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NEURALLY ADJUSTED VENTILATORY ASSIST: FROM ANIMAL STUDIES TO CLINICAL PRACTICE

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NEURALLY ADJUSTED VENTILATORY ASSIST: FROM ANIMAL STUDIES TO CLINICAL PRACTICE

Thesis for Doctoral Degree (Ph.D.)

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TO MY FAMILY

...”Homme libre, toujours tu chériras la mer!”…

Charles Beaudelaire, L´Homme et la Mer

ABSTRACT

Patients in the Intensive Care Unit (ICU) undergoing ventilator treatment may experience asynchrony with the ventilator, which has been associated with increased need of sedation, sleep disruption, prolonged mechanical ventilation and unsuccessful weaning from the ventilator. The search for new strategies to improve patient-ventilator interaction is ongoing. Neurally Adjusted Ventilatory Assist (NAVA) is a recently developed ventilator support that uses the Electrical Activity of the diaphragm (EAdi) as an input signal to control the ventilator. Each breath is delivered in proportion to the EAdi amplitude and follows the timing of the EAdi start and ending. NAVA may potentially improve patient-ventilator synchrony, which could be beneficial, especially in paediatric ICU patients. Further, NAVA use could be of interest during surgery and general anaesthesia, potentially reducing risk for intraoperative atelectasis formation and postoperative complications. However, the feasibility of NAVA during general anaesthesia has not been investigated. There is some uncertainty among clinicians on how to set NAVA bedside. A pragmatic strategy, targeting NAVA to different levels of muscle unloading has not been studied, but could be a means of standardizing the approach to unload. Finally, it is not known if lower unloading could improve the distribution of ventilation in favour of the dorsal regions of the lungs.

Patient-ventilator synchrony and diaphragm unloading with NAVA compared to Pressure Support (PS) were evaluated in Acute Lung Injured (ALI) rabbits with increasing level of assist and in rats breathing with an added dead space in the respiratory circuit. The feasibility and efficacy of NAVA was investigated in small species, close in weight to the smallest viable human being. The pattern of breathing in NAVA and PS was studied in small species, when dead space was added. The feasibility of NAVA in sedation and anaesthesia with sevoflurane and propofol and the impact of these drugs on the pattern of breathing and muscle contractility were investigated in a big animal model. The possibility to target NAVA to different levels of respiratory muscle unloading was evaluated in Neurosurgical Intensive Care (NICU) patients and the effect of moderate unloading on the distribution of ventilation was investigated with Electrical Impedance Tomography.

Synchrony with NAVA was maintained and diaphragm unloading increased when raising the assist level, while for high PS the synchrony and unloading worsened, due to wasted inspiratory efforts causing a larger work of breathing. With NAVA, oxygenation and ventilation remained in the physiologic range in small species and when dead space was added, similar $PaCO_2$ was achieved with a lower increase in respiratory rate and minute ventilation, compared to PS. Sedation and anaesthesia with sevoflurane and propofol, during NAVA in pigs, preserved the EAdi signal and spontaneous breathing, keeping the gas exchange in the physiologic range. The tidal volume variability in NAVA was preserved both with sevoflurane and propofol, being higher in propofol, due to more frequent sighs, followed by post-sigh apnoea. With sevoflurane Neuro-mechanical and Neuro-Ventilatory Efficiency (NVE) were higher, suggesting that sevoflurane could better preserve muscle

contractility, compared to propofol. In NAVA it was feasible to set the assist at different levels of respiratory muscle unloading in NICU patients, by means of the NVE. Lower muscle unloading was shown to redistribute ventilation towards the dorsal regions of the lungs.

In conclusion this thesis demonstrates that NAVA improves patient-ventilator synchrony for increased assist levels and unloads the respiratory muscles with lower pressures and volumes compared to PS. NAVA is feasible and efficacious in small species, close in weight to the lowest viable human being and appears to be more efficient than PS in eliminating the CO_2. NAVA is feasible during sedation and anaesthesia with sevoflurane and propofol in pigs and preserves the natural variability in pattern of breathing. Propofol in combination with NAVA is associated with more sighs and post-sigh apnoea than sevoflurane. Sevoflurane appears to preserve muscle contractility in NAVA better than propofol. NAVA can be targeted to different levels of muscle unloading by means of the NVE and lower unloading redistributes ventilation towards the dorsal regions of the lungs, providing the premise for a better matching of ventilation and perfusion.

Key words: Neurally Adjusted Ventilatory Assist, Electrical Activity of the diaphragm, Patient-ventilator synchrony, Pressure Support, Sevoflurane, Propofol, Neuro-Ventilatory Efficiency, Respiratory muscle unloading.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals as indicated below:

I. **Improved Sinchrony and Respiratory Unloading by Neurally Adjusted Ventilatory Assist (NAVA) in Lung-Injured Rabbits.**
Jennifer Beck, Francesca Campoccia, Jean-Christophe Allo, Lukas Brander, Fabrice Brunet, Arthur S. Slutsky, Christer Sinderby.
Pediatric Research 2007; 61(3):289-294

II. **Neurally Adjusted Ventilatory Assist and Pressure Support Ventilation in Small Species and the Impact of Instrumental Dead Space.**
Francesca Campoccia Jalde, Abdul Raoof Almadhoob, Jennifer Beck, Arthur S. Slutsky, Michael S. Dunn, Christer Sinderby
Neonatology 2010; 97(3):279-285

III. **Neurally Adjusted Ventilatory Assist Feasibility during Anaesthesia. A randomised crossover study of two anaesthetics in a large animal model.**
Francesca Campoccia Jalde, Fredrik Jalde, Peter V. Sackey, Peter J. Radell, Staffan Eksborg, Mats K.E.B. Wallin
European Journal of Anaesthesiology 2016; 33(4):283-291

IV. **Target Unloading of Respiratory Muscles during Neurally Adjusted Ventilatory Assist. A pilot study in ICU patients.**
Francesca Campoccia Jalde, Fredrik Jalde, Mats K. E. B. Wallin, Fernando Suarez-Sipmann, Peter J. Radell, David Nelson, Staffan Eksborg, Peter V. Sackey
Manuscript

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LIST OF ABBREVIATIONS

ALI	Acute Lung Injury
ARDS	Acute Respiratory Distress Syndrome
BL	Baseline
C_{dyn}	Dynamic Compliance
COPD	Chronic Obstructive Pulmonary Disease
CoV	Centre of Ventilation
CT	Computed Tomography
CV_{vt}	Coefficient of Variation of the tidal volume
DS	Dead Space
EAdi	Electrical Activity of the diaphragm
$EAdi_{peak}$	Peak of the Electrical Activity of the diaphragm
EAdi-tp	Electrical Activity of the diaphragm-time product
EIT	Electrical Impedance Tomography
EMG	Electromyogram
F	French
ICU	Intensive Care Unit
IQR	Interquartile range
NAVA	Neurally Adjusted Ventilatory Assist
$NAVA_{40\%}$	NAVA at 40% respiratory muscle unloading
NICU	Neurosurgical Intensive Care Unit
NIV-NAVA	Non-invasive NAVA
NME	Neuro-Mechanical Efficiency
nRR	Neural Respiratory Rate
MV	Mechanical Ventilation
NVE	Neuro-Ventilatory Efficiency

Paw	Airway Pressure
Paw_{mean}	Mean Airway Pressure
PBW	Predicted Body Weight
PC	Pressure Control
Pdi	Transdiaphragmatic Pressure
Pdi-tp	Pdi-time product
PEEP	Positive End Expiratory Pressure
Pes	Esophageal Pressure
Pga	Gastric Pressure
Pmusc	Muscular pressure
PS, PSV	Pressure Support
PVBC	Patient Ventilator Breath Contribution
Pvent	Pressure delivered by the ventilator
ROI	Regions of Interest
RR	Respiratory Rate
SD	Standard Deviation
T_i	Inspiratory Time
VC	Volume Control
V_E	Minute Ventilation
Vt	Tidal Volume

PROLOGUE

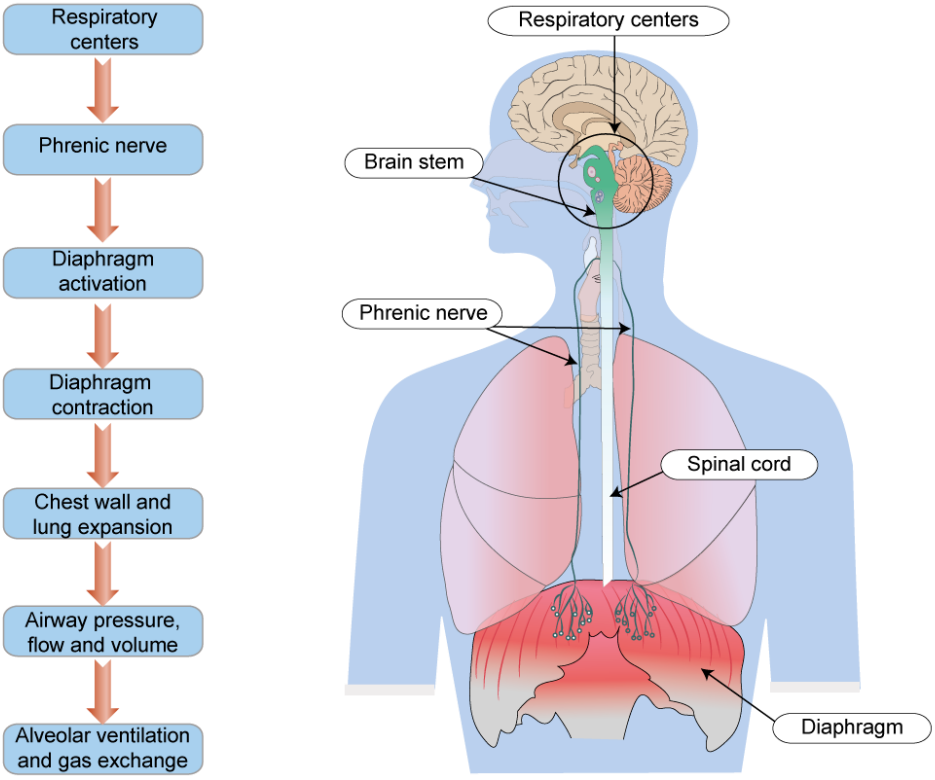
Neurally Adjusted Ventilatory Assist (NAVA) begins…
It was in 1993 in the Italian Alps, during a Swedish-Italian meeting between Christer Sinderby and Paolo Navalesi, that the idea of NAVA was born.
The first patent application related to NAVA, "Diaphragm Electromyography Analysis Method and System", by Sinderby et al., was filed in 1995 [1].
The first article to introduce NAVA was published in Nature medicine in 1999 (Sinderby et al., 1999).
The first patient to be ventilated in NAVA with the SERVO-i ventilator was at St Michael´s Hospital Intensive Care Unit, in Toronto, Canada on the 13th of May 2006 and I had the honour to be bedside for the 24h run. And this was the second Swedish-Italian meeting…

INTRODUCTION

Breathing anatomy, physiology and pathophysiology

From the moment we are born until we die, breathing keeps us alive by providing oxygen, vital to aerobic metabolism and eliminating the CO_2 produced by the cells of the body.
Each breath is initiated in the respiratory centres located in the brainstem, responsible of generating automatic and rhythmic breathing. Voluntary control of breathing from the cortex is also possible to some extent. Furthermore, emotional states may influence the pattern of breathing.
The output message is transferred to the respiratory muscles, especially to the diaphragm, considered to be the primary inspiratory muscle. Through the phrenic nerves, directed to the diaphragm and through other nerves, innervating the other respiratory muscles, the signal is transmitted to the neuromuscular junction and the motor end plate of the respiratory muscle fibres. The respiratory muscles are thereby electrically activated and contract. Such contraction determines chest wall displacement and lung inflation (Fig. 1).

Figure 1. Generation of a breath



Adapted From Maquet Clinical Image Collection. Simplified picture of anatomy and sequence of events generating a breath.

Lung expansion warrants the movement of fresh air with oxygen through the airways into the lungs. The inhaled air is filtered, humidified and warmed through the conducting airways, which do not participate to gas exchange.
The pulmonary capillaries deliver the blood-borne CO_2 produced by the cells´ metabolism into the alveoli.
The gas exchange occurs at the alveolo-capillary interface, a huge surface area providing tight contact between the pulmonary capillaries and the alveoli, the ultimate ideal configuration for such function. During expiration, air enriched with CO_2 is eliminated from the body through the airways.
In order to maintain arterial O_2 and CO_2 within a tight range, the entire process of breathing is efficiently regulated by a complex integration of information coming from central and peripheral receptors, as well as by influence from the brain cortex.
Diseases that damage the lung tissue may impair the gas exchange, thus leading to hypoxemic respiratory failure, such as in Acute Respiratory Distress Syndrome (ARDS). Pathologic processes that cause the alveoli to collapse, consolidate or get filled with oedema fluids, ultimately lead to increased shunt. Shunt and ventilation-perfusion mismatch are the most common mechanisms underlying hypoxemia.
Diseases such as asthma, impose an increased load to the respiratory muscles, in this case due to airway obstruction. Ventilatory failure may develop, if the alveolar ventilation is not sufficient to eliminate the CO_2 produced by the cells´ metabolism.
Critically ill patients may thus need intubation and mechanical ventilation to unload the respiratory muscles and to provide adequate gas exchange. Intubated patients may receive ventilator support with controlled ventilation, when the ventilator provides breaths with predefined volumes or pressures, regardless of the patient´s own breathing pattern. High doses of sedatives are often required to tolerate controlled ventilation. In some cases muscle paralysis is added, in order to maximize ventilator synchrony [2]. With assisted ventilation instead, spontaneous breathing is a central feature. In assisted ventilation, each breath is supported and the ventilator performs a portion of the respiratory work in every breath. Lower doses of sedatives are required on assisted ventilation than with controlled ventilation.

Mechanical ventilation-Historical perspective and rationale for its use

In 1543 the Anatomy Professor Vesalius writes in the “De Humani Corporis Fabrica”:“But that life may be restored to the animal, an opening must be attempted in the trunk of the trachea, into which a tube of reed or cane should be put; you will then blow into this, so that the lung may rise again and take air”. This is the first reference to positive pressure ventilation, currently applied to intensive care patients with respiratory failure [3].
It was not until mid-18th century that mouth-to-mouth resuscitation, a form of positive

pressure ventilation, was described and used [4].
In the late 19th century, to replace the patients´ respiratory muscles, subatmospheric pressure was applied around the body by an "iron lung" during inspiration. The "iron lung" was also used to rescue drowning victims and, later on, to treat patients with polio [5]. During the Scandinavian polio epidemic of 1952, hundreds of patients with respiratory failure were hand-ventilated for weeks by medical students and doctors [6].
Positive pressure ventilators proved to be more efficient in improving gas exchange than negative pressure ventilators [3]. Since then, mechanical ventilation has shifted from replacing and supporting the neuromuscular pump to additionally providing gas exchange assistance. At the time of the polio epidemic, ventilatory support developed on a large scale and became the treatment of choice in severe respiratory failure, reducing mortality in polio from 87 to 40%. At the same time, the intensive care unit developed as the place to provide dedicated ward to critically sick patients.
The technical development of improved trigger function, together with an increased focus on patient-ventilator interaction and growing awareness of ventilator induced diaphragm dysfunction has led to a greater use of assisted modes of ventilation since the 1980-1990s [3].

Challenges and complications of mechanical ventilation

Ventilator and tube intolerance - Sedation

The delivery of full artificial positive pressure ventilation implies that the patient´s airways are bypassed by an endotracheal tube. Intubation and mechanical ventilation generate discomfort. To tolerate such treatments, patients often need sedation and occasionally muscle paralysis.

