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Different drug cocktails in the immobilization of the White Rhinoceros (*Ceratotherium simum simum*)

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Aos meus pais,

“The process by which an unsuspecting rhinoceros can be interrupted in its daily habits, attacked in the centre of the territory which it has painstakingly marked out with a trail of urine and faeces, rendered unconscious, bound and then airlifted to a distant wilderness, there to be released and encouraged to resume the daily grind, is swift, extremely expensive and unmarred by self-criticism as the building of the Ark.”

Marnham, 1980

ABSTRACT

The capture of wild free-ranging white rhinoceros has evolved greatly from the initial phencyclidine and gallamine captures in the early 50's to the more elaborate chemical immobilization protocols of today. Nevertheless, rhinoceros anesthesiology continues to be a subject in its development, and those who do it have the ongoing responsibility to explore better procedures that minimize the animal's distress and the risk of morbidity and mortality, thus getting, step by step closer to the concept of anesthesia and further from the concept of immobilization. Such is the objective of this thesis, through a pharmacological evaluation of the different drug cocktails used and the physiological interpretation of its outcomes upon the immobilized rhinoceros.

The eight rhinos used in this thesis were immobilized during a butorphanol course held at Tswalu Kalahari Game Reserve in August (20 and 21st) 2009. The animals were knocked down using five sets of different immobilization cocktails: etorphine plus butorphanol and midazolam, etorphine plus azaperone, medetomidine plus butorphanol, etorphine plus midazolam, and finally etorphine plus nalbuphine. Immobilization times varied between five and half minutes and twenty-five minutes. During the monitoring period, all but the rhino immobilized with medetomidine and butorphanol showed signs of different levels of hypoxia. Hypercapnia and acidosis, in turn, was also recorded in some of the immobilized rhinos. Finally, a good and uneventful recovery was achieved through the administration of naltrexone or naltrexone plus atipamezole in the rhino immobilized with butorphanol plus medetomidine.

In summary, this thesis not only provides a further understanding to the current knowledge of the different tolls that capture and anesthesia take in wild free-ranging white rhinoceros, but also provides useful information regarding the pharmacological activities of butorphanol.

RESUMO

A captura de rinocerontes selvagens tem evoluído bastante desde a fenciclidina e a galamina, fármacos utilizados para a sua captura nos anos 50, até aos elaborados protocolos utilizados actualmente. Estes, no entanto, continuam a ser um tema em desenvolvimento, havendo uma responsabilidade, de quem os pratica, de explorar melhores procedimentos que minimizem tanto o stress como risco de morbilidade e mortalidade dos animais. Ficando mais perto de alcançar o conceito de anestesia e não o de imobilização, propriamente dita. O objectivo desta tese é assim, a avaliação farmacológica das diferentes combinações anestésicas usadas e a interpretação de um ponto de vista fisiológico dos seus efeitos nos rinocerontes.

Nesta dissertação, os resultados foram obtidos da observação da captura de oito rinocerontes, tendo sido imobilizados no curso de butorfanol na Tswalu Kalahari Game Reserve em Agosto (dias 20 e 21) de 2009. Os animais foram anestesiados usando cinco combinações farmacológicas: etorfina com butorfanol e midazolam, etorfina com azaperona, medetomidina com butorfanol, etorfina com midazolam e finalmente etorfina com nalbufina. Os tempos de imobilização variaram de cinco minutos e meio até vinte e cinco minutos. Todos os animais imobilizados, excepto o rinoceronte imobilizado com a combinação de medetomidina com butorfanol, apresentaram sinais de hipoxia. Por outro lado, foi verificado em alguns rinocerontes hipercapnia e acidose. Finalmente foi alcançado uma boa recuperação anestésica, por parte dos rinocerontes, através da administração de naltrexona isoladamente ou em conjunto com atipamezole, quando a combinação farmacológica usada para a imobilização foi butorfanol com medetomidina.

Em sumário, este estudo teve como objectivo, não só facultar conhecimentos da forma de captura e das diferentes combinações farmacológicas utilizadas nos rinocerontes brancos selvagens, mas também fornecer informação útil em relação a actividade farmacologia do butorfanol.

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LIST OF ABBREVIATIONS AND ACRONYMS

% - Percent

ABG – Arterial Blood Gas

ANS – Autonomic Nervous System

ATP - Adenosine-5'-triphosphate

ATPase - Adenosine-5'-triphosphatase

BP – Blood Pressure

bpm – Beats per minute

bpm – Breaths per minute

BZ – Benzodiazepine

CBF – Cerebrospinal Fluid

CNS – Central Nervous System

D_2 - Dopamine Subtype 2

D_3 - Dopamine Subtype 3

D_4 – Dopamine Subtype 4

dexMDT - Dexmedetomidine

e.g. – Example

Fig. - Figure

FQ – Orpharin

GABA – Gamma Aminobutyric Acid

$GABA_A$ – Gama Aminobutyric Acid Receptor Subtype A

GPCR – G Protein Coupled Receptor

GVA – General Visceral Afferent

GVE – General Visceral Efferent

h – Hour

HR – Heart Rate

IM – Intra-muscular

IV – Intra-venous

K^+_{ATP} – Potassium Sensitive ATP

levoMDT - Levomedetomidine

M99 – Commercial name of etorphine hydrochloride

MBM – M99 Butorphanol Midazolam

mm Hg - Millimeter of Mercury

mmol/l – Milimolar per liter

N - Nociceptin

N/OFQ – Nociceptin/Orpharin

ND – Non Detectable

nm - Nanometer

NMDA – N-Methyl-D-Aspartate

NTS – Nucleus Tractus Solitarii

O_2Hb – Oxygenated Hemoglobin

O_2Sat – Oxygen Saturation

$P_{(a-A)}CO_2$ –Arterial Alveolar Carbon Dioxide Partial Pressure Diference

P_ACO_2 – Alveolar Carbon Dioxide Partial pressure

$PaCO_2$ – Arterial Carbon Dioxide Partial Pressure

PaO_2 – Arterial Oxygen Partial Pressure

PCV – Packed Cell Volume

$P_{Et}CO_2$ – End Tidal Carbon Dioxide Partial Pressure

pKa - Acid Dissociation Constant

PO_2 – Oxygen Partial Pressure

pulse-ox – Pulse Oxymeter

RBC – Red Blood Cell

RHb – Reduced Hemoglobin

RR – Respiratory Rate

SaO_2 – Arterial Oxygen Saturation

SBP – Systemic Blood Pressure

U/l – Units per liter

V_A/Q – Ventilation/Perfusion

α – Alpha adrenoreceptor

α_1 – Alpha Subtype 1

α_2 – Alpha Subtype 2

β – Beta

β_2 – Beta Subtype 2

δ - Delta

δ_1 – Delta Subtype 1

δ_2 – Delta Subtype 2

ε - Epsilon

ζ – Zeta

ι - Iota

κ - Kappa

κ_1 – Kappa Subtype 1

κ_2 – Kappa Subtype 2

κ_3 – Kappa Subtype 3

λ - Lambda

μ - Mu

μ_1 – Mu Subtype 1

μ_2 – Mu Subtype 2

μ_3 – Mu Subtype 3

μm - Micrometer

CHAPTER I – BIBLIOGRAPHIC REVIEW

1 – CONCEPTS

1.1 – Rhino capture and rhino conservation hand in hand

Rhinocerotidae are truly living fossils, a remnant of an archaic mammalian family represented only by five species in four genera restricted to Africa and Asia[6].

Fig.1 – Paraceratherium.

Represented by a far more expanded group of organisms than what exist today, rhinos once occupied a dominant place among organismic groups with over 150 fossil discovered by paleontology across four continents[7]. But the present day grew grim for the Rhinocerotidae family. With a marked decline from a time when Perissodactyla was an order spread over four continents with great numbers coupled with great diversity, to today, a time where four of the five rhinoceros's species are critically endangered because of poaching and loss of habitat[1-2]. Regardless of decline being due to normal natural climate and geographical changes or, most recently, to mankind (poaching), rhinoceros population numbers reached dangerous, near extinction numbers with only about 100 white rhinos in South Africa in 1929[8]. Nowadays South Africa is the last stronghold of white rhinos in Africa, already harboring more than 14,500 animals, in 2005[9]. Additionally, worldwide there is a collection of around 730 white rhinos in captivity[10].

In the early days, the words "capture operations" when applied to african rhinoceros were a synonym for ropes and a chase vehicle. For this reason, huge mortality rates, both on the animals as well as on the members of the capture team were normally a rule in those captures due to the huge stress and danger involved. Although dangerous to the operator and stressful to the animal, some teams in East Africa became remarkably proficient at this form of capture[11].

However, in the early 1960's physical capture began to fade away as a direct consequence to the usage of anesthetic drugs as a safer immobilization option. Chemical capture was first attempted using dissociative anesthetic, phencyclidine, and the curariform muscle relaxant, gallamine triethiodide[6, 8]. Although with better success rates than physical capture the use o phencyclidine still possessed serious side effects on the animal. That is why this drug also fell in disuse when modern synthetic opioids began to be manufactured. Initially, morphine and dimethylthiambutene (themalon) were the opioids of choice[12], quickly followed by

considerably more potent drugs: etorphine; fentanyl and carfentanil[6, 8]. Over the past 40 years, etorphine HCl (M99) became the standard opioid for capture of the African and Asian rhinoceroses[6]. As a result, current field anesthesia protocols of white rhinos nowadays are based on highly effective reversible opioid combinations.

Nevertheless, these immobilizations still come with an unwanted big risk to the animals due to the rhino's great sensitivity to opioids (e.g. respiratory depression; hypertension)[13-16] and particular anatomy. Additionally, the inability to evaluate the animal's exact weight and health status (nutrition, parasite load, infection, estrus, pregnancy, dehydration…) prior to the immobilization period makes it impossible to formulate an exact dosage with the different cocktail drugs in the immobilization dart. That is why anesthetic techniques must always and continuously be improved, both on efficacy and safety for the animals and human personnel involved. For now, field rhinoceros anesthesia still remains a dangerous endeavor only to be conducted by experienced personal that can cope with the many eventualities that accompany this animal's immobilization.

So on a retrospective analysis field anesthesia is what made possible the rhinoceros conservation success stories of the twentieth century[17, 18] and remains a critical tool for proactive rhinoceros management programs incorporating translocation, ear-notching, radio-telemetry, microchip implantation, and other techniques designed to secure the conservation of both African and Asian species[19, 20]. This, coupled with intensive management and anti-poaching laws, explains the incredible comeback of the specie from around 100 in 1929 to a estimated total population of southern white Rhinoceros or Square-lipped rhinoceros (*Ceratotherium simum simum)* of about 17,480 (wild-living animals at the end of 2007 according to IUCN 2008).

1.2 – Applied physiology in the capture of white rhinoceroses

When one wants to dart rhinoceros, one must consider many aspects: environmental, physical, anatomical, pharmacological, behavioral and ballistician.

In this section, several of these aspects that take a toll in the decision of what drugs to use in rhinoceros, and why, are going to be laid out.

- White rhinoceros are members of the Perissodactyla [21] order (odd-toed ungulates) and like the horse, zebra and donkeys, rhinos are hind-gut fermenting mammals[15, 22];

- At maturity, mean body mass varies strongly between sexes with about 2100kg for a bull (max 2400kg) and 1650kg for a cow (max 1800kg)[23]. On the other hand, mean shoulder height is fairly similar between bull (1,8m) and cow (1,77m)[24];.
- The skin of a white rhinoceros, although extremely sensitive, is incredibly rough and can have up to, or even more than 1cm thickness[24];
- Rhinos have very bad eyesight, and like many prey species, their eyes are on opposite sides of their heads, giving them binocular-like vision;
- These animals are not good swimmers, due to their inability to lift their head high, and therefore can drown easily[4];
- During the rainy season, when there is abundance of freely available water, white rhinos will drink twice a day (with an average need of 72 liters a day), however, in the dry season they can go up to four days without water[23];
- Although white rhinos feed mainly during the day, they are also active feeders at night with a feeding spectrum of grass and forbs (they do not browse like the black rhino)[4, 23];
- These are gregarious animals that usually occur in small family groups with a mean size of 2.3 animals constituted mainly by the cow and her calf plus a sub-adult (that can be either her previous calf or not), two cows[4, 23], or a group of sub-adults forming what is called the "club house"[4]. As a rule, adult bulls are generally solitary[4, 23];
- Although they are not seasonal breeders, having a peak in mating season scattered throughout the year, their normal calving peak season is in March and April[23]. With this in mind, and since the female normally has a gestation period of 16 months[24], if the female is sexually mature and found to be without an accompanying calf, or with an 18-month calf by its side, it should always be assumed that the cow is bearing a calf within[4]. Normal maturity age of a cow is 4-5 years, having therefore their first mating between 4,5-6 years and thus its first calf at 6-7 years[4];
- Finally, the life expectancy of an average white rhino is 35-40 years.

Field chemical capture of wild rhinoceros has been for several decades now, associated with various deleterious effects on the rhinoceros physiology[11, 13, 15, 23, 25]. However, in order to be able to properly evaluate the anesthetic status of the immobilized animal, it is necessary to have some reference ranges of cardiopulmonary parameters to which the obtained data can be compared[3].

Physiologic parameter	Mean	SE	Range	Min	Max	25%	75%
Heart rate (bpm)[a]	39	0.8	10	32	23	38	41
Respiratory rate (bpm)[b]	19	0.6	7	16	42	17	20
Indirect systolic pressure (mm Hg)[c]	135	2.7	34	123	157	128	140
Indirect systolic pressure (mm Hg)[d]	160	2.9	37	146	183	155	167
Indirect diastolic pressure (mm Hg)[c]	78	2.2	23	66	89	73	84
Indirect diastolic pressure (mm Hg)[d]	104	2.3	29	88	117	100	111
Indirect mean pressure (mm Hg)[c]	102	3.1	38	92	130	95	106
Indirect mean pressure (mm Hg)[d]	124	2.2	27	108	135	119	129
EtCO2 (mm Hg)	45.1	0.7	6.3	41.7	48.0	42.7	46.9
Rectal temperature (ºC)	36.8	0.1	0.6	36.6	37.2	36.7	37.0

[a] bpm, beats/min; [b] bpm, breaths/min; [c] Coccygeal blood pressures uncorrected to heart level; [d] Coccygeal blood pressures corrected to heart level.

Table 1. Reference physiologic data from 12 healthy, standing, unrestrained captive white rhinoceroses (*Ceratotherium simum*)[3].

Arterial blood parameter	Mean	SE	Range	Min.	Max.	25%	75%
pH	7.391	0.006	0.085	7.346	7.431	7.382	7.399
paO_2 (mm Hg)	98.2	1.3	18.4	90.2	108.6	95.9	100.2
$paCO_2$ (mm Hg)	49.0	0.8	9.3	44.4	53.7	46.7	50.7
Base excess (mmol/L)	3.5	0.4	4.0	1.9	5.9	2.3	4.9
HCO_3 (mmol/L)	29.3	0.4	4.9	27.3	32.2	28.4	30.1
SaO_2 (%)	97.2	0.1	1.4	96.6	98.0	96.9	97.4

Table 2. Reference arterial blood pH, paO_2, $paCO_2$, SaO_2, HCO_3, and base excess from 12 healthy, standing, unrestrained captive white rhinoceroses (*Ceratotherium simum*)[3]

Further hematological parameters can also be collected in order to obtain a better understanding regarding the general health status of the animal or the physiological implications of immobilization.

