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PARAMETRI CARDIOVASCOLARI E RESPIRATORI DEI MACACHI (*MACACA MULATTA*) ANESTETIZZATI PER PROCEDURE NEUROCHIRURGICHE

CARDIOVASCULAR AND RESPIRATORY PARAMETERS IN ANESTHETIZED RHESUS MONKEYS (*MACACA MULATTA*) UNDERGOING NEUROSURGICAL PROCEDURES

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Summary

Abstract

Background: Chemical restraint in non-human primates (NHPs) is routinely done in laboratory settings. This study evaluated the effects of sedation with ketamine and medetomidine on cardiovascular and respiratory parameters in 30 macaques undergoing neurosurgical procedures.

Methods: Thirty adult macaques were sedated with ketamine (4.5 mg/kg) and medetomidine (50 µg/kg) before undergoing neurosurgical procedures. The macaques were divided into four groups: Isoflurane delivered in 100% oxygen (OI), Isoflurane delivered in a mixture of 1:1 oxygen and medical air (OMI), Use of thiopental to perform intubation (T), and No thiopental to perform intubation (NT). Cardiovascular parameters and respiratory parameters were monitored and evaluated at designated time points during the surgical procedure.

Results: Our study revealed a significant delay in the reduction of MAP in the T group compared to the NT group with significant difference at T30 (p = 0.016), suggesting a potential influence of thiopental on macaque cardiovascular dynamics during neurosurgical procedures. Furthermore, the OI group exhibited a notable increase in respiratory rate (RR) and a simultaneous decrease in $EtCO_2$ levels from baseline values from T180, indicating a significant impact of oxygen administration on respiratory function in macaques undergoing surgery.

Hypotension may occur and could be treated with the administration of 0.2 µg/kg/h of noradrenaline.

Conclusion: This protocol is safe and useful for macaques undergoing neurosurgical procedures. Cardiovascular and respiratory parameters remain within physiological range even though mild hypotension may occur.

Background: Il contenimento farmacologico nei primati è eseguito routinariamente in contesto laboratoriale. Questo studio ha valutato gli effetti della sedazione con ketamina e medetomidina sui parametri cardiovascolari e respiratori in 30 macachi sottoposti a procedure neurochirurgiche.

Metodi: Trenta macachi adulti sono stati sedati con ketamina (4,5 mg/ kg) e medetomidina (50 µg/ kg) prima di sottoporsi a procedure neurochirurgiche. I macachi erano divisi in quattro gruppi: Isoflurano vaporizzato in 100% di ossigeno (OI), Isoflurano vaporizzato in una miscela di 1:1 ossigeno e aria medicale (OMI), Uso di tiopentale per eseguire intubazione (T), e non utilizzo di tiopentale per eseguire l'intubazione (NT). I parametri cardiovascolari e i parametri respiratori sono stati monitorati e valutati in determinati momenti durante la procedura chirurgica.

Risultati: Il nostro studio ha rivelato un ritardo significativo nella riduzione della MAP nel gruppo T rispetto al gruppo NT con differenza significativa a T30 (p = 0.016), suggerendo una potenziale influenza del tiopentale sulla dinamica cardiovascolare del macaco durante le procedure neurochirurgiche. Inoltre, il gruppo OI ha mostrato un notevole aumento della frequenza respiratoria e una diminuzione simultanea dei livelli di $EtCO_2$ dai valori di base da T180, indicando un impatto significativo della somministrazione di ossigeno sulla funzione respiratoria nei macachi sottoposti a chirurgia.

Possono verificarsi fenomeni ipotensivi che possono essere trattati con la somministrazione di 0.2 µg/kg/h di noradrenalina.

Conclusione: Questo protocollo è sicuro e utile per i macachi sottoposti a procedure neurochirurgiche. I parametri cardiovascolari e respiratori rimangono entro limiti fisiologici anche se può verificarsi una lieve ipotensione.

Introduction

Non-human primates (NHPs) have played a pivotal role in advancing scientific understanding across a spectrum of disciplines, with particular significance in the field of neuroscience. The use of NHPs in research is due to their remarkable phylogenetic proximity to humans, offering valuable insights into complex biological and neurological processes that underlie cognition, behaviour, and disease. In neuroscience, NHPs have emerged as a crucial model system for investigating fundamental questions related to brain structure and function. Their brain architecture, complexity, and social behaviours closely resemble those of humans, making them an invaluable resource for studying neural mechanisms underlying complex cognitive processes, emotional responses, and neurological disorders. (Mitchell et al., 2018)

However, the ethical considerations surrounding NHP research have led to the establishment of the "three R's" principles—Replacement, Reduction, and Refinement—as guiding principles for ethical animal research.

1) Replacement: The principle of Replacement advocates for the replacement of NHPs with alternative methods whenever possible. Advances in technology and the development of alternative models, such as in vitro and computer simulations, have allowed researchers to reduce their reliance on NHPs for certain experiments. By adopting innovative approaches, scientists aim to minimize the number of NHPs used in research.
2) Reduction: Reduction focuses on minimizing the number of NHPs used in experiments while maximizing the information obtained from each individual. Refinements in experimental design, statistical methodologies, and data analysis have contributed to reducing the sample size needed for meaningful results. Additionally, longitudinal studies and sharing of data across research institutions can help maximize the utility of each NHP in scientific investigations.
3) Refinement: The principle of Refinement emphasizes the optimization of procedures and care practices to enhance the welfare of NHPs. This includes improvements in housing, handling, and anesthesia protocols to minimize stress and discomfort. Moreover, the development of non-invasive techniques for data collection and the use of positive reinforcement training contribute to refining NHP research methodologies.

Ketamine is the main drug used to induce deep sedation in Macaca mulatta; there are studies of the combination of ketamine and medetomidine which are often of low ketamine and high medetomidine dosages. Isoflurane is the most common volatile anesthetic used for NHPs.

The aim of this thesis is to evaluate the effects on the cardiovascular and respiratory parameters of ketamine, medetomidine and isoflurane in Rhesus macaques undergoing neurosurgical procedures.

Chapter 1

1.1 Taxonomy

NHPs have been utilized in many areas of biomedical, behavioural and evolutionary research because their phylogenetic and physiologic similarity to humans make them uniquely valuable animal models.

NHPs belong to the order Primates which contain two suborders: Strepsirrhini and Haplorrhini. The first one contains three infraorders: Lemuriformes (lemurs), Chiromyifromes (aye-aye) and Lorisiformes (lorises and galagos) that are seldom utilized in biomedical research.

In the Haplorrhini order there are two infraorders: Tarsiiformes (tarsiers) and Simiiformes (simian primates).

In the Simiiformes order the most common utilized monkeys in biomedical research are:

- Platyrrhini (New World monkeys)
    - Cebidae family (marmosets, tamarins, capuchins and squirrel monkeys)
- Catarrhini (Old World monkeys)
    - Cercopithecidae family (macaques, baboons, African green monkeys, mangabeys)
    - Hominidae family (chimpanzees) (Fortman Jeffrey D. et al., 2018)

1.2 Rhesus macaque *(Macaca mulatta)*

Macaca mulatta is an Old-World monkey that belongs to the Cercopithecidae family; there are currently six recognized subspecies.

Rhesus macaques have a life span of about 30 years; the body weight is 5-8 kg for the females and 8-15 kg for the males. They are 47 cm and 52 cm long, respectively: Figure 1. (Van Wagenen & Catchpole, 1956; Lewis & Prongay, 2015)

Figure 1 - External appearance of the male (A) and the female (B) rhesus monkey. (Casteleyn & Bakker, 2021)

Rhesus macaques are characterized by brown to grey fur with a lighter underside (from beige to white). They have medium length tail, pinkish elongated muzzle with narrowly spaced, down turned nostrils, cheek pouches (Figure 2) for storing food, prominent ischial callosities, marked sexual dimorphism. (Fortman Jeffrey D. et al., 2018) Females possess a pair of pectoral mammary glands (Figure 1 [B]).

Figure 2 - Rhesus macaque with full cheek pouches. (Fortman et al., 2018)

Rhesus monkeys possess nails (unguis) on each finger and toe. The palms of the hands and the soles of the feet are keratinized with epidermal ridges. (Casteleyn & Bakker, 2022)

The skin covering and surrounding the genitals is also devoid of hairs. In males, the

scrotum is non-pendulous and contains a pair of testes that measure 4 cm in length. The penis is normally retracted within the preputium, only extracted during mating. (Casteleyn & Bakker, 2022)

They are found in a wide range of habitats, even in rural agricultural or urban settings.

They are omnivores feeding on a wide variety of plant and animal items.

In nature, rhesus monkeys live in large groups; the females tend to remain in their natal group where they form dominance hierarchies based on matrilineal kinships: when the matriarch dies, she is replaced by her youngest daughter. The males leave their natal group shortly after reaching puberty.

In research, they are used mainly for reproductive biology, behaviour, neuroscience, immunology and infectious disease studies.

Physiologic parameters in rhesus macaque are reported in the figure 1. (Fortman Jeffrey D. et al., 2018)

Parameter	Units	RH[a]
Weight	kg	
Adult male		5.5–12
Adult female		4.4–10.9
Lifespan	years	20–30
Respiratory rate	breaths/min	35–50
Heart rate	beats/min	98–122[b]
MAP	mmHg	113 ± 13[b]
Blood volume	mL/kg	50–80
Body temperature	°F	98.6–103.1
	°C	37–39

Note: MAP, mean arterial pressure.
[a] Magden et al. (2015) and Buck (2015).
[b] Measured via telemetry.

Table 1 - Normative physiological and biological data of Rhesus (RH). Map has been detected by telemetry (Fortman, 2018)

1.3 Anatomy and physiology of the cardiovascular system in Rhesus macaque

In *M. mulatta* the heart has an oval shape rather longer than broad with rounded apex formed by the left ventricle, a shallow anterior interventricular sulcus, a small right appendicular bulge anterior to a single anterior vena cava and a rounded conus (Figure 3). The right ventricular papillary muscles arise from the anterior and posterior free wall and the septum. There is a complex intertwining network of trabeculae on the free wall and the septum. Three pulmonary venous stems with short myocardial sleeves enter the roof of the left atrium separately. Left ventricular papillary muscle has several heads, one or more of which separate from others. The septum is heavily trabeculated except in the subaortic region; it has a large pars membranacea and small right and left cartilaginous trigones. (Rowlatt, 1990)

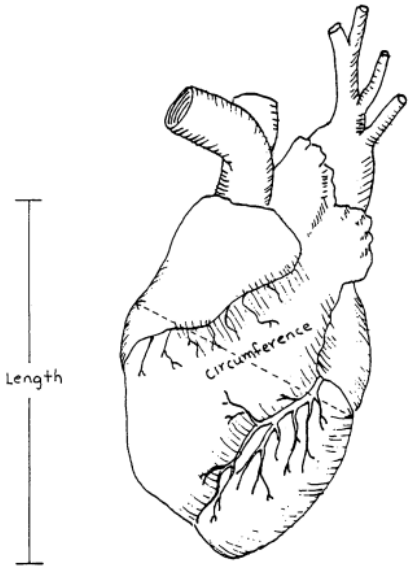

Figure 3 - Ventral view of the heart (Swindle et al., 1986)

Mean heart weights ranged from 3.3 g for males under one month of age to 31.5 g in a 10-year-old. In females they ranged from 3.1 to 26.0 g (Figure 4). (Cupp & Ventura, 1981)

In Table 2 are described measurements of the heart in normal rhesus monkeys. (Swindle et al., 1986)

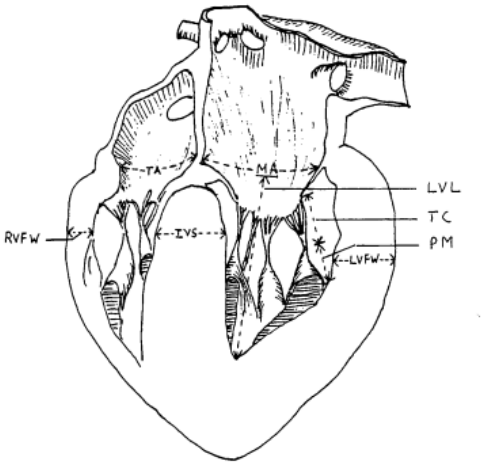

Figure 4 - Tangential section through the heart demonstrating the four chambers and areas for making measurements. RVFW, right ventricular free wall; IVS, interventricular septum; LVFW, left ventricular free wall; TA, tricuspid annulus; MA, mitral annulus; LVL, left ventricular length; TC, tendinous chordae; OM, papillary muscle. (Swindle et al., 1986)

	No. of monkeys				
	Male	Female	Total	Mean ± SD	Range
Body weight	11	16	27	.56 ± .16	.32–.9
L/C	12	20	32	.32 ± .09	.31–.8
IVS/LVFW	13	26	39	.80 ± .24	.44–1.0
MAC/TAC	8	15	23	.92 ± .14	.62–1.18
LVL/PM (AMV)	5	13	18	4.34 ± 2.39	2.50–13.0
LVL/PM (PMV)	5	13	18	3.47 ± 1.69	1.68–8.12
LVL/PM + TC (AMV)	5	13	18	3.73 ± 1.83	1.79–8.0
LVL/PM + TC (PMV)	5	13	18	3.15 ± 1.65	1.66–8.0
PM + TC + AMVW/LVL	5	11	16	.65 ± .16	.32–.95
PM + PMVW/LVL	5	11	16	.54 ± .14	.26–.76
AMVW/MAC	11	21	32	.22 ± .09	.09–.42
PMVW/MAC	11	21	32	.14 ± .06	.06–.26

[1]Definitions of abbreviations:
% body weight, weight of heart/body weight; L/C, length of heart/circumference of heart; IVS/LVFW, interventricular septum thickness/left ventricular free wall thickness; MAC/TAC, mitral annular circumference/tricuspid annular circumference; LVL/PM (AMV), left ventricular length/papillary muscle length (anterior mitral valve); LVL/PM (PMV), left ventricular length/papillary muscle length (posterior mitral valve); LVL/PM + TC (AMV), left ventricular length/papillary muscle length + tendinous chordae length (anterior mitral valve); LVL/PM + TC (PMV), left ventricular length/papillary muscle length + tendinous chordae length (posterior mitral valve); PM + TC + AMVW/LVL, papillary muscle length + tendinous chordae length + anterior mitral valve width/left ventricular length; PM + PMVW/LVL, papillary muscle length + posterior mitral valve width/left ventricular length; AMVW/MAC, anterior mitral valve width/mitral annular circumference; PMVW/

Table 2 - Normal Morphometric Parameters of the Rhesus Monkey Heart (Swindle et al., 1986)

The mitral annulus circumference to tricuspid annulus circumference (MAC/TAC) ratio is used as an indicator of ventricular dilatation. There is a difference in the MAC/TAC ratio between sexes: unlike humans, male rhesus macaques seemed to have a larger value compared with females.

For the first time, vertebral heart score (VHS) was established for NHPs with and without cardiac disease by A. R. Williams et al. (2020). A total of 150 rhesus macaques was enrolled in the study and underwent echocardiography and thoracic radiography (right lateral, [RL]; dorsoventral, [DV]; ventrodorsal, [VD]) (Figure 5) under ketamine sedation (10 mg/kg intramuscularly [IM]) with or without dexmedetomidine (0.025-0.075 mg/kg IM).

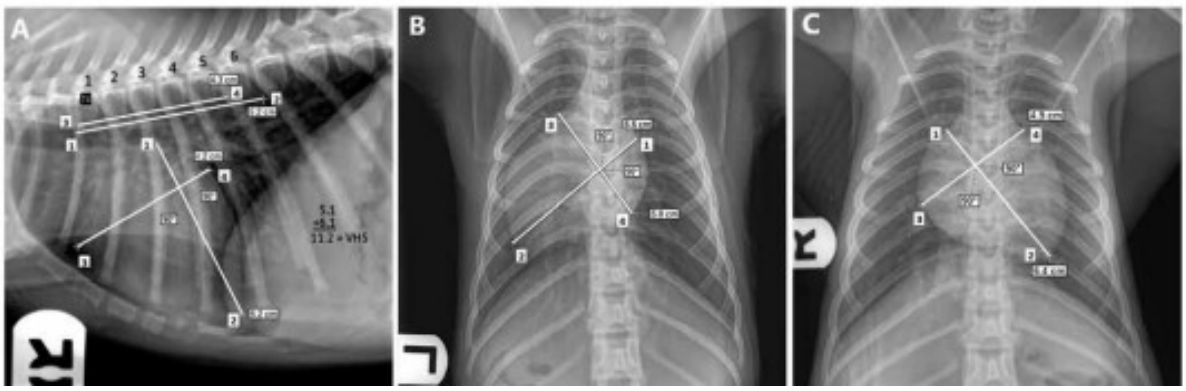

Figure 5 - (A) RL, (B) DV, and (C) VD radiographic views of the thorax in healthy rhesus macaque without significant cardiac disease (control). Each view illustrates the measurements of long (point 1 to point 2) and short (point 3 to point 4) axes of the cardiac silhouette. (Williams et al., 2020)

For the control animals (n=121) mean heart rate (HR) was 115 ± 22 beats per minute (bpm).

VHS reference intervals for the three radiographic views are reported in Table 3.

View	Minimum	Maximum	Mean	Median	Standard deviation	Lower limit	90% CI	Upper limit	90% CI
Right lateral	7.2	11.6	9.79	9.8	0.71	8.39	8.21–8.58	11.19	11.00–11.37
Dorsoventral	7.1	11.6	10.2	10.3	0.71	8.8	8.61–8.99	11.62	11.43–11.80
Ventrodorsal	8.2	12	10.4	10.4	0.67	9.13	8.96–9.30	11.75	11.58–11.92

Table 3 - Reference intervals of VHS in 121 healthy rhesus macaques without significant cardiac disease (Williams et al., 2020)

VHS did not differ significantly between male and female control macaques in all three radiographic views.

Sixteen monkeys aged from 17 to 20 years and six monkeys aged 7-8 years were evaluated by ultrasonography under ketamine sedation (10 mg/kg IM). (Tang et al., 2008)

The measurements of cardiac structure in Table 4 showed that values of older monkeys are significantly higher than that of adults, whereas the right ventricular diameter at the end diastole is lower. This can be explained by the decreasing in physiological function of the heart and in the elasticity of ventricular muscle; this can lead to compensatory enhancement with adaptational hypertrophy.

The older monkeys exhibited markedly a decrease in the left ventricular compliance compared with young adults.

In Table 5 mean blood pressure (MAP) was reported (Tang et al., 2008).

	Young adult monkey	Older adult monkey	*P*-value
Vp(cm/s)	65.43 ± 3.82	62.90 ± 15.90	0.7928
E(cm/s)	81.76 ± 17.23	58.42 ± 9.30*	0.0017
A(cm/s)	66.98 ± 13.99	48.04 ± 10.10*	0.0067
E/A	1.22 ± 0.05	1.11 ± 0.23	0.3651
E/VP	1.23 ± 0.34	0.99 ± 0.23	0.1666
DecT(ms)	55.72 ± 13.93	106.40 ± 21.00*	5.4E-05
EVI(cm)	6.22 ± 2.08	6.02 ± 1.63	0.8360
AVI(cm)	2.47 ± 1.00	3.33 ± 0.85	0.0944
EVI/AVI	2.57 ± 0.25	1.71 ± 0.42*	0.0019
TVTI(cm)	8.69 ± 3.07	9.35 ± 1.35	0.5093
RFF	0.72 ± 0.02	0.64 ± 0.09	0.1130
AFF	0.28 ± 0.02	0.36 ± 0.09	0.1013
Er/Ar	1.16 ± 0.08	1.02 ± 0.16	0.1096
LVVmax(cm/s)	97.23 ± 15.15	79.47 ± 10.70*	0.0082
PVS(cm/s)	33.33 ± 3.76	36.56 ± 5.45	0.2815
PVD(cm/s)	61.28 ± 11.03	41.28 ± 3.65*	8.89E-06
S/D	0.55 ± 0.06	0.90 ± 0.11*	2.37E-05
AR(cm/s)	42.60 ± 4.50	27.93 ± 4.77*	3.41E-06
ARd(ms)	67.22 ± 6.74	82.62 ± 9.27*	0.0335
Ad(ms)	63.00 ± 8.08	84.50 ± 9.59*	0.0050
ARd/Ad	1.08 ± 0.14	0.88 ± 0.11*	0.0458
Ea(cm/s)	12.96 ± 0.74	7.68 ± 2.16*	0.0011
Aa(cm/s)	6.39 ± 0.38	7.52 ± 1.52*	0.2297
Ea/Aa	2.03 ± 0.07	1.09 ± 0.39*	0.0017
MVS(cm/s)	6.58 ± 0.20	7.14 ± 1.23	0.3366
IVRT(ms)	73.33 ± 2.72	79.62 ± 19.10	0.5314
E/Ea	7.19 ± 0.10	9.52 ± 1.70	0.0918

*$P < 0.05$, compared with the adult monkeys.

Table 4 - Doppler echocardiographic parameters (Tang et al., 2008)

	Young adult monkey	Older adult monkey	*P*-value
Blood Pressure			
SBP(mmHg)	117.39 ± 9.83	122.83 ± 18.09	0.2317
DBP(mmHg)	65.33 ± 7.88	75.10 ± 13.81*	0.0063
MBP(mmHg)	91.36 ± 6.65	98.97 ± 15.10*	0.0062

Table 5 - Ultrasonic parameters of blood vessels (Tang et al., 2008)

Ultrasonic technique was also used to investigate arterial compliance, vessel resistance and blood flow of the vessels including the major easily detectable arteries: common carotid artery, external carotid artery, internal carotid artery and abdominal aorta. (Table 6) (Tang et al., 2008)

In older monkeys, rigidity of the blood vessels increases while compliance and elasticity decrease, probably due to senescence.

Anatomy of the forelimb veins is described for Cercopithecoidea (including *M. mulatta*) (Thiranagama et al., 1989).

Rhesus monkeys have both radial and ulnar *venae comitantes* and in the upper arm veins accompany both the superficial and the deep brachial artery. The superficial brachial vein is continuous with the radial *venae comitantes*. The two brachial vein unit around the middle part of the upper arm giving rise to a single brachial vein which continued as the axillary vein: Figure 6.

These veins receive the veins accompanying the major arterial branches. They also received muscular branches. The radial *venae comitantes* receive perforating veins, usually in the cubital and wrist regions.

	Young adult monkey	Older adult monkey	*P*-value
R-VA	0.70 ± 0.07	0.66 ± 0.09	0.4187
L-CCA	0.82 ± 0.06	0.81 ± 0.05	0.8769
L-ICA	0.69 ± 0.11	0.65 ± 0.08	0.3500
L-ECA	0.85 ± 0.06	0.78 ± 0.08	0.1006
L-VA	0.74 ± 0.09	0.68 ± 0.07	0.1451
Compliance			
R-CCA	17.70 ± 6.85	8.54 ± 4.14*	0.0010
R-BCA	12.83 ± 4.35	8.91 ± 4.02*	0.0398
R-ICA	16.30 ± 7.15	8.93 ± 4.46*	0.0083
L-CCA	17.82 ± 4.18	9.04 ± 3.21*	3.74E-05
L-BCA	16.00 ± 3.82	8.39 ± 4.08*	0.0008
L-ICA	14.16 ± 4.07	9.36 ± 3.75*	0.0166
AB-AO	0.01 ± 0.01	0.02 ± 0.01*	0.0050
Rigidity index β			
R-CCA	2.98 ± 0.84	6.67 ± 4.53*	0.0065
R-BCA	4.51 ± 2.21	5.71 ± 2.72	0.3456
R-ICA	5.32 ± 6.89	5.59 ± 2.02	0.8865
L-CCA	2.79 ± 0.51	5.10 ± 1.56*	0.0022
L-BCA	3.21 ± 0.81	6.20 ± 2.67*	0.0148
L-ICA	3.78 ± 0.99	5.54 ± 3.52	0.2475
Elasticity index			
R-CCA	0.35 ± 0.11	0.23 ± 0.11*	0.0413
R-BCA	0.50 ± 0.16	0.51 ± 0.23	0.8890
R-ICA	0.28 ± 0.15	0.23 ± 0.14	0.5258
L-CCA	0.40 ± 0.10	0.25 ± 0.08*	0.0020
L-BCA	0.72 ± 0.23	0.41 ± 0.21*	0.0066
L-ICA	0.22 ± 0.09	0.18 ± 0.09	0.3545

*$P < 0.05$, compared with the adult monkeys.

Table 6 - Ultrasonic parametes of blood vessels (Tang et al., 2008)

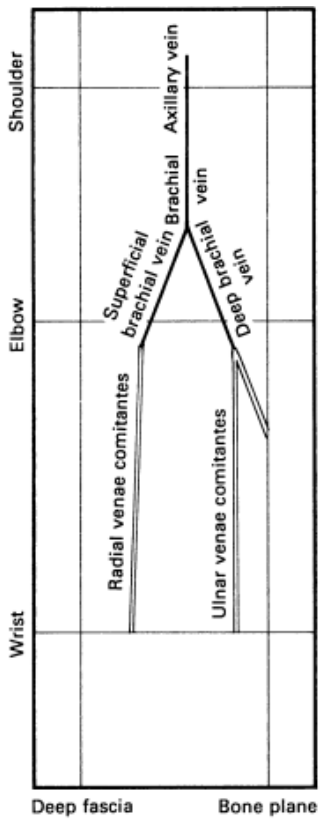

Figure 6 - Deep venous channels in the cercopithecoid forelimb. (Thiranagama et al., 1989)

In the proximal part of the thigh, the external iliac artery continues as the femoral artery, which is suitable for palpation of the pulse, after the a. profunda femoris has branched off (Figure 7). This artery gives origin to the lateral circumflex artery, which branches supply the vasti muscles. The femoral artery then divides into the saphena artery and the popliteal artery. Saphena artery emerges in the angle formed by the sartorius and gracilis muscles and runs superficially to the medial side of the tibia. She subsequently migrates to the cranial aspect of the tarsus to become the a. dorsal pedis (superficialis et profunda). From the popliteal artery branches the a. tibialis cranialis. She becomes the a. tibialis caudalis at the level of the lower leg. At the level of the foot, the a. tibialis caudalis divides into the a. plantaris lateralis et medialis. (Casteleyn & Bakker, 2022)

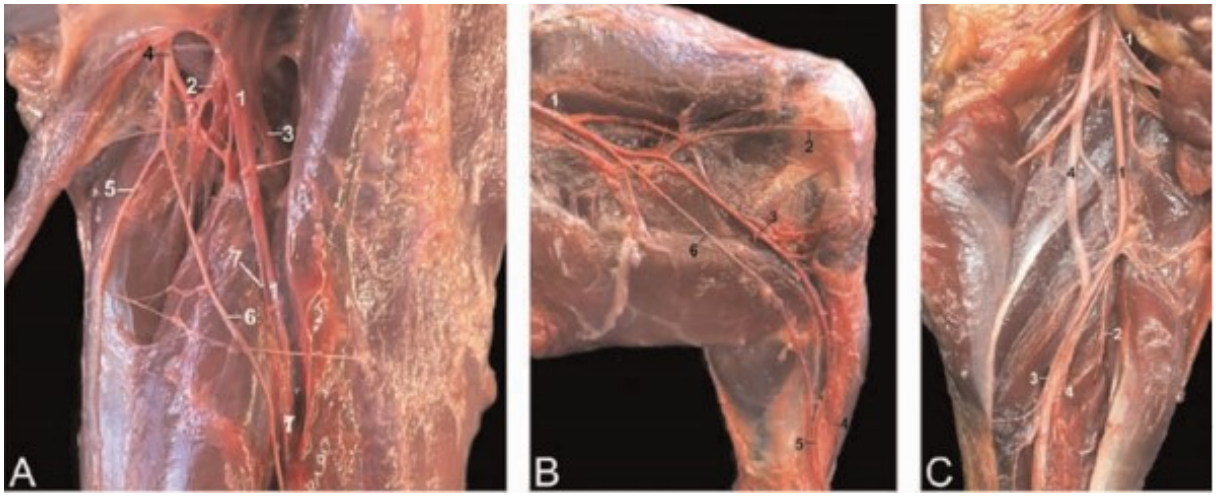

Figure 7 - Vasculature and nerves of the pelvic limb. A: Dorsomedial view of the right upper leg with 1: a. femoralis, 2: a. circumflexa femoris lateralis, 3: a. profunda femoris, 4: n. femoralis, 5: rami cutanei craniales, 6: n. saphenus. B: Medial view of the thigh and knee of the left leg with 1: a. femoralis, 2: a. genus proximalis, 3: a. saphena: 4: a. dorsalis pedis profunda: 5: a. dorsalis pedis superficialis, 6: n. saphenus. C: Caudal view of the popliteal region of the left leg with 1: a. poplitea, 2: a. tibialis cranialis, 3: a. tibialis caudalis, 4: n. tibialis. (Casteleyn & Bakker, 2022)

In analogy with the thoracic limb, the venous drainage of the pelvic limb is mainly effectuated by the vv. *comitantes*. The vv. *marginalis* medialis et lateralis pedis drain the dorsal side of the foot. The v. *marginalis* medialis pedis drains into the superficially located v. *saphena* magna that proximately flows into the femoral vein. The v. *marginalis lateralis pedis* drains into the v. *saphena parva*.