Ventilator Induced Lung Injury (VILI)

Although ventilators made it possible to save the life of patients with respiratory failure, their use has been associated with many adverse effects. Before the awareness of the detrimental effects of mechanical ventilation, the goal of ventilation was to speedily correct blood gas derangements with intubation and, if needed, by applying higher pressures.
Already at the polio epidemic time, structural changes in the lungs of patients treated with mechanical ventilation were observed. In 1967, post-mortem histological changes found in mechanically ventilated patients were described as the "respirator lung"[7].
In the last thirty years, great attention has been directed to understanding the underlying mechanisms involved in lung injury associated to mechanical ventilation, the so called Ventilator Induced Lung Injury or VILI. VILI is known to prolong the time of stay in the intensive care unit (ICU) and in the hospital.

ARDS lungs are inhomogeneous, with collapsed lung regions alternating with aerated areas. *Atelectrauma* is the mechanism of injury for which, the cyclical opening and closing of alveolar units, between inspiration and expiration, exposes the lung tissue to stretch forces, leading to local tissue damage [8].
Barotrauma indicates the injury occurring when ventilator high pressures, combined with an already damaged lung, lead to alveolar rupture and air leaks as pneumothorax, pneumo-mediastinum and subcutaneous emphysema.
Volutrauma describes the regional overdistention as being injurious for the lung [9].
*Biotrauma***.** In the injured lung the alveolar space is invaded by inflammatory cells, citokines are released and translocate in the systemic circulation, inducing distant organ failure [10, 11].
Many studies, aimed at defining the optimal lung protective strategy in ventilating ARDS patients, have been performed by the international scientific community. Opening lung units with lung recruitment manoeuvers renders ARDS patients´ lung parenchyma less inhomogeneous. Striving to keep the lung open thereafter during the expiratory phase, by the use of high PEEP levels, aims to avoid lung collapse and atelectrauma [12]. A study published in year 2000 increased clinical awareness that a protective strategy to ventilate lungs, based on low tidal volumes, could reduce mortality of ARDS patients [13]. “Permissive hypercapnia” [14, 15] implies that a certain degree of respiratory acidosis (by tolerating lower pH or higher $PaCO_2$ targets), is accepted, as long as it does not apparently harm the patient, in order to provide a less injurious ventilation.
The state of the art today is to ventilate ICU patients with lower tidal volume, high PEEP, allowing higher CO_2.

Ventilator Induced Diaphragm Dysfunction (VIDD)

Prolonged Mechanical ventilation is associated with diaphragm weakness, defined as VIDD. VIDD occurs frequently in ICU patients and leads to adverse outcome. VIDD has early onset and develops more quickly than disuse atrophy in limb muscles, since diaphragm atrophy and contractile dysfunction takes place within 24-48h of Mechanical Ventilation [16, 17]. Multiple underlying mechanisms have been identified, among them oxidative stress, damaging contractile proteins and thus reducing muscle fibres sensitivity to calcium [18] and the activation of different protease, resulting in destruction of sarcomere architecture and mitochondrial function impairment [16].
Maintaining spontaneous breathing has been found to reduce diaphragm weakness and dysfunction [19], making ventilator strategies that keep the diaphragm active and avoid overassist of some interest.

Atelectasis and lung collapse

CT scan studies of the chest, performed during general anaesthesia and under controlled mechanical ventilation, have demonstrated the occurrence of lung atelectasis early after anaesthesia induction (in up to 90% of anesthetized adults), causing pulmonary shunt. This is especially true in obese patients where a higher pressure from the ventilator is needed to expand the stiff chest wall. The collapsed areas of the lungs can be re-opened by performing a recruitment manoeuver, thus improving oxygenation [20].
In ARDS patients, the dependent parts of the lungs are prone to gravity dependent collapse, since they have injured and heavy lungs. Lung recruitment and high PEEP levels, in order to prevent the development of new collapse have been shown to be beneficial [21].
Assisted modes of ventilation do part of the work of breathing and they are better tolerated than control modes of ventilation, thus requiring less sedation [22]. Furthermore, maintaining spontaneous breathing increases aeration of the dependent lung regions [23], thereby improving ventilation/perfusion matching and gas exchange [24, 25]. In this perspective, strategies that keep the diaphragm active may prove beneficial both in anaesthesia and intensive care, to avoid and counteract the occurrence of atelectasis and lung collapse. In an Electrical Impedance Tomography study, NAVA was shown to induce a more homogenous distribution of ventilation compared to PS [26].

Patient-ventilator interaction-Asynchrony

Asynchrony between the patient and ventilator can be described as the mismatch of patient and ventilator inspiratory and expiratory time [27]. Patient-ventilator asynchrony has been observed in around 25% of patients during mechanical ventilation. The Asynchrony Index (AI), expressed as a percent, quantifies the asynchronous breaths in relation to global respiratory rate (including the ventilator respiratory rate and the wasted efforts). AI above 10% represents a high incidence of asynchrony and it has been associated with prolonged duration of mechanical ventilation [27]. Patient-ventilator asynchrony during weaning have been shown to contribute to unsuccessful weaning [28]. Patient-ventilator asynchrony has been further associated with increased need for sedation, sleep disruption and fragmentation [29-31], ultimately increasing morbidity and mortality in ICU patients [27, 32].
In order to initiate a ventilator assisted breath, the muscle pressure (Pmusc) has to overcome the elastic recoil of the respiratory system and the threshold of the inspiratory trigger. The ventilator assistance is intended to provide a rapid response to patients´ effort in order to reduce the work of breathing. However, delays present between patient effort and the delivery of ventilator assist are responsible for more energy expense. Variations in assist level, in respiratory drive and in intrinsic PEEP may all affect the amount of work necessary to trigger the ventilator [33]. If the patient is overinflated, the chest wall configuration becomes suboptimal, thus putting the diaphragm in mechanical disadvantage [34] and the work to start a breath may become significant. High levels of PS have been shown to be associated

with large tidal volumes and prolonged ventilator inspiratory time [33]. Due to the Hering-Breuer reflex, in response to large volumes, patients tend to shorten their neural inspiratory time. The discrepancy between the neural and the mechanical inspiratory time results in an inspiratory flow that continues into the neural expiration, thus generating hyperinflation. The now elevated elastic recoil pressure of the respiratory system might at times become too high to overcome and the patient might fail to trigger the next breath. Such attempts to breathe, that do not succeed to trigger the ventilator, are defined as ***Wasted inspiratory efforts*** or ***Ineffective triggering*** [33, 35-37], they represent a mechanism of asynchrony and generate extra cost in terms of work of breathing. Factors associated to a high frequency of ineffective triggering are low trigger sensitivity, high assist level, high tidal volume, alkalosis, high bicarbonate level and background disease chronic obstructive pulmonary disease (COPD)[27], intrinsic PEEP, muscle weakness and deep sedation [38].

Ventilator expiratory time is determined by the tidal volume size and by compliance and resistance of the respiratory system. If the patient increases the respiratory rate, in order to get the next breath, the patient will have to shorten the expiratory time by active expiration or increase the effort during inspiration [33]. Ineffective triggering has been shown to be by far the most common mechanism of asynchrony observed [27].

Delayed cycling occurs when the ventilator´s inspiration prolongs into patients effort to expiration and it is revealed clinically by an active exhalation generated with contraction of abdominal muscles. Such asynchrony may lead to high Vt and insufficient expiratory time and thereby air-trapping.

Premature cycling and ***Double triggering***. Premature cycling is another described asynchrony occurring when the ventilator´s set termination criterion does not match patients' effort. Mechanical inspiration thus ends prematurely while patient is still inspiring. Asynchrony increases the resulting work of breathing for the patient [39]. If the effort made by the patient is strong enough it will directly succeed to trigger a new breath, thus causing double triggering. Factors associated with a higher frequency of double triggering have been identified as being too short inspiratory time, more severe lung injury and higher ventilator drive [27, 38]. Double triggering can lead to undesired large tidal volumes [38], particularly undesirable in ARDS patients, where a lung protective ventilation strategy is considered important.

Autotriggering occurs when the ventilator provides a breath that is not initiated by an inspiratory effort by the patient. This may happen if the trigger sensitivity is very high. Examples of stimuli that may start a breath, when the trigger is sensitive, are the cardiogenic oscillations as observed in post-cardiac surgery patients [40] or the presence of leaks in the respiratory circuit [27].

Optimization of the settings of PS and PEEP with an individualised approach has been recommended in order to reduce the incidence of patient-ventilator asynchrony, thus

avoiding undesired energy expenditure, achieving a shorter duration of mechanical ventilation [27] and an improved quality of sleep [41].

Paediatric considerations

Patient-triggered ventilation in infants has been shown to induce changes in the respiratory pattern, in terms of improved tidal volume and reduced respiratory rate; such changes corresponded to a reduced work of breathing [42]. Comparing Synchronised Intermittent mandatory Ventilation (SIMV) to Intermittent Mandatory Ventilation (IMV) was resulting in oxygenation improvement [43]. PAV was shown to reduce transpulmonary pressure and peak airway pressures, thus reducing the risk for long term damage to lung tissue, while gas exchange was maintained [44]. Besides these advantages, no effect on mortality was demonstrated by using patient-triggered ventilation. One possibility is that not enough synchrony between ventilator and patient effort had been reached. Paediatric patients tolerate mechanical ventilation poorly, typically requiring deep sedation. Critically ill children are more prone to patient-ventilator asynchrony due to the presence of leaks in the respiratory circuit, due to small tidal volumes and high respiratory rates [45].

Assisted modes of ventilation

Since the first ventilators that only could provide controlled mechanical ventilation, assist modes supporting patients own breathing have been developed. Such modes include among others, SIMV, Pressure Support (PS), Proportional Assist Ventilation, of which, PS is the most commonly used.

Pressure Support (PS)

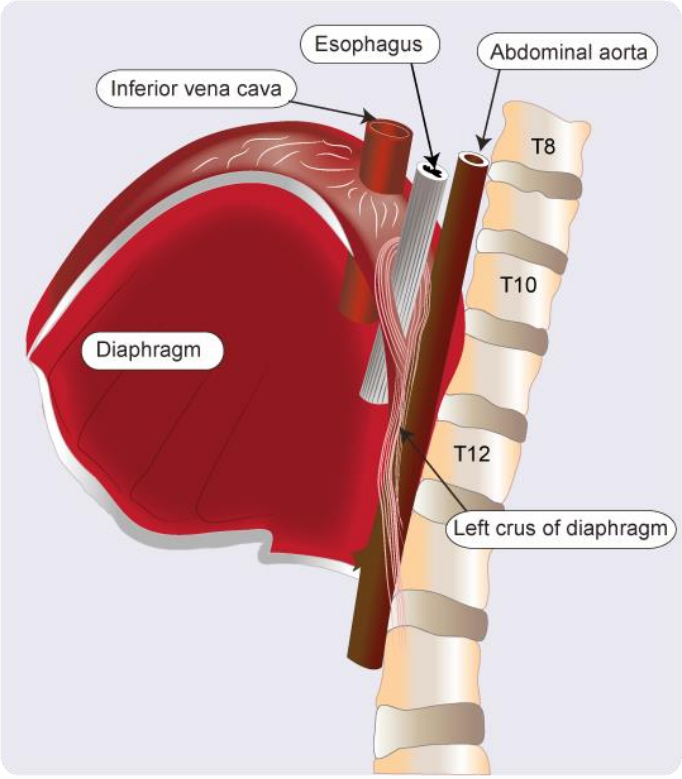
Pressure Support is an assist mode of ventilation widely used for ICU patients. The criteria to start a breath is set by the caregiver and is based on pneumatic (pressure or flow) thresholds that the patient has to overcome. A sensitive trigger implies that the threshold to start a breath is low and does not require a high inspiratory effort from the patient. PS is a pressure targeted mode that implies that the ventilator delivers a predefined level of pressure for each triggered breath [46], independent of the effort applied by the patient and of the respiratory drive. The resulting tidal volume depends on the mechanic properties of the respiratory system (compliance and resistance) together with the contribution of the patient´s own respiratory muscles. The switch to expiration is based on the cycle-off criteria. Inspiration cycles to expiration when a predefined percentage of the peak inspiratory flow, set by the caregiver, is reached. A low percent implies prolonged inspiration, while a high percent results in early switch to expiration. Increasing PS is believed to unload progressively respiratory muscles. However, for high assist, the work to trigger ventilator may increase [33, 35], as discussed in the patient-ventilator interaction section.

Neurally Adjusted Ventilatory Assist (NAVA)

Neurally Adjusted Ventilatory Assist is a recently developed assist mode that uses the Electrical Activity of the diaphragm (EAdi) to control the ventilator. The use of the EAdi as a control input signal for the ventilator requires integrity of respiratory centre, phrenic nerve and neuromuscular junction.

In normal subjects, the diaphragm is considered to be the main inspiratory muscle [47]. The diaphragm movement during inspiration has been described as a widening piston [48]. The diaphragm consists of a crural and costal region that contribute to different movements of the diaphragm. The costal part is inserted in the inner surface of the six lowest ribs and contributes to the outward movement of the lower ribcage and shifts the abdominal content downward. The crural part instead, is inserted to the anterior surface of the first 3 lumbar vertebrae and determines the abdominal expansion during inspiration. The crural and costal parts both merge in the central tendon. The crural and the costal diaphragm act as a single functional unit [47, 49, 50] controlled from the respiratory centres. The crural diaphragm forms a muscular tunnel covering the esophagus at the level of the esophageal hiatus (Fig. 2) [51].

Figure 2. Diaphragm



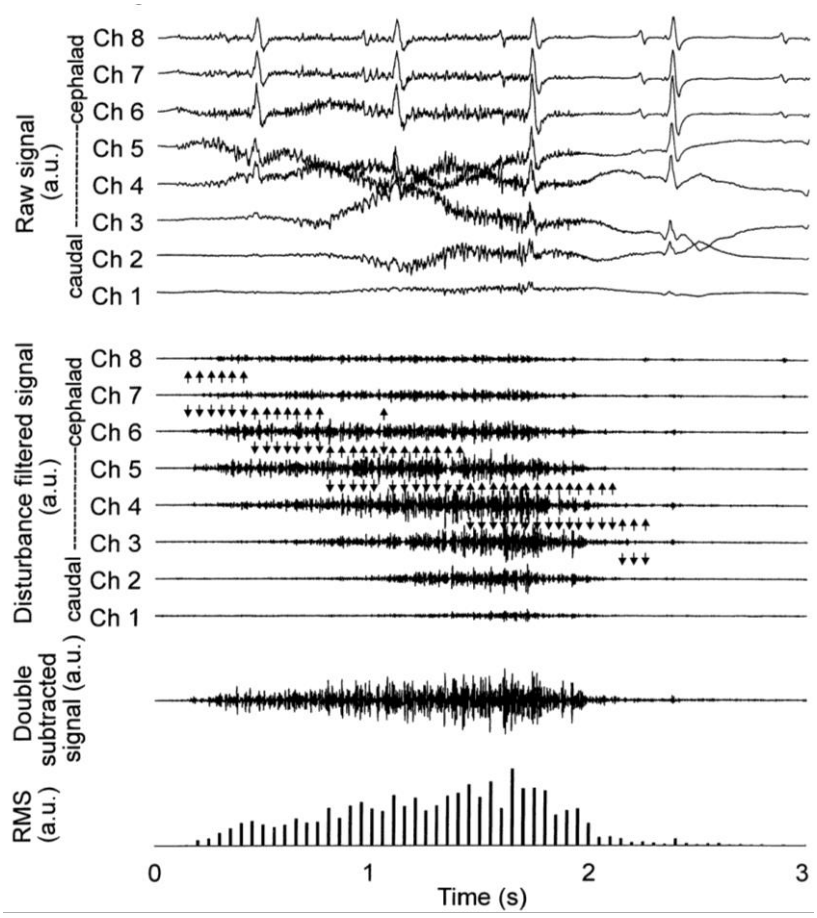
Adapted From Maquet Clinical Image Collection. Side picture of the diaphragm and of the crural region in relation to the thoracic vertebrae, the vena cava, the abdominal aorta and the esophagus.