Parameter	Mean	Standard deviation	Range
Red cell count ($x10^{12}$/l)	6.17	0.49	5.6-6.96
Hemoglobin (g/l)	13.78	1.32	12.1-15.9
Packed cell volume	37.60	3.98	33.0-43.4
Mean corpuscular volume	61.00	5.26	55.0-70.9
Mean corpuscular hemoglobin (pg)	22.37	1.81	20.2-25.6
Mean corpuscular Hb conc. (g/dl)	36.80	0.75	35.4-38.0
Platelet count ($x10^{9}$/l)	483	152	255-696
White cell count ($x10^{9}$/l)	15.14	2.55	11.1-19.5
Neutrophils (%)	26.70	9.10	13.0-38.0
Lymphocytes (%)	61.20	10.70	48.0-79.0
Monocytes (%)	34.0	-	1-10
Eosinophils (%)	5.50	-	1-12
Basophils (%)	0.70	-	0-2

Table 3. Reference haematology parameters of white rhinoceroses (*Ceratotherium simum*)[4]

Component	Mean value	Standard deviation
Albumin g/l	26.1	3.7
Alanine transaminase U/l	8.6	3.7
Alkaline phosphatase U/l	127	33.2
Aspartate dehydrogenase U/l	40	14.6
Chlorine mmol/l	94.2	3.05
Creatine kinase U/l	48	14.1
Cortisol mmol/l	26.2	32.4
Gammaglutamyl transferase U/l	7.6	2.8
Lactate dehydrogenase U/l	526	126
Potassium mmol/l	5.4	2.6
Sodium mmol/l	129.6	4.2
Total proteins g/l	92.7	9.0

Table 4. Reference blood chemistry parameters of white rhinoceroses (*Ceratotherium simum*)[4]

Ever since rhinoceros have been darted and their immobilization periods analyzed and documented in an anesthetic point of view, respiratory depression (with hypoxia and hypercapnia) coupled with serious alterations of the cardiovascular system (e.g. severe hypertension, tachycardia), hyperthermia and acid-base imbalances have been an historical constant[26, 27]. However, little is still known regarding the rhino's physiology, and since these

animals are a part of the Perissodactyla order and a close relative to Equidae family, its members would be expected, up to a certain point, to experience the same adverse effects of recumbency and anesthesia as horses do[26]. As such, in this paper, the horse will become the model animal for cardiorespiratory physiological comparisons.

1.2.1 – Respiratory physiology

The rate and depth of respiration is modified by the nervous system from two different types of chemoreceptors: central or medullary and peripheral or arterial[28]. Central receptors respond to a reduction in pH, by increasing the respiratory rate, being it directly from an increase of hydrogen ions or indirectly by a boost in carbon dioxide[28]. This indirect mechanism consists on the CO_2 crossing the blood brain barrier, binding with water, forming HCO_3^- and consequently releasing H^+, therefore stimulating the central respiratory chemoreceptor to increase alveolar ventilation [28, 29]. On the other hand, a respiratory suppression can also be induced by these receptors in response to an increase in pH[28]. Regarding the peripheral receptors, these are also called arterial, for they are located in the carotid and aortic bodies and in contrast with the medullary receptors, are more sensitive to anoxia (low PaO_2) rather than hypercapnia (high $PaCO_2$) in arterial blood [28]. These, under normal circumstances, do not affect respiration since their actions are generally over-imposed by the central chemoreceptor control[28]. However, in horses, it has been reported that if PaO2 levels fall below 60 mmHg, the arterial chemoreceptors stimulation will be sufficient to induce an increase in respiration [28]. Smale and colleagues in a study[30] have indicated that in standard conditions a 60mmHg of PaO_2 in an equine is equivalent to a SaO_2 of 93%.

Respiratory depression, although normally associated with the drugs used in the procedure, is a result of innumerous factors that affect the animal during its immobilization. Hypoxia and hypercapnia can be pointed as a consequence of already existing pulmonary conditions such as tuberculosis[31-34], severe pulmonary emphysema[34, 35], respiratory located neoplasias[36], blood parasites such as babesiosis[34, 37, 38], trypanossomiasis[39, 40] and theileriosis[38] or other systemic pre-conditions (e.g. anemia[41, 42]) that during the immobilization period aggravate the drug induced respiratory impairment. In addition, several reports in horses have been made stating a pulmonary ventilation-perfusion mismatch[28, 43-45] that while in some cases can be position-related during general anesthesia[28, 45-47], in other circumstances can be exercise-induced[44, 48-50], having though in both cases a greater respiratory impairment as the outcome. And even though no report has been made proving this

V_A/Q mismatch happening in rhinoceros, the extensive similarities between these two animals would dictate so.

The degree in which a pulmonary ventilation-perfusion mismatch affects the level of hypoxia and hypercapnia in a field situation is very difficult to assess. It cannot be determined by the tidal volume or respiratory rate, but it can be reflected in blood gas values for PaO_2 and $PaCO_2$, should they be measured[28, 51]. Due to asymmetries in the airways and vascular geometry, and due to differences in ventilation and perfusion between the top and the bottom of the lung, innumerous V_A/Q ratios throughout the lung are going to exist[28, 52]. These ventilation-perfusion ratio values can vary from $V_A/Q=\infty$ (alveolar dead space) to $V_A/Q=0$ (shunt)[2, 51, 53], and although the normal overall mean ratio is in the order of 0,8, a $V_A/Q=1$ is the ideal ratio for gas exchanges between blood flow and alveolar ventilation [52, 54].

A non-normal immobilization derived low or equal to zero V_A/Q ratio situation consists of a continuous airway closure with alveolar collapse[43, 55] caused by an increase of pleural pressure gradient[56] resultant from a drug induced muscular relaxation that allows thoracic cavity compression by the abdominal contents[43, 45, 55, 56]. On the other hand, and possibly at the same time, in the non dependent areas of the lung, if pulmonary arterial pressure happens to be less than alveolar pressure, these vessels collapse and the alveoli that these vessels transverse will receive little or no blood flow ($\uparrow V_A/Q$ or $V_A/Q=+\infty$)[52, 57, 58]. It is mainly because of these events that recumbency has been so extensively associated with gas exchange impairments both in anesthetized equines[43, 45, 47, 58] as in immobilized rhinoceros[14, 26] However, it is important to mention that, although both circumstances lead to arterial hypercapnia and hypoxia, they do not do so in equal manner or extent. The $\downarrow V_A/Q$ regions in the lower respiratory system account for the areas that give origin to the right-to-left shunt where a fraction of the total blood does not exchange gases and thus enters the oxygenated blood downstream of the lungs with the same CO_2 and O_2 content of mixed venous blood[43, 45, 51, 54]. Regarding the pulmonary areas where the V_A/Q ratio is high or even at an infinity level, no blood perfusion of the affected alveoli is taking place[43, 51, 58] and therefore, in spontaneously breathing animals, without any oxygen supplement, the maintenance of the same tidal volume will result in a lower O_2 intake. Since the expired air is composed by a mixture of air originating from exchanging alveolar units and non-exchanging alveoli, as the physiological dead space grows, so does the gross underestimation of blood CO_2 by monitoring methods such as the Capnometer[2].

Some authors report that these respiratory impairment events start as early as 5 minutes after induction[56] and that the abdominally located weight has a pronounced effect on the efficacy of gas exchange[59] (with the food of non-starved animals, such as the captured ones in

this trial, contributing to the overall abdominal weight[46]. Further aspects to take into consideration are the possible correlations between body mass, shape of thorax and abdomen and/or animal's age in the degree of VA/Q mismatch. Schatzmann (1982), Stegman and Littlejohn (1987), Moens (1989) as well as Juliet and Eddie (2008) have shown that in the anesthetized horse a positive correlation between body mass and the degree of ventilation-perfusion mismatch exists[47, 57, 60, 61]. However, while Moens (1995) described abdominal shape as being a more relevant factor than body mass (with round-bellied horses suffering from more pronounced VA/Q mismatch, than flat bellied-horses)[59], Juliet and Eddie managed to associate both lines of thought by demonstrating that oxygenation during anesthesia takes place at better levels of efficiency when the thoracic dimension per unit body mass increases[47].

Finally although V_A/Q inequality has already been shown to increase with age, the magnitude of its effect is on average physiologically small and clinically not significant[62]. Nonetheless, increase of body mass during growth[47], age-related respiratory diseases[63, 64] and other age-associated potential processes have been described as possible causes leading to an impairment of gas transportation from blood to the alveoli and vice-versa.

As said before, the animal's position[43, 45, 46] and the level of exercise[44, 49] underwent by the animal also take part in the V_A/Q mismatch extent. Regarding animal positioning, during a capture operation it is not always possible to place the animal in an ideal position, and if enough space and manpower for animal manipulation exists, there is still no such thing as an immobilization sound position, but rather a less harmful immobilization position. Although several studies have shown that for large animals such as horses and rhinos sternal recumbency induces a higher level of shunt[29, 32] (with up to one third of the blood pressure able to pass through the lung without coming into contact with ventilated parenchyma[43]), others have established that sternal recumbency is responsible for a better level of oxygenation than lateral recumbency[14, 26, 53]. This may, perhaps, be due to the higher shift of the diaphragmatic outline that takes place with lateral recumbency in comparison to sternal recumbency[46].

A major objective driving free-ranging wildlife anesthesiology evolution is the everlasting need to reduce knock down periods in field capture operations. Although the main purpose of such an objective has been to reduce the incidence of capture myopathy that may arise from long periods of intensive exertion[14, 21, 65], other factors may also be in play. Hypoxemia, even though through processes not yet entirely understood, has also been associated with extenuating exercise[44, 48-50]. And although such event has not yet been described in rhinoceros, the fact that this condition has already been appraised in a number of species,

including humans and equines[49, 50, 66, 67] (never forgetting the existing similarities between rhinos and horses), makes it sufficiently relevant to be considered, even if only in capture scenarios with a long knock down time. Several articles have been published suggesting possible explanations towards this phenomenon [48-50, 66, 68]. The most defended theories are: 1) "relative" alveolar hypoventilation[68], 2) right-to-left shunt, 3) ventilation-perfusion mismatch and finally 4) diffusion limitation impeding alveolar-end capillary gas equilibration [48-50, 68]. In these studies, while the first possible explanation is in fact observed with exercise, through the continuous increase of $PaCO_2$, despite the accompanying significant increase of alveolar ventilation, this mechanism does not account for the entire decrease in arterial O_2 tension observed in exercising horses[68]. Regarding a presumed shunt involvement, although it has not yet been laid aside the possibility, several authors have already discarded this theory[48, 49, 66].

Because ventilation, during exercise, increases relatively more than what cardiac output does, the V_A/Q distribution is going to be shifted to a higher range of V_A/Q ratios[50] and as such does not explain arterial hypoxemia, for it should increase arterial PO_2 since it raises alveolar oxygen tensions[50]. However, other causes may lead to a degradation of V_A/Q. Considering the remaining offered explanation, this O_2 alveolar-capillary diffusion limitation is thought to be caused hemodynamically by a significant shortening of the transit time for blood in the pulmonary capillaries[51, 68, 69] and by the development of a transient interstitial edema in the lung[44, 49, 50], thus worsening the V_A/Q ratios[49]. Finally, and for emphasizing purposes only, these events based on current literature are nothing short of speculation once applied in rhinoceros.

1.2.2 – Cardiovascular physiology

The function of the cardiovascular system is to ensure that the tissues of the body are adequately supplied with blood flow in order to satisfy their requirements for oxygen and energy substrates while guaranteeing the removal of metabolic waste products[70].

The cardiovascular system includes not only an extremely complex pump, the heart, impelling blood through a widespread network of blood vessels, but is also comprised by an extensive neurological and endocrinal component that controls it. Although endowed with an enormous plasticity[71-73], the more relevant adaptive features of the cardiovascular system for this paper are the immediate ones arising from strenuous exercise and from short immobilization periods. These changes in the cardiovascular system are largely accomplished by the autonomic nervous system (ANS)[71] through the neural and humoral components of the sympathetic and

parasympathetic systems[71, 74-77]. Nevertheless, this pursuit for homeostasis is also enabled through ascending information from the body, via sensory neurons of the General Visceral Afferent (GVA) pathways, towards higher neurological control centers located in the hypothalamus, midbrain, pons and medulla[77, 78]. The GVA system is comprised, amongst many others, by baroreceptors and chemoreceptors[79, 80].

Baroreceptors are constituted by nervous endings of afferent fibers located in the adventitia of the carotid sinus and aortic arch, running along branches of the glosso-pharyngeal and vagus nerves, respectively[79, 81] and provide information regarding blood pressure. Chemoreceptors, as mentioned before, are divided into peripheral chemoreceptors of the aortic[80] and carotid bodies (that respond primarily to hypoxemia and arterial carbon dioxide tension (PCO2)) and into central chemoreceptors of the brain stem (that respond to hypercapnia) [80, 82-85]. After the information is integrated in the cardiac center within the *nucleus tractus solitarii* (NTS)[75, 78, 81, 83, 85-87], new commands are relayed through the general visceral efferent neurons (GVE) that enervate smooth muscle associated with blood vessels and visceral structures, glands, and cardiac muscle[81, 88, 89]. The NTS, mainly because of its connections with regions of the brain, such as the *area postrema* that do not have a blood brain barrier, becomes subjected to opioids, α-adrenergic agonists and other similar agents[81].

Furthermore as the disruptions in homeostasis require an integrative response at a multi-systemic organ level, other mechanisms regulating renal function and respiration are also going to have a profound effect on the cardiovascular system[75, 76].

Lastly, not only do these capture-induced cardiovascular changes start as early as the animal senses the helicopter searching, but also they do not proceed in an equal manner throughout the exercise and immobilization fractions of the capture, and as such are going to be divided into pre-immobilization and immobilization changes.

1.2.2.1 – Pre-immobilization cardiovascular changes

As the animal is disrupted from his customary daily habits by a stressful stimulus, several neuroendocrine mechanisms rise up in an attempt to maintain cardiovascular homeostasis while ensuring that the body is able to cope with the higher demand of blood flow by the working muscles throughout the following exercise[72].

This is achieved by a withdrawal from parasympathetic control and by an increase in sympathetic activity [73, 84, 90] that starts even before the animal begins to run[73, 91]. The

"fight or flight" response is thus invoked resulting in a swift and harsh alteration of resting cardiovascular functions[92].

Through ANS nervous endings in the sinoatrial-node and myocardium, the increase in neural sympathetic tone and the release of catecholamines (especially adrenaline and noradrenaline) coupled with a decrease in parasympathetic tone, has a positive chronotropic and inotropic activity upon the heart[70, 76, 81]. This initial cardiac rate and contractibility boost is coupled with prompt blood redirection from non-essential tissues to the musculoskeletal system through a dual action of the sympathetic system[81] that affects great arteries (aorta and pulmonary artery), arterioles, capillaries, venules, great veins and in the lymphatics[76, 81]. In other words, while neural stimulus in the vascular smooth muscle[81] lead to vasoconstriction in some tissues (such as the kidneys and gut[93]), a humoral control through the increase of circulating catecholamines causes a vasodilatation in skeletal muscle arteries and arterioles[73, 75]. Regardless of this, a persistent blood flow to the brain is still maintained[94]. Therefore an increase in cardiac preload therefore takes place, since these adjustments in vascular resistance create a greater return of blood from blood pools in the venules and great veins[72, 81]. An even further increase in cardiac preload then comes from the adrenergic stimulation[91, 95] (acting most likely upon α_1-adrenoreceptors[91, 96, 97] although some studies also refer an α_2-adrenoreceptor involvement[98]) over the spleen during this excitement phase causing it to contract and release its blood reserves[91, 99]. This superior preload then, through the Frank-Starling phenomenon, induces a higher contractibility in the cardiac muscle, thus increasing furthermore the stroke volume and finally the cardiac output[76, 99, 100]. The combined elevation of both cardiac output and vascular resistance results lastly in a rise of blood pressure (BP)[81, 84].

