water (2ml) (intravenously, "Control"), an intervention with butorphanol only (intravenously, "But"), a combination of butorphanol (intravenously) and nasotracheal oxygen insufflation ("But + O_2"), or only nasotracheal oxygen insufflation ("O_2"). The brackets indicate significant differences between interventions (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; for Tremor intensity - Friedman's test and *post-hoc* Dunn's tests; PaO_2 - one-way ANOVA and *post-hoc* Dunnett's test). The lower panel graphs represent the Spearman rank-order correlation between the mean PaO_2 and the median tremor intensity (score) for the different interventions (▼ - Control; ● - Butorphanol; ▲ – Butorphanol + O_2; ■ – O_2) at the various time points.

The data from the different interventions (butorphanol only, butorphanol and nasotracheal oxygen insufflation and nasotracheal oxygen insufflation only) when compared to the control (saline solution) resulted in significant changes to the $PaCO_2$ at the 10 minute ($p < 0.0001$, $F_{3, 21} = 40.8$, Fig. 4.1.3 F), 15 minute ($p < 0.0001$, $F_{3, 21} = 81.67$, Fig. 4.1.3 G) and 20 minute ($p < 0.0001$, $F_{3, 21} = 65.84$, Fig. 4.1.3 H) time points. Butorphanol administered at 6 minutes led to a significantly lower $PaCO_2$ compared to the control (5 minutes – mean $PaCO_2$ = 83.13 mmHg, 10 minutes – mean $PaCO_2$ = 87.75 mmHg, 15 minutes – mean $PaCO_2$ = 85.75 mmHg & 20 minutes – mean $PaCO_2$ = 82.13 mmHg) at 10 minute ($p < 0.0001$, mean $PaCO_2$ = 66.5 mmHg, Fig. 4.1.3. F), 15 minute ($p = 0.0007$, mean $PaCO_2$ = 67.3 mmHg, Fig. 4.1.3 G) and 20 minute ($p < 0.0001$, mean $PaCO_2$ = 68 mmHg, Fig. 4.1.3 H) time points. The rhinoceros, however, remained hypercapnic throughout the immobilisation period, even though the $PaCO_2$ had decreased compared to when no intervention (control) was administered (normal mean $PaCO_2$ = 49 mmHg, range = 44.4 - 53.7 mmHg; Citino & Bush 2007). During the combination intervention of butorphanol and nasotracheal oxygen insufflation, no significant change was reported for the $PaCO_2$ at the 10 minute time point, 4 minutes after the intervention was administered, when compared to the $PaCO_2$ at the 5 minute time point (mean $PaCO_2$ = 74.9 mmHg, Fig. 4.1.3 F). Although the $PaCO_2$ tended to be lower than that of the control, it was significantly lower than the control only at 15 minute ($p = 0.048$, mean $PaCO_2$ = 75.4 mmHg, Fig. 4.1.3 G) time interval and the rhinoceros remained hypercapnic despite this slight improvement. When the nasotracheal oxygen insufflation was administered on its own, the $PaCO_2$ was significantly higher than the control intervention, at all the time points (10 minute - $p = 0.007$, mean $PaCO_2$ = 106.4 mmHg; 15 minutes - $p = 0.002$, mean $PaCO_2$ = 116.9 mmHg & 20 minutes - $p = 0.0004$, mean $PaCO_2$ = 121.8 mmHg, Fig. 4.1.3 F, G & H); during the entire immobilisation period, the rhinoceros were severely hypercapnic (normal mean $PaCO_2$ = 49 mmHg, range = 44.4 - 53.7 mmHg; Citino & Bush 2007). During the interventions at the different time points it seemed that when the tremor intensity (score) was at its highest, the $PaCO_2$ was also very high. However, there was no correlation between the $PaCO_2$ and tremor intensity over the interventions at the different time points (Fig. 4.1.3 I – L).

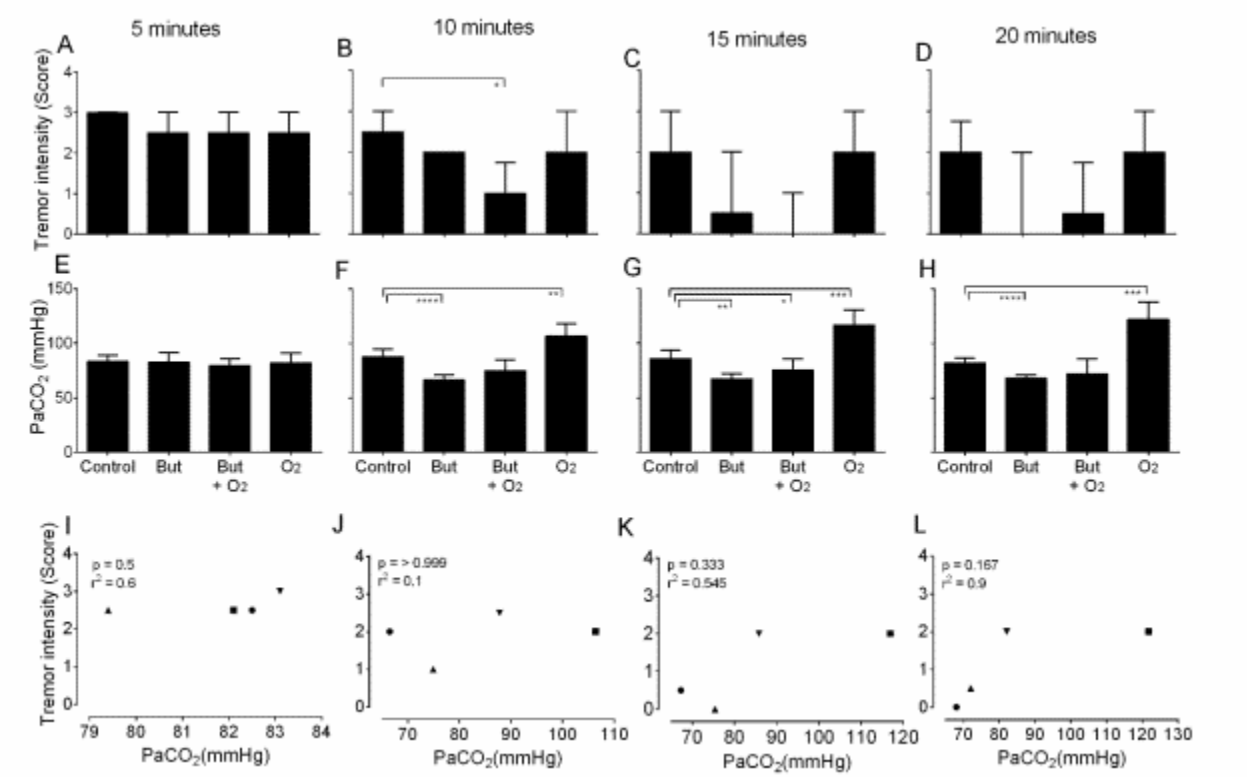

Figure 4.1.3 The median visual tremor intensity scores with interquartile ranges and the mean arterial partial pressure of carbon dioxide (± standard deviation) ($PaCO_2$) at the 5 minutes (left panel), 10 minutes (panel second from the left), 15 minutes (panel second from the right) and 20 minutes (right panel) in 8 white rhinoceros, which were chemically immobilised with etorphine and azaperone. On four separate occasions and at 6 minutes after being placed into lateral recumbency, the rhinoceros received either, sterile water (2ml) (intravenously, "Control"), an intervention with butorphanol only (intravenously, "But"), a combination of butorphanol (intravenously) and nasotracheal oxygen insufflation ("But + O_2"), or only nasotracheal oxygen insufflation ("O_2"). The brackets indicate significant differences between interventions (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; for Tremor intensity - Friedman's test and *post-hoc* Dunn's tests; $PaCO_2$ - one-way ANOVA and *post-hoc* Dunnett's test). The lower panel graphs represent the Spearman rank-order correlation between the mean $PaCO_2$ and the median tremor intensity (score) for the different interventions (▼ - Control; ● - Butorphanol; ▲ – Butorphanol + O_2; ■ – O_2) at the various time points.

The blood pH significantly changed at the 10 minute ($p < 0.0001$, $F_{3, 21} = 26.54$, Fig 4.1.4 F), 15 minute ($p < 0.0001$, $F_{3, 21} = 58.85$, Fig 4.1.4 G) and 20 minute ($p < 0.0001$, $F_{3, 21} = 81.97$, Fig 4.1.4 H) time points, when the different interventions (butorphanol only, butorphanol and nasotracheal oxygen insufflation and nasotracheal oxygen insufflation only) were compared to the control (saline) intervention. Once the butorphanol only intervention was administered, there was a significant increase in the blood pH (10 minutes - $p = 0.005$, mean pH = 7.31, 15 minutes - $p = 0.002$, mean pH = 7.31 & 20 minutes - $p = 0.007$, mean pH = 7.32, Fig 4.1.4 F - H), when compared to the control intervention (10 minutes - mean pH = 7.23; 15 minutes - mean pH = 7.23 & 20 minutes - mean pH = 7.24). The acidaemia corrected as the blood pH increased (mean pH = 7.30; Citino & Bush 2007). The rhinoceros showed mild acidaemia (mean pH = 7.30; Citino & Bush 2007) after the combination intervention of butorphanol and nasotracheal oxygen insufflation was administered at 6 minutes peri-recumbency and when the combination intervention was compared to the control intervention (10 minutes - $p = 0.035$, mean pH = 7.27, 15 minutes - $p = 0.011$, mean pH = 7.27 & 20 minutes - $p = 0.02$, mean pH = 7.28, Fig 4.1.4 F - H). However, when nasotracheal oxygen insufflation was administered on its own, the blood pH significantly decreased (10 minutes - $p = 0.035$, mean pH = 7.16; 15 minutes - $p = 0.011$, mean pH = 7.12 & 20 minutes - $p = 0.02$, mean pH = 7.13, Fig 4.1.4 F - H), thereby resulting in a severe acidaemia (mean pH = 7.30; Citino & Bush 2007). No correlation over the interventions occurred for the blood pH and the tremor intensity (score) at the different time points (5 minute - $p = 0.5$, $r^2 = 0.067$, Fig. 4.1.4 I; 10 minute - $p = 0.5$, $r^2 = 0.9998$, Fig. 4.1.4 J and 15 minute - $p = 0.167$, $r^2 = 0.545$, Fig. 4.1.4 K) except at the 20 minute time point ($p < 0.0001$, $r^2 = 0.9$, Fig. 4.1.4 L).

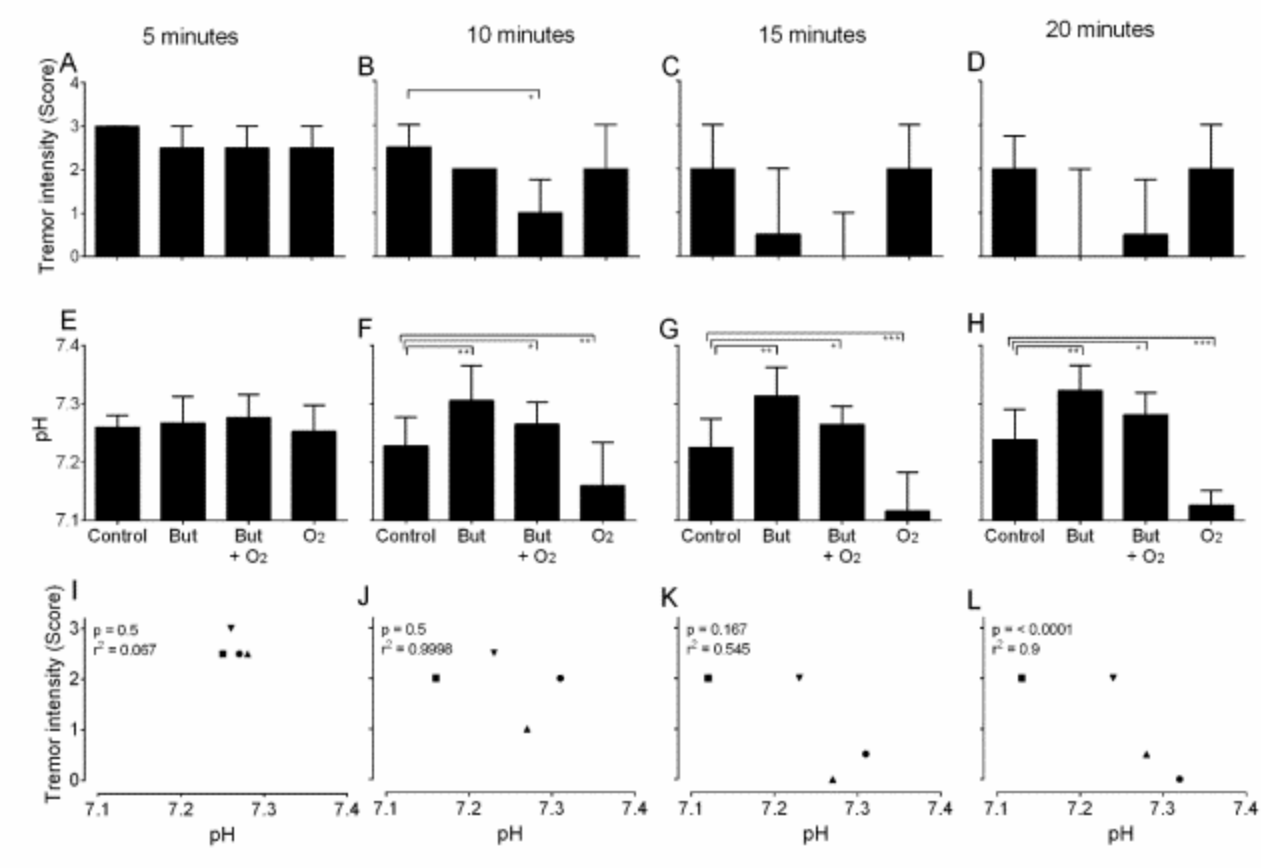

Figure 4.1.4 The median visual tremor intensity scores with interquartile ranges and the mean pH (± standard deviation) at the 5 minutes (left panel), 10 minutes (panel second from the left), 15 minutes (panel second from the right) and 20 minutes (right panel) in 8 white rhinoceros, which were chemically immobilised with etorphine and azaperone. On four separate occasions and at 6 minutes after being placed into lateral recumbency, the rhinoceros received either, sterile water (2ml) (intravenously, "Control"), an intervention with butorphanol only (intravenously, "But"), a combination of butorphanol (intravenously) and nasotracheal oxygen insufflation ("But + O_2"), or only nasotracheal oxygen insufflation ("O_2"). The brackets indicate significant differences between interventions (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; for Tremor intensity - Friedman's test and *post-hoc* Dunn's tests; pH - one-way ANOVA and *post-hoc* Dunnett's test). The lower panel graphs represent the Spearman rank-order correlation between the mean pH and the median tremor intensity (score) for the different interventions (▼ - Control; ● - Butorphanol; ▲ – Butorphanol + O_2; ■ – O_2) at the various time points.

4.2 Measurement of tremor intensity using activity loggers (objective measure) and visual observations (subjective measure) methods for an individual white rhinoceros in the field

The combination of butorphanol and nasotracheal oxygen insufflation was chosen as the supportive treatment for chemically immobilised rhinoceros in the field, based on the positive cardiorespiratory effects observed in the boma-managed rhinoceros. The tremor intensity was recorded for each individual rhinoceros using the same visual (subjective) observational scoring scale as was used in the boma study (subjective tremor intensity scores, see Table 3.3), but at 1 minute time points and by using activity data loggers (objective measure).

Figure 4.2.1 shows tremor intensity data from an individual rhinoceros using both subjective (Fig 4.2.1 A) and objective (Fig 4.2.1 B & C) measurements. From the moment that the rhinoceros was placed into lateral recumbency, until it received the intervention of butorphanol and nasotracheal oxygen insufflation at the 6 minute time point, the rhinoceros had mild tremors that resulted in shoulder and chest, and slight leg and foot movements (tremor intensity score = 2, Fig. 4.2.1 A). After the administration of butorphanol and nasotracheal oxygen insufflation, the tremors decreased to slight tremors resulting in leg and foot movements only (tremor intensity score = 1). After the 10 minute time point, the tremors decreased to no tremors being observed (tremor intensity score = 0) for the remainder of the study. The higher tremor intensity that was observed subjectively before the administration of butorphanol and nasotracheal oxygen insufflation was also detected by both of the activity loggers that were used to measure tremor objectively. The Sigma-Delta logger recorded a high activity (tremor intensity) between the peri-recumbency and pre-intervention period (1 to 5 minute: tremor intensity counts/min range = 30 – 42, Fig. 4.2.1 B). Once the butorphanol and nasotracheal oxygen insufflation was administered, the tremor intensity decreased within 1 minute after the intervention (tremor intensity counts/min = 15) and remained at a lower tremor intensity for the remainder of the immobilisation period (tremor intensity counts/min range = 0 - 9). The Actical activity logger also recorded a high tremor

intensity during the pre-intervention period, with the tremors fluctuating at each time interval (1 to 5 minute - tremor intensity counts/min range = 527 - 931, Fig. 4.2.1 C). The tremor intensity that was recorded by the Actical activity logger decreased to zero counts/min directly after the administration of butorphanol and nasotracheal oxygen insufflation. According to the Actical activity logger, once butorphanol and nasotracheal oxygen insufflation was administered, the tremors remained at zero throughout the immobilisation period, apart from some minor tremor activity spikes at 10, 11 and 21 minutes (10, 11 & 21 minutes - tremor intensity counts/min = 13). Both Sigma-Delta and Actical loggers recorded high tremor intensities in the pre-intervention period and then lower tremor intensities post-intervention, which corresponds with the changes in visual tremor intensity scores.

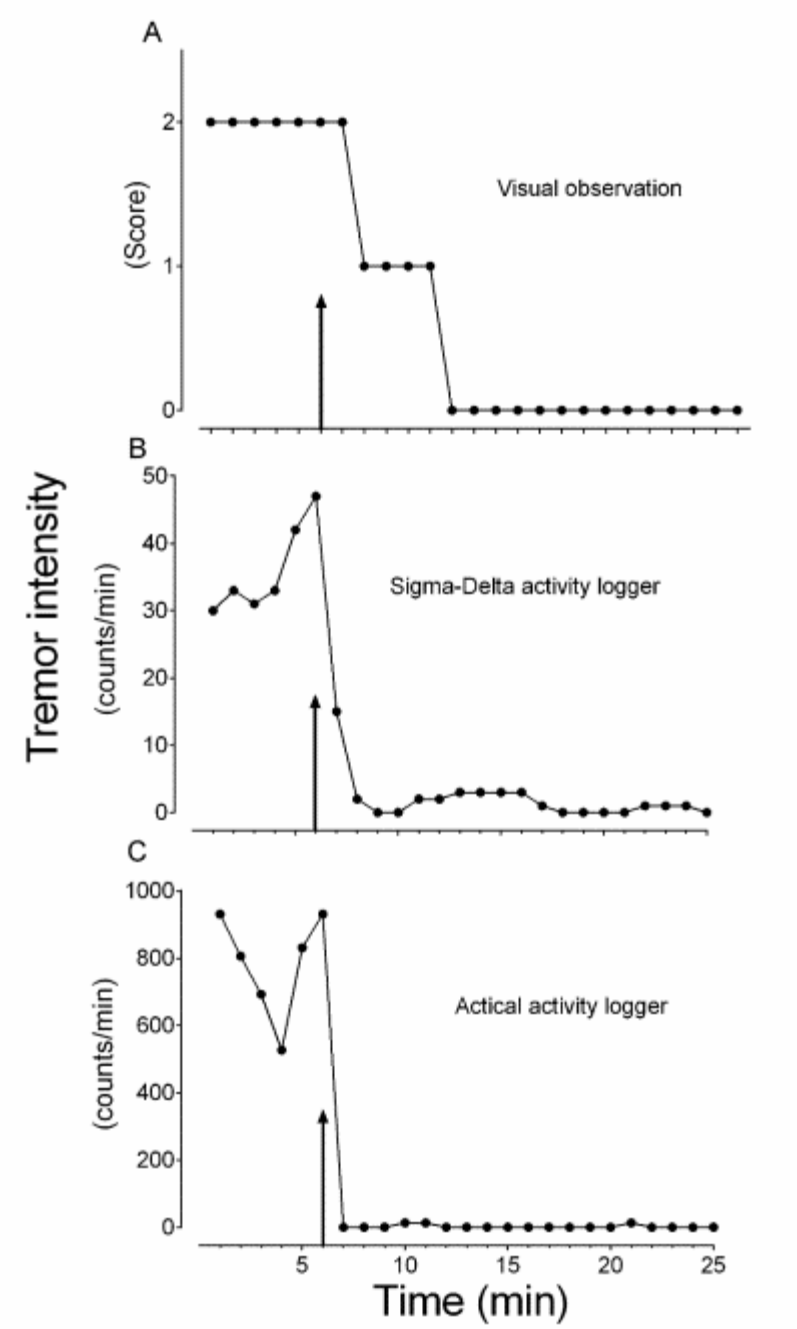

Figure 4.2.1 The tremor intensity was recorded for an individual white rhinoceros that was chemically immobilised with etorphine and azaperone, using visual observations (A – tremor intensity score), a Sigma-Delta logger (B – tremor intensity counts/min) and an Actical logger (C – tremor intensity counts/min). Six minutes after the rhinoceros had been placed into lateral recumbency, it received the combination of butorphanol and nasotracheal oxygen insufflation (represented by the arrow).

4.3 Comparison of visual observation (subjective measure) and activity logger (objective measure) methods for measuring tremor intensity

4.3.1 Visual observations (subjective measure) of tremor intensity of immobilised rhinoceros in the field

The visually observed tremor intensity significantly decreased ($p < 0.0001$, $F_5 = 30.77$, Fig 4.3.1) over time during the immobilisation. During the pre-intervention period (1 to 5 minutes), the rhinoceros had mild tremors that resulted in shoulder and chest movement with leg and foot movements (median tremor intensity score = 2). However, during this time, there were variations among the individuals, as some rhinoceros had slight tremors, resulting in leg and foot movements only (median tremor intensity score = 1) while others had moderate tremors that resulted in gross shoulder, chest, leg and foot movements (median tremor intensity score = 3). During this period, no significant difference was reported for the tremor intensity between the 1 minute and 5 minute time points. At the 8 minute time point, which was 2 minutes after the administration of butorphanol and initiation of nasotracheal oxygen insufflation, the tremors ranged from slight (median tremor intensity score = 1) to mild (median tremor intensity score = 2). By the 10 minute time interval, the tremors were recorded as slight tremors (median tremor intensity score = 1). Even though there was a trend towards lower tremor intensities, there was no significant difference between the 10 minute and 1 minute time points. At the 15 and 20 minute time points, the tremor intensity varied amongst individuals, and was observed to range from slight tremors to no visible tremors (median tremor intensity score = 0 to 1). The tremor intensity at the 15 minute time interval was not significantly different to the 1 minute time interval, while the tremor intensity at the 20 minute time interval was significantly different ($p = 0.001$) to the 1 minute time interval. At 25 minutes, no tremors were observed (1 minute vs 25 minutes - $p < 0.0001$, median tremor intensity score = 0). Compared to tremor intensity just before the butorphanol and nasotracheal oxygen insufflation were administered (5 minute) tremors decreased at the 15 minute ($p = 0.001$), 20 minute and 25 minute ($p < 0.0001$), but not the 10 minute time points. The tremor intensity significantly decreased ($p = 0.043$) from slight

tremors at 10 minutes (median tremor intensity score = 1) to no visible tremors at 25 minutes (median tremor intensity score = 0).

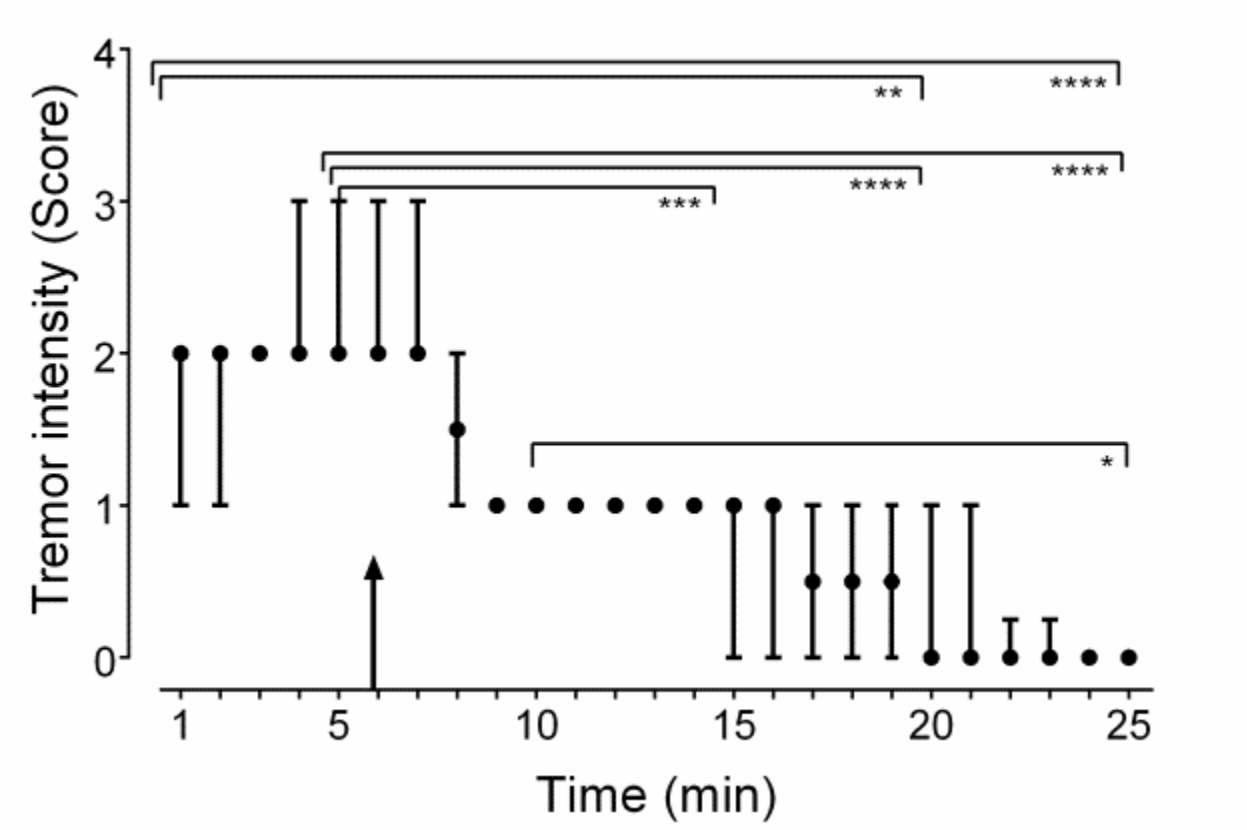

Figure 4.3.1 The median tremor observation with interquartile ranges of 14 white rhinoceros, which were all chemically immobilised, with a mixture of etorphine and azaperone. The arrow represents the time point at which the partial opioid agonist/antagonist butorphanol was administered and nasotracheal oxygen insufflation initiated. The brackets indicate which time points is significantly different from the other (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; Friedman's with *post-hoc* Dunn's tests).