It is an important vein as it drains the larger part of the hind leg and is suitable for venipuncture at the caudal aspect of the calf. In the popliteal fossa, she drains into the popliteal vein. This vein is also suitable for venipuncture. (Figure 8) The femoral vein proximally drains into the external iliac vein that in turn flows into the common iliac vein (Figure 9). (Casteleyn & Bakker, 2022)

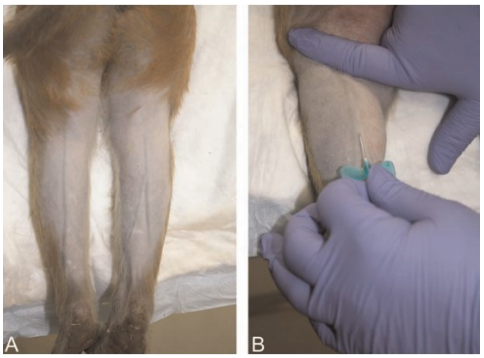

Figure 8 - Superficial veins of the pelvic limb. A: Subcutaneous localization of the v. saphena parva. B: Catheterization of the v. saphena parva. (Casteleyn & Bakker, 2022)

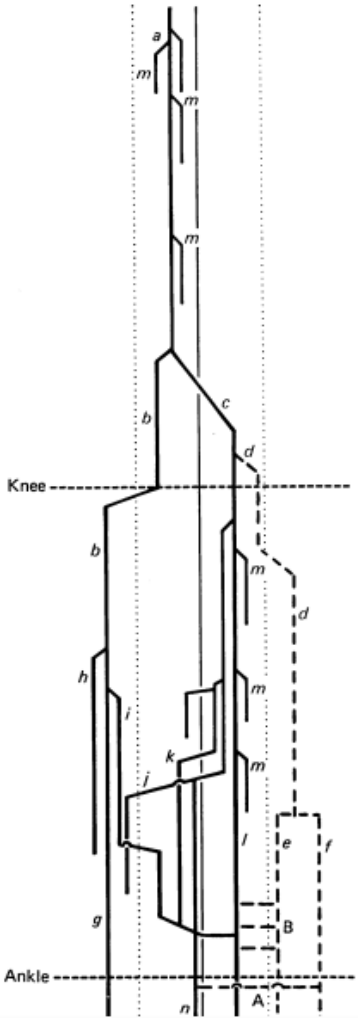

Figure 9 - Distribution of main venous channels in hind limb of Macaca fascicularis. Horizontal interrupted lines indicate planes of knee and ankle joints. Heavy dashed lines represent autonomous veins and the solid lines, venae comitantes. Diagram not to scale: (a) femoral vessels; (b) venae comitantes of main trunk of arteria saphena; (c) popliteal vessels; (d) short saphenous veins (s.s.v.); (e) medial tributary of s.s.v.; (f) lateral tributary of s.s.v.; (g) main continuation of venae comitantes of arteria saphena; (h) venae comitantes of posterior branch of arteria saphena; (i) venae comitantes of anterior or deep branch of arteria saphena; (i) posterior tibial vessels;(m) muscular vessels; (n) dorsalis pedis vessels. (A) Constant perforator connecting lateral tributary of short saphenous vein (f) with venae comitantes of the deep branch of the arteria saphena. (B) Constant perforators connecting short saphenous vein with venae comitantes of the posterior tibial artery. (Chapple & Wood, 1980)

1.4 Anatomy and physiology of the respiratory system in Rhesus macaque

The respiratory system of *M. mulatta* is similar to the respiratory system of other mammals, in particular the human species. The lungs are contained in the region from the 2nd to the 8th intercostal space.

The right lung consists of cranial, middle, caudal and accessory lobes, and the left lung has two lobes: cranial and caudal lobes. (Figure 10) (Casteleyn & Bakker, 2022)

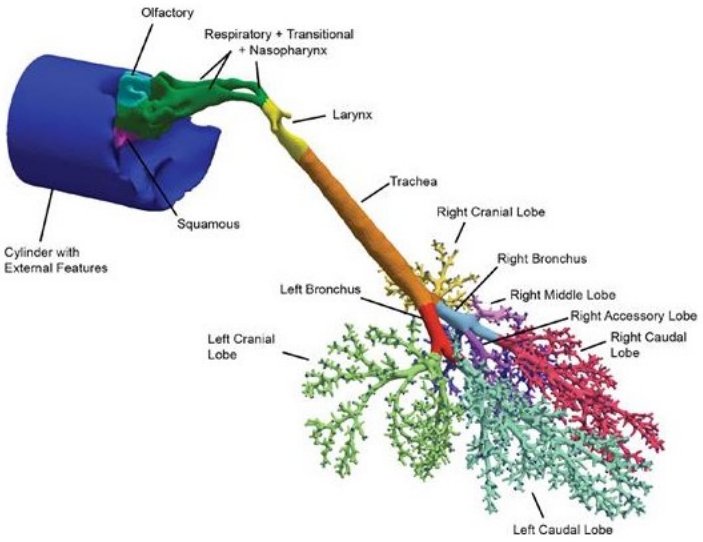

Figure 10 - Surface maps of hybrid CFD/PBPK models for male Rhesus monkey showing specific regions of the respiratory airways categorized by epithelial cell type or anatomic regions indicated by different surface colours. (Corley et al., 2012)

Each lung lobe is ventilated by a principal bronchus. The trachea counts approximately 27 cartilaginous ring and measures approximately 10 cm in length and 1 cm in diameter. (Casteleyn & Bakker, 2022)

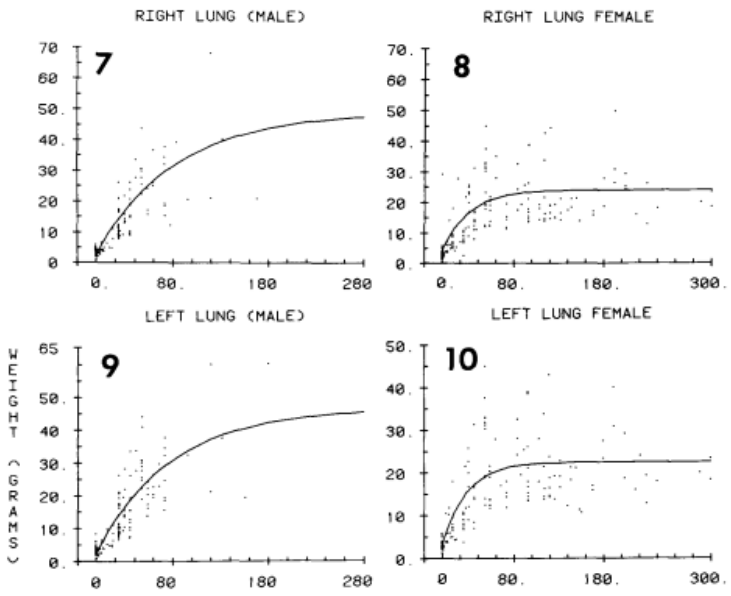

Figure 11 - Growth curve of lungs weights plotted over the individual measurements. (Cupp & Ventura, 1981)

The lung growth shows marked differences between males and females. (Cupp & Ventura, 1981) Female exhibited steep weight increases in both right and left lungs up to six years of age, as shown in Figure 11 (boxes 8 and 10). Lung weights then remained unchanged for the rest of the life. In males, both right and left lungs increased markedly throughout the entire lifespan of the animals. (Figure 11: boxes 7 and 9) The right lung was significantly heavier than the left in females but not in males.

Lung volume (V_L) is the sum of the volume of the trachea and all the airway volumes proceeding distally from the trachea down until terminating at an alveolar sac (Figure 12). V_L can be estimated with the measurement of the total lung capacity (TLC). (Asgharian et al., 2012)

Hyde et al. (2007) determined the measurements of V_L as a function of body weight.

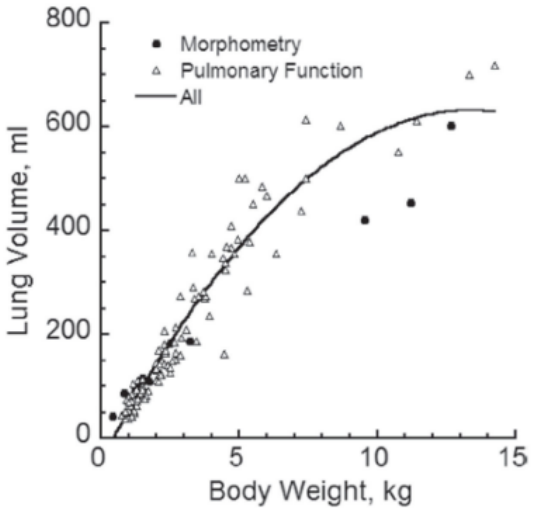

Figure 12 - Lung volume as a function of body weight in male rhesus monkeys. The open triangles are total lung capacity measurements of lung volume from pulmonary function studies. The closed circles are lung volume data from the morphometry study of Hyde et al. (2007). A quadratic fit to all the data is shown. Asgharian et al., 2012)

Geometry model of the conducting airways for rhesus monkeys was developed based upon postmortem CT images of a six-month-old rhesus macaque lungs. (Asgharian et al., 2012) Physiologic variables were established as follows: route, rate and depth of breathing, the volume of the upper respiratory tract (URT), and the volume of the lungs at functional residual capacity (FRC).

The volume of the URT is reported in the following Table 7:

			Nasal + maxillary sinus		Nasal only		
Group	Age (days)	BWT (kg)	Volume (cm^3)	Surface area (cm^2)	Volume (cm^3)	Surface area (cm^2)	SA to volume ratio
Air	180	1.3	1.06	20.6	0.96	18.73	19.55
	179	1.498	1.28	27.8	1.18	25.93	22.01
	176	1.55	2.44	76.2	2.34	74.33	31.79
	177	1.39	2.54	82.6	2.44	80.73	33.11
Ozone	179	1.548	1.18	26.8	1.08	24.93	23.12
	178	1.32	1.24	27.4	1.14	25.53	22.43
	182	1.363	2.12	54.4	2.02	52.53	26.03
	177	1.43	2.62	98.6	2.52	96.73	38.41
Ave. & Std. Air		1.43 ± 0.11			1.73 ± 0.77	49.93 ± 32.11	26.62 ± 6.83
	Ozone	1.42 ± 0.10			1.69 ± 0.70	49.93 ± 33.75	27.50 ± 7.44
	All Animals	1.42 ± 0.10			1.71 ± 0.68	49.93 ± 30.50	27.06 ± 6.63

Table 7 - Nasal plus maxillary sinus and only nasal volumes and surface areas of 6-month-old male rhesus monkeys exposed to clean air or cyclic ozone for 6 months. (Asgharian et al., 2012)

The relationship between URT volume and the body weight of rhesus monkeys from 1.79 kg to 10 kg is highly linear over this body weight range, as reported in Figure 13.

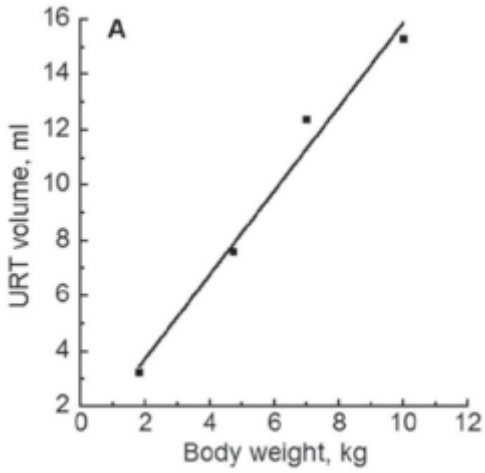

Figure 13 - Relationship of body weight of rhesus monkeys to the volume of the upper respiratory tract (Asgharian et al., 2012)

This study compared physiological values from three different studies (Binns et al., 1972; Brooks et al., 1957; Karel & Weston, 2015).

For animals weighing up to 4.5 kg there is no relationship between respiratory frequency and body weight for either sex. The overall breathing frequency for the males and females was 39.7 and 39.6 acts per minute (apm), respectively.

There is a significant variability in the values of the minute volume because body weight and sex seem to significantly influence these values. Therefore, logarithmic models were suggested to calculate the minute volume. Nevertheless, another study did not specify sex and they were grouped together, and the same parameters were examined (Table 8).

				Body weight (kg)			Breathing Frequency (bpm)			
Study	**Anesthesia**	**Sex**	**n**	**Range**	**Mean**	**Standard deviation**	**Range**	**Mean**	**Standard deviation**	**Minute Volume (L/min)**
Karel & Weston (1946)	No	Male	19	1.99–3.55	2.58	0.42	20–49	38.8	6.9	1.133
	No	Female	19	2.19–3.54	2.65	0.34	27–48	36.8	6.5	0.937
Binns et al. (1972)	No	Male	7	2.4–4.3	3.48	0.59	33–51	38.3	5.8	1.481
	No	Female	8	2.8–3.9	3.33	0.38	29–57	39.8	9.7	1.820
Brooks et al. (1957)	Yes	Male	5	3.44–5.35	4.05	0.78	28–53	47.6	5.0	2.010
	Yes	Female	9	3.07–4.5	3.81	0.52	32–71	45.3	14.6	1.907
Crosfill & Widdicombe (1961)[a]	Yes	NS[b]	4	1.8–3.05	2.45	NS	27–47	33.0	NS	0.700
Guyton (1947)	No	NS	6	2.05–3.08	2.68	NS	31–52	40.0	NS	0.863
Lees et al. (1965)	Yes	NS	14	4.2–6.7	5.80	0.86	31–52	42.7	7.0	1.791
Liu and DeLauter (1977)	No	Male	4	3.6–4.5	NS	NS	NS	28.0	6	1.100[e]
Howell (1995)	No	Both	6[c]	7–14.8	NS	NS	NS	21.4	1.6	2.000
Howell et al. (1995)	No	Both	3[d]	7.2–14.2	NS	NS	NS	18.9	1.7	2.000
						Overall values		39.0	7.8	
						Sample size			91	

[a] The authors did not state the monkeys were rhesus but Liu and DeLauter (1977) included their data in a table for rhesus monkeys.

[b] NS: not reported by the author(s).

[c] Five males and one female.

[d] Two males and one female.

[e] Value is mean after 1 hr; the 6 hr mean was 1.082.

Table 8 - Body weight and physiological respiratory variables for rhesus monkeys (Asgharian et al., 2012)

Tidal volume can then be indirectly estimated by minute volume and respiratory frequency. Tidal volume is approximately 10 ml/kg. Total lung capacity in function of body weight was

analysed by taking in account of the three mentioned studies and may be approximately estimated in 621 mL for a 10 kg rhesus macaque. Also, functional residual capacity was studied in function of body weight and may be approximately estimated in 413 mL for a 10 kg rhesus macaque. (Asgharian et al., 2012)

Chapter 2

2.1 Pre-operative assessment

Pre-operative assessment includes proximate and remote history, physical examination, laboratory findings and the evaluation of the influence of the current experimental protocol on anaesthetic management. The animal's clinical and experimental history should be reviewed with respect to current or recent treatments or procedures; previously applied anaesthetic regimes should be recorded.

A thorough physical examination in the awake animal is not possible but it is important to detect any sign of illness prior to any subsequent procedure such as unusual posture or behaviour, anorexia and abnormal urine or feces. Obtaining bodyweight and temperature, auscultation for heart rhythm and bilateral lung sounds, palpation of femoral artery and observation of the animal's mucosal colour and perfusion will provide additional information.

It is not necessary to perform additional clinical laboratory testing in animals that are in good physical condition. However, in anticipating the impact of an experimental protocol on the animal's health additional laboratory data may be meaningful.

Preoperative fasting is preferably at least of 12 hours to decrease the risk of pulmonary aspiration. (Popilskis Sulli J. et al., 2008).

The prolonged withholding of water neither reduces gastric volume nor increases pH and doesn't increase the risk of aspiration of gastric contents when compared to 3 hours of withholding fluids so the latter is sufficient. (Popilskis et al., 1992)

Some studies report the use of IM histamine receptor antagonists such as cimetidine (10 mg/kg) or ranitidine (1.5 mg/kg) 30-40 minutes prior induction in *Papio spp*. to provide adequate protection against aspiration pneumonia (Popilskis et al., 1992).

A more recent study suggests the pre-operative use of NK1 receptor antagonists to reduce the risk of post-operative vomiting and aspiration pneumonia in *M. mulatta* undergoing neurosurgeries. The incidence of post-operative vomiting with 1 mg/kg of subcutaneous (SC) maropitant is reduced from 11% to 1% in a total of 32 macaques. The plasma concentration reaches the proposed plasma level for clinical efficacy 20 minutes after maropitant SC administration. (Steinbach et al., 2018)

2.2 Chemical restraint

In NHPs primates the delivery of anaesthesia requires a restraint that includes physical (squeeze back cage, manual restraint or chair/tube restraint) (Figure 14) or chemical means which can be associated with cooperative training such as presenting limb for intravenous or intramuscular injection.

The method of restraint should be planned to ensure the safety of both the personnel and the animal.

The safest method of restraint for the staff and least stressful for the animal is the pharmacological one. Chemical restraint is obtained using various sedative agents usually administered through intramuscular injection in large muscle mass such as quadriceps femoris. (Figure 14) To avoid muscle necrosis in the site of injection after repeated administration with low pH drugs (e.g., ketamine) is preferable to change the site (e.g., using the opposite limb). (Popilskis Sulli J. et al., 2008)

NHPs are often non-compliant, and thus it is mandatory that sedation is profound. Consequently, pharmacological restraint is commonly based on the administration of preanesthetics combined with drugs usually used for general anesthesia (e.g., ketamine and alfaxalone).

Figure 14 - Squeeze-back cage demonstrating the proper positioning of a nonhuman primate for injection. The side of the animal faces the front of the cage allowing injection into the muscles of the upper arm or thigh. (Fortman et al., 2018)

2.2.1 Ketamine

Ketamine, an analogue of phencyclidine (Figure 15), has been widely used for chemical restraint in NHPs since the 1970s. It is a low pH molecule that is generally included in preparations with a preservative agent such as benzethonium hydrochloride. it is a racemic compound with equal parts of the two stereoisomers S (+) and R (-) of which the S isomer can produce hypnosis for the double of the time and a much more profound analgesia compared to the other.

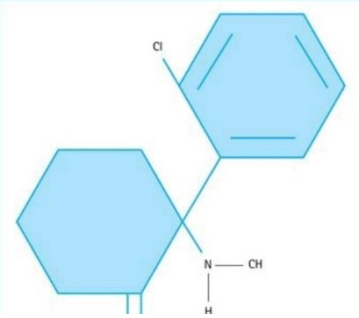

Figure 15 - Ketamine chemical structure. (Bufalari et al., 2012)

Ketamine produces a dissociative anaesthesia with functional and electrophysiological separation or dissociation between the limbic system and the thalamic-neocortical system that are respectively associated with the integration and elaboration of nociceptive stimuli and the mediation of painful responses, the influence of the pain's emotional components and the behavioural outputs.

Ketamine produces a catalectic state characterized by hyper tone, not pain stimulated twitching, the permanence of the laryngeal and palpebral reflexes, central pupil and nystagmus.

It has analgesic properties primarily due to the non-competitive antagonist action against NMDA glutamate receptors, even at subanaesthetic doses. It can produce more somatic analgesia compared with visceral analgesia.

Ketamine influences the central nervous system (CNS) incrementing cerebral perfusion, intracranial pressure, cerebrospinal fluid pressure due to cerebral vasodilatation, and increased systemic blood pressure.

It indirectly stimulates the cardiovascular system increasing the HR, cardiac output, blood pressure and the oxygen consumption by stimulation of the central adrenergic control structures or inhibition of the neuronal reuptake of catecholamines.

Ketamine needs to be used with caution in patients with cardiac problems because the partial increase in the coronary flux cannot be sufficient to compensate for the myocardia oxygen demand.

After administration of ketamine in hypovolemic patients, there is a rise in HR and blood pressure not associated with a rise in cardiac output.

The respiratory function is maintained with the use of ketamine. High doses can induce apneustic breathing. (Bufalari et al., 2012)

Ketamine determines an increase of salivary and tracheobronchial secretions; these effects may be prevented by administration of atropine at a dose of 0.02-0.05 mg/kg IM prior the ketamine injection. (Popilskis Sulli J. et al., 2008)

In NHPs ketamine has a wide range of safety.

Ketamine alone can be used as a sedative agent at 5-20 mg/kg IM; sedation is achieved in about 5 minutes after injection providing 15-30 minutes of chemical restraint. Complete recovery occurs within 40-60 minutes. (Popilskis et al., 2008)

It is described that doses from 5 to 12 mg/kg can induce chemical restraint while doses from 10 to 30 mg/kg determine surgical anaesthesia. (Beck & Dresner, 1972; Ochsner, 1977; Colillas, 1978)

There are some disadvantages in utilizing ketamine alone such as poor muscle relaxation, spontaneous movement and hypersalivation, and long and rough recovery. ((Banknieder et al., 1978) F. J. Sun et al., 2003) Moreover, ketamine used for repeated sedations can induce tolerance in the patient; the time to ataxia and subsequent loss of the righting reflex in 8 rhesus macaques increases significantly after the first IM injection of ketamine of 10 mg/kg (Figure 16). (Settle et al., 2010)

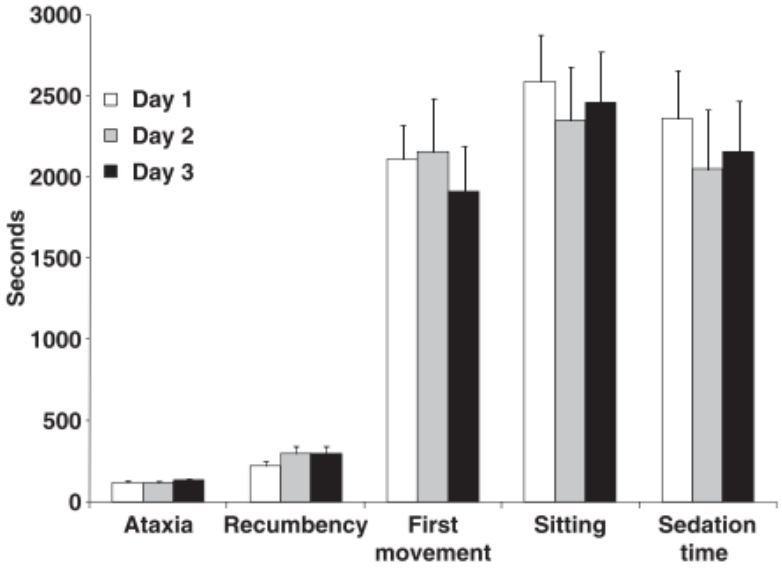

Figure 16 - Anesthesia time points (s) (mean ± SE) for ketamine by day. (Settle et al., 2010)

For these reasons, ketamine is rarely used alone, and in literature where reported different protocols in which including ketamine are combined whit various sedatives for chemical restraint of described in *M. mulatta*.

2.2.2 A2 agonists

Each α2-agonist has different selectivity for α2 receptors and α1 receptors with different effects on the organism. They are sedative agents with anxiolytic effect. Depending on the emotional state of the patient they exert their sedative action with different efficacy: in case of pre-existent stress, agitation, fear, pain or excitement there is an increasing in circulating endogenous catecholamines that undermine the reduction in the releasing of excitatory neurotransmitters induced by the α2-agonists.

If the patients sedated with α2 agonist are stimulated with noises or painful stimuli a sudden awakening may result.

They have analgesic properties primarily by spinal and supraspinal action on $\alpha 2_{A/D}$ receptors, located in the cortex and in the spinal cord. Analgesia is dose-dependent and has shorter duration compared with the sedative effect.

A2-agonists have an important impact on the cardiovascular system: in fact, they induce a first increase in the peripheral vascular resistance with transient hypertension, after that the action on the α2 receptors causes bradycardia and moderate hypotension.

The respiratory parameters are not significantly affected, with a modest reduction in the RR.

A2 agonists effects can be reversed with the administration of atipamezole (Bufalari et al., 2012).

When chemical restraint of NHPs is required, α2 agonists are always used combined with other drugs, otherwise they only have tranquilizing effects. (Naccarato & Hunter, 1979)

Ketamine/xylazine

This combination provides sedation, muscle relaxation and analgesia sufficient for minor surgical procedures. It is reported 10 mg/kg ketamine and 0.25 mg/kg xylazine can produce 45 minutes anaesthesia; by increasing the dose of xylazine to 2 mg/kg, the anaesthesia duration is prolonged to 138 minutes. This combination produces a significant decrease in MAP and HR compared to ketamine alone.

It is considered the minimum effective IM dosages of ketamine and xylazine to be 2.5 mg/kg and 0.25 mg/kg respectively. (Capuano III et al., 1999; Naccarato & Hunter, 1979)

Ketamine/medetomidine

The advantages of this combination compared to ketamine/xylazine are similar, such as analgesia, anesthesia and muscle relaxation. Medetomidine is a more selective and specific α2 agonist. It cannot be used alone to immobilize rhesus macaques, even with intravenous (IV) dosages of 0.2 mg/kg. (Capuano et al., 1999) Another study confirms this finding in Japanese macaques (*Macaca fuscata*) sedated with IM medetomidine at a dosage of 120 μg/kg. (Miyabe et al., 2001)

Medetomidine (0.15 mg/kg) combined with ketamine (3 mg/kg) in rhesus macaques induces a deeper plan of anaesthesia of longer duration compared to ketamine alone. (F. J. Sun et al., 2003)

Rhesus monkeys anesthetized with 2 kg/kg of IM ketamine and 0.075 mg/kg of IM medetomidine had a HR of 135 at 5 minutes after injection declining to 115 at 25 minutes after injection. MAP were 70 and 65 mmHg, respectively. By adding medetomidine to ketamine, there is a marked depressive effect on the cardiovascular apparatus, with a negative trend of the HR and of the MAP. At 20 minutes there is evidence of the decreasing of circulating ketamine concentrations, with the overriding depressive effect of medetomidine. There is also a clear decreasing trend in temperature beyond 25 minutes. It is suggested that to reverse this effect can be administered atipamezole or reduced the dose of medetomidine. (Settle et al., 2010)

Doses of ketamine lower than 2 mg/kg may not be adequate to achieve anesthesia, even in combination with other agents. (West Gary et al., 2014)

The doses of ketamine and medetomidine produce different effects also according to the species. In fact, a study demonstrates that 2 mg/kg of ketamine combined with 50 μg/kg of medetomidine is insufficient for immobilization of cynomolgus monkeys. (Young et al., 1999)

For most NHPs species, the researchers recommend a ketamine dose of 5-8 mg/kg combined with a low dose of medetomidine (0.02-0.04 mg/kg) for induction or short duration anesthesia. (West Gary et al., 2014)

Ketamine/dexmedetomidine

The combination of ketamine (5 mg/kg) and medetomidine (0.02 mg/kg) administered intramuscularly is described in 7 rhesus macaques in comparison with IM ketamine combined with diazepam (10 mg/kg and 1 mg/kg, respectively) and with IM Telazol (5 mg/kg) (Vaughan et

al., 2014). There were no significant differences in RR, systolic blood pressure (SBP) and diastolic blood pressure (DBP) between groups. HR was significantly lower (p < 0.001) in the ketamine/diazepam group compared with the other two groups, as reported in Figure 17.

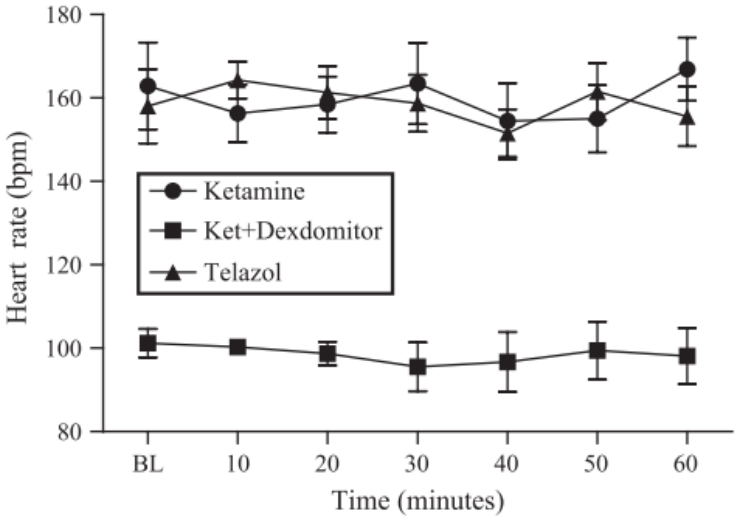

Figure 17 - Mean ± SD heart rate during three anesthesia conditions across seven time points during one-hour intravenous glucose tolerance testing (n = 7 animals in each condition). (Vaughan et al., 2014)

Two other protocols for IM sedation are described in literature: 3 mg/kg ketamine and 0.05 mg/kg dexmedetomidine (D. C. Turner et al., 2019) and 4 mg/kg ketamine and 0.05 mg/kg dexmedetomidine (Richardson et al., 2017) whereas the sedation lasted 40-70 minutes.

2.2.3 Benzodiazepines

Benzodiazepines are tranquilizers whose target is GABA receptors. They have anxiolytic, sedative, hypnotic, amnesic, myorelaxant, and anticonvulsant effects. Flumazenil is the specific antagonist of this class of drugs.

The clinic response to benzodiazepines administration is often unpredictable, especially when used alone. They can induce agitation in the patient that can interfere with the action of other injectable agents. Nevertheless, they are useful and safe in clinically compromised patients.

Benzodiazepines have not analgesic properties. (Bufalari et al., 2012)

Ketamine/Diazepam

Diazepam may be administered alone intramuscularly at 1 mg/kg or intravenously at 0.25-0.50 mg/kg. It is important to underscore that IM injection of diazepam may be painful, with

unreliable absorption; multiple doses may lead to prolonged recovery due to its long elimination half-life. (Popilskis Sulli J. et al., 2008)

The addition of a benzodiazepine to ketamine can provide muscle relaxation and anticonvulsant activity but in macaques is insufficient to allow endotracheal intubation. (Popilskis et al., 2008)

The doses reported in adult male *Papio* during a dental study are 10 mg/kg of ketamine combined with 0.2-0.35 mg/kg of diazepam given intramuscularly. (Woolfson et al., 1980)

Ketamine/Midazolam

Compared to diazepam, midazolam is better absorbed after IM injection, provides more effective anxiolytic sedation, and has a shorter elimination half-life.(Jacobs et al., 1993)

The combination of ketamine and midazolam at doses of respectively 10 mg/kg and 1 mg/kg IM in common marmosets and black-tufted marmosets produces rapid immobilization and good muscle relaxation for 30-45 minutes. (Table 9) (Furtado et al., 2010)

Group	Induction (min)	Immobilization (min)	Recovery (min)
CJR (n = 5)	2.9 ± 2.1	38.6 ± 31.5	53.8 ± 29.7
CJS (n = 5)	2.1 ± 0.5	32.8 ± 8.9	35.4 ± 8.2
CPR (n = 5)	1.6 ± 1.0	44.4 ± 15.1	46.0 ± 17.7
CPS (n = 5)	1.7 ± 0.9	28.8 ± 14.4	33.8 ± 24.9

[a] CJR, common marmosets receiving racemic ketamine; CPR, black-tufted marmosets receiving racemic ketamine; CJS, common marmosets receiving (S+) ketamine; CPS, black-tufted marmosets receiving (S+) ketamine.