In summary, the initial rise in BP as well as heart rate can be related to a simultaneous parasympathetic withdrawal and sympathetic stimulation[72, 74, 75]. On the other hand, if longer periods of strenuous exercise are to be experienced, heart rate and blood pressure fall into more complex control mechanisms. The maintenance of exercise evokes high levels of blood pressure and heart rate during long periods of exercise[73]. Although, the reason behind the mentioned not yet fully understood, this is thought to occur due to a "functional resetting"[75, 101, 102] of the classical arterial baroreflex input–output that would, in resting conditions, lead to a return of cardiovascular parameters to normal ranges[81, 101]. Present studies[75, 101-103], though, speculate a neural feedback from somatic afferents, stimulated by muscle contraction that project to and activate a cascade of events in the NTS that ultimately result in local release of GABA wich selectively inhibits barosensitive neurons. These somatic afferents

are part of a group of peripheral chemoreceptors and it is by responding mainly to hypoxia[71] that they are activated and supposedly modulate the baroreflex response to be able to operate at higher blood pressures while at the same time directly exciting sympathetic pre-motor neurons in the rostral ventro-lateral medulla, thus causing a further increase in blood pressure and heart rate[83, 101, 102].

While these hemodynamic changes are brought up in order to comply with higher exercise-induced necessities, they can also be responsible for stirring up several unwanted physiological needs and processes that can further affect the immobilized animal.

An increase in systemic blood pressure is essential with the onset of exercise, for it determines blood flow towards the tissues[28], however, an exaggerated BP in pulmonary circulation has been reported to become more detrimental than beneficial due to an increase in pulmonary transmural pressure (intra-capillary minus perivascular –alveolar- pressures[69]). In several reports performed in horses while undergoing exercise[49, 50, 69], the exercise generated pulmonary hypertension has been shown to lead to hypoxia through the formation of interstitial pulmonary edema (low V_A/Q ratio), thus impairing gas exchanges. Hypoxia can even be further exacerbated if pulmonary transmural pressures reach high enough values capable of inducing capillary rupture[50, 68, 99, 104, 105]. Nevertheless, it must be noted that these studies were performed in racehorses and that equine alveolar capillary wall has been shown to be able to withstand higher transmural pressures (up to 100mmHg before rupture[99, 105, 106]) than any other studied animal[106, 107]. Although the reasons for this are not yet fully understood, and no studies in rhinoceros alveolar capillary wall resistance to different transmural pressures have yet been done, pulmonary hypertension leading to pulmonary hemorrhage in a rhino not only remains possible[108] but probably occurs at lower transmural pressures, since a rhinoceros is not an athlete bred animal. In elephants, for example, this same process has already been witnessed due to the use of powerful opioids such as etorphine (severe hypertensive side-effect) and is denominated “Pink foam syndrome”[109, 110].

An additional source of exercise induced arterial hypoxia can also be traced to the increase in pulmonary capillary transmural pressure. The increase in pulmonary BP causes the capillaries to distend (augments the diameter) and therefore reduces the vascular resistance in these capillaries[111]. This outcome, when coupled with a dramatical increase of cardiac output, results in an exaggerated blood velocity through alveolar capillaries[48, 51, 68], thus reducing the blood’s capacity to fully oxygenate[68]. In such a situation of short blood transit times through the alveolar capillaries, a lower PaO_2 and a higher $PaCO_2$ are to be expected, despite the significant increase in alveolar ventilation[69] (“relative hypoventilation”).

Oxygen delivery to the tissues depends not only upon cardiac output, but also upon arterial oxygen content, and for this the spleen is of paramount importance[73]. Although most rhino immobilization reports do not publish necessary data that would confirm a sympathetic-derived splanchnic contraction, such takes place in several species[91, 95, 98, 112, 113] including horses[73, 99, 114-117], and thus cannot be discarded as an invalid premise. In a report published by Richard A. Kock[118], PCV fluctuations in black rhinos were observed, but since the post-capture reduction in PCV could have been due to more than one cause, splanchnic resequestration was only mentioned as a probable cause. It has been shown that the equine spleen upon contraction is able to discharge several liters of red cells (12 L or even more) into circulation depending on the spleen's weight and on total blood volume[73]. A further study done in humans also suggests that the amount of erythrocytes release by the spleen is in close relation with the undergone exercise intensity[95] and hypoxic level[119]. Regardless of this, it is said that the equine spleen, at rest, stores around 50% of total red blood cells[95]. Therefore, the spleen contraction not only significantly raises the number of circulating erythrocytes[73], but also greatly enhances oxygen transport and improves aerobic performance[91, 95, 98, 113]. However, the increase of circulating red blood cells also greatly increases blood viscosity[73, 98, 99], and thus, depending on the hydration status of the animal and the actual amount of erythrocytes that are release to the blood stream, the increase of blood viscosity may or may not have undesired adverse effects. Amongst these, the possible contribution to augment pulmonary hypertension (as it has been reported in horses[73, 99]), systemic hypertension and finally an increase in cardiac workload[98] can be pointed out.

During exercise, heart rate, cardiac contractibility and arterial blood pressure, both systemic and pulmonary, increase[72, 73]. However, the rise in these variables and the degree into which they augment (according to the exercise intensity and duration) creates different increases in the myocardial needs. It is important to note that myocardial oxygen consumption increases whenever there is tachycardia and may increase as much as fivefold in the transition from rest to exercise[120, 121]. Furthermore, and to make matters worse the myocardium has a very limited anaerobic capacity[91, 121, 122]. As such, any increase in oxygen demand must be met by an increase in coronary blood flow[73, 121]. The dominant control of coronary blood flow is via local metabolic factors that match coronary blood flow to myocardial oxygen consumption[120]. Although the processes that lead to a coronary vasodilation and thus an increase in coronary blood flow has not yet been fully understood, the current postulated mechanisms include adenosine, K^{+}_{ATP} channels and the direct effects of O_2 and CO_2[91, 120-122]. It is currently speculated that hypoxia directly[122], or through adenosine liberation or

ATP-sensitive potassium channels[91, 121] leads to the relaxation of vascular smooth muscle and thus to an increase in vascular diameter and to a decrease in vascular resistance[91, 121].

Nevertheless, coronary blood vessels have a rich dual parasympathetic and sympathetic innervations[91, 120], and as such are still under adrenergic control, even throughout exercise[120-123]. While β-adrenoreceptor (mostly β_2 under the effect of noradrenaline[91]) stimulation contributes to arterial vasodilatation[121, 123], α-adrenoreceptor (mainly through α_1-receptor, as it has been shown in dogs[91]) causes vasoconstriction[120, 121]. However, they are not in direct competition, since vasodilation or vasoconstriction of a given vessel depends on its size[121] with coronary arteries > 100 μm undergoing vasoconstriction, while the smaller ones experience the opposite[91, 120, 121].

Regarding the larger coronary arteries that also dilate in response to exercise, the α-adrenergic control purely limits the extent of this dilation rather than causing an actual reduction in diameter[121]. The objective behind this α-adrenergic vasoconstriction is to preserve blood flow to the inner layers of the left ventricle during the cardiac cycle[120] by decreasing intra-myocardial vascular capacitance and wasteful antigrade-retrograde flow oscillations[122]. The special importance regarding left ventricular subendocardium vascularization lies on the combination of tremendous compressive forces generated during systole in this area throughout exercise and the considerable distance between subendocardium capillaries and the supplying artery[120]. As a result from this, the left ventricular subendocardium only receives blood flow during diastole (when compressive forces are minimal), thus being at risk whenever the heart experiences severe tachycardias[120]. This situation, however, has not yet been confirmed in rhinos, nor does it happen in horses since they possess no gradation of blood flow across the myocardial wall[73].

Exercise and anesthesia derived fluctuations in arterial PCO_2 and PO_2, besides being responsible for several modifications in the heart circulatory patterns, has also been reported to impose changes in cerebral hemodynamics in a wide variety of species [124-126]. However, at present, cerebral blood flow (CBF) in rhinos has not yet been quantified and as such the effects of drugs and ventilation on cerebral perfusion must be inferred through extrapolation from data in other species. Nevertheless, considering the rhinoceros long history of chemical capture induced respiratory depression with concomitant hypercapnia and hypoxia[16, 26, 65], an increase in CBF could be considered the animal's homeostatic response to avoid cerebral ischemia and thus brain damage. Although recent studies indicate hypoxia as being a decisive factor in increasing CBF[94, 126], CO_2 remains still the most powerful stimulant of CBF [94, 124, 125], with even small increases in PCO_2 (on the order of 5 to 6mmHg) capable of

generating appreciable changes on perfusion[127]. Through CO_2 stimulation of central chemoreceptors, hypercapnia induces a cerebral vasodilation of smaller capillaries (through the activation of K^+_{ATP} channels in vascular smooth muscle[127]) that in turn, by reducing overall vascular resistance, increases blood velocity and thus cerebral blood flow[126, 128, 129]. An increase in CBF then augments CO_2 diffusion from the cerebrospinal fluid and the brain extracellular fluid to the cerebral vessels while enhancing cerebral oxygenation[94], hence avoiding ischemic damages to cerebral tissue.

1.2.2.2 – Immobilization cardiovascular changes

During this period, the cardiovascular parameters will become a dynamic reflection of the several interfering elements described. Hypoxia, hypercapnia and blood pH, as stated previously, are going to have a very determinant say in the different cardiovascular (and respiratory) parameters witnessed in the immobilized rhino, mainly due to the already described exercise pressor reflex[102] (or muscle metaboreflex[71]).

1.2.3 – Acid-base physiology

A detailed analysis of acid–base balance provides a biochemical and physicochemical description of the state of the organism, or of individual organs and tissues within the body[130]. Furthermore, severe acid–base disturbances are often associated with high-intensity or prolonged-duration exercise[130], with respiratory drug, exercise or pathological impairments[131], and finally, hyperthermia[132]. Therefore, an understanding of the origins of acid–base disturbances is of interest to both basic and clinical physiologists[130].

Regarding metabolic acidosis, it has become common knowledge that high-intensity muscle contraction causes intracellular acidification that, in turn, generates an extracellular systemic acidosis in the whole organism[130]. The mechanism, in which the stated takes place, has however been the theme of innumerous discussions in the past few years. It was, for a long time, widely acknowledged that an increase in mechanical load past the anaerobic threshold would result in an increase in lactic acid generation, since the oxygen demand by the muscle would exceed its supply and the muscle cells would, in turn, switch to anaerobic respiration so that they could maintain their function[133-136]. Lactic acid then, through the release of a proton (H^+), to convert into acid salt lactate, would therefore be the culprit of metabolic acidosis (lactic acidosis)[137]. However, a recent study presented by Robergs and colleagues[137]

contradicts the lactic acidosis conjecture by stating that metabolic acidosis is in fact the consequence of an increased reliance on non-mitochondrial ATP turnover that occurs when the rate of ATP hydrolysis, and therefore the rate of ATP demand, exceeds the rate at which ATP is produced in the mitochondria. In this new conception, lactate production supports continued ATP regeneration through glycolysis, consumes protons (retarding acidosis), and finally, also facilitates proton removal from muscle[136-138]. Regardless of the true acidosis origin, the end result still remains the same, being it a drop of pH resultant from a imbalance between the high proton (H^+) release and the body's proton buffering and removal capacity[134, 137, 139]. In addition, the metabolic increase in PCO_2 further aggravates metabolic acidosis due to the reaction with bicarbonate buffer through the following equation $H^+ + HCO_3^- \rightarrow H_2O + CO_2$ [130, 134, 137-139]. Respiratory acidosis, on the other hand, develops when alveolar capacity to eliminate CO_2 decreases, thus increasing PCO_2 level and lowering systemic pH[28, 140, 141].

Whether acidosis is respiratory or metabolically derived, it nevertheless causes, amongst many detrimental alterations, one very important immediate physiological change that helps the animal to cope with hypoxic and hypercapnic conditions. This physiological change consists on a pH dependent reduction in the affinity of hemoglobin for oxygen (Bohr effect) that immediately follows the development of acidosis[142-144]. Hemoglobin, then, by having a decreased affinity for oxygen, enhances tissue oxygenation, since it gives up oxygen more easily during the microcirculation[143, 145].

One electrolytic imbalance that was previously associated with acidosis is hyperkalemia. It was believed that in acidemic conditions the higher circulating H^+ would cause a cellular exchange of intra-cellular K^+ with the extra-cellular H^+ [28, 146-148], thus causing hyperkalemia. However, recent reports[149, 150] have proven that the increased circulating potassium occurs immediately following exercise and is attributed to both net water losses from plasma and rapid net release of K^+ from contracting muscle cells in proportion to the exercise intensity. Nonetheless, there is no doubt that severe hyperkalemia can be fatal[28, 151]. The ratio of extracellular to intracellular potassium concentration largely determines the cell membrane resting electrical potential and in turn regulates the function of excitable tissues (cardiac and skeletal muscle, and nerve)[151]. Even a small absolute change in the extracellular K^+ concentration will have large effects on that ratio, and consequently on the function of excitable tissues [151]. In other words, hyperkalemia affects the excitation conduction system of the heart, hence it being associated with risks of severe arrhythmia[152] and even death through cardiac function suppression[28]. Furthermore, high potassium may result in paraesthesias and weakness progressing to a flaccid paralysis that typically spares the diaphragm[151]. However, circulating

levels of K^+ upon cessation of exercise quickly return to normal due to high rates of Na^+/K^+-ATPase activity in the previously contracting muscles, high intramuscular Na^+ concentration and elevated levels of circulating catecholamines[150].

1.2.4 – Temperature physiology

Hyperthermia in rhinoceros during chemical immobilization has been the subject of concern for the past few decades, with recorded temperature sometimes reaching levels higher than 41ºC[16]. The reason for this being the increase in muscular workload experienced throughout the capture process, either initially because of the fleeing attempt or secondarily by a lack of an adequate muscular relaxation. Regardless of the reason, muscular contraction causes a marked increase in metabolic rate (can be 20 fold the resting value[153]) that in turn leads to an elevation of body temperature[154], since approximately 80% of the energy produced through the ATP pathways is liberated as heat[153]. Furthermore, considering that the temperature regulatory center is located in the hypothalamus[153, 155, 156], any drug used in the knock out cocktail that may suppress its functions directly impairs the animal's ability to regulate body temperature, thus increasing its predisposition to both hypothermia or, most commonly, hyperthermia[28]. This increase in body temperature then can be accompanied by either detrimental or beneficial physiological responses in the animal.

On the one hand, hyperthermia increases metabolic activity and cellular oxygen consumption[15, 157] (10% for each ºC degree in human)[28]. In mammals at body temperatures above 41ºC, oxygen consumption surpasses oxygen supply, in normal ventilation, initiating hypoxic cellular damage with brain, kidneys, liver[28] spleen, stomach and intestines being the most susceptible[158]. In addition, hyperthermia also redirects blood supply from the viscera to the skin in an attempt to dissipate heat (through sweat)[153, 155, 159-161] leading to a drop in central venous pressure predisposing the animal to hypotension, hypovolemic shock and organ shutdown due to loss of perfusion[28]. However a few current reports[162, 163] defend an override of thermoregulation demands in short-term high-intensity exercise, by showing a redistribution of blood away from the skin to the exercising muscle in order to maintain work output, thus increasing heat storage. Additionally, the evaporation of sweat leads to dehydration, electrolyte imbalance (mainly due to Na^+, Cl^- losses in the case of equine sweat[164]) and to hemoconcentration[28]. Ultimately, hyperthermia may affect the CNS (cerebral anoxia) causing convulsions episodes, collapse rapidly followed by death if core temperature rises to and remains at 42 or 43ºC[16, 28].

With this in mind and considering that a high gradient between body temperature and ambient temperature means a greater rate of heat loss[28, 155], captures should not be carried out at temperatures over 25ºC[13, 15, 28].

On the other hand, an increase in body temperature heightens the peripheral chemoreceptors sensitivity towards O_2 and the central chemoreceptors sensitivity towards CO_2, leading to a reduction in cardiorespiratory response time in the face of hypoxia and hypercapnia[29]. In addition, the ascension in core temperature is accompanied by a right shift in the oxyhemoglobin dissociation curve causing a reduction in the O_2 affinity for hemoglobin and therefore an increase in oxygen extraction from the blood[28, 29, 153, 156, 165].