4.3.2 Measurement of tremor intensity using an activity logger (objective measure) of immobilised rhinoceros in the field

The tremor intensities for 14 rhinoceros were recorded by a Sigma-Delta activity logger (Fig. 4.3.2). The tremor intensity significantly decreased ($p < 0.0001$, $F_5 = 44.68$) during the immobilisation period, with rhinoceros showing high tremor intensities before the interventions were administered and then lower intensities after administration, a similar pattern to that which was visually observed (Fig. 4.3.1). During the pre-intervention period, there was no significant difference in the tremor intensities between the 1 minute (median tremor intensity counts/min = 20.5) and the 5 minute time points (median tremor intensity counts/min = 20). However, throughout this initial time period, there was substantial variability among individual rhinoceros, as indicated by the large interquartile ranges (3 minutes - 5.74 – 48.77 counts/min interquartile range; 5 minute - 9.31 – 47.79 counts/min interquartile range). Once butorphanol and the continuous flow of nasotracheal oxygen insufflation had been administered, the tremors decreased. Even though the tremors where lower, at the 7 minute time interval compared to the pre-intervention period, large variability remained in the tremor intensity (5.64 - 42.40 counts/min interquartile range), but at the 9 minute time interval, the variability was much lower (1.47 - 7.84 counts/min interquartile range). At the 10 minute time interval (median tremor intensity counts/min = 3), which was 4 minutes post-intervention, no significant difference in tremor intensity occurred between the 10 minute time point and the 1 minute and 5 minute time points. The tremor intensity decreased to 3 counts/min, at 9 minutes, and remained below 3 counts/min during the rest of the immobilisation. When compared to the 1 minute time point, tremor intensity at the 15 minute ($p = 0.002$), 20 minute ($p < 0.0001$) and 25 minute ($p = 0.003$) time points was significantly lower. Similarly, tremor intensities at the 15, 20 and 25 minute time points were different to the tremor intensity at 5 minutes (5 minutes vs 15 minutes - $p = 0.002$; 5 minutes vs 20 minutes - $p < 0.001$; 5 minutes vs 25 minutes - $p = 0.004$).

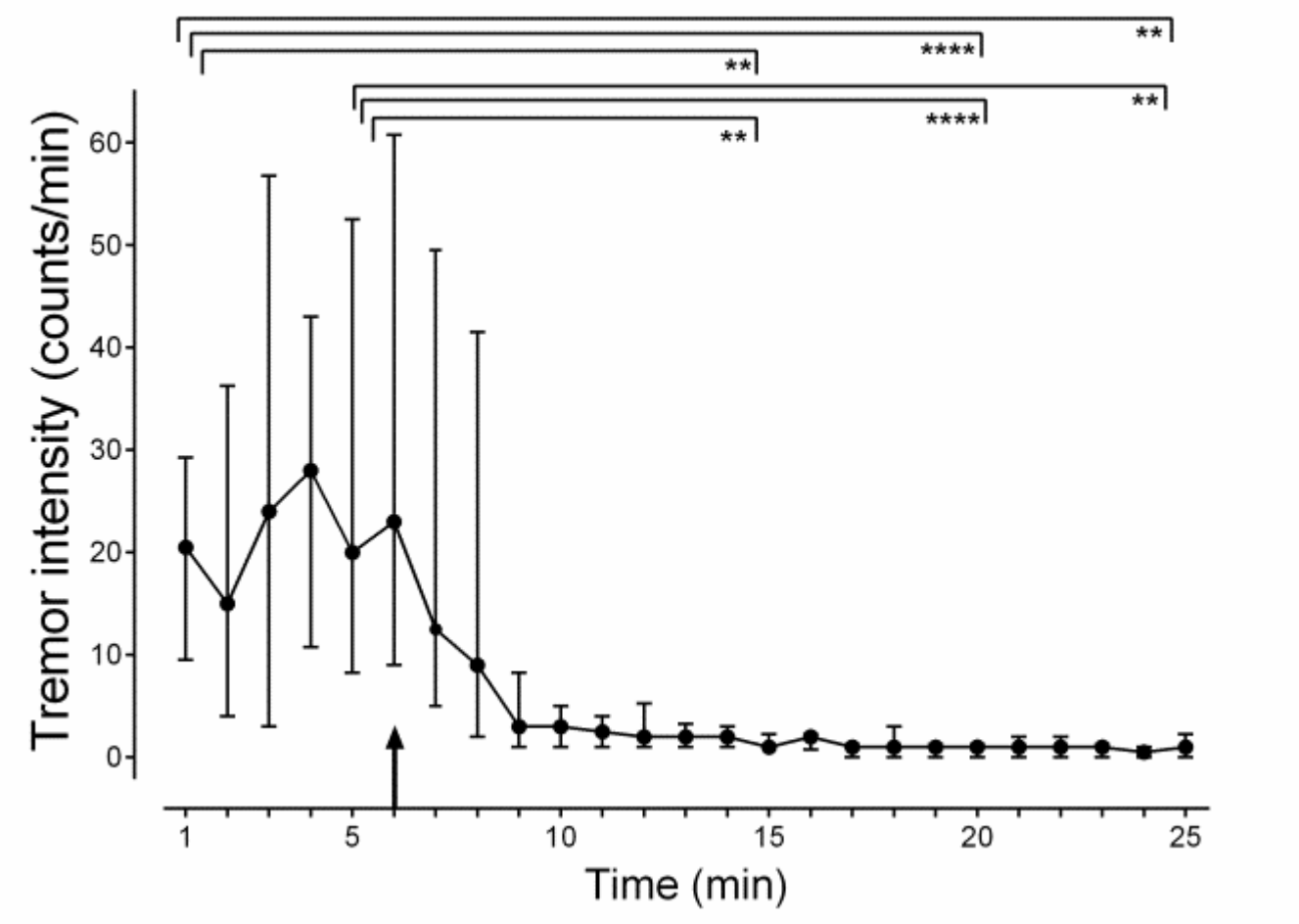

Figure 4.3.2 Median tremor intensity with interquartile range, for 14 white rhinoceros, which were chemically immobilised with a mixture of etorphine and azaperone, over the 25 minutes while the rhinoceros were chemically immobilised. The arrow represents the time point at which butorphanol and nasotracheal oxygen insufflation were administered. The brackets indicate which time points were significantly different from one another (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; Friedman's with *posthoc* Dunn's tests).

4.3.3 The relationship between activity loggers (objective measure) and visual observation (subjective measure) methods of measuring tremor intensity

Visual observations (subjective) and activity loggers (objective) reflected similar changes in the tremor intensities during the immobilisation period. Both of the methods detected high tremor intensities before the administration of butorphanol and nasotracheal oxygen insufflation, and lower tremor intensities shortly after the intervention was administered, until very low or no tremors were recorded by the end of the immobilisation period. However, the activity logger indicated that there were some low-intensity tremors that were scored as zero by visual observations. Between the 10 minute and 15 minute time points, the observed tremor intensity remained constant (median tremor intensity score = 1), while the activity loggers recorded a finer scale in changes in activity levels between the 10 minute (median tremor intensity counts/min = 3) and 15 minute (median tremor intensity counts/min = 1) time points. Comparison of the two methods, were significantly correlated with one another ($p < 0.0001$, $r^2 = 0.9$, Fig. 4.3.3; Spearman rank-order correlation). The Sigma-Delta logger was able to record a finer scale of tremor intensity thereby giving a better comparative grading between slight and severe tremors compared to the visual (subjective) observations. However, visual observations were also adequate for assessing general trends in changes in tremor intensities in chemically immobilised rhinoceros.

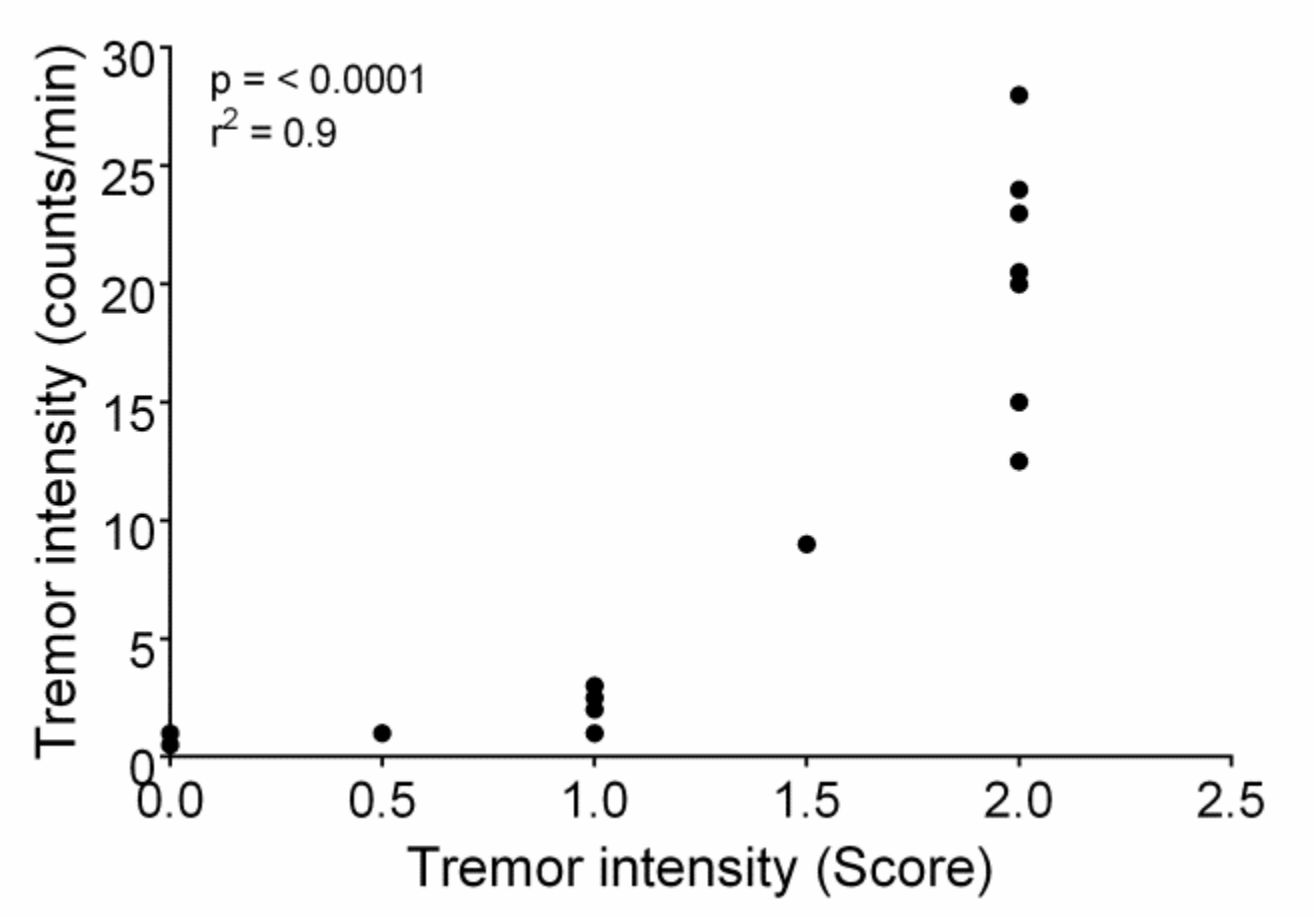

Figure 4.3.3 Spearman rank-order correlation of the median tremor intensity (counts/min) that was recorded by the activity loggers to the median tremor intensity (score) that was observed in 14 white rhinoceros that were chemically immobilised with a combination of etorphine and azaperone. Six minutes after the rhinoceros had been placed into recumbency, they received the intervention of butorphanol combined with nasotracheal oxygen insufflation. At a 1 minute time interval, the tremor intensities were recorded using both the visual observations (subjective) and the activity loggers (objective). Each point of intersection represents the median tremor intensity and the corresponding median tremor intensity score at each minute for 14 white rhinoceros.

4.3.4 Comparison of two limb locations and two types of activity loggers to record tremor intensity

Actical and Sigma-Delta activity loggers were placed in packs at two anatomical locations on the rhinoceros's legs (see Fig. 3.3 & Fig. 3.4). All the loggers, whether from the upper or the lower pack (Fig. 4.3.4) recorded a similar pattern of tremor intensity with high intensities before the butorphanol and nasotracheal oxygen insufflation administration, at 6 minutes, and lower intensities after administration. During the pre-intervention periods, the Sigma-Delta activity logger (Fig. 4.3.5 A) recorded median tremor intensities ranging between 13.2% - 23.6%, while the Actical loggers (Fig. 4.3.5 B) recorded a median tremor intensity range between 0.32% - 7.7%. Four minutes post-intervention, at the 10 minute time interval, all the activity loggers recorded a median tremor intensity that was lower than 11.8%. However, after the 10 minute time interval until the end of the study, the Actical loggers recorded a median tremor intensity of mostly 0% while the Sigma-Delta loggers recorded some movement (median tremor intensity < 2.7%). The Sigma-Delta loggers recorded the lower-intensity tremors, while the Actical loggers did not record these tremors. For comparisons with other data from the study, the Sigma-Delta activity logger was chosen because it was more sensitive than the Actical logger in recording finer tremors. The data that was recorded by the activity loggers in both the upper and lower packs were similar (Fig. 4.3.5). However, out of the two packs that contained the activity loggers, the loggers in the lower packs on the foot were influenced more by non-tremor associated movements. These movements included paddling or general leg movements by the rhino and movement caused by handlers pulling on a rope that was tied around the rhinoceros' foot; this rope was placed on the foot for security purposes and to try limiting major paddling movements. Figure 4.3.6 illustrates how activities measured by the loggers in the lower pack were affected by non-tremor associated movements. Therefore, the activity loggers that were in the upper pack were selected as they were less influenced by non-tremor associated movements.

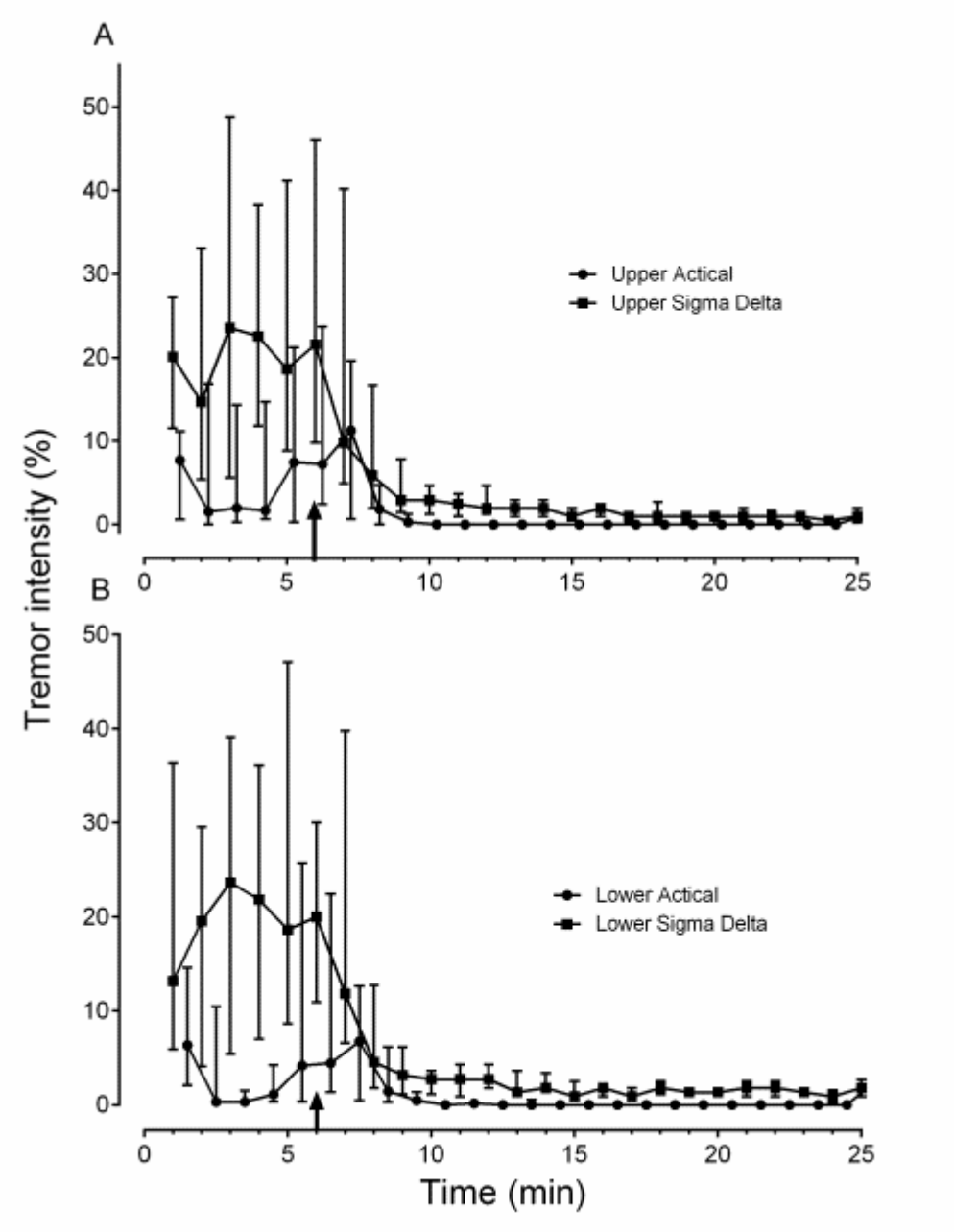

Figure 4.3.4 Median tremor intensity with interquartile ranges, (% of recorded counts compared to maximum counts for each logger) recorded by the Actical and Sigma-Delta activity loggers from the upper (A) and lower (B) packs for 14 white rhinoceros that were chemically immobilised with a combination of etorphine and azaperone. Six minutes after the rhinoceros were placed into recumbency (indicated by the arrow), they received a combination intervention of butorphanol and nasotracheal oxygen insufflation.

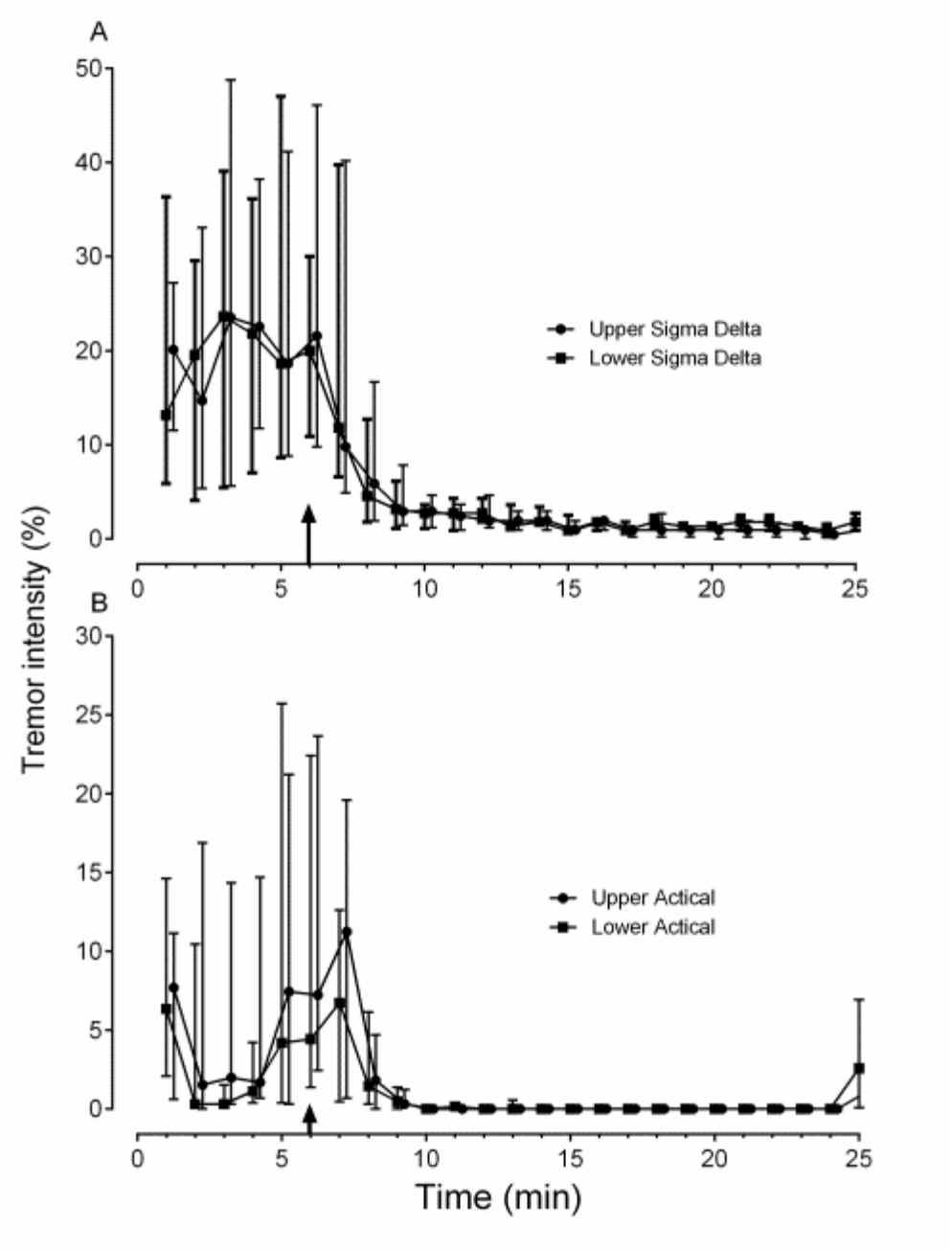

Figure 4.3.5 Median tremor intensity with interquartile ranges, (% of recorded counts compared to maximum counts for each logger) that were recorded by the Sigma-Delta activity loggers from the upper and lower packs (A) and the Actical activity logger from the upper and lower packs (B) for 14 white rhinoceros that were chemically immobilised with a combination of etorphine and azaperone. Six minutes after the rhinoceros were placed into recumbency (indicated by the arrow), they received a combination intervention of butorphanol and nasotracheal oxygen insufflation.

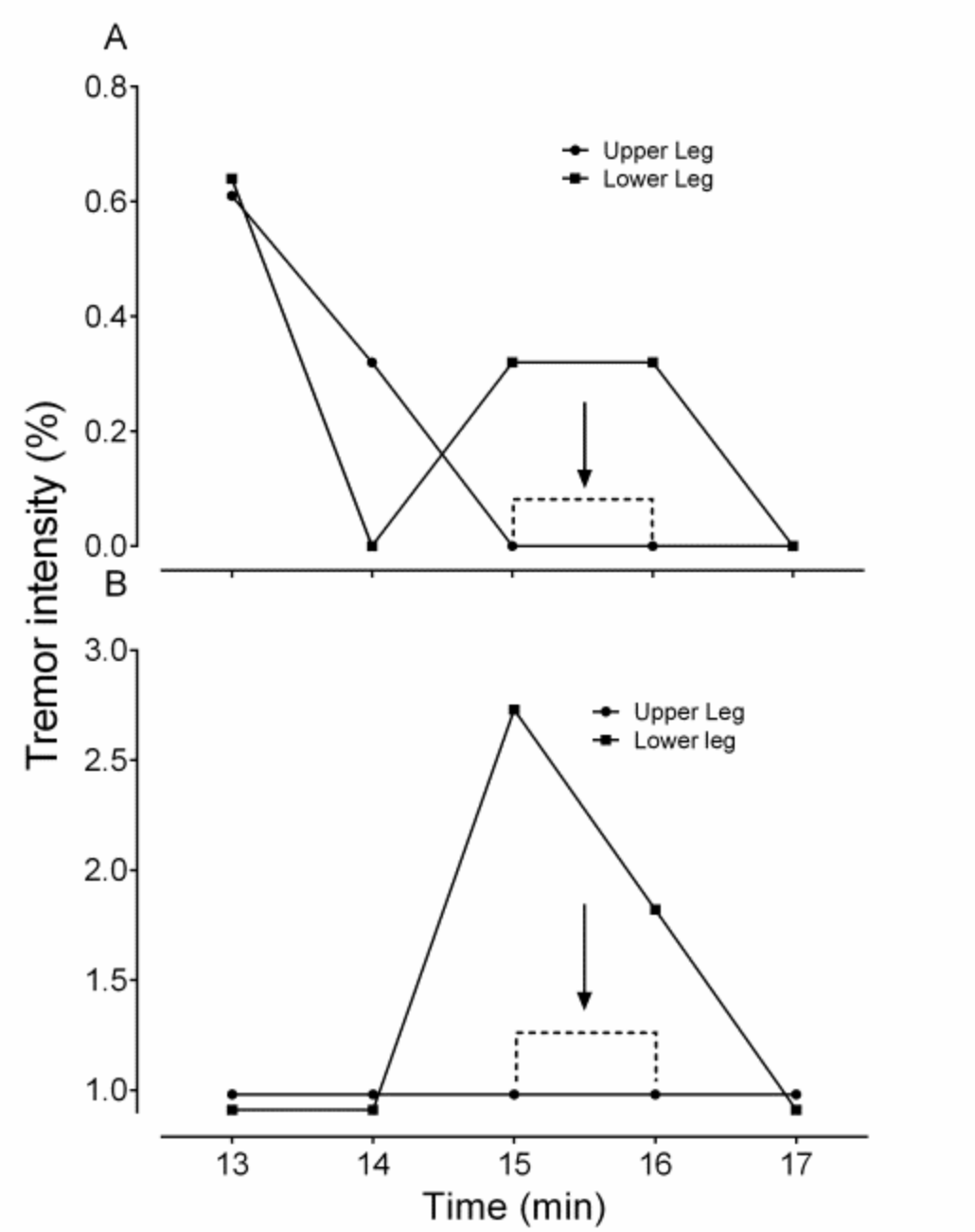

Figure 4.3.6 The tremor intensity of Actical activity loggers (A - % of recorded counts compared to maximum counts for each logger) and Sigma-Delta (B - % of recorded counts compared to maximum counts for each logger) from the upper and lower packs that were placed on the legs of an individual white rhinoceros over a time-span of 5 minutes. The time points when the rhinoceros moved its legs by pulling it up or moving it forward, is represented by the arrow and dashed bracket.