Table 9 - Mean (±SD) duration of induction, immobilization, and recovery after administration of either midazolam and racemic ketamine (CJR, CPR), or midazolam and S (+) ketamine (CJS and CPS) in *Callithrix jacchus* (CJR, CJS) and Callithrix penicillata (CPR, CPS). (Furtado et al., 2010)

It has been described also the administration of oral midazolam (1 mg/kg) with IM ketamine (8 mg/kg) in 8 macaques, but unfortunately this protocol did not provide adequate sedation and did not improve muscle relaxation (Table 10). (Lee Vanessa K et al., 2010)

	KetMed (n = 8)			KetMid (n = 8)			Ket (n = 7)		
	T0	T10	T20	T0	T10	T20	T0	T10	T20
Jaw tone	4.125 (0.295)	4.125 (0.295)	4.000 (0.267)	1.750 (0.366)	1.286 (0.184)	1.286 (0.286)	1.714 (0.421)	1.714 (0.286)	1.714 (0.286)
Spontaneous movement	4.625 (0.375)	5.000 (0.0)	5.000 (0.0)	4.125 (0.441)	2.286 (0.522)	1.500 (0.267)	3.857 (0.634)	2.714 (0.606)	2.714 (0.606)
Palpebral reflex	4.250 (0.412)	4.750 (0.250)	4.625 (0.375)	2.000 (0.378)	1.375 (0.263)	1.286 (0.286)	2.429 (0.612)	2.000 (0.535)	2.000 (0.535)
Limb manipulation	4.750 (0.250)	4.875 (0.125)	5.000 (0.0)	2.571 (0.369)	1.500 (0.224)	1.167 (0.167)	3.429 (0.429)	2.714 (0.522)	2.429 (0.571)
Pedal reflex	4.875 (0.125)	4.875 (0.125)	4.750 (0.250)	2.625 (0.460)	1.571 (0.369)	1.143 (0.143)	3.714 (0.474)	3.571 (0.571)	2.429 (0.612)

Values are indicated as mean (SEM).

Table 10 - Scores for depth of anesthesia. (Lee et al., 2010)

Medetomidine/midazolam

13 Japanese macaques (*M. fuscata*) were successfully and profoundly sedated with the combination of IM medetomidine and midazolam (30 µg/kg and 0.3 mg/kg, respectively) (Miyabe et al., 2001). The onset and the duration of action are reported in the following table (Table 11). One macaque did not become recumbent, probably for biological variability. Authors suggested to administer higher doses or to give a half dose of the same protocol.

	Sedative onset	Time to lateral recumbency	Lateral recumbency
Medetomidine-midazolam	4 ± 1	12 ± 5 (5/6)	74 ± 37 (5/6)

Table 11 - Onset and duration of action (minutes) of medetomidine-midazolam to take effect and the duration of lateral recumbency. Data are expressed as mean ± standard deviation (n=6). (Miyabe et al., 2001)

Physiological parameters were not evaluated. No adverse effects occurred during the sedation with this combination.

2.2.4 Tiletamine/Zolazepam

Tiletamine is a dissociative agent chemically like ketamine with similar anesthetic and analgesic properties. Tiletamine has longer duration and more marked side effects (apneustic breathing, muscle rigidity, clonic-tonic movements) compared to ketamine. Like in ketamine anesthesia, most of the reflexes are maintained. Moreover, tiletamine produces dose-dependent respiratory depression.

Tiletamine is commercially available in combination with zolazepam (Zoletil® 1:1 ratio), which is a benzodiazepine with myorelaxant and anticonvulsant properties able to counteract the side effects of tiletamine.

In various animal species, the difference in the metabolism of the two molecules influences the quality of the recovery. In the dog, zolazepam is metabolized before tiletamine so dysphoria is a common side effect during the recovery. In the cat, tiletamine has a shorter duration thus the recovery is usually uneventful. (Bufalari et al., 2012)

In NHPs tiletamine/zolazepam at a recommended dosage of 4-6 mg/kg IM can maintain anesthesia for about 45-60 minutes. (Cohen & Bree, 1978) High doses produce longer duration of anesthesia and cause reduced rectal temperature in cynomolgus monkeys compared with ketamine/acepromazine combination. (López et al., 2002) Tiletamine/zolazepam can also cause

overheating that lasts for about 24 hours after induction and can cause convulsions and ataxia during recovery.

It is reported a tiletamine/zolazepam sensitivity in some NHP which lead to some clinical signs such as rigid muscles, exaggerated movements of the limbs, torticollis and bruxism. (Marion et al., 2022)

Tiletamine/Zolazepam/Medetomidine

A study (Fahlman et al., 2006) described the use of tiletamine/zolazepam (0.8-2.3 mg/kg IM) combined with medetomidine (0.02-0.06 mg/kg IM). This mixture results in rapid and smooth induction in about 1-7 minutes, good muscle relaxation and anesthesia for gibbons and macaques, that had a RR of 20-56 breaths per minute, 68-160 beats per minute and SpO2 of 88-99%. Some animals presented hypotension and hypoxemia (Table 12).

Parameter	0–10 min	11–20 min	21–30 min	31–40 min
RR (breaths/min)	41 ± 11 (24–60) *n* = 10	47 ± 23 (26–112) *n* = 12	48 ± 25 (22–120) *n* = 11	38 ± 10 (24–54) *n* = 8
HR (beats/min)	84 ± 15 (64–114) *n* = 11	90 ± 13 (68–112) *n* = 12	88 ± 13 (64–104) *n* = 10	85 ± 13 (66–106) *n* = 8
Rectal temp. (°C)	37.2 ± 0.7 (35.7–38.4) *n* = 9	37.2 ± 0.8 (35.4–38.3) *n* = 11	36.8 ± 1.1[bc] (34.7–38.6) *n* = 9	36.1 ± 0.6[bc] (35.0–36.9) *n* = 6
Tympanic temp. (°C)	37.1 ± 0.8 (35.4–38.0) *n* = 8	37.0 ± 0.8 (35.5–38.4) *n* = 9	36.7 ± 1.0[bc] (34.8–38.3) *n* = 9	36.4 ± 1.3[bc] (34.7–38.6) *n* = 6
SAP (mmHg)	125 ± 12 (111–148) *n* = 5	106 ± 22[b] (74–134) *n* = 6	94 ± 14[b] (71–118) *n* = 7	92 ± 11[bc] (80–111) *n* = 5
SpO_2 (%)	88 ± 0 (88–88) *n* = 2	92 ± 1.3 (90–93) *n* = 4	92 ± 1.0 (82–93) *n* = 4	92 ± 2.0 (89–94) *n* = 4

[a] RR = respiratory rate; HR = heart rate; SpO_2 = pulse oximetry derived hemoglobin oxygen saturation; SAP = systolic arterial blood pressure measured noninvasively. Values expressed as mean ± SD (range).
[b] Significant difference from the 0–10 min value.
[c] Significant difference from the 11–20 min value.

Table 12 - Physiological parameters for Bornean orangutans anesthetized with medetomidine-zolazepam-tiletamine at the Sepilok Orangutan Center, Malaysia. (Fahlman et al., 2006)

2.2.5 Phenotiazines

Acepromazine belongs to the phenothiazine class that antagonizes the dopamine action on the basal ganglia and forebrain limbic zones. Acepromazine has a high therapeutic index. Low doses of acepromazine reduce spontaneous movements without influencing spinal reflexes and reactions to stimuli. High doses induce a cataleptic state with abnormal positions, indifference to most of the stimuli, maintaining of conscience, and retraction reaction to painful stimuli.

Acepromazine preserves from substances that bind dopaminergic receptors in the Chemoreceptor Trigger Zone in the medulla oblongata, and thus that are able to induce vomit, with antiemetic effect. This sedative agent has a high therapeutic index.

On the cardiovascular apparatus it causes vasodilatation and hypotension. (Bufalari et al., 2012)

Phenothiazines have not analgesic properties.

It has been reported a combination of ketamine and acepromazine in a 10:1 mixture at 0.12 ml/kg that has been compared with 6 mg/kg of Telazol and a combination of alfaxalone (5 mg/kg) and midazolam (0.3 mg/kg) administered intramuscularly in 15 rhesus macaques and 15 cynomolgus monkeys undergoing a plethysmography study. Ketamine/acepromazine had the lowest anesthesia quality score in both species (Figure 18). (Marion et al., 2022)

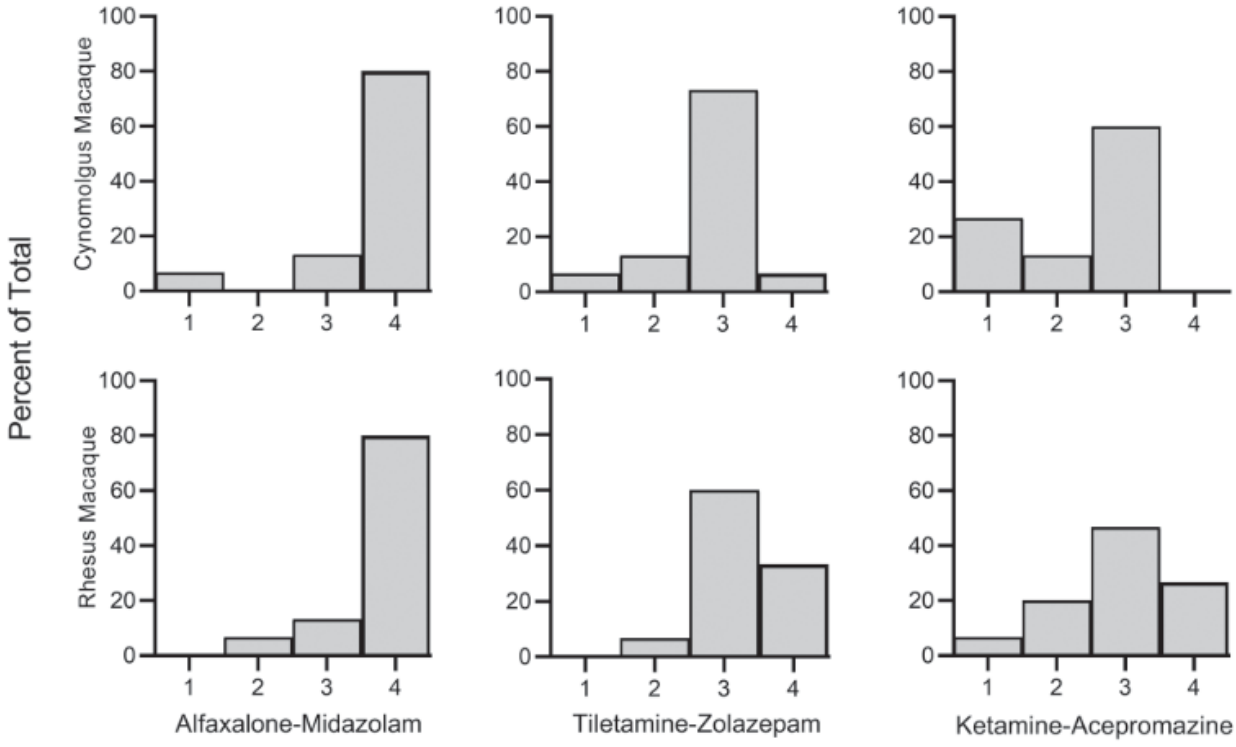

Figure 18 - Percentage of 15 NHPs per species scored for quality of anesthesia. Quality score are (1) no periods of steady state minute volume (SSMV) achieved, (2) one period of SSMV achieved at less than 20 minutes, (3) 2 periods of SSMV achieved with the first attempt sustained for less than 20 min, and (4) first attempt at SSMV sustained for 20 min. (Marion et al., 2022)

2.2.6 Alfaxalone

Alfaxalone is a synthetic neuroactive steroid that binds to type A gamma-aminobutyric acid receptors.(Martín Bellido & Vettorato, 2022).

Alfaxalone has minimal cardiovascular effects with dose-dependent decrease in RR, non-invasive blood pressure (NIBP), SpO2 and rectal temperature in 6 cynomolgus monkeys sedated with several alfaxalone doses administered intramuscularly (5 mg/kg, 7.5 mg/kg and 10 mg/kg), as described in Table 13. (Wada et al., 2020)

Valuables	Treatments	Minutes after the IM drug administration						
		10	20	30	45	60	90	120
RR (breaths/min)	CONT	38 (36–40)	37 (36–40)	34 (34–40)	36 (36–36)	36 (35–38)	34 (34–37)	35 (34–41)
	ALFX0.625	36 (35–36)	33 (32–36)	35 (34–36)	36 (36–36)	38 (34–38)	36 (35–38)	35 (34–36)
	ALFX1.25	35 (34–39)	36 (35–36)	38 (35–40)	35 (34–38)	37 (35–38)	38 (36–42)	35 (34–36)
	ALFX2.5	35 (29–40)	35 (28–36)	36 (30–38)	38 (30–40)	39 (32–40)	39 (35–42)	39 (34–42)
	ALFX5	27 (23–30)[a]	26 (23–31)[a]	25 (21–29)	29 (25–32)	30 (29–35)	31 (30–37)	30 (30–38)
	ALFX7.5	28 (26–33)[a]	25 (23–26)[a]	22 (21–25)[a]	23 (22–27)[a]	23 (21–26)[a]	29 (27–32)	33 (29–34)
	ALFX10	25 (21–30)[a]	24 (19–28)[a]	23 (21–26)[a]	23 (20–28)[a]	22 (20–29)[a]	25 (23–31)	33 (26–37)
HR (beats/min)	CONT	237 (232–242)	224 (215–245)	224 (212–230)	227 (201–244)	233 (224–240)	225 (214–233)	219 (217–221)
	ALFX0.625	233 (226–238)	232 (228–235)	221 (218–235)	222 (213–236)	216 (201–225)	227 (219–232)	204 (198–212)
	ALFX1.25	238 (237–241)	230 (215–244)	233 (212–244)	232 (214–234)	227 (213–234)	224 (214–228)	217 (206–219)
	ALFX2.5	234 (233–235)	223 (219–225)	226 (221–225)	217 (215–227)	231 (223–234)	225 (220–231)	225 (217–234)
	ALFX5	232 (228–235)	223 (213–202)	209 (201–235)	205 (191–208)	200 (194–213)	201 (195–216)	207 (195–213)
	ALFX7.5	243 (236–246)	233 (230–198)	208 (206–236)	200 (191–203)	205 (192–215)	208 (197–213)	205 (191–211)
	ALFX10	246 (239–253)	241 (239–207)	211 (208–244)	194 (185–205)	192 (189–207)	202 (193–216)	199 (188–212)
NMABP (mmHg)	CONT	100 (93–107)	98 (89–106)	97 (90–98)	101 (95–104)	95 (92–100)	95 (89–101)	92 (89–95)
	ALFX0.625	97 (94–101)	101 (94–103)	98 (90–101)	99 (96–103)	103 (99–104)	103 (98–105)	98 (94–102)
	ALFX1.25	100 (95–103)	95 (89–106)	93 (91–103)	102 (95–111)	99 (92–104)	97 (89–103)	102 (101–105)
	ALFX2.5	99 (97–103)	90 (84–95)	86 (86–94)	91 (89–95)	106 (101–110)	103 (101–109)	104 (99–107)
	ALFX5	89 (82–99)	100 (86–103)	99 (95–101)	98 (94–104)	107 (102–108)	108 (105–112)	106 (97–112)
	ALFX7.5	82 (79–92)	82 (71–84)	76 (62–78)[a]	78 (74–81)	93 (83–105)	99 (88–104)	103 (98–106)
	ALFX10	76 (70–79)[a]	71 (68–78)[a]	71 (64–84)[a]	76 (70–80)	101 (92–103)	98 (92–101)	98 (92–106)
SpO_2 (%)	CONT	98 (97–99)	98 (97–98)	98 (98–98)	98 (97–98)	98 (97–98)	98 (98–98)	98 (97–98)
	ALFX0.625	98 (97–98)	98 (98–99)	97 (97–99)	98 (97–98)	98 (98–98)	98 (98–99)	98 (98–99)
	ALFX1.25	98 (97–99)	98 (97–98)	98 (97–98)	97 (97–97)	98 (97–98)	99 (98–99)	98 (97–99)
	ALFX2.5	97 (95–97)	97 (96–97)	97 (97–98)	97 (97–97)	98 (97–99)	98 (98–98)	98 (98–98)
	ALFX5	95 (93–96)	93 (92–94)	94 (93–96)	96 (95–97)	96 (96–97)	97 (94–98)	97 (96–99)
	ALFX7.5	93 (92–94)[a]	92 (91–93)[a]	91 (90–92)[a]	93 (92–94)[a]	94 (93–96)[a]	96 (95–97)	98 (96–98)
	ALFX10	92 (91–93)[a]	90 (89–92)[a]	90 (89–91)[a]	92 (91–93)[a]	94 (93–95)[a]	96 (94–97)	97 (96–98)
RT (°C)	CONT	38.5 (38.4–38.6)	38.6 (38.5–38.9)	38.7 (38.6–38.8)	38.7 (38.6–38.9)	38.5 (38.4–38.5)	38.5 (38.2–38.7)	38.6 (38.5–38.8)
	ALFX0.625	38.5 (38.4–38.6)	38.5 (38.4–38.6)	38.5 (38.3–38.7)	38.5 (38.4–38.5)	38.5 (38.5–38.6)	38.6 (38.6–38.7)	38.4 (38.4–38.6)
	ALFX1.25	38.4 (38.3–38.6)	38.5 (38.4–38.5)	38.5 (38.4–38.5)	38.6 (38.5–38.6)	38.6 (38.4–38.7)	38.4 (38.3–38.5)	38.5 (38.4–38.6)
	ALFX2.5	38.4 (38.3–38.7)	38.1 (38.1–38.3)	37.8 (37.4–38.1)	37.8 (37.7–38.0)	38.1 (38.0–38.2)	38.4 (38.2–38.6)	38.6 (38.5–38.7)
	ALFX5	38.0 (37.8–38.2)	37.2 (37.0–37.2)[a]	36.9 (36.8–37.1)[a]	36.8 (36.6–36.8)[a]	36.6 (36.4–36.8)	37.3 (36.8–37.5)	37.7 (37.5–37.9)
	ALFX7.5	37.4 (37.3–37.6)[a]	36.6 (36.3–36.8)[a]	36.0 (35.9–36.2)[a]	35.3 (35.1–35.3)[a]	35.1 (35.0–35.3)[a]	35.1 (35.0–35.5)[a]	36.4 (35.8–36.7)[a]
	ALFX10	37.5 (37.0–37.7)[a]	36.3 (36.3–36.7)[a]	35.9 (35.7–36.1)[a]	35.4 (35.2–35.4)[a]	34.8 (34.7–34.9)[a]	34.8 (34.3–35.4)[a]	35.8 (35.2–36.6)[a]

Table 13 - Change in cardiorespiratory variables after intramuscular (IM) drug treatment in six cynomolgus monkeys. Data are expressed as medians (interquartile ranges) obtained from six cynomolgus monkeys. HR: heart rate, RR: respiratory rate, NMABP: non-invasive mean arterial blood pressure, SpO2: percutaneous oxygen saturation of haemoglobin, RT: rectal temperature. a) Significant difference from control (CONT) treatment at each time point (p<0.05). HPCD: 2–hydroxypropyl–beta–cyclodextrin. (Wada et al., 2020)

In other 6 cynomolgus macaques with the same protocols, deep level of sedation was rapidly achieved and maintained for 20 minutes with the lower dose; the monkeys were immobilized for over 40 minutes and endotracheal intubation was feasible with 7 and 10 mg/kg, as reported in Table 14. (Wada et al., 2020)

	IM drug treatments (n=6)						
	CONT	ALFX0.625	ALFX1.25	ALFX2.5	ALFX5	ALFX7.5	ALFX10
Number of animals became lateral recumbency	0	0	0	2	6	6	6
Number of animals accepted endotracheal intubation	0	0	0	0	3	6	6
Induction score	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	1 and 1	1.5 (1.0–2.0)	3.5 (3.0–4.0)[a)]	4.0 (3.3–4.0)[a)]
Recovery score	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	3 and 4	4.0 (3.3–4.0)	3.5 (3.0–4.0)	3.0 (3.0–3.8)
Maximum total sedation score	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	2.0 (1.3–9.5)	16.0 (15.0–20.0)	24.0 (22.5–24.0)	25.0 (25.0–25.0)[a)]
Time to recumbency (min)	N.C.	N.C.	N.C.	5.0 and 8.0	6.5 (5.3–7.8)	4.0 (4.0–4.0)	3.0 (3.0–3.8)[a)]
Duration of immobilization (min)	N.C.	N.C.	N.C.	5.0 and 19.0	27.5 (19.0–33.8)	56.0 (42.3–60.8)	74.5 (62.8–78.0)[a)]
Recovery time (min)	N.C.	N.C.	N.C.	4.0 and 19.0	12.0 (8.0–14.5)	12.5 (11.3–13.0)	10.5 (7.8–14.0)

Table 14 -Time and scores related to sedative effect after intramuscular (IM) drug treatment in 6 cynomolgus monkeys. Data are expressed as number of animals, value obtained, or median (interquartile range) obtained from six cynomolgus monkeys. N.C.: not calculated. Time to recumbency: time from the completion of IM administration (time 0) to the onset of lateral recumbency. Duration of immobilization: duration from the onset of lateral recumbency to the first appearance of spontaneous movement. Recovery time: duration from the first appearance of spontaneous movement to unaided sitting or standing in animals showing lateral recumbency after IM drug administration. (Wada et al., 2020)

There are some disadvantages on the use of IM alfaxalone alone.

The following side effects may appear during the recovery in different species: nausea, vomiting, ataxia, muscle tremors, opisthotonos-like postures, pronounced limb extension and paddling occurred, although the only remarkable clinical sign was tremors probably due to the low body temperature. (Diehl et al., 2001)

Large injection volumes are needed for IM sedation (0.75-1 ml/kg), and this make clinical use more difficult since by the European Federation of Pharmaceutical Industries and Associations and the European Centre for the Validation of Alternative Methods fixed the preferable IM volume to 0.25 ml/kg and the maximum dose volume to 0.5 ml/kg, as reported in Table 15. (Diehl et al., 2001)

Species	Route and volumes (ml kg^{-1})					
	Oral	s.c.	i.p.	i.m.	i.v. (bolus)	i.v. (slow inj.)
Macaque	5 (15)	2 (5)	[c] (10)	0.25 (0.5)	2	[c]

Table 15 - Administration volumes considered good practice (and possible maximal dose volumes). [c]Data not available. (Diehl et al., 2001)

Alfaxalone/Midazolam

Combination of alfaxalone (5 mg/kg) and midazolam (0.3 mg/kg) given intramuscularly in 15 rhesus monkeys and 15 cynomolgus monkeys produce better quality anesthesia compared with tiletamine/zolazepam and ketamine/acepromazine (see Figure 18) (Marion et al., 2022). The average anesthesia duration was 87 minutes in cynomolgus monkeys and 62 minutes in rhesus macaques.

The most common side effect was muscle twitching in response to touch. At lower doses of alfaxalone caution is essential because abrupt recovery with little warning can happen. Also, these authors pointed out that another negative aspect involving primarily the alfaxalone is the large volume required to obtain and adequate sedation. (Marion et al., 2022)

Alfaxalone/Midazolam/Medetomidine

Good muscle relaxation and deep surgical plane anesthesia of 7 rhesus macaques was achieved by SC administration of 20 µg/kg medetomidine, 0.3 mg/kg midazolam and 2 mg/kg alfaxalone. (Bertrand, Sandersen, et al., 2017) Anesthestia lasted 20 minutes. The low dose of medetomidine appeared to have slightest effects on blood pressure and HR. A mild depression of the ventilation with decrease in RR and increase in end tidal CO_2 ($EtCO_2$) has been reported (Figure 19). (Bertrand, Sandersen, et al., 2017)

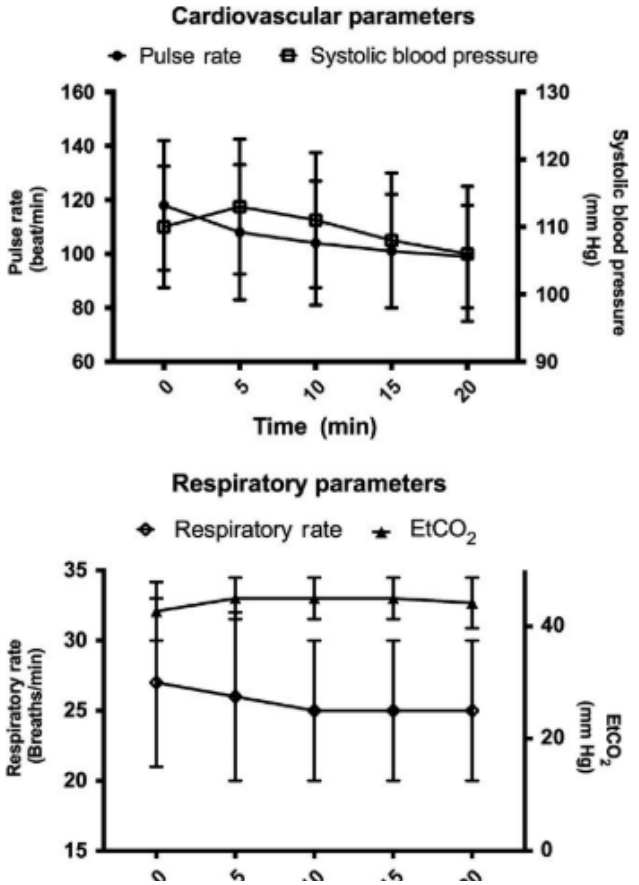

Figure 19 - Physiological parameters over the time. The data are represented as mean ± 1 SD; Pulse rate, 0 min = 118 ± 24, 5 min = 108 ± 25, 10 min = 104 ± 23, 15 min = 101 ± 21, 20 min = 99 ± 19; Systolic blood pressure, 0 min = 110 ± 9, 5 min = 113 ± 10, 10 min = 111 ± 10, 15 min = 108 ± 10, 20 min = 106 ± 10; Respiratory rate, 0 min = 27 ± 6, 5 min = 26 ± 6, 10 min = 25 ± 5, 15 min = 25 ± 5, 20 min = 25 ± 5; $EtCO_2$, 0 min = 43 ± 5, 5 min = 45 ± 4, 10 min = 45 ± 4, 15 min = 45 ± 4, 20 min = 44 ± 4. The painful stimuli were applied after the first time-- point (0 min). (Bertrand et al., 2017)

2.2.7 Opioids

Opioids are drugs whose primary target receptors are μ, k and δ receptors.

M receptors have different subtypes: μ1, responsible for supraspinal analgesia; μ2, distributed in the spinal cord and with less affinity for the opioids, responsible for the opioids' respiratory depression; μ3, with immunosuppressive action.

The k receptors are k1, that induce spinal analgesia, and k3, that induce supraspinal analgesia.

Sigma receptors are not clinically relevant. At sub analgesic doses of δ agonist, there is enhancement of the analgesia caused by morphine. They are also responsible for mydriasis and dysphoria.

Opioids produce analgesia through neuronal inhibition; they can reduce or interrupt the transmission of the nociceptive stimulus from the periphery to the CNS in a dose-dependent manner in the spinal cord. Analgesia has different duration and efficacy secondary to type of drug, dose and route of administration.

Opioids have a sedative effect due to the action on the μ receptors.

Effects on the cardiovascular system are minimal: agonist opioids can cause vasodilatation and mild systemic hypotension. The stimulation of the vagal tone can induce bradycardia that can be treated with atropine administration.

Opioids reduce the activity of the pontine and bulbar respiratory centres which results in reduced sensibility for hypercapnic and hypoxic conditions. They induce bradypnea up to transient apnea in a dose-dependent way.

Opioids can also induce vomit due to stimulation of the Chemoreceptor Trigger Zone. (Bufalari et al., 2012)

Fentanyl/Midazolam/Medetomidine

Comparison of fentanyl/midazolam/medetomidine (10 μg/kg – 0.5 mg/kg – 20 μg/kg) and ketamine (10 mg/kg) is described for sedation of rhesus macaques (Bertrand et al., 2016). The combination has a rapid onset, but longer than ketamine, due to the different absorption rate of the components. It produces sedation comparable to surgical anesthesia, enough to enable intubation. There is a marked negative influence on the cardiovascular and respiratory functions due to fentanyl and medetomidine effects, as reported in Table 16.

	Treatment groups										*p-value*
	Ketamine					Fentanyl-Midazolam-Fentanyl					
Time	0 *n = 8*	5 *n = 8*	10 *n = 8*	15 *n = 7*	20 *n = 7*	0 *n = 8*	5 *n = 8*	10 *n = 8*	15 *n = 8*	20 *n = 8*	
HR*(beat/min)*	134 ± 17	121 ± 18	117 ± 17	110 ± 14	112 ± 18	97 ± 17	90 ± 17	87 ± 18	86 ± 18	85 ± 17	0.0066*
SpO_2*(%)*	98.2 ± 1.0	99.8 ± 0.7	100 ± 0	99.9 ± 0.4	100 ± 0	96.1 ± 7.8	99.6 ± 0.7	99.8 ± 0.5	99.8 ± 0.7	99.6 ± 0.7	0.7254
RR*(breaths/min)*	36 ± 8	41 ± 8	36 ± 6	40 ± 11	43 ± 11	30 ± 10	28 ± 12	28 ± 11	29 ± 11	29 ± 10	0.0416*
$EtCO_2$*(mmHg)*	36 ± 8	32 ± 9	32 ± 9	34 ± 9	32 ± 8	39 ± 12	44 ± 11	43 ± 11	44 ± 10	42 ± 10	0.0462*
BPsyst*(mmHg)*	111 ± 10	111 ± 9	107 ± 9	107 ± 11	108 ± 13	101 ± 9	102 ± 10	97 ± 10	93 ± 9	90 ± 8	0.0313*
SD	12 ± 3	12 ± 2	11 ± 3	11 ± 3	11 ± 4	17 ± 1	18 ± 1	18 ± 1	18 ± 1	18 ± 1	0.0009*

Table 16 - Physiological parameters recorded over 20 min for each treatment group. Data are shown as mean ± 1 SD. n represents the number of animals that provide data at each time point. An asterisk next to the p-value indicates a significant statistical difference at α threshold of 5%. (Bertrand et al., 2016)

Recovery time with fentanyl/midazolam/medetomidine is significantly shorter than ketamine alone and the quality of recovery is significantly better. (Bertrand et al., 2016)

2.3 Anticholinergics

Anticholinergics drugs such as atropine or glycopyrrolate are useful in case of reflex bradycardia or α2 agonists-induced bradycardia, especially in young nonhuman primates because the cardiac output is heart rate-dependent. Inclusion of anticholinergics is not always necessary or indicated due to the arrhythmogenic properties that may predispose to ventricular bradycardia and bigeminal patterns.