During capture, body temperature is normally assessed in the animal's rectum due to its straightforward accessibility. Besides providing real time information regarding the animal status under chemical restraint, the collected temperature values allow blood-gas adjustment based on established dissociation constants that vary with temperature[166]. However, it must be noted that a body temperature distribution inequality exists during exercise, with muscle temperature normally reaching the highest values followed by venous, rectal and finally skin temperature[166]. Also of great importance is the notion that although the rate of increase of the core temperature is mirrored by the rate of increase of the deep rectal temperature, the changes in rectal temperature characteristically lag behind core temperature and as such seldom represent the present fluctuating temperature[159, 166]. Therefore, the blood gas temperature adjustments based on rectal temperatures may lead to under or overestimation of blood gas parameters[166].

1.3 – Applied pharmacology

1.3.1 – Opioids

There are three classes of opioid receptors that have been extensively studied in current literature (μ, δ and κ)[167-169]. These are normally stimulated by endogenous opioid peptides, falling themselves also into three distinct families: *enkephalins*, *endorphins* and *dynorphins[167]*. New developments, though, suggest the existence of a fourth opioid receptor group named N/OFQ, stimulated by a peptide called nociceptin (N) by a group of researchers or orpharin (OFQ) by another group[167]. Furthermore, based on several assays, other subtypes have been proposed such as: **ε**[170], **ι**[171], **λ**[172] and **ζ**[173]. Each major opioid receptor has a specific physiological response and anatomical distribution in the brain, spinal cord and periphery.

Receptor	Subtypes	Location	Function
Mu (μ)	**μ_1, μ_2, μ_3**	• Brain -Cortex (laminae III and IV) -Thalamus -Striosomes -Periaqueductal gray • Spinal cord -*Substantia gelatinosa* -Intestinal tract	• Spinal and supraspinal analgesia; • Sedation; • Immunomodulation; • Physical dependence; • Respiratory depression; • Miosis/midriasis; • Euphoria; • Reduced GI motility; • Nausea/vomiting;
Kappa (κ)	**κ_1, κ_2, κ_3**	• Brain -Hypothalamus -Periaqueductal gray -*Claustrum* • Spinal cord -*Substantia gelatinosa*	• Spinal and supraspinal analgesia; • Sedation; • Miosis/midriasis; • Inhibition of ADH release;
Delta (δ)	**δ_1, δ_2**	• Brain -*Pontine nuclei* -Amygdala -Olfactory bulbs -Deep cortex	• Spinal and supraspinal analgesia; • Antidepressant effects; • Physical dependence; • Increase appetite; • Immunomodulation.

Table 5. Opioid-receptors locations and functions

The N/OFQ system, is mainly involved in a complex behavioral profile, including effects on drug reward and reinforcement, stress responsiveness, feeding behavior, interplay with the stress system, learning and memory process[167].

Opioids are characterized by the type of interaction with the opioid receptor(s) with which they interact and by the effect elicited upon binding. Opioids may be full agonists, partial agonists, antagonists and combinations thereof. The mode in which a full agonist opioid acts is by creating a dose dependent effect upon the activated opioid-receptor that eventually plateaus in unconsciousness and analgesia[168]. On the other hand, a partial agonistic binds to the opiate receptor only to plateau at a submaximum response (lower than an agonist) regardless of an increase of dosage[168]. Finally, an opioid antagonist competes for the binding site in the opioid receptor, hence causing the displacement of the agonists, not resulting in an activation of the receptor. They are considered for this to be competitive antagonists[168].

Despite the different anatomical spread and physiological response elicited upon stimulation, all four opioid-receptor types belong to the GPCR family[167]. As such, when an opioid agonist binds with one of the respective(s) μ, κ or δ-receptor(s) it activates both G-coupled proteins (which inhibit adenylyl cyclase) and receptor linked K^+ ion channels while suppressing voltage gated Ca^{2+} channels[167, 168, 174]. The current theories then defend that the K^+ current activation coupled with the Ca^{2+} influx suppression leads to a hyperpolarization of the membrane potential, thus explaining the opioid blockage of neurotransmitter release and pain transmition in various neuronal pathways[167, 168].

1.3.1.1 – Etorphine

Etorphine is a semi-synthetic, opioid-derivative, non-selective full agonist, first prepared in 1963 by Bentley & Hardy[167, 168, 175, 176]. Several thousand times the potency of morphine[13, 167, 168, 177-180], etorphine coupled with the development of reliable dart delivery systems revolutionized the ability of biologists and veterinarians to safely capture and restrain many species that previously could not be handled.

Extensively used in wild and free-ranging animals[181, 182], etorphine is currently one of the most powerful opioid derivatives[13, 167-169, 180, 182], mainly due to the strong agonistic inhibitory action it simultaneously produces upon all μ, δ and κ-opioid receptors[167, 168, 178, 180, 183-185] and its extremely high lipid solubility[13, 168, 181] (300 times that of morphine[181]) that enables it to be rapidly absorbed through transdermal, transmucosal, subcutaneous and intramuscular routes (by order of speed of absorption, from the lowest to the highest) and distributed to the more perfused organs such as CNS (quickly crossing the blood-brain barrier)[186]. In addition, the molecular structure of etorphine, by causing it to have around 20 times the affinity of morphine towards the opioid receptor, further increases its potency[181]. Current studies[187-189] even postulate that the high potency of etorphine results also from its involvement in yet another opioid receptor: ε-opioid receptor. However, the very notion that these studies have only been performed in laboratory mice and that significant inter-species opioid-receptor differences exist[190-194] makes any ε-opioid receptor related conclusions in rhinoceros currently almost impossible and extremely precarious to make.

Etorphine, by being one of the most potent opioid available, carries a high associated risk of complications, especially considering that the relative μ:δ:κ-opioid receptors affinity ratio of etorphine is of 10:2:1[195]. The more severe side effects of etorphine use can include hyperexcitation upon administration, hyperthermia, exertional myopathy, severe respiratory

depression, muscular tremors, tachycardia, hypertension and finally death [13, 14, 24, 25, 108, 169, 177, 179-182, 196-199].

Induction time, as a relative pharmacokinetic measurement of the velocity in which etorphine is absorbed through the darted muscle and distributed across the blood-brain barrier into the CNS, remains a hard and inexact value to come across with. However, some authors[13, 180, 199] suggest that after a successful IM administration of etorphine, induction should take place in a matter of 3-10 minutes. Then again, if this is a sign of fast distribution of etorphine, due to its high liposolubility, towards the CNS, a swift redistribution from the CNS to other tissues (such as muscle and fat) based on the same principle, should also be expected. Such, however, is not the case with registered periods of immobilization in non-reversed rhinos of up to 7 hours[181].

With peak plasma levels, normally occurring at 15-30 minutes post IM injection[179, 181] etorphine starts declining thereof through what is suspected to be an hepatic metabolisation by glucoronide conjugation reactions via the oxidative cytochrome P450 mediated metabolism[168] forming mostly etorphine glucoronide and some other not so relevant metabolites[181, 182]. Afterwards, and in opposition to most other opioids, etorphine's metabolites are then mainly voided in the bile amongst the feces with only a small amount being passed in the urine through glomerular filtration[200, 201].

One of the greatest advantages of the usage of opioids such as etorphine is their ability to be reversed, giving them a reliable safety margin. Etorphine can be reversed by using any of the following drugs: diprenorphine (3-5 times the dosage of etorphine), naltrexone (40 times the dosage of etorphine) or naloxone (0.04-0.07 mg/kg)[108, 179, 180]. However, the use of etorphine has been shown to cause renarcotization after the administration of its reversal agent[13, 179, 181, 202]. The reason for this may lie in a redistribution of the drug from the adipose depots towards the CNS[13, 179, 181, 203, 204] or in the entero-hepatic circulation of etorphine in which etorphine glucoronide is hydrolyzed in the cecum by bacteria derived β-glucuronidase, forming and thus partially absorbing the recycled etorphine[13, 179, 181, 204]. Nevertheless, renarcotization can also be the result of an antagonistic under-dosage or of the use of an antidote that possesses a shorter half-life than etorphine[181, 203]. Renarcotization can occur between 2-72h and the effects are variable and somewhat mimic the signs seen during induction with an underdose of opioid[181].

1.3.1.2 – Butorphanol

Butorphanol is a morphine congener[167] that besides being widely used in small animal and equine veterinary medicine for its sedative and analgesic properties, has been recently implicated in the chemical restraint of wild and free-roaming animals.

Butorphanol is a synthetic opiate mixed agonist-antagonist, exhibiting an antagonistic activity upon the μ-opioid receptors and agonist activity at the κ and δ-opioid receptors[167, 168, 180, 205-207]. It is said to possess around 5-7 times the κ-receptor agonistic potency of morphine[168, 208], 20 times that of pentazocine[208, 209] and about 40 times the κ-receptor agonistic actions of meperidine[209]. On the other hand, the μ-receptor antagonistic activity of butorphanol has been reported to be of about one-fortieth of naloxone's potency, equivalent to nalorphine and approximately 30 times stronger than pentazocine[210]. However, the overall lack in comparative butorphanol agonistic/antagonistic potency studies in rhinos combined with possible significant opioid-receptor interspecies differences hinders, to a certain degree, the acknowledgement of the cited opioid potency relationships in rhinoceroses. Nonetheless, the fact that it produces both superficial and visceral analgesia[211-214] while possibly antagonizing the respiratory depression of other μ-receptor stimulating opioids makes it useful for a balanced anesthesia[65, 180, 215], thus explaining its rising popularity in wildlife anesthesiology.

Butorphanol is a weak base with a pKa of 8.6[168] and is highly lipophilic[168, 210]. As such, following IV administration, butorphanol has a fast onset of action[168, 206], since it undergoes a rapid distribution across the blood-brain barrier towards the CNS. Also IM administration of butorphanol, through the same physical principle, has been shown to have a very rapid absorption in horses (absorption half life of around 6 minutes)[216]. However, through IM administration, the butorphanol's systemic bioavailability in adult horses has been reported to significantly drop to 34-37%[214, 216]and in foals to around 66%[214].

Following IM delivery, butorphanol in equines has a rapid and broad distribution which is typical for opiates[206], with the highest levels (of the parent compound and metabolites) found in excretory organs[210], such as the liver, kidneys, and intestine[217]. In addition, concentrations in the lungs, endocrine tissues, spleen, heart, fat tissue and blood cells also reach higher levels than those found in the plasma[217]. Finally, butorphanol has even been found to cross the placental barrier[217, 218].

Regardless of IV or IM administration, the half-life of butorphanol in horses is short[206, 214, 219], with the drug undergoing an extensive metabolisation in the liver[206, 210, 218] forming biologically inactive metabolites such as Hydroxybutorphanol[210, 218]. These

metabolites and the parent compound are mainly excreted into the urine[206, 217] with a small dose passed into the bile and eliminated along with the feces[206, 217].

Acute life-threatening overdoses with butorphanol should be unlikely. However, the use of butorphanol has been reported to cause some side effects such as ataxia, excitement and stimulation of the locomotor system[206, 208, 211, 214, 220], probably due to dopamine release in the *nucleus accumbens* through δ-receptor stimulation[167, 211, 220]. Nonetheless, if an overdose occurs, a narcotic antagonist, such as naloxone or naltrexone, can be given to reverse the effects of butorphanol.

1.3.1.3 – Nalbuphine

Nalbuphine, a morphine derivative, is a synthetic narcotic of the phenanthrene series[221], structurally similar to the narcotic antagonist naloxone[167, 222, 223] that derives its analgesic and sedative effects through an agonistic κ-opioid receptor binding activity while being a μ-opioid receptor antagonist[223, 224]. Therefore, as with butorphanol, nalbuphine could be used in a balanced anesthesia to antagonize the respiratory depressant effects of opioid agonists, whilst still maintaining analgesia[168, 221, 225]. It is said to have approximately 3-4 times the potency of pentazocine[222] and to have the same analgesic potency as morphine[167, 168, 223], being thus normally used for the relief of moderate to severe pain. However, it is neither a well studied drug in equines nor is it commonly used in the chemical immobilization of rhinoceroses.

Nevertheless, studies performed in healthy human subjects indicate that after an IM administration it takes about 30 min for nalbuphine to be absorbed [222, 226], reaching an average bioavailability in the levels of the 81-83%[222]. Also in human studies nalbuphine is metabolized in the liver[167] and its mean elimination half-life has been reported to vary between 2.2 and 2.6 h[167, 222].

Reversal, like the other opioids mentioned can be achieved through the administration of several opioid competitive antagonists such as naloxone, diprenorphine and naltrexone.

1.3.1.4 – Naltrexone

Naltrexone is a synthetic pure opioid antagonist[180, 198, 227, 228], with activity at the μ, κ and δ-receptors, often used to reverse the effects of very potent opioids such as carfentanil and etorphine[168, 229] by returning the resting conformation of the receptor upon binding[230].

Due to its high affinity to[230, 231], and superior concentration at the opioid receptor site, naltrexone not only reverses the effects of administered opioids but also actively inhibits the actions of endogenous opioids[167, 198, 227]. In addition to its antagonistic power the substantially longer half-life (up to 10 h [13]) that naltrexone possesses over other opioid antagonists, in rhinos, greatly reduces the possibility of renarcotization after its use[13, 203].

Therefore, not only is naltrexone currently considered to be one of the most powerful existent opioid antagonist but also the opioid antidote of choice to use when the animal is to be release into an environment where it has to cope with predators[180, 199, 203, 229].

The dose, as mentioned before, to reverse the effects of etorphine is 40 mg per mg of used etorphine[180] and in rhinoceroses it should be administered IM since sudden arousal and short periods of excitement during recovery have been noted following the intravenous use of naltrexone[13]. After IM administration, naltrexone is rapidly absorbed[204] and distributed to the CNS with the animals generally standing up within 2-4 minutes[203, 204, 232, 233].

Naltrexone is metabolized in the liver and originates 6-natrexone that is then excreted mainly through glomerular filtration[167]. Nonetheless, this weaker metabolite is still biologically active and with an average 13-hour half-life (in humans)[167] further reduces the possibility of renarcotization happening with the use of naltrexone.

1.3.2 – Adrenergic agonists and antagonists

1.3.2.1 – Medetomidine

Medetomidine is a potent alpha-2 agonist widely used in animals to rapidly produce a dose dependent sedation, analgesia, muscle relaxation and to assist in the chemical restraint[234-236]. It is comprised by a racemic mixture of two optical enantiomers with different biological activities[235]. While dexmedetomidine (dexMDT) is the active component of medetomidine, its enantiomer, levomedetomidine (levoMDT), may act as competitive inverse agonist against dexMDT, thus causing it to possess a lower pharmacological effect[234, 235].

Seeing as medetomidine is a very specific α_2-adrenoreceptor stimulant with a $\alpha 2{:}\alpha 1$ selectivity ratio of 1620:1[234, 237], the undesired α_1-adrenoreceptor side effects that normally accompany other not so specific α_2 agonists like xylazine and detomidine[234, 238] are less likely to take place[234, 238]. On the other hand, due to the dihydro-imidazole ring attached to its structure, medetomidine possesses some agonistic activity at the imidazoline receptors that

currently are thought to play a major role in mediating central hypotension and antiarrhytmogenesis[234].

In order to create analgesia and sedation, medetomidine acts through G protein linked α-2-adrenoreceptors mainly in the *locus coeruleus* and spinal cord[234, 238-240]. Upon binding, it inhibits the positive feedback mechanism for the release of noradrenaline from presynaptic nerve endings through an efflux of K^+[235] membrane hyperpolarization and reduction of calcium conductance at N-type calcium channels[234]. The following decrease in sympathetic tone due to a noradrenaline attenuation then decreases arousal (sedation) and inhibits the afferent pain pathways[234]. Furthermore, the presence of α_2-adrenoceptors in various other non-neuronal sites[235, 241, 242] and the concomitant action of medetomidine upon them, explains the several other pharmacodynamic effects witnessed with its use[87, 241, 243-246]. Finally, α_2-adrenergic agonists reduce drug tolerance and act synergistically with opioids[234].