4.4 The relationship between total distance run (meters), and the dose of etorphine, azaperone and butorphanol, with the measured tremor intensity (counts/min)

The amount of physical exercise that the rhinoceros's were doing before they were placed into recumbency may have influenced tremor intensity. However, there was no correlation between the total distance run (meters) and the tremor intensity (counts/min) at the 1 minute ($p = 0.47$, $r^2 = 0.042$; Spearman rank-order correlation) and 5 minute ($p = 0.88$, $r^2 = 0.002$; Spearman rank-order correlation) time points in the 14 immobilised rhinoceros. Similarly, there was no correlation between the dose of etorphine (mg/kg) and the tremor intensity (counts/min) at 1 minute ($p = 0.23$, $r^2 = 0.12$) or 5 minute ($p = 0.87$, $r^2 = 0.003$; Spearman rank-order correlation) after the 14 rhinoceros became immobile. Additionally, the dose of azaperone (mg/kg) and the tremor intensity were also not correlated (1 minute - $p = 0.23$, $r^2 = 0.12$; 5 minute - $p = 0.87$, $r^2 =0.003$; Spearman rank-order correlation).

Although tremor intensity decreased after the administration of butorphanol and nasotracheal oxygen insufflation the tremor intensity after administration was not correlated with the dose of butorphanol (mg/kg) that was used in the 14 immobilised rhinoceros (10 minute - $p = 0.49$, $r^2 = 0.045$; 15 minute - $p = 0.84$, $r^2 = 0.002$; 20 minute - $p = 0.77$, $r^2 = 0.015$; Spearman rank-order)

4.5 The relationship between measured physiological variables and tremor intensity in white rhinoceros in the field

4.5.1 The relationship between the partial pressure of arterial blood gases and tremor intensity

The tremor intensity that was recorded by the activity loggers was high during the pre-intervention period, and then decreased once the butorphanol and nasotracheal oxygen insufflation were administered (Fig. 4.5.1 A). There was also a change in the arterial

partial pressure of oxygen (PaO_2, Figure 4.5.1 B) and carbon dioxide ($PaCO_2$, Figure 4.5.1 C) following the administration of butorphanol and nasotracheal oxygen insufflation.

The PaO_2 of 14 rhinoceros increased significantly over the course of the immobilisation period ($p < 0.0001$, $F_{13, 39} = 47.12$, Fig. 4.5.1 B). Five minutes after the rhinoceros were placed in recumbency, they were severely hypoxic, in that the PaO_2 was at the lowest recorded level (mean PaO_2 = 35 ± 6.56 mmHg). PaO_2 then increased significantly at 10 minutes ($p < 0.0001$, mean PaO_2 = 69 ± 7.24 mmHg), 4 minutes after the administration of butorphanol and initiation of nasotracheal oxygen insufflation. However, the rhinoceros were still hypoxaemic (normal mean PaO_2 = 98.2 mmHg, range = 90.2 - 108.6 mmHg; Citino & Bush 2007) despite the significant increase in PaO_2 from pre-intervention values. Similarly, PaO_2 was significantly higher at the 15 minute ($p < 0.0001$, mean PaO_2 = 79 ± 12.11 mmHg) and 20 minute ($p < 0.0001$, mean PaO_2 = 82 ± 23.15 mmHg) time points in comparison to the PaO_2 at the 5 minute time interval, but normoxia was not achieved. The PaO_2 continued to increase until the 15 minute time interval (10 minute vs 15 minute - $p = 0.024$), before it reached a plateau at the 20 minute time interval (10 minute vs 20 minute - $p = 0.024$).

The $PaCO_2$ of the 14 free-ranging rhinoceros (Fig. 4.5.1 C) decreased during the immobilisation period ($p = 0.0002$, $F_{13, 39} = 10.93$). At 5 minutes the rhinoceros were hypercapnic, with the $PaCO_2$ at the highest recorded value in the entire immobilisation period (mean $PaCO_2$ = 63 ± 7.70 mmHg, normal $PaCO_2$ = 49 mmHg, range = 44.4 - 53.7 mmHg; Citino & Bush 2007). Four minutes after butorphanol and nasotracheal oxygen insufflation had been administered, at the 10 minute time interval, the $PaCO_2$ decreased significantly ($p = 0.017$, mean $PaCO_2$ = 55 ± 8.47 mmHg) compared to the pre-intervention period (5 minutes). The $PaCO_2$ in the pre-intervention period (5 minutes) was also found to be significantly higher than at the 15 minute ($p = 0.001$, mean $PaCO_2$ = 53.5 ± 6.88 mmHg) and 20 minute ($p = 0.001$, mean $PaCO_2$ = 55.6 ± 4.93 mmHg) time points. During the post-intervention periods, the $PaCO_2$ did not

change significantly among the 10 minute and the 15 minute (mean $PaCO_2$ = 53.5 mmHg) and 20 minute (mean $PaCO_2$ = 55.6 mmHg) time points.

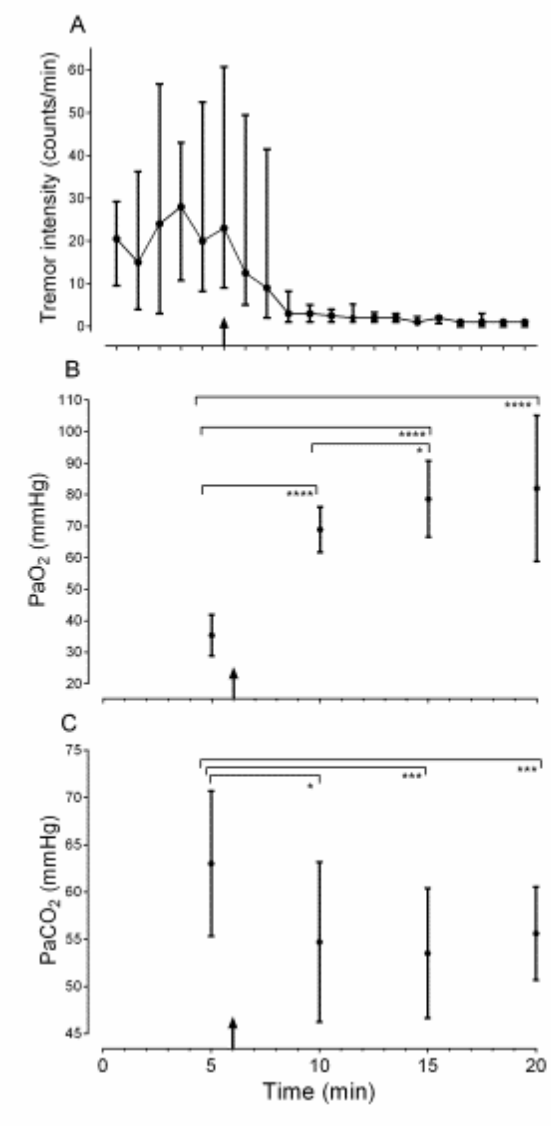

Figure 4.5.1 The median tremor intensity with interquartile range, (A) over the course of the study, and the mean (± SD) plasma arterial partial pressure of the oxygen (PaO_2) (B) and carbon dioxide ($PaCO_2$) (C) of 14 rhinoceros that were chemically immobilised with a combination of etorphine and azaperone. Six minutes after the rhinoceros were placed in lateral recumbency, they received a combination of butorphanol and nasotracheal oxygen insufflation, which is represented by the arrow. The brackets indicate the significant differences between the time points (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; arterial partial pressure: one-way ANOVA with *posthoc* Tukey's tests).

When the PaO_2 was at its lowest, which was at the 5 minute time interval, the tremor intensity was high. Once butorphanol and nasotracheal oxygen insufflation were administered, the PaO_2 increased as the tremor intensity decreased, resulting in a significant, negative correlation between PaO_2 and tremor intensity ($p = 0.0003$, $r^2 = 0.9995$, Fig. 4.5.2 A; Pearson product-moment correlation). Similarly, when the $PaCO_2$ was at its highest, which was at the 5 minute time point, the tremor intensity was also high. After butorphanol and nasotracheal oxygen insufflation were administered, the tremor intensity and the $PaCO_2$ decreased during the remainder of the immobilisation period. There was a positive, but non-significant relationship between the tremor intensity and the $PaCO_2$ ($p = 0.064$, $r^2 = 0.877$, Fig. 4.5.2 B; Pearson product-moment correlation). These relationships between the arterial blood gases and the tremor intensity are similar to the findings previously reported for rhinoceros that were kept in the boma, in that there were correlations between tremor intensity scores and PaO_2 but not $PaCO_2$ across the interventions (Fig. 4.1.2 & Fig. 4.1.3).

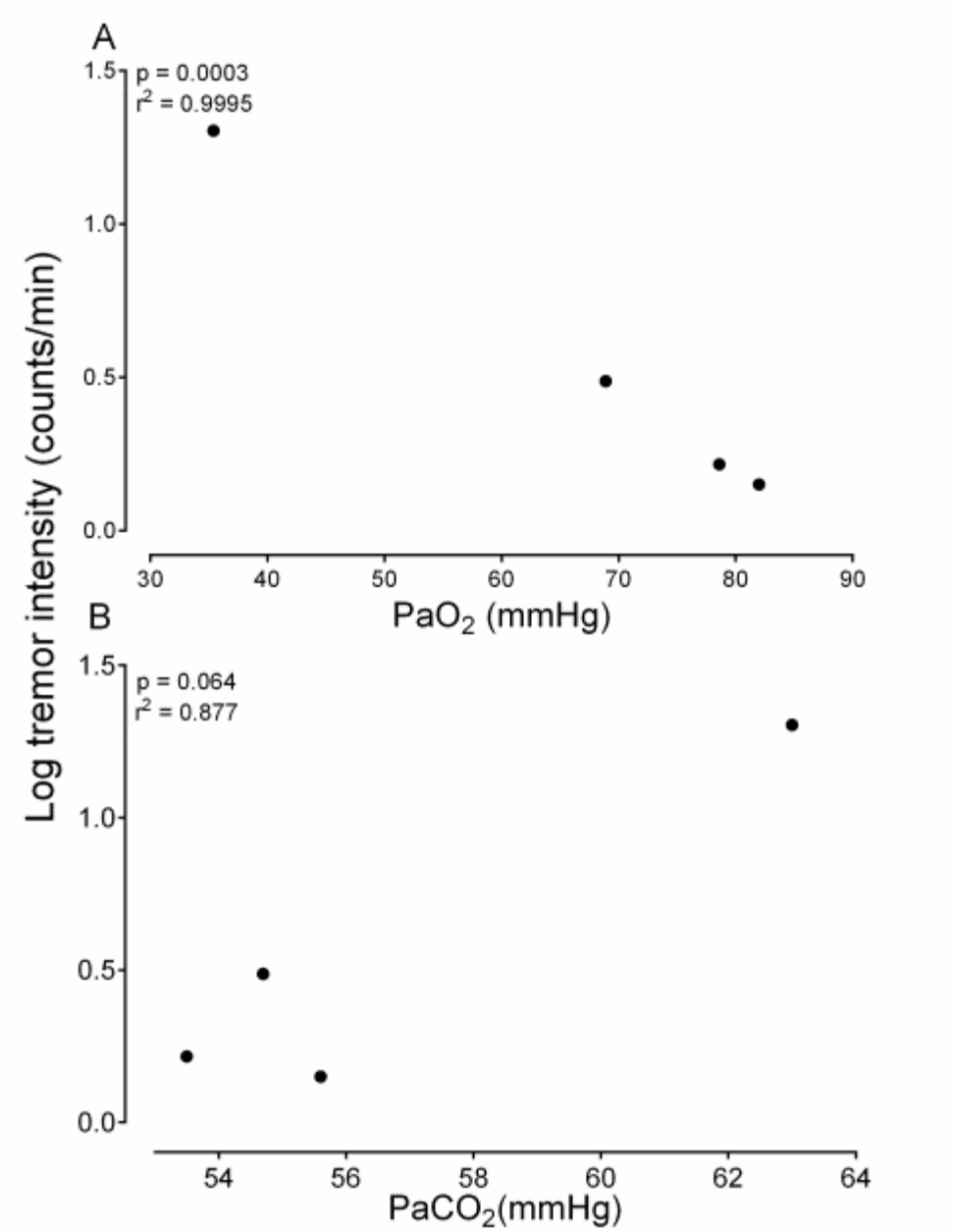

Figure 4.5.2 The log mean tremor intensity of the 14 chemically immobilised white rhinoceros was correlated, using Pearson product-moment correlation, with the mean plasma arterial partial pressure of oxygen (PaO_2) (A) and carbon dioxide ($PaCO_2$) (B). The rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation, 6 minutes after the rhinoceros were placed in lateral recumbency.

4.5.2 The relationship between plasma catecholamines (adrenaline and noradrenaline) and tremor intensity

The tremor intensity was greater before the intervention of butorphanol and nasotracheal oxygen insufflation was administered than after (Fig. 4.5.3 A). Both plasma adrenaline (Fig. 4.5.3 B) and plasma noradrenaline (Fig. 4.5.3 C) concentrations followed a similar pattern, as the tremor intensity, over the course of the immobilisation period. Plasma adrenaline concentration was initially high and decreased significantly ($p < 0.0001$, $F_4 = 49.21$, Fig. 4.5.3 B) over the immobilisation period. For plasma adrenaline concentration, no significant difference occurred between the 1 minute (median adrenaline = 1.23 ng/ml) and 5 minute (median adrenaline = 1.58 ng/ml) pre-intervention periods. At the 10 minute time point, 4 minutes after butorphanol and nasotracheal oxygen insufflation had been administered, plasma adrenaline concentration decreased significantly ($p = 0.02$, median adrenaline = 0.56 ng/ml) compared to the 5 minute time point, but not compared to the 1 minute time point. By the 15 minute time point (median adrenaline = 0.2 ng/ml), plasma adrenaline concentration was still decreasing, resulting in a significant difference among the 15 minute time point and the 1 minute ($p = 0.0013$) as well as the 5 minute ($p < 0.0001$) time point, but not with the 10 minute time interval. The plasma adrenaline concentration at the 20 minute time interval (median adrenaline = 0.2 ng/ml) was also significantly different from the 1 minute and 5 minute ($p < 0.0001$) time points.

A significant decrease over the course of the immobilisation period was also observed for plasma noradrenaline concentrations ($p < 0.0001$, $F_4 = 50.51$, Fig. 4.5.3 C). The highest concentration was during the pre-intervention period, at the 1 minute (median noradrenaline = 3.63 ng/ml) and 5 minute (median noradrenaline = 3.87 ng/ml) time points, and no difference was reported between these two time points. At the 10 minute time interval, 4 minutes after butorphanol and nasotracheal oxygen insufflation had been administered, plasma noradrenaline declined (median noradrenaline = 1.92 ng/ml), but its concentration was not significantly different from the 1 minute or the 5 minute time points. The plasma noradrenaline concentration at the 15 minute time point

(median noradrenaline = 0.98 ng/ml) was significantly lower than at the 1 minute ($p = 0.02$) and the 5 minute ($p < 0.0001$) time points. Although there seemed to be lower plasma noradrenaline concentration at the 20 minute time point (median noradrenaline = 0.72 ng/ml) than what was recorded for the 15 minute time point, no significant difference was reported. However, plasma noradrenaline concentration at 20 minutes was significantly different from the concentration at the 1 minute, 5 minute ($p < 0.0001$), and 10 minute ($p = 0.001$) time points.

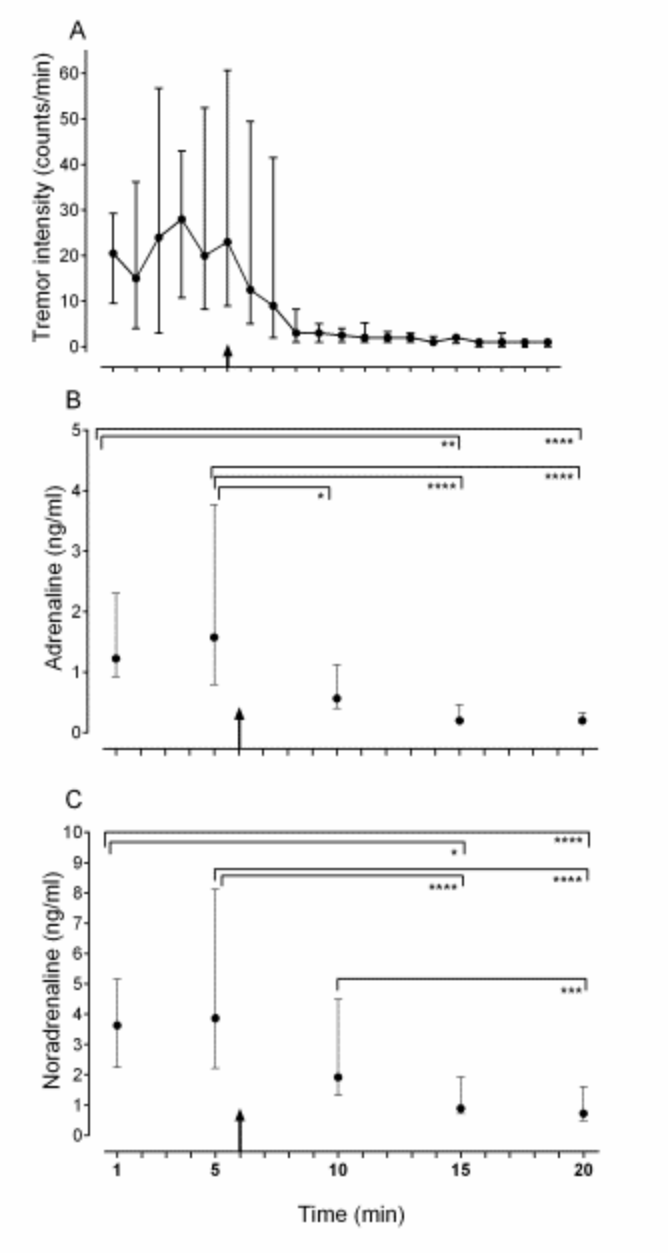

Figure 4.5.3 Fourteen white rhinoceros were chemically immobilised with a combination of etorphine and azaperone. The median tremor intensity (A), median plasma adrenaline (B) and median plasma noradrenaline (C) concentrations with interquartile ranges, were sampled at pre-selected time points. Butorphanol and nasotracheal oxygen insufflation were administered 6 minutes after the rhinoceros were placed in lateral recumbency, which is represented by the arrow. The brackets indicate the significant differences between the time points (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; adrenaline and noradrenaline: Friedman's with *post-hoc* Dunn's tests).

Both plasma adrenaline ($p = 0.003$, $r^2 = 0.96$, Fig. 4.5.4 A) and plasma noradrenaline ($p = 0.02$, $r^2 = 0.89$, Fig. 4.5.4 B) concentrations were significantly and positively correlated with tremor intensity (Pearson product-moment correlation) over the immobilisation period. The plasma adrenaline concentration had a stronger relationship than the plasma noradrenaline concentration with tremor intensity. Directly after capture, at the 1 minute time interval, which was shortly after the rhinoceros were placed into recumbency, there was no correlation among the tremor intensity and the plasma adrenaline ($p = 0.84$, $r^2 = 0.003$, Fig. 4.5.5 A) and plasma noradrenaline ($p = 0.996$, $r^2 = 0.0000024$, Fig. 4.5.5 B) concentrations in the 14 immobilised rhinoceros.

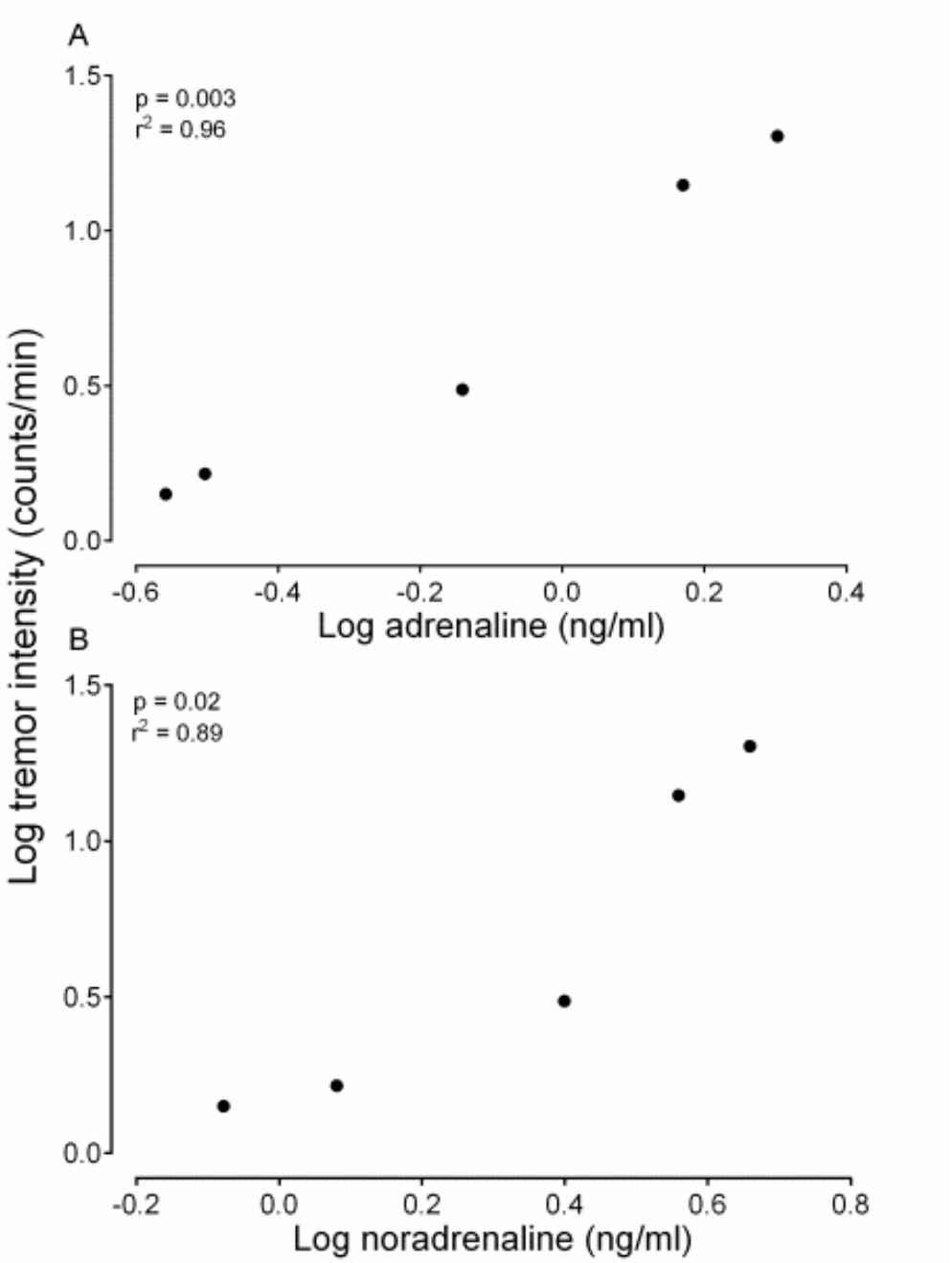

Figure 4.5.4 The log mean tremor intensity of 14 white rhinoceros that were chemically immobilised with a combination of etorphine and azaperone was correlated, using Pearson product-moment correlation, with the log mean adrenaline (A) and log mean noradrenaline (B) concentrations. The rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation 6 minutes after they were placed in lateral recumbency.