The effective dose of atropine for reverse the bradycardia is 0.02-0.05 mg/kg. (Popilskis Sulli J. et al., 2008)

Chapter 3

3.1 Injectable anesthetics

3.1.1 Propofol

Propofol (Figure 20) is an injectable anesthetic that induce hypnosis through is action on the GABA-A receptors. It is characterized by the rapidity of peak concentration in the plasma and the rapidity of redistribution and metabolism. There are not alterations in the pharmacokinetics of the drug in patients with renal or hepatic problems. (Bufalari et al., 2012)

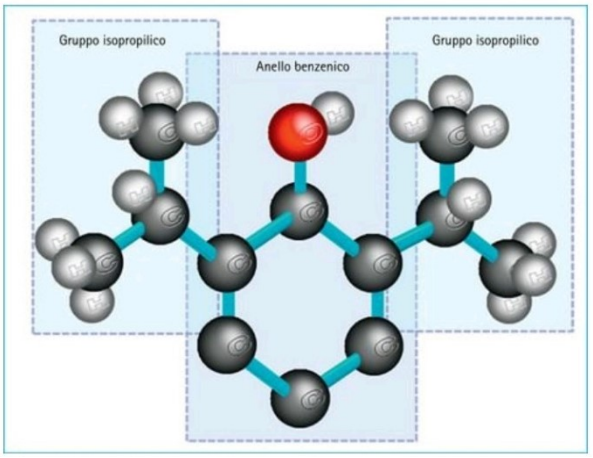

Figure 20 - 2,6-Diisopropylphenol chemical structure. (Bufalari et al., 2012)

Propofol has a hypnotic effect with depression of the CNS functions; it reduces the endocranial pressure and has antiemetic properties. (Borgeat & Stirnemann, 1998)

The most important effect on the cardiovascular system is reduction in arterial blood pressure due to peripheral vasodilatation and negative inotropic effect. In humans the reduction is about 25-40% even without cardiovascular pathologies and there is not the compensatory increase of the HR, probably due to inhibition of the baroreceptor reflex.

Propofol determines respiratory depression through depression of the respiratory centre and reduced response of chemoreceptors to PaCO2 variations with reduction of tidal volume and RR. It determines depression of the pharyngeal laryngeal reflexes. It induces bronchodilation and at high doses there is a reduction of the calibre of the upper airway.

Propofol does not have analgesic properties.

The induction of anesthesia is fast and smooth. IV administration of propofol can be painful. It is important to note that the depth of the anesthesia can be reversed suddenly in an on-off manner, so a close monitoring of the animal is vital. (Bufalari et al., 2012)

Propofol is ideal for procedures of short duration. The induction dose in macaques and *Papio* species is 2-4 mg/kg IV with good muscle relaxation. (Popilskis Sulli J. et al., 2008) A slow injection of propofol can reduce the occurrence of apnoea, that is seldom seen in rhesus monkeys. In case of apnea or hypoventilation intermittent single assisted breaths (sighs) can be beneficial.

Propofol can be used for maintenance of anesthesia as CRI or in boluses. In Table 17 it is reported an example of propofol infusion rates. Repeated boluses of 2-5 mg/kg IV can be administered to extend the duration of anesthesia without delaying recovery. (Fowler et al., 2001; Popilskis et al., 2008)

Propofol infusate (3.0 mg/ml)		(C) Infusion rate by drops (60 drops/ml) (drops/kg/min)
(A) Dose (mg) (mg/kg/min)	(B) Dose (ml) (ml/kg/min)	
0.1	0.033	2
0.2	0.066	4
0.3	0.099	6
0.4	0.133	8
0.5	0.167	10
0.6	0.200	12
0.7	0.233	14

Table 17 - Propofol dose rate chart. (Fowler et al., 2001)

Based on the results obtained by bolus administration the suggested infusion rate of 0.3-0.4 mg/kg/min is sufficient in macaques. In Table 18 and Table 19 are reported the heart rates and respiratory rates, respectively, of various doses of propofol. (Sainsbury et al., 1991)

The most effective dosage rate for maintenance of anesthesia with propofol is 0.4-0.6 mg/kg/min.

Rhesus macaques received larger doses when compared with that used in other species (Table 20). (Fowler et al., 2001)

			Heart rate (beats/min)						
Dose of propofol* (mg/kg bwt)	Sample size	Prior to dose	Time after dose (min) 1	2	3	4	5	10	20
2.5	4	146	158	–	–	158	152	143	143
5	5	139	147	121	115	124	135	124	116
7.5	3	150	161	168	149	150	153	139	118
10	5	143	158	168	169	153	152	138	128
15	2	162	183	179	185	181	177	155	135
20	4	165	159	173	171	169	167	166	153
All dose rates	23	149	158	162	159	155	153	142	133

Table 18 - The effect of various doses of propofol on heart rate (mean values). *Each dose category includes results where the exact dose rates were up to 1.0 mg/kg bodyweight higher or lower than the figure given. (Sainsbury et al., 1991)

				Respiratory rate (breaths/min)			
Dose of propofol* (mg/kg bwt)	Sample size	Prior to dose	Imm. after admin.	Time after dose (mins) 5	10	15	20
2.5	4	30	29	31	30	–	–
5	5	26	26	24	24	27	28
7.5	3	31	17	17	21	21	21
10	5	34	26	25	26	30	24
15	2	19	14	21	16	18	18
20	4	38	44	42	45	48	47
All dose rates	23	31	27	27	28	30	30

Table 19 - The effect of various doses of propofol on respiratory rate (mean values). *Each dose category includes results where the exact dose rates wereup to 1.0 mg/kg bodyweight higher or lower than the figure given. (Sainsbury et al., 1991)

Species	Rate (mg/kg/min)	Comments
Human	0.1–0.2	Supplemental nitrous oxide and oxygen (60–70%)
Dog	0.4	Premedication with acepromazine and supplemental oxygen
Dog	0.15	Premedication with medetomidine
Cat	0.51	Propofol given as increments and calculated as mean maintenance dose
Rabbit	0.87	Unpremedicated

Table 20 - Species comparison for administration rates of propofol in maintenance of anesthesia. (Fowler et al., 1991)

There are also large individual variations in the dose response to propofol with no relationship between physiological parameters or body weight. (Fowler et al., 2001) Anesthetic recovery from constant rate infusion of propofol (CRI) with different rates in 27 rhesus monkeys was uneventful and occurred within 5-10 minutes from stopping of the infusion.

In chair trained rhesus macaques, it was compared the induction of anesthesia with thiopental (19 mg/kg) and to that with propofol (9 mg/kg) prior to maintenance with isoflurane 1.5% mixed with oxygen. (Choi et al., 2016) Induction in each group was smooth and intubation was easily performed and no adverse effects was observed. There was a decrease in HR, RR, temperature and blood pressure in induction in both groups (Figure 21).

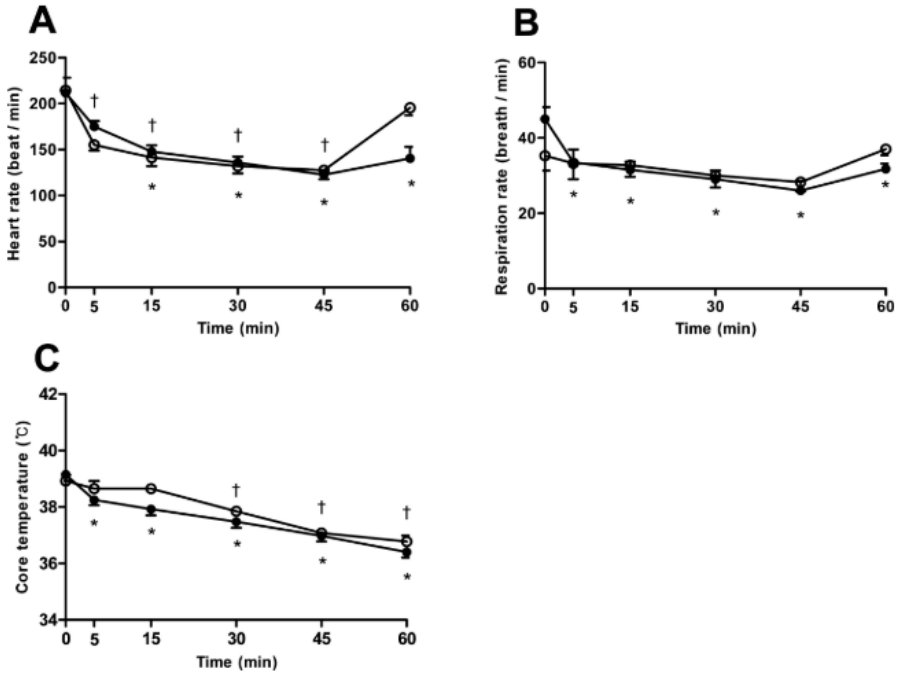

Figure 21 - The variation of (A) heart rate, (B) respiratory rate, (C) core temperature after induction with thiopental (closed circle) or propofol (open circle) following maintenance of isoflurane anesthesia by 45 minutes. Data are presented as the mean±SD. *p<0.05 in thiopental group and †p<0.05 in propofol for significant different from baseline. (Choi et al., 2016)

Blood pressure variations (Figure 22) in the propofol group under isoflurane were minimal compared with the thiopental group, in which the SBP was significantly lower.

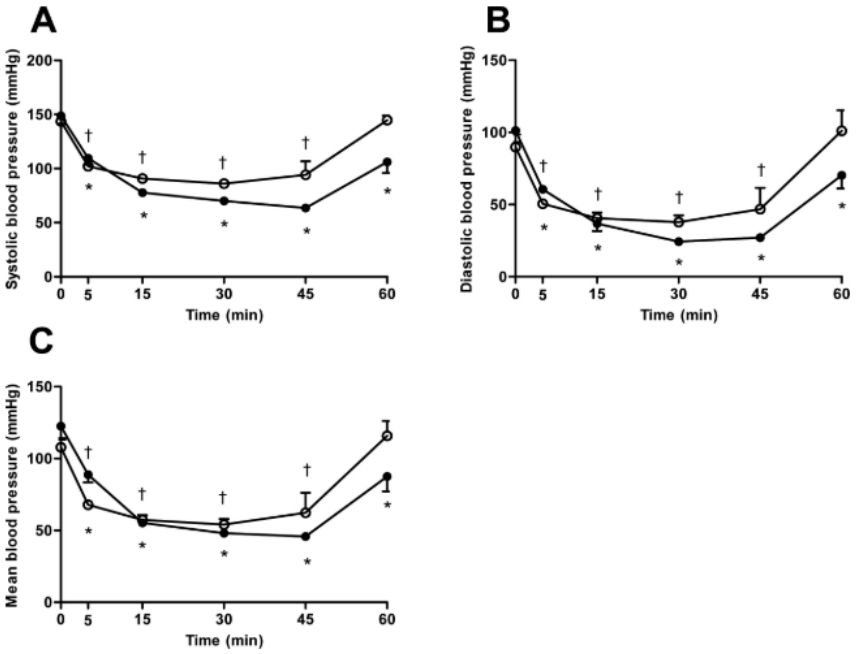

Figure 22 - The variation of (A) systolic, (B) diastolic, and (C) mean blood pressure after induction with thiopental (closed circle) or propofol (open circle) following maintenance of isoflurane anesthesia by 45 minutes. Data are presented as the mean±SD. *p<0.05 in thiopental group and †p<0.05 in propofol group for significant different from baseline. (Choi et al., 2016)

Body temperature in the propofol group was significantly decreased until 60 minutes. (Choi et al., 2016)

Expeditious preparation and heat insulation of the animal could prevent hypothermia generated by propofol. (Fowler et al., 2001)

3.1.2 Thiopental

Thiopental is an ultra-short acting barbiturate (Bufalari et al., 2012). The onset is about 30-40 seconds and the duration is about 5-20 minutes with recovery in 10-15 minutes. Barbiturates are barbituric acid derivatives that are sedative hypnotic agents without analgesic properties. They cause a CNS depression with increase in the inhibitory action on the GABA receptors. Barbiturates must be administered intravenously, else there is perivascular damage.

On the cardiovascular apparatus thiopental cause a peripheral vasodilatation secondary to depression of the vasomotor centre, with decrease of blood pressure. Arrhythmia is common after thiopental administration.

Barbiturates frequently cause reduction in tidal volume and short periods of apnoea. They can affect the sensibility of the respiratory centre to CO2 concentrations and of aortic and carotid chemoreceptors with reduced respiratory response to high PaCO2 levels. (Bufalari et al., 2012)
In NHPs thiopental is used to facilitate intubation at 10-15 mg/kg IV and if the animal previously received ketamine the dosage is about 5-7 mg/kg. (Popilskis Sulli J. et al., 2008)
Chemical restraint of *Papio ursinus* is achieved with 15-17 mg/kg/h of thiopental infusion with stable physiological parameters (reduction in HR, respiration, body temperature and blood pressure) for 90 minutes and the recovery is within 20 minutes after discontinuation of the infusion. (Goosen et al., 1984)

3.1.3 Pentobarbital

Pentobarbital is a short acting barbiturate that is used as sedative and anesthetic agent.
The usual dosage in NHPs induction of anesthesia is 20-30 mg/kg IV with patient-to-patient variability. The duration of surgical anesthesia is between 30 and 60 minutes. Pentobarbital anesthesia is induced by delivering approximately one-half the calculated dosage as a bolus and then delivering the additional amounts to effect.
Pentobarbital has a depressive effect on the respiratory system with inability to modulate the depth of anesthesia and has a long recovery period (more than 3 hours). Pentobarbital is most common used in neurosurgeries due to the minimal impact on the CFS pressure with minimal decrease of CBF and metabolic rate. (Popilskis Sulli J. et al., 2008)
In 50 rhesus macaques, 20-25 mg/kg of pentobarbital over 10-15 minutes induced a significant reduction in MAP due to venodilatation and negative myocardial contractility that was similar to induction with 3 mg/kg of propofol followed by CRI at 0.2-0.4 mg/kg/min and with induction with isoflurane 4% supplemented with oxygen followed by maintenance with 1-2% isoflurane. (Hom et al., 1999) In contrast, ketamine anesthetized monkeys showed a basal MAP comparable to conscious monkeys, as illustrated in Table 21. Arterial blood gas analyses were similar among monkeys anesthetized with ketamine, pentobarbital, propofol and isoflurane.

	No anesthetic (n=25)	Ketamine (n=34)	Pentobarbital (n=50)	Propofol (n=7)	Isoflurane (n=8)
Mean arterial pressure (mm Hg)	99 ± 10.30	96 ± 12.97	79 ± 8.39*	71 ± 8.04*	62 ± 5.05
Heart rate (beats/min)	110 ± 11.51	134 ± 14.72	131 ± 11.44	125 ± 27.72	121 ± 10.93

Table 21 - Heart rate and mean arterial pressure (mean standard ± deviation) in rhesus monkeys after various anesthetic regimens. (Hom et al., 1999)

Evaluation of activation of parts of brain involved in pain perception during the application of noxious stimuli was done in 8 *M. fascicularis* under either propofol or pentobarbital sedation. For the first group sedation was achieved with 20 or 30 mg/kg/h; for the second group pentobarbital was administered as a single bolus (20 mg/kg). (Shirai et al., 2020) Pentobarbital is generally thought of as having no intrinsic analgesic activity and at sedating doses of either propofol or pentobarbital did not suppress noxious stimuli. Current findings underscore the need for appropriate analgesic treatment during painful procedures using these agents.

3.1.4 Alfaxalone

The use of alfaxalone an induction agent in macaques is not reported in literature. It is safely used in various species, domestic and exotica animals, mammals and reptiles. Consequently, in the future it could also find valid clinical application in NHPs.

As an induction agent in dogs alfaxalone determines good-excellent quality of induction regardless of the animal's temperament, its health status, the use of pre-anesthetic medication and sedation obtained. Myoclonus, twitching, excitement, tremors, head shaking, blinking, vocalisations and paddling are repoted when it is used for induction of anesthesia. While no correlation between the incidence of these events and the pre-anesthetic medications administered was found by some authors, others suggested that profound sedation could reduce their occurrence. The type of pre-anesthetic medications administered may play an important role in the overall hemodynamic impact of alfaxalone. (Martín Bellido & Vettorato, 2022)

Alfaxalone used for induction in unpremedicated dogs (mean dose 2.8 ± 0.3 mg/kg) is smooth and uneventful with decrease in SAP and MAP, increase in HR that lasted for the experiment duration, probably due to baroreceptor response, and no reported arrythmia. (Hampton et al., 2019) Alfaxalone causes a decrease in systemic vascular resistance index.

No clinically important respiratory depression was noted even though apnea may occur after induction, above all if the IV injection is too fast or the dose is high.

Alfaxalone commonly reduces the RR compared with propofol at induction (Table 22). (Maney et al., 2013)

	Baseline (n = 8)	After Induction (n = 8)	+5 minutes (n = x)
Temperature (°C)			
Alfaxalone	38.9 ± 0.6	38.6 ± 0.6**	38.8 ± 0.5 (n = 6)
Propofol	38.9 ± 0.4	38.8 ± 0.6	38.7 ± 0.4 (n = 5)
Pulse rate (beats minute^{-1})			
Alfaxalone	121 ± 25	144 ± 46	145 ± 33 (n = 7)
Propofol	123 ± 24	139 ± 29	111 ± 20 (n = 5)
Respiratory rate (breaths minute^{-1})			
Alfaxalone	66 ± 31	33 ± 17**	32 ± 19 (n = 7)
Propofol	62 ± 37	31 ± 17	39 ± 29 (n = 5)
Systolic arterial pressure (mmHg)			
Alfaxalone	149 ± 29	139 ± 23	108 ± 23 (n = 7)
Propofol	141 ± 24	138 ± 46	110 ± 16 (n = 6)
Diastolic arterial pressure (mmHg)			
Alfaxalone	75 ± 11	83 ± 9	64 ± 9 (n = 7)
Propofol	78 ± 13	79 ± 23	68 ± 5 (n = 6)
Mean arterial pressure (mmHg)			
Alfaxalone	102 ± 11	106 ± 10	81 ± 11 (n = 7)
Propofol	102 ± 11	102 ± 29	85 ± 5 (n = 6)

*Values are mean ± SD. **Values significantly different within drug treatment between baseline and post-induction time points ($p < 0.03$).

Table 22 - Temperature, pulse, respiration, and arterial blood pressures before (= baseline) and after anesthetic induction with alfaxalone or propofol in female mixed-breed dogs. (Maney et al., 2013)

In dogs the hypoventilation and the hypoxemia reported after administration of alfaxalone as an induction agent were dose dependent. (Martín Bellido & Vettorato, 2022)

3.1.5 Ketamine

Although ketamine is primarily used in premedication it can be used as induction agent due to its characteristics that ensure a rapid blood-brain equilibration and clinical onset. Rat models demonstrates that its half-life is just about 2 min (Morris et al., 2009). It is most often used in companion animals as an induction agent. In 13 unrestrained cats, (Child et al., 1972) ketamine at different dosages (4, 8, 16, 32 and 64 mg/kg) was studied: a startle response before loss of consciousness after 20-25 seconds occurred at 4, 8 and 16 mg/kg with subsequent rise in blood pressure within 125-135% of the control values within 1-5 min of the start of injection. The HR also increased but not to a significant extent. At 32 mg/kg ketamine induced a reduction in the RR. Profound respiratory and circulatory depression occurred at the highest dose.

In NHPs ketamine has a wide therapeutic index. (McCarthy et al., 1965)Even though ketamine has a negative inotropic effect, it acts as a sympathomimetic in intact autonomic nervous system, so it increases the HR, arterial pressure and cardiac output. There is also the preservation of baroreflexes responses. Ketamine maintains MAP even in animal models of endotoxin induced shock with prevention of metabolic acidosis and cytokine responses in a

dose dependent manner. (Tweed W. A. et al., 1972; Hoka et al., 1988; Gelissen Harry P. M. M. et al., 1996; Taniguchi et al., 2003)

IV ketamine bolus injected rapidly can cause a brief period of apnoea that is usually self-limited and it can be avoided by administering ketamine over 30-60 seconds. However, ketamine has been found useful with airways that are known or predicted to be anatomically difficult or animals that may benefit from continuous breathing with signs of physiologic difficulty or with low tolerance of brief period of apnoea, as reported in humans. (Merelman et al., 2019)

Ketamine is also thought to have intrinsic action as a bronchodilator and is the preferred induction agent for patients being intubated for obstructive lung disease. (Goyal & Agrawal, 2013)

3.1.6 Etomidate

Etomidate is a carboxylate derivative of imidazole. It is present in two formulations: with acid solution of 35% propylenic glycol and with lipidic solution. The first one is characterized by irritation around injection area, and it can cause collateral effects in CRI or in case of altered elimination. The second one is safer.

Etomidate is a sedative and hypnotic agent; the mechanism of action is not yet determined. It does not have analgesic properties. Duration of hypnosis is dose dependent.

The rapid hydrolysis is useful for repeated boluses or in CRI.

Etomidate reduces the intracranial pressure, cerebral blood flow (CBF) and oxygen consumption. It has minimal hemodynamic impact and it is not arrhythmogenic.

At induction it can cause transient apnea and hypoxia, but PaCO2 is unchanged. The respiratory depression is dose dependent, influenced by administration's velocity.

Etomidate does not produce muscle relaxation with the occurrence of muscle hyper tone, tremors and involuntary movements due to subcortical disinhibition. These effects appear to be dose dependent.

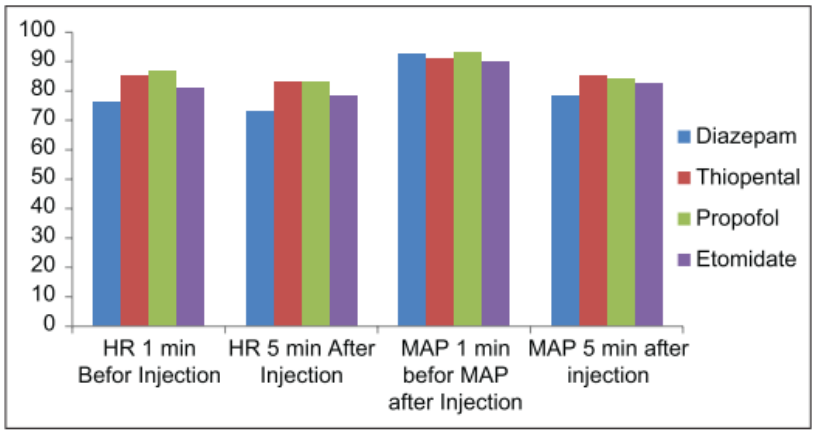

Figure 23 - Mean heart rate and MAP index 1 min before and 5 min after injection by study groups. (Abdi et al., 2022)

It is suggested the use of sedatives prior to induction with etomidate. The association of the drug with benzodiazepines or/and opioids can reduce the dose required for induction and maintenance. (Bufalari et al., 2012)

Many studies in humans confirms that there is no significant difference in hemodynamic parameters between propofol and etomidate in induction. (Figure 23) (Abdi et al., 2022)

Cardiovascular responses to etomidate and propofol was compared in rhesus macaques. (Fanton et al., 2000) The two anesthetic protocols were 2 mg/kg of propofol in induction followed by CRI at 0.2 mg/kg/min and 1 mg/kg of etomidate followed by CRI at 0.1 mg/kg/min. Both protocols decreased MAP as seen in Figure 24, but in the etomidate group the reduction was equal to 24% from baseline, which is uncommon compared with results in other human studies. It was noted a reduction in HR, probably due to resetting of baroreceptors. The stroke volume remained unchanged in both groups with a decreasing myocardial contractility.

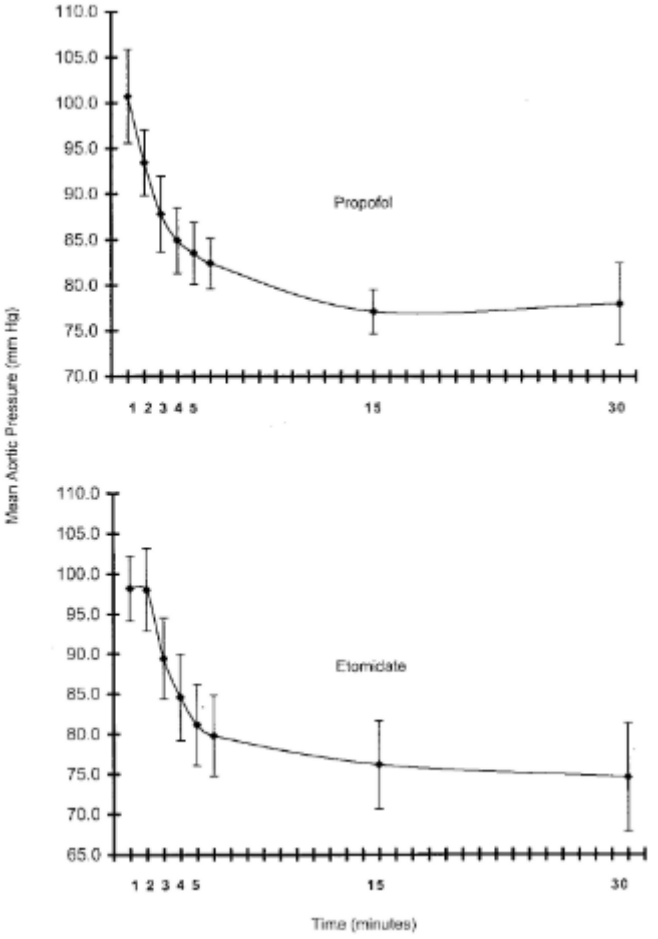

Figure 24 - Effect of infusion of propofol or etomidate on aortic pressure in rhesus monkeys. Data are expressed as mean ± SEM. (Fanton et al., 2000)

3.2 Inhalational anesthetics

3.2.1 Isoflurane

Isoflurane is a halogenated anesthetic with low solubility in blood, therefore induction and recovery are fast. It has a negative inotropic effect and a periphery vasodilatory effect; thus, it causes dose-dependent hypotension with compensatory increasing in HR. There is also a dose-dependent respiratory depression in which the RR is diminished but the tidal volume is maintained. Isoflurane is not ideal for mask induction because it is irritating on the airway. (Bufalari et al., 2012)

Isoflurane does not sensibilize the myocardium to the arrhythmogenic properties od circulating catecholamines.

Minimal alveolar concentration (MAC) value for isoflurane in *M. fascicularis* is 1.28%. (Tinker John H. et al., 1977) Similar concentration (1.2 ± 0.2%) is utilized in a study for anesthesia maintenance in 12 *M. mulatta* undergoing neurosurgeries after sedation with ketamine (10 mg/kg IM) and induction of anesthesia with propofol (8 mg/kg IV). HR and SBP decreased after administration of isoflurane, as reported in Table 23, with mean values of 113 ± 13 beats/min and 88 ± 7 mmHg, respectively. Mean RR was 25 ± 7 breaths/min and mean body temperature was 37,5 ± 0.4 °C.

	Isoflurane
Total anaesthesia duration (min)	462 ± 130
Maintenance EtGas (%)	1.2 ± 0.2
Heart rate (beats/min)	113 ± 13
Systolic blood pressure (mmHg)	88 ± 7
$EtCO_2$ (mmHg)	34.3 ± 0.7
Respiration rate (breaths/min)	25 ± 7
Core body temperature (°C)	37.5 ± 0.4
Crystalloid bolus administration*	1.5 ± 0.8
Ephedrine bolus†	0.3 ± 0.5
Colloid bolus§	0 ± 0

Table 23 - Anesthesia data. The data were collected over the maintenance period of anaesthesia and are reported as mean±1SD. *Crystalloid bolus dose of 10 mL/kg over 10 min; †Ephedrine bolus of 0.1 mg/kg intravenously; §Colloid bolus dose of 3 mL/kg. $EtCO_2$: end-tidal carbon dioxide; EtGas: End-tidal anaesthetic gas concentration

The recovery after discontinuation of isoflurane administration is smooth and uneventful with longer times compared with the recovery after sevoflurane anesthesia (2%). There is no significant difference in quality of recovery in both cases. (Bertrand, Springer, et al., 2017)

Isoflurane has a cerebral vasodilatory effect; it is reported a reduction in CBF in combination with hypotension and hypocapnia that did not result in hypoxia during isoflurane and nitrous oxide anesthesia in 5 rhesus monkeys so in clinically normal doses isoflurane was found safe from cerebral oxygenation point of view. There was no evidence of a generally inadequate CBF, however during isoflurane anesthesia pronounced regional differences in CBF were noted as

metabolism-flow coupling was impaired. (Enlund et al., 1997) Isoflurane has a dose and duration-dependent and regional specific effect on CBF, as described by Li & Zhang (2018) (Figure 25).

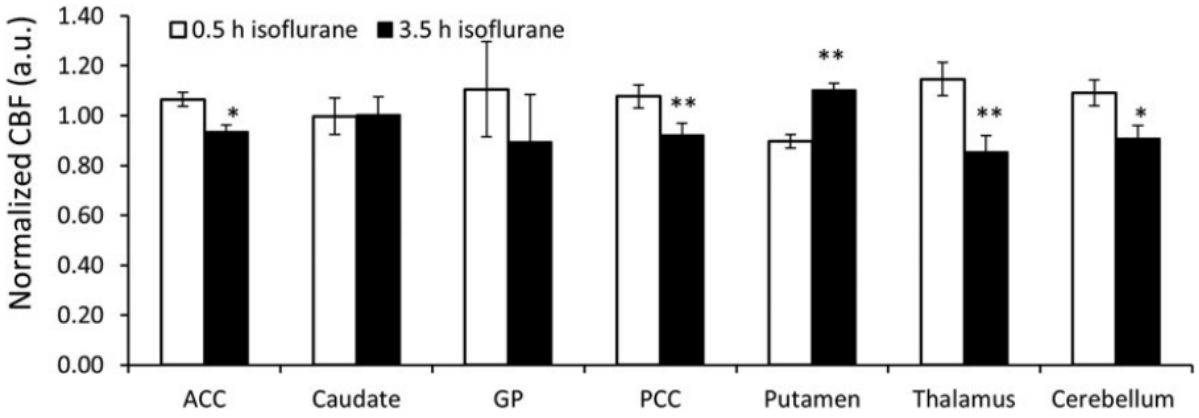

Figure 25 - CBF changes in selected ROIs of normal macaque monkeys maintained with 1% isoflurane administration (n = 5); error bar indicates standard deviation. $^{**}p < 0.01$; $^{*}p < 0.05$ versus 0.5 h. h, hour. (Li & Zhang, 2018)

3.2.2 Sevoflurane

Sevoflurane is a halogenated anesthetic with lower blood solubility compared with isoflurane so induction, recovery and changing in anesthesia plane are even more rapid. Cardiovascular and respiratory effects are comparable with those of isoflurane, with minor myocardial depression and hypotension and lower HR. (Bufalari et al., 2012)

Sevoflurane has a less pungent odour than isoflurane and it is a potent bronchodilator. (Popilskis Sulli J. et al., 2008) It can create “compost A” after reaction with soda lime that can be the cause of kidney failure.

In cynomolgus monkeys it was not detected any signs of effects on renal function after multiple administration of sevoflurane. (Soma et al., 1995) The production of compost A can increase with low flow anesthesia, with increase in temperature in the anesthetic circle system and with high concentration of sevoflurane. In circulatory systems the concentration of compost A is 5 to 10 times less than the toxic dose. (Popilskis Sulli J. et al., 2008)

Sevoflurane causes a dose-dependent increase in CBF and in rhesus monkeys the autoregulation of CBF is compromised at 2.0% sevoflurane concentration. (Kaneko et al., 1997)

Chapter 4

4.1 Analgesia in neurosurgery

NHPs are wild animals, and consequently they tend to display few signs of discomfort and pain, especially after surgical procedure or injuries; so, animals that demonstrate signs of pain likely are experiencing severe pain.