The high liposolubility, and the fact that medetomidine is a weak acid, enables it to be rapidly absorbed with plasma peak levels at 15-30 minutes post IM administration (in dogs, cats and rats)[237, 240, 247, 248] and distributed towards well perfused tissues such as the brain[240, 247, 248], liver, kidney, lung and adrenal gland[249]. This rapid absorption and distribution coupled with an elevated affinity of medetomidine towards α-2 adrenoreceptors[239, 240] results in a swift onset of clinical signs[247]. Elimination half-life has been reported to be low ($51.3 \pm 51,3 \pm 13.09$ in horses[250] with medetomidine undergoing metabolisation in the liver[248]. However, LevoMDT has been shown to have significant drug interactions and to cause hepatic enzyme inhibition[251, 252], thus prolonging the half-life of various drugs such as Ketamine[235]. Excretion of its pharmacologically deprived metabolites then takes place mainly through glomerular filtration in the kidneys[237] with a small amount being passed in the feces[247].

Finally, medetomidine, although well tolerated in doses up to 5X (IV) and 10X (IM) the maximum dose, its side effects may include bradycardia, occasional atrio-ventricular blocks, decreased respiration, hypothermia, urination, vomiting, hyperglycemia, and pain on injection (IM)[253]. In any case, the effects of medetomidine can be cancelled by the use of α_2-antagonists such as idazoxa, tolazoline, yombine and finally atipamezol[180, 234].

1.3.2.2 – Atipamezole

Atipamezole hydrochloride is an injectable synthetic alpha$_2$-adrenergic antagonist, indicated for the reversal of the sedative and analgesic effects of medetomidine[234, 238]. It is only licensed for intra-muscular injection since the preservative methylparaben following IV administration can induce histamine release and a consequential marked hypotension[234].

It is a competitive antagonist that not only reverses the effects of α2 agonists by having a higher affinity towards the receptors (α_2:α_1 selectivity of 8526[234, 237, 238]) but also because it is normally given at a higher dosage (5-6 times the medetomidine dose[15, 254]), thus having a superior concentration at the receptors. Reversal is normally smooth and fast, with the animals normally standing up in less than 5 minutes[15, 237, 254].

Atipamezole has no activity in other receptor sites such as alpha$_1$-adrenergic, 5HT, histaminergic, muscarinic or dopaminergic[234], thus reducing possible side effects upon administration. However, some authors consider that such a powerful reversal may produce an abrupt reversal of sedation (especially if administered IV[180]) and, presumably, analgesia with possible scenarios of delirium and apprehensive or aggressive behavior[237] probably due to a sudden and significant increase in sympathetic tone[180, 234]. Furthermore, and in spite of atipamezole's higher α_2-adrenergic affinity and reported half-life (2-3 hours[255]) a return of medetomidine's effects after atipamezol reversal has been described as possible[234, 237, 241, 256].

1.3.3 – Cyclohexylamines: Ketamine

Ketamine, first described in 1965[257], is a derivative of phencyclidine hydrochloride and a part of a group of drugs called cyclohexylamines[258]. These constitute the second most used group of knock down drugs in current chemical restrain which can be used in broad range of mammal, bird and reptile species[179, 180]. Ketamine is supplied as a racemic mixture even though the S-ketamine alone has 4 times the potency of its enantiomer (R-ketamine), with fewer side effects[257, 259-261]. The state induced by ketamine is termed "dissociative anesthesia" for it produces a state resembling catalepsy, in which the eyes remain open and sensory inputs seem to reach cortical sensory areas but are not perceived due to suppression of the association areas between the thalamocortical and limbic systems [179, 180, 257, 258].

Ketamine is a dissociative anesthetic that acts through a non-competitive inhibition of N-Methyl-D-Aspartate (NMDA) excitatory ligand-gated ion channels[257, 258, 262-266]. It

connects to the NMDA receptors at the phencyclidine binding site thus preventing the binding of the excitatory neurotransmitter glutamate, resulting therefore in a low activity at the thalamocortical and limbic system with a further depression of the nuclei in the reticular activating system[258]. Additionally, ketamine has also been reported to modulate the activity of nicotinic acetylcholine, muscarinic and opioid receptors (μ and κ[258, 259, 267]) as well as voltage-gated ion channels, but only at significantly higher concentrations than the one needed to block NMDA receptors[258, 263, 265-267]. Regarding the gamma-aminobutyric acid (GABA) receptors, although the major current consensus indicate that ketamine has no activity upon them[258, 268], Wulf Hevers and colleagues report an interaction between ketamine and some $GABA_A$ receptor subtypes[266]. Also Ketamine has been reported to enhance the effect of muscle relaxants by decreasing the sensitivity of the motor end-plate[257]. Finally, like other dissociative agents, ketamine causes an increase of noradrenaline via central adrenergic stimulation and decrease reuptake, resulting in an increase of sympathetic tone that affects all organs under adrenergic control[258, 259, 269].

Following ketamine parenteral administration, the onset of clinical signs is normally quite fast (<1 minute through IV administration in humans[269] and between 3 to 5 minutes through IM injection in most animals with immobilization occurring between 5-10 minutes[179, 180]), mainly due to the drug's high liposolubility[257, 265], bioavailability (90%[257]) and low plasma protein binding capacity (around 50% in several species[270-274]) that enables a fast ketamine brain uptake[257] (brain to plasma ketamine concentration ratio in rats is suspected to round 6.5:1[265]). Ketamine is afterwards rapidly distributed into all body tissues including the fetus (if present[257]) with highest levels found in the brain, liver, lung, and fat[179].

Ketamine, with an accounted half life of about 1 hour (in equines)[264] is metabolized in the liver[179, 259-261] mainly through N-demethylation into norketamine, which then often undergoes transformation into glucuronide derivatives[258, 259, 261, 264]. Norketamine is an active metabolite of ketamine that retains 10-30% activity of the parent drug[258]. Finally, these conjugated metabolites along with a small portion of unchanged ketamine are mostly voided in the urine (>90%), with the rest being eliminated through the bile[179, 258, 259, 264].

Ketamine, although reported to be quite safe to use[258, 269], has been reported to cause several undesirable situations such as emergence delirium[257, 259], muscle rigidity, myoclonic jerking or uncoordinated muscle movements[179, 180, 258] that may arise if ketamine is used on its own. Furthermore, dissociative anesthetics, when used alone, do not produce a surgical depth of anesthesia (induces anesthetic stages I and II, but not stage III)[258]. As such, ketamine

should always be used along with other agents (opioids, α2-agonists, benzodiazepines or others)[258, 259].

There is no reversal for the effects of cyclohexylamines and the animal may be affected for several hours[180]. The end of ketamine's activity is not so much due to its metabolism and excretion but rather to a redistribution of the drug from out of the CNS[258]. The duration of anesthesia depends on the animal and on dosage used to knock down that animal, seeing as an increase in ketamine dose increases the duration of anesthesia, not the intensity, with already recorded recumbency periods of up to 5 hours[179].

1.3.4 - Benzodiazepines: Midazolam

Benzodiazepines, such as midazolam, are sedative-hypnotic drugs normally used in veterinary medicine for its anticonvulsant and muscle relaxation activities and to decrease anesthetics dose requirements[234, 275, 276]. However, due to a persistent state of awareness[275] and possible transient periods of excitation created by the sole use of benzodiazepines[234], midazolam is generally used with other agents such as opioids, α_2-agonists or cyclohexylamines in order to produce a more reliable and predictable sedation[234, 275, 277]. Benzodiazepines also potentiate the analgesic effects of other agents (opioids and α_2-agonists) but have none themselves[234, 278].

Benzodiazepines work synergistically with gamma-aminobutyric acid (GABA), which is an inhibitory neurotransmitter[275, 279-281]. Midazolam is a central acting skeletal muscle relaxant that selectively depresses the transmission of impulses at the interneurons of spinal cord, brain stem and sub-cortical regions of the brain[278]. It acts by binding to and activating the benzodiazepine receptor binding site (BZ receptor) located on the gamma subunit of the gamma-aminobutyric acid receptor subtype A ($GABA_A$)[234, 275, 280, 281]. Midazolam agonism at the BZ binding site on $GABA_A$ receptors modulates the GABA stimulation, increasing the frequency in which the chloride ion channel opens, thus augmenting the inward flux of Cl^-, leading to a post-synaptic neuron hyperpolarization that consequently decreases the neuronal transmition[234, 275, 279, 280].

These receptors, although found in a greater number in the cerebral cortex, very few exist outside of the CNS, hence the midazolam's minor to none effects in the cardiovascular and respiratory systems[234, 275, 281, 282]. However, midazolam in larger doses has been reported to cause a reduction in the cerebral blood flow and an even greater reduction in oxygen assimilation[283, 284]. Furthermore, a sole substantial IV administration of midazolam has also

been shown to induce a drop in blood pressure and an increase of heart rate, mainly due to a decrease in peripheral vascular resistance[275].

Within physiological pH, midazolam becomes highly liposoluble[277, 280, 282], being rapidly and completely absorbed, from an IM administration site, towards the CNS[275, 277]. This rapid distribution combined with a high potency and BZ receptor affinity (twice as much as diazepam[234]) significantly reduces the lag time until the onset of action[275]. However, the fast uptake of midazolam (on behalf of the CNS) is quickly followed by an outward redistribution of midazolam to other tissues, especially muscle and fat[275, 279]. Although midazolam is considered to be a short-acting sedative[280, 285] elimination half-life is very species and dosage related[278, 286]. It undergoes a hepatic metabolisation[287] primarily by hydroxylation[280] with the resulting metabolites still experiencing glucuronidation before they are excreted in the urine[234, 279, 287]. Amongst these metabolites, 1-hydroxymidazolam has been shown in humans to be almost as equipotent as the parent compound[288-290].

Reversal can be obtained through the IV use of flumazenil, which is believed to be a specific benzodiazepine antagonist[234, 275, 280].

1.3.5 – Butyrophenones: Azaperone

Azaperone is a butyrophenone frequently used as an opioid synergist for the chemical immobilization of wildlife or on its own as a short acting neuroleptic agent capable of providing a short duration tranquilization (4-6 h) in rhinoceroses[13, 291, 292]. It has been extensively used in recent years in combination with etorphine for the chemical restraint of free-ranging and confined black and white rhinoceroses[13, 108, 293, 294], mainly due to its muscular relaxation activities[65, 234] and because of its capacity to counteract, to a certain point, the hypertension side-effect of etorphine [179, 180, 295].

The main CNS effects of azaperone are mediated through a selective dopaminergic receptor antagonism performed upon D_2-like receptors[234]. The D_2-like receptor group is comprised by D_2, D_3 and D_4 receptors subtypes[296] and like all the dopamine receptor subtypes these are members of the G protein coupled receptor (GPCR) superfamily[297]. In addition, azaperone has also been implied to have a relative antagonism at α_1-adrenergic, histaminergic and cholinergic receptors[198, 234].

Azaperone does not provide any type of analgesia[234, 298] nor does it cause a profound cortical depression (unconsciousness), however, it does suppress spontaneous movements while sparing spinal reflexes and unconditioned pain reflexes[299, 300]. In rhinoceroses, while a small

dose of around 60mg induces sedation, doses from 200mg up to 400mg normally already lead to recumbency[13]. On the other hand, azaperone has been reported to induce transient periods of excitation in horses following an IV dosage[301] and in the white rhino[302], before the animal becomes sedated. Extra-pyramidal effects such as restlessness, catalepsy and increase in aggression are sometimes seen[198].

Azaperone is considered to have a fairly rapid onset of action following IM administration (5·10 minutes[180, 234, 303, 304]) with a peak effect at approximately 30 minutes post injection[234]. Metabolism occurs in the liver[234] through several biochemical pathways, resulting in more than one metabolite[305, 306]. The produced metabolites afterwards suffer glucoronide conjugation in order to become more water-soluble compounds and are in their majority excreted in the urine[234, 305-307] with only a small portion being excreted in the feces[234].

There is currently no known reversal for azaperone[180, 291].

1.4 – Monitoring

Monitoring is essential for immobilization and anesthesia. Its goals are to detect physiological changes in time to correct irreversible injuries and to ensure adequate anesthetic depth.

A procedure, before it can be considered to be anesthetic must obey several parameters, these being: loss of conscience; muscle relaxation; amnesia; analgesia and to the best of its capabilities secure the animal basic functions. Since amnesia is a standard currently not possible to access in non sentient beings and analgesia, although many times secured due to the use of powerful opioid agonists, is not a normal starting field immobilization objective, it is going to be the remaining variables that are going to be the basis for anesthetic evaluation.

1.4.1 – Loss of conscience

Regarding consciousness, or loss or conscience, in anesthesiology this is assessed by observing a patient's alertness and responsiveness, and can be seen as a continuum of states ranging from alert and oriented to time and place through disorientation and delirium ending with loss of movement in response to painful stimulation [308]. In a capture situation where an animal is initially aroused and stressed by the helicopter, one very important goal is to try to reduce to the maximum the time that goes from the moment that the animal is darted to the

moment that the animal is down, in other words, unconscious. For that is the moment of total absence of control, when the animal experiences harsh, serious and significant physiological changes while under the imminent risk of suffering from several harmful situations, such as physical trauma and drowning, since the animal is in a mental transition between awareness and unconsciousness.

From darting until the animal is down several behavioral changes occur, normally species-specific that indicate the onset of immobilization as well as the successful/unsuccessful administration and/or absorption of the immobilization drug and therefore should be noted. The speed in which the animal progresses from one stage to the other not only indicates the rate in which the drug is being absorbed but also the likelihood of a successful immobilization. These changes in animal behavior may be divided into several stages:

Stage	Animal behavior
1	Alarm reaction to the noise and impact of the dart
2	Wariness
3	Initial barely perceptible changes in behavior
4	Obvious abnormal behavior
5	Initial slight change in gait which becomes progressively worse resulting in an obvious ataxia (Fig.1)
6	Loss of balance, stumbling and apparent blindness. The animal adopts a high-stepping trotting gait with its head held up and back, commonly called the "**hackey gait**" (Fig. 2), or
7	Animals usually go down in a sternal or lateral recumbency. Some animals will walk into a bush or tree and lean against it remaining standing there. (Fig. 4)
8	Loss of reflexes

Table 6. Stages of induction[5]

Fig.2 – Initial clinical signs.

Fig.3 – "Hackey gait" (kindly provided by Matilde Burel).

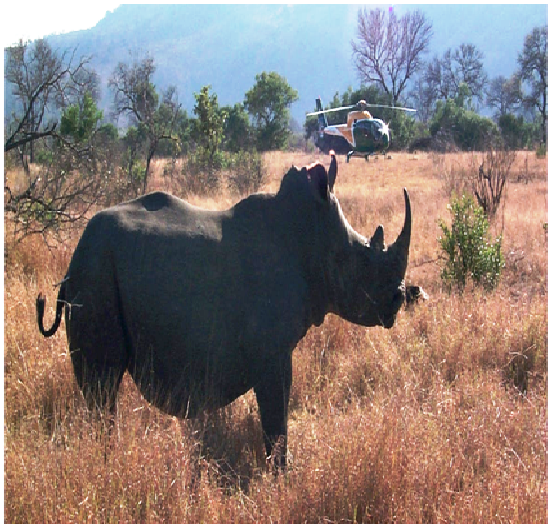

Fig.4 – Typical opioid stance (photo kindly provided by Dr. Peter Buss).

Fig.5 – Immobilized rhinoceros in sternal recumbency (kindly provided by Matilde Burel).