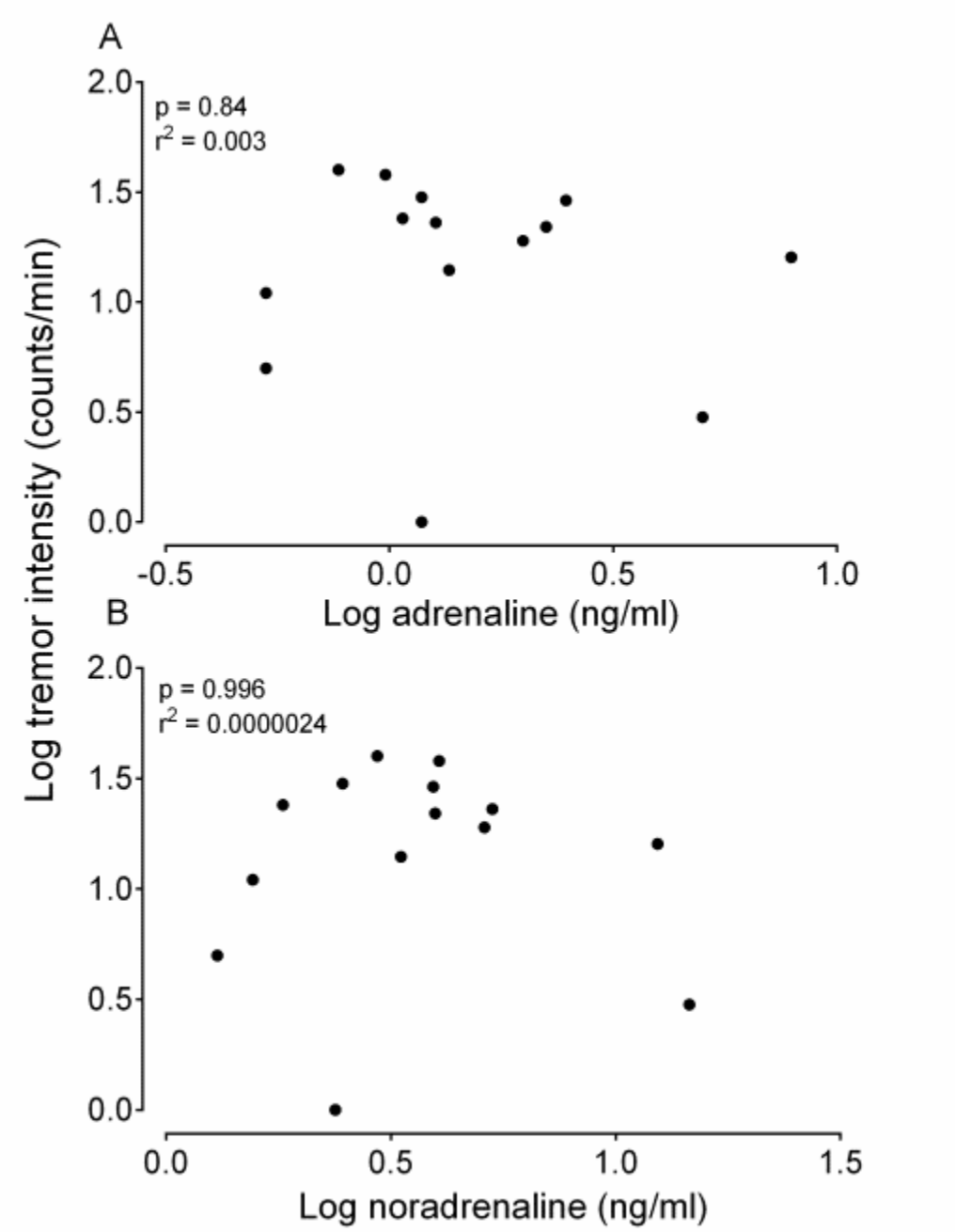

Figure 4.5.5 The log mean tremor intensity, at 1 minute peri-recumbency, of 14 white rhinoceros that were chemically immobilised with a combination of etorphine and azaperone was correlated, using Pearson product-moment correlation, with the log mean adrenaline (A) and log mean noradrenaline (B) concentrations. The rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation 6 minutes after they were placed in lateral recumbency.

4.5.3 The relationship between blood electrolytes and tremor intensity

The concentration of blood potassium (K^+) decreased significantly over the course of the immobilisation period ($p = 0.0014$, $F_{3, 39} = 11.51$, Fig. 4.5.6 A), and displayed a similar change over time as the tremor intensity that was recorded by the activity logger (Fig. 4.3.2). The blood potassium concentration was highest at the 5 minute time point (mean potassium = 4.2 ± 0.407 mmol/L), before the butorphanol and nasotracheal oxygen insufflation was administered. At the 10 minute time point, 4 minutes after butorphanol and nasotracheal oxygen insufflation had been administered, blood potassium concentration decreased significantly ($p = 0.031$, mean potassium = 3.7 ± 0.31 mmol/L) compared to the pre-intervention period (5 minutes). At 15 minutes, the blood potassium concentration was not significantly different compared to the concentration at 10 minutes (mean potassium = 3.5 ± 0.402 mmol/L). However, blood potassium concentration at 15 minutes was significantly different from that at the 5 minute time point ($p =0.015$). Similarly, at the 20 minute time point (mean potassium = 3.5 ± 0.268 mmol/L), no significant difference in blood potassium concentration was reported compared to the 10 minute or the 15 minute time points, but it was significantly different from the pre-intervention period (5 minutes) ($p = 0.007$).

The plasma sodium (Na^+, $p = 0.93$, $F_{3, 39} = 0.083$, Fig. 4.5.6 B), plasma chloride (Cl^-, $p = 0.875$, $F_{3, 39} = 0.0714$, Fig. 4.5.6 C) and plasma calcium (Ca^{++}, $p = 0.691$, $F_{3, 39} = 0.435$, Fig. 4.5.6 D) concentrations did not change significantly over the course of the immobilisation period.

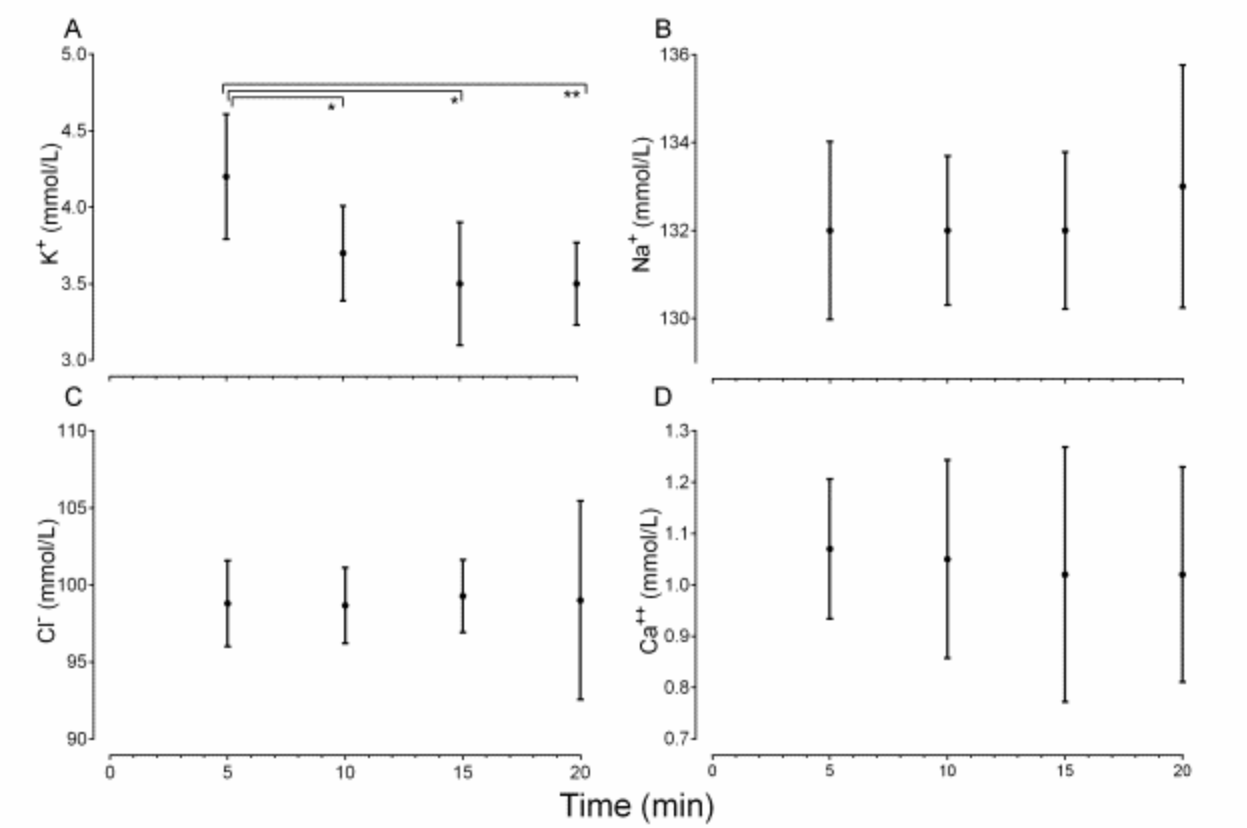

Figure 4.5.6 The mean blood potassium (K^+) (A), sodium (Na^+) (B), chloride (Cl^-) (C) and calcium (Ca^{++}) (D) concentrations (± standard deviaiton) of 14 white rhinoceros that were chemically immobilised with a combination of etorphine and azaperone. Six minutes after the rhinoceros were placed in lateral recumbency they received a combination of butorphanol and nasotracheal oxygen insufflation, which is represented by the arrow. The brackets indicate the significant differences between the time points (* $p < 0.05$; ** $p < 0.01$; one-way ANOVA with *post-hoc* Tukey's tests).

The blood potassium concentration was positively correlated with the tremor intensity ($p = 0.001$, $r^2 = 0.997$, Fig. 4.5.7 A; Pearson product-moment correlation); as the blood potassium concentrations decreased, so did the tremor intensity. The tremor intensity was not correlated with the blood sodium concentration ($p = 0.511$, $r^2 = 0.239$, Fig. 4.5.7 B; Pearson product-moment correlation) or with the blood chloride concentration ($p = 0.449$, $r^2 = 0.304$, Fig. 4.5.7 C; Pearson product-moment correlation). There was also no correlation between the blood calcium concentration and tremor intensity ($p = 0.06$, $r^2 = 0.886$, Fig. 4.5.7 D; Pearson product-moment correlation).

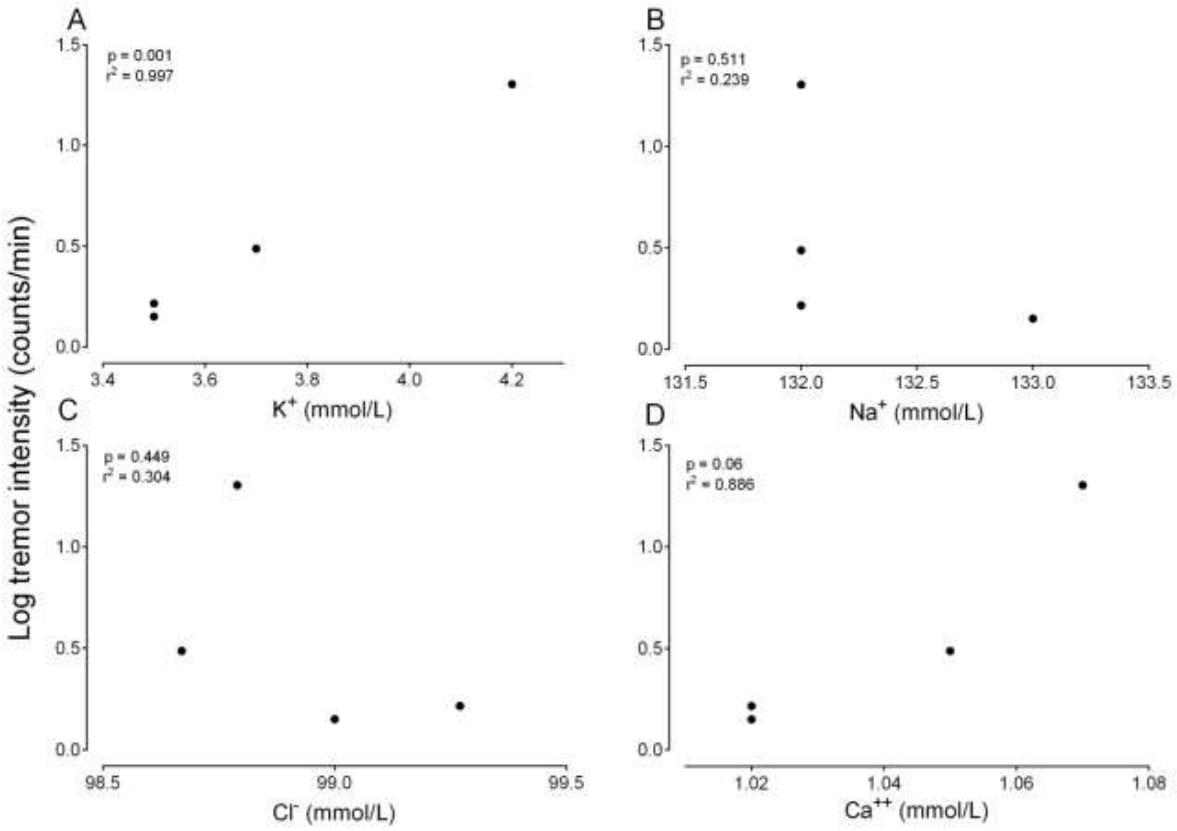

Figure 4.5.7 The log mean tremor intensity of 14 white rhinoceros that were chemically immobilised with a combination of etorphine and azaperone was correlated, using Pearson product-moment correlation, with the blood potassium (K^+) (A), sodium (Na^+) (B), chloride (Cl^-) (C) and calcium (Ca^{++}) (D) concentrations. The rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation 6 minutes after they were placed in lateral recumbency.

4.5.4 The relationship between blood pH, blood glucose and tremor intensity

After the combination intervention of butorphanol and nasotracheal oxygen insufflation was administered, the blood pH for the 14 rhinoceros increased significantly during the immobilisation period ($p < 0.0001$, $F_{3, 39} = 28.97$, Fig. 4.5.8 A). The blood pH was lowest (mean pH = 7.083 ± 0.145) at the 5 minute time point, which was 1 minute before the administration of the intervention of butorphanol and nasotracheal oxygen insufflation. At the 10 minute time point, which was 4 minutes after the intervention was administered, the blood pH increased significantly ($p = 0.01$, mean pH = 7.146 ± 0.144) compared to the 5 minute time point. The blood pH continued to increase and, at the 15 minute time point (mean pH = 7.162 ± 0.134), it was significantly higher than the blood pH at the 10 minute ($p = 0.017$) and at the 5 minute time points ($p = 0.001$). Similarly, the plasma pH at the 20 minute time interval (mean pH = 7.187 ± 0.112) was also higher than that at the 5 minute ($p < 0.0001$), 10 minute ($p = 0.013$) and 15 minute ($p = 0.01$) time points.

The blood glucose concentration increased significantly ($p = 0.01$, $F_{3, 39} = 6.12$, Fig. 4.5.8 B) during the chemical immobilisation period. During the pre-intervention period (5 minutes), the blood glucose concentration was at its lowest (mean glucose = 8.8 ± 1.77 mmol/L), before it increased 4 minutes post-intervention, at the 10 minute time point (mean glucose = 10.2 ± 1.95 mmol/L). However, there was no significant difference for the blood glucose between the 5 and 10 minute time points. At the 15 minute time interval, the plasma glucose concentration changed (mean glucose = 9.9 ± 1.69 mmol/L), but a significant difference was reported only with the 5 minute ($p = 0.016$) and not with the 10 minute time point. At the 20 minute time point (mean glucose = 9.9 ± 1.92 mmol/L), the blood glucose concentration was not significantly different from that measured at the other time points.

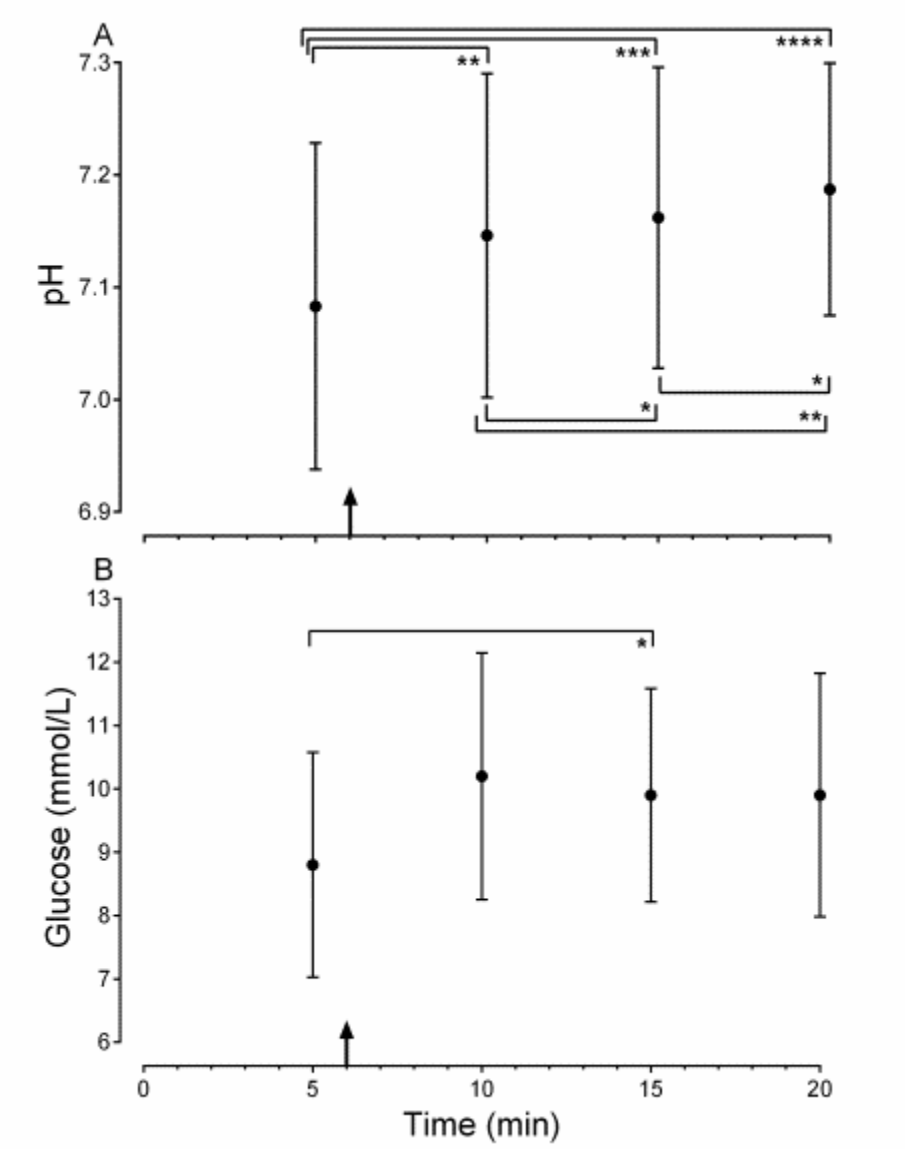

Figure 4.5.8 Mean blood pH (A) and glucose (B) concentrations (± SD) of 14 white rhinoceros that were chemically immobilised with a combination of etorphine and azaperone. Six minutes after they were placed in lateral recumbency, the rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation, which is represented by the arrow. The brackets indicate the significant differences between the time points (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; one-way ANOVA with *post-hoc* Tukey's tests).

The blood pH was negatively correlated with tremor intensity ($p = 0.02$, $r^2 = 0.97$, Fig. 4.5.9 A; Pearson product-moment correlation). Even though the blood glucose concentration changed over the course of the immobilisation period, it was not correlated with the tremor intensity ($p = 0.126$, $r^2 = 0.76$, Fig. 4.5.9 B; Pearson product-moment correlation).

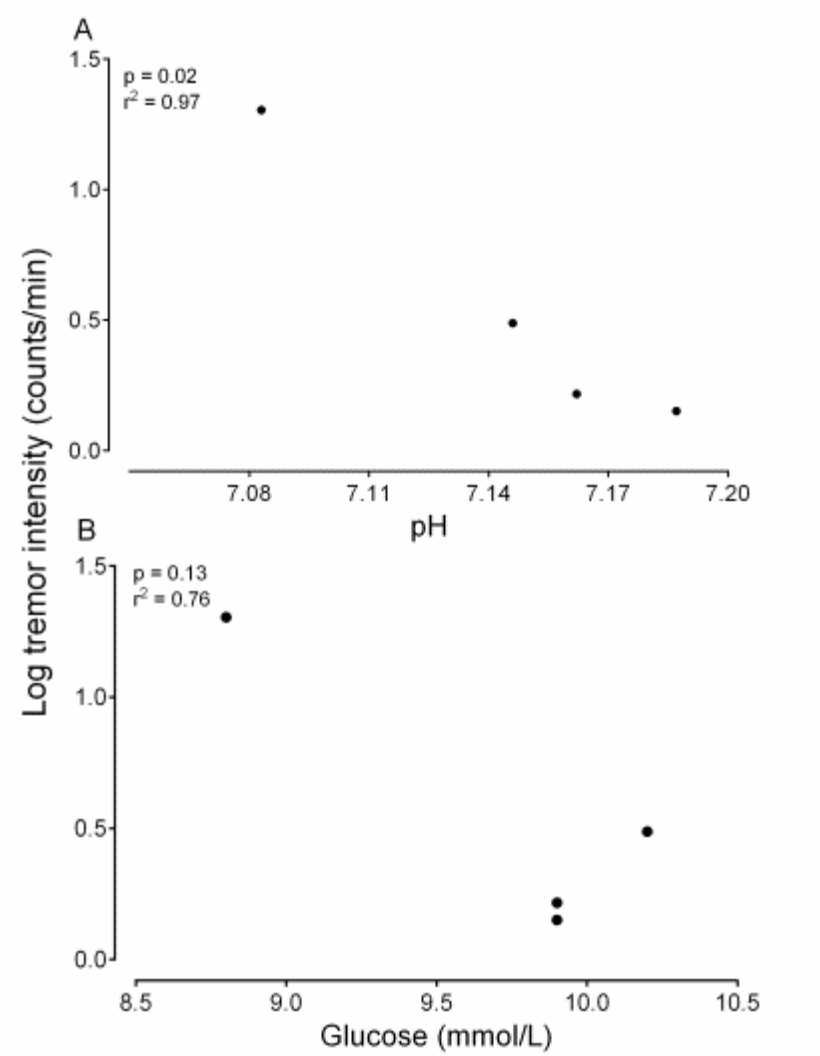

Figure 4.5.9 The log mean tremor intensity of 14 white rhinoceros that were chemically immobilised with a combination of etorphine and azaperone was correlated, using Pearson product-moment correlation, with the blood pH (A) and glucose (B) concentrations. Six minutes after they were placed in lateral recumbency, the rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation.

4.5.5 The relationship between the blood lactate concentration and tremor intensity

The median blood lactate concentration significantly changed during the chemical immobilisation period ($p = 0.001$, $F_3 = 15.60$, Fig. 4.5.10); there was a significant decrease between the 10 minute and 20 minute ($p = 0.001$) time points. No correlation occurred between the blood lactate concentration and the tremor intensity ($p = 0.49$, $r^2 = 26$, Fig. 4.5.11) over the immobilisation period.

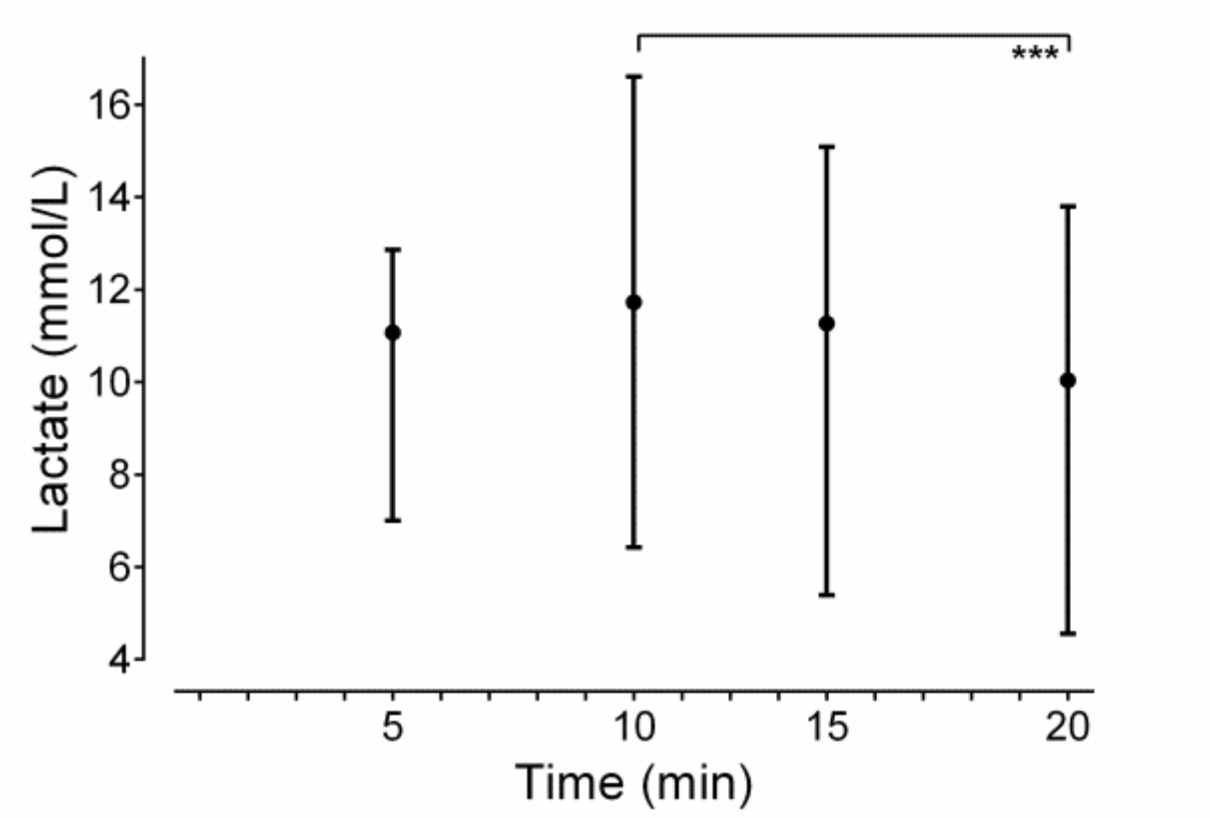

Figure 4.5.10 Median blood lactate concentrations with interquartile range, of 14 white rhinoceros that were chemically immobilised with a combination of etorphine and azaperone. Six minutes after they were placed in lateral recumbency, the rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation, which is represented by the arrow. The brackets indicate the significant differences between the time points (*** $p < 0.001$; Friedman's with *post-hoc* Dunn's tests).