NHPs may show pain by reduction of appetite and food and water intake, by stop grooming, hair loss and lethargy, by reduced mobility, isolation and state of alertness, by vocalization and gnashing of teeth, change in posture (crouched and huddled), focusing attention on a body part (including biting, scratching, self-mutilating and licking).

A score system for the recognition of pain was ideated by Wolfensohn and Honess (2005). (Table 24)

Parameter	Animal	Score
Appearance	Normal	0
	Absence of grooming	1
	Loss of hair, nasal or ocular mucus	2
	Piloerection, apathetic state	3
Food and water intake	Normal	0
	5% weight loss	1
	10-15% weight loss	2
	Refusal of food and water	3
Clinical parameters	Normal temperature and heart rate and respiratory rate	0
	Little changes in these parameters	1
	Temperature ± 1°C, 30% difference from baseline values of heart rate and respiratory rate	2
	Temperature ± 2°C, 50% difference from baseline values of heart rate and respiratory rate or remarkable reduction	3
Behaviour	Normal	0
	Small behavioural changes	1
	Reduced mobility, isolation and state of alert	2
	Vocalizations, self-mutilations, extreme restlessness	3
Responses to behavioral stimulation	Normal	0
	Moderate depression or exaggerated response to stimulation	1
	Changes in expected behavioural responses	2
	Violent or excessively reduced reaction, precomatous state	3
If score 3 is reached more than once 1 point must be added for every score 3 reached.		2-5
Total		0-20

Table 24 - Score system for the recognition of pain in NHPs. 0-4: normal; 5-9: To be monitored carefully, consider administering analgesics and other treatments; 10-14: Animal suffering, continuous monitoring, administering treatments can provide relief; 15-20: Serious suffering, consider euthanasia. (Wolfensohn and Honess, 2005)

NHPs are usually subjected to repeated surgeries and this can lead to the “wind up” effect that is an increased responsiveness of pain receptors that may heighten pain response. (DiVincenti Louis Jr, 2013)

Neurosurgical procedures can cause in the postoperative period moderate to severe pain according to human patients and this pain is often undertreated, especially if the patient is an animal. Analgesics for animals in laboratory settings are often underused due to the lack of pain assessment techniques available for many species, to the withholding of analgesics when signs of pain are not recognized and to a reduced duration of analgesic administration with inappropriate analgesic coverage. (Coulter et al., 2009)

The pain in neurosurgeries as craniotomies is described in humans as a superficial pain therefore with a somatic origin rather than visceral. Moreover, the severity of pain is associated with the surgical approach. (Flexman et al., 2010) Type C fibres involved in the innervation of the scalp are the more affected when there are disruption of the temporal muscles and soft tissues. (Warner et al., 2001) These fibres when stimulated determine a poorly localized burning that persists beyond the painful stimulus.(Lamont, 2008) In addition tissue injuries cause cell damage with inflammatory cells recruitment thus peripheral sensitization secondary to modifications in nociceptor responses. Direct post operative complications secondary to inadequate pain management are agitation, hypertension, vomiting with possible intracranial bleeding. (Türe et al., 2009)

The most important analgesic approach is the multimodal analgesia that is a pharmacologic method of pain management which combines various group of medications for pain relief. The most commonly combined medication group include LA, opioids, NSAIDs, ketamine, corticosteroids, and alpha-2 agonists. Multimodal approach during the perioperative period takes benefit from different synergistic effects determined by different mechanisms of action: it reduces the doses required for individual drug, it reduces the requirements for maintenance of anesthesia, it improves recovery time and minimize side effects from high doses of volatile anesthetics.(DiVincenti Louis Jr, 2013)

4.2 Pre-emptive analgesia

Pre-emptive analgesia is an antinociceptive treatment that prevents the activation of nociceptors. Administration of analgesics in addition to general anesthesia prevent the sensation of pain during the surgery, thereby preventing the formation of scars caused by

changes in the central nervous system during surgery. Pre-emptive analgesia treats pain prior to its onset to modulate pain response and prevent maladaptive pain. There are different classes of drugs with analgesic properties that can be used prior to surgery to reach peak blood concentration in the moment of the administration of painful stimuli. Pre-emptive techniques include parenteral administration of systemic analgesics, infiltration of a suture line with local anesthetics and epidural administration of analgesics.

4.2.1 Local anesthetics

Local anesthetics (LA) agents act as a sodium channel inhibitor. LA have greater affinity for receptors within sodium channels during their activated and inactivated states than when they are in their resting states. Neural fibres having more rapid firing rates are most susceptible to LA action. Tiny, rapid-firing autonomic fibres are most sensitive, followed by sensory fibres and finally somatic motor fibres. After spinal anesthesia, patients first regain voluntary motor function, then sensation and finally autonomic control. Sensory fibres have different diameters and firing rates. Pain fibres are more sensitive than those carrying pressure and proprioception. A patient may remain more disturbed by a sense of pressure despite complete anesthesia of pain fibres.

Duration of local anesthesia is influenced by the time a LA remains near neural fibres. Sequestration of highly lipid-soluble anesthetics locally may allow for continuous release to the neuronal membranes, prolonging duration but constriction of neighbouring vasculature is more significant in this regard. Vasopressors such as epinephrine are then added to LA to prolong their duration of action by their vasoconstriction mediated by activation of α1 adrenergic receptors. They can be useful not only to delay anesthetic absorption but also to reduce the risk for systemic toxicity and prolongation of anesthesia. (Becker & Reed, 2012)

Most used LA are lidocaine, ropivacaine and bupivacaine. LAs are CNS depressants so large doses can cause hypotension and seizures due to loss of central inhibitory tracts. At clinical doses adverse effects are rare but LA can potentiate any respiratory depression associated with sedatives and opioids. Furthermore, serum concentrations required to produce seizures are lower if hypercarbia is present.

LAs are generally used in neurosurgeries for local scalp infiltrations and regional scalp blocks. (DiVincenti Louis Jr, 2013) Analgesic effects of LAs seem to last long after the supposed drug's duration of action. (Warner et al., 2001; Bala et al., 2006)

In addition to reducing postoperative pain, LAs modulate hemodynamic effects of surgery and improve circulatory stability in human craniotomy patients, as seen in Figure 26, Figure 27 and Figure 28. (Bloomfield et al., 1998; Mohammadi S. S. et al., 2009; Pakulski et al., 2001)

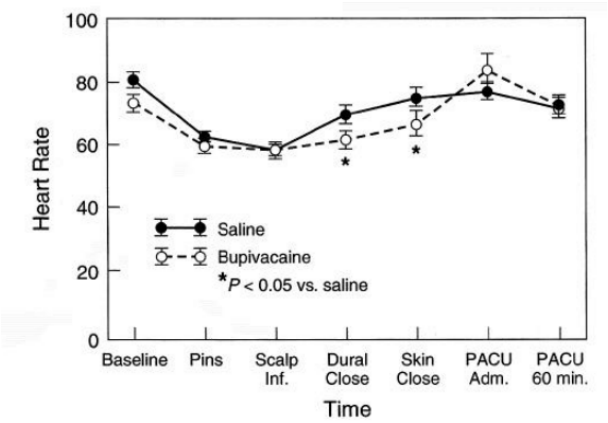

Figure 26 - Mean heart rates (bpm) in patients receiving bupivacaine (n=18) or saline (n=18) intraoperatively and postoperatively. PACU=postanesthesia care unit. Error bars are defined as SEM. *p<0.05 (Bloomfield et al., 1998)

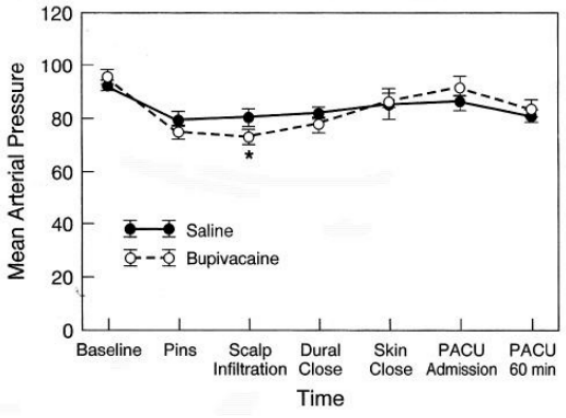

Figure 27 - Average mean arterial pressures (mmHg) in patients receiving bupivacaine (n=18) or saline (n=18) intraoperatively and postoperatively. PACU= postanesthesia care unit. Error bars are defined as SEM. *p<0.05. (Bloomfield et al., 1998)

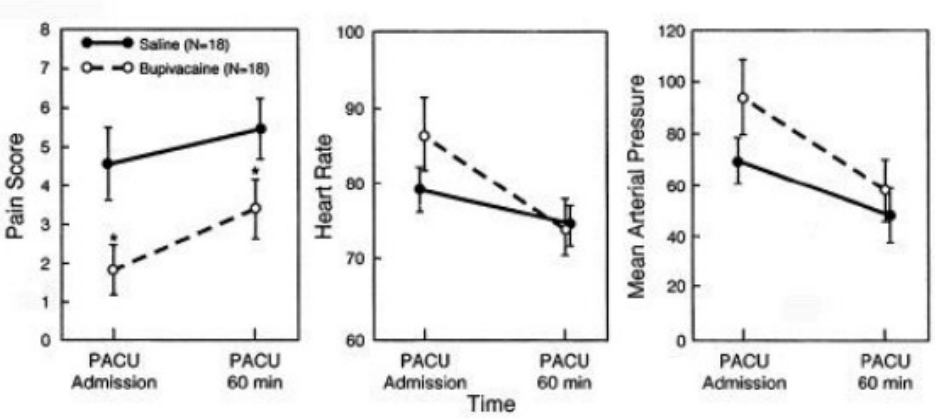

Figure 28 - Pain scores for patients on admission to the PACU and at 1h postadmission with correlation of heart rate (bpm) and mean arterial pressure (mmHg). Error bars are defined as SEM. (Bloomfield et al., 1998)

Lidocaine

Lidocaine is a water-soluble LA that for its molecular structure and its pKa can easily spread in the tissues. Lidocaine has an intermediate duration of action (about 70-90 minutes). It has vasodilatory action: coadministration of adrenaline can prolong the duration of lidocaine's action and can improve its potency by limiting its blood concentration. High concentrations and prolongate infusions with spinal catheters can determine neurological damages because lidocaine is one of the most neurotoxic LA. Spinal analgesia with lidocaine is therefore not recommended. (Bufalari et al., 2012) In humans, there is evidence of lidocaine toxicity at

concentrations >5 μg/mL but convulsive seizures happen at concentrations >10 μg/mL. (Becker & Reed, 2012)

Many studies reported the use of lidocaine as anesthetic agents in NHPs. Lidocaine has been used for infiltration of subcutaneous tissues, muscles, incision line and perineural tissues. In neurosurgeries 2% lidocaine is most often used in infiltrations at the incision site or as irrigation of the tissues. (D'Arceuil et al., 2006)

Bupivacaine

Bupivacaine is a LA that has a greater protein-binding capacity (79-90%) compared to lidocaine. It is characterized by long latency period and long duration of action.

It is one of the least neurotoxic LA and one of the most used for spinal and epidural anesthesia. (Bufalari et al., 2012) However, it should be noted that bupivacaine exhibits greater potential for direct cardiac toxicity by its greater affinity for the inactive and resting sodium channel configurations and by the delayed dissociation from these channels. Cardiac tissue is therefore more susceptible to arrythmias.(Becker & Reed, 2012)

Ropivacaine

Ropivacaine has intermediate liposolubility compared with lidocaine and bupivacaine and a protein-binding capacity similar to bupivacaine. Injected in the peridural its half-life is about 4 hours. Clinical effects are comparable to bupivacaine with a motor block less pronounced and of shorter duration of action. (Bufalari et al., 2012)

In NHPs LAs can be also administered by an epidural catheter. Placement of epidural catheter is a relatively simple technique in NHPs such as macaques. It can be done by introducing an 18-gauge Tuohy epidural needle with a curved distal end at lumbar 5^{th}-6^{th} or 6^{th}-7^{th} interspace. Once the epidural needle penetrates the infraspinous ligament a syringe with a freely movable plunger is attached and the needle is advances into the epidural space. Entry into the epidural space is confirmed by ease of depression of the plunger in the syringe. The nylon catheter is then inserted, and the epidural needle removed and to ascertain the proper placement it is advanced 5-7 cm from the introduction site. (Popilskis et al., 2008)

Single-punture administration of a LA around a nerve, plexus, or in a fascial space is of limited duration. In humans, cessation of its effect may be associated with acute rebound that worsen

postoperative recovery. (Lavand'homme, 2018) Adding an adjuvant drug to LAs can prolong the sensory blockade and the analgesic effect.

4.2.2 Adjuvant to local anesthetics

Dexmedetomidine

Dexmedetomidine in combination with LAs prolongs their analgesic effect through direct effect on peripheral fibres: inhibition of action potential generation in unmyelinated C-fibres and cell hyperpolarisation through activation of voltage-dependent channels without α2 receptors activation since their expression in peripheral nerves is absent. (Nguyen et al., 2017; Weerink et al., 2017)

Dexmedetomidine prolongs duration of intravenous regional anesthesia, peripheral nerve blocks and intradural anesthesia. Potential systemic effects can occur so intraoperative monitoring is needed. (Fernández Martin et al., 2023) In humans in most studies a dose of 1 µg/kg both at peripheral nerve level and at the fascial space level is reported. (Q. Sun et al., 2019; Zhao et al., 2020) In a randomized controlled trial 17 cats received intraperitoneal bupivacaine 0.25% (2 mg/kg) with either epinephrine (2 µg/kg) or dexmedetomidine (1 µg/kg) before undergoing ovariohysterectomy. Pharmacokinetic parameters and pain scores were not different between treatments (p>0.05) and intraperitoneal bupivacaine with epinephrine or dexmedetomidine produced concentrations below toxic levels and similar analgesic effects, as reported in Figure 29 and in Figure 30. (Benito et al., 2018)

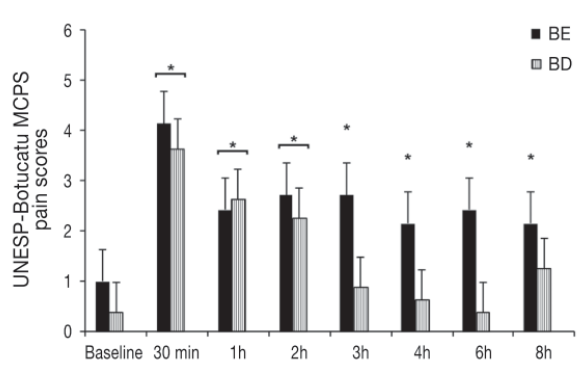

Figure 29 - Mean ± standard error of mean (SEM) pain scores using the multidimensional composite pain scale (UNESP-Botucatu MCPS). BE=intraperitoneal bupivacaine (bupivacaine 0.25%, 2 mg/kg BW) and epinephrine (2 mg/kg BW). BD = intraperitoneal bupivacaine (bupivacaine 0.25%, 2 mg/kg BW) and dexmedetomidine (1 mg/kg BW). * Significantly increased when compared with baseline values. (Benito et al., 2018)

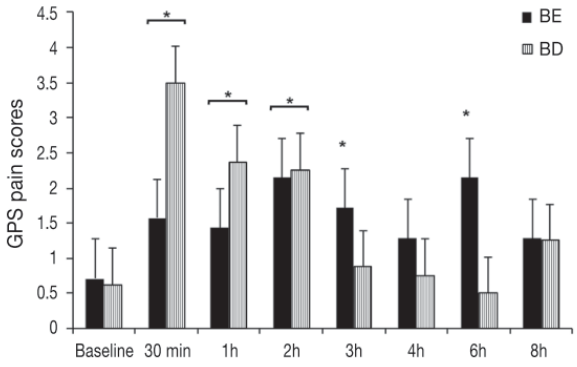

Figure 30 - Mean ± standard error of mean (SEM) pain scores using the Glasgow feline composite pain scale (GPS). BE=intraperitoneal bupivacaine (bupivacaine 0.25%, 2 mg/kg BW) and epinephrine (2 mg/kg BW). BD = intraperitoneal bupivacaine (bupivacaine 0.25%, 2 mg/kg BW) and dexmedetomidine (1 mg/kg BW). * Significantly increased when compared with baseline values. (Benito et al., 2018)

Dexamethasone

Prolonging of the duration of a block after perineural administration of dexamethasone is due to different mechanisms: suppression of the activity of unmyelinated C-fibres, inhibition of potassium channels, vasoconstriction effect, promotion of hyperpolarisation of peripheral nerve fibres, blockade of synaptic transmission and reduction of perineural inflammation. Perineural dexamethasone administration compared with intravenous administration in humans undergoing upper or lower limb surgery resulted in a longer duration of the sensory block (3 hours) not accompanied with a reduction in postoperative pain intensity or opioid consumption, so the longer duration of the block was unlikely to be clinically significant. (Pehora et al., 2017)

Regarding neurotoxicity in vivo studies show that dexamethasone attenuates the cytotoxicity of bupivacaine in mouse neuroblastoma cells or of ropivacaine in the medullary posterior horn but could crystallise with the latter at the nerve level. (Pehora et al., 2017; Polderman et al., 2018)

Opioids

Opioids as adjuvant to LAs are used but it is difficult to determine if their analgesic effect is due to stimulation of peripheral receptors or to central effect following redistribution to the central compartment. (Mousa et al., 2001)

Administration of epidural opioids produce effective and safe post-operative analgesia with minimal sympathetic blockade and no sedation or respiratory depression.

Morphine is characterized by low lipid solubility relative to other opioids which make systemic absorption less likely to occur from the epidural site. A dose of 0.1 mg/kg of morphine is reported for pain relief after thoracotomy and hysterotomy in baboons. (Popilskis et al., 2008)

Buprenorphine has been shown to prolong the duration of analgesia after perineural administration with LA in humans. It exerts its action through concentration-dependent blockade of sodium-dependent voltage-gated channels, inhibiting the generation of action potentials and interacting with μ receptors in unmyelinated C-fibre axons. (Leffler et al., 2012)

Side effects of neuroaxial opioids can be itch or pruritus, nausea, vomiting, urinary retention and respiratory depression with itch having an incidence of 20 to 100% of cases in humans. Pruritus arises shortly after analgesia and reduces the efficacy of neuroaxial opioids for pain relief. Intrathecal morphine (10-320 μg) produced profound scratching responses for several hours in rhesus monkeys (Figure 31). (M. C. H. Ko & Naughton, 2000) Different molecules are tested to reduce morphine-induced itch: the most effective drugs able to counteract this response maintaining analgesia are partial agonists such as nalbuphine and butorphanol, K opioid receptor (KOP) agonists such as nalfurfine, even if they produce dysphoria, and mixed KOP/MOP agonists such as butorphanol and pentazocine and they do not produce dysphoria or euphoria. (M. C. Ko, 2015)

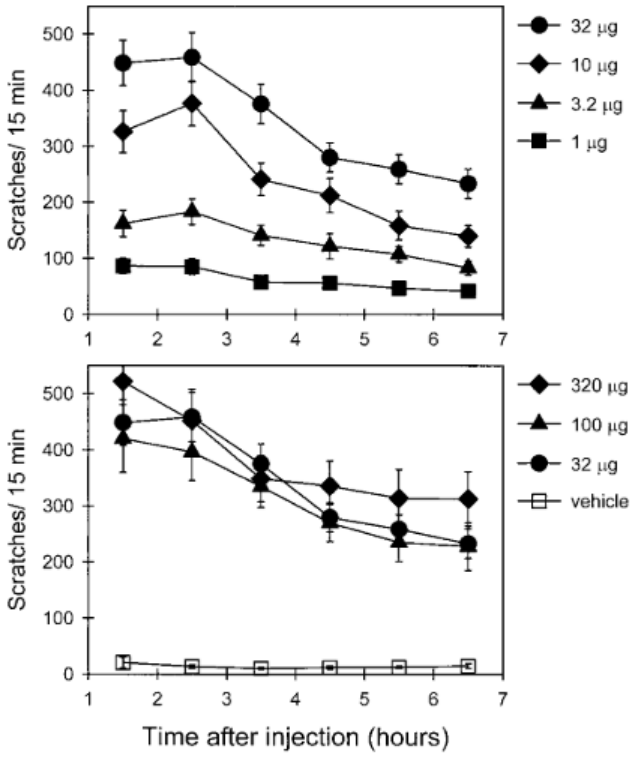

Figure 31 - Average time course of intrtechal morphine-induced scratiching responses in monkeys (n=8). Each value represents the mean SEM (two to three replications). Solid symbols represent different doses (micrograms) of intrathecal morphine, and open squares represent the intrathecal vehicle (saline) injection. (M. C. H. Ko & Naughton, 2000)

4.3 Intraoperative analgesia

4.3.1 Fentanyl

Fentanyl is a μ, k, δ receptor agonist with analgesic properties and high therapeutic index. The analgesic effect is about 5-15 minutes long according to dose and to route of administration. Peak effect is in 2-5 minutes after IV administration. Fentanyl tends to accumulate in the body after 2 hours of CRI, so the dose per minute should be reduced after the second hour.

Fentanyl induces dose-dependent respiratory depression associated with reduction in RR and tidal volume and increase in $EtCO_2$. Respiratory activity should be monitored in the recovery period. (Bufalari et al., 2012)

A dose of 8 μg/kg of fentanyl IV produced in 6 rhesus monkeys undergoing isoflurane anesthesia minimal changes in breaths clinically relevant. Two hours after fentanyl administration there was an increase in RR that was concomitant with a substantial decrease in plasma fentanyl concentrations as reported in Table 25. PaCO2 did not vary at any time point during the study. (Valverde Celia R. et al., 2000)

	Variable					
Time (min)	Temp (C)	RR–T (breaths/min)	RR–VC (breaths/min)	pHa (units)	$Paco_2$ (mm Hg)	Pao_2 (mm Hg)
Baseline	37.3 ± 0.2	12.7 ± 7.2	8.8 ± 2.2	7.51 ± 0.04	31.9 ± 4.3	446.1 ± 107.1
1	37.2 ± 0.3	9.0 ± 2.2	8.7 ± 2.7	7.49 ± 0.04	30.0 ± 3.7	416.9 ± 94.5
2	37.2 ± 0.3	8.2 ± 2.6	8.2 ± 2.6*	7.50 ± 0.03	31.2 ± 3.7	415.9 ± 91.3
5	37.3 ± 0.3	8.0 ± 2.5	7.7 ± 2.0*	7.50 ± 0.04	31.6 ± 2.5	422.5 ± 110.3
15	37.2 ± 0.3	6.8 ± 1.7	6.3 ± 0.8*	7.48 ± 0.05	31.3 ± 4.3	412.6 ± 105.3
30	37.2 ± 0.3	6.3 ± 0.8	6.3 ± 0.8*	7.46 ± 0.04*	34.6 ± 4.4	433.2 ± 94.8
60	37.1 ± 0.3	7.5 ± 2.6	6.3 ± 0.8*	7.49 ± 0.03	32.5 ± 2.9	450.4 ± 104.7
120	37.2 ± 0.4	13.5 ± 3.6	7.0 ± 1.7*	7.48 ± 0.05	32.3 ± 5.4	456.9 ± 97.3
180	37.5 ± 0.2	18.0 ± 5.1*	7.7 ± 2.0	7.49 ± 0.04	29.6 ± 4.3	465.7 ± 56.7
240	37.4 ± 0.1	23.8 ± 4.8*	7.5 ± 1.6	7.51 ± 0.05	30.5 ± 2.2	456.9 ± 70.2
300	37.4 ± 0.2	23.3 ± 6.6*	6.8 ± 1.2*	7.49 ± 0.03	32.9 ± 2.5	482.9 ± 71.2
360	37.4 ± 0.3	26.6 ± 5.2*	7.0 ± 1.7*	7.49 ± 0.03	30.6 ± 2.3	474.2 ± 75.9
420	37.5 ± 0.3	28.3 ± 5.6*	6.7 ± 1.8*	7.50 ± 0.03	29.9 ± 2.7	468.5 ± 66.2
480	37.4 ± 0.4	29.7 ± 9.2*	6.7 ± 1.8*	7.53 ± 0.03	29.2 ± 3.0	472.4 ± 71.3

Table 25 – Haemodynamic and hemtologic values for 6 monkeys anesthetized with isoflurne prior to and after IV administration of fentanyl. Data are expressed as mean SD. * Significant (p<0.05) difference from baseline value. HR: heart rate, bpm: Beats per minute, SAP: Systolic arterial pressure, DAP: Diastolic arterial pressure, MAP: mean arterial pressure. (Valverde et al., 2000)

At clinical doses, fentanyl can cause sinus bradycardia by increasing in vagal tone and no other significant cardiac effect. (Bufalari et al., 2012)

In a trial, six male rhesus monkeys in isoflurane anesthesia received progressive fentanyl bolus injections from 2 to 128 µg/kg (cumulative dose of 214 µg/kg) with 10 minutes between doses. (Nussmeier Nancy A. et al., 1991) As in humans, plasma fentanyl concentration producing analgesia was approximately 3 ng/mL and detectable respiratory depression as reduction in RR and increase in PaCO2 occurred at the same plasma concentration of 2-3 ng/mL. (Mcclain et al., 1980; Andrews et al., 1983; Jaffe Todd B. & Ramsey Frederic M., 1983) Below 40 ng/mL there were no significant changes in MAP, cardiac output, stroke volume, pulmonary arterial and central venous pressures and systemic vascular resistance, comparable to studies in humans. (Stanl & Webster, 1978; Mcclain et al., 1980; Bovill et al., 1984; Wynands et al., 1984; Bailey et al., 1985) Above this threshold there was a significant difference in MAP and cardiac output; these results are not consistent with human studies. The maximum analgesic, cardiovascular, respiratory effects were attained at 40 ng/mL plasma fentanyl concentration.

It is reported intranasal (IN) fentanyl administration in rhesus monkeys as a considerable alternative route of administration (Saccone et al., 2016). IN fentanyl administration was compared with IM administration with the same dosages (0.010-0.032 mg/kg) in three adult male subjects (Figure 32).

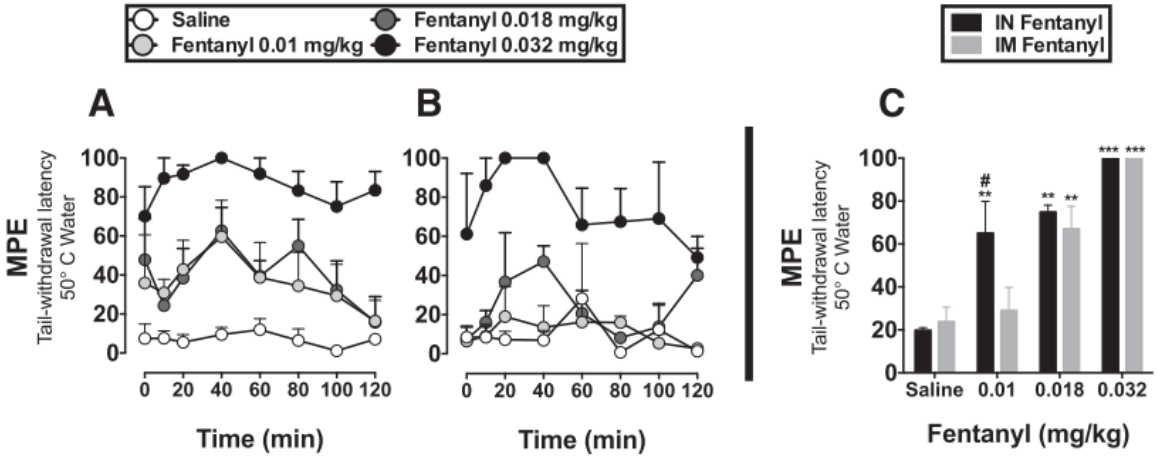

Figure 32 - The effect of fentanyl IN (A) and IM (B) on mean (6S.E.M.) tail-withdrawal latency in 50°C water plotted as a function of dose and time in rhesus monkeys (n = 3). Averaged (6 S.E.M.) peak measurement of tail-withdrawal latency taken from the time-course data for fentanyl administered IN (black bars) and IM (grey bars). Significant differences between fentanyl and saline are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Significant differences between routes of administration are indicated by #$p < 0.05$. (Saccone et al., 2016)

Dose of 0.032 mg/kg produced similar magnitudes and durations of effects and a comparable onset. Smaller doses of IN fentanyl (0.010 and 0.018 mg/kg) produced increases in tail-withdrawal latency whereas IM injections were not significantly different from control (saline). IN fentanyl administrations tend to have a faster onset of action compared to IM administrations. 0.018 mg/kg IN fentanyl produced a 50% increase in tail-withdrawal latency at the 0 time-point whereas the same IM dose reached the same magnitude of effect at 40 minutes after administration. (Saccone et al., 2016)

4.3.2 Ketamine

Ketamine can be used in CRI at sub anesthetic doses to ensure intraoperative analgesia; it was considered safe, with better postoperative analgesia, reduced need for additional opioid administration and reduced requirements for general anesthetics. There are no reported studies for ketamine used intraoperatively in *M. mulatta*.