1.4.2 – Muscle relaxation

The implications of muscular work increase have already been discussed in greater detail previously in this paper. As such, only a superficial approach to the topic in matter will be made at this stage.

Muscle relaxation in a chemical capture acts as a preemptive maneuver to avoid further metabolic needs (particularly O2 and glucose[309, 310]) and complications (acidosis[130], hyperthermia[132, 153]) that may arise from a high muscular tone throughout the immobilization period (twitching, paddling, convulsions or plain increase in muscular tone[15]). In addition, a

high muscular tone can constitute a significant respiratory impediment since chest wall muscle rigidity hinders the thoracic movements and thus inspiration and expiration (inhibits deep ventilation)[13, 15]. On the other hand, muscular relaxation may also actively contribute to a higher respiratory impairment by allowing an abdominal content compression over the lungs[43, 45, 47].

Nevertheless it must be noted that the presence of an adequate muscle relaxation does not necessarily imply unconsciousness or analgesia[311].

1.4.3 – Monitoring of Basic animal functions

Currently, in anesthesia as well as in immobilization procedures, an inherent risk is always present. This risk is further on worsened by the notion that no pre-immobilization animal evaluation is done. As a result, a thorough and continuous assessment of the respiratory system as well as of the cardiovascular system and body temperature during the immobilization period should always take place in order to avoid or predict any unwanted situations still within time of intervention. Also, the collection of this data will allow a proper classification of the immobilization period.

1.4.3.1 – Respiratory system

In this trial, the methods chosen to analyze the respiratory system were visual count of respiratory rate and depth, pulse-oximetry, collection of arterial blood for blood gas analysis, direct visual evaluation of the colour of collected venous blood and measurement of end tidal CO_2 through the use of a Capnometer.

a. Respiratory rate and depth

Respiratory rate can be easily and fairly accurately determined through visual count. However, respiratory depth or tidal volume, without proper monitoring equipment, can only be roughly estimated as large or small through a visual assessment of thoracic wall movement or by feeling the amount of air moving in and out of the nostrils with each breath of air[28]. Therefore, any conclusion in respiratory minute volume (volume of air that the animal inhaled or exhaled by the animal in one minute) under these conditions would have to be considered subjective.

Furthermore, in field circumstances, a respiratory system evaluation based purely on respiratory rate and depth, even if very precise, does not translate the true oxygenation status of the animal since it doesn't evaluate alveolar ventilation, ventilation/perfusion mismatches, gaseous exchanges, account for the amount of recumbency induced lung impairments nor does it appraise blood O_2 or CO_2 transport. Consequently, the need to use further monitoring techniques, such as a pulse-oxymeter machine, becomes practically mandatory.

b. Pulse-oximetry

Pulse oximetry is a standard non-invasive technique used world-wide for monitoring arterial hemoglobin oxygen saturation that has found numerous applications in anesthesiology, postoperative recovery and critical care[312, 313].

The principle of pulse-oximetry lies in the different light absorption characteristics of hemoglobin in its oxygenated (O_2Hb) and reduced state (RHb)[312, 314]. This is then evaluated through the placement of a pulsating arterial vascular bed between a two-wavelength light emitting diode and a detector (photodiode)[312]. While O_2Hb absorbs more of the 940 nm wavelength (infrared), RHb absorbs ten times more of the 660 nm wavelength (red) than O_2Hb[2, 312, 314, 315].

Fig.6 – Pulse oxymetry attached to the ear after the superficial pigmented layers were scraped off (kindly provided by Matilde Burel).

Although a valuable monitoring apparatus capable of detecting early hypoxic changes in sedated or anesthetized animals[15], pulse-oximetry comes not without its flaws. There are several factors that can adversely influence the pulse oximeter readings. These include changes in the strength of the arterial pulse, body movements or motion artifacts[314], optical interferences (dyshemoglobinemias, plasma lipids and bilirubin, color interferences), venous pulsations, and various other physical factors (such as ambient light or signal-to-noise ratio)[312, 314] and/or health conditions (e.g. Anemia)[314].

Strength of the arterial pulse	Any factor affecting the normal perfusion, and therefore altering the arterial pulsation (hypothermia, hypotension), will undoubtedly reduce the machine's capability to detect, properly analyze and, thus, to calculate the arterial oxygen saturation (low signal-to-noise ratio)[312, 314-316].
Body movements (motion artifacts)	In spite of the fact that many of the recent pulse-ox machines are able to tolerate small amounts of movements, exaggerated mobility has been associated with intermittent absorbance changes, thus compromising the monitoring accuracy[314]. Shivering and muscle twitching, as an example, can influence both pulse rate and overestimate O_2 hemoglobin saturation reading by generating a signal artifact[312, 314, 317]. This artifact consists on an optical shunt, generated by light from the LEDs that reach the photo-detector without passing through an arterial bed[314].
Bilirubin	Hyperbilirubinemic animals tend to have an overestimated measurement of O_2 hemoglobin saturation, seeing as they increase blood concentration of CO upon the breakdown in Hb[314].
Dyshemoglobine-mias	Methemoglobin, carboxyhemoglobin, and or sulfhemoglobin can result in imprecise pulse oximeter readings due to absorbance of one or both wavelengths produced[312]. • Carboxyhemoglobin may absorb as much of the 660-nm wavelength as O_2Hb[312, 314] leading to an overestimation of pulse-ox readings. • Methemoglobin will absorb as much of the 660-nm light as RHb, but absorbs the 940-nm light to even a greater degree[312, 314] leading to an erroneously pulse-ox readings. Since the pulse-oximeter instrument does not differentiate between different forms of hemoglobin, the readings it provides are not considered to be of oxyhemoglobin itself but of a "functional oxyhemoglobin", since its values result from a percentage of the sum of oxyhemoglobin and deoxyhemoglobin[2].
Venous pulsations	Arteriovenous anastamoses and glomuses are unusual features of the cutaneous circulation. Nevertheless, if present(eg. in venous congestion situation[314]), arterial blood is shunted into a vein, which also becomes pulsatile, artificially causing falsely low O_2 hemoglobin saturation readings[312].
Ambient light Pigmentation and thickness of skin	Ambient light is potentially a major source of interference leading to overestimations of saturation in animals with a true O_2 hemoglobin saturation of less than 85% due to the mentioned optical shunt created[314].
Pigmentation and thickness of skin	Some authors consider that dark pigmentation acts somewhat like an obstacle for light penetration, with significantly more signal detection failures[314]. However, others claim that in general, the pulse-ox instrument accommodates a wide range of tissue thickness and skin pigmentation[312].
Health conditions	Anemia has been considered to cause an underestimation of oxygen saturation by pulse oximetry[314]

Table 7. Possible pulse-oxymeter error sources

Although all of these factors may take a toll in the final reading values, they are not always present and if so, they may be only transient. However, a constant and ever present conditioning factor is that the pulse-ox machine merely monitors the changes in arterial hemoglobin oxygen saturation of the peripheral tissues(SpO_2)[312], which in this case consists of the ear.

One other unavoidable limiting feature of pulse oximeters is that these machines are only as accurate as their empirical calibration curves[314]. Now having in mind that there does not currently exist a calibration curve for oxygen-hemoglobin dissociation in rhinoceros and that the used curve derived from human volunteers that have only been tested until $SaPO_2$ levels of 75-80%[314], the registered levels of arterial blood oxygenation must not be considered to be a true value of SpO_2, but an approximation number from where the oxygenation status of the animal can be extrapolated[15], with obvious implications on the accuracy of pulse oximetry at low oxygen saturations. For this reason, correction factors for variables like temperature, pH, and 2,3-diphosphoglycerate concentration must be inserted for accurate results[314].

Regardless of its limitations and sources of possible errors, pulse-oximetry still remains as a useful tool for anesthesia monitoring. However, it further bears mentioning that the pulse oximeter assesses the adequacy of oxygenation, but in no way measures the adequacy of alveolar ventilation[314, 318-320].

c. End tidal CO2

Fig. 7 – Capnometry from expired air sample collected in the right nostril (kindly provided by Matilde Burel).

Since the lung is the only body compartment in which carbon dioxide normally and continuously accumulates, the presence of cyclic exhaled CO_2 can be used to assess airway patency and pulmonary ventilation[54]. In this trial $P_{Et}CO_2$ was obtained by means of a portable capnometry machine that uses a sidestream PCO_2 analyzer, measuring PETCO2 through the absorption of infra-red light at a wavelength of 4.26 μm[321, 322]. The portion of respired gases, that were analyzed, was diverted by a suction pump from the animal's nostril to the sensor through a sampling tube.

The principle behind capnometry is based on the gas exchange system occurring at an alveolar level in the lungs. As new air enters to the alveoli, a gas exchange gradient is going to exist between alveolar and capillary CO_2 tensions. It is then this $P_{(a-A)}CO_2$ that is going to be the engine behind normal CO_2 diffusion from the bloodstream to the alveolar space, that continues at a rate proportional to the difference of PCO_2 between the two[1] (alveolar and capillary). As can be seen in the fig. 8, in a normal physiological situation, without any pulmonary pathophysiological limitations (the one to the left) a complete equilibration between P_aCO_2 and P_ACO_2 occurs in the end of gas the diffusion period[1, 2, 321]. Having this in mind and considering that capnometry only measures PCO_2 in the end of expiration (since it is the only time that pure alveolar air is available for sampling), $P_{ET}CO_2$ can be used as a proxy to arterial PCO_2 oscillations[1, 2].

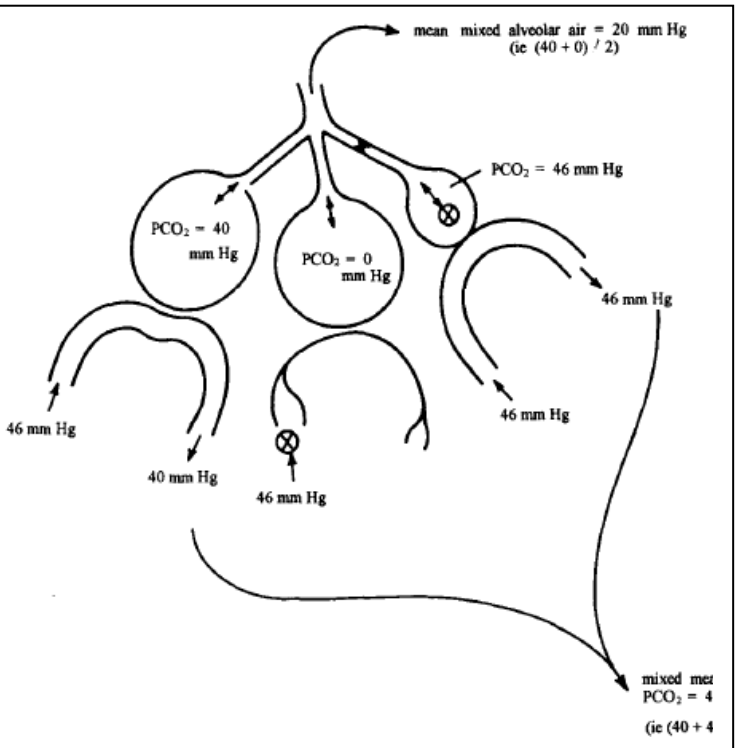

Fig. 8 – An approximated model of gas exchange at an alveolar level[1]

However, in an extenuating circumstance such as in a field chemical immobilization, several factors also take effect, compromising the validity of capnometry. Capnometry comes not without its flaws, especially in the field, being presented with several problems, such as mucous plugging that either lead to a complete obstruction of the sampling tube, or to a partial obstruction, resulting in no readings or inaccurately low readings of $P_{ET}CO_2$[1, 2]. Another important limitation is the condensation of water vapor that can affect the readings either by falsely increasing the CO_2 readings through condensation on the window of the sensor cell,

hence absorbing part of the IR light[322, 323], and by a phenomenon called "collision broadening"[323], or by erroneously lowering he CO_2 values through obstruction of the nose catheter[1], or by diluting the CO_2[323]. In addition, other limitations such as noisy signals and poor or unstable calibration can also add up to unreal and inconsistent results[1].

Fig. 9 – End-Tidal reading from the animal's nostril through the use of a sampling tube.

Furthermore, one must never forget that alveoli are emptied essentially simultaneously, rather than sequentially, and as such gas anywhere from actively exchanging "ideal" lung units to non-exchanging alveoli combine in parallel rather than in series throughout expiration[2]. Therefore, capnometry, as the measurement and display of CO_2 concentrations on a digital or analog indicator at the end of the expiration phase[322, 324], does not account for alveolar dead space (from where the air expired has the same CO_2 content as the inspired air[2, 45, 316]) or pulmonary shunting[2, 54]). This may result in a significant widening of the arterial-end-tidal CO_2 gradient, with a serious underestimation of $PaCO_2$ by $P_{ET}CO_2$ [1, 2] questioning therefore the true validity of capnometry both in horses[45] as in rhinoceros . Therefore, in the further attempt to better evaluate the respiratory status of an animal under the effect of several anesthetical drugs, one can attempt to seek additional information through capnograms and very importantly through blood gas analyses.

d. Collection of arterial blood for blood gas analysis

Fig. 10 – Blood being collected from the right medial auricular artery.

Fig. 11 – Blood sample (kindly provided by Matilde Burel).

Arterial blood analyses although in this case provided no immediate information during the immobilization period, it allowed a further academical understanding, in an anesthetical point of view, of the immobilization period undergone, in this case, by the knocked-down rhinos. The collected data in this trial, being it O_2 saturation, PaO_2, $PaCO_2$ and pH casts an additional light to the degree of respiratory depression suffered by the immobilized rhinoceros, since it provides the most accurate information concerning gas exchange at a pulmonary level. Nevertheless, measurement errors are still a possible reality with blood gas analysis, compromising therefore its reliability, even if only to a small scale.

Seeing as the accuracy of blood gas analyzers is widely recognized, the most important sources of error in interpretation of blood gases most likely come from pre-analytical factors[325]. Amongst these, syringe type[325-330], temperature[325, 328-332], delay time[329, 330, 333] and the presence of air bubbles[332, 333] are considered to be the most influential factors in the end values.

e. Direct evaluation of collected venous blood

Through visual evaluation of the collected venous blood one can have a rough assessment regarding the oxygenation status of the immobilized animal[15, 27]. The principle behind this concept lies in the different light absorption characteristics of deoxyhemoglobin (660nm) and oxyhemoglobin (940nm)[2, 312, 314]. A dark red, almost black blood indicates poor oxygenation, whereas a lighter red color is normal, correlates well with normal mucous membrane color[15, 229] and suggests a more oxygenated blood[334].

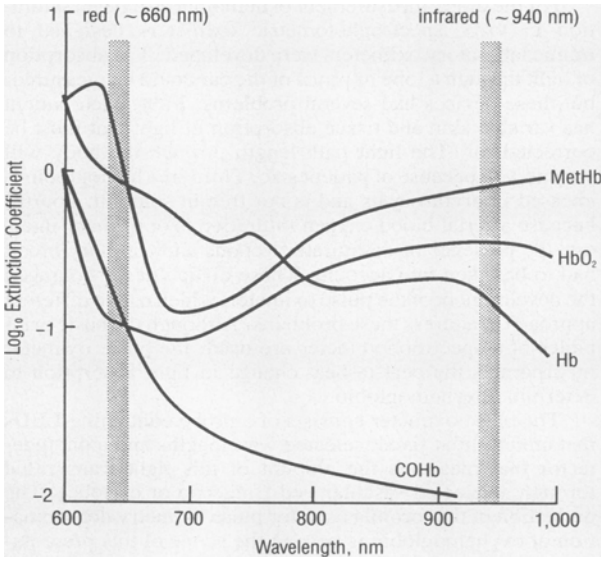

Fig. 12 – Absorption spectra[2]

1.4.3.2 – Cardiovascular system

While in the pre-immobilization period, when the rhinoceros are still in transition between awareness and unconsciousness, and physiological changes taking place cannot be assessed during the knock down period, several monitoring techniques can be applied in order to ascertain the rhino's ongoing cardiovascular parameters.