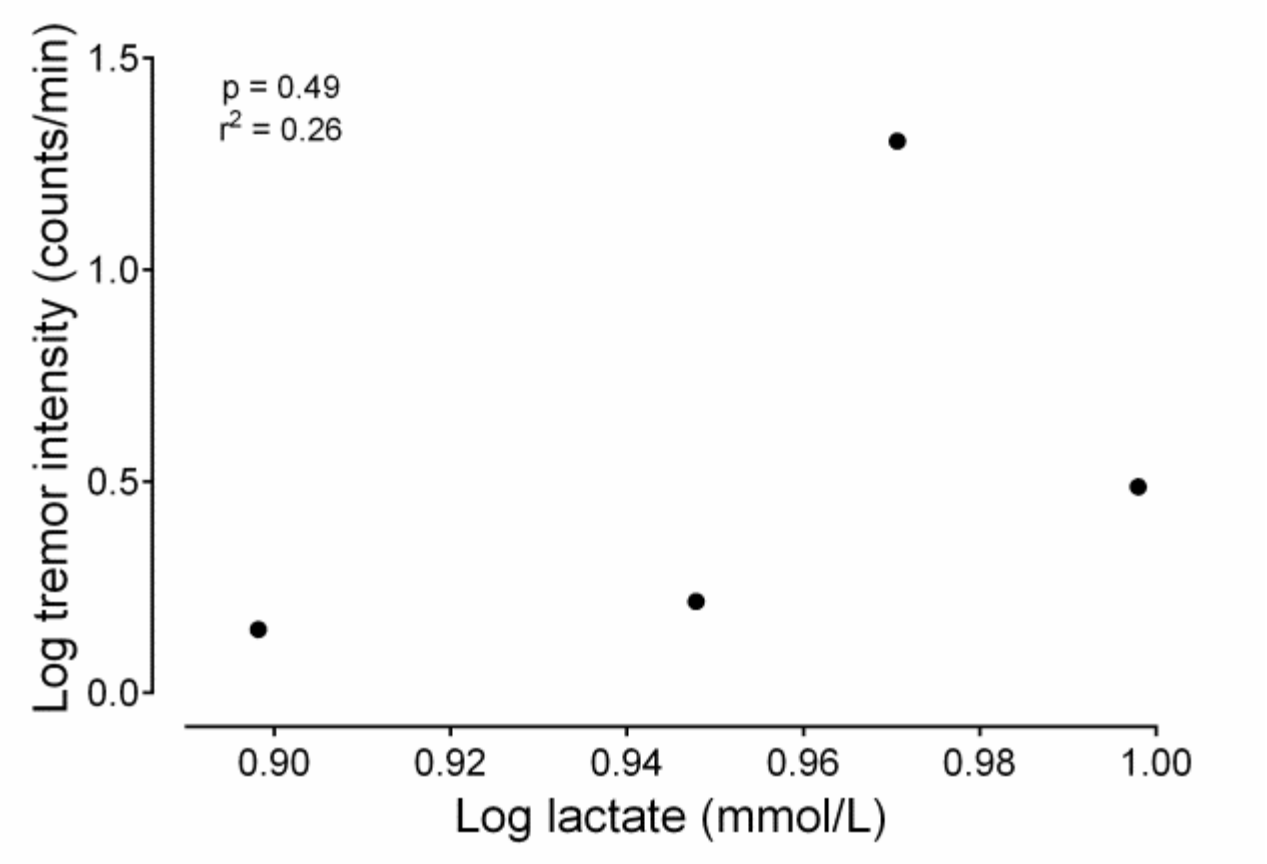

Fig 4.5.11 The log mean tremor intensity of 14 white rhinoceros that were chemically immobilised with a combination of etorphine and azaperone was correlated, using Pearson product-moment correlation with the log mean blood lactate. Six minutes after they were placed in lateral recumbency, the rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation.

4.6 The association between plasma adrenaline, plasma noradrenaline and measured physiological variables

4.6.1 The relationship between arterial blood gases with plasma adrenaline and plasma noradrenaline

There was a correlation between plasma adrenaline concentration and the arterial partial pressure of oxygen (PaO_2) ($p = 0.025$, $r^2 = 0.95$, Fig. 4.6.1 A; Pearson product-moment correlation). This correlation was a negative correlation, in that when the plasma adrenaline concentration was at its highest, the PaO_2 was at its lowest. The plasma adrenaline concentration had no correlation with the arterial partial pressure of carbon dioxide ($PaCO_2$) ($p = 0.137$, $r^2 = 0.75$, Fig. 4.6.1 B; Pearson product-moment correlation).

The PaO_2 was not correlated with noradrenaline ($p = 0.073$, $r^2 = 0.86$, Fig. 4.6.1 C; Pearson product-moment correlation). However, when the PaO_2 was at its lowest, the noradrenaline concentration was at its highest concentration, thus displaying a similar pattern to the relationship found between PaO_2 and adrenaline. Plasma noradrenaline, like the plasma adrenaline concentration, was not correlated with $PaCO_2$ ($p = 0.248$, $r^2 = 0.57$, Fig. 4.6.1 D; Pearson product-moment correlation).

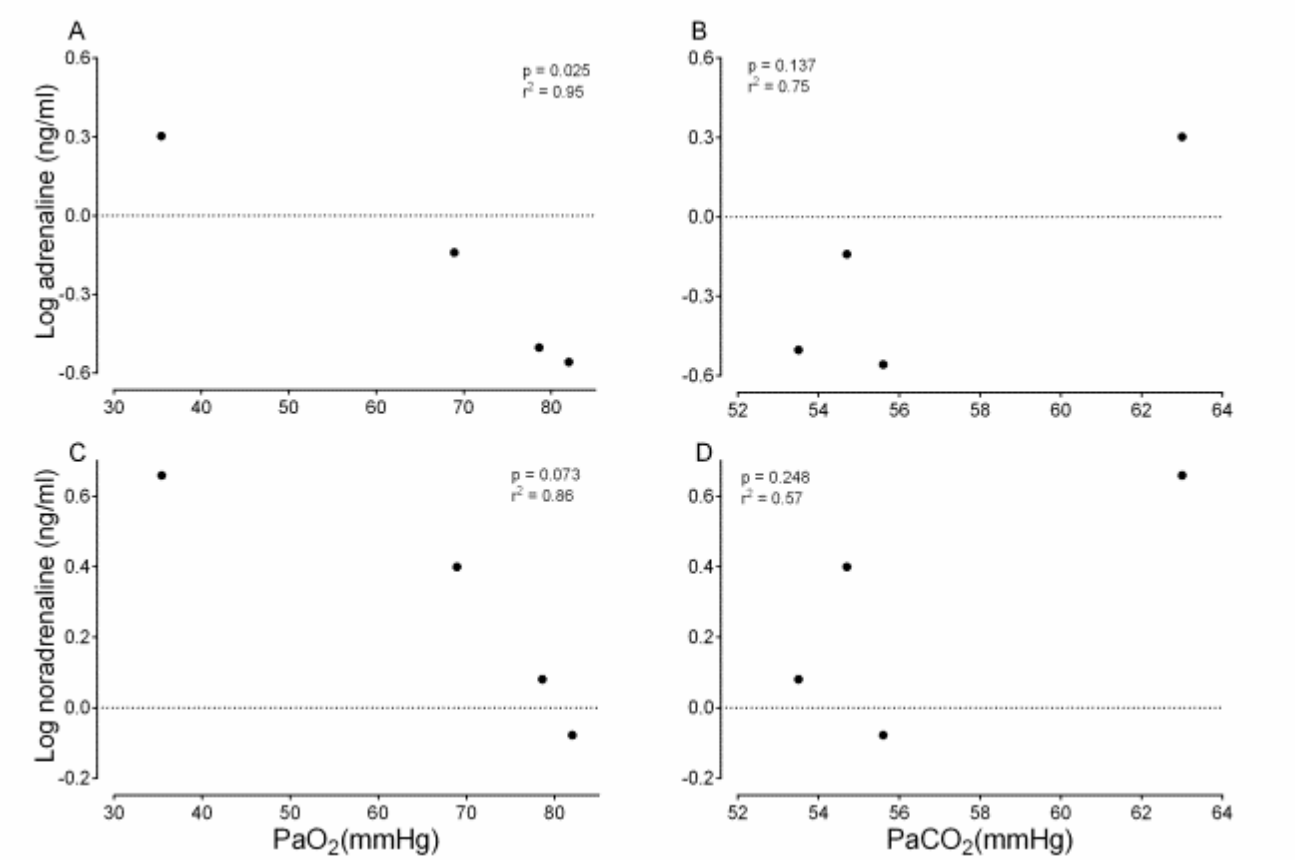

Figure 4.6.1 The log mean adrenaline concentration of the 14 white rhinoceros was correlated, using Pearson product-moment correlation, with the mean PaO_2 (A) and $PaCO_2$ (B). The mean PaO_2 (C) and $PaCO_2$ (D) were also correlated with the log mean noradrenaline concentration. All 14 rhinoceros were chemically immobilised with a combination of etorphine and azaperone. The rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation 6 minutes after they were placed in lateral recumbency.

4.6.2 The relationship between the blood electrolytes and the plasma adrenaline and plasma noradrenaline

Plasma adrenaline concentration was positively correlated with blood potassium concentration ($p = 0.02$, $r^2 = 0.96$, Fig. 4.6.2 A; Pearson product-moment correlation), while blood sodium concentration ($p = 0.441$, $r^2 = 0.31$, Fig. 4.6.2 B; Pearson product-moment correlation) and blood chloride concentration ($p = 0.319$, $r^2 = 46$, Fig. 4.6.2 C; Pearson product-moment correlation) did not to correlate with adrenaline concentration. The blood calcium concentration was however positively correlated with the plasma adrenaline concentration ($p = 0.011$, $r^2 = 0.98$, Fig. 4.6.2 D; Pearson product-moment correlation).

Plasma noradrenaline concentration, had no significant correlation with blood potassium concentration ($p = 0.073$, $r^2 = 0.86$, Fig. 4.6.3 A; Pearson product-moment correlation), plasma sodium concentration ($p = 0.305$, $r^2 = 0.48$, Fig. 4.6.3 B; Pearson product-moment correlation) and blood chloride concentration ($p = 0.319$, $r^2 = 0.49$, Fig. 4.6.3 C; Pearson product-moment correlation). Blood calcium concentration however, had a significant correlation with the plasma noradrenaline concentration ($p = 0.02$, $r^2 = 0.96$, Fig. 4.6.3 D; Pearson product-moment correlation). As the blood calcium concentration decreased, so did the plasma noradrenaline concentration.

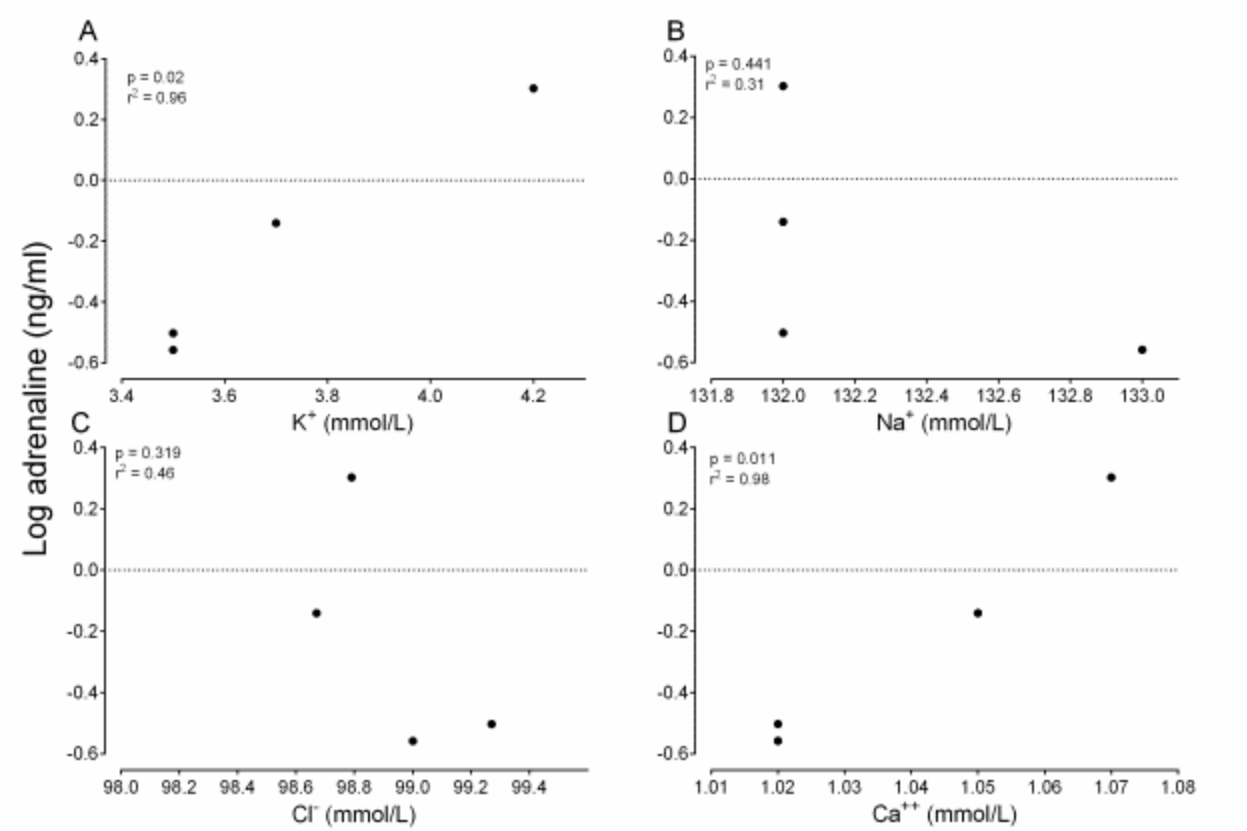

Figure 4.6.2 The log mean adrenaline concentration of the 14 white rhinoceros was correlated, using Pearson product-moment correlation, with the mean blood potassium concentration (K^+) (A), mean blood sodium concentration (Na^+) (B), mean blood chloride concentration (Cl^-) (C) and mean blood calcium concentration (Ca^{++}) (D). All 14 rhinoceros were chemically immobilised with a combination of etorphine and azaperone. The rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation 6 minutes after they were placed in lateral recumbency.

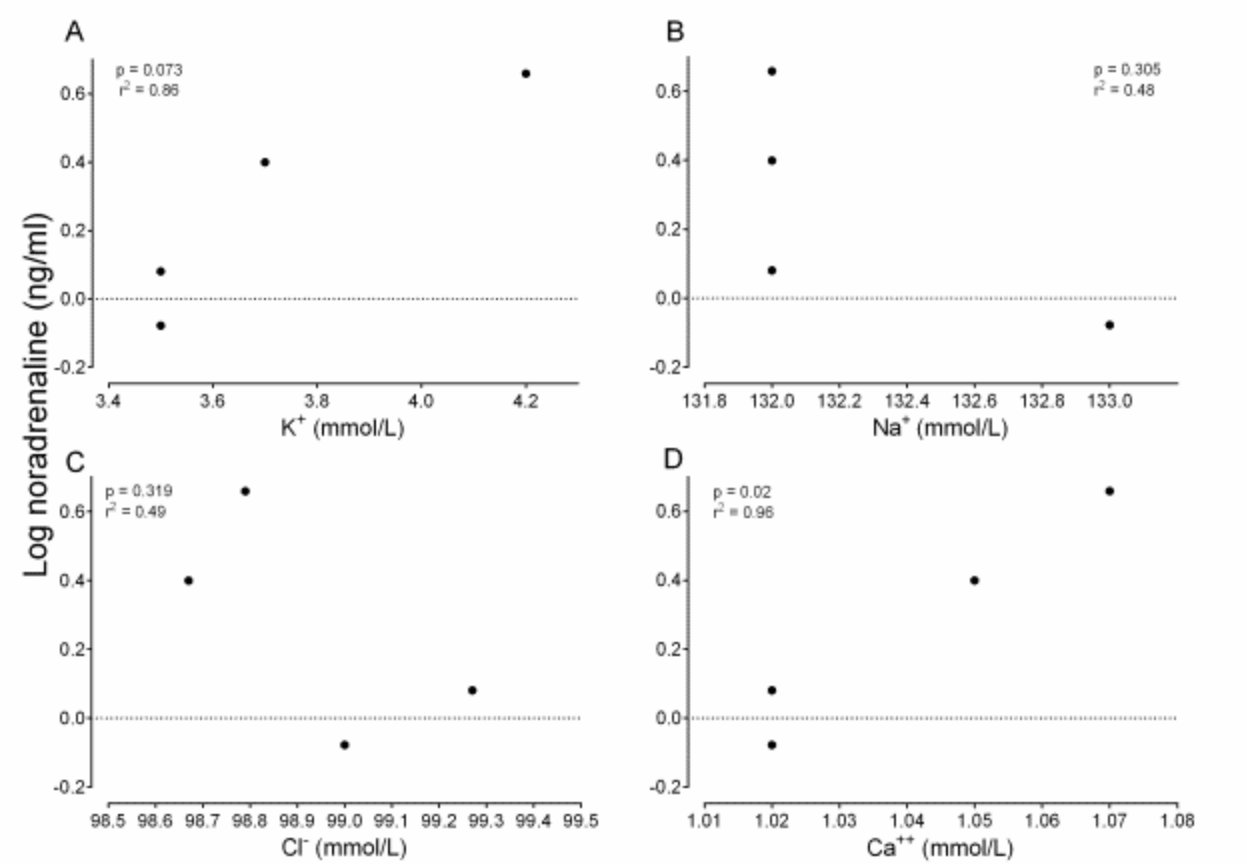

Figure 4.6.3 The log mean noradrenaline concentration of the 14 white rhinoceros was correlated, using Pearson product-moment correlation, with the mean blood potassium concentration (K^+) (A), mean blood sodium concentration (Na^+) (B), mean blood chloride concentration (Cl^-) (C) and mean blood calcium concentration (Ca^{++}) (D). All 14 rhinoceros were chemically immobilised with a combination of etorphine and azaperone. The rhinoceros received a combination of a butorphanol and nasotracheal oxygen insufflation 6 minutes after they were placed in lateral recumbency.

4.6.3 The relationship between blood pH, blood glucose, plasma adrenaline and plasma noradrenaline

Plasma adrenaline concentrations had a significant, negative relationship with the blood pH ($p = 0.03$, $r^2 = 0.94$, Fig. 4.6.4 A; Pearson product-moment correlation). The blood pH increased and became less acidic as the plasma adrenaline concentration decreased. The blood glucose concentration, however, had no significant relationship with adrenaline concentration ($p = 0.245$, $r^2 = 0.57$, Fig. 4.6.4 B; Pearson product-moment correlation). Following similar changes to those of plasma adrenaline concentration, plasma noradrenaline concentration had a significant, negative relationship with blood pH ($p = 0.044$, $r^2 = 0.91$, Fig. 4.6.4 C; Pearson product-moment correlation). The blood glucose concentration had no significant correlation with the plasma noradrenaline concentration ($p = 0.354$, $r^2 = 0.42$, Fig. 4.6.4 D; Pearson product-moment correlation).

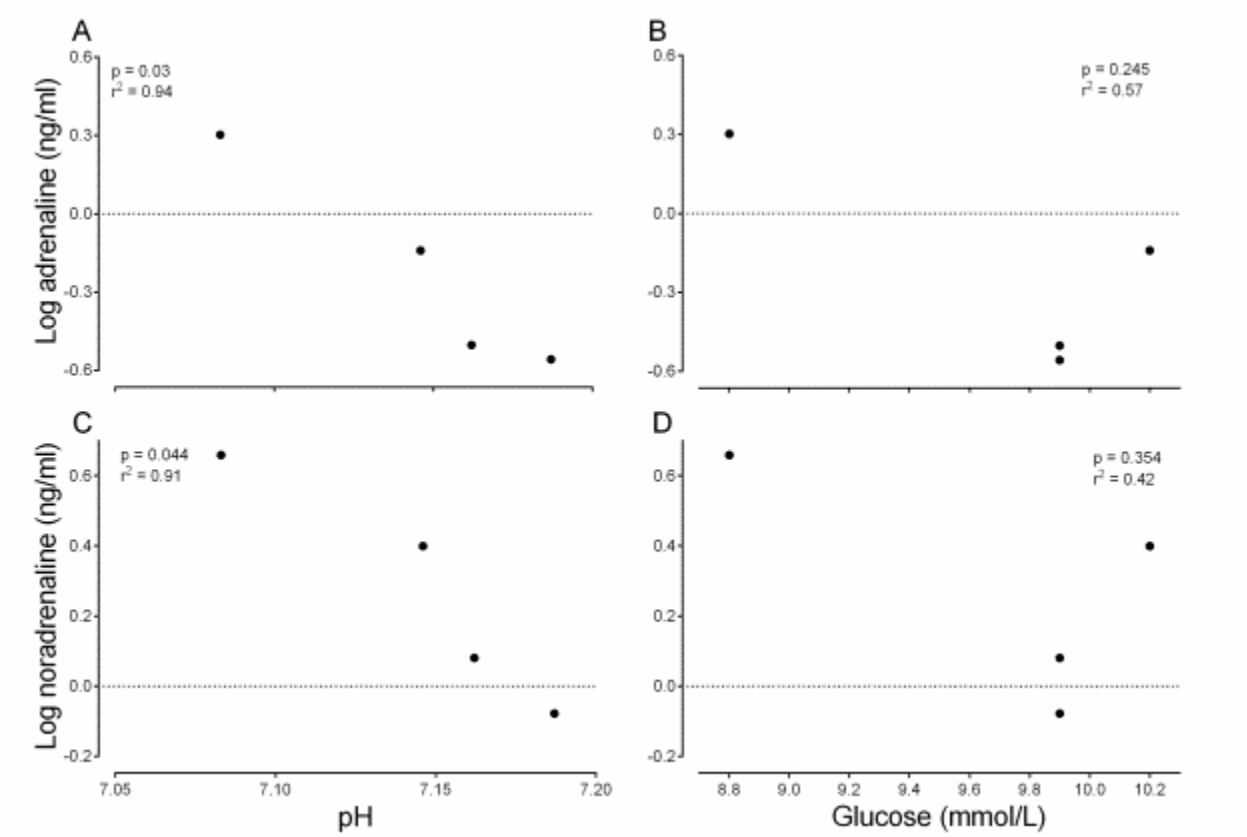

Figure 4.6.4 The log mean adrenaline concentration of the 14 white rhinoceros was correlated, using Pearson product-moment correlation, with the mean blood pH (A) and glucose concentration (B). The mean pH (C) and glucose concentration (D) were also correlated with the log mean noradrenaline concentration. All 14 rhinoceros were chemically immobilised with a combination of etorphine and azaperone. The rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation 6 minutes after they were placed in lateral recumbency.

4.6.4 The relationship between the blood lactate, plasma adrenaline and plasma noradrenaline

There was no correlation between the blood lactate and both the plasma adrenaline ($p = 0.35$, $r^2 = 0.42$, Fig. 4.6.5 A; Pearson's correlation) and plasma noradrenaline ($p = 0.22$, $r^2 = 0.78$, Fig. 4.6.5 B; Pearson's correlation) concentrations.

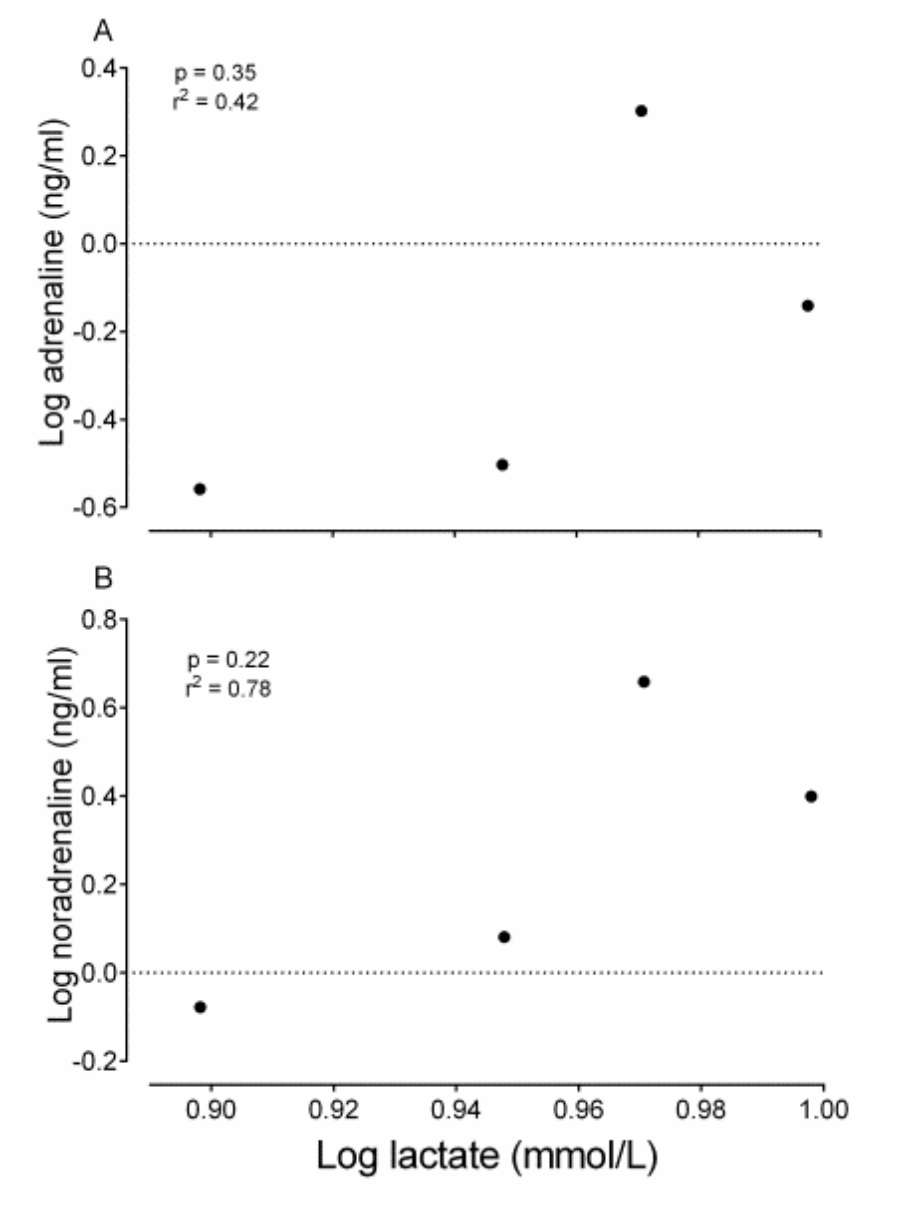

Figure 4.6.5 The log mean blood lactate concentration of the 14 white rhinoceros was correlated, using Pearson product-moment correlation, with the log mean adrenaline concentration (A) and log mean noradrenaline concentration (B). All 14 rhinoceros were chemically immobilised with a combination of etorphine and azaperone. The rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation 6 minutes after they were placed in lateral recumbency.