Due to the difficulties related to crosstalk of the abdominal and intercostal muscles and to the invasiveness of needle electrodes to pick the costal diaphragm, a method measuring the EMG of the crural diaphragm by means of esophageal electrodes has instead been developed. The crural EMG has been shown to be in phase with the transdiaphragmatic pressure changes during inspiration [52] and it represents a sample of the motor unit population of the whole diaphragm [53]. Thus crural EMG reflects the global activation of diaphragm [53].

EAdi signal

The EAdi is derived from the electromyogram (EMG) of the crural diaphragm and it corresponds to the neural activation of the diaphragm. Diaphragm activation is determined by the combination of recruited motor units and their firing frequency [53]. In other terms, the signal is a spatial and temporal summation of action potentials derived from recruited motor units in the diaphragm at their firing rate [54]. A specially designed nasogastric tube with an array of microelectrodes detects the EAdi, the electrodes being perpendicular to the muscle fibre direction [51]. The location of the centre of activation is identified by cross-correlation of the EMG detected by pairs of electrodes along the array. Being an electrical signal, the EAdi may be contaminated by other electrical activity nearby, coming from the heart (ECG), esophagus (peristalsis) or other muscles. Furthermore, the signal may be affected by disturbances such as the background electrical noise and electrode motion artefacts. Computer algorithms have been developed that control for such contamination and filter the signal [55]. Once filtered from ECG and motion artefacts, the signal is processed and amplified [55] (Fig. 3). Finally the EAdi is sent to the SERVO-i ventilator in order to control the ventilation.

Figure 3. Filtration of EMG

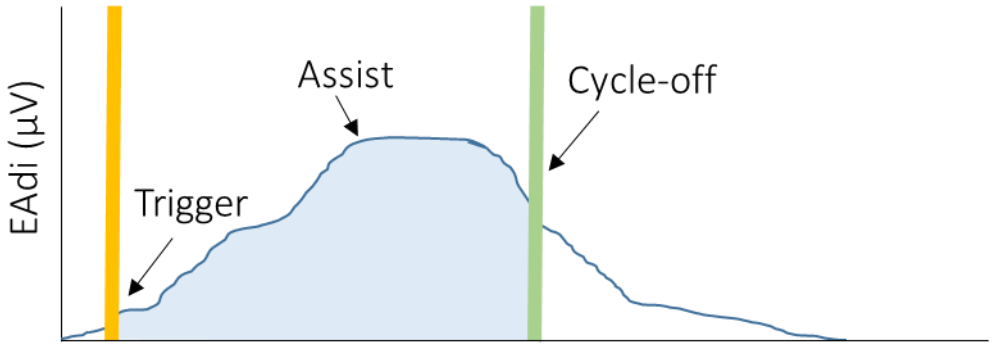


Adapted from J Appl Physiol. 1998 Dec; 85(6):2146-58. Steps in filtration of the EMG signal. RMS corresponds to EAdi.

Trigger and cycle-off

The sensitivity of the trigger is adjusted to a level above the background noise. In the event that no EAdi is detectable, the ventilator is provided with a backup trigger based on the flow or pressure. Thereby triggering takes place based on whichever signal comes first, EAdi or flow/pressure ("first come first served"). Both the neural and pneumatic trigger are adjustable, but the neural cycle-off criteria is fixed and expiration starts when the EAdi drops below 70% of EAdi peak (Fig. 4).

Figure 4. Trigger, Cycle-off and Assist in NAVA



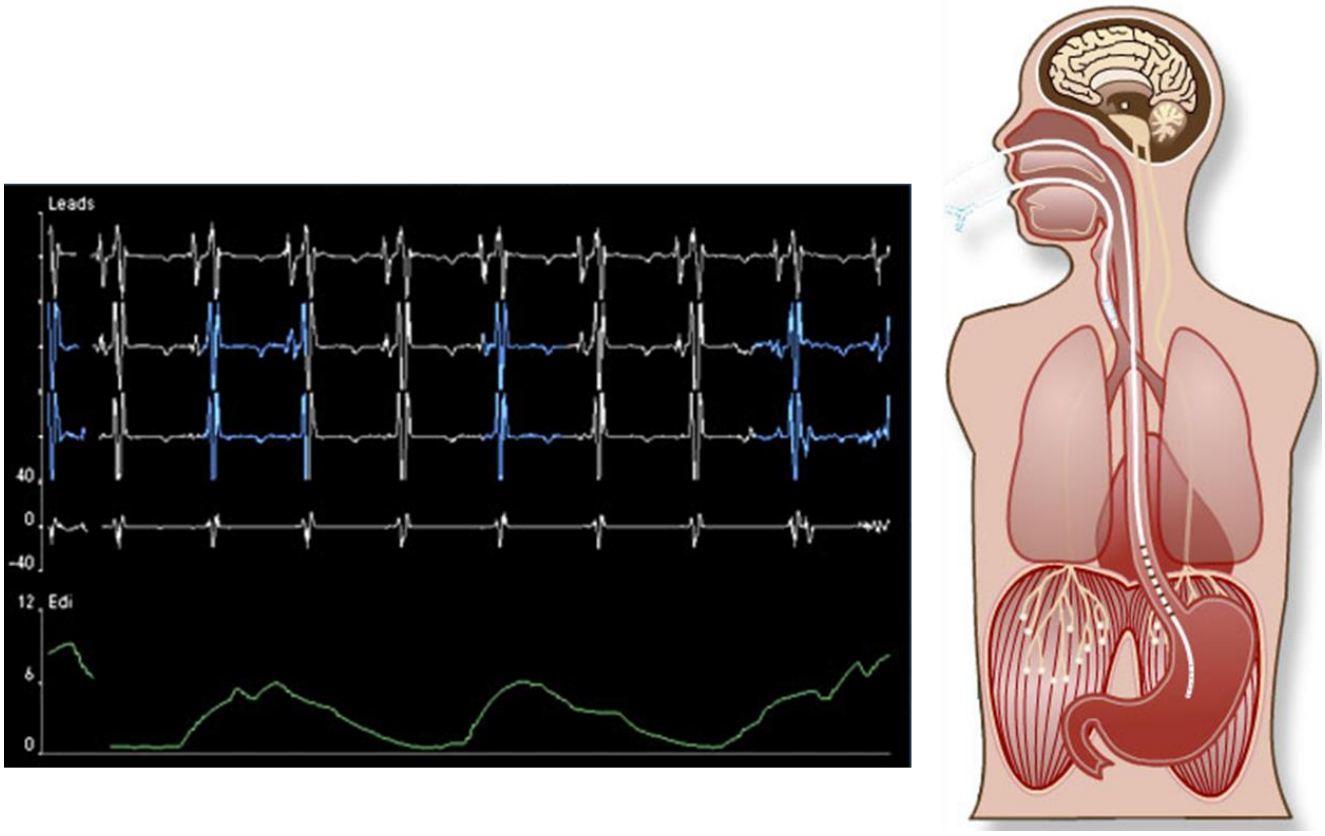
The yellow line indicate when the trigger threshold is reached and the ventilator assist starts. The green line shows the ventilator cycle-off to expiration when EAdi drops below 70% of peak EAdi. The blue area under the curve shows the diaphragm activation (EAdi) during inspiration. The pressure assist (above PEEP) delivered from the ventilator during inspiration corresponds to this activation (blue area) multiplied by the NAVA level.

EAdi catheter positioning

The esophageal electrode array is equipped with nine microelectrodes in order to cover for diaphragm respiratory movements and to permit slightly suboptimal position of the catheter. Furthermore, the array has to accommodate to varying diaphragm thickness, due to anatomic differences among subjects. For such reasons, the size of the EAdi catheter, the inter electrode distance and thereby even the length of the electrode array are optimized for patient height and for age group. The centre of the array should be placed at the level of the crural diaphragm.

An estimate on how deep to insert the EAdi catheter in the esophagus is provided by the modified nose-earlobe-xiphoid process distance (NEX). The position is then fine-tuned based on the ECG waveforms picked up by the electrodes, which are visualized in the EAdi positioning window of the SERVO-i. The optimal position is obtained when p wave disappears in the lowest (deepest) lead as well as the QRS decreases in amplitude (Fig. 5). Every 16 ms, the software algorithm in the ventilator selects the electrode pair that is closest to the crural EMG, based on cross correlation.

Figure 5. EAdi catheter positioning



Adapted from Maquet Clinical Image Collection and J. Beck collection. On the left side, four leads in the EAdi positioning window of the SERVO-I (Maquet Critical Care, Sweden). The ECG p wave disappears and the QRS decreases in amplitude in the lowest and deepest lead, indicating a correct position of the EAdi catheter. The EMG is marked in blue in the lead that detects the highest amplitude of the crural diaphragm EMG signal. The EAdi signal controlling the ventilator is displayed at the bottom as a green line. On the right, schematic anatomic view and relationship between the EAdi catheter array of electrodes to the diaphragm.

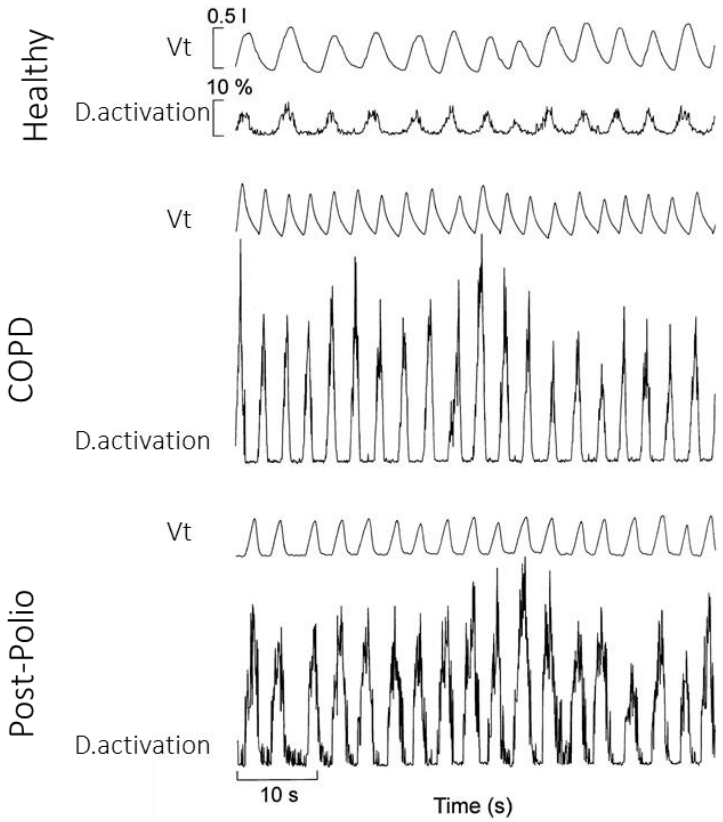
NAVA assist

The EAdi is expressed in μV. In NAVA the EAdi determines the start, the end and the amplitude of each breath. Increased ventilatory demand corresponds to increased EAdi and there is proportionality between them.

The pressure delivered by the ventilator is the resultant of the EAdi multiplied by the NAVA level ($cmH_2O/\mu V$), P_{vent} = EAdi*NAVA level. The NAVA level is a proportionality factor and is set by the caregiver. Increasing the NAVA level decreases the EAdi if the neural feedback loops are preserved [56].

To achieve similar tidal volumes, the electrical activation of the diaphragm during quiet breathing can be more than five times higher in a patient with a poor muscle function (such as COPD and post-polio patients) when compared to the EAdi in healthy subjects [57](Fig. 6). Thus, the response to a weaker mechanical output or to an increased respiratory load is an increase in the EAdi. The same NAVA level results in different delivered pressures from the ventilator, depending on the EAdi amplitude.

Figure 6. EAdi in healthy subjects and patients with poor muscle function



Examples of differing diaphragm activation. Adapted from Sinderby et al, J Appl Physiol. 1998 Dec; 85(6):2146-58. Vt= tidal volume and D. activation= diaphragm activation obtained during 60 s of breathing at rest in 1 healthy subject, 1 COPD patient, and 1 Post-Polio patient.

Assessment of respiratory muscle function during mechanical ventilation

ICU patients' vital functions are monitored in order to guide therapies and interventions. However respiratory muscle function is not commonly assessed, even though respiratory muscle dysfunction may prolong mechanical ventilation, increasing morbidity and mortality [58]. Assessing respiratory muscle performance in intensive care patients helps predict weaning success and estimate patient long term outcome [59]. Respiratory muscle function can be assessed to determine muscle strength and to measure the work of breathing.

Maximal Inspiratory Pressure is a measure of muscle strength and is obtained during an occluded inspiration [60] and for predicting weaning failure.

Maximal Sniff manoeuver is used as a measure of the global muscle respiratory function [61], however low values may also reflect poor technique, rendering it unreliable [58].

The ***Esophageal pressure (Pes)*** swings are measured during inspiration alone or combined with the gastric pressure (Pga), in order to obtain the ***Transdiaphragmatic pressure*** (Pdi = Pga-Pes), by means of a special nasogastric tube provided with esophageal and gastric balloon. Pes is used to determine the work of breathing of the respiratory muscles, while Pdi monitors more specifically the diaphragm. Pes is also used to monitor patient-ventilator asynchrony [62]. Pes measurement requires specific equipment, it may present difficulties in the interpretation of the curves if expiratory muscle are active during inspiration and it is not of routine use [58].

In the present project (Paper I), Pdi was used to measure the work of breathing, calculated as minute pressure-time product (Ptp_{di}= Pdi*Ti*RR).

With ***Magnetic twitch stimulation of the diaphragm*** [63], considered the golden standard, a magnetic stimuli is applied to the phrenic nerve and the corresponding pressure developed by the diaphragm contraction is measured as airway pressure or Pdi change. Such technique requires technical expertise and is not routinely performed, but rather considered a research tool.

By ***Ultrasonography (US)*** , it is possible to measure diaphragm excursion or to determine diaphragm thickening that is proportional to patient inspiratory effort [64] and has been used as a weaning index [65]. However US does not provide a continuous measurement [58].

Using the ***EAdi*** provides a continuous measure of the respiratory drive and of the diaphragm activity. EAdi allows the detection of patient-ventilator asynchrony [58]. Furthermore, power spectrum analysis of the EMG helps identifying patterns of fatigue [66, 67].

Neuro-Ventilatory Efficiency (NVE) is an index derived from the EAdi. It quantifies the inspired volume in response to the electrical output of the diaphragm and it is expressed as ml/μV. It is obtained by zeroing the assist in one breath. The NVE has been used as index to monitor during weaning [68, 69] and to investigate the physiologic response to PEEP [70]. It is affected by the respiratory system mechanical properties. So far, the NVE is not available in the current NAVA software and is used as research tool.

Patient-Ventilator Breath Contribution (PVBC) is an index based on the NVE and expresses the proportion of the respiratory work done in each breath by the patient, as opposed to the ventilator contribution [71, 72].

Neuro-Mechanical Efficiency (NME) is an index derived from the EAdi. It is a measure of the mechanical output of the diaphragm in response to its electrical activation and is expressed as $cmH_2O/\mu V$. NME is obtained while the subject makes an inspiratory effort with the airway being occluded. NME has been used as a predictor of the outcome of spontaneous breathing trials [68] and to evaluate pharmacological effects on diaphragm efficiency during loaded breathing [73]. NME is not available in the current NAVA software and is used as a research tool.