In 17 dogs undergoing mastectomy were assigned three different protocols: ketamine bolus of 0.5 mg/kg and CRI of 20 µg/kg/h; fentanyl bolus of 5 µg/kg and CRI of 2 µg/kg/h; ketamine-fentanyl with the aforementioned doses. (de Moura et al., 2022) The infusions did not trigger significant cardiovascular changes in any of the groups and ensure analgesia during the intraoperative period. There were no significant differences between groups in the postoperative period. In the ketamine-fentanyl group there was a reduction in HR as reported in Table 26 that was expected considering the inhibition of the voltage-dependent calcium channels determined by the activation of µ opioid receptors. It did not trigger significant

		Postoperative period (hours)								
Variable	**Group**	**Baseline**	**0.25**	**0.5**	**1**	**2**	**4**	**6**	**8**	**12**
HR (beats minute^{-1})	GK	104 ± 13	107 ± 14^{a}	96 ± 20	87 ± 10$^{a\#}$	95 ± 14^{a}	86 ± 13	86 ± 13	98 ± 39	101 ± 14
	GKF	110 ± 22	104 ± 13^{a}	97 ± 9	89 ± 7^{a}	87 ± 14$^{a,b\#}$	90 ± 13	90 ± 7	95 ± 14	94 ± 16
	GF	97 ± 35	82 ± 24^{b}	71 ± 13	70 ± 20$^{b\#}$	75 ± 21$^{\#}$	65 ± 17$^{\#}$	79 ± 14	83 ± 18	83 ± 17
f_R (breaths minute^{-1})	GK	30 ± 10	40 ± 23	36 ± 15	43 ± 12$^{\#}$	47 ± 11$^{\#}$	37 ± 18	32 ± 10	30 ± 10	43 ± 47
	GKF	34 ± 14	44 ± 41	39 ± 23	45 ± 23	52 ± 39	35 ± 13	36 ± 11	29 ± 10	35 ± 15
	GF	28 ± 4	54 ± 21	44 ± 25	68 ± 37	43 ± 27	29 ± 17	33 ± 20	38 ± 19	27 ± 7
SAP mmHg	GK	136 ± 25	137 ± 9^{a,b}	139 ± 12	136 ± 15	136 ± 24	148 ± 17	144 ± 17	140 ± 22	143 ± 16
	GKF	153 ± 13	153 ± 15^{a}	155 ± 18	151 ± 17	170 ± 28	160 ± 15	160 ± 21	151 ± 12	143 ± 21
	GF	136 ± 13	132 ± 23^{b}	163 ± 12	148 ± 8	138 ± 35	136 ± 15	141 ± 10	158 ± 7$^{\#}$	147 ± 3

Table 26 - Mean ± standard deviation values of cardiorespiratory parameters, in dogs treated with ketamine (GK: bolus 0.5 mg -kg−1; CRI 20 µg -kg−1 -min−1 in intra- and postoperative periods), fentanyl (GF: bolus 5 µg -kg−1; intraoperative CRI 5 µg -kg−1 -h−1 and postoperative CRI 2 µg -kg−1 -h−1), or combination of ketamine-fentanyl (GKF: aforementioned doses) in postoperative period. a, b, c Significant difference between group. # Significant difference in treatment with a basal time point. HR Heart rate, fR RR, SAP systolic arterial pressure (de Moura et al., 2022)

hemodynamic changes.

Ketamine infusion maintained HR at a level close to baseline values due to the activation of adrenergic receptors. MAP was maintained at > 60 mmHg without the use of vasoactive drugs in any of the animals, probably due to a lower requirement for isoflurane.

4.4 Post-operative analgesia

4.4.1 Opioids

Buprenorphine

Buprenorphine is a partial opioid agonist-antagonist with long onset (30 minutes) and long duration of about 8-12 hours.

At clinical doses buprenorphine acts as a pure μ agonist. It has high μ receptor affinity such as antagonists that, consequently, are not able to reverse its effects.

In humans, opioids are effective and safe for the management of post craniotomy patients. (Na et al., 2011) Buprenorphine is not described as a single analgesia agent in human neurosurgery, but it has been used for postoperative pain after orthopaedic and abdominal surgery in people with adequate analgesia. In veterinary medicine buprenorphine alone or in combination with nonsteroidal anti-inflammatory drugs (NSAIDs) is described for various procedures. Doses are reported in Table 27. (DiVincenti Louis Jr, 2013)

Opioids		
	Buprenorphine	0.01–0.03 mg/kg IM every 12 h
	Fentanyl	0.5-0.15 μg/kg IM as needed
		7–10 μg/kg/h IV constant-rate infusion
	Morphine	1–2 mg/kg IM or SC every 4 h
	Oxymorphone	0.15 mg/kg IM every 4-6 h
NSAID		
	Carprofen	2–4 mg/kg IV or SC every 8-12 h
	Flunixin meglumine	1 mg/kg IM every 12 h
	Ketoprofen	5 mg/kg IM every 24 h
	Meloxicam	0.2 mg/kg PO every 24 h

Table 27 – A summary of suggested drugs, doses, and routes of administration for macaques. (DiVincenti Louis Jr, 2013)

It has been used alone and in combination with NSAIDs for neurosurgeries in NHPs such as craniotomies for different purposes: placement of deep-brain stimulation electrodes, implantation of headposts and microelectrode assays. The reported dose of buprenorphine for mild-moderate pain in *S. sciures* is 0.015 mg/kg. (Morris, personal communication, 2005)

Buprenorphine has the least respiratory depressant potential among all opioids and at low analgesic doses (below 3 mg/kg) it does not cause respiratory depression. (Ohtani et al., 1997)

It is reported the ability of buprenorphine to antagonize the respiratory effects of pure μ agonists in rhesus monkeys. (Liguori et al., 1996)

In case of coadministration of sedative and anesthetic drugs, especially benzodiazepines, the risk of significant respiratory depression is increased. (Johnson et al., 2005; Vadivelu & Anwar, 2010)

Potential adverse effects of opioids are generally not clinically significant and are easily controlled. Reduction in HR but not blood pressure has occurred secondary to buprenorphine in dogs and rats, but no haemodynamic alterations are noted in anesthetized cats. (Cowan' et al., 1977)

In case of buprenorphine-induced hypotension intravenous fluid administration is sufficient to restore physiological blood pressure.

To reverse the adverse respiratory effects of buprenorphine administration of doxapram instead of naloxone is indicated since the latter is not effective because buprenorphine has a higher receptor-binding capacity.

Sex differences for low to moderate efficacy μ opioid receptor (MOR) ligands such buprenorphine are described in rhesus monkeys. In a behavioural study including 3 males and 3 females rhesus macaques, buprenorphine, similarly to fentanyl, caused dose-dependent decreases in responding only in female monkeys suggesting a sex differences in MOR pharmacology that depends upon both the efficacy of the MOR ligand and the behavioural endpoint. (Schwienteck et al., 2019)

Transdermal fentanyl solution

Fentanyl is the ideal drug for transdermal delivery since it has a molecular mass less than 500 Da, a high lipophilicity and a low required daily dosage.

Long-acting single-administration topical fentanyl has been studied in six rhesus macaques both males and females. (Gregory W Salyards et al., 2017) Two formulations applied to the clipped dorsal skin were reported: one of 1.3 mg/kg and the other of 2.6 mg/kg of fentanyl. No adverse reaction was noted.

Mean fentanyl plasma concentration above the minimum effective concentration (MEC) reported in humans (0.2 ng/mL) was achieved one hour after the administration of the higher dose and two hours after the administration of the lower one. It remained above this threshold for 10 days and 7 days, respectively.

Mean plasma concentration of fentanyl remained above the average reported effective concentrations of 0.6 ng/mL from 8 hours through 4 days after administration for the 1.3 mg/kg dose and from 4 hours through 6 days after administration for the 2.6 mg/kg dose and of 2.6 ng/mL from 4 hours through 4 days for the 2.6 mg/kg dose, as described in Figure 33. Since the 1.3 mg/kg dose did not exceed the threshold of 1.2 ng/mL, the authors suggested carefulness in using this formulation. Terminal half-lives of both formulations were comparable to each other, and an absorption-dependent kinetics resulted from this study in which the rate of drug absorption is slower than elimination resulting in a terminal half-life that is reflective of absorption instead of elimination.

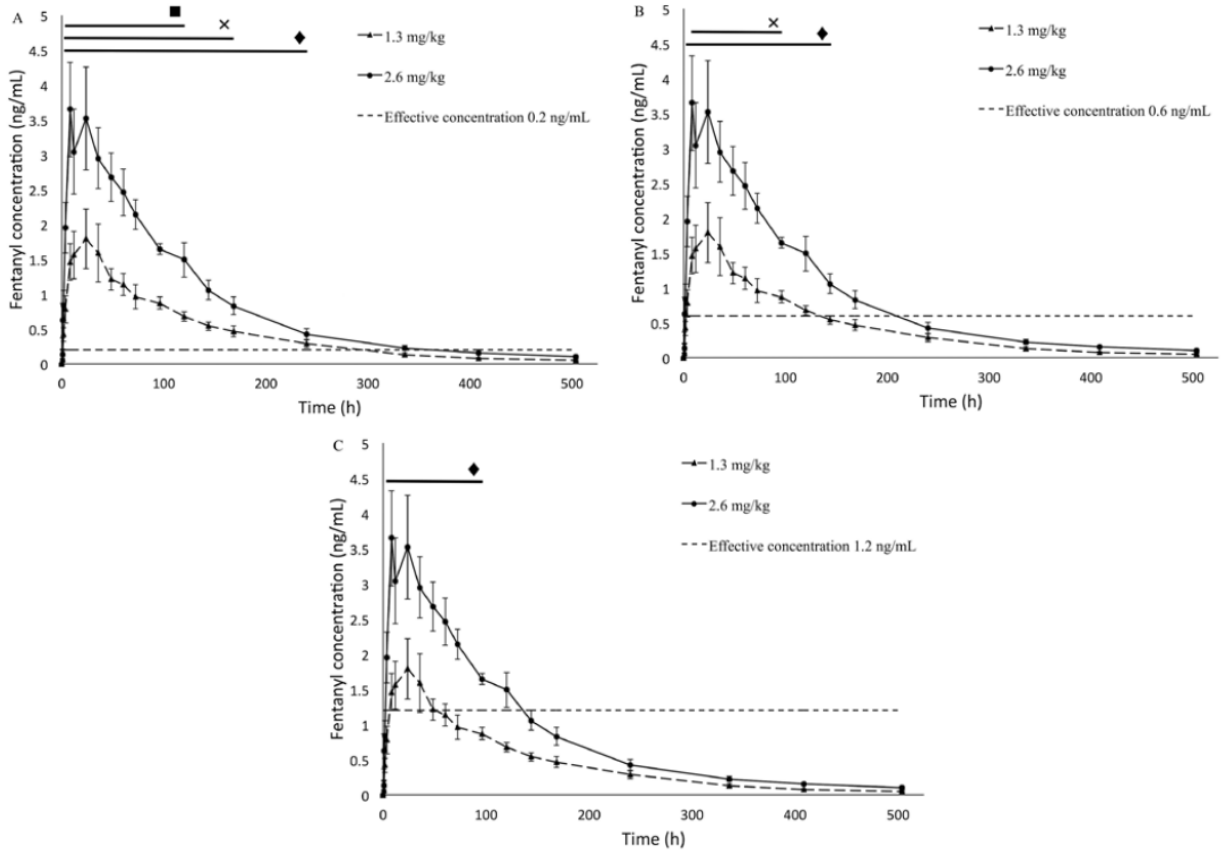

Figure 33 - Mean plasma fentanyl concentrations (ng/mL) over 504 h (21 d) in rhesus macaques administered TFS at a dose of either 1.3 or 2.6 mg/kg by means of a single topical administration as compared with the range of minimum effective concentrations (MEC) in humans undergoing abdominal laparotomy. (A) MEC = 0.2 ng/mL. (B) MEC = 0.6 mg/mL. (C) MEC of 1.2 ng/mL. Error bars represent 1 SEM above and below the data point; ×, time points at which fentanyl concentrations for the 1.3-mg/kg dose were significantly ($p < 0.05$) above the respective MEC; ♦, time points at which fentanyl concentrations for the 2.6-mg/kg were significantly ($p < 0.05$) above the respective MEC; ▪, time points at which fentanyl concentrations differed significantly between doses (panel A only). (Gregory W Salyards et al., 2017)

In males, plasma concentrations were significantly lower than in females suggesting a sex-specific difference probably related to the distribution of fat. Other possible causes can be differences in cytochrome P450 concentrations, sex-steroid effects, differences in drug binding, health status or social habits of the individual.

Tramadol

Tramadol is an analgesic drug similar to codeine. It is a racemic compound: the enantiomers interact with opioid, α2, 5-HT3 and serotonin receptors.

Pharmacokinetics of tramadol was analysed in 4 rhesus macaques after either oral (3 mg/kg) and intravenous administration (1.5 mg/kg). No significant adverse effects were noted after both administrations. Terminal half-life of IV tramadol was 111 minutes while that of oral tramadol was 133 minutes. Serum concentrations of tramadol over time are described in the following figure (Figure 34). (Kelly et al., 2015)

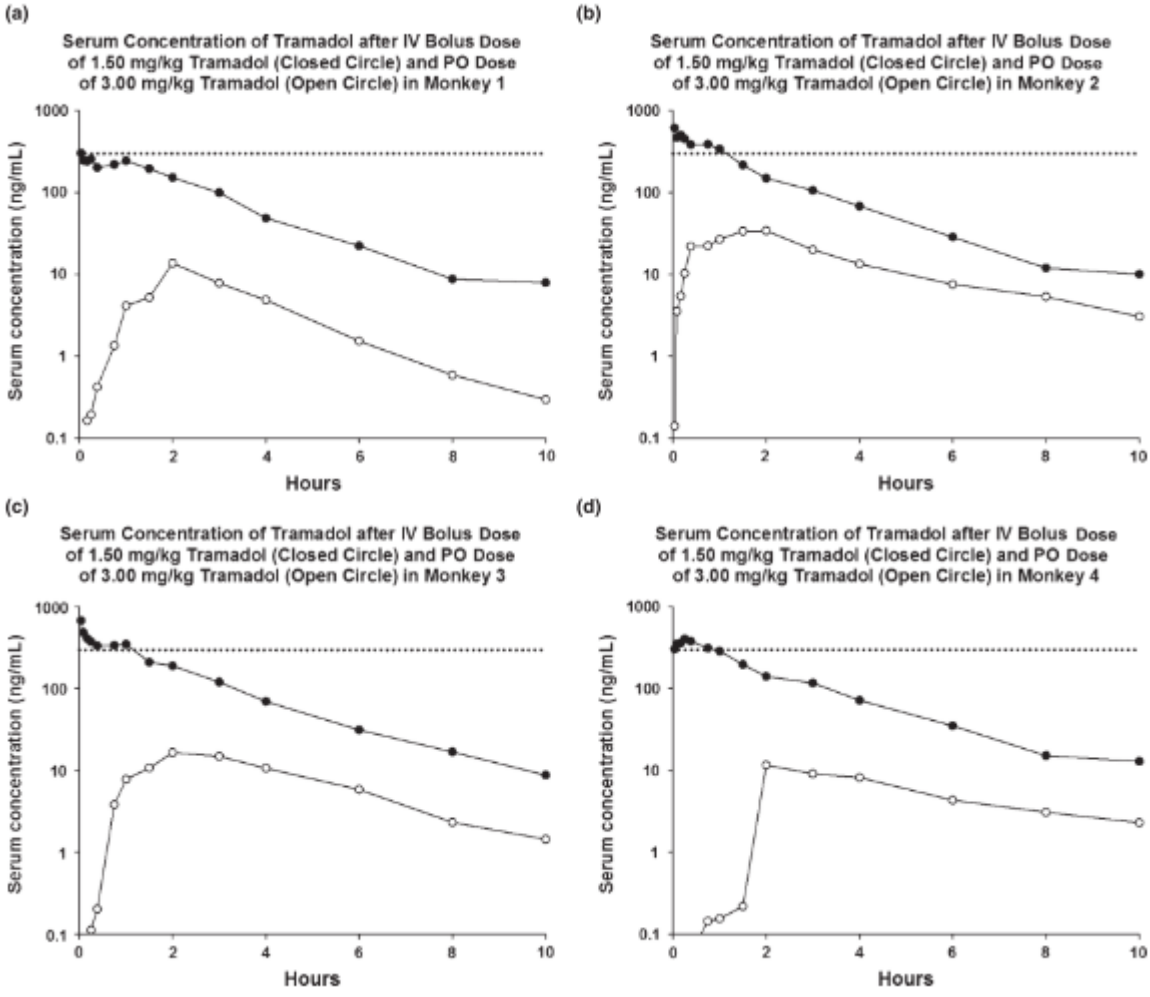

Figure 34 - (a–d) Serum tramadol concentrations in four rhesus macaques (Macaca mulatta) after IV bolus doseof 1.50 mg/kg tramadol (closed circles) and PO dose of 3.00 mg/kg tramadol (open circles). Horizontal dotted line reflects minimum target analgesia concentration of 298 ng/mL reported in humans (Lehmann et al., 1990). (Kelly et al., 2015)

Oral tramadol for post orthopaedic surgery analgesia is described in macaque ad a dosage of 2.5 mg/kg BID. (Uchihashi Mayu et al., 2015)

4.4.2 Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs prevent the formation of prostanoids, that are a subgroup of prostaglandins that mediate inflammation, pain and pyrexia, through the inhibition of the cyclooxygenase (COX). There are two isoforms of COX: COX-1 that is normally present in many tissues such as the gastrointestinal, renal and cardiovascular tissues and COX-2 that is expressed in response to inflammatory prostaglandins.

This class of drugs include many drugs such as aspirin, carprofen, ibuprofen, ketoprofen and ketorolac.

NSAIDs inhibits the inflammatory mediators so there is a ceiling effect that limits their analgesic potential. (Lamont, 2008) Analgesia from NSAIDs alone is not sufficient for major surgery. (Rao & Gelb, 2009)

Side effects of NSAIDs are secondary to the inhibition of COX-1: loss of vascular tone, loss of cytoprotective mechanisms in the gastrointestinal mucosa, loss of normal blood flow to the kidney and loss of thromboxane formation by platelets that lead to GI ulceration and haemorrhage, reduced glomerular filtration rate (GFR), kidney disease and coagulopathies.

Fluxine-meglumine is a more potent inhibitor of COX1 than COX2 so it is more likely to have adverse effects. Its use has been described in NHP neurosurgery at a dosage of 1.0 mg/kg BID for 3-5 days after the operation. (Tu et al., 2011)

Carprofen has an intermediate selectivity for COX2 and can be used for mild-to-moderate pain (2-4 mg/kg SC, IV q8h-q12h). Ketoprofen and meloxicam are the most selective for COX2 and their use has been described in neurosurgeries.(Saleem & Tanaka, 1996)

Ketoprofen (3 mg/kg OS) in 3 cynomolgus monkeys has a peak in ketoprofen plasma concentration at 20 minutes after administration and an elimination half-life of 2.32 ± 0.36 h. (Mauleon et al., 1994)

Meloxicam was evaluated in 12 cynomolgus monkeys (*M. fascicularis*) at different dosages and routes of administration. (Bauer et al., 2014) Dosages were based on subjective observations of animals that had been clinically treated with meloxicam and in which pain attenuated based on observed behaviours. Macaques were divided in two groups: one received SC meloxicam (0.6 mg/kg) followed 6 weeks by oral meloxicam (0.1 mg/kg). The second group received IM meloxicam (0.2 mg/kg) followed 6 weeks by SC meloxicam (0.6 mg/kg). After dosing peak plasma concentration occurred at 4h after a single oral dose, half-hour after a single IM

injection and half-hour after SC injection (Table 28). Absorption of meloxicam was independent of the dose.

Species	Route	Dosage	N	AUC_{∞} (ng×h/mL)	CL (mL/kg/h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	T_{max} (h)	Vz/f (mL/kg)	MRT (h)	V_d mL/kg
Cynomolgus	IM	0.2 mg/kg × 3 doses	4	109758.0 ± 31297.0	1.9 ± 0.6	2467.5 ± 630.5	13.6 ± 2.1	42.0 ± 12.0	37.3 ± 8.4	41. 0 ± 3.0	79.2 ± 22.6
Cynomolgus	IM	0.2 mg/kg	6			2134.2	13.6 ± 2.1	0.4			
Cynomolgus	PO	0.1 mg/kg × 3 doses	3	31900.6 ± 2485.5	3.1 ± 0.2	507.9 ± 80.3	14.1 ± 2.0	29.3 ± 22.8	63.9 ± 10.7	49.7 ± 7.7	156.1 ± 23.7
Cynomolgus	PO	0.1 mg/kg	6			440.7	14.1 ± 2.0	4			
Cynomolgus	SR1	0.6 mg/kg	6	80995.8 ± 13224.5	7.6 ± 1.2	3183.2 ± 447.8	13.1 ± 2.9	4.2 ± 2.2	140.1 ± 14.6	20.80 ± 6.30	152.21 ± 58.67
Cynomolgus	SR2	0.6 mg/kg	12	70421.1 ± 20520.6	9.1 ± 2.4	3942.2 ± 711.3	12.4 ± 1.7	2.3 ± 2.9	161.6 ± 40.5	16.57 ± 3.53	145.37 ± 26.63

Table 28 - Pharmacokinetics values for meloxicam in Cynomolgus monkeys. AUC_{∞}; AUC from time 0 to infinity with extrapolation of the terminal phase; CL, clearance; C_{max}, peak plasma concentration; F, bioavailability; IM, intramuscular; NS, not significant; PO, oral; SR, sustained release; T_{max}, time to peak plasma concentration; V_z, volume of distribution; V_d, volume of distribution at steady state.

Ibuprofen (7 mg/kg) is a mild analgesic that has been used in NHPs so it can be used only for mild postsurgical pain. Ketorolac (0.5-1 mg/kg IM) provides analgesia for moderate postoperative pain in NHPs and because it can cause prolong bleeding it should not be used in subjects with acquired or natural coagulopathies. (Popilskis et al., 2008)

4.4.3 Dexmedetomidine

Dexmedetomidine is an α2 agonist that can be used for its analgesic properties in the recovery period since its effects in the dorsal horns of the spinal cord reducing the activity of ascending nociceptive neurons. (Bao & Tang, 2020)

An experimental study in which auditory and somatosensory evoked potentials have been evaluated in 8 dogs demonstrated that dexmedetomidine in CRI has analgesic effect at higher doses (3 µg/kg/h) than sedative effects that occurred at a dosage of 1 µg/kg/h. (van Oostrom et al., 2011) Clinical signs of sedation are therefore not indicative of analgesia. However, at doses of 3 µg/kg/h dexmedetomidine may exert its side effects such as 2nd degree AV block type II due to reduction of the sympathetic tone. (Uilenreef et al., 2008) Dexmedetomidine infusion seems to have a cardioprotective role since the reduction in cardiac oxygen requirements secondary to the reduction in sympathetic tone. (Willigers et al., 2003)

4.4.4 Dexamethasone

Dexamethasone is a corticosteroid drug that is used in neurosurgeries to control cerebral edema and to reduce inflammation through suppression of prostaglandin synthesis. Dexamethasone suppresses bradykinin, releases other neuropeptides from nerve endings and has a direct inhibitory effect on C fibres in injured nerves. (Holte & Kehlet, 2002)

It also reduces post-operative pain: in humans a single dose of perioperative IV dexamethasone produces lower pain scores and decreased opioid consumption compared with placebo.(De Oliveira et al., 2011; Waldron et al., 2013) Intermediate (0.11-0.20 mg/kg) and high doses (>0.20 mg/kg) of dexamethasone resulted effective in reducing pain and opioid consumption compared with low dose dexamethasone (<0.10 mg/kg). (De Oliveira et al., 2013)

Chapter 5

5.1 Intraoperative monitoring and support

5.1.1 Depth of anesthesia

Parameters that can be used to assess the depth of anesthesia are loss of palpebral and corneal reflexes (Table 29), degree of muscle relaxation, rate and depth of breathing and lack of somatic response to surgical stimuli. If muscle relaxants are used during the maintenance of anesthesia monitoring autonomic responses to surgical events must be done to assess the depth of anesthesia.

Righting ability	When lost, the animal is unable to assume normal postures. This is the first reflex to be lost during anesthesia.
Swallowing/gag/ cough reflex	When lost, the placement of an endotracheal tube is possible. Dissociative anesthetics, such as ketamine, may not eliminate this reflex.
Palpebral reflex	When lost, the eyelids do not move when the corner of the eye is lightly touched. Dissociative anesthetics, such as ketamine, interfere with the interpretation of this reflex.
Withdrawal reflex	When lost, a toe that is firmly squeezed does not produce limb withdrawal. Anesthesia is sufficient to perform painful procedures when this reflex is lost.
Pupillary reflex	When lost, pupils do not constrict in response to light. Pupillary dilation and lack of light response are indicative of dangerously deep anesthesia.

Table 29 - Reflexes commonly used to monitor depth of anesthesia. (Fortman et al., 2018)

Increases in HR and SBP over 20% from baseline values can be interpreted as inadequate depth of anesthesia. Lacrimation and attempts to breathe out of synchrony with a ventilator might also indicate inadequate anesthesia. Patients generally lose consciousness at around 0.25-0.40 isoflurane MAC. MAC that abolishes voluntary movement is above the MAC that determines loss of consciousness and memory so the animal can be sufficiently anesthetized even though spontaneous movements can persist. (Popilskis et al., 2008)

5.1.2 Cardiovascular monitoring

Continuous HR and heart rhythm can be assessed through the positioning of an electrocardiogram (ECG). In larger NHPs, the insertion of an esophageal stethoscope can facilitate the detection of changes in HR and rhythm. (Popilskis Sulli J. et al., 2008)

Arterial blood pressure can be monitored through non-invasive methods such as cuff placement on the lower arm or leg or through invasive methods. Direct arterial pressure can be monitored after percutaneous cannulation or cutdown of the femoral or metatarsal artery. To cannulate the femoral artery in NHPs over 10 kg can be inserted an 18-gauge catheter; for smaller animals

can be used a 20-gauge catheter. Placement of a three-way stopcock between the catheter and the arterial tubing can be useful for serial blood sampling.

For monitoring of central venous pressure for the management of fluid replacement the external jugular is easily accessible using a 20-gauge catheter for medium-large sized macaques. Through a pulmonary artery catheter (5 French paediatric Swan-Ganz in adult macaques), cardiac output and calculation of systemic and pulmonary vascular resistance can be assessed. Placement of the pulmonary artery catheter into the right internal jugular vein represents the most direct approach to the right heart. It can be performed through catheterization of the femoral artery, but it requires fluoroscopic guidance. (Popilskis et al., 2008)

5.1.3 Respiratory monitoring

Assessment of oxygenation can be ascertained by the colour of the mucous membranes; other methods for quantitative pulmonary evaluation can be blood gas analysis, pulse oximetry, $EtCO_2$ monitors or mass spectrometry.

Pulse oximetry provides continuous measurement of arterial oxygen saturation. It can be attached to the tongue, finger, or ear. The tongue seems to be the site less influenced by intraoperative conditions or the colour of the skin.

Airway patency and adequacy of ventilation can be determined by measurements of expired CO2. Monitors for $EtCO_2$ sample the gas from the animal's airway and generate CO2 levels and CO2 waveform.

Airway pressure created by assisted or controlled ventilation is measured by a pressure manometer within the airway circuit. Peak inspiratory pressures of 15-30 cm H20 are usually sufficient to expand the lungs. High inspiratory peak pressures can be associated with kinked endotracheal tube, endobronchial intubation, or an insufficiently opened pop-off valve. High peak inspiratory pressure may lead to barotrauma and eventually pneumothorax or subcutaneous emphysema. (Popilskis et al., 2008)

5.1.4 Body temperature

NHPs have a small body mass and are prone to losing body temperature rapidly if care is not taken to minimize evaporative, conductive and radiation heat loss. Especially very young and small-size animals are particularly at risk for hypothermia. Insertion of a temperature probe into

either the oesophagus or the rectum is useful for assessing intraoperative body temperature. Rectal temperature is less accurate when changes in temperature are very rapid.

A tympanic probe in the external auditory canal can be used to assess brain temperature and is useful during cerebral procedures. (Popilskis et al., 2008)

Prevention of hypothermia is essential so warmed fluid bags, circulating warm water blankets and warm air blankets can be useful to maintain an adequate body temperature during anesthesia. Blankets or thick towels can be put under the animal to avoid heat loss from direct contact with the metal surfaces. (Fortman Jeffrey D. et al., 2018)

5.1.5 Urinary monitoring

Catheterization of the urinary bladder, especially for animals undergoing abdominal prolonged surgery, can be done to prevent urination during surgery. It helps prevent contamination of the surgical site and prevent heat loss through moisture evaporation. (Fortman Jeffrey D. et al., 2018)

5.1.6 Positioning

Type of procedure dictates the positioning of the animal on the operating table. Padding should be used under the animal to avoid pressure on bony areas.

In neurosurgeries the animal is often positioned in a stereotactic frame to keep the head still, so the animal is in a sternal position.

5.1.7 Fluid therapy

IV infusion of crystalloid solutions during surgery helps maintain normovolemia. The total amount of intraoperative fluids and blood loss depends by the site and duration of surgery. Isotonic electrolyte solutions such as Ringer solution can be sufficient at maintaining normal fluid composition at rates of 5-10 ml/kg/min. If haematocrit falls below 20% can be useful blood administration from heterologous or autologous sources. In macaques, initial blood transfusions do not normally cause any of the symptoms associated with acute transfusion reaction because performed isoantibodies to erythrocyte antigens are absent. (Popilskis et al., 2008)

5.1.8 Vasoactive drugs

Effects of prolonged hypotension are multifocal myoclonus, depressed electroencephalographic activity, rises in cisternal cerebrospinal (CSF) pressure, respiratory depression, and characteristic changes in serum and cisternal CSF glucose. Hypoxia and acidosis can occur during insult or recovery periods rather than hypotension itself.

Phenylephrine is a pure α agonist which increases blood pressure by increasing vascular resistance; this determines increases in afterload and reduction in HR and cardiac output. (Mon et al., 2017)

Twenty-one late-juvenile rhesus monkeys were rendered profoundly hypotensive for 15-, or 30-minute periods by means of infusion of trimethaphan camsylate. Blood pressure was then restored to prehypotensive levels with phenylephrine infusions. (Gamache & Myers, 1975) In another study has been described CRI of phenylephrine at 25 µg/kg/min IV. (J. K. Williams et al., 1988)

To treat isoflurane-induced hypotension a bolus of phenylephrine (1-2 µg/kg/min) followed by a CRI of 0.5-1.0 µg/kg/min is described. (Popilskis Sulli J. et al., 2008)

There are no current studies on the dosage of norepinephrine in rhesus macaque: in anesthetized dogs infusion doses of 0.1-1.5 µg/kg/min cause a dose-dependent increases in MAP without a significant change in HR and systemic vascular resistance. (Melchior et al., 1987; Bakker & Vincent, 1993)

When hypotension is accompanied by bradycardia a 2.5 mg IV bolus of ephedrine, repeated if needed, will increase blood pressure and improve cardiac output. (Popilskis Sulli J. et al., 2008)

To help suppress premature ventricular contractions a bolus of IV lidocaine (1.0-2.0 mg/kg) followed by a CRI (20-50 µg/kg/min) may be needed. Seizures occur at a dosage of IV lidocaine of 13.8 ± 3.0 mg/kg in unanesthetized monkeys. (Munson & Wagman, 1969)

Dobutamine is a sympathetic activator with β1-adrenoreceptor activity on myocytes mediating inotropic effect, β2-adrenoreceptor activity on vascular smooth muscle cells causing vasorelaxation, thus reducing peripheral vascular resistance, and α1-adrenoreceptor activity mediating chronotropic effect. In 66 *M. fascicularis* with dysmetabolic and diabetic conditions accompanied by proteinuria dobutamine infusions were evaluated (5, 10, 20, 30 and 40 µg/kg/min for 7 minutes each). They resulted in a dose-dependent increase in cardiac output, stroke volume and HR compared with control. (Wikstrom et al., 2021)

In case of cardiovascular collapse with decreased cardiac output and markedly reduced blood pressure dopamine and norepinephrine infusions can be used. (Popilskis Sulli J. et al., 2008)

5.1.9 Muscle relaxant

Vecuronium is described in rhesus macaques at a dosage of 0.04 mg/kg/h IV in the intraoperative period. In anesthetized subjects vecuronium helps facilitate mechanical ventilation and reduced skeletal muscle tone.