Fig. 13 – Heart rate and blood pressure reading (kindly provided by Matilde Burel)

Fig. 14 – Heart rate and blood pressure reading (kindly provided by Matilde Burel)

In this trial, cardiovascular status was monitored through regular heart rate and blood pressure readings coupled also with the mentioned blood gas measurements. While heart rate was monitored through digital compression over the medial auricular artery, pulse oxymeter machine and through the use of a sphygmomanometer, blood pressure was measured through the use of the mentioned sphygmomanometer.

The use of a sphygmomanometer, however useful as means of obtaining rough readings of blood pressure in field immobilized rhinoceroses, does not provide 100% reliable values. First of all, it must be noted that blood pressure values are not equally distributed throughout the body, and as such, all reading must be corrected to a heart level according to the formula: distance in cm from center of cuff on tail to heart base/1.36 + actual coccygeal blood pressure[3]. Furthermore, the use of uncalibrated sphygmomanometers may also be the cause of clinically significant over and under-estimation of the actual blood pressure[335, 336]. Finally, the fact that the used sphygmomanometer was built for humans may present yet another error source. Considering that a sphygmomanometer, in order to be able to obtain a blood pressure reading, needs to inflate its cuff to a level above arterial pressure[337, 338], the significantly rougher, thicker and more solid skin of a rhino may present a mechanical obstruction towards the

sphygmomanometer induced compression. As such, the use of a human intended sphygmomanometer may lead to clinically significant blood pressure overestimations.

CHAPTER II – FIELD STUDY – MONITORIZATION OF WHITE RHINOCEROS AFTER DART IMMOBILIZATION

1. MATERIAL AND METHODS

Ten white rhinoceros were immobilized at Tswalu Kalahari Reserve (August 20th and 21st 2009) during a course for the Wildlife Group, by Dr. Douw Grobler, Dr. Scott Citino and Dr. Mitchell Bush, from where the data of these eight animals was collected. All of these animals were immobilized from a R44-helicopter by Dr. Douw Grobler using a Dan-Inject system, 1.5 cc dart and a 60 mm long 2.2 diameter smooth needle. No mortality was observed during the capture, immobilization period or in the observed post immobilization period of these animals.

Different drug cocktails were used in order to access which one would provide the safest and more effective immobilization. The following data was collected from the captured animals, and kindly provided by Dr. Mitchell Bush and Dr. Scott Citino.

2. RESULTS

Rhinoceros	1	2	3
Animal description	Sub-adult male weighing about 1160 kg	Sub--adult female weighing about 1230 kg	Sub-adult male weighing about 1330 kg
Drugs used in the dart	**M99** - 2mg **Butorphanol** - 40 mg **Midazolam** - 25 mg	**M99** - 3mg **Butorphanol** - 60 mg **Midazolam** - 30 mg	**M99** - 3mg **Butorphanol** - 40 mg **Midazolam** - 25 mg
Butorphanol: M99 ratio	20:1	20:1	13:1
First Signs	3 minutes	6 minutes	3 minutes
Recumbency	22 minutes	12 minutes	8 minutes
Reversal agent	**Naltrexone** - 100 mg I.M.	**Naltrexone** - 100 mg I.M.	**Naltrexone** - 100 mg I.M.
A summary of the physiological monitoring showed the following averages	RR = 8 bpm HR= 72 bpm SBP= 121 mm Hg Pulse Ox = 84% $P_{Et}CO_2$ = 55 mm Hg pH = 7.283 PO_2 = 53 mm Hg O_2Sat = 89%	RR = 9 bpm HR= 65 bpm SBP = 139 mm Hg Pulse Ox = 88% $P_{Et}CO_2$ = 46 mm Hg pH = 7.347 PO_2 = 50 mm Hg O_2Sat = 89%	RR = 9 bpm HR= 74 bpm SBP = 142 mm Hg Pulse Ox = 90% $P_{Et}CO_2$ = 48 mm Hg pH = 7.365 PO_2 = 50 mm Hg O_2Sat = 89%
Remarks	The animal had to be pulled down at the 22 minutes. At the end of the monitoring period the animal stood following being prodded. Good and uneventful reversal	Following the administration of Naltrexone the animal made a good and uneventful recovery.	The animal went down by himself. The rhino was walked in the end with an IV administration of 10mg of butorphanol. Good and uneventful reversal.

Rhinoceros	**4**		**5**	**6**	
Animal description	Sub-adult female weighing about 1660 kg.		Sub-adult male weighing about 900 kg	Sub-adult male weighing about 980 kg.	
Drugs used in the dart	**M99 -** 3mg **Azaperone -** 25mg		**Butorphanol** - 60mg **Medetomidine –** 4mg	**M99 –** 3.5mg **Midazolam** – 30 mg	
Additional administrations	**Butorphanol** – 35mg + **Midazolam** – 10 mg I.V.			**Butorphanol**– 30mg I.V.	
First Signs	3 minutes		5 minutes and 30 seconds	3 minutes	
Recumbency	12 minutes		16 minutes.	6 minutes	
Reversal agent	**Naltrexone** - 100 mg I.M.		**Naltrexone** - 100 mg I.M. **Atipamezol -** 20mg I.M.	**Naltrexone** - 100 mg I.M.	
A summary of the physiological monitoring showed the following averages	Before **Butorphanol** and **Midazolam**	After **Butorphanol** and **Midazolam**	RR= 10 bpm HR= 37 bpm SBP= 106 mm Hg	Before **Butorphanol**	After **Butorphanol**
	RR= 11 bpm Pulse Ox= 74 bpm $P_{Et}CO_2$= 60 mm Hg O_2Sat = 74% PO_2=39 mm Hg	Pulse Ox= 90% $P_{Et}CO_2$= 48 mm Hg O_2Sat = 92% PO_2=58 mm Hg	Pulse Ox= 93% $P_{Et}CO_2$= 44 mm Hg pH= 7.423 PO_2= 89 mm Hg O_2Sat = 96%	RR= 15 bpm HR= 104 bpm O_2Sat =ND $P_{Et}CO_2$= 67 mm Hg PO_2= 18 mm Hg	HR= 72 bpm O_2Sat = 89% $P_{Et}CO_2$= 48 mm Hg PO_2= 52%
Remarks	The animal went down by himself. Both butorphanol and midazolam were administered within the first monitoring period and the obtained changes were maintained throughout the remaining monitoring period. Good and uneventful reversal.		The animal went down by himself. Was very stable physiologically and very relaxed. The animal after being reversed with atipamezol and naltrexone recovered well and uneventfully.	The animal went down by himself. Before the administration of butorphanol there were marked muscle tremors. These subsided after the adm. of butorphanol (4 minutes after arriving at the animal). Good and uneventful reversal.	

Rhinoceros	**7**		**8**
Animal description	Small sub-adult female/calf weighing about 730 kg		Sub-adult female weighing about 1460 kg
Drugs used in the dart	**M99 –** 1,5mg **Nalbuphine** – 20 mg		**M99** - 3mg **Butorphanol** - 30 mg **Midazolam** - 30 mg
Butorphanol: M99 ratio			10:1
Additional administrations	**Butorphanol** – 15mg I.V		**Ketamine** – 200mg I.V.
First Signs	3minutes and 30 seconds		Not observed
Recumbency	10 minutes		25 minutes
Reversal agent	**Naltrexone** - 100 mg I.V.		**Naltrexone** - 100 mg I.V.
A summary of the physiological monitoring showed the following averages	Before **Butorphanol**	After **Butorphanol**	RR= 9 bpm HH= 78 bpm SBP= 158 mm Hg Pulse Ox= 88% End tidal CO_2= 44 pH= 7.387 PO_2= 53 mm Hg O_2Sat = 90%
	HH= 100 bpm SBP= 150 mm Hg Pulse Ox= 63% $P_{Et}CO_2$= 63 mm Hg PO_2= 33 mm Hg PCO_2= 74 mm Hg pH= 7,29 O_2Sat= 64%	HH= 68 bpm SBP= 96 mm Hg Pulse Ox= 86% $P_{Et}CO_2$= 56 mm Hg PO_2= 54 mm Hg PCO_2= 60 mm Hg pH= 7,34 O_2Sat= 90%	
Remarks	The animal went down by himself. Butorphanol was administered within 4 minutes after arriving at the animal and the resulting changes maintained in the remaining monitoring period. Good and uneventful reversal.		At 23 minutes the animal was still up. After the K**etamine I.V.** the animal was down in 2 minutes. Slight muscle tremors observed. The rhino was walked in the end with 10mg of butorphanol I.V.. Good and uneventful reversal, without any signs of ketamine .

Table 8. Immobilization data

CHAPTER III – DISCUSSION

All of the performed immobilizations were made with the dual purpose of identifying the animals and to evaluate the anesthetic toll of the different drug cocktails in the animal's normal physiological parameters. The data was collected by Dr. Scott Citino and Dr. Mitchell Bush with the help of everyone enrolled in the butorphanol course while members of Tswalu Kalahari Game Reserve proceeded with the ear-notching and microchip implantation, both in the hump and in the horn.

In order to be able to fully evaluate the immobilization procedure of the animals involved, in an anesthetical point of view, several immobilization records were collected. These consist in dosages used, immobilization times, and general animal evaluations along with several samples taken from each animal at three different moments during the procedure. However, since only a small part of this data is available for this thesis, none of the immobilization protocols were exactly repeated in other similar animals and all of the eight rhinoceros differed at least in weight and age, the purpose of this thesis will not be to state as fact the various pharmacological interactions witnessed with the use of the different immobilization cocktails, but rather to suggest feasible explanations to some of the observed reactions in the immobilized rhinos during this butorphanol course.

Due to the considerable differences between rhinos, the drugs used to immobilize them (except rhinos 1, 2, 3 and 8) and the set of collected data from each one of them, a general conjunct line of comparison between all of the different monitoring periods becomes impractical at best. Therefore, besides the group of rhinos immobilized with etorphine, butorphanol and midazolam, an individual approach to each of the knock down animals will be made.

In the MBM group (M99, butorphanol and midazolam), several aspects must be noted. First of all, the animals are all sub-adults and there does not exist a marked difference in weights between them. As such, the different drug doses and butorphanol:M99 ratios used in the cocktails have, as it would be expected, different effects upon the rhinos. One such difference lies in the induction times with rhinos 1 and 8 not only taking the longest to go down but also having to be either pulled down (rhino 1) or toped up with ketamine (rhino 8). The explanation behind the long immobilization period of rhino 1 may lie in a drug underdose [24, 339], seeing as the increase in butorphanol (20mg) and M99 (1mg) dosage in rhino 2 caused the animal to go down by himself in a significantly shorter time. Then again, since butorphanol in the presence of strong opioid-receptor agonists like etorphine acts as a mixed agonists-antagonist stimulating κ-receptors and antagonizing μ-receptors[65, 167, 168], a long immobilization period might have

also been the consequence of an excessive and simultaneous competition for the μ-opioid receptors ligant site by butorphanol and etorphine [65]. This would then clarify the further reduction in induction time witnessed in rhino 3 comparing to rhino 2 since a lower butorphanol:M99 ratio was used in rhino 3. On the other hand, since rhino 8 had the lowest IM administered butorphanol:M99 ratio, and it still had to be toped up with ketamine in order to be able to go down, other not quantified factors may also interfere. Amongst these, individual differences[340, 341], different and individual stress responses[342, 343], genetic[344, 345], sex[346-351] and age related differences [352-354] have recently been identified as possible factors that might dramatically influence opioid-receptor functions. Of particular importance are the age related differences in both distribution and concentration of opioid receptors that have been shown to exist in laboratory animals between neonates and adults[353, 354]. Although not yet evaluated in rhinos, these differences may be the reason behind the disparity in doses used between a calf rhino (100kg→1mg etorphine [4, 15, 23]) and an adult rhino (1800kg→4mg etorphine [4, 23, 24, 108]), suggesting a higher opioid tolerance by young rhino calves. However, the implications of a possible opioid concentration and distribution inequality in sub adult rhinoceroses still remain an enigma. Furthermore, considering that none of the other features have ever been tested in rhinoceros to determine if in reality they affect the animal's opioid response, they cannot be accounted as being determinative, but nevertheless should not be yet discarded as non-influential. Finally, the speed in which ketamine induced recumbency in rhino 8 must be noted. This could be explained by its IV administration, by the fact that the animal was already under the effect of strong opioids or by ketamine's capacity to also act upon μ, δ and κ-opioid receptors, thus operating as an excellent opioid synergist [16, 355-358] and hastening the attainment of recumbency[13, 233].

The immobilization periods of these 4 MBM immobilized rhinos can be classified as good (rhino 1 and 8), good to excellent (rhino 2) and excellent (rhino 3). The reason for this may reside in the conjunct administration of butorphanol that although might not occur systemically at the same ratios as the IM administered (37% bioavailability in horses[214, 216]), it still nonetheless seems to retain enough μ-opioid antagonistic activity to reverse the respiratory depressive effects of etorphine. This comes in accordance with several studies performed in different species, where butorphanol successfully or partially reversed the strong opioid depressive effects of fentanyl[359] or sufentanyl[215] in rats, oxymorphone in dogs[360-362] and fentanyl in rabbits[363]. On the other hand, Sandra Wenger and colleagues[65], in a study performed in white rhinoceros, did not observe any significant advantages with the additional use of butorphanol in the immobilization mixture (etorphine, azaperone, detomidine and

hyaluronidase). However, in that study, the butorphanol's lack of influence over the rhinoceros immobilization parameters could be pointed to the relatively low butorphanol:M99 ratio used (less than 10:1).

The animals subjected to the MBM drug cocktail experienced a relatively similar immobilization period. These, despite exhibiting a slower RR (8-9 breaths/minute) in comparison to the recorded normal 19 breaths/minute[3], possessed high levels of hemoglobin O_2 saturations as was indicated by ABG analysis and corroborated by the field pulse-oxymeter (with only a slight deviation). In addition, close to normal values of $P_{Et}CO_2$ values were recorded (except for rhino 1 probably due to etorphine's induced hypoventilation[26, 65] or/and a higher metabolic rate induced by the capture[310]). However, PaO_2 levels still indicated hypoxemia (PaO_2<70mm Hg[65]) with values rounding the 50mmHg which would nevertheless come in accordance with the observed 89-90% O_2Sat if one would consider the rhinoceros to have a similar oxyhemoglobin dissociation curve as horses[364, 365]. The hypoxemia in these cases is impossible to be traced to a specific origin since there are not enough records, such as $PaCO_2$ (evaluating possible lung impairments) and the distance ran during the 22 minutes before the rhino went down (involving exercise-induced respiratory limitations). However, it could undoubtedly be pointed as a possible consequence of recumbency due to a thoracic cavity compression by abdominal content (of non-starved animals)[13, 21, 22, 26] aggravated by the muscle relaxation capacities of midazolam or/and due to a respiratory center depression by etorphine [13, 14, 16]. The increase of cardiovascular parameters in rhino1 (HR), 2 and 3 (HR and SBP) could then be due to a chemoreceptor (central and peripheral) stimulation in response to the ongoing hypoxia[83]. Rhino 8, although probably under the same chemoreceptor control, might also have been under the concomitant indirect noradrenaline increasing effects of ketamine[258, 366], hence its higher SBP. On the other hand, tachycardia and hypertension could have also been due to the stress and exercise undergone by these 4 animals before recumbency[73, 367, 368] or due to the use of etorphine, appearing as side-effects[16, 108]. Finally, through a pH evaluation of these 4 animals only rhino 1 was acidemic, possibly due to the exercise it underwent before going down[21] or/and due to recumbency[14] (although the other 3 animals did no present acidosis with recumbency), thus indicating a good tissue oxygenation with the use of a MBM combination.