Rationale for the present thesis

Prior to the start of this PhD project, Neurally Adjusted Ventilatory Assist was a promising new ventilator mode, but there was a number of unanswered questions regarding its use.
It was postulated that NAVA might reduce the presence of asynchrony, but no studies comparing NAVA and Pressure Support on patient-ventilator synchrony had been performed. NAVA offered the potential to increase patient-ventilator synchrony even in paediatric patients. Children are of smaller size, have higher respiratory rate and higher heart rate compared to adults, which could present difficulties for the detection of the EAdi, for the filtering and the replacement of the ECG signal. Therefore, a first necessary step was to prove the feasibility of detecting the EAdi signal with the esophageal catheter even in small species, close in weight to the smallest viable human being. Next, the feasibility to use the EAdi signal to ventilate small animals with NAVA needed to be investigated. Third, it was necessary to confirm that NAVA could contribute to unloading of the respiratory muscles in small patients. With increasing data supporting the use of spontaneous breathing during anaesthesia, with regard to lung mechanics, it could be of value to consider NAVA in this setting. However no studies had systematically investigated how commonly used anaesthetics (such as sevoflurane and propofol) affect the EAdi and muscle performance, or whether spontaneous and partially assisted breathing would be possible with the use of such drugs in therapeutic concentrations.
Despite the description and use of different methods to set the assist in NAVA, setting the NAVA level was still associated with uncertainty. No standardized approach to the setting of NAVA based on different levels of respiratory muscle unloading had been evaluated. Potentially, such titration of unloading could reduce ventilator support to the lowest level necessary. Such reduction could theoretically improve distribution of ventilation towards the dorsal regions of the lungs, but had not been evaluated previously.

AIMS

The specific aims of the present studies were to investigate if:

1. NAVA improves patient-ventilator synchrony and unloads the respiratory muscles compared to Pressure Support, with increasing levels of assist (Paper I-II).

2. The use of NAVA is feasible in small species, close to the smallest viable human being (Paper II).

3. Additional instrumental dead space impacts the pattern of breathing in NAVA compared to Pressure Support (Paper II).

4. NAVA is feasible during sedation and general anaesthesia with propofol and sevoflurane (Paper III).

5. The choice of anaesthetic agent affects the pattern of breathing and contractility during NAVA (Paper III).

6. It is feasible to target different levels of respiratory muscle unloading in NAVA (Paper IV).

7. Lower muscle unloading in NAVA improves the distribution of ventilation (Paper IV).

MATERIALS AND METHODS

The following section briefly describes and discusses material and methods used in the present thesis. More detailed information is provided in Paper I-IV.

Ethical considerations

Animal studies (Paper I-III)

St Michael´s Hospital Animal Care and Use Committee, Toronto, Canada approved study I and II.
The Animal Research Committee of Uppsala University, Sweden, approved study III.

Human study (Paper IV)

The Regional Ethics Committee in Stockholm, Sweden approved study IV. Informed consent was obtained from a next of kin before recruiting the patient in the study.

Experimental animals

In study I, twelve adult male New Zealand white rabbits weighing 3.3 ± 0.9 kg were used.
In study II, nine adult male Sprague-Dawley rats with an average weight of 385 ± 4 g were used.
In study III, nine juvenile mixed country breed male pigs with a median body weight of 27 (26; 31) kg were used.

Patients

In study IV, ten patients were enrolled at the Neurosurgical Intensive Care Unit. At study entry, patients had been intubated and ventilated for more than 48 hours and had started weaning from mechanical ventilation. Patients with unstable intracranial pressure and with severe respiratory, hemodynamic or bleeding disorders were excluded.

Sedation and anaesthesia

In study I, the rabbits were anaesthetized with an intramuscular bolus of ketamine hydrochloride (35mg/kg), followed by a continuous intravenous infusion of ketamine hydrochloride (10 mg/kg/h) and xylazine (2mg/kg/h). The animals were sedated, but spontaneously breathing.
In study II, the rats were anesthetized with isoflurane in 100% oxygen until they were unresponsive to stimuli. The rats received then an intraperitoneal injection of ketamine hydrochloride (100 mg/kg) and xylazine (10mg/kg). Continuous intravenous infusion of ketamine hydrochloride (25 mg/kg/h) and xylazine (2,5mg/kg/h) was provided via the tail vein throughout the study. The animals were sedated, but spontaneously breathing.
In study III, pigs were pre-anesthetized with an intramuscular injection of ketamine (10mg/kg). Before instrumentation, propofol (2mg/kg) was injected intravenously. Low dose ketamine (5mg/kg/h) was provided by intravenous infusion throughout the study. The pigs received sevoflurane and propofol in random order at sedation and anaesthetic level with and without remifentanil. The sedation and anaesthesia levels were set according to stimuli of standardized and predefined intensity in a previous pilot study. Sedation implied that the animal could tolerate the prick of a needle in the leg, but not the pinching of the claw with tongs. At anaesthesia, the animal could tolerate even the pinching of the claw without any sign of distress. The remifentanil infusion dose used was 0.1μg/kg/min, which corresponded to the maximum dose not causing apnoea. The remifentanil infusion was followed by a wash out period of 40 min.
Since the SERVO-i (Maquet Critical Care, Solna, Sweden) used in the present study is not equipped with a vaporizer, the sevoflurane was provided via an external flow through an activated carbon filter at the Y piece. Since the Anaconda filter would have resulted in a too large dead space in our animal model, a filter of 55ml dead space was specially designed by Maquet Critical Care´s research department, by adapting a heat and moisture exchanger (SERVO Filter Humidifier 172, Maquet Critical Care, Solna, Sweden).
In study IV patients were sedated according to the clinical setting before study entry. Sedation was kept unchanged throughout the study.

Interventions and monitoring

Study I-III. An arterial line was inserted for monitoring of blood pressure and for obtaining blood gas samples. Oxygen saturation was monitored with pulse oximetry. Body temperature was continuously measured with a rectal probe and kept within physiologic range by keeping animals on a surgical heating table or by a heating pad.
In Study I-II, the animals were tracheotomised and ventilated by a modified SERVO ventilator 300 (Maquet Critical Care, Solna, Sweden).

In Study I, the rabbits were initially ventilated in Volume Control (VC) with a Vt of 6ml/kg, a RR of 20/min and a PEEP of 2 cmH_2O. After the ALI, the PEEP level was titrated in order to reduce tonic activity of the diaphragm [72] and thereafter kept constant along the study protocol, resulting in average of 7.8 ± 2.4 cmH_2O. F_IO_2 was set at 0.5 in all but 2 animals that had 0.4.
In Study II a PEEP level of 1.5 cmH_2O and F_IO_2 of 0.5 were kept all along the study.
In Study III the pigs were intubated and ventilated by SERVO-i ventilator (Maquet Critical Care, Solna, Sweden). A PEEP level of 3 cmH_2O and F_IO_2 of 0.4 were kept all along the study. During instrumentation and until EAdi was detectable, pigs were ventilated in VC with a Vt of 6ml/kg and a RR of 20/min. When EAdi was back, ventilation was switched to NAVA.
In study IV an arterial line was already on place according to the routine of the NICU and used for blood gas sampling and blood pressure monitoring. The patients were ventilated in PS and in NAVA by a SERVO-i ventilator (Maquet Critical Care, Solna, Sweden). PEEP was kept according to the clinical setting at study entry. F_IO_2 was increased by 10 % if needed, to avoid the oxygen saturation to drop below 95%.

NAVA in the studies

In study I, 8-French (F) EAdi catheters provided with gastric and esophageal balloons (Neurovent Research, Toronto, Canada) were used, in order to obtain the transdiaphragmatic pressure Pdi as Pdi = Pga-Pes. Correct esophageal balloon position was checked with the occlusion method [73].
In study II, 5.6-F size were used (Neurovent Research, Toronto, Canada).
In study III, 16-F EAdi catheters were used (Neurovent Research, Toronto, Canada). A NAVA level of 0.5 $cmH_2O/\mu V$ was chosen and kept unchanged during the study.
In study IV, 16-F size were used (Maquet Critical Care, Solna, Sweden).
Cycle-off criteria: In study I-II, the ventilator cycled to expiration when the EAdi fell to 80% of the inspiratory peak. In study III-IV the cycle-off criteria was as by default in the SERVO-i, at 70% of the inspiratory peak EAdi.

PS in the studies

Trigger criteria: in study I-II, the flow trigger was set to medium sensitivity.
Inspiratory rise time in study I-II was set at 5% of the respiratory cycle time.
Cycle-off criteria: In study I-II, the ventilator cycled to expiration by default at 5% of the peak inspiratory flow (nonadjustable in the SERVO 300, Maquet Critical Care, Sweden).
In study IV trigger sensitivity, rise time and cycle-off criteria were kept according to clinical setting.

Electrical Impedance Tomography (EIT)

EIT is a non-invasive technique that allows to assess the distribution of ventilation in the lungs bedside. In the present project, study IV, Swisstom BB2 EIT Device (Swisstom AG, Landquart; Switzerland) was used to compare the distribution of ventilation in the lungs at the different levels of muscle unloading.

The Swisstom Sensor Belt has 32 active microelectrodes imbedded in the belt, which is positioned around the thoracic cage, under the mammillary level. The impedance images are created at a frequency of 50 images per second, by analysing the difference in impedance between inspiration and expiration. With the Sensor Belt, a lung volume about 5cm above and below the belt is visualized.

In study IV, EIT was used to compare the distribution of ventilation in the lungs at the different levels of muscle unloading.

Experimental protocols

In study I, the rabbits were provided deep sedation or, if needed, muscle relaxant, until EAdi was undetectable in order to measure respiratory system compliance. Acute lung injury (ALI) was then induced by instillation of hydrochloric acid (2ml/kg, at pH 1.5) in the trachea, followed by a recruitment manoeuver with CPAP at 25 cmH_2O for 5 s. The injury severity was assessed by measuring the respiratory system compliance and the PaO_2/F_IO_2 ratio. The animals were ventilated in VC until they recovered the EAdi. They went through incremental PS and NAVA, the order of the mode being randomized. The low PS level was set at 4cmH_2O and increased in four steps of 4 cmH_2O. With NAVA, the lowest assist level was set to match PS 4cmH_2O, and increased thereafter in four steps of 0.2-0.4 cmH_2O/arbitrary units of EAdi in NAVA level. A blood gas sample was drawn for analysis at the end of each study step.

In study II, a pneumotachograph (1.3ml dead space) was added between the tracheostomy and the Y-piece. The rats were first ventilated in NAVA and then in PS without (Baseline, BL) and with an additional dead space of 0.8ml (Dead Space, DS), the order between Baseline and Dead Space being randomized. The NAVA level was set to 0.8 cmH_2O/arbitrary units of EAdi and the PS level was chosen to match NAVA in peak inspiratory pressure. A blood gas sample was drawn for analysis at the end of each study step lasting 10min.

In study III, the study has a randomized crossover design. When steady state was reached for each sedation level, the animals were ventilated in NAVA for 15min. During the last five minutes of each sedation step, ventilator parameters were recorded and blood gas samples collected and analysed. Before proceeding to next sedation step, the animals were exposed to a 30 s expiratory hold to measure the NME. If a $PaCO_2$ below 7 kPa could not be reached, the animals were switched to VC and sedation was reduced in 10% steps, in order to fulfil

both the CO_2 target and the clinical sedation criteria. The animal was excluded if both criteria could not be fulfilled, which happened in one animal out of ten.
In study IV, patients were ventilated in PS according to the clinical setting (PS_{cli1}). PS was followed by NAVA delivered at 3 levels of assist, respectively corresponding to 40% respiratory muscle unloading ($NAVA_{40\%}$), 60% ($NAVA_{60\%}$) and matching the unloading during PS_{cli1}. In the last study step patients were ventilated in PS, with the same settings as in PS_{cli1} (PS_{cli2}). The order between the 3 NAVA levels was randomized. Patients were ventilated for 30 min in each study step in order to reach a steady state in gas exchange and ventilation distribution. NVE was calculated every 10 min and thereby even the unloading based on the NVE. If needed, the NAVA level was adjusted to maintain the unloading to the predefined target. At the end of each step, NME was also measured during an occlusion manoeuver. A blood gas sample was drawn for analysis in the last 5min of each study step and EIT imaging recorded.

Data collection and analysis

In study I, the last minute recordings for each 5 min steps were analysed breath by breath in an off-line tool. The tool could be set to show curves from only some of the recorded signals. In this way, the analysis was performed on the EAdi and the Pdi curves, being blinded of the airway pressure and flow signal. Also, the analysis of the ventilator parameters was done being blinded of the EAdi and Pdi signal. This functionality made it possible to perform unbiased synchrony calculation.
Indexes of diaphragm energy expenditure per minute were calculated off line and were based on the EAdi and the Pdi signal. The EAdi-time product was calculated as EAdi-tp= mean insp EAdi*neural Ti*neural RR.
The trigger delay was calculated as the difference between the onset of the neural and of the mechanical inspiration. The cycle-off delay was determined by the difference between the onset of the neural and mechanical expiration.
In study II, the respiratory variables during the last minute recording were analysed. A breath by breath analysis was performed on the EAdi, flow and airway pressure curve signal.
In Study I-II ineffective inspiratory efforts were identified by the presence of EAdi signal without triggering of the ventilator.
In study III, the SERVO Tracker (version 3.33, Maquet Critical Care) software program collected signals from the SERVO-i ventilator (EAdi, Airway Pressure and Flow). The Acknowledge (version 3.9.1, Maquet Critical Care) software program collected both the SERVO Tracker signals and the end tidal sevoflurane signal for simultaneous recording. Ventilator parameters in the last five minutes of each sedation step were analysed. The variability of the Vt was expressed with the coefficient of variation (SD/mean) as a percentage. The sighs were defined as breaths higher than twice the average Vt and they

were counted manually in the 5min periods and expressed as sighs/h. Apnoea longer than 5s following the sighs were counted as apnoea/h. The dynamic compliance of 5 breaths before and after the sighs was also measured (Cdyn). The NME at occlusion was calculated at the end of each sedation step. Since we were comparing NVE between different sedation strategies, the NAVA level was kept constant throughout the protocol and a modified NVE (NVE_{NAVA}) was determined over each 5min period, without zeroing the assist.

In study IV, the SERVO Trend Tool (Maquet Critical Care, Solna) software program was used for detecting the zero assist and the end-expiratory hold manoeuvres necessary for determining the NVE, the NVE Unloading breath by breath and the NME. The SERVO Tracker (version 4.2, Maquet Critical Care) software program collected all other ventilator parameters from the SERVO-i ventilator.

The Swisstom BB2 EIT Device (Swisstom AG, Landquart; Switzerland) was used for recording the images of the ventilation distribution breath by breath in the last 5 min of each study step. The specific software Ibex (Swisstom AG) determined the localization of the Centre of Ventilation (CoV) in each study period. The CoV indicates where the ventilation is mostly distributed in the lung in the ventral-dorsal axis. A value of 50 indicates that the ventilation is equally distributed between ventral and dorsal regions of the lungs. Values above 50 mean that the ventilation is more towards the dorsal areas of the lungs.

Furthermore, the software divided the EIT image recorded in 4 Regions of Interest (ROI), each representing 25% of the ventro-dorsal axis and named respectively Ventral, Mid-Ventral, Mid-Dorsal and Dorsal (Fig. 7).