Vecuronium was also described as a single IV injection (10 μg/kg) in 4 anesthetized rhesus macaques and recovery without any reversal agent happened in about 23 minutes. The use of a reversal agent like sugammadex (1.0 mg/kg) shortened the recovery to 4 minutes after the second administration of vecuronium. (Staals et al., 2011)

5.1.10 Eye protection

Ophthalmic lubricant should be used routinely to protect the corneas from desiccation, especially in ketamine anesthetized animals whose eyes remain open thereby increasing the potential for dissection or irritation. (Fortman Jeffrey D. et al., 2018)

5.2 Post-operative care

Vomit is a common event when NHPs emerge from anesthesia. Extubation should be delayed until the animal regains the swallowing reflex or other signs of voluntary movements, e.g head movement and chewing. However, given the non-domestic nature of NHPs, it is advisable to remove the endotracheal tube just before the reappearance of the swallowing reflex to avoid shaking or gagging. Before extubation the oropharynx and the trachea should be suctioned if there are secretions, in particular for smaller NHPs because respiratory passages can be easily obstructed with bronchial secretions. If vomiting occurs after extubation the head should be positioned lower than the body in a prone position. Arterial and venous catheters should be maintained in place until sufficient consciousness has been regained. Nevertheless, given the non-domestic nature of NHPs, it is advisable to remove the catheters a few minutes after extubation, after verifying that the patient is properly ventilated. (Popilskis Sulli J. et al., 2008)

5.2.1 Environmental considerations

NHPs recovering from anesthesia should be kept warm in order to maintain an adequate body temperature. Placement of recovering animals in cages with blankets or thick towels in rooms with increased ambient temperatures, supplemental heat sources, can be methods to maintain body temperature. Recovery areas should be quiet and preferably separate to other animals.

NHPs should be maintained in a position that allows normal breathing and minimizes swelling or soiling of surgical sites. It is preferable a lateral recumbency to avoid accumulation of saliva in the back of the throat and excessive extension or flexion of the head and neck. Either of these situations can lead to respiratory compromise. (Fortman Jeffrey D. et al., 2018)

5.2.2 Monitoring

Vital signs should be checked until animals start to return to consciousness. Once the animal exhibits voluntary movement and swallow reflex, visual checks may be all that are safe for the caregiver to perform. (Popilskis Sulli J. et al., 2008)

Chapter 6

Materials and methods

6.1 Animals

Nine Rhesus macaques (*M. mulatta*), (2 females and 7 males, aged between 5 and 11 years) mean age of 8.6 years weighting between 8.45 and 17 kg (mean weight of 10.9 kg) underwent neurosurgical procedures (n=30) from 2019 to 2023.

Animals were enrolled in the two research projects:

- "Substrati neurali di funzioni motorie e socio-cognitive nella scimmia: dal laboratorio alle interazioni sociali libere", Aut Min 802/2018-PR del 15/10/2018, supported by ERC StG Grant "WIRELESS" (n. 678307), grant "Fare" MIUR, project "GANGLIA" (n. R16PWSFBPL), head of project Prof. Luca Bonini (University of Parma);
- "Meccanismi anatomo-fisiologici soggiacenti il recupero della consapevolezza visiva nella scimmia con cecità corticale", Aut Min 803/2018-PR del 15/10/2018, supported by ERC Consolidator grant "LIGHTUP" (n. 772953); project manager: Prof. Marco Tamietto (University of Turin); director of experiments: Prof. Luca Bonini.

Animals were housed in the following animal facility, A75A0 section of the Medicine and Surgery Department, Neuroscience Unit, University of Parma. (Aut. Min. della Salute (DGSAF) n. 10/2019-UT released the 19/03/2018 ai sensi dell'art. 20 del D.lgs 26/2014)

All animals were housed in stainless steel cages in pairs in accordance with 2010/63/CE and Dlgs 26/2014, regarding the space and environmental enrichment. These cages allowed for physical restrain of the macaques to perform IM injections.

Room was on a 12:12 light cycle. Temperature (20-24°) and humidity (30-60°) were controlled and maintained at optimal environmental conditions.

Diet consisted of pellet feed (about 200 g/day) or flour pellet flour mesh, peanut butter, fresh fruits, fruit juices, fresh vegetables. During training the liquids were regulated according to the frequency of training they perform; when they are at rest, they are given about 1 L of water/day.

6.2 Anesthetic equipment

Anesthesia machine used in procedures from 2017 to 2022 was an anesthesia system on stand with a non-rebreathing circuit and with a rebreathing circuit Komesaroff CO2 absorber canister.

From March 2022 the anesthesia machine used was Mindray® V60 with a pressure-/volume-control ventilator Mindray® Veta 5 (Figure 35) connected with a non-rebreathing circuit. Depending on the size of the animal the rebreathing bag used varied from 0.5 to 1.5 L.

A multiparameter monitor (Mindray® iMEC8 Vet) was used for the intraoperative control of the following physiological parameters: HR, ECG, NIBP, RR, $EtCO_2$, haemoglobin saturation and oesophageal temperature (Figure 35).

A fluid pump was utilized for the fluid therapy during the intraoperative period.

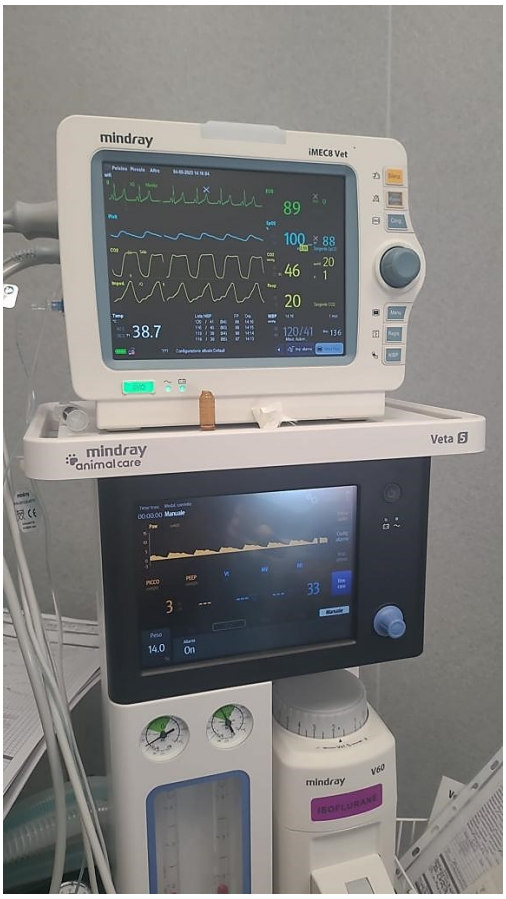

Figure 35 – Mindray® V60 anesthesia machine, Mindray® Veta 5 ventilator and Mindray® iMEC8 Vet multiparameter monitor.

6.3 Anesthetic procedure

Before surgery, animals fasted for about 12 hours to reduce the probability of vomit at the moment of sedation or recovery.

0.03 mg/kg of atropine (Atropina solfato®) was administered intramuscularly in the bicep femoris prior to the administration of the sedation mixture to reduce salivary secretions. After about 10 minutes, animals were sedated with 4.5 mg/kg of ketamine (Lobotor®) combined with 50 µg/kg of medetomidine (Domitor®) mixed in the same syringe given intramuscularly in the bicep femoris.

When profound sedation was achieved the animals were then transported from the cage to the preparation room. Trichotomy of the head and insertion of cannula in the external saphenous vein using either a 20G or 22G cannula depending on the size of the animal were performed: Figure 36.

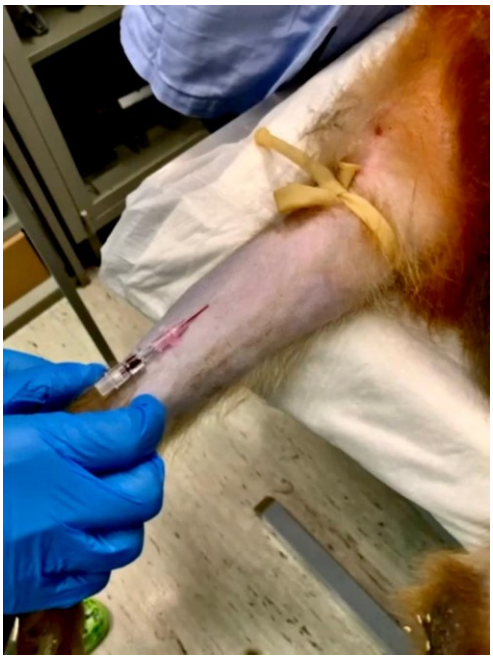

Figure 36 - Cannula insertion in the external saphenous vein.

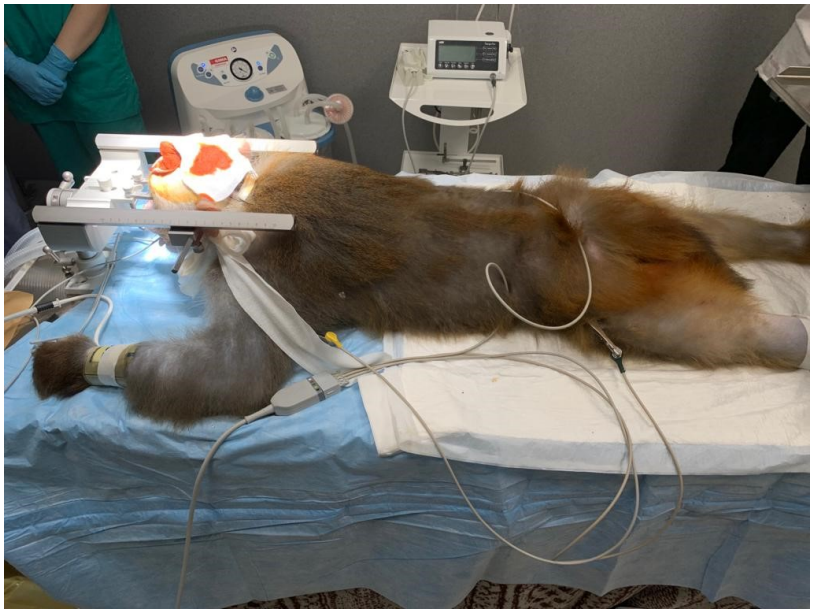

Figure 37 – Positioning in sternal recumbency.

Once the preparation of the animal was done, it was transported on the operating table and positioned in sternal recumbency (Figure 37). If the level of sedation reached was light, an IV bolus of thiopental 1.25% (Pentothal sodium®) of 0.3 mg/kg was administered to perform intubation.

A splash of 0.2-0.4 mg/kg lidocaine (Lidocaina 2%®) on the glottis was performed at 2 minutes before intubation (Figure 38).

The endotracheal tube, lubricated with lidocaine gel (Luan 2.5% gel®), was inserted into the trachea with the aid of a laryngoscope (Macintosh blades) (Figure 39). The tube was then secured with a gauze to the neck and cuffed.

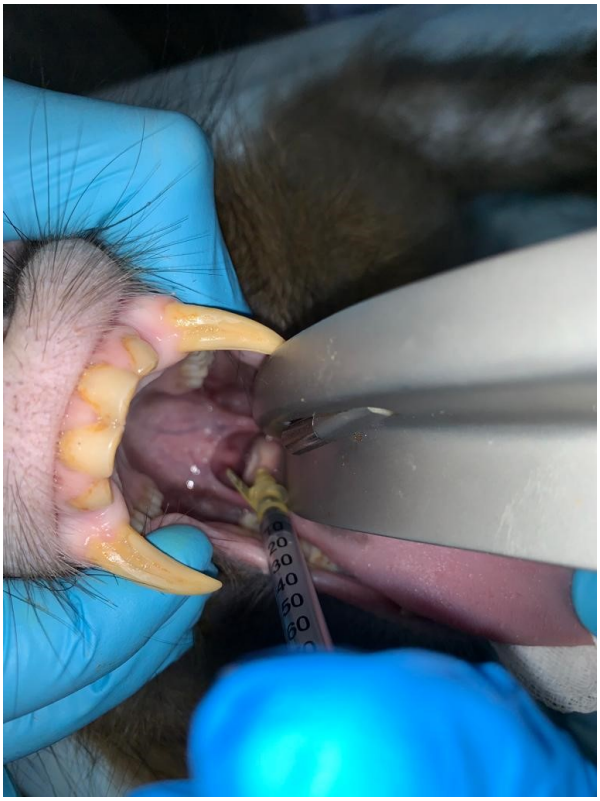

Figure 38 - Splash of lidocaine on the glottis.

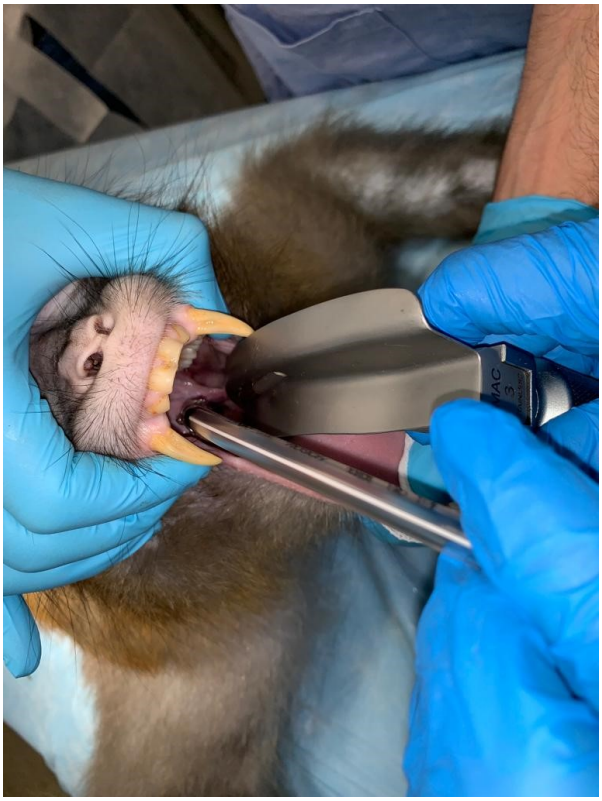

Figure 39 - Intubation.

The animal was placed on a thermic support (heating pad or forced-air warming device, Mistral-Air® MA1200) on the surgical table. The animal was positioned in the stereotactic frame in sternal recumbency (Figure 40) and attached to the breathing circuit and anesthesia was maintained with isoflurane (Isoflo®) vaporized in a mixture of oxygen and medical air (1:1) at a flow rate of 0.3 L/kg.

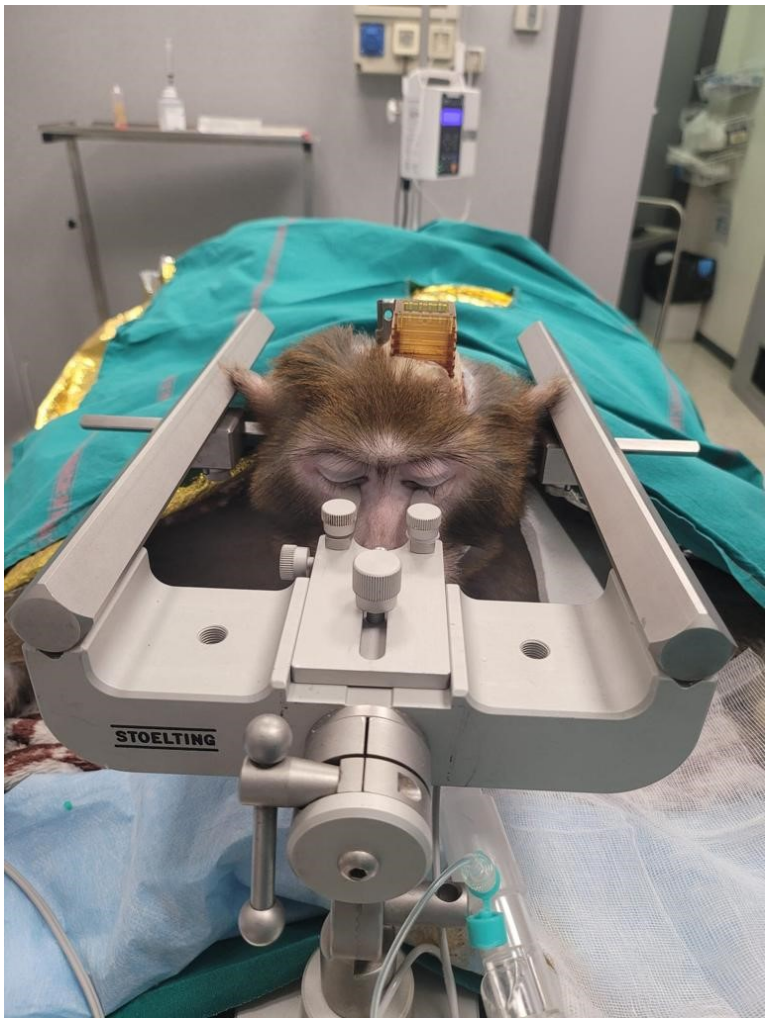

Figure 40 - Positioning in the stereotaxic frame.

6.4 Anesthesia monitoring and support

Pulse oximeter was attached either at the tongue or at the finger (thumb or forefinger) of the animal (Figure 41**Errore. L'origine riferimento non è stata trovata.**). Ringer's lactate solution was used for crystalloid infusion at 4-5 ml/kg/hr (Figure 42).

Pressure cuff for NIBP was positioned on the arm (Figure 43) and ECG electrodes were placed.

An esophageal probe was inserted into the oesophagus to monitor body temperature (°C).

The animal was covered with an isothermal blanket. (Figure 43)

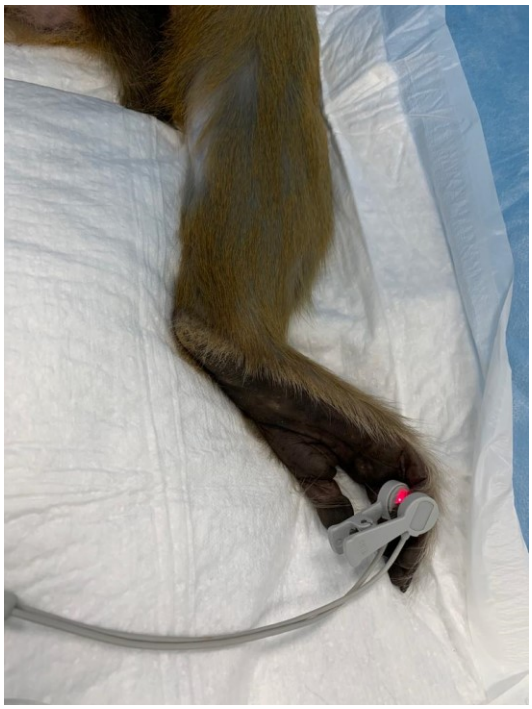

Figure 41 - Pulse oximeter placement on the finger.

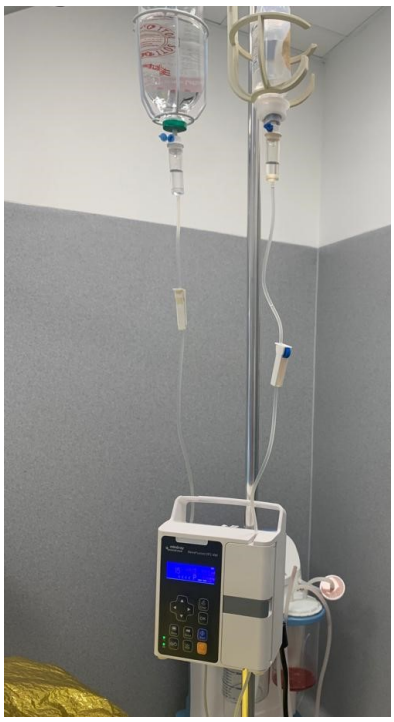

Figure 42 - Ringer's lactate solution infusion and norepinephrine infusion.

Figure 43 - NIBP cuff positioning. Detail of the isothermal blanket.

Monitoring of HR (beats per minute, bpm), RR (acts per minute, apm), SAP, MAP, DAP (mmHg), oxygen saturation (SpO2, %), end tidal carbon dioxide concentration ($EtCO_2$, mmHg), temperature, gas settings (%) was recorded at 15 minutes intervals on the anesthesiology record.

If MAP dropped below 60 mmHg for more than 5 minutes an IV infusion of norepinephrine (Noradrenaline tartrate®) at 0.1-0.3 µg/kg/min (Figure 42**Errore. L'origine riferimento non è stata trovata.**) or an IV infusion of dobutamine at 2-5 µg/kg/min was performed.

If required, the animal was mechanically ventilated with pressure- or volume- control ventilator in order to maintain an $EtCO_2$ of about 40 mmHg.

Dexamethasone (Soldesan®) 0.5 mg/kg was administered IM to avoid cerebral edema at 20 minutes before surgery and at the end of the surgical procedure. Ketoprofen (Orudis®) was administered IM at the end of the procedure in case of necessity (3 mg/kg). Benzatinic benzylpenicillin (50000 UI/kg) combined with dihydrostreptomycin (16 mg/kg) (Rubrocillina forte®) was administered IM at the end of surgery.

6.5 Surgical procedures

Every surgical procedure was performed in aseptic conditions: the surgical sites were shaved and disinfected. The placement in stereotaxic frame was crucial for most of the procedures, in particular the electrodes placements where precise stereotaxic coordinates were essential to the insertion of the electrodes in target brain regions (Figure 44).

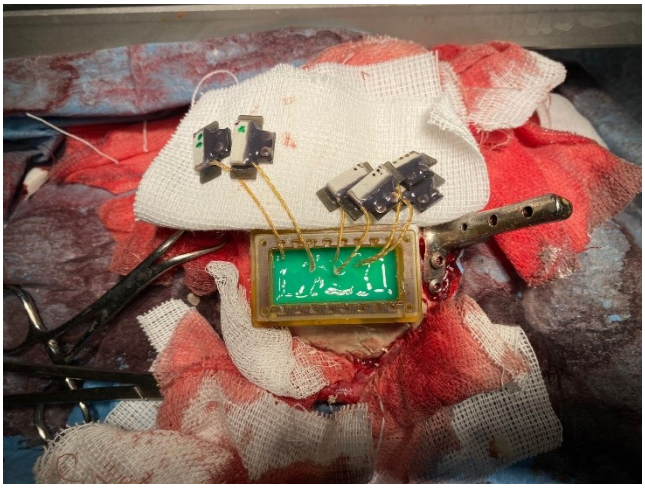

Figure 44 - Chamber and electrodes placement.

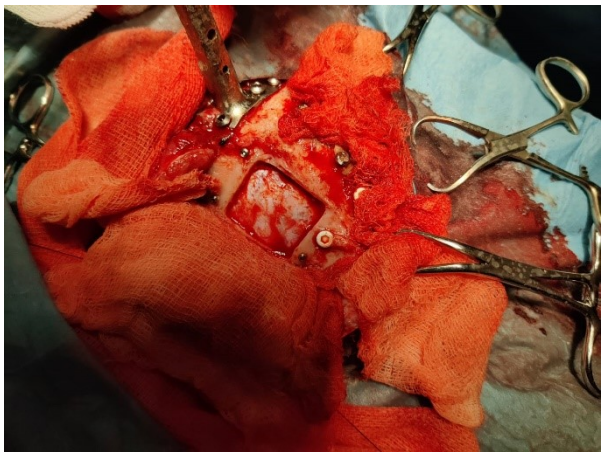

Figure 45 - Craniotomy.

Headpost placements and craniotomies for chamber placement (Figure 45) were performed in according to the neurosurgical standard procedures. The muscle fascial planes were

sutured; then, an intradermal suture and a simple interrupted sutures of the skin were performed using a reabsorbable material. (Gindrat et al., 2015)

6.6 Post-operative period

The animal was maintained on the thermic support until the removal of the endotracheal tube.

The orotracheal tube was removed about at 5 minutes after discontinuation of isoflurane administration and oxygen was delivered approximately for 5 minutes after extubation. Then, the intravenous cannula was removed, and the animal was transported from the surgery room to the recovery cage. The animal was placed in lateral recumbency, and respiratory pattern and rate were monitored.

If there were no spontaneous movements within 10 minutes, it was given atipamezole (Antisedan®) at the dose of 20-30 µg/kg IM. The animal was monitored visually until recovery of the ability to climb. If the animal showed dysphoria, it was treated with medetomidine 0.5-1 µg/kg.

Chapter 7

Results

A total of 30 neurosurgical procedures was done: they included implants of headposts (n=3), recording chamber placements (n=8), electrodes placements (n=13), implants revisions (n=3), implants removals (n=2) and wound curettage (n=1). Mean duration of procedures was 4 hours 27 minutes (range from 35 minutes to 10 hours 25 minutes) (Table 30).

Procedure	**Headpost placement**	**Recording chamber placement**	**Electrodes placement**	**Implants revision**	**Implants removal**	**Wound curettage**
Mean time	215 min	246 min	347 min	91 min	237 min	65 min

Table 30 - Procedures mean duration

With the selected protocol (ketamine 4.5 mg/kg, medetomidine 50 µg/kg), profound sedation was achieved in the first 5 minutes in 28 macaques. In two animals, profound sedation was achieved between 10 to 30 minutes.

Intubation was performed with endotracheal tubes with sized ranging from 3.5 to 6 mm.

To perform intubation in 21 macaques a dose of 0.3 mg/kg of thiopental 1.25% was necessary.

In 27 anesthetic procedures ventilation was spontaneous; for 3 of 30 animals volume- or pressure-controlled ventilation was necessary (mean peak airway pressure 10 mmHg).

MAP was maintained above 60 mmHg. There were multiple hypotensive phenomena (MAP < 60 mmHg for more than 5 minutes) that needed the use of a vasoactive drug. For all the cases (n=5) 0.1-0.3 µg/kg/min norepinephrine infusion was started at a mean time of 80 minutes after the beginning of the monitoring (range 5 to 120 minutes). Infusions were continued until the end of all procedures.

During electrodes placements in four macaques and during chamber placement in one macaque a mannitol CRI (1.5 g/kg in 20 minutes) was needed to reduce the possible increase in intracranial pressure.

HR, RR, $EtCO_2$, MAP and oesophageal temperature values were considered as physiological parameters of this study. These were taken in consideration at different time points: T0 (= 10 minutes after intubation), T15, T30, T60, T120, T180, T240 and T300.

Macaques were divided into 4 groups:

- Isoflurane delivered in 100% oxygen (OI) (n=22)

- Isoflurane delivered in a mixture of 1:1 oxygen and medical air (OMI) (n=8)
- Use of thiopental to perform intubation (T) (n=8)
- No thiopental to perform intubation (NT) (n=22)

The recorded data for all parameters were compared as follows: the OI group was compared with the OMI group whereas the T group was compared with the NT group using the student t-test.

HR was not significantly different between T and NT groups (p>0.05) while the same parameter resulted significantly increased at T30 in the OI group compared with the OMI group (p=0.008).

At T240 the RR of the T group was significantly increased compared with the NT group (p=0.0397). The respiratory rates of T120, T180 and T240 of the OMI group were significantly lower than the same parameters at the same time points of the OI group (p_{120}=0.0489; p_{180}=0.0238; p_{240}=0.0449).

There was no significative difference in $EtCO_2$ at any time point (p>0.05).

MAP resulted significantly increased at T30 in the T group compared with the NT group (p=0.0163) while other time points did not show any significant difference (p>0.05).

Oesophageal temperature showed a mild decrease in all groups, but no significant differences were recorded. Using a thermal support, body temperature remained within physiological values.

In the following table (Table 31) it is reported mean values, levels of significance (p) and degree of freedom for each comparison.

		T0	T15	T30	T60	T120	T180	T240	T300
HR									
T/NT mean values		118.71-113.57	106.67 - 109	110 - 106.09	109.83 – 100.43	102 – 97.8	101.25 - 99.24	101.67-99.93	112.5-102.44
p		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
df									
OI/OMI mean values		116.95-111.64	117.5 - 100.8	109.96 - 99.38	105.21-96.13	100.38-94.75	100.79-97.29	101.27-97.86	104.13-104.67
p		> 0.05	> 0.05	0.0077	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
df				28					
RR									
T/NT mean values		27.14-26.9	25 - 29	28 – 30.53	29.5 – 28.32	30 - 28.28	35.33 - 28.2	37.5 – 25.7	37 - 25.33
p		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	0.0397	> 0.05
df								13	
OI/OMI mean values		27.5 - 24.6	28.25 - 26.5	29.72 - 26	29.5 - 25	30.18 – 23.2	31.43 - 22.25	29.64 – 20.75	28.5 – 24.67
p		> 0.05	> 0.05	> 0.05	> 0.05	0.0489	0.0238	0.04449	> 0.05
df						20	16	13	
	$EtCO_2$								
T/NT mean values		46 -46.9	51-42.67	48.71 – 46.1	48.33 - 44.05	46.75 - 42.17	46.33 – 41.53	45 - 40	44 – 38.33
p		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
df									
OI/OMI mean values		47.09 – 44.8	47.25 - 41	47.09 – 45.4	45.5 – 43.4	43.29 - 42	42.36 – 42.25	40.36 – 41.5	39.5 - 39
p		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
df									
MAP									
T/NT mean values		95.17 - 88.71	83.5 - 89	79.57 - 66.15	71.5 - 66.65	69 - 65.94	61 - 66.31	65.5 – 64.55	66 -68.5
p		> 0.05	> 0.05	0.0163	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
df				25					
OI/OMI mean values		90.24 – 89.83	81.25 - 95.67	70.59 – 65.4	68.63 – 64.5	65.82 - 70.67	64.39 - 73.5	63.7 - 68	67.57 - 70
p		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
df									

Table 31 – T-test between T and NT groups and between OI and OMI groups. It is reported the level of significance (*p*) and the degree of freedom (df) for each comparison.

Then, student t-test was performed for every parameter in each group comparing its T0 value to every other time point value, as reported in Table 32, Table 33, Table 34 and Table 35.

	T15	**T30**	**T60**	**T120**	**T180**	**T240**	**T300**
HR mean value T0: 118.71	106.67	110	109.83	102	101.25	101.67	112.5
p	> 0.05	> 0.05	> 0.05	0.01906	0.009	0.0241	> 0.05
RR mean value T0: 27.14	25	28	29.5	30	35.33	37.5	37
p	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
$EtCO_2$ mean value T0: 46	51	48.71	48.33	46.75	46.33	45	44
p	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
MAP mean value T0: 95.16	83.5	79.57	71.5	69	61	65.5	66
p	> 0.05	> 0.05	0.01293	0.03058	0.04774	> 0.05	> 0.05

Table 32 - T-test between T0 and different time points reporting the p value and the mean value for each parameter in the T group.