Regarding rhino 4, this animal was successfully and rather rapidly (12 minutes) immobilized with a drug combo of M99 and Azaperone. However, considering the animal's weight, probably a higher dose of etorphine should have been used (4mg)[23, 24, 108] in order to shorten the immobilization time. Due to the significant signs of hypoxia and hypercapnia

presented by the animal (pulse-ox of 74% plus $P_{Et}CO_2$ of 60mm Hg), a mixture of butorphanol and midazolam was decided to be administered in the ear vein. This caused a fast improvement of the hypoxic state of this rhino (pulse-ox of 90% and $P_{Et}CO_2$) that was maintained throughout the remaining monitoring process. The suffered hypoxia not only may have been a direct consequence of etorphine's μ-receptor agonism[14, 21, 65, 179, 233], by lowering the central respiratory center sensitivity and thus responsiveness to the increasing CO_2[198, 302], but may have also been aggravated by the simultaneously administered azaperone. Azaperone, by acting also as an α_1-adrenoreceptor antagonist, counteracts the hypertensive effects of etorphine through the induction of vasodilation[13, 108, 369]. Additionally, azaperone has even been mentioned to counteract some of the respiratory depression caused by opioids[181, 302]. Then again, because of the same α_1-antagonism, azaperone may actively reduce the blood's O_2 carrying capacity, since it can cause the splanchnic capsule to relax, thus reclaiming within the once sympathetically released RBC (lower PCV) [13, 304]. To make matters worse, if an azaperone α_1-antagonism takes place in the smooth muscle of cardiac arteries, then hypothetically serious endocardial ischemic lesions may occur in the presence of marked tachycardias. Situations that, should not occur with the use of a highly specific α_2-agonist, such as medetomidine due to its extremely limited α_1 effects[234, 238]. However, Harry H. Jalanka in his report[237] suggested a medetomidine as the cause for the observed decrease in PCV, due to its adrenolytic properties. After the IV administration of butorphanol and midazolam, O_2Sat and PaO_2 values increased while tachycardia subsided (104 to 72 beats/minute), further supporting the μ-opioid receptor antagonistic capacities of butorphanol to, at least partially, reverse the respiratory depressive effects, and consequently cardiovascular responses of strong opioids such as etorphine.

Rhino 5, besides the long immobilization period (16 minutes to go down), experienced what appears to be an excellent immobilization period with several collected parameters averaging closely to the normal physiological ones (HR=37 beats/minute; $P_{Et}CO_2$=44 mm Hg; PaO_2=89 mm Hg and O_2Sat=96%). This long immobilization time, and the dangers that accompany it (such as capture myopathy[14, 16, 65, 181, 198]), may be the result of a massive sympathetic stimulation undergone by the animal during the capture procedure, since medetomidine acts upon adrenergic receptors[198, 234, 237]. If a significant sympathetic stimulation occurs, a competition between noradrenaline and medetomidine for the adrenergic receptors emerges, thus hardening the onset of medetomidine's actions and prolonging the immobilization time[370, 371]. The same concept can be applied to explain the sudden arousal described in several procedures with medetomidine[237]. Additionally, because butorphanol has been reported to be an opioid not only rather incapable of producing profound sedation in adult

horses[214, 372] and rhinos[65], but also capable of inducing periods of paradoxical excitation [168, 169, 206, 373], its conjunct administration in the dart for the immobilization of free-ranging wild rhinos may be somewhat inappropriate. On the other hand, immobilizations using butorphanol in combination with an α_2-agonist such as medetomidine or detomidine in zoos not only has been performed successfully[15, 232, 374] but has been considered quite ideal for captive situations[15]. The reason for the increasing popularity of this combination lies in the capacity of medetomidine and butorphanol to apparently induce an oxygenated, relaxed and physiologically stable anesthesia (as witnessed in rhino 5) without the hypertensive and respiratory depressive side-effects of strong opioids such as etorphine seen in countless other rhino immobilizations [14, 25-27, 65, 233, 375]. However, this seemingly optimal combination does not come without its hazards. Medetomidine, for instance, induces a biphasic cardiovascular response characterized by an initial bradycardia and hypertension (reduced sympathetic flow and baroreflex-induced peripheral vasoconstriction) followed by a period of bradycardia and hypotension due to a baroreflex shift or inhibition[234, 235, 240]. While this may explain the found hypotension (106 mm Hg) and "normal" heart rate (37 beats/minute) in rhino 5, the question would then be if the outstanding PaO_2 and O_2Sat levels, also recorded in rhino 5, are sufficient enough to avoid the possible appearance of ischemic lesions that can arise from hypotension and bradycardia. Furthermore, if one considers that an initial hypertension as high as 200 mm Hg has already been recorded in horses with detomidine[376], then the use of medetomidine might, therefore, be able to induce pulmonary edema and/or hemorrhage due to the generation of exaggerated pulmonary transmural pressures. Then again, the collected PaO_2 and O_2Sat values in this rhino refute the stated conception (probably due to the IM route used[377]). Additionally, butorphanol, without the presence of a strong μ-opioid receptor agonist, has been reported to have weak μ-agonistic properties[168, 302]. However, it does not induce a dose dependent cardiorespiratory depression[208, 210]. Not only does it have minor effects on the respiratory system[65, 206, 208, 214], but also the effects that butorphanol does have do not pass a certain point regardless of the administered dose ("ceiling effect"[65, 210, 302]), conferring thus safety to its use. Added benefits that can arise from the use of medetomidine in an immobilization cocktail are: the redistribution of blood flow from nonessential areas (muscle, intestine, skin) to vital ones (kidney, lung, heart)[241]; excellent muscle relaxation[234, 240](as observed in rhino 5); potentiates opioid CNS depression[244] and it also inhibits the further release in catecholamines from the adrenal glands[234]. In summary, the combination butorphanol and medetomidine presented itself as an extraordinary

combination and further studies on it should be performed both under zoo and free-ranging conditions.

While the previous immobilizations were performed with demonstrative purposes, the following two were merely experimental. Nevertheless, they still contributed to an even greater understanding of butorphanol's μ-antagonistic capacities.

The M99 midazolam combination used in rhino 6, induced a severe respiratory depression, regardless of the 15 breaths/minute, with unreadable pulse-ox values (below the detection level) and further on confirmed by an unbelievably low PaO_2 of 18 mm Hg through ABG analysis, thus probably explaining the elevated heart rate. This, although having innumerous explanations, may have been, most likely, due to an excessive etorphine dose[118, 375] (considering that rhino 4, 680 kg heavier, only needed 3mg of etorphine to go down) combined with the sole administration of midazolam that not only does not balance the respiratory depressive side effects of etorphine but may have further exacerbated the CNS depressive actions of it[13, 234, 378]. In addition, marked muscle tremors were observed with the use of this combination, which would not be expected due to the excellent muscle relaxation inducing capacities of midazolam[13, 15, 27, 108, 198]. Then again, opioids, and in particular etorphine, have been reported to cause excitement, muscle hypertonicity, shivering and convulsions in a number of species[168, 198], including rhinos[15, 26, 27, 108, 379]. The mechanism in which etorphine causes these muscle tremors and muscle hypertonicity has not yet been fully understood, however, a possible explanation could be an opioid derived neuronal excitation that culminates in the release of excitatory neurotransmitters (dopaminergic or/and adrenergic)[168] or in a decreased activity of the inhibitory neurotransmitter GABA[168, 208]. In addition, the release of acetylcholine has also been suggested to cause this opioid derived excitation[380]. Midazolam, then, by not being able to efficiently suppress the potentially etorphine-induced seizures on its own, may result in the observed marked muscle tremors[167]. An increase in muscular work then further endangers the animal physiologically by causing an elevation of core temperature (hyperthermia)[13, 15, 155]. The observed muscle hypertonicity, in turn, by increasing the oxygen metabolic demands and the production of CO_2 could partially shed some light into the low levels of PaO_2 and high $P_{Et}CO_2$ values collected[15, 27]. Furthermore, the fact that a high muscle tone affecting the thoracic wall respiratory muscles is able to hinder deep ventilation[13, 15, 65], the animal in this case could have been reduced to a shallow and fast breathing pattern[27], hence the high $P_{Et}CO_2$ recorded number (67 mm Hg) and the high RR (in comparison to the other immobilized rhinos). Regarding the IV administration of butorphanol 4 minutes after arriving at the animal, a partial antagonism of the depressive effects

of etorphine was observed, with an overall oxygenation status improvement and a reduction of the hypercapnic degree. However, the fact that a low butorphanol:M99 ratio was able to induce such enhancements in several recorded parameters and still improve muscle relaxation cannot be considered as a precedent for the usage of low butorphanol:M99 ratios, but rather a sign of higher bioavailability of butorphanol through an IV administration route[214]. A post hoc evaluation between rhinos 1, 2, 3, 6 and 8 would then suggest that an addition of butorphanol to the anesthetic combination of etorphine and midazolam (in the dart) leads to a better immobilization period with enhanced muscle relaxation, oxygenation and improved physiological parameters.

Finally, rhino 7 was the smallest captured animal during the course (730kg) and not only was it rapidly immobilized with the smallest dose of M99 administered in this course (1,5mg) but a partial agonist with μ-opioid receptor antagonistic activities was also administered with it in the dart. Nonetheless, respiratory depression was still observed with a marked hypoxia (pulse-ox= 63%, O_2Sat= 64% and PaO_2= 33mm Hg), hypercapnia ($PaCO_2$= 74 mm Hg and $P_{Et}CO_2$= 63 mm Hg) and metabolic acidosis (pH= 7,29), which may have been caused by the use of etorphine coupled with a lack of an effective μ-antagonistic activity of nalbuphine to counteract its depressive side-effects. Although described as a reversal for opioid respiratory depression in rhinos[21, 27, 108], nalbuphine in this case may have not been able to perform as such, perhaps due to a etorphine relative underdose of nalbuphine, a much delayed period of nalbuphine absorption from the injection site (30 minutes in humans[222, 226]) and/or due to an insufficient μ-antagonistic potency, or concentration at the receptors, of nalbuphine in comparison to etorphine. On the other hand, the IV administration of butorphanol may have been the responsible factor in inducing, at least partially, a turnaround in the respiratory depression that marked the initial monitoring period[15]. The fact that, quickly following the IV administration of butorphanol a higher oxygenation status could be observed, with higher PaO_2 (54 mm Hg), O_2Sat (90%) and pulse-ox (86%) values may suggest a superior μ-antagonistic capacities of butorphanol over nalbuphine, due to a higher μ-opioid receptor affinity of butorphanol in comparison to nalbuphine[381]. Then again, if the non-attenuation of etorphine's side effects by nalbuphine was solely due to a slow absorption, then not only a potency comparison between butorphanol and nalbuphine cannot be achieved in this case but also the butorphanol's μ-antagonistic effects may have been potentiated by nalbuphine. Moreover, the evidences showing a drop in both SPB, from 150 to 96 mm Hg and HR, from 100 to 68 beats/minute, after the oxygenation status improvement that followed the IV administration of butorphanol, promoted the idea that in this case hypertension and tachycardia may have worked as an organism

protective mechanism from ischemia under hypoxic conditions[16, 21, 26] However, there are evidences that the cardiovascular effect of etorphine in ungulates can also be caused by increased activity of the sympathetic nervous system[13, 25, 108, 198]. In a study by Bogan and colleagues[382], etorphine-induced tachycardia and hypertension was accompanied by a six-fold plasma noradrenaline increase. Furthermore, through an $P_{Et}CO_2$ and $PaCO_2$, evaluation in this animal a discrepancy can be noted between the arterial CO_2 tensions and the CO_2 tension of the exhaled air, thus suggesting either hypoventilation[65] or possible lung impairment (areas of ventilation/perfusion imbalance). This although, being highly likely to be recumbency induced[13, 14, 26, 375], exercise induced respiratory impairments cannot be discarded, since the walked distance of rhino 7 was not recorded. On the other hand, since rhino 7 was the lightest in this group, it should have the lowest predisposition towards thoracic compression by the abdominal content (especially by the digestive system)[15, 47, 65]. Finally, the pH value also rose following the improvement in oxygenation status, suggesting that in this case acidosis may have been most likely the consequence of opioid respiratory depression[375].

All immobilized rhinoceroses were placed in sternal recumbency. Since it allows a better oxygenation status[14, 26], some of the respiratory parameters obtained may have been enhanced by this position, thus appearing superior to other trials where the animals were placed in lateral[26, 65, 232, 233] or dorsal recumbency[22]. However, on the downside, lateral recumbency allows a superior blood flow to the legs, in detriment to sternal positioning, with muscles having a higher chance to get oxygen and to get rid of the carbon dioxide and heat generated while running[383]. Additionally, if sub-adult white rhinos possess lower values of hemoglobin than adult ones, as is the case with black rhinos[118], than the animals in this course may have had lower oxygen carrying capabilities. Nevertheless, the high intrinsic oxygen affinity of hemoglobin in rhinoceros (the low half saturation pressure ,or P_{50}, is of about 20 mm Hg in rhinos [384]) coupled with the lower tissue metabolic rate of large mammals equipes the rhinoceros with a higher capacity to provide adequate tissue oxygenation with lower PaO_2 than other smaller animals[65].

Once the monitoring period was finished, both naltrexone and atipamezole were administered. Seing as they were capable of reversing the effects of opioids and medetomidine in a brief couple of minutes, as expected from previous reports[13, 15, 25, 27, 302, 375, 385-387], naltrexone and atipamezole, respectively, presented themselves as optimum reversal agents. Regarding rhino 8, no signs of ketamine upon awakening, such as emergence delirium[257, 259], were witnessed probably due to the fact that the midazolam administered within the dart was not reversed[198, 259, 279]. Midazolam in the other rhinos (except rhino 4 and 7), as well as

azaperone (rhino 4) were not reversed, probably contributing, through their respective sedative[234, 280, 388] and tranquilizing[13, 291] effects, to the smooth and calm reversal periods of these rhinos. However, it must be noted that reversal was only evaluated in the short minutes following the animal's awakening, seeing as they all ran away into the velt and therefore renarcotization could not be assessed. Furthermore, the animals showed no difficulty in rising indicating that the sternal recumbency they were positioned in did not lead to severe blood supply occlusions, which normally occur when the animal lies on its back legs for extended of periods of time, thus appearing reluctant to stand upon antidote delivery[108].

Finally, and unfortunately, several protective mechanism that might have taken place during these immobilizations, such as the increase in cerebral blood flow in response to hypercapnia[126, 128] (or ketamine induced[257, 389], in rhino 8) and an increase in circulating RBC due to a sympathetic spleen contraction[91, 95], could not be assessed with the available collected data, and thus remain a speculation.

CHAPTER IV– CONCLUSION

The data collected in this butorphanol course, although allowed for a superficial approach into the pharmacodynamic effects of butorphanol, in combination with other CNS depressive agents, for the immobilization of white rhinoceroses, the offered explanations to the observed reactions cannot be considered as definitive due to their statistically deprived condition. Furthermore, all findings must be positioned in time (August 20th and 21st 2009) and in place (Tswalu Kalahari Game Reserve) due to annual and geographical variations in, amongst others, food and water availability, disease spread, parasite load and other factors that may affect the individual response to the different drugs used in the immobilization cocktail.

Regarding the different cocktails used in this course, it is of the author's opinion, based on the available data and on butorphanol pharmacokinetic studies, that butorphanol should only be administered IV once the animal is already recumbent and not in the dart combination.

As a final point, further studies should be performed with some of the combinations used during this course: etorphine plus butorphanol and midazolam in the dart cocktail; etorphine and azaperone as the immobilization agents in the dart plus butorphanol IV when the animal is immobilized, and finally, medetomidine and butorphanol administered in combination within the dart, since it presented itself as an exceptional immobilizing cocktail during this butorphanol course and was the closest one to the concept of anesthesia.

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