Figure 7 Regions of Interest

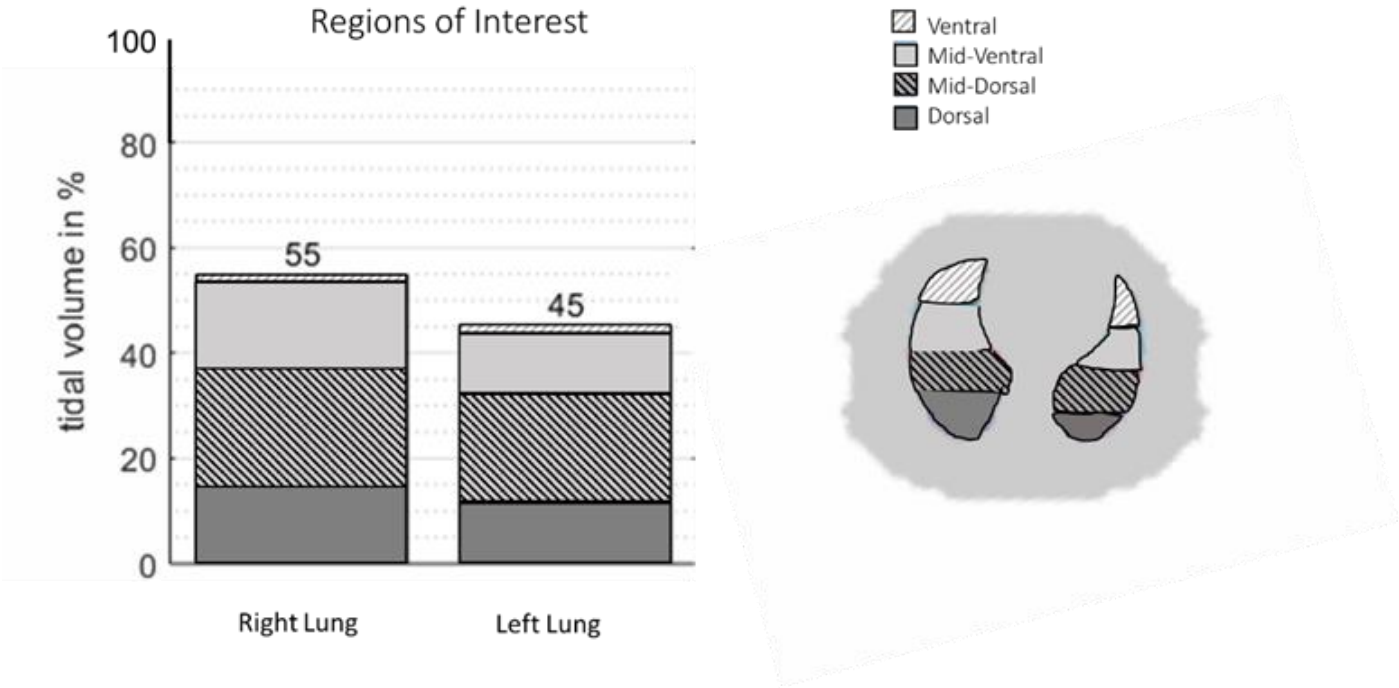


Figure modified from Ibex Software output picture. In the present analysis, the right and the left lung regions were summed and their relative contribution was expressed as % ventilation.

Statistical methods

Data are presented as median and interquartile range (25^{th} and 75^{th}).

In paper I, where each mode was analysed in three steps, the data were divided in three groups, according to the increasing level of assist, both for NAVA ($NAVA_{lo}$, $NAVA_{med}$ and $NAVA_{hi}$) and for PS (PS_{lo}, PS_{med} and PS_{hi}).

In paper I-II, animals were ventilated at different assist levels (Paper I) or exposed/not exposed to dead space (Paper II) during PS and NAVA, and variables were analysed by using the Repeated Measures (RM) Analysis of Variance for non- parametric data, or Friedman Repeated Measures ANOVA on ranks (Sigmastat, Jandel Scientific, San Rafael, CA). All pairwise multiple comparisons were then performed with the Student-Neuman-Keuls method.

In paper III, each animal was exposed to different sedation steps. The variables were analysed with the Wilcoxon Signed Rank Test for related samples for non-parametric data (IBM SPSS Statistics version 20.0 for Windows, SPSS Inc., Chicago, Illinois, USA).

In paper IV, since each patient was ventilated at different levels of respiratory muscle unloading, the variables were analysed with the Friedman Repeated Measures ANOVA. The multiple comparisons of the related samples in PS_{cli1}, $NAVA_{40\%}$, $NAVA_{60\%}$, and PS_{cli2} were performed with Dunn´s test.

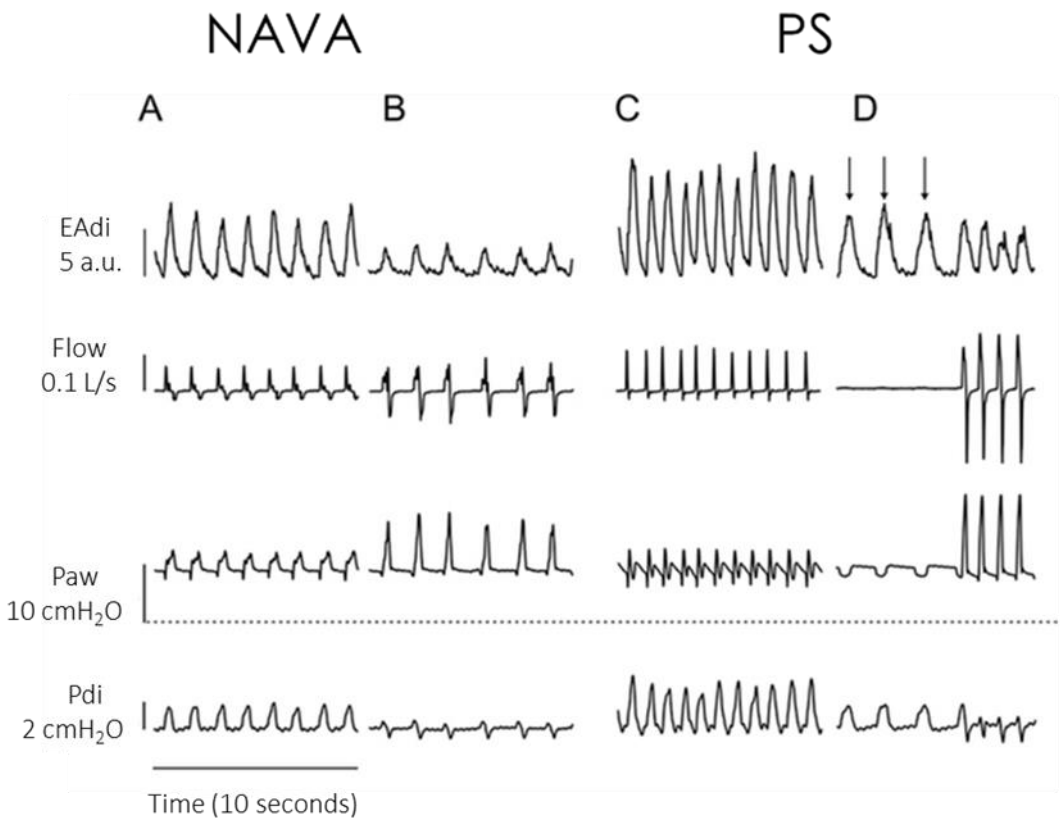
For all studies the significance level was set at p values below 0.05 (2-tailed tests).

SUMMARY OF RESULTS

Asynchrony

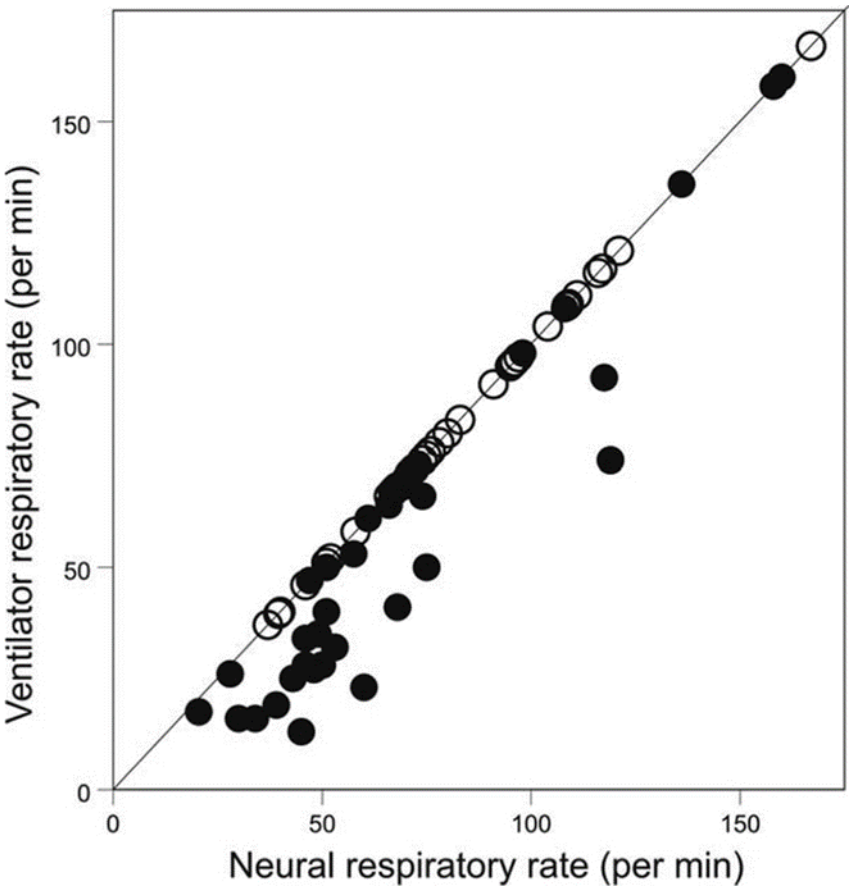
Patient-ventilator synchrony in PS and NAVA was subject of investigation in paper I-II. In ALI rabbits (paper I), wasted inspiratory efforts were observed for high levels of PS. When increasing NAVA level instead, the synchrony was maintained (Fig 8).

Figure 8. Patient-ventilator synchrony



Example of tracings obtained in one rabbit (paper I) breathing on $NAVA_{lo}$ and $NAVA_{hi}$ and PS_{lo} and PS_{hi}. EAdi (top tracings), airway pressure (Paw, measured at the Y piece), and flow tracings (measured at the ventilator) and Pdi (bottom tracings) are demonstrated for one rabbit at $NAVA_{lo}$ (A), $NAVA_{hi}$ (B) and PS_{lo} (C) and PS_{hi}.

Figure 9. Neural and Ventilator RR



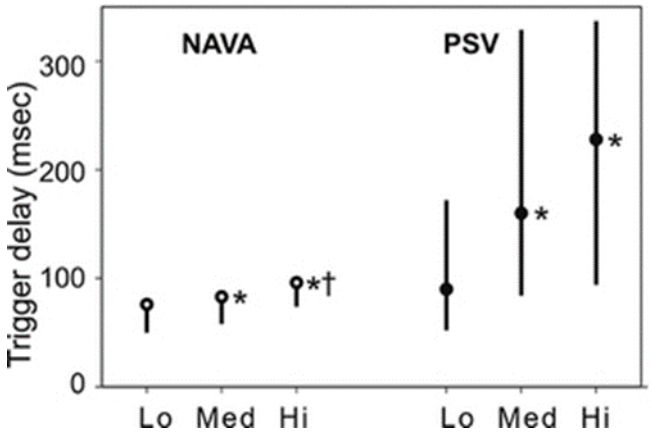
Comparison of neural respiratory rate to ventilator rate in all rabbits during PS and NAVA (paper I). For each animal at each level of assist, neural respiratory rates were plotted against ventilator rate, for both PS (solid circles) and NAVA (open circles).

At low PS level (PS_{lo}), 95% of the neural inspiratory efforts triggered the ventilator, while at high PS level (PS_{hi}) only 66% of neural breaths succeeded to trigger the ventilator. Fig.9 shows the relationship between the neural and the ventilator respiratory rate (RR) for all the assist levels in NAVA (empty circles) and in PS (solid circles) in all the rabbits.

In paper II, 2.5 wasted inspiratory efforts per min occurred in four out of nine rats, during ventilation with PS, without dead space. In NAVA, no wasted efforts were observed.

In paper I, the delay between the onset of the neural and mechanical inspiration, or Trigger Delay, was calculated in PS and NAVA. The Trigger Delay increased more for increasing level of PS (90-228 ms) than with NAVA (76-96 ms), see Fig. 10.

Figure 10. Trigger Delay

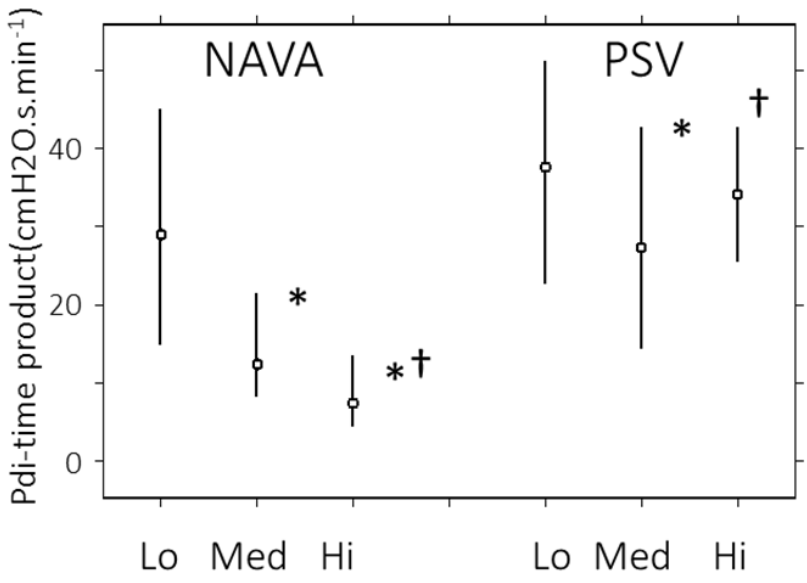


Trigger delay (paper I) for PS_{lo}, PS_{med}, and PS_{hi} and $NAVA_{lo}$, $NAVA_{med}$, and $NAVA_{hi}$. Group data (median with quartiles). *Significantly different from low level, and † significantly different from medium level, $p < 0.05$.

Respiratory muscle unloading and respiratory parameters

In study I, for increasing NAVA level, a progressive decrease in mean inspiratory EAdi and Pdi was observed, as shown in Fig. 8, point A->B. Similarly, the energy expenditure, calculated as Pdi-time product, progressively decreased at increasing NAVA level, indicating successful muscle unloading (Fig. 11). In PS instead, the EAdi and the Pdi corresponding to wasted efforts had higher amplitude (Fig. 8, point D) compared to the successfully triggered breaths (Fig. 8, point C). In PS, there was an initial reduction of Pdi-time product at intermediate level PS (PSmed), followed by an increase for PShi (Fig. 11).