4.6.5 The relationship between blood pH, blood potassium (K^+) and blood calcium (Ca^{++})

The blood pH can affect or be affected by a number of different variables, including a change in the electrolyte concentration. However, a significant, negative correlation occurred only between the blood pH and blood potassium ($p = 0.027$, $r^2 = 0.95$, Fig. 4.6.6 A; Pearson product-moment correlation). The blood pH increased and became less acidic as the blood potassium concentration inversely decreased. A similar pattern was observed between blood calcium concentration and the blood pH, with the plasma pH becoming less acidic as the calcium concentration tended to decrease. However no correlation occurred between blood pH and blood calcium concentration ($p = 0.07$, $r^2 = 0.87$, Fig. 4.6.6 B; Pearson product-moment correlation).

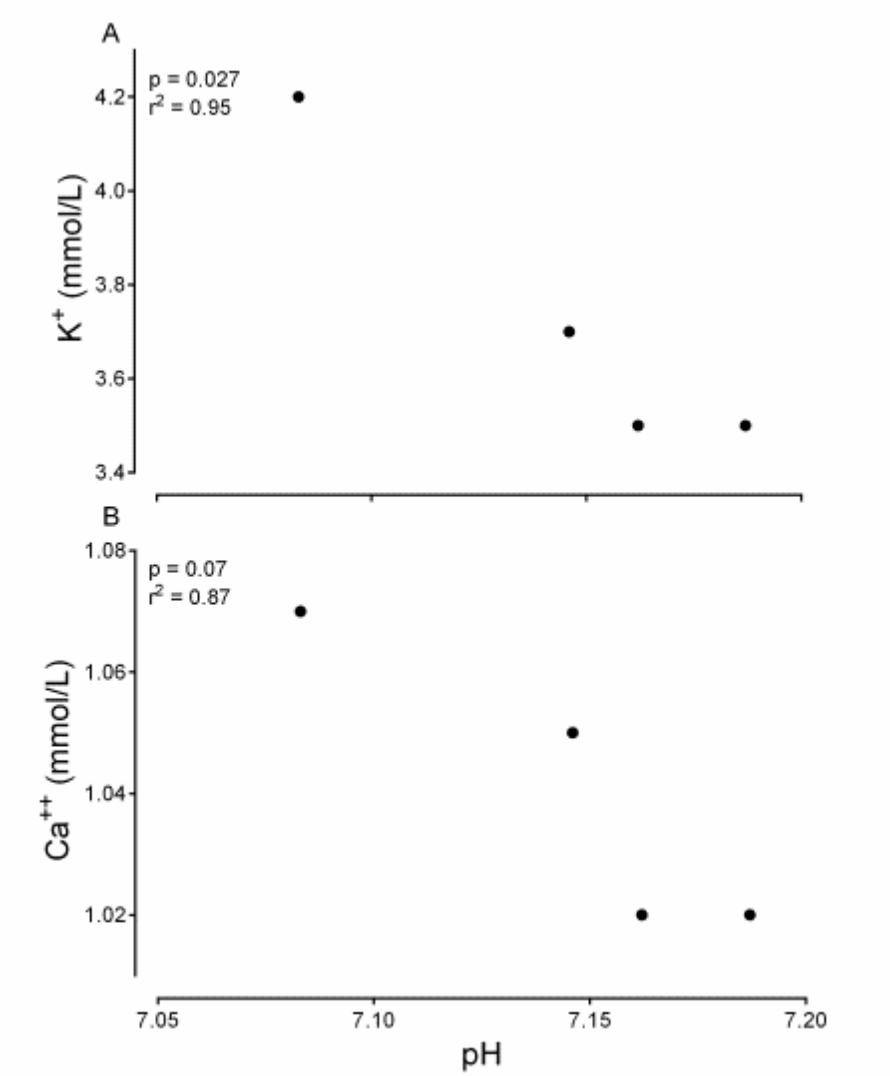

Figure 4.6.6 The mean blood potassium concentration (K^+) (A) and calcium concentration (Ca^{++}) (B) of the 14 white rhinoceros that were chemically immobilised with a combination of etorphine and azaperone was correlated, using Pearson product-moment correlation, with the mean blood pH. The rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation 6 minutes after they were placed in lateral recumbency.

4.6.6 The relationship between blood pH and blood lactate

There was no correlation between the blood pH and the blood lactate ($p = 0.34$, $r^2 = 0.37$, Fig. 4.6.7; Pearson product-moment correlation).

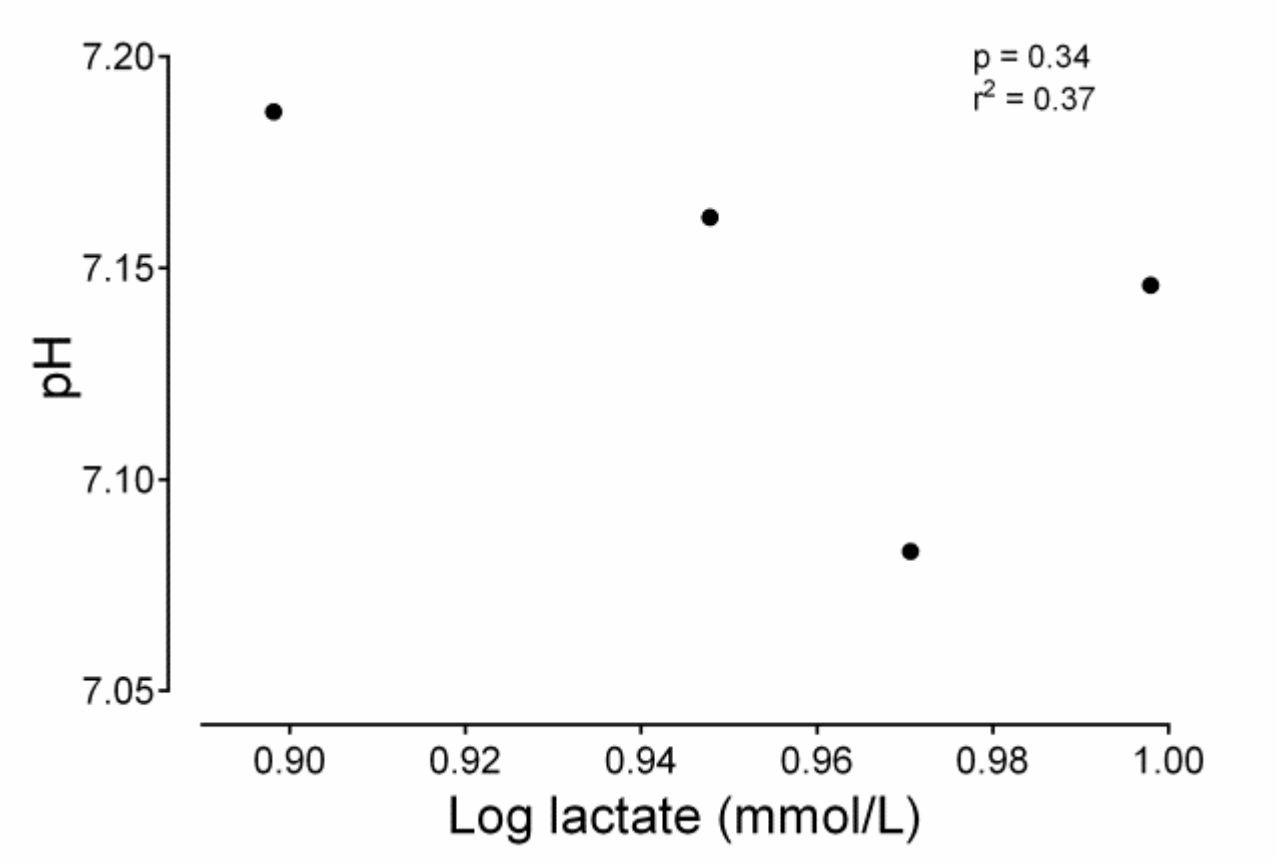

Figure 4.6.7 The log mean blood lactate concentration of the 14 white rhinoceros was correlated, using Pearson product-moment correlation, with the mean blood pH. All 14 rhinoceros were chemically immobilised with a combination of etorphine and azaperone. The rhinoceros received a combination of butorphanol and nasotracheal oxygen insufflation 6 minutes after they were placed in lateral recumbency.

Chapter 5

Discussion and Conclusion

5.1 Discussion

When white rhinoceros were chemically immobilised with the same drug combination and similar doses, both in a boma setting as well as in the field, they developed muscle tremors. In the free-ranging rhinoceros, the high tremor intensity was associated with high blood catecholamine concentrations, low arterial partial pressure of oxygen (PaO_2) and low blood pH. By administering a partial opioid agonist/antagonist, butorphanol, with continuous nasotracheal oxygen insufflation to the rhinoceros, the PaO_2 and blood pH increased and catecholamines and tremor intensity decreased. The rhinoceros were chemically immobilised with a mixture of etorphine and azaperone, both in the boma and field. In both environments, all the rhinoceros experienced initial high intensity muscle tremors once they were immobilised, with the tremors resulting in pronounced shaking of the chest, shoulder, legs and foot of the rhinoceros. A current theory is that these tremors are a result of the direct pharmacological action of the immobilising drugs. However, we found that there was no dose-response relationship among the doses of etorphine or azaperone and the tremor intensity. Another school of thought is that high levels of exercise during the capture event may predispose rhinoceros to high tremor intensities. However, there was no correlation between the total distance that the rhinoceros ran before they were placed into lateral recumbency and the tremor intensity. This finding indicates that the amount of exercise undertaken by the rhinoceros before being placed in lateral recumbency was not associated with the severity or intensity of muscle tremors.

Field practitioners have also noticed that tremor intensity decreases when butorphanol is administered to the immobilised rhinoceros (Moreira, 2010; Burroughs *et al*. 2012b; M. Hofmeyr, personal communication 2013). Butorphanol is often administered during the chemical immobilisation of rhinoceros, mainly because it is thought to reduce the

opioid-induced respiratory depression that results from etorphine. In this study on the boma-managed rhinoceros, there was a decrease in the tremor intensity (according to visual observation scores) after butorphanol was administered to the immobilised rhinoceros. However, this decrease was not significantly different to when the rhinoceros did not receive butorphanol (i.e. during the control intervention when the rhinoceros only received sterile water IV). This finding is in contrast to what has previously been observed by field practitioners, but it does not mean that these previous observations should be ignored or overlooked. If an objective method, like the use of activity loggers, was used to measure tremor intensity, rather than subjective observations, a decrease in tremor intensity may have been detected following butorphanol administration. The study in the boma-managed rhinoceros did not include an objective measure as the primary focus of this study was on investigating the cardiorespiratory effects of the drugs used during chemical immobilisation of rhinoceros. When nasotracheal oxygen insufflation was administered on its own in the boma-managed rhinoceros, there was no decrease in tremor intensity over time or compared to the different interventions at the different time points.

In the boma study, the rhinoceros were all initially severely hypoxic, hypercapnic and acidaemic before they received the various interventions at 6 minutes into the immobilisation period. When only nasotracheal oxygen insufflation was administered, the hypoxia improved only slightly but the hypercapnia and acidaemia worsened. The small increase in the PaO_2 during this intervention may be because the nasotracheal oxygen insufflation resulted in atelectasis and intrapulmonary shunting (Haw *et al.* 2014). By administering only butorphanol the hypoxia, hypercapnia and acidaemia improved only moderately and the rhinoceros remained compromised throughout the remainder of the immobilisation period. The combination intervention of butorphanol and nasotracheal oxygen insufflation corrected the hypoxia, improved the acidaemia, but it did not correct the hypercapnia. There was a negative correlation when comparing the PaO_2 (that was altered by the various interventions) to tremor intensity at the 5, 10 and 15 minute time points, suggesting that the severity of the rhinoceros hypoxic state may be an important variable influencing tremor intensity. Since oxygen insufflation on its

own did not cause an increase in PaO_2, we were unable to completely determine the effects of oxygen and butorphanol on tremor intensity and therefore we unfortunately cannot definitively explain oxygen and butorphanol's individual effects on tremor intensity. The blood pH had a negative correlation with the visual tremor intensity only at the 20 minute time interval, potentially indicating that pH may also play a role in influencing tremor intensity. On the other hand, the $PaCO_2$ showed no relationship with the visually observed tremor intensity between the interventions at the different time points suggesting that hypercapnia did not affect the tremor intensity.

Despite there being relationships among the blood pH, PaO_2 and the observed tremor intensity in the boma study, we considered that a more sensitive and objective measure of tremor intensity was required for a more thorough investigation into the relationship between tremor intensity and some important physiological variables in immobilised rhinoceros. In the field study, accelerometer loggers were used as an objective method of measuring the magnitude of tremor intensity, in addition to subjective visual observations, in the rhinoceros. The significant positive relationship between the visual observations of the tremor intensity and the accelerometer logger supported the use of activity loggers to record tremor intensity. Moreover, accelerometer loggers were able to record finer movements and a better scale of tremor intensity than an observer using visual observations. Each individual rhinoceros may also react differently to being captured. Some rhinoceros may have been captured before and therefore could have been more familiar with helicopters or the stress of being approached by humans, while others might not have been. Therefore, only the mean log tremor intensity counts of 14 rhinoceros at the time points (1 minute, 5 minute, 10 minute, 15 minute & 20 minute) were considered when attempting to understand the relationship between tremors and various measured variables.

Similar to the findings in the boma-managed rhinoceros, when the field-immobilised rhinoceros received the combination administration of butorphanol and nasotracheal oxygen insufflation there was an increase in the PaO_2 and blood pH and both variables

were associated with tremor intensity. The $PaCO_2$ decreased in both studies but its change was not associated with tremor intensity in either study.

As far as we are aware, there are currently no blood catecholamine values for rhinoceros at rest. Since the horse and the rhinoceros share a common ancestor (Perissodactyla) (Moreira 2010), known normal catecholamine values from the horse were compared to the rhinoceros' to get an indication of the magnitude of the response that occurred in the rhinoceros. A previous study used a similar analysis protocol, using high-performance liquid chromatography (HPLC), to what we used in our rhinoceros, to analyse catecholamine concentrations in horses (Nagata *et al.* 1999). The plasma adrenaline (plasma adrenaline = 1.70 ± 3.91 ng/ml) and plasma noradrenaline concentrations (plasma noradrenaline = 3.65 ± 5.042 ng/ml) in the rhinoceros in this study were substantially higher than those recorded in horses at rest (normal plasma adrenaline = 0.055 ± 0.005 ng/ml & normal plasma noradrenaline = 0.124 ± 0.011 ng/ml; Nagata *et al.* 1999). There was a positive correlation among the plasma adrenaline and plasma noradrenaline concentrations, and the tremor intensity in the rhinoceros. Plasma adrenaline concentration was also negatively correlated with PaO_2, while both plasma adrenaline and plasma noradrenaline had a positive correlation with the blood pH. These relationships show that when the rhinoceros were severely hypoxic and acidaemic, they had the highest plasma adrenaline and plasma noradrenaline concentrations as well as greatest tremor intensities. We hypothesise that the relationships among the blood oxygen, pH and the catecholamines were caused by hypoxia and acidaemia. The hypoxia and acidaemia activate the sympathetic nervous system, thereby stimulating a release of catecholamines into the blood (Perry *et al.* 1989; Wasser & Jackson 1991; Prabhakar 2000; Solomon 2000), which could potentially result in an increase in the tremor intensity.

Changes in physiological variables such as electrolytes and glucose concentrations of rhinoceros can be measured objectively with a blood test, but an objective method of measuring the tremor intensity in rhinoceros still needed to be investigated. As far as we know, this is the first study on the use of an objective method of measuring tremor

intensity in rhinoceros during chemical immobilisation. However, we found that the multidirectional accelerometer loggers, which have accelerometers that record movement in multidirectional planes, were more effective at detecting finer scale tremor movements than the omnidirectional loggers. In a previous study on hand tremors in humans; a good logarithmic relationship was determined between visual observations and activity data recorded by an accelerometer (Elble *et al*. 2006). These findings are similar to what we found when comparing the visual observation to the multidirectional logger in the rhinoceros in the field.

In this study in field animals, the rhinoceros ran before they were placed into recumbency. Exertion may have resulted in multiple physiological changes in the rhinoceros, including changes in blood electrolyte (Koeppen & Stanton 2000a) and glucose (Rose & Richter 2005) concentrations, which in turn may have caused tremors (Carithers 1995). In the field-immobilised rhinoceros, no significant change occurred in the blood calcium concentration. In endurance horses before and after the endurance ride, and also after a 30 minute resting period, their plasma calcium concentrations did not significantly differ (Rose *et al*. 1977). No relationship was observed in our rhinoceros between blood calcium and tremor intensity. Hypocalcaemia is known to cause tremors in dogs (Carithers 1995). The captured rhinoceros in our field study were not hypocalcaemic, but more likely hypercalcaemic (blood calcium = 1.06 ± 0.20 mmol/L; normal serum calcium = 0.66 ± 0.05 mmol/L; Miller & Buss 2012). Therefore, changes in calcium in our rhinoceros probably did not play a role in causing the tremors during immobilisation. Blood sodium concentrations (blood sodium = 132 ± 2.05 mmol/L) were similar to those previously reported as normal for rhinoceros (serum sodium = 134 ± 5 mmol/L; Miller & Buss 2012), while blood chloride concentrations (blood chloride = 99 ± 3.8 mmol/L) were slightly higher in our study compared to those reported as normal values (serum chloride = 95 ± 4 mmol/L; Miller & Buss 2012). Blood sodium and chloride concentrations did not change over the immobilisation period and were not related with the tremor intensity. Hyperkalaemia is also known to potentially cause tremors (Carithers 1995). Depending on the amount and intensity of exercise, blood potassium concentrations increase as a result of potassium being released from

skeletal muscle cells (Koeppen & Stanton 2000b). During high intensity exercise on treadmills, researchers (Harris & Snow 1988) determined that there was an increase in the plasma potassium concentration in horses. Once the exercise had been completed, there was a decrease in the plasma potassium concentration during the recovery period. In another study, when comparing plasma potassium samples of endurance horses before the ride to shortly after the ride, a group of researchers (Rose *et al.* 1977) determined that there was a significant increase in their plasma potassium concentrations. Thirty minutes after the endurance ride, the plasma potassium was still higher than before the ride, but lower than shortly after the endurance ride. The potassium concentration in men can decrease exponentially within a very short time period after intense exercise (Medbø & Sejersted 1990). As we were unable to measure the plasma potassium before and during the time period that the rhinoceros ran, we cannot show whether plasma potassium increases during exercise or the time it takes for it to decrease in rhinoceros. The blood potassium concentrations (3.7 ± 0.4 mmol/L) in our rhinoceros however, were lower than those reported as normal for rhinoceros (serum potassium = 4.7 ± 0.8 mmol/L; Miller & Buss 2012). Similarly to other studies (Rose *et al.* 1977; Medbø & Sejersted 1990), we found a decrease in the plasma potassium concentration, when comparing the time interval shortly after exercise (5 minute) to the 10 minute, 15 minute and 20 minute time points. In our study, potassium was the only electrolyte in the blood that had a significant positive relationship with the tremor intensity. However, we don't believe there was direct association between blood potassium and tremors as our rhinoceros were not hyperkalaemic; their blood potassium concentrations were lower than what is believed to be normal for this species (Miller & Buss 2012). This relationship may simply have been a reflection of the effect of the changes in blood pH, as blood pH was also correlated with tremor intensity, as it also strongly influences the movement of potassium in and out of the extracellular space, and hence influences blood potassium concentrations (Burnell *et al*.1956).

Electrolytes, specifically calcium and potassium, play an important role in the contractibility of the muscles (Clegg 2004). These electrolytes are also affected by a change in pH (Burnell *et al.* 1956), but only potassium had a significant relationship with

the pH in our study. The blood pH of the rhinoceros in the field was lower (pH range = 6.863 – 7.326) than that previously recorded for conscious rhinoceros at rest (7.341 – 7.346; Citino & Bush 2007). Although there was a strong correlation between blood pH and tremor intensity in the rhinoceros in the field, it is not clear from the data we collected whether this relationship arose primarily from a respiratory or metabolic acidosis, as neither $PaCO_2$ nor lactate were individually correlated with tremor intensity. It is likely that the cumulative effect of both respiratory and metabolic acidosis in the rhinoceros played a role in causing the correlation among blood pH, tremor intensity and catecholamine concentrations. When a stressor stimulates the sympathetic nervous system there is a release of catecholamines, which increases gluconeogenesis, and in turn results in an increase in blood glucose (Reeder & Kramer 2005). It has been reported that hypoglycaemia can result in tremors (Carithers 1995), while other researchers (Tan *et al*. 2006) determined that hyperglycaemia can cause tremors in humans, although its occurrence is rare. The rhinoceros in our study were not hypoglycaemic, but rather hyperglycaemic (blood glucose = 10.0 ± 1.86 mmol/L vs. normal blood glucose = 5.39 ± 2.17 mmol/L; Miller & Buss 2012), but no relationship occurred between the blood glucose concentrations and tremor intensity was observed in the rhinoceros.

Physiological changes are not the only variables that can affect muscle tremors. Opioids could possibly have a direct effect on muscles and therefore muscle tremors, as opioid receptors occur on skeletal muscles (Kurz & Sessler 2003). Tremors have been visually observed in previous studies on rhinoceros that were chemically immobilised with etorphine and azaperone (Radcliffe *et al.* 2000; Atkinson *et al.* 2002; Portas 2004; Fahlman 2008; Moreira 2010). A combination of etorphine and azaperone was used to chemically immobilise the rhinoceros in our study. Worth noting is that rhinoceros and the horse, of the order Perissodactyla, appear to be particularly prone to develop muscle tremors following opioid administration (Haigh 1990; Moreira 2010). Etorphine has been shown to be a potent agonist at all the opioid receptors, but could possibly have a more potent effect at the μ-receptor (Haigh 1990). During chemical immobilisation with etorphine, muscle tremors have been observed to occur in a variety

of rhinoceros (Haigh 1990; Bush *et al*. 2004; Portas 2004; Mentaberre *et al*. 2010; Moreira 2010). Rest tremors, which often resemble Parkinson's tremors, frequently occur after drugs that block dopamine receptors (Deuschl *et al.* 2001), such as metaclopramide, are administered (Morgan & Sethi 2005). Azaperone (a butyrophenone), which is a tranquilizing drug, exerts most of its effects by dopaminergic adrenergic blockage (Mentaberre *et al.* 2010). We found no correlation among the drug doses of etorphine and azaperone that we used and tremor intensity. This study was however not specifically designed to test for the effects of drugs on tremors. Therefore, further pharmacological studies are needed to adequately assess the role that drug-induced receptor activation or deactivation may play in immobilisation-induced tremors.

Based on pure observations, butorphanol, in combination with azaperone, has been reported to reduce chest rigidity in opioid immobilised captive white rhinoceros (Radcliffe *et al*. 2000), which may occur by the activation of receptors that are found on the membranes of different structures, including skeletal muscles (Kurz & Sessler 2003). Additionally, it was previously observed that a decrease in tremor intensity occurred when butorphanol was administered to white rhinoceros that had tremors during etorphine immobilisation (Moreira 2010; Burroughs *et al.* 2012b; M. Hofmeyr, personal communication 2013). Butorphanol, which is a partial opioid agonist/antagonist, is hypothesised to have both an agonistic and antagonistic effect on the μ-receptor and κ-receptor respectively (Radcliffe *et al*. 2000; Prado *et al*. 2008; Knych *et al*. 2012). Even though our findings did not support what has been previously observed, in that there was no difference in tremor intensity between the control and butorphanol only interventions, it should not be overlooked. It could be that the pharmacological effects of butorphanol may have contributed to the decreased tremor intensity that was recorded when the combination intervention of butorphanol and nasotracheal oxygen insufflation was administered. Drug doses did not appear to influence tremor intensity as there was no relationship between drug doses and tremor intensity. However, an increase in etorphine has been proposed to cause an increase in tremors (Heard *et al.* 1992; Kock *et al.* 1995), and if the activation of μ-receptors causes this effect, one can theorize that butorphanol would decrease tremors by partially

reversing the effects of etorphine on this receptor. However, with the data we are unable to clarify whether this theory is correct.