	T15	**T30**	**T60**	**T120**	**T180**	**T240**	**T300**
HR mean value T0: 113.57	109	106.09	100.43	97.8	99.24	99.93	102.44
p	> 0.05	0.0204	0.0001	1.4E-05	0.0008	0.0017	
RR mean value T0: 26.9	29	30.53	28.32	28.28	28.2	25.69	25.33
p	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
$EtCO_2$ mean value T0: 46.9	42.67	46.1	44.05	42.17	41.53	40	38.33
p	> 0.05	> 0.05	> 0.05	0.0298	0.0213	0.0052	0.0039
MAP mean value T0: 88.71	89	66.15	66.65	65.94	66.31	64.55	68.5
p	> 0.05	0.0001	0.0001	0.0001	0.0006	0.0006	0.0255

Table 33 - T-test between T0 and different time points reporting the p value and the mean value for each parameter in the NT group.

	T15	T30	T60	T120	T180	T240	T300
HR mean value T0: 116.94	117.5	109.95	105.21	100.38	100.79	101.73	104.13
p	> 0.05	0.02648	0.00171	7.5E-05	0.00046	0.00146	0.01415
RR mean value T0: 27.5	28.25	29.73	29.5	30.18	31.43	29.64	28.5
p	> 0.05	> 0.05	> 0.05	> 0.05	0.04139	> 0.05	> 0.05
$EtCO_2$ mean value T0: 47.09	47.25	47.09	45.5	43.29	42.36	40.36	39.5
p	> 0.05	> 0.05	> 0.05	> 0.05	0.04	0.00995	0.01225
MAP mean value T0: 90.24	81.25	70.59	68.63	65.82	64.38	63.7	67.57
p	> 0.05	0.00082	0.00016	6.6E-05	0.00025	0.00077	0.01242

Table 34 - T-test between T0 and different time points reporting the p value and the mean value for each parameter in the OI group.

	T15	T30	T60	T120	T180	T240	T300
HR mean value T0: 111.63	100.8	99.38	96.13	94.75	97.29	97.86	104.67
p	> 0.05	0.00761	0.00224	0.00066	0.00025	0.018	0.0244
RR men value T0: 24.6	28.25	26	25	23.2	22.25	20.75	24.67
p	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
$EtCO_2$ mean value T0: 44.8	41	45.4	43.4	42	42.25	41.5	39
p	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
MAP mean value T0: 89.83	95.67	65.4	64.5	70.67	73.5	68	70
p	> 0.05	0.00497	0.00508	0.03655	> 0.05	0.01293	-

Table 35 - T-test between T0 and different time points reporting the p value and the mean value for each parameter in the OMI group.

Compared to baseline values (=T0) in the T group (Table 32) a significant reduction in HR is evident at T120, T180 and T240 (p_{120}=0.019; p_{180}=0.009; p_{240}=0.0241) associated with a significant reduction in MAP values at T60, T120 and T180 (p_{60}=0.013; p_{120}=0.024; p_{180}=0.045) remaining in physiologic range.

In the NT group (Table 33) there is a significant reduction in HR values from T30 to T240 (p_{30}=0.02; p_{60}=0.0001; p_{120}=1.4E-05; p_{180}=0.0008; p_{240}=0.0017) compared with T0 values. MAP significantly decreased from T30 to T300 compared with T0 values (p_{30}=0.0001; p_{60}=0.0001; p_{120}=0.0001; p_{180}=0.0006; p_{240}=0.0006; p_{300}=0.0255). $EtCO_2$ has a negative trend with significantly different mean values from T120 compared with T0 (p_{120}=0.03; p_{180}=0.021; p_{240}=0.0052; p_{300}=0.0039).

HR in the OI group (Table 34) significantly decreased compared with T0 values (p_{30}=0.02648; p_{60}=0.00171; p_{120}=7.5E-05; p_{180}=0.00046; p_{240}=0.00146; p_{300}=0.01415).

A reduction in MAP values compared with baseline values was also seen (p_{30}=0.0008; p_{60}=0.0002; p_{120}=6.6E-05; p_{180}=0.0003; p_{240}=0.0008; p_{300}=0.0124). $EtCO_2$ values have a negative trend from T180 to T300. (p_{180}=0.04; p_{240}=0.00995; p_{300}=0.01225).

At T180 an increased in RR from baseline values is evident (p_{180}=0.04139).

From T30 a significant reduction in HR values was shown in the OMI group (Table 35) compared with baseline values (p_{30}=0.00761; p_{60}=0.00224; p_{120}=0.00066; p_{180}=0.00025; p_{240}=0.018; p_{300}=0.0244). It was accompanied by a significant reduction in MAP values compared with baseline values (p_{30}=0.00497; p_{60}=0.00508; p_{120}=0.00066; p_{180}=0.03655; p_{240}=0.01293).

If there was any significant variation between T0 and a specific time point, the point value in question would be further analysed. The previous and the successive time points were compared with the time point that had shown a significant difference in the previous inquiry using the student t-test.

In the NT the MAP has shown a significant decrease between T15 and T30 (p=0.0025) from 89 to 66.15 mmHg.

MAP had also shown a significant decrease between T15 and T30 in the OMI group (p=0.0189) from 95.67 to 65.4 mmHg.

To evaluate the trend of every group, mean values for every parameter at any time point were compared and showed in the following graphs (Figure 46, Figure 47, Figure 48 and Figure 49).

All macaques recovered with no complication. Mean time between the extubation and the recovery of climb ability is 17 minutes with a range between 15 and 27 minutes.

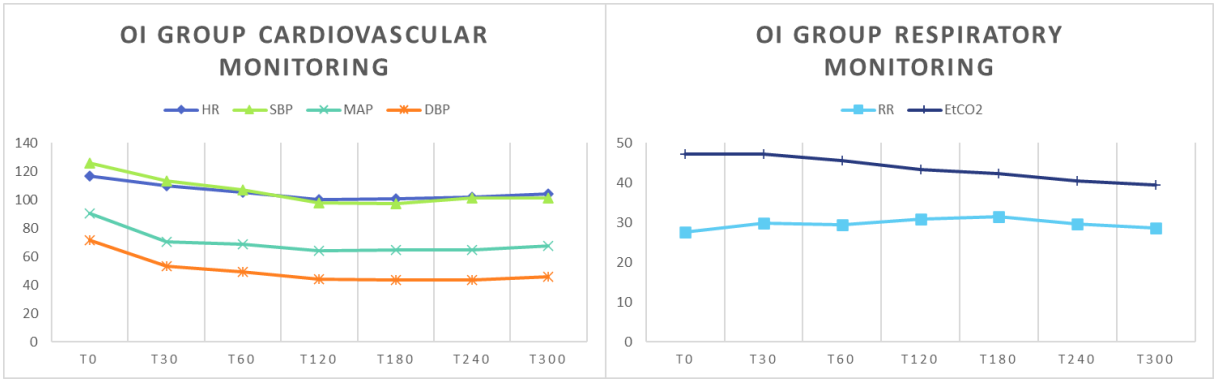

Figure 46 - (A) Cardiovascular parameters trend of the OI group. (B) Respiratory parameters trend of the OI group. HR: heart rate; SBP: systolic blood pressure; MAP: mean arterial pressure; DBP: diastolic blood pressure; RR: respiratory rate; $EtCO_2$: end tidal CO2.

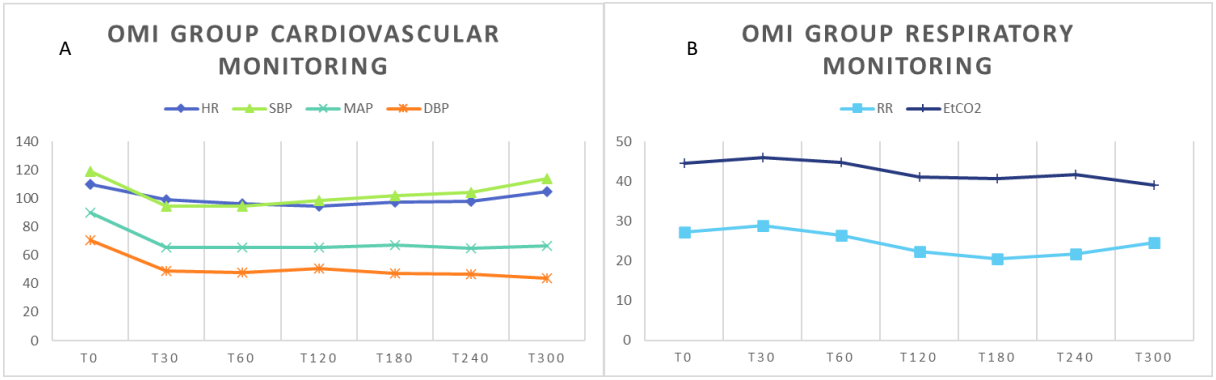

Figure 47 - (A) Cardiovascular parameters trend of the OMI group. (B) Respiratory parameters trend of the OMI group. HR: heart rate; SBP: systolic blood pressure; MAP: mean arterial pressure; DBP: diastolic blood pressure; RR: respiratory rate; $EtCO_2$: end tidal CO2.

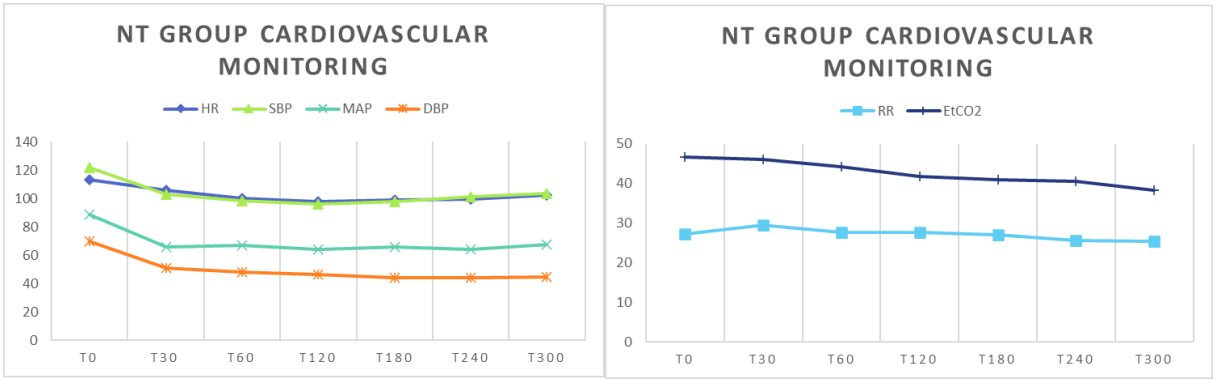

Figure 48 - (A) Cardiovascular parameters trend of the NT group. (B) Respiratory parameters trend of the NT group. HR: heart rate; SBP: systolic blood pressure; MAP: mean arterial pressure; DBP: diastolic blood pressure; RR: respiratory rate; $EtCO_2$: end tidal CO2.

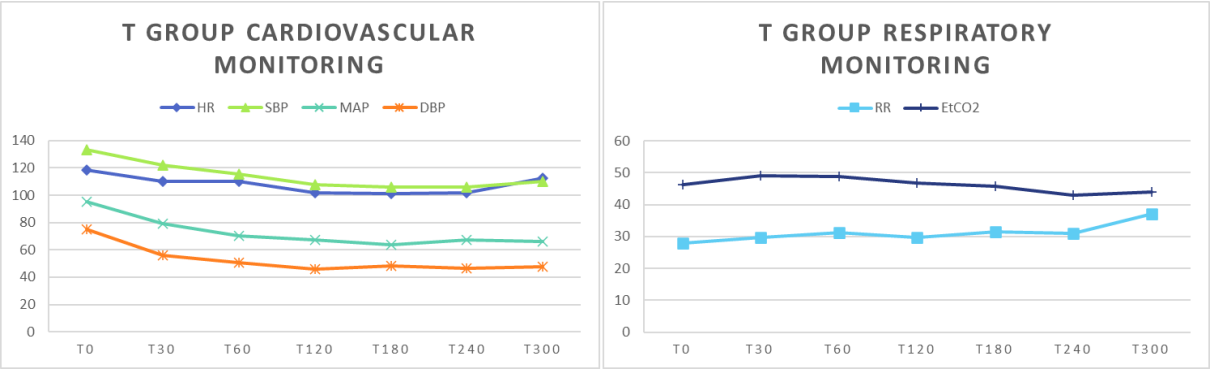

Figure 49 - (A) Cardiovascular parameters trend of the T group. (B) Respiratory parameters trend of the T group. HR: heart rate; SBP: systolic blood pressure; MAP: mean arterial pressure; DBP: diastolic blood pressure; RR: respiratory rate; $EtCO_2$: end tidal CO_2.

Chapter 8

Discussion

The sedative protocol used in the present study (ketamine 4.5 mg/kg and medetomidine 50 µg/kg) was successful in achieving deep sedation in all macaques in about 5 minutes. Cardiovascular and respiratory parameters for about 300 minutes after the sedation and maintenance of anesthesia with isoflurane were evaluated.

A reduction in HR and MAP from baseline values was seen in every group in the current study, probably due to the physiological effect of the drugs on these variables. Medetomidine is an α2-agonist; it is one of the sedative agent of choice due to its potency, requiring decreased total volume that is administered, to the possibility of antagonism through atipamezole and to its predictable side effects (bradycardia, hypotension and hypothermia). (Savola et al., 1986; MacDonald et al., 1988; Virtanen et al., 1988; Virtanen, 1989)

IV medetomidine alone (50, 100, 150 and 200 µg/kg) was administered in 15 rhesus macaques (Capuano et al., 1999); it was reported a reduction in HR at all dosages, probably due to inhibition of norepinephrine release, to vagomimetic effect and to the sedation. (Mroczek et al., 1973; de Jonge et al., 1981; MacDonald et al., 1988)

Unlike other species (Jalanka & Roeken, 1990), blood pressure after IV medetomidine in rhesus macaques did not show a biphasic trend characterized by a short period of hypertension followed by a longer period of hypotension. The decrease in MAP was gradual, as reported in Figure 50, with maximal effect at 65 min after administration of the 50

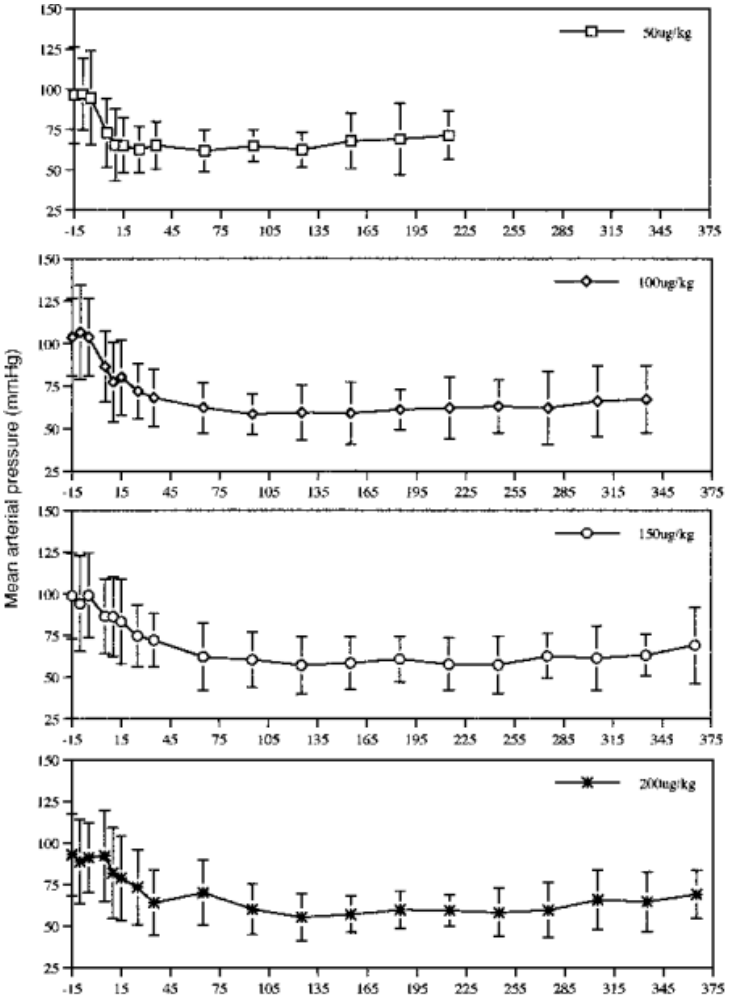

Figure 50 - Mean arterial pressure before and after intravenous administration of medetomidine. Data are expressed as mean ± SD. (Capuano et al., 1999)

µg/kg dosage, at 95 min for the 100 µg/kg dosage and at 125 min for the 150 and 200 µg/kg dosages. (Capuano et al., 1999) As these findings, in the current study it was not reported a biphasic trend of blood pressure, but rather a gradual reduction of this parameter.

Ketamine is one of the most used agents for chemical restraint in NHPs. It is a phencyclidine derivative that produces rapid onset of anesthesia with significant analgesia action. It stimulates the cardiovascular system increasing the HR, cardiac output, blood pressure and the oxygen consumption by stimulation of the central adrenergic control structures or inhibition of the neuronal reuptake of catecholamines. With ketamine sedation (10 mg/kg) HR and MAP both declined after IM injection in 8 rhesus macaques. (Settle et al., 2010)

20-25 minutes after ketamine injection, a positive trend was noted with MAP significantly increasing, probably due to the decline in plasma concentration.

Comparing this protocol with ketamine-medetomidine (2 mg/kg – 75 µg/kg) (Settle et al., 2010) the HR reduction was more pronounced in the KM group and MAP at 20-25 minutes continued to decline, as aforementioned. (Figure 51) In accordance with these data, current findings showed a significant decrease in both HR and MAP at T30 with a stable trend (Figure 48).

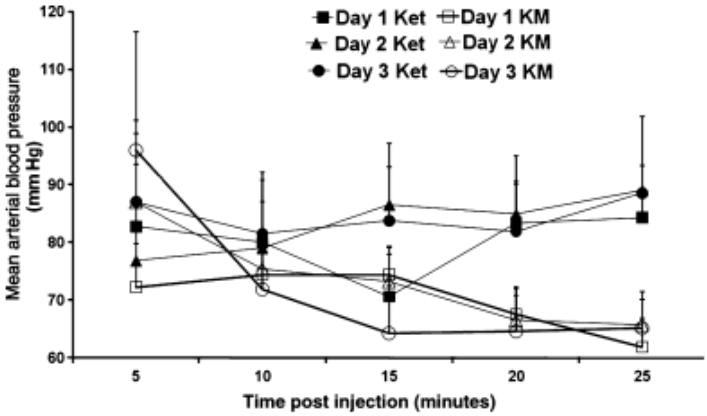

Figure 51 - The effect of time, drug group and day on mean (±SE) arterial blood pressure (mmHg). (Settle et al., 2010)

The use of thiopental was at discretion of the anesthesist: when the plane of anesthesia was considered light to safely perform intubation, 0.3 mg/kg of thiopental 1.25% was administered.

Thiopental sodium is a short acting barbiturate agent that has been commonly used as induction agent to facilitate intubation prior to inhalation anesthesia. (Brainard et al., 2007; Enouri et al., 2008; Chang et al., 2011)

Disadvantages of the use of thiopental are distinct respiratory suppression at the dosages required for analgesia, inability to modulate depth of anesthesia and a long period of recovery. (D. M. Turner & Ilkiw, 1990; Kojima et al., 2002)

The effect of thiopental as an induction agent in macaques sedated with the current protocol is not yet studied.

In 8 unsedated rhesus macaques, induction with thiopental (19.41 ± 0.53 mg/kg) followed by maintenance with 1.5% isoflurane with oxygen supply caused a significant reduction in HR and RR up to 45 minutes following injection. Blood pressure rapidly decreased from 5 to 45 minutes; in particular SBP was significantly lower compared with propofol induction (9.32 ± 1.02 mg/kg) but restored to baseline after withdrawal from isoflurane. (Choi et al., 2016)

In dogs premedicated with IV medetomidine (10 µg/kg) and IV hydromorphone (0.05 mg/kg) anesthesia was induced with thiopental and maintained with isoflurane in oxygen. (Enouri et al., 2008) Minimal additional changes in the measured hemodynamic variables occurred after induction with thiopental, as seen in Figure 52 and Figure 53. Medetomidine was probably responsible for the sever cardiovascular changes detected after administration of preanesthetic medications, as it did not appear to further compromise cardiovascular function.

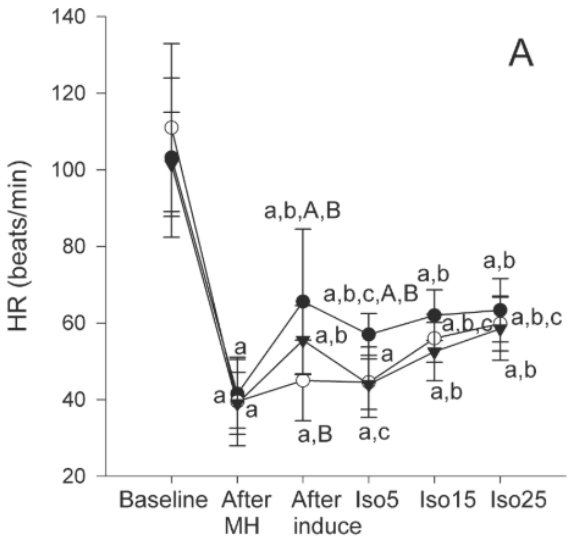

Figure 52 – Mean ± SD values for heart rate measured before administration of preanesthetic medications (baseline), after preanesthetic medications (after MH), 10 minutes after induction of anesthesia (after induce) and during maintenance of anesthesia with isoflurane at 5, 15 and 25 minutes (iso5, iso15 and iso25, respectively). Thiopental induction regimens is caharcterized by inverted black triangles. a Within an induction regimen, value differs significantly ($p < 0.05$) from the value for baseline. b Within an induction regimen, value differs significantly ($p < 0.05$) from the value for After MH. c Within an induction regimen, value differs significantly ($p < 0.05$) from the value for After induce. A Within a time point, value differs significantly ($p < 0.05$) from the value for propofol. B Within a time point, value differs significantly ($p < 0.05$) from the value for thiopental. (Enouri et al., 2008)

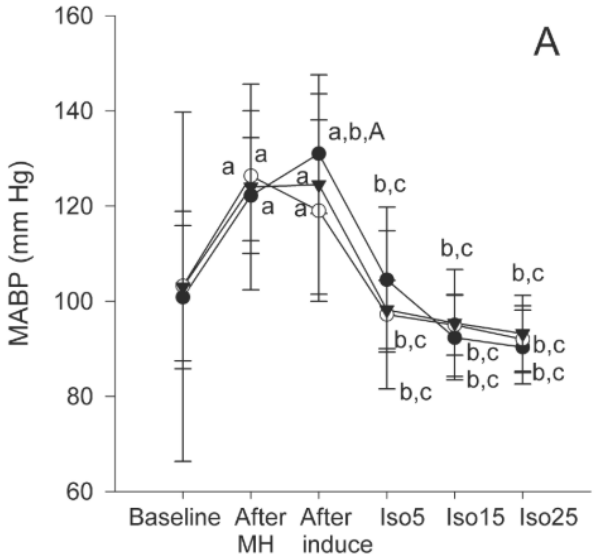

Figure 53 - Mean ± SD values for MABP measured before administration of preanesthetic medications (baseline), after preanesthetic medications (after MH), 10 minutes after induction of anesthesia (after induce) and during maintenance of anesthesia with isoflurane at 5, 15 and 25 minutes (iso5, iso15 and iso25, respectively). Thiopental induction regimens is caharcterized by inverted black triangles. a Within an induction regimen, value differs significantly ($p < 0.05$) from the value for baseline. b Within an induction regimen, value differs significantly ($p < 0.05$) from the value for After MH. c Within an induction regimen, value differs significantly ($p < 0.05$) from the value for After induce. A Within a time point, value differs significantly ($p < 0.05$) from the value for propofol. B Within a time point, value differs significantly ($p < 0.05$) from the value for thiopental. (Enouri et al., 2008)

In 6 Thoroughbred horses 6 μg/kg medetomidine and 20 μg/kg midazolam were used as sedative protocol followed by induction of anesthesia with 4 mg/kg thiopental. (Table 36) (Wakuno et al., 2017) One of the six horses showed slight muscular rigidity or limb movement during transition to lateral recumbency. HR did not decrease significantly after premedication and during anesthesia, although thiopental administration is commonly associated with cardiovascular depression. (Lees & Tavernor, 1969) Blood pressure remained approximately at baseline values.

	Baseline	After premedication	5 min	10 min	15 min
HR (beats/min)	31 (24-48)	24 (20-29)	26 (23-30)	24 (23-27)	25 (22-31)
SAP (mmHg)	n/a	n/a	129 (111-137)	127 (111-135)	124 (111-133)
MAP (mmHg)	n/a	n/a	106 (94-114)	106 (95-110)	98 (92-99)
DAP (mmHg)	n/a	n/a	95 (82-99)	88 (81-96)	82 (77-90)

Table 36 - Heart rate (HR), systoli arterial blood pressure (SAP), mean arterial blood pressure (MAP), and diastolic arterial blood pressure (DAP) in 6 horses before premedication (baseline), after premedication and at 5, 10 and 15 min after administration of thiopental. Data are expressed as median (range). (Wakuno et al., 2017)

In the current study the T group showed significant differences both in HR and in MAP: HR decreased from baseline values from T120 while MAP decreased from baseline values from T60. In the NT group these parameters compared with baseline values decreased significantly from T30. Comparing the T and NT groups, MAP was significantly higher in the T group at T30. Despite the reported negative cardiovascular effect of thiopental that is not always consistent in the aforementioned studies, the difference in time of the decrease in HR and MAP between T and NT group can be attributed to the delay in the metabolism of medetomidine, that is primarily responsible for the decreases in MAP and HR, due to the overriding pharmacokinetics of thiopental.

$EtCO_2$ significant reduction compared with baseline values was seen in the OI group from T180. There is a statistically not significant negative trend from T180 that has been shown in Figure 46 that is accompanied with a statistically relevant increase in RR at the same timepoint. RR returned to baseline values at T240. There are not significant differences in $EtCO_2$ between groups at any time points whereas RR of the OI group was significantly increased compared with the OMI group.

Increase in RR and decrease in $EtCO_2$ are consistent with a possible decrease in tidal volume due to pulmonary atelectasis; to maintain minute volume the organism compensates with an increase in RR that influences the values of $EtCO_2$. The possible reasons for the development of pulmonary atelectasis can be the prolonged sternal recumbency in the stereotaxic frame or/and the delivery of isoflurane in 100% oxygen.

Studies concerning in particular NHPs prolonged sternal recumbency and the possible respiratory implications are not present in the literature; for the similar anatomical conformation of the macaques chest human studies regarding these topics are taken into consideration.

Atelectasis is a major complication associated with general anesthesia. Anesthesia-induced atelectasis impairs gas exchange by shunting pulmonary blood flow; it decreases the oxygen reservoir resulting in intra- and postoperative hypoxaemia. There is a strong correlation between atelectasis, oxygen desaturation and poorer clinical outcomes. (Hedenstierna et al., 1986)

The prone position can increase intrathoracic and abdominal pressure by external compression and decrease respiratory compliance and peripheral aeration, in particular for pediatric patients whose lung elastic recoil decreases, and compliance increases with an increase with age. (Jang et al., 2020) Moreover it seems that in adults dynamic compliance did not change between supine and prone position. Spaeth et al. (2016) observed that to avoid intratidal de-recruitment PEEP of 6 cm H_20 was not sufficient if the patient was in prone position compared with the supine position. They had to increase PEEP to 9 cm H_20 to reduce end-expiratory alveolar collapse during prone ventilation.

Another study showed that recruitment manoeuvres in prone position improve lung aeration and oxygenation compared with the supine position, in particular of the dorsal areas. Dorsal alveolar recruitment is accompanied by an improved dorso-basal ventilation/perfusion relationship. (Martinsson et al., 2021)

Given these contrasting studies prone positioning of the animal cannot be a possible explanation for the changing respiratory parameters.

Hyperoxia that is inspiratory oxygen fraction (FiO_2) of 0.80 can be another factor for respiratory parameters changes. Adverse effects of hyperoxia are increased oxidative stress due to ROS, hyperoxic vasoconstriction and resorption atelectasis. (Weenink et al., 2020) Clinically, data in

the cited review did not show a signal of harm due to perioperative hyperoxia such as increased mortality, pulmonary complications and cardio- and cerebrovascular complications.

Perioperative high FiO_2 seemed to not cause increasing in the incidence of postoperative atelectasis or other pulmonary complications, but it is demonstrated that hyperoxia can cause absorption atelectasis which may increase pulmonary shunting. (Tokics et al., 1987; Edmark et al., 2003) In healthy subjects breathing 1.0 FiO_2 atelectasis combined with inhibition of hypoxic pulmonary vasoconstriction doubles intrapulmonary right-to-left shunt, compared to subjects breathing room air. (Calzia et al., 2010) The authors of the review suggested to reduce the FiO_2 to 0.80 to substantially reduce the atelectasis and to use positive end-expiratory pressure of 10 cm H_20 to prevent it. (Neumann et al., 1999; Edmark et al., 2003; Weenink et al., 2020)

Another study did not find any consistent correlation between 0.80 FiO_2 and a significant risk of harm compared with 30-35% oxygenation. (Mattishent et al., 2019)

Due to the inconsistent findings more studies are needed to evaluate the respiratory implications of prolonged sternal recumbency in the stereotaxic frame of the macaques and the anesthesia management of the modifications in respiratory parameters found in this study.

Chapter 9

Conclusions

NHPs, in particular *M. mulatta*, are keys in the biomedical and neuroscience research. In literature there are lots of studies dealing with this animal model, however the effects of anesthesia protocol are underrepresented and protocols for specifically rhesus monkeys are often extrapolated by other primates or even other species of mammals.

Ketamine and medetomidine are described in different ratios for the sedative protocol of rhesus macaques. These drugs in the dosages used in this study were proved effective in the proper sedation of the animals, with hypotensive events in 5 cases of 30 that needed the administration of vasoactive drugs.

There were regurgitation and vomit episodes in the perioperative period in two cases. The administration of maropitant can be implemented to effectively reduce the incidence of regurgitation.

In case of prolonged prone position or of 100% oxygen delivery, studies about respiratory parameters and breathing characteristics are needed to evaluate the possible resorption pulmonary atelectasis.

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