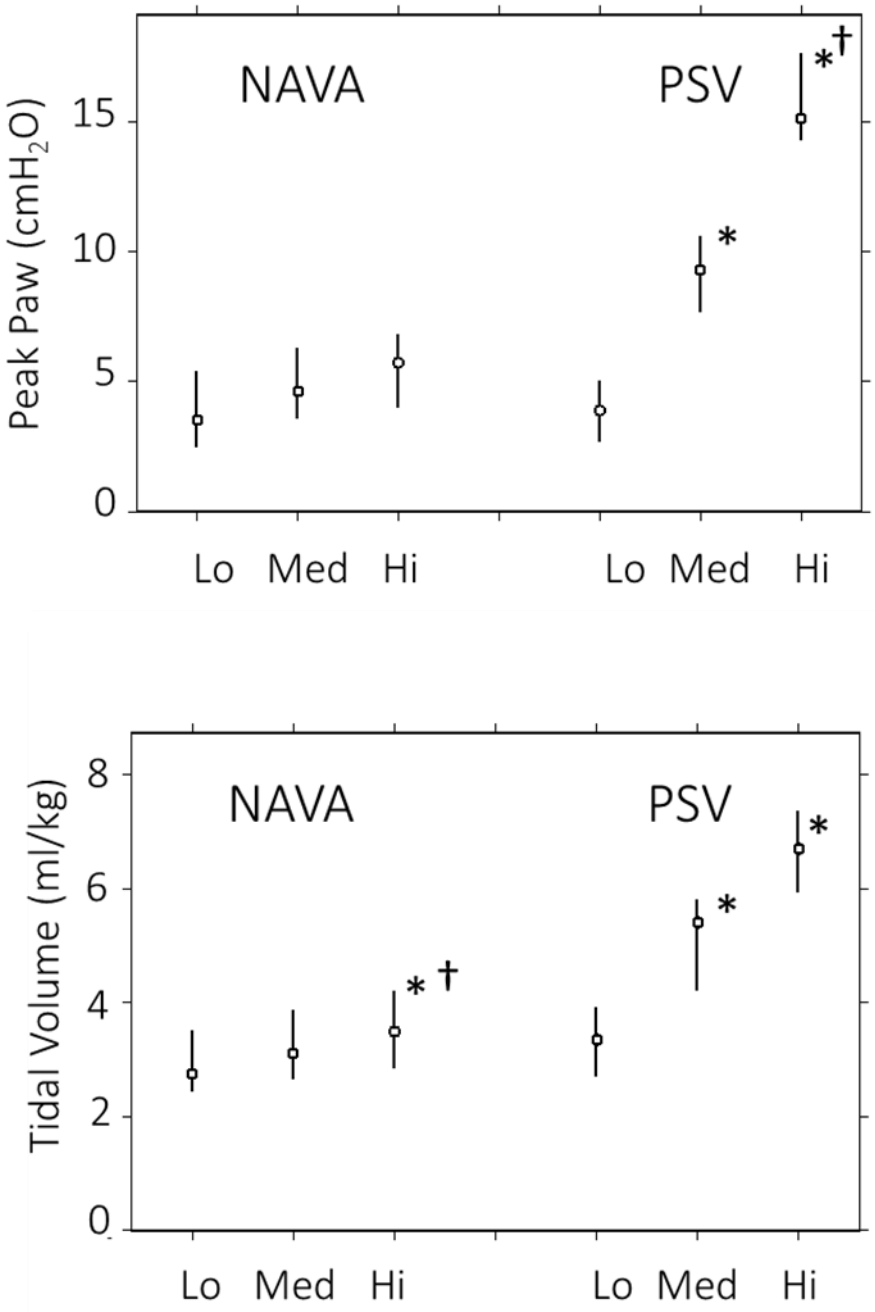
Figure 11. Pdi-time product



Pdi-time product (paper I) for PS_{lo}, PS_{med}, PS_{hi} and $NAVA_{lo}$, $NAVA_{med}$, and $NAVA_{hi}$. Group data (median with quartiles). *Significantly different from low level, and †indicates significantly different from medium level, $p < 0.05$.

Tidal volumes and Airway pressures increased much more when raising the assist in PS compared to NAVA (Fig. 12).

Figure 12. Airway Pressure and Tidal Volume



Peak Paw = Peak Airway pressure (above PEEP) and Tidal Volume for $NAVA_{lo}$, $NAVA_{med}$, and $NAVA_{hi}$ and PS_{lo}, PS_{med}, and PS_{hi}. Group data from paper I (median with quartiles). *Significantly different from low level, and †indicates significantly different from medium level, $p < 0.05$.

Feasibility in small species

In paper II, PS and NAVA were applied to tracheotomised rats weighing around 400g, close in weight to the smallest viable human being. The EAdi is evident even in small species and has an amplitude sufficient to ventilate in NAVA. Oxygenation and ventilation did not differ between PS and NAVA (Tab. 1).

Table 1. Neural breathing pattern and arterial CO_2 for the different ventilatory conditions

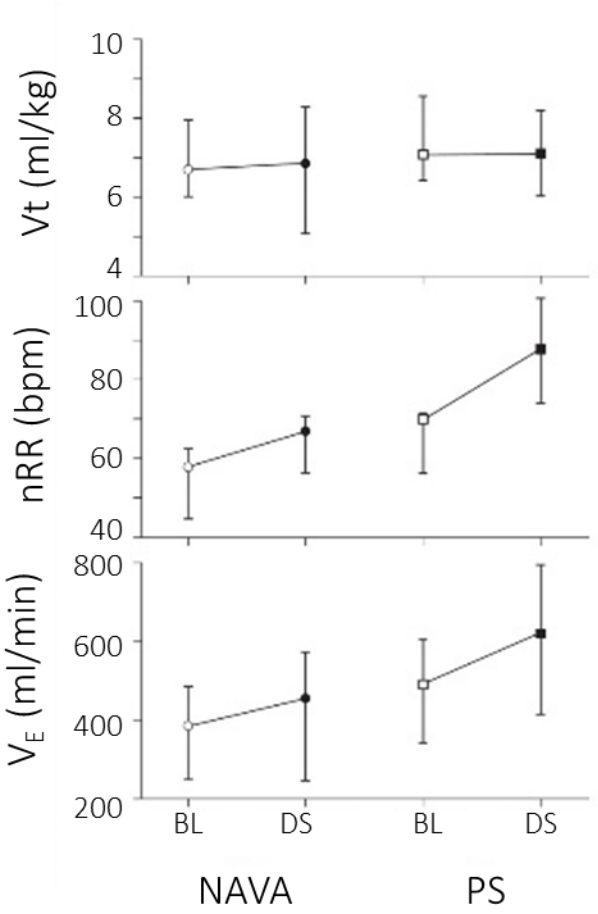
Variable	NAVAbl	NAVAds	PSVbl	PSVds	ANOVA p value
PaCO2, mm Hg	48.5 (44; 54)	55.5 (53.5; 65)[c]	51 (46; 55)	59.5 (59; 61.5)[d]	0.002
IXEAdi, AU	8.5 (7.4; 12.3)	11.8 (8.9; 14.6)[c]	10.4 (7.3; 13.6)	12.9 (9.0; 17.3)[d]	0.015
Ti, ms	474 (450; 512)	428 (391; 486)[c]	340 (306; 382)[a]	266 (235; 294)[b], [d]	<0.001
Ti/Ttot	0.53 (0.47; 0.60)	0.52 (0.49; 0.61)[c]	0.41 (0.38; 0.49)[a]	0.41 (0.34; 0.44)[b]	0.002
Vt/Ti, l/sec	5.3 (4.4; 6.2)	6.4 (5.9; 6.8)[c]	7.7 (6.7; 8.8)[a]	9.6 (9.4; 11.2)[b], [d]	<0.001

Values from paper II are reported as medians (IQR). bl = Baseline condition; ds = dead space condition; $PaCO_2$= partial pressure of carbon dioxide in the arterial blood; IXEAdi = mean inspiratory diaphragm electrical activity; AU = arbitrary units. [a]Post hoc comparison of $NAVA_{bl}$ vs PSV_{bl} $p < 0.05$. [b]Post hoc comparison of $NAVA_{ds}$ vs PSV_{ds} $p < 0.05$. [c]Post hoc comparison of $NAVA_{bl}$ vs $NAVA_{ds}$ $p < 0.05$. [d]Post hoc comparison of PSV_{bl} vs PSV_{ds} $p < 0.05$.

Pattern of breathing with and without dead space

In paper II, in terms of pattern of breathing, no differences were observed regarding the Vt between PS and NAVA. However, we noticed a shorter inspiratory time (T_i) in PS than in NAVA, both at baseline (BL) and after the dead space (DS) was added (Tab.1). The addition of the ds generated a higher increase in RR and minute ventilation (V_E) in PS compared to NAVA to achieve similar levels of $PaCO_2$ (Fig. 13).

Figure 13. Vt, nRR and V_E in PS and NAVA



Vt = tidal volume, nRR = neural respiratory rate and V_E = minute ventilation during NAVA baseline periods (circles), PS (squares) baseline periods (empty symbols) and during periods with dead space (solid symbols). Data from paper II are presented as medians with 25^{th} and 75^{th} quartiles.

Feasibility during sedation and anaesthesia

In study III, we investigated the feasibility of ventilating with NAVA intubated pigs that were sedated and anesthetized with sevoflurane and propofol in randomized order, with and without remifentanil. EAdi, oxygenation and ventilation were preserved with both anaesthetics in this animal model, even when low dose remifentanil was added. Furthermore, oxygenation and $PaCO_2$ did not differ between the two anaesthetics (Tab. 2).

Table 2. Baseline respiratory parameters and blood gas analyses

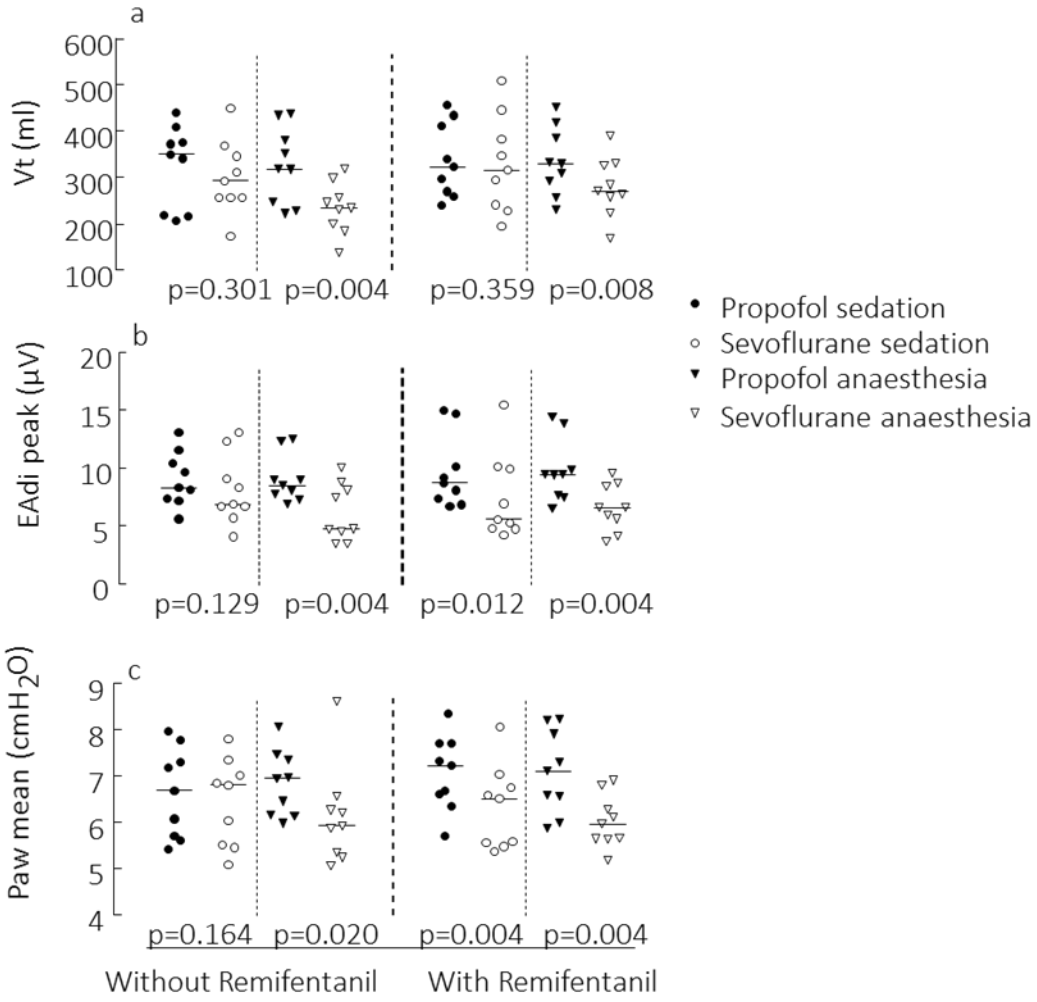
	Without remifentanil					
	Sedation		p	Anaesthesia		p
	Propofol	Sevoflurane		Propofol	Sevoflurane	
Vt(ml)	350 (217; 392)	294 (257; 357)	0.301	318 (238; 408)	235 (192; 278)	0.004
RR(bpm)	23 (19; 25)	26 (23; 27)	0.214	21 (15; 23)	24 (22; 26)	0.097
EAdi peak (µV)	8.3 (7.3; 10.9)	6.9 (6.2; 10.7)	0.129	8.4 (7.5; 10.6)	4.7 (3.9; 8.4)	0.004
$Paw_{,mean}(cmH_2O)$	6.7 (6; 7)	6.8 (5.5; 7.2)	0.164	6.9 (6.1; 7.4)	5.9 (5.3; 6.4)	0.020
PaO_2(kPa)	24 (19; 27)	23 (22; 24)	0.734	23 (18; 26)	22 (18; 22)	0.570
$PaCO_2$(kPa)	5.5 (5.3; 5.9)	5.5 (5.2; 5.6)	0.496	6 (5.7; 6.2)	6 (5.6;6.3)	0.496
	With remifentanil					
Vt(ml)	323 (265; 422)	316 (235; 415)	0.359	329 (275; 402)	270 (240; 329)	0.008
RR(bpm)	20 (17; 24)	26 (24; 27)	0.012	16 (13; 22)	22 (19; 25)	0.021
EAdi peak (µV)	8.7(7.1; 12.4)	5.6 (4.7; 10)	0.012	9.4 (7.5; 11.8)	6.5 (4.8; 8.5)	0.004
$Paw_{,mean}(cmH_2O)$	7.2 (6.5; 7.7)	6.5 (5.5; 6.9)	0.004	7.1 (6.3; 8.0)	5.9 (5.6; 6.5)	0.004
PaO_2(kPa)	22 (18; 25)	22 (21; 25)	0.203	20 (19; 21)	22 (19; 24)	0.734
$PaCO_2$(kPa)	6 (5.5; 6.3)	5.4 (5; 5.6)	0.008	6.2 (6.1; 6.8)	6.3 (6; 6.5)	0.300

Data from paper III are median (IQR). EAdi peak, electrical activity of diaphragm inspiratory peak value; Paw_{mean}, mean airway pressure; RR, respiratory rate; Vt, tidal volume

Pattern of breathing with different anaesthetics

In study III, the pattern of breathing in NAVA was observed in pigs with different anaesthetics. During sevoflurane anaesthesia, EAdi, airway pressures and tidal volumes were lower with sevoflurane than with propofol (Fig. 14). RR did not differ between the anaesthetics.

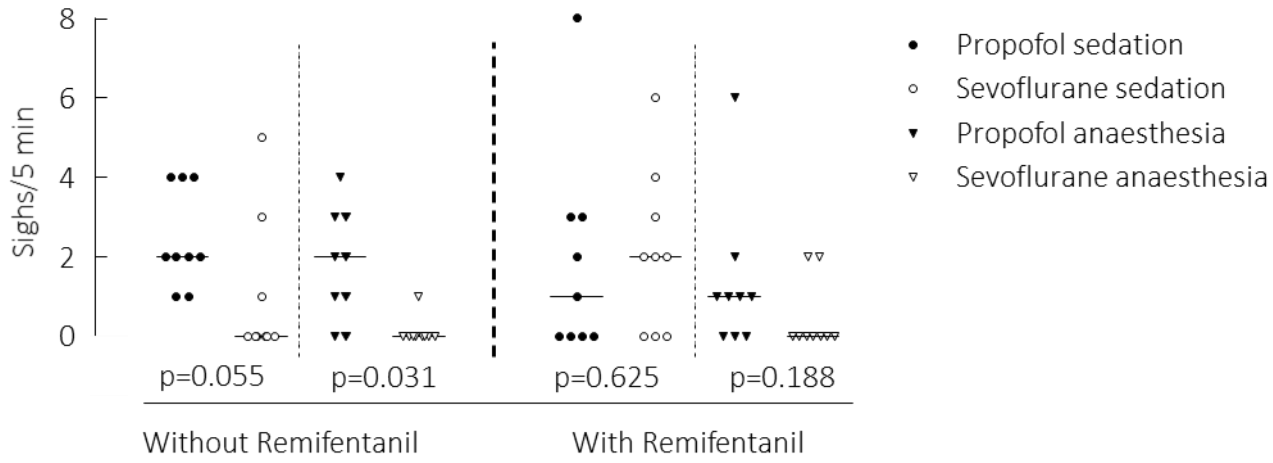
Figure 14. EAdi, Paw and Vt



Changes in tidal volume, EAdi and airway pressure with propofol and sevoflurane. (a–c) Values of tidal volume (Vt), peak electrical activity of the diaphragm (EAdi peak), mean airway pressure (Paw mean) during sedation (dots) and anaesthesia (triangles) from paper III. Left, propofol or sevoflurane alone; right, propofol or sevoflurane combined with remifentanil. *p* values refer to comparison between propofol and sevoflurane at each sedation level. Median value represented by horizontal line.