It has been shown that game-ranched rhinoceros that were hypoxic, hypercapnic and acidaemic after they were chemically immobilised with etorphine and azaperone, but once they received butorphanol, the PaO_2, $PaCO_2$, and pH improved (Boardman *et al*. 2014). A previous study hypothesised that butorphanol's effects on improving blood gases could be due to butorphanol decreasing muscle rigidity and metabolism (Miller *et al*. 2013). If this hypothesis is validated, it would provide the evidence that butorphanol has a direct effect on muscle fibres. This in turn could lead to possible evidence that muscle tremors are the result of the direct pharmacological action of etorphine. However, even though the tremors decreased when only butorphanol was administered, this decrease was not significantly different to the control, when the tremor intensity was recorded using the visual observations. Therefore, we believe that etorphine may possibly cause an indirect pharmacological effect that result in tremors. By using objective data loggers, future research is required to properly clarify these effects.

What is evident from this study is that the indirect effects of etorphine and azaperone are likely to influence the tremor intensity. Similar to both of the boma-managed and field captured rhinoceros in this study, rhinoceros in other studies, in which etorphine and azaperone were used and tremors were observed, also developed hypoxaemia, hypercapnia and acidaemia (Radcliffe *et al.* 2000; Atkinson *et al.* 2002; Portas 2004; Fahlman 2008; Moreira 2010). In healthy humans, finger tremors increased when subjects were exposed to hypoxic conditions (Kraus *et al.* 2000). In contrast, another study found that hypoxia inhibited shivering in hypothermic patients (De Witte & Sessler 2002). Since our rhinoceros were not hypothermic, it is unlikely that the tremors observed are related in any way to shivering and hence hypoxia did not reduce tremor intensity but rather exacerbated it. In another study on human patients, it has been reported that when hypoxia and hypercapnia were combined it resulted in an increase in tremor intensity, even higher than when compared to only hypoxic conditions (Kraus *et al.* 2000). If the tremor intensity in our rhinoceros had decreased without butorphanol,

but when oxygen was supplemented on its own, it would have better clarified that hypoxia is one of the primary causes of muscle tremors in chemically immobilised rhinoceros, but administration of oxygen only in our rhinoceros did correct hypoxaemia. An earlier study in rats, cats, ferrets and rabbits showed that a change in intra-cellular pH may have played an important role in reducing the tension (contraction) of cardiac muscle during acidosis. The change in pH can have a direct effect on the contractile proteins in muscle which results in a change in cardiac muscle tension and also the time between contractions and their magnitude (Allen & Orchard 1983). Our rhinoceros however had an increase in muscle contractions in the limbs during acidaemia. These differences between our study and that of Allen & Orchard (1983) may be due to the different types of muscle tissue and the mechanisms that stimulate their contractions. In a previous study in humans muscle pH significantly decreased as lactate and pyruvate concentrations increased (Sahlin *et al.* 1976). However, it has been hypothesised that the changes in muscle pH are not only determined by these metabolic by-products but also by the by-products of glycolysis and ATP hydrolysis (Robergs *et al*. 2004). These hypotheses may provide an explanation why the blood pH and tremor intensity were not associated with lactate concentration. Another study in rats showed that a lower intracellular pH can result in an increased uptake of calcium into the muscle cells (Fabiato & Fabiato 1978), but we found no relationship among the blood pH, tremor intensity and blood calcium concentrations. Although hypoxia and acidaemia have direct effects on muscle and its contraction we could find no clear description of a mechanism that could directly link hypoxia and acidaemia to muscle tremors. However, we believe that the relationships found in this study among tremor intensity and hypoxia and acidaemia are most likely the result of an indirect effect. We believe that this effect is most likely brought about by the activation of chemoreceptors (Berne & Levy 2000; Prabhakar 2000) with a resultant central nervous system response that results in tremors.

The relationship among PaO_2, $PaCO_2$ and catecholamines has been studied before, as well as the importance of both hypoxaemia and hypercapnia in the release of catecholamines into the circulatory system (Fishman 1976; Perry *et al*. 1989; Yates *et*

al. 2012). It has been shown that plasma adrenaline and noradrenaline concentrations increased when foetal sheep were exposed to hypoxaemia (Yates *et al.* 2012). Other vertebrates, including fish, dogs and turtles, also experience an increase in adrenaline and noradrenaline concentration during hypoxia (Rose *et al*. 1977; Perry *et al.* 1989; Wasser & Jackson 1991). Hypoxia has been found to stimulate the release of multiple neurotransmitters, including noradrenaline and acetylcholine (Prabhakar 2000; Solomon 2000). There are different pathways that hypoxia can stimulate the release of neurotransmitters, which still need to be fully investigated. These hypothesized pathways however include the direct and indirect activation of calcium and potassium channels on glomus cells, which are a type of chemoreceptor tissue (Prabhakar 2000). The relationship between the PaO_2 and adrenaline in our study suggests that hypoxia may be an important driving force for the high adrenaline and therefore the high tremor intensity. The relationships that we reported between tremor intensity and hypoxia as well as among hypoxia and plasma adrenaline and plasma noradrenaline in our rhinoceros are likely to be a result of an indirect relationship between these variables through the stimulation of the sympathetic nervous system. Hypercapnia leads to increased circulating hydrogen ions which results in acidosis (Fishman 1976). Chemoreceptors that are activated by a change in hydrogen concentration are found on the brainstem (Lassen 1990). Wasser and Jackson (1991) showed that different vertebrates, including fish (Perry *et al.* 1989) had elevated concentrations of catecholamines when they were acidaemic. A relationship between both the plasma adrenaline and plasma noradrenaline with blood pH was also found in our rhinoceros. We believe that this release of catecholamines in turn resulted in an increase in the tremor intensity (Frishman 2003; Cazzola & Matera 2012).

When a rhinoceros is interrupted from its daily habits as a result of a stressful stimulus, numerous neuroendocrine mechanisms are activated to retain cardiovascular homeostasis. At the same time the body needs to manage the large amount of blood flow to the muscles. This occurs when the parasympathetic control decreases and the sympathetic activity increases and before the rhinoceros even starts running. This response is known as the “fight or flight” response and results in a fast change in the

resting cardiovascular function (Moreira 2010; Fitzgerald 2012). A part of the response is the release of catecholamines and the redirection of blood from the non-essential organs to the skeletal muscular system (Frishman 2003; Moreira 2010; Fitzgerald 2012). Various types of physiological stressors such as hypoxia, hypercapnia and acidaemia can result in an increase of catecholamines in the circulatory system (Perry *et al.* 1989). A previous study determined that there was no direct activation of skeletal muscle fibres by the sympathetic nervous system. Any catecholamine related effect that does occur in the muscle is likely due to the diffusion of the circulating catecholamines or from the vascular system or from sympathetic neurons where adrenergic transmitters diffuse into the muscle fibres (Bowman 1981). An increase in catecholamine concentration in the blood has been associated with an increase in muscle tremors (Frishman 2003; Cazzola & Matera 2012). Another study, which was conducted on human patients that had pre-existing tremors due to Parkinson's or essential tremors, showed that the administration of additional adrenaline resulted in an increase in tremor intensity. They also found that the administration of additional noradrenaline had different effects, with the occasional increase, decrease or no change to tremor intensity (Marshall & Schnieden 1966). The relationship between tremor intensity and the plasma adrenaline and noradrenaline concentrations in our rhinoceros indicated that these two catecholamines may play an important role in determining the severity of the tremors.

The increased catecholamine concentration that initially occurred in the immobilised rhinoceros may have been from the psychological stress of the capture. However, the physiological stress, caused by drug-induced respiratory depression, could also have played a role in causing an increase in catecholamine concentrations, through the activation of different types of adrenergic receptors. There are different types (α- and β-) and sub-types (α_1-, α_2, β_1-, β_2- and β_3-) of adrenergic receptors throughout the body which are stimulated by adrenaline and noradrenaline (Fitzgerald 2012), and result in different effects. Adrenaline stimulates both α- and β-receptors to almost the same extent, while noradrenaline, which can also stimulate both types of receptors, but has a greater effect on the α-receptors (Guyton & Hall 1997; Glick 2010). Both horses (Yovich *et al*. 1984) and humans (Cazzola & Maltera 2012) develop tremors when they are

given β_2-receptor agonists. Frishman (2003) found that by administering a β-blocker the catecholamines ability to activate adrenergic receptors, which occurs throughout the body, is minimized. Specifically, β_2-blockers are able to decrease tremors in humans (Lakie 2010). Therefore it is plausible to consider that in chemically immobilised rhinoceros the activation of the sympathetic nervous system, by hypoxia and acidaemia, stimulates an increase in plasma catecholamine concentration, which could activate β_2-receptors and cause muscle tremors.

This study has shown that there are multiple variables that need to be taken into consideration when investigating tremor intensity in rhinoceros during chemical immobilisation. It would have been ideal to use the activity loggers in the boma study to better clarify the effects of the drugs on muscle tremors. However, due to a different pre-determined study design, the use of respective measurement equipment was not feasible. To determine whether the tremors occur directly at the level of the muscle fibre or due to a sympathetic nervous system response, one could potentially use electromyography (EMG) in a boma setting. It may also be beneficial to draw blood samples for catecholamine analysis in a boma setting, as the stress induced by the helicopter would have been eliminated, and therefore the effects physiological stressors, such as drug-induced hypoxia and acidaemia, could be better defined. Additionally, instead of administering the oxygen through nasotracheal insufflation, the oxygen could be administered by ventilating the rhinoceros to ensure that the arterial partial pressure of oxygen (PaO_2) returns to normoxia. It would also be possible to treat rhinoceros with bicarbonate to tease out the role that acidaemia plays in tremor intensity.

5.2 Conclusion

When rhinoceros are chemically immobilised they develop tremors. These tremors don't appear to be caused directly by the pharmacological effects of the immobilising drugs, but rather by their indirect effects which result in pathophysiology. The pathophysiological effects of the drugs which were most strongly associated with tremors were hypoxaemia and acidaemia. The relationship between tremor intensity and the degree of hypoxaemia and acidaemia can be explained by their activation of the sympathetic nervous system which results in an increase in plasma catecholamine concentrations, which appears to be the direct cause of the rhinoceros' tremors. The administration of the combination of butorphanol and nasotracheal oxygen insufflation corrected this hypoxaemia and acidaemia and abolished tremors during chemical immobilisation. Therefore, tremors can be effectively treated in immobilized rhinoceros if an animal's blood oxygen levels and pH are corrected, currently this can be achieved by administering butorphanol and nasotracheal oxygen insufflation. Lastly, if tremors occur during chemical immobilisation of white rhinoceros their intensity can be used to assess the extent of an animal's respiratory and metabolic compromise.

Addendum

Addendum A

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Tremors in the white rhinoceros *(Ceratotherium simum simum)* during chemical immobilization**
PROJECT NUMBER	**V087-13**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Ms. S de Lange**

STUDENT NUMBER (where applicable)	**280 310 84**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**White rhinoceros *(Ceratotherium simum simum)***	
NUMBER OF ANIMALS	**25**	
Approval period to use animals for research/testing purposes		**February 2014-December 2014**
SUPERVISOR	**Dr. L Meyer**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 25 November 2013
CHAIRMAN: UP Animal Ethics Committee	Signature

Addendum B

AESC 2012 M&E

Please note that only typewritten applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND

ANIMAL ETHICS SCREENING COMMITTEE
MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: Anna Haw

b. School and email address: Physiology

Anna.Haw@students.wits.ac.za

c. Experiment to be modified / extended

		AESC NO	
Original AESC number	2012	23	04
Other M&Es :			

d. Project Title: The use of partial-opioid antagonism combined with oxygen insufflation to support the cardiorespiratory physiology of the anaesthetized white rhinoceros (*Ceratotherium simum*)

		No.	Species
e.	Number and species of animals originally approved:	28	*White rhino*
f.	Number of additional animals previously allocated on M&Es:	0	
g.	Total number of animals allocated to the experiment to date:	28	White rhino
h.	Number of animals used to date:	8	White rhino

i. Specific modification / extension requested:
Addition of another principle researcher, Stephanie De Lange, to be added to the clearance
Additional venous blood samples to be taken (20 ml)
Additional 5 animals to be added to the total number of animals needed for the study

j. Motivation for modification / extension:
Stephanie is a new MSc student who will be using data from the measurements that are taken and have already been approved for my study. In addition, she will need some extra venous samples and therefore more venous blood will need to be drawn. Therefore, I would like to add her name to the list of researchers involved in this project and request that extra venous blood be allowed.
In addition, it has come to light that there is extensive variability in the field and therefore, in order to make sound conclusions, a further 5 animals may be required in order to increase the sample size.

Date: 07/02/2013 Signature:

RECOMMENDATION
Approved: addition of co-worker; additional blood samples and extra animals
Date: 14 February 2013

Signature:

Chairman, AESC

Addendum C

South African
NATIONAL PARKS

AGREEMENT

BETWEEN

SOUTH AFRICAN NATIONAL PARKS

herein represented by **Dr. Freek Venter**

in his capacity as **GM: Conservation Management**

(hereinafter referred to as “SANParks”)

AND

Ms. Anna Haw

Id/Reg No _____

(hereinafter referred to as "the Researcher" or any other person appointed by the Researcher and approved by SANParks)

WHEREAS the Researcher submitted a research application to SANParks to conduct a research on **"**The use of partial-opioid antagonism combined with oxygen insufflation to support the cardiorespiratory physiology of the anaesthetized white rhinoceros (*Ceratotherium simum*)**"** ("research") in the Kruger National Park ("the Park");

AND WHEREAS SANParks accepted the Researcher's application to conduct a research subject to the terms and conditions as stipulated hereunder:

THE PARTIES AGREE AS FOLLOWS

1. PERIOD OF AGREEMENT

1.1. This Agreement shall commence on the date of the last signature hereto and shall expire on 31 July 2015

1.2. Either party may terminate this agreement by giving the other party at least 2 (two) months written notice.

2. THE RESEARCH

Crucial to the conservation of this species is the ability to safely immobilize rhinoceroses for translocation, monitoring and anti-poaching procedures or medical interventions. While the potent opioid agonist, etorphine hydrochloride (M99®, Novartis), has proved extremely useful in the immobilization of large wild herbivores, the deleterious side-effects can result in potentially lethal complications. The white rhinoceros in particular, is extremely sensitive to etorphine-induced respiratory depression (Heard, Olsen & Stover 1992; Burroughs, Morkel, Kock, Meltzer & Hofmeyr 2006), with all rhinos suffering from some degree of hypoxia when under etorphine immobilization (Burroughs *et al*. 2006). This respiratory depression is dose dependent (Portas 2004) and therefore rhinoceroses immobilized in the field are generally more vulnerable to this side-effect as higher doses of etorphine are needed to shorten induction times.

Hypoxaemia, defined as abnormally low oxygen partial pressures in arterial blood (PaO_2), is therefore seen as one of the most significant and life-threatening side effects of potent narcotic anaesthetic agents in rhinoceroses (Heard *et al.* 1992; Rodgers 1993; Kock, Morkel, Atkinson & Foggin 1995). Severe hypoxaemia with PaO_2 values well below 60 mm Hg has been reported in captive as well as free-ranging white rhinoceroses anaesthetized with etorphine (Heard *et al*. 1992; Hattingh & Knox 1994; Bush, Raath, Grobler & Klein 2004).

Decreasing the risk of complications during white rhino immobilization and improving the ability to walk sedated rhino will benefit all those responsible for the care and management of these animals in national parks, zoological gardens and in game farm collections. Although a number of studies have already evaluated these parameters, this study is the first to critically evaluate a number of different interventions and the additional measurements may be of benefit and offer different options. Measuring cardiorespiratory parameters provides objective criteria to determine clear values for comparison under different conditions that will allow the investigators to provide recommendations for protocols that may provide additional benefits in reducing risk during immobilizations and enabling rhino to be safely walked into a crate.

3. THE RESEARCHER'S OBLIGATION

3.1. The Researcher acknowledges that he (assistance or team included) will work entirely at own risk.

3.2. The Researcher will obtain written permission from SANParks authorities to take out of the Park any specimens related to the research and restricted to the total number that will give sufficient results of the research. Furthermore, the researcher shall obtain any other necessary permits from relevant authorities for the transportation of specimens.

3.3. The Researcher shall sign both the agreement and the indemnity form before work can begin and shall ensure that all co-workers sign the indemnity form before coming to work in the Park.

3.4. The Researcher shall carry a signed copy of the research authorization when working in the Park.

3.5. The Researcher shall contact the SANParks Liaison to arrange their visit to the park, well in advance.

3.6. The Researcher shall adhere to tourist traveling times and park rules and regulations when doing fieldwork in the Park. Where sampling has to be done at night, the Researcher shall obtain relevant permission from SANParks authorities.

3.7. The Researcher shall complete and submit a Ranger Notification form to the Science Liaison well in advance if field work is planned.

3.8. The Researcher shall obtain approval from the relevant local ranger to camp at the sampling site, if necessary. This will be subject to weather conditions and other activities in the area.

3.9. Where necessary, the Researcher shall be accompanied by a game guard during their fieldwork within the park, and they will pay for use of game guard (including a daily fee, overtime and subsistence & travel costs).

3.10. Where the Researcher has obtained KNP Proficiency Certificate, he will be responsible for his own safety in the field.

3.11. The Researcher shall submit an annual report after a format has been sent to him.

3.12. The Researcher shall submit duplicate samples at the Skukuza Herbarium (if biological material is collected).

3.13. Within a reasonable time period after completion of the research, the Researcher will provide a well-organized documented electronic copy of raw data sets generated from this

study, with the prescribed metadata files (See Appendix 1) allowing SANParks to use data for further research purposes.

3.14. It is agreed between the parties that issues relating to benefit sharing of the proceeds of the Intellectual Property developed from the Research will be discussed as they arise, and appropriate sharing proportions will be formalized in addenda to this agreement.

3.15. The Researcher shall make available copies of publications, reports or theses arising from this study to the SANParks liaison.

3.16. The Researcher shall acknowledge SANParks staff in any publication ensuing from such data; in the case of significant assistance, due consideration to co-authorship should be given.

3.17. The Researcher shall not disclose the details of the Research Project to the Press, until it has provided SANParks with a copy of any proposed Press release. SANParks shall provide comment on any proposed release within 21 days of receipt. However, SANParks shall not have the right to prohibit academic publications.

4. PERMIT TO COLLECT NATURAL RESOURCES MATERIAL IN THE KRUGER NATIONAL PARK

In compliance with section 4(1) of the National Environmental Management: Protected Areas Act No. 57 of 2003, permission is hereby granted to the person named in this contract to collect natural resources material in the Kruger National Park

4.1 Blood may be collected, duplicate samples to the VWS Biobank

The researcher is responsible for obtaining any other permits required

5. OBLIGATIONS OF SANParks

5.1. SANParks shall afford the Researcher (and his assistant or team) free park entry.

5.2. SANParks shall provide discounted accommodation (when available) to the Researcher (and his assistant or team) at the research camps (Skukuza, Phalaborawa, Shingwediz, Twee Rivieren) while doing research work in the Park.

5.3. Any other accommodation required in the park for the purpose of sample collection will have to be booked and paid for by the researcher at normal tourist rates.

5.4. Where deemed necessary, SANParks shall provide a game guard to accompany the Researcher and his assistant (team) during field work, provided SANParks is notified well in advance and subject to availability.

5.5. Where required, SANParks will supply the Researcher with a SANParks vehicle decals (at a refundable cost of R100 per pair after approval) if fieldwork will be in view of tourists.

5.6. SANParks liaison shall initially inform in advance the local Rangers of the activities of the Researcher in their sections. However, it is very important that the researcher ***MUST*** contact the Section Ranger at least one day before they go out into the field.

5.7. Where available, SANParks shall provide basic laboratory facilities which shall not be exclusive to The Researcher.

5.8. Where no conflict of interest arises, SANParks shall make available existing datasets (including GIS data layers) subject to the Researcher signing a data user agreement form. These datasets should not be distributed to other parties. Some datasets (including lead-time and copyright protected datasets) will not be available to the Researcher.

6. BREACH OF AGREEMENT

6.1. Should any party commit a breach of any of the provisions of this Agreement and fail to remedy the breach within a period of 7 (seven) business days after receipt of the notice by the injured party to remedy the breach, the injured party shall at its discretion and without prejudice to any other rights be entitled to terminate the Agreement.

7. INDEMNITY

7.1. SANParks shall not be liable and the Researcher hereby indemnifies SANParks against liability for any claim for damages, loss or injury which the Researcher or any of his assistant (team) may suffer as a result of this Agreement.

8. AMENDMENT

8.1. This document constitutes the entire Agreement between two parties and no amendment thereof shall have any effect unless reduced to writing and signed by both parties.

8.2. No indulgence on the part of either party shall constitute a waiver of rights in terms of this Agreement.

8.3. The Researcher shall not be entitled to cede or assign this Agreement, nor in any other way transfer any of its rights or obligations under this Agreement.

9. DOMICILIUM CITANDI ET EXECUTANDI

9.1. The parties choose as their *domicilium citandi et executandi* for all purposes under this Agreement the following addresses:

SANParks	**The Researcher**
Manager: Legal Services	Anna Haw
643 Lleyds Street	University of Witwatersrand
MUCKLENEUK	Faculty of Health Sciences,
PRETORIA	School of Physiology
0001	WITS

Tel: (012) 426-5000 Tel +27 79 629 5439

Fax: (012) 343-0155 Fax 012 529 8304

9.2. Any notice or communication required or permitted to be given in terms of this Agreement shall be valid and effective only if in writing.

9.3. Either party may by written notice to the other party change the physical address chosen as its *domicilium citandi et executandi* to another physical address where postal delivery occurs, provided that the change shall become effective on the seventh business day from the deemed receipt of the notice by the other party.

9.4. Any notice to a party –

9.4.1. Sent by prepaid registered post (by airmail if appropriate) in a correctly addressed envelope to it at the address chosen as its *domicilium citandi et executandi* to which post is delivered shall be deemed to have been received on the fifth business day after posting (unless the contrary is proved);

9.4.2. Delivered by hand to a responsible person during ordinary business hours at the physical address at is *domicilium citandi et executandi* shall be deemed to have been received on the day of the delivery.

9.5. Notwithstanding anything to the contrary herein contained a written notice of communication actually received by a party shall be adequate written notice of communication to it notwithstanding that it was not sent to or delivered at its chosen *domicilium citandi et executandi*.

SANPARKS

SIGNED AT _____ ON THIS _____ DAY OF _____

_____

Dr. F. Venter

AS WITNESS

1. _____ 2. _____

RESEARCHER

SIGNED AT _____ ON THIS _____ DAY OF _____

_____

A. Haw

AS WITNESS

1. _____ 2. _____

Appendix 1 - Data and Metadata requirements

We are busy establishing a data catalogue that will be available through the internet. We have already added the KNP datasets and would like to add the research datasets as the projects are completed. For us to be able to do this efficiently could you please submit the original unprocessed data and metadata in the following way

General metadata required for the whole studies data:

1. The final report needs to be completed as requested.
2. Abstract for the dataset.
3. Geographic coverage. Area of the study needs to be stipulated e.g. Entire KNP or where you are working with transects the beginning and end point coordinates need to be given. If points are used then a GPS point for each should be given.
4. Temporal coverage. The dates that the data was collected
5. Keywords
6. Taxonomic coverage of the dataset. Please provide the genus and specie name of the individuals that were sampled in your dataset. This can be provided in a table format.
7. Data Usage rights. Enter a paragraph that describes the intended usage rights of the data. Specifically include any restrictions (scientific, technical, and/or ethical) to sharing your data within the public scientific domain. If your dataset is lead time protected please include the length of this period.
8. Access control .If you do want to restrict the dataset but have certain people that you would like to be able to access this data they should be mentioned here
9. Methods. The methods of the study should be discussed here. If you already have them in your project proposal please just copy and paste them.
10. People and organizations. Please supply the contact details of the people that you would like to be associated with the dataset and also the role that they played on the dataset e.g. metadata provider, principal investigator.

The metadata needed for each dataset is as follows

1. **GIS data and Imagery**
   Each shape file needs to be submitted with a FGDC xml metadata document that can be made via the metadata tool of Arc catalogue.

   Any imagery needs to be accompanied by a text file that indicates the level of processing of the image.

2. **Spreadsheet or column data**
   Excel spreadsheet and any other column data (e.g. Access tables) need to be exported as text files. For each column in the text file the following information is needed.

` 1. Column heading

   2. Column description

3. Type of variable i.e. numeric, date/time, enumerated (i.e. if you have codes you need to describe all the codes used. This description may be in another text file then just indicate that here.

4 Measurement unit e.g. mm, parts per million (ppm) etc.

5. Precision of the measurement i.e. if your measurements are in meters and your precision is 1 it means that your measurement is accurate to the nearest meter.

6. Bounds if the variable that you measured can only take on certain values stipulate them e.g. if a value can only be between 0 1 and 1 say min =0 max = 1.

This data and metadata need to be submitted to Judith.botha@sanparks.org. If your data does not fit in any of the above categories please contact judith.botha@sanparks.org for help.

References

Allen, D.G. & Orchard, C.H., 1983, 'The effects of changes of pH on intracellular calcium transients in mammalian cardiac muscle', *The Journal of Physiology* 335, 555-567.

Anderson, M.G. & Aitken, M.M., 1977, 'Biochemical and physiological effects of catecholamine administration in the horse', *Research in Veterinary Science* 22, 357-360.

Arbaizar, B., Gómez-Acebo, I. & Llorca, J, 2008, 'Postural induced-tremor in psychiatry', *Psychiatry and Clinical Neurosciences* 62, 638-645.

Atanasovska, T., Petersen, A.C., Rouffet, D.M., Billout, F., Ng, I. & McKenna, M.J., 2014, 'Plasma K^+ dynamics and implications during and following intense rowing exercise', *Journal of Applied Physiology* 117, 60-68.

Atkinson, M.W., Hull, B., Gandolf, A.R. & Blumer, E.S., 2002, 'Repeated chemical immobilization of a captive greater one-horned rhinoceros (*Rhinoceros unicornis*), using combinations of etorphine, detomidine, and ketamine', *Journal of Zoo and Wildlife Medicine* 33(2), 157-162.