The variability of the Vt, expressed by the coefficient of variation (CV_{Vt}%) was higher with propofol than sevoflurane both at sedation [34 (26; 35)% vs. 13 (7; 27)%] and anaesthesia [27 (19; 29)% vs. 11 (10; 20)%]. A higher frequency of sighs and apnoea longer than 5s was observed with propofol than sevoflurane without remifentanil (Fig. 15).

Figure 15. Sighs per 5 min



The number of sighs per 5 min during sedation (dots) and anaesthesia (triangles), from paper III. Left, propofol or sevoflurane alone; right, propofol or sevoflurane combined with remifentanil. *p* values refer to comparison between propofol and sevoflurane at each sedation level. Median value represented by horizontal line.

When remifentanil was added, no more differences in sighs and in Vt variability were observed between drugs. RR was lower with propofol-remifentanil than sevoflurane-remifentanil.

Feasibility of targeting unloading

In study IV, respiratory muscle unloading was determined from the NVE measurement in NICU patients and used to target NAVA assist to different levels of unloading (respectively 40% and 60%). Since the unloading in PS_{cli1} was too high (Tab.3) to be adequately matched with corresponding unloading in $NAVA_{cli}$, it was necessary to reduce the unloading during $NAVA_{cli}$. The study step $NAVA_{cli}$ was therefore excluded from the analysis. The respiratory muscle unloading achieved in each study period is reported in Tab. 4. To keep the target unloading constant in each step, some adjustments in the NAVA level were necessary and are reported in Tab 4.

Table 3. Clinically set PS (PS_{cli1})

Patient	PS_{cli1} (cmH_2O)	$Unloading_{NVE}$ in PS_{cli1} (%)	$Unloading_{NME}$ in PS_{cli1} (%)
1	10	87 (79; 93)	86 (84;87)
2	10	77 (62; 87)	77 (76; 80)
3	7	46 (35; 55)	48 (43; 52)
4	12	88 (78; 95)	89 (87; 92)
5	12	79 (67; 85)	85 (83; 87)
6	4	60 (45; 71)	25 (20;30)
7	10	77 (66; 85)	65 (56; 70)
8	8	64 (54; 72)	61 (58; 63)
9	9	86 (76; 94)	87 (83; 89)
10	8	63 (54; 76)	38 (32; 43)

The $Unloading_{NVE}$ reported is based on the entire PS_{cli1} period and expressed as median and interquartile range (paper IV).

Table. 4 Unloading based on NVE and NME

	$Unloading_{NVE}$ %	$Unloading_{NME}$ %	NAVA level ($cmH_2O/\mu V$)	N_0 of NAVA level adjustments/step#
PS_{cli1}	70 (57; 85)	71 (45; 86)	--	--
$NAVA_{40\%}$	43 (32; 60)	41 (33; 62)	0.8 (0.6;1.2)	2.5 (1;5)
$NAVA_{60\%}$	60 (47; 69)	65 (56; 68)	2.6 (2.0;3.7)	3.5 (3;6)
PS_{cli2}	71 (54; 93)	58 (36;87)	--	--

Number of NAVA level adjustments made to keep the unloading constant during each study step (paper IV). No changes in NAVA level were necessary during the last 5 minutes in each study step, corresponding to the recording period of ventilator parameters, EIT and blood gas analyses.

Oxygenation and ventilation were unchanged throughout the study, even with moderate unloading. As expected, the EAdi increased for lower levels of unloading (Tab. 5). No signs of distress or discomfort was observed in NAVA for moderate levels of muscle unloading.

Table 5. Ventilatory Parameters

Ventilatory Parameters	PS_{cli1}	$NAVA_{40\%}$	$NAVA_{60\%}$	PS_{cli2}
Paw_{mean} (cmH_2O)	12.2 (8.3; 13)	11.4 (8.5; 11.5)	11.6 (10; 12.2)$	12.1 (8.3;13.2)*
RR (bpm)	15 (11; 17)	16 (13; 18)	16 (11; 19)	15 (10; 18)
Vt (ml)	525 (488; 642)	530 (408; 552)	525 (427; 585)	535 (486; 559)
MV (L/min)	8.4 (5.7; 9.2)	8.2(5.9; 8.9)	8.3 (5.6; 9.1)	8.4 (5.6; 9.9)
$EAdi_{peak}$ (µV)	2.7(1.6; 4.7)	5.3(4.2; 6.9)**	4.7(2.8; 5.3)	3.9(1.7; 7.3)
PaO_2/FIO_2 (KPa)	44 (38; 49)	40 (39; 45)	42 (37; 46)	41 (37; 47)
$PaCO_2$ (KPa)	5.4 (5.1; 5.9)	5.5 (5.1; 5.9)	5.5 (5; 5.7)	5.3 (5.1; 5.7)

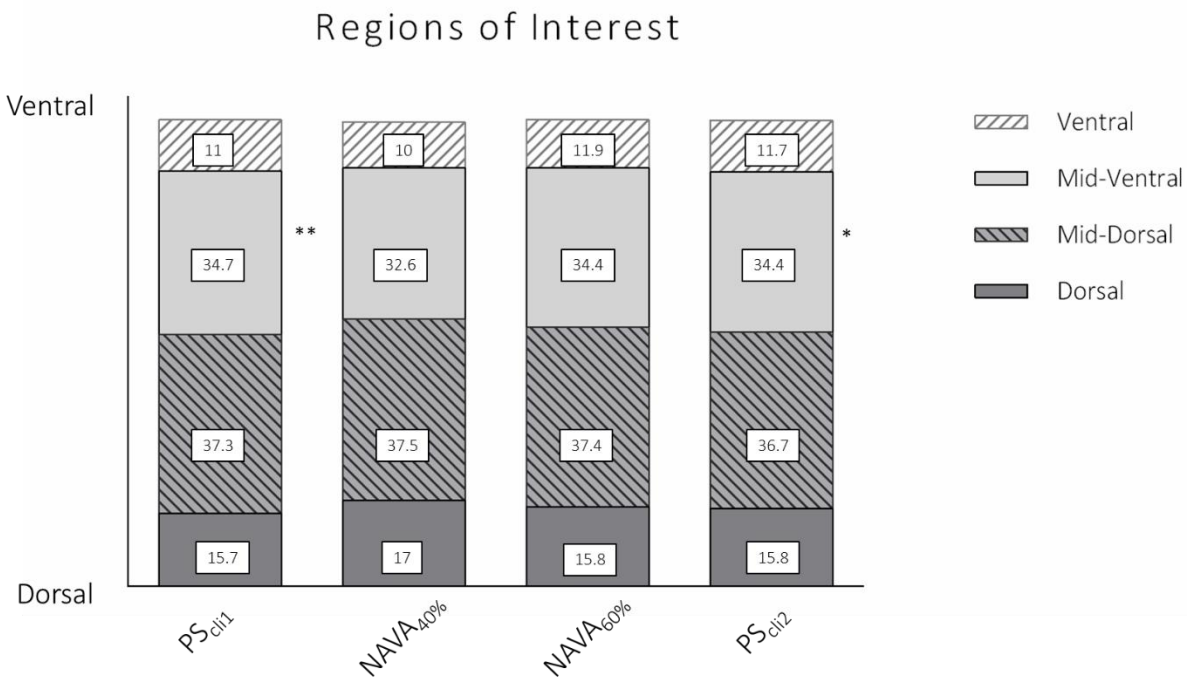
Data from paper IV. Mean Airway Pressure (Paw_{mean}); Respiratory Rate (RR); Tidal Volume (Vt), Minute Volume (MV); Peak EAdi ($EAdi_{peak}$). Friedman RM Anova Paw_{mean} p= 0.007. Friedman RM Anova $EAdi_{peak}$ p= 0.03. * p < 0.05 PS_{cli2} vs $NAVA_{40\%}$; $ p< 0,05 $NAVA_{40\%}$ vs $NAVA_{60\%}$. ** p < 0.05 PS_{cli1} vs $NAVA_{40\%}$.

Distribution of ventilation

In study IV, the distribution of ventilation was studied by means of EIT, for different levels of respiratory muscle unloading applied to ICU patients. The Centre of Ventilation (CoV) was used to compare ventilation distribution, a higher value reflecting more dorsal distribution. Reducing muscle unloading led to a shift in ventilation distribution towards the dorsal areas of the lungs. More specifically, the CoV was at 55% (51; 56) in $NAVA_{40\%}$ versus 52% (48; 55) in PS_{cli2} and 53% (51; 56) with $NAVA_{60\%}$.

Furthermore, the ventilation distribution was expressed in Regions of Interest (ROI), each representing 25% of the ventro-dorsal diameter of the lungs. For each level of unloading, the relative contribution of the ROI was quantified and compared. The contribution to ventilation distribution of the mid-ventral region decreased between PS_{cli1} and PS_{cli2} to $NAVA_{40\%}$ (Fig. 16).

Figure 16. Regions of Interest



The ventilation distribution is described in 4 lung regions in the ventro-dorsal axis of the lungs in supine position (paper IV). Friedman RM Anova for Mid-Ventral region p= 0.02. ** $p<0.05$ PS_{cli1} vs $NAVA_{40\%}$; * $p<0.05$ PS_{cli2} vs $NAVA_{40\%}$.

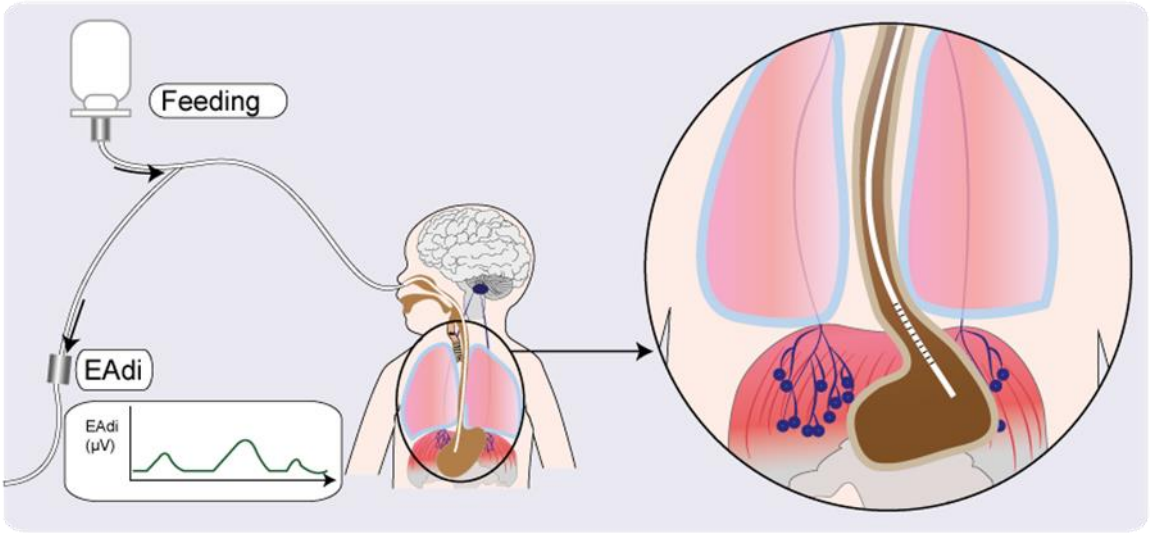
DISCUSSION

In the present thesis, the feasibility of NAVA was investigated in different situations resembling clinical scenarios.

NAVA can be used in small individuals

In study I and II, it was possible to ventilate with NAVA, despite the small size of the species used. These studies, together with other animal studies [74-76], were the first steps towards the use of NAVA in paediatric intensive care patients. At that time, the EAdi catheter design was tailored and tested to fit different patients' size. Contemporarily, the algorithms for processing the EMG signal were developed and evaluated. Today, NAVA is successfully used even as NIV-NAVA in preterm babies as small as 23 weeks gestational age, weighing around 500 g [77] (Fig. 17).

Figure 17. EAdi recording in small children



Adapted From Maquet Clinical Image Collection. EAdi catheter picking the EAdi signal and used for enteral feeding in a small child.

Compared to conventional modes of ventilation controlled by pneumatic signals, NAVA presents the advantage of not being affected by air leaks, which may be common while ventilating paediatric patients either invasively with un-cuffed endotracheal tubes or non-invasively [78, 79].

Furthermore, in study I, performed in rabbits with acute lung injury, tidal volumes and airway pressures increased much more with raised assist level in Pressure Support compared to NAVA. Similarly, both in paediatric [80-82] and adult patients [83, 84] ventilated with NAVA,

airway pressures and tidal volumes have been shown to be lower compared to conventional modes of ventilation. With increasing lung distention, the feedback signal from the stretch receptors in the lungs to the respiratory centres, down-regulates the EAdi, leading to earlier transition from inspiration to expiration, thus avoiding lung over-distention [56]. In a short-term experimental animal model of acute lung injury, NAVA was shown to be as protective (on lung tissue and other organs) as the low tidal volume strategy in absence of spontaneous breathing [85]. In a crossover study on ARDS patients ventilated with Pressure Control (PC), PS (both targeted to deliver Vt of 6ml/kg PBW) and with NAVA, it was shown that NAVA was as lung protective as PC and PS, in terms of Vt size and lung distending pressures [86]. These findings suggest that NAVA may be a potential lung protective mode of assisted ventilation, when regulating feedbacks and respiratory centres are intact and when pH is not too low. Indeed, maintaining acid base homeostasis is primary to the human body and the response to a very low pH with increased respiratory drive may lead to exceeding tidal volumes and transpulmonary pressures [86, 87]. In early severe ARDS, in spontaneously breathing patients, a very high respiratory demand and mechanic load burden the inspiratory muscles, thus leading to a large increase in oxygen consumption and to the activation of expiratory and inspiratory accessory muscles. The adoption of assisted ventilation in severe ARDS patients has been debated [88] and some studies have shown improved survival when muscle paralysis is applied during the first 48h in patients with severe ARDS [2]. The authors suggested the use of muscle paralysis as beneficial to improve patient-ventilator synchrony and the maintenance of lung protective strategy, limiting the occurrence of lung collapse and regional over-distention.
However, considering the benefits associated with assisted ventilation, a strategy applying NAVA in patients with severe ARDS after the first critical 48 hours may be of benefit.

Patient-ventilator interaction is improved with NAVA

In study I-II, patient-ventilator synchrony was shown to improve with NAVA compared to Pressure Support in small animals for the first time. Since, our findings have been corroborated by many clinical studies, demonstrating improved patient-ventilator interaction with NAVA, compared to conventional modes of ventilation. This has been demonstrated both in paediatric [89, 90] and adult patients [86, 91-94]. In study I-II, synchrony was maintained with NAVA even for increasing levels of assist, progressively unloading the diaphragm, while in Pressure Support, wasted inspiratory efforts were observed, especially for high levels of assist, thus failing to unload the diaphragm. Furthermore, in study I, the delay in inspiratory trigger increased much more for rising levels of Pressure Support compared to NAVA. Besides confirming better synchrony with NAVA regarding wasted efforts and trigger delays [92, 95], clinical studies have also shown an improved interaction when it comes to avoiding auto-triggering and asynchronies when inspiration cycles to expiration, as

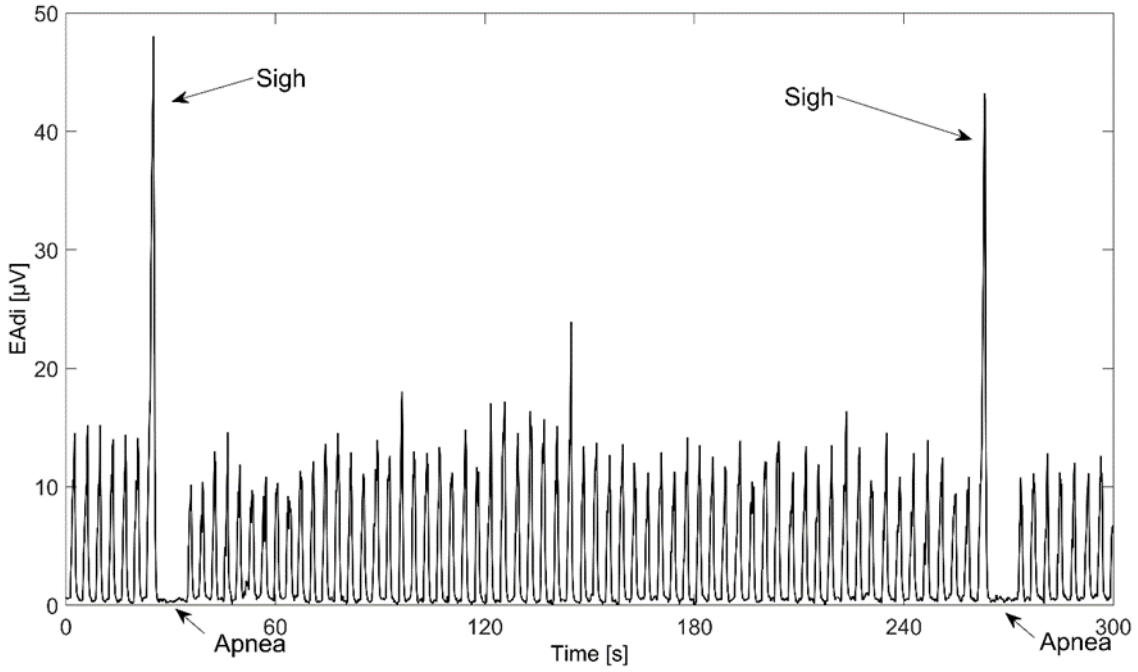
both premature and delayed cycling-off are reduced with NAVA [91, 96, 97].
As previously mentioned, a high degree of asynchrony is associated with prolonged mechanical ventilation [27], risk for unsuccessful weaning [28], increased need for sedation, disrupted sleep [30] and ultimately increased morbidity and mortality for ICU patients [27, 32]. Thus improved patient-ventilator synchrony has relevant clinical implications. In paediatric patients, better synchrony achieved with NAVA improves patient comfort [98, 99] and leads to lower sedative requirement [100]. In adults ventilated with NAVA, the quality and quantity of sleep appear to improve [101].
The EAdi signal itself has been used for monitoring and has improved the possibility to detect the presence of asynchrony bedside [102], while airway pressure and flow waveforms have low sensitivity in asynchrony detection [103]. Thus, EAdi monitoring may support the clinician in optimizing ventilator settings even in other modes of ventilation. An automated method to analyse breath by breath patient-ventilator interaction has recently been developed [104], however its use in daily practice is not yet evaluated [58].
In study II, we observed the pattern of breathing before and after placing additional dead space in the respiratory circuit in small species. No differences in tidal volume were observed between the two modes, while a shorter inspiratory time was observed in Pressure Support compared with NAVA, both before and after adding dead space. By adding dead space, a higher increase in respiratory rate and minute volume was seen in PS compared to NAVA, but reaching similar levels of $PaCO_2$. We believe that the worse efficiency in eliminating the CO_2 with PS was not due to differences in the inspiratory efforts, since the EAdi was similar with PS and NAVA, but it could be due to several other reasons. First, PS and NAVA differ in the way the assist is delivered. In PS the target pressure is reached early by providing high inspiratory flow, while in NAVA the assist is provided instantly in proportion to patient´s effort. Second, as described above, the inspiratory time was shorter in PS and a shorter inspiratory time has been associated with worse CO_2 exchange [105, 106]. Third, PS and NAVA differ in the cycling-off algorithms. In PS, in the present study, the cycle-off at 5% of the peak inspiratory flow appeared to be too early, compared to the neural cycle-off.
Although our study shows how extra dead space may challenge respiratory drive and determine different pattern of breathing, while ventilated with different support modes, however our findings in small species might not meet the response observed in premature babies.

NAVA as a mode of ventilation during anaesthesia and surgery

The third study focuses on NAVA as a potential mode of ventilation beyond the Intensive Care setting, into the operating theatre. Our study showed that NAVA is feasible in a big animal model during sedation and anaesthesia, with the commonly used anaesthetics

propofol and sevoflurane, suggesting the possibility to use NAVA even in patients in the operating room. There are numbers of surgical procedures that do not require muscle relaxation or high opioid doses and where the use of NAVA may be beneficial, keeping the diaphragm active and thus potentially reducing the intraoperative atelectasis formation and postoperative complications. However, in this work we did not study the potential effect of NAVA on reducing atelectasis formation and such aspects need to be further investigated.
In our short-term investigation in study III, we did not observe differences in ventilation and oxygenation and the EAdi was preserved with both anaesthetics, even when they were combined with low dose remifentanil (0.1μg/kg/min). Remifentanil in higher doses depressed the respiratory centre causing apnoea.
These findings regarding the anaesthetic effect on the EAdi are promising, but need to be confirmed in human trials.
In the version of NAVA present in the SERVO-i and SERVO-u ventilators, a backup mode is provided in case no EAdi signal is detected. Such a situation may occur either if the patient is apnoeic (due to increasing levels of opiate analgesia) or if the NAVA catheter is displaced or pulled out. The ventilator then provides Pressure Support followed by Pressure Control if no breathing attempts are sensed.
The use of the EAdi signal for continuous monitoring has been suggested when deep sedation is needed, while providing partial support [107]. Indeed, deep propofol sedation was shown to increase asynchrony in ICU patients during Pressure Support ventilation, but not with NAVA [107].
Some studies have previously demonstrated that a variable breathing pattern is beneficial when it comes to lung mechanics, oxygenation and ventilation distribution [108]: these authors have then artificially induced random variability in the breathing pattern by changing the PS level, in the so called Noisy Pressure Support. [109, 110]. In study III, NAVA applied to our animal model, maintained tidal volume variability similar to the natural variability observed in resting healthy individuals [111]. Such variability observed in NAVA has been associated with positive effects in gas exchange [83] and ventilation distribution [26]. In NAVA, tidal volume variability reflects the activity of the respiratory centre, while in Noisy PS it is artificially induced.
In study III, the Vt variability was maintained both with propofol and sevoflurane. With propofol the Vt variability was higher than with sevoflurane, due to a larger frequency of sighs (Fig. 18).

Figure 18. Sighs



Example of sighs in one pig during anaesthesia with propofol (paper III). The tracing shows the EAdi curve and two sighs during 5-min period. Each sigh is followed by an apnoeic period >5 s long.

In resting individuals, the frequency of sighs has been reported to be around 10 per hour [112]. In our animal study, the sigh frequency with sevoflurane was similar as in healthy individuals, while with propofol it increased to 30 per hour. We did not find any evidence that sighs had a lung-recruiting effect when observing the dynamic compliance of the breaths preceding and following the sighs. Some previous studies introduced artificial periodic sighs, while ventilating critically ill patients, in order to improve respiratory mechanics and gas exchange [113]. However in our study no oxygenation improvement was observed in relation to sighs, to support their physiologic purpose. Monitoring lung aeration with imaging techniques such as EIT might have provided more valuable information about end expiratory lung volume changes during the different study steps and in relation to sighs [114]. Neuro-Mechanical and Neuro-Ventilatory Efficiency were higher with sevoflurane than propofol, suggesting that muscle contractility may be better preserved with sevoflurane. Sevoflurane doses associated with negative inotropic effect on the diaphragm are well above the clinical recommended dose [115, 116]. Propofol on the other hand has been shown to partially depress muscle contractility when given at clinical concentrations [117-119].

Standardised NAVA titration during ventilator treatment

Study IV investigated the possibility to have a pragmatic and standardised approach to the abstract concept of the NAVA level, often felt difficult to set and hard to understand by the clinician bedside. We investigated an alternative possibility to set the assist in NAVA, according to predefined levels of diaphragm unloading, based on the Neuro-Ventilatory Efficiency. Up to now, different methods have been used to set the NAVA level, with some studies aiming at matching the peak airway pressure achieved in PS [86, 96]. Since EAdi varies from breath to breath and is under the influence of neural feedback, the pressure predicted for NAVA, according to the overlay window during PS, may not reflect the pressure achieved once ventilation in NAVA is started. Some other studies have focused on the inflection point identified during a titration manoeuver, performed by increasing stepwise the NAVA level, until airway pressure and tidal volume reach a plateau [76, 120]. This procedure is time-consuming and the inflection point is not always clear and may generate uncertainties bedside. Other researchers have titrated the NAVA level to a specific target EAdi [121].

A perhaps more pragmatic and quantitative approach, taking into account the proportions of respiratory work done by the patients respiratory muscles and by the ventilator, could be more intuitive for the clinician. A prerequisite to this approach is the integrity of the neural feedback loop in NAVA, which warrants a reflex reduction of EAdi activation when the assist is increased [56, 74, 122]. Setting the assist to target predefined unloading, based on the NVE, proved to be feasible (study IV). Zero assist manoeuvers are required to obtain NVE at regular intervals of time, in order to quantify and regularly recalibrate the level of unloading. We observed that some adjustments of the NAVA level were needed to keep the unloading constant. The measure of patient ventilator breath contribution (PVBC) during NAVA has been developed [72] and used in weaning patients [71]. In our study, a similar concept was used to instead quantify how much the ventilator unloads the respiratory muscles. Such an approach could be of use not only to set the ventilator in NAVA, but more generally as a tool to monitor muscle unloading during other support modes.

In our study, the PS level, unchanged from the clinical setting, was found to provide very high unloading in patients without lung injury, leading to a very low EAdi, sometimes almost completely suppressed. This is an interesting finding for clinical practice, where a tool could make the caregiver aware of the unloading associated with a certain level of ventilator support and individualize targets of unloading to specific patients.

These findings from study IV warrant further studies, investigating the long terms effects of moderate unloading in lung injured patients.

Distribution of ventilation – improved by reduced unloading?

In study IV, monitoring ventilation distribution by means of the Electrical Impedance Tomography (EIT), we observed that ventilation was more dorsally distributed when the assist was targeted to moderate levels of respiratory muscle unloading ($NAVA_{40\%}$) compared to higher unloading (PS_{cli} and $NAVA_{60\%}$). Distribution of ventilation was quantified with the Centre of Ventilation (CoV), previously shown to have high reproducibility [123]. Our finding indicates that for lower unloading, the diaphragm is more active, thereby shifting ventilation relatively more towards the dorsal regions of the lungs. Similar to our finding, another EIT study comparing NAVA and PS [26] observed that the CoV was located more dorsally for lower levels of assist in PS and NAVA. In the representation of the lung in Regions of Interest, the change in location of the CoV from PS_{cli} to $NAVA_{40\%}$ corresponded to a reduction in ventilation of the mid-ventral region and an increase in ventilation of the dorsal region. With this stated, the differences observed in our study are small and probably not so relevant from a clinical point of view. We believe that one reason that the differences in EIT measurements, gas exchange and physiologic parameters were small, could be ascribed to the fact that the patients enrolled in the study were not lung injured. Per study inclusion criteria, they required a relatively low F_IO_2, PEEP and had a low EAdi compared to acute respiratory failure patients ventilated in NAVA in other studies [83, 84, 124]. Studies aiming to investigate the effects of moderate unloading with NAVA in more lung injured patients are warranted in order to identify if greater differences and advantages are present.

FUTURE CLINICAL AND RESEARCH PERSPECTIVES

NAVA has been shown to improve the patient-ventilator interaction and to reduce the risk of over-assistance in animal and human studies, provided that respiratory centres and regulating reflexes are intact and pH is not too low. An approach considering the use of NAVA in ARDS patients, after the first 48h (during which controlled ventilation may be preferable), might be of interest as it may reduce the risk of Ventilator Induced Diaphragm Dysfunction. However, clinical trials investigating the long term use of NAVA in critically ill patients should be encouraged to investigate if the promising results observed in animal studies and in short-term human studies improve patient outcomes, compared to conventional lung protective strategies.

The application of EAdi monitoring in daily practice may be useful to optimize ventilator settings, in order to improve patient-ventilator interaction, not only when patients are ventilated with NAVA, but even with other modes of ventilation. Systems providing online breath by breath analysis of the relationship between neural and mechanical cycles may be a useful tool for the clinician bedside.

Furthermore, the EAdi signal may be of interest as an on-line monitor for patients requiring deep sedation, while ventilated with assisted modes of ventilation. A very low or suppressed EAdi signal might make care-providers more aware of excessive sedation and serve as a warning signal for reduction of sedative doses.

The use of NAVA for surgical patients in the operating room needs to be investigated. By keeping the diaphragm active, NAVA has the potential to reduce atelectasis formation already at anaesthesia induction and such beneficial effect may extend even to the postoperative period, reducing the incidence of respiratory complications. NAVA could be used during surgical procedures that do not require high opioid doses or muscle relaxation, or with neuroaxial blockades as a good alternative to preserve respiratory drive. Furthermore, studies comparing NAVA to other modes of ventilator support during surgical procedures should also be subject of investigation in order to investigate if there are specific advantages with NAVA intraoperatively and in the postoperative period.

Human trials assessing the anaesthetic effects on the EAdi and on neuromuscular coupling during NAVA are also warranted in order to identify potential clinically relevant differences among them. Long-term effects of propofol and sevoflurane, when used for ICU sedation in NAVA may be of clinical interest. The tidal volume variability and the effects of sighs on lung

recruitment and on gas exchange during NAVA should be investigated by means of lung imaging techniques in humans and with a longer observation time than in the studies in this thesis.

Using the NVE and unloading indices to determine respiratory unloading may be a useful approach for the clinician, not only as a guide while setting the ventilator in NAVA, but also to monitor diaphragm unloading and avoiding EAdi suppression with other support modes.

CONCLUSIONS

1. With increasing levels of assist, Neurally Adjusted Ventilatory Assist maintains patient-ventilator synchrony and unloads the diaphragm at lower levels of applied pressure and volume compared to Pressure Support.

2. NAVA is feasible and efficacious in small species, close in weight to the smallest viable human being, maintaining oxygenation and ventilation in the physiologic range.

3. The addition of dead space in the respiratory circuit in small species leads to lower increase in breathing frequency and minute ventilation with NAVA compared to Pressure Support, indicating a more efficient elimination of CO_2 with NAVA.

4. NAVA is feasible during sedation and general anaesthesia with sevoflurane and propofol, even when combined with a low dose remifentanil, in a big animal model.

5. The tidal voume variability is higher with propofol than sevoflurane, due to more frequent sighs followed by post-sigh apnoea. Sevoflurane maintains Neuro-mechanical and Neuro-Ventilatory Efficiency better than propofol, suggesting better preserved muscle contractility with sevoflurane.

6. In NAVA the assist can be targeted to different levels of respiratory muscle unloading, by titrating the assist, using Neuro-Ventilatory Efficiency-based unloading.

7. Reduced NAVA unloading, targeted with NVE, redistributes ventilation towards the dorsal regions of the lungs.

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