Bain, P.G., 1998, 'Clinical measurement of tremor', *Movement Disorders* 13(3), 77-80.

Berne, R.M. & Levy, M.N., 2000, 'Cardiovascular system – Peripheral circulation and its control', in R.M. Berne & M.N. Levy (eds.), *Principles of Physiology*, pp. 256 – 264, Mosby, Missouri.

Boardman, W.S.J., Caraguel, C.G.B., Raath, J.P. & Van Zijll Langhout, M., 2014, 'Intravenous butorphanol improves cardiopulmonary parameters in game-ranged white

rhinoceros (*Ceratotherium simum*) immobilized with etorphine and azaperone', *Journal of Wildlife Disease* 50(4), 849-857.

Bowman, W.C., 1981, 'Effects of adrenergic activators and inhibitors on the skeletal muscles', in L. Szekeres (ed) Adrenergic activators and inhibitors, pp.47-128, Springer Berlin Heidelberg.

Burnell, J.M., Villamil, M.F., Uyeno, B.T. & Scribner, B,H., 1956, 'The effect in humans of extracellular pH change on the relationship between serum potassium concentration and intracellular potassium', *Journal of Clinical Investigation* 35(9), 935-939.

Burroughs, R., Meltzer, D. & Morkel, P. 2012a., 'Applied Pharmacology' in M.D. Kock and R. Burroughs (eds.), *Chemical and Physical restraint of wild animals: a training and field manual for African species*, pp. 53-80, International Wildlife Veterinary Services (Africa), South Africa.

Burroughs, R., Hofmeyr, M., Morkel, P., Kock, M.D., Kock, R. & Meltzer, D., 2012b, 'Chemical immobilization – Individual species requirements', in M.D. Kock and R. Burroughs (eds.), *Chemical and Physical restraint of wild animals: a training and field manual for African species*, pp. 143-264, International Wildlife Veterinary Services (Africa), South Africa.

Bush, M., Custer, R.S. & Smith, E.E., 1980, 'Use of dissociative anesthetics for the immobilization of captive bears: blood gas, hematology and biochemistry values', *Journal of Wildlife Diseases* 16(4), 481-489.

Bush, M. & De Vos, V., 1987, 'Observations on field immobilization of free-ranging giraffe (*Giraffa camelopardalis*) using carfentanil and xylazine', *The Journal of Zoo Animal Medicine* 18(4), 135-140.

Bush, M., Raath, J.P., Grobler, D. & Klein, L., 2004, 'Severe hypoxaemia in field-anaesthetised white rhinoceros (*Ceratotherium simum*) and effects of using tracheal insufflation of oxygen', *Journal of the South African Veterinary Association,* 75(2), 79-84.

Carithers, R.W., 1995, 'Shiver and Tremble' in S.J Ettinger & E.C. Feldman (eds.) *Textbook of Veterinary Internal Medicine*, pp. 143-145.

Catterall, W.A. 2011. Voltage-gated calcium channels. *Cold Spring Harbor Perspectives in Biology*, 3(8), 1-24.

Cazzola, M. & Matera, M.G., 2012, 'Tremor and β_2-adrenergic agents: Is it a real clinical problem?', *Pulmonary Pharmacology and Therapeutics* 25, 4-10.

Citino, S.B. & Bush, M., 2007, 'Reference cardiopulmonary physiologic parameters for standing, unrestrained white rhinoceros (*Ceratotherium simum*)', *Journal of Zoo and Wildlife Medicine* 38(3), 375-379.

Clegg, C.J. (ed), 2004, 'Support and movement', in *Introduction to advanced biology*, pp 304-320, John Murray, London.

Coetzee, D., 2006, 'The effects of a tyrosine hydroxylase activator on the monoamine metabolism in rabbits exposed to tobacco smoke', MSc dissertation, School of Pharmacy, University of the North-West, Potchefstroom, South Africa.

Culp, W.T.N., Mayhew, P.D. & Brown, D.C., 2009, 'The effect of laparoscopic versus open ovariectomy on postsurgical activity in small dogs', *Veterinary Surgery* 38, 811–817.

Dalvi, A. & Premkumar, A., 2011, 'Tremor: Etiology, Phenomenology and Clinical Features', *Disease-A-Month* 57(3), 109-126.

Deuschl, G., Raethjen, J., Lindemann, M. & Krack, P., 2001, 'The pathophysiology of tremors', *Muscle & Nerves* 24(6), 716-735.

De Villiers, A.S., Russell, V.A., Carstens, M.E., Aalbers, C., Gagiano, C.A., Chalton, D.O. & Taljaard, J.J.F., 1987, 'Noradrenergic function and hypothalamic-pituitary-adrenal axis activity in primary unipolar major depressive disorder,' *Psychiatry Research* 22(2), 127-140.

De Witte, J. & Sessler, D.I., 2002,'Perioperative Shivering', *Anestesiology* 96, 467-484.

Diaz, M. & Becker, D.E., 2010, 'Thermoregulation: Physiological and clinical consideration during sedation and general anesthesia', *Anesthesia progress* 57(1), 25-33.

Elble, R.J., Pullman, S.L., Matsumoto, J.Y., Raethjen, J., Deuschl, G. & Tinter, R., 2006, 'Tremor amplitude is logarithmically related to 4- and 5-point tremor rating scales', *Brain* 129(10), 2660-2666.

Evans, A.A.L., Tunnicliffe, G., Knights, P., Bailey, C.J. & Smith, M.E., 2001, 'Delta opioid receptor mediate glucose uptake in skeletal muscles of lean and obese-diabetic (ob/ob) mice', *Metabolism* 50(12), 1402-1408.

Fabiato, A. & Fabiato, F., 1978, 'Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles', *The Journal of Physiology* 276(1), 233-255.

Fahlman, A., 2008, 'Advances in wildlife immobilization and anaesthesia: Clinical and physiological evaluation in selected species', Ph.D thesis, Swedish University of Agricultural Sciences.

Findley, L.J., 1996, 'Classification of tremors', *Journal of Clinical Neurophysiology* 13(2), 122-132.

Fishman, A.P., 1976, 'Hypoxia on the pulmonary circulation. How and where it acts', *Circulation Research* 38(4), 221-231.

Fitzgerald, P.J., 2012, 'Beta-blockers, norepinephrine, and cancer: an epidemiological viewpoint', *Clinical Epidemiology* 4, 151-156.

Frishman, W.H., 2003, 'Beta-adrenergic blockers', *Circulation* 107(18), 117-119.

Foggin, C., Masterson, C. & Hoare, R., 2012, 'Legal and ethical considerations in the use of immobilizing drugs, using Zimbabwe and South Africa as examples', in M.D. Kock and R. Burroughs (eds), *Chemical and Physical restraint of wild animals: A training and field manual for African species*, pp. 1-11, International Wildlife Veterinary Services (Africa), South Africa.

Glick, D.B., 2010. 'The Autonomic Nervous System' in R.D. Miller, L.I. Eriksson, L.A.Fleisher, J.P. Wiener-Kronish & W.L. Young (eds.), *Miller's Anesthesia*, pp. 261-304, Philadelphia, Pennsylvania.

Grayson, J. & Oyebola, D.D.O., 1983, 'The effect of catecholamines on intestinal glucose and oxygen uptake in the dog', *The Journal of Physiology* 343(1), 311-322.

Guyton, A.C & Hall, J.E. (eds.), 1997, 'The autonomic nervous system; cerebral blood flow; and cerebrospinal fluid', in *Human Physiology and Mechanisms of Disease*, pp. 495-507, Sounders Company, Pennsylvania.

Haigh, J.C., 1990, 'Opioids in zoological medicine', *Journal of Zoo and Wildlife Medicine* 21(4) 391-413.

Harris, P. & Snow, D.H., 1988, 'The effects of high intensity exercise on the plasma concentration of lactate, potassium and other electrolytes', *Equine Veterinary Journal* 20(2), 109-113.

Haw, H., Hofmeyr, M., Fuller, A., Buss, P., Miller, M., Fleming, G. & Meyer, L., 2014, 'Butorphanol with oxygen insufflation corrects etorphine-induced hypoxaemia in chemically immobilized white rhinoceros (*Ceratotherium simum*)', *BMC Veterinary Research* 10(1), 253-261.

Hawker, S. & Waite, M. (eds.), 2007, 'Tremor' in *Oxford Paperback Dictionary and Thesaurus*. Oxford University Press. 2nd Edition, pp. 975.

Heard, D.J., Olsen, J.H. & Stover, J., 1992, 'Cardiopulmonary changes associated with chemical immobilization and recumbency in a white rhinoceros (*Ceratotherium simum*)', *Journal of Zoo and Wildlife Medicine* 23(2), 197-200.

Heil, D.P., 2006, 'Predicting Activity energy expenditure using the Actical® activity monitor', *Research Quarterly for Exercise and Sport* 77(1), 64-80.

Heistad, D.D. & Abboud, F.M., 1980, 'Dickinson W. Richards Lecture: Circulatory adjustments to hypoxia', *Circulation* 61(3), 463-470.

Hetem, R.S., Strauss, W.M., Fick, L.G., Maloney, S.K., Meyer, L.C.R., Shobrak, M., Fuller, A. & Mitchell, D., 2010, 'Variation in the daily rhythm of body temperature of free-living Arabian oryx (*Oryx leucoryx*): does water limitation drive heterothermy?', *Journal of Comparative Physiology B*, 180(7) 1111-1119.

Hetem, R.S., Strauss, W.M., Fick, L.G., Maloney, S.K., Meyer, L.C.R., Shobrak, M., Fuller, A. & Mitchell, D., 2011, 'Does size matter? Comparison of body temperature and activity of free living Arabian oryx (*Oryx leucoryx*) and the smaller Arabian sand gazelle

(*Gazella subgutturosa*) in the Saudi desert', *Journal of Comparative Physiology B*. 182(3), 437-449.

Hetem, R.S., Strauss, W.M., Fick, L.G., Maloney, S.K., Meyer, L.C.R., Fuller, A., Shobrak, M., & Mitchell, D., 2012, 'Selective brain cooling in Arabian oryx (*Oryx leucoryx*): a physiological mechanism for coping in aridity?', *The Journal of Experimental Biology* 215(22), 3917-3924.

Holzer, P., 2009, 'Opioid receptors in the gastrointestinal tract', *Regulatory Peptides* 155(1), 11-17.

Huizinga, J.D. & Lammers, W.J.E.P., 2009, 'Gut peristalsis is governed by a multitude of cooperation mechanisms', *American Journal of Physiology - Gastrointestinal and Liver Physiology* 296(1), 1-8.

Jeffers, L.A., 1986, 'The effects of acidosis on cardiovascular function', *Journal of the Association of Nurse Anesthetists* 54(2), 148-150.

Kania B.F., 1985, 'Neurochemical changes in the brain and spinal cord of sheep: a basis for the immobilizing action of etorphine', *Journal of the South African Veterinary Association* 56(2), 89-92.

Kanaya, N., Zakhary, D.R., Murray, P.A. & Damron, D.S., 1998, 'Differential effects of fentanyl and morphine on intracellular Ca^{2+} transients and contraction in rat ventricular myocytes', *Anesthesiology* 89(6), 1532-1542.

Knych, H.K., Casbeer, H.C., McKemie, D.S. & Arthur, R.M., 2012, 'Pharmacokinetics and pharmacodynamics of butorphanol following intravenous administration to the horse', *Journal of Veterinary Pharmacology and Therapeutics* 36(1), 21-30.

Kock, M.D., La Grange, M. & Du Toit, R., 1990a, 'Chemical immobilization of free-ranging black rhinoceros (*Diceros bicornis*) using combinations of etorphine (M99), fentanyl and xylazine', *Journal of Zoo and Wildlife Medicine* 21(2), 155-165.

Kock, M.D., Du Toit, R., Kock, N., Morton, D., Foggin, C. & Paul, B., 1990b, 'Effects of capture and translocation on biological parameter in free-ranging black rhinoceros (*Diceros bicornis*) in Zimbabwe', *Journal of Zoo and Wildlife Medicine* 21(5), 414-424.

Kock, M.D., 1992, 'Use of hyluronidase and increased etorphine (M99) doses to improve induction times and reduce capture-related stress in the chemical immobilization for the free-ranging black rhinoceros (*Diceros bicornis*) in Zimbabwe', *Journal of Zoo and Wildlife Medicine* 23(2), 181-188.

Kock, R.A. & Garnier, J., 1993, 'Veterinary management of the three species of rhinoceroses in zoological collections', *Rhinoceros biology and conservation, San Diego: San Diego Zoological Society,* 325-345.

Kock, M.D., Morkel, P., Atkinson, M. & Foggin, C., 1995, 'Chemical immobilization of free-ranging white rhinoceros (*Ceratotherium simum simum*) in Hwange and Matobo National Parks, Zimbabwe, using combinations of etorphine (M99), fentanyl, xylazine, and detomidine', *Journal of Zoo and Wildlife Medicine* 26(2), 207-219.

Koeppen, B.M. & Stanton, B.A., 2000a, 'Renal system – Potassium, Calcium and phosphate homeostasis', in R.M. Berne & M.N. Levy (eds.), *Principles of Physiology*, pp. 455 – 468, Mosby, Missouri.

Koeppen, B.M. & Stanton, B.A., 2000b, 'Renal system – Role of the kidneys in acid-base balance', in R.M. Berne & M.N. Levy (eds.), *Principles of Physiology*, pp. 469 – 481, Mosby, Missouri.

Kraus, W.L., Leiter, J.C., Tenney, S.M. & Daubenspeck, J.A., 2000, 'Acute hypoxia activates human 8-12 Hz physiological tremor', *Respiration Physiology* 123(1), 131-141.

Kugelberg, E., 1947, 'Electromyograms in muscular disorders', *Journal of Neurology, Neurosurgery and Psychiatry* 10(3), 122-133.

Kurz, A. & Sessler, D.I., 2003, Opioid-induced bowel dysfunction: Pathophysiology and Potential new therapies, *Drugs* 63(7), 649-671.

Lakie, M., 2010, 'The influence of muscle tremor on shooting performance', *Experimental Physiology* 95(3), 441-450.

Lascelles, B.D.X., Hansen, B.D., Thomson, A., Pierce, C.C., Boland, E. & Smith, E.S., 2008, 'Evaluation of a digitally integrated accelerometer-based activity monitor for the measurement of activity in cats', *Veterinary Anaesthesia and Analgesia* 35(2), 173–183.

Lascurain, A.A.G., Lopez, H.S., Steffey, E.P., Doherty, P.S. & Hernandez, E.N., 2006, 'The influence of butorphanol dose on characteristics of xylazine-butorphanol-propofol anesthesia in horses at altitude', *Veterinary Aneasthesia and Analgesia* 33(2), 104-110.

Lassen, N.A., 1990, 'Is central chemoreceptor sensitivity to intracellular rather than extracellular pH', *Clinical Physiology* 10(4), 311-319.

Lees, P. & Tavernor, W.D., 1970, 'Influence of halothane and catecholamines on heart rate and rhythm in the horse', *British Journal of Pharmacology* 39(1), 140-159.

Lesku, J.A., Meyer, L.C.R., Fuller, A., Maloney, S.K., Dell'Omo, G., Vyssotski, A.L. & Rattenborg, N.C., 2011, 'Ostriches sleep like platypuses', *PloS one* 6(8), 1-7.

Liang, B.T. & Gross, G.J. 1999. Direct preconditioning of cardiac myocytes via opioid receptors and K_{ATP} Channels *Journal of the American Heart Association: Circulation Research*. 84: 1396-1400.

Lin, J.Y., Hung, L.M., Lai, L.Y. & Wei, F.C., 2008, 'Kappa-opioid receptor agonist protects the microcirculation of skeletal muscle from ischemia reperfusion injury', *Annals of Plastic Surgery* 61(3), 330-336.

Moratinos, J. & Reverte, M., 1993, 'Effects of catecholamines on plasma potassium: the role of alpha- and beta-adrenoceptors', *Fundamental Clinical Pharmacology* 7(3-4), 143-153.

Marshall, J. & Schnieden, H., 1966, 'Effect of adrenaline, noradrenaline, atropine, and nicotine on some types of human tremor', *Journal of Neurology, Neurosurgery and Psychiatry* 29(3), 214-218.

McFarland, R., Hetem, R.S., Fuller, A., Mitchell, D. Henzi, S.P. & Barrett, L., 2013, 'Assessing the reliability of biologger techniques to measure activity in a free-ranging primate', *Animal Behaviour* 85(4), 861-866.

Medbø, J.I. & Sejersted, O.M., 1990, 'Plasma potassium changes with high intensity exercise', Journal of Physiology 421(1), 105-122.

Meltzer, D. & Kock, N., 2012, 'Stress and capture-related death', in M.D. Kock and R. Burroughs (eds.), *Chemical and physical restraint of wild animals: A training and field manual for African species,* pp. 81-88, International Wildlife Veterinary Services (Africa), South Africa.

Mentaberre, G., López-Olvera, J.R., Cases-DÍaz, E., Marco, I. & LavÍn, S., 2010, 'Haloperidol and azaperone in drive-net captured Southern Chamois (*Rupicapra pyrenaica*)', *Journal of Wildlife Diseases* 46(3), 923-928.

Miller, M.A. & Buss, P.E., 2012, 'Rhinoceridae (Rhinoceroses)', in R.E. Miller & M.E. Fowler (eds.), Zoo and Wild Animal Medicine, pp. 538-547, Elsevier – Saunders, Missouri.

Miller, M., Buss, P., Joubert, J., Mathebula, N., Kruger, M., Martin, L., Hofmeyr, M. & Olea-Popelka, F., 2013, 'Use of butorphanol during immobilization of free-ranging white rhinoceros (*Ceratotherium simum*)', *Journal of Zoo and Wildlife Medicine* 44(1), 55-61.

Mitchell, D., Maloney, S.K., Laburn, H.P., Knight, M.H., Kuhnen, G. & Jessen, C., 1997, 'Activity, blood temperature and brain temperature of free-ranging springbok', *Journal of Comparative Physiology B* 167(5), 335-343.

Moreira, L.P.R., 2010, 'Different drug cocktails in the immobilization of the white rhinoceros (*Ceratotherium simum simum*)', MSc thesis, University of Tras -os- Montes and Alto Douro.

Morgan, J.C. & Sethi, K.D., 2005, 'Drug-induced tremors', *The Lancet Neurology* 4(12), 866-876.

Morkel, P., 1994, 'Chemical immobilization of the black rhinoceros (*Diceros bicornis*)', *Proceedings of a Symposium on Rhinos as Game Ranch Animals,* Onderstepoort, 9 & 10 September, pp. 128-135.

Nagata, S., Takeda, F., Kurosawa, M., Mima, K., Hiraga, A., Kai, M. & Taya, K., 1999, 'Plasma adrenocorticotropin, cortisol and catecholamines response to various exercises', Equine Veterinary Journal 30, 570-574.

Owen, N.E., 1986, 'Effect of catecholamines on Na/H exchange in vascular smooth muscle cells', *The Journal of Cell Biology* 103(5), 2053-2060.

Papailiou, A., Sullivan, E. & Cameron, J.L., 2008, 'Behaviors in rhesus monkeys (*Macaca mulatta*) associated with activity counts measured by accelerometer', *American Journal of Primatology* 70(2), 185–190.

Paul, D.R., Kramer, M., Moshfegh, A.J., Baer, D.J. & Rumpler, W.V., 2007, 'Comparison of two different physical activity monitors', *BMC Medical Research Methodology* 7(1), 26-31.

Perry, S.F., Kinkead, R., Gallaugher, P. & Randall, D.J., 1989, 'Evidence that hypoxemia promotes catecholamine release during hypercapnic acidosis in rainbow trout (*Salmo gairdneri*)', *Respiration Physiology* 77(3), 351-364.

Portas, T.J., 2004, 'A review of drugs and techniques used for sedation and anaesthesia in captive rhinoceros species', *Australian Veterinary Journal* 82(9), 542-549.

Prabhakar, N.R., 2000, 'Oxygen sensing by the carotid body chemoreceptors', *Journal of Applied Physiology* 88(6), 2287-2295.

Prado, T.M., Doherty, T.J., Boggan, E.B., Airasmaa, H.M., Martin-Jimenez, T. & Rohrbach, B.W., 2008, 'Effects of acepromazine and butorphanol on tiletamine-zolazepam anesthesia in llamas', *American Journal of Veterinary Research* 69(2), 182-188.

Primmett, D.R.N., Randall, D.J., Mazeaud, M. & Boutilier, R.G., 1986, 'The role of catecholamines in erythrocyte pH regulation and oxygen transport in rainbow trout (*Salmo gairdneri*) during exercise', *Journal of Experimental Biology* 122(1), 139-148.

Raath, J.P., 1994, 'Anaesthesia of the white rhino', *Proceedings of a Symposium on Rhinos as Gave Ranch Animals,* Onderstepoort, 9 & 10 September, pp. 119-127.

Radcliffe, R.W., Ferrell, S.T. & Childs, S.E., 2000, 'Butorphanol and azaperone as a safe alternative for repeated chemical restraint in captive white rhinoceros (*Ceratotherium simum*)', *Journal of Zoo and Wildlife Medicine* 31(2), 196-200.

Radcliffe, R.W. & Morkel, P.vdB., 2008, 'Rhinoceroses' in G. West, D. Heard and N. Caulkett (eds) *Zoo animal and wildlife immobilization and anesthesia*, pp. 543–565.

Reeder, D.M. & Kramer, K.M., 2005, 'Stress in free-ranging mammals: integrating physiology, ecology, and natural history', *Journal of Mammalogy* 86(2), 225-235.

Robergs, R.A., Ghiasvan, F. & Parker, D., 2004, 'Biochemistry of exercise-induced metabolic acidosis', American Journal of Physiology – Regulatory, Integrative and Comparative Physiology 287(3), 502-516.

Rose, R.J., Purdue, R.A. & Hensley, W., 1977, 'Plasma biochemistry alterations in horses during an endurance ride', *Equine Veterinary Journal* 9(3), 122-126.

Rose, A.J. & Richter, E.A., 2005, 'Skeletal muscle glucose uptake during exercise: How is it regulated?', *Physiology* 20(4), 260-270.

Sahlin, K. Harris, R.C., Nylind, B. & Hultman, E., 1976, 'Lactate content and pH in muscle samples obtained after dynamic exercise', *Pflügers Archiv* 367(2) 143-149.

Sessler, D.I., 2011, 'Temperature Monitoring: Consequences and Prevention of mild perioperative hypothermia', *American Society of Anesthesiologists Annual Meeting*., 109-116.

Snow, D.H. & Machenzie, G., 1977, 'Some metabolic effects of maximal exercise in the horse and adaptations with training', *Equine Veterinary Journal* 9(3), 134-140.

Solomon, I.C., 2000, 'Excitation of phrenic and sympathetic output during acute hypoxia: contribution of medullary oxygen detectors', *Respiration Physiology* 121(2), 101-117.

Sternini, C., Patierno, S., Selmer, I.S. & Kirchgessner, A., 2004, 'The opioid system in the gastrointestinal tract', *Neurogastroenterology and Motility* 16(2), 3-16.

Storr, M., Geisler, F., Neuhuber, W.L., Schusdziarra, V. & Allescher, H.D., 2000, 'Endomorphin-1 and -2, endogenous ligands for the μ-opioid receptor, inhibit striated and smooth muscle contraction in the rat oesophagus', *Neurogastroenterology and Motility* 12(5), 441-448.

Tan, J.H., Chan, B.P.L., Wilder-Smith, E.P.& Ong, B.K.C., 2006, 'A unique case of reversible hyperglycemic Holmes' tremor', *Movement Disorders* 21(5), 70-709.

Taylor, S.H. & Meeran, M.K. 1973. Different effects of adrenergic beta-receptor blockade on heart rate response to mental stress, catecholamines, and exercise. *British Medical Journal* 4(5887), 257-259.

Ventura, C., Spurgeion, H., Lakatta, E.G., Guarnieri, C. & Capogrossi, M.C., 1992, 'Kappa and delta opioid receptor stimulation affects cardiac myocyte function and Ca^{2+} release from an intracellular pool in myocytes and neurons', *Circulation Research* 70(1), 66-81.

Wasser, J.S. & Jackson, D.C., 1991, 'Effects of anoxia and graded acidosis on the levels of circulating catecholamines in turtles', *Respiration Physiology* 84(3), 363-377.

Wenger, S., Boardman, W., Buss, P., Govender, D. & Foggin, C., 2007, 'The cardiopulmonary effects of etorphine, azaperone, detomidine and butorphanol in field anesthetized white rhinoceros (*Cerathotherium simum*)', *Journal of Zoo and Wildlife Medicine* 38(3), 380-387.

Williams, R.J., Marlin, D.J., Smith, N., Harris, R.C., Haresign, W. & Davis Morel, M.C., 2002, 'Effects of cool and hot humid environmental conditions on neuroendocrine responses of horses to treadmill exercise', *The Veterinary Journal* 164(1), 54-63.

Williams, S.H., Vinyard, C.J., Glander, K.E., Deffenbaugh, M., Teaford, M.F. & Thompson, C.L., 2008, 'Telemetry system for assessing jaw-muscle function in free-ranging primates', *International Journal of Primatology* 29(6), 1441-1453.

Yates, D.T., Macko, A.R., Chen, X., Green, A.S., Kelly, A.C., Anderson, M.J., Fowden, A.L. & Limesand, S.W., 2012, 'Hypoxaemia-induced catecholamine secretion from adrenal chromaffin cells inhibits glucose-stimulated hyperinsulinaemia in fetal sheep', *The Journal of Physiology* 590(21), 5439-5447.

Yovich, J.V., Horney, F.D. & Hardee, G.E., 1984, 'Pheochromocytoma in the horse and measurement of norepinephrine levels in horses', *The Canadian Veterinary Journal* 25(1), 21-25.

Zhang, Y., Chen, Q. & Yu, L.C., 2008, 'Morphine: A protective or destructive role in neurons?', *Neuroscientist* 14(6), 561-570.