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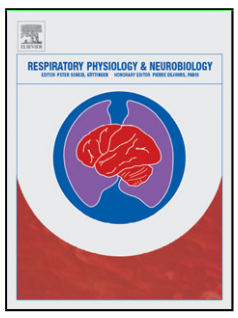

Title: NEURAL CONTROL OF VENTILATION PREVENTS BOTH OVER-DISTENSION AND DE-RECRUITMENT OF EXPERIMENTALLY INJURED LUNGS

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NEURAL CONTROL OF VENTILATION PREVENTS BOTH OVER-DISTENSION AND DE-RECRUITMENT OF EXPERIMENTALLY INJURED LUNGS

Running title: Continuous neurally adjusted ventilatory assist (cNAVA)

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Highlights

Scientific Knowledge on the Subject:

The Hering-Breuer inflation and deflation reflexes, discovered over 140 years ago, limit the volume of inspiration (so called Hering-Breuer inflation reflex) and prevent de-recruitment of the lung during exhalation (Hering-Breuer deflation reflex). We reasoned that if these vagally-mediated reflexes were permitted to guide mechanical ventilation by adjusting the positive airway pressure delivered during in- and expiration using a neurally driven pressure delivery device (i.e. continuous neurally adjusted ventilatory assist, cNAVA), their functional impact on lung recruitment and volume delivery could be studied.

What This Study Adds to the Field:

When using cNAVA in early experimental lung injury, vagally mediated reflexes a) inhibit the neural inspiratory activity of the diaphragm (i.e. the signal controlling both, the respiratory muscles and the ventilator) such that lung-distending pressure is limited during inspiration, and b) increase the neural activity of the diaphragm during exhalation such that lung de-recruitment is prevented. This study presents the first in situ and in vivo model to clarify neurally mediated lung protective mechanisms during application of mechanical ventilation before and after ALI and highlights the importance of functioning vagal lung reflexes and spontaneous breathing during mechanical ventilation.

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Abstract

Background: Endogenous pulmonary reflexes may protect the lungs during mechanical ventilation. We aimed to assess integration of continuous neurally adjusted ventilatory assist (cNAVA), delivering assist in proportion to diaphragm's electrical activity during inspiration and expiration, and Hering-Breuer inflation and deflation reflexes on lung recruitment, distension, and aeration before and after acute lung injury (ALI).

Methods: In 7 anesthetised rabbits with bilateral pneumothoraces, we identified adequate cNAVA level ($cNAVA_{AL}$) at the plateau in peak ventilator pressure during titration procedures before (healthy lungs with endotracheal tube, [HL_{ETT}]) and after ALI (endotracheal tube [ALI_{ETT}] and during non-invasive ventilation [ALI_{NIV}]). Following titration, $cNAVA_{AL}$ was maintained for 5 minutes. In 2 rabbits, procedures were repeated after vagotomy ($ALI_{ETT+VAG}$). In 3 rabbits delivery of assist was temporarily modulated to provide assist on inspiration only. Computed tomography was performed before intubation, before ALI, during cNAVA titration, and after maintenance at $cNAVA_{AL}$.

Results: During ALI_{ETT} and ALI_{NIV}, normally aerated lung-regions doubled and poorly aerated lung-regions decreased to less than a third ($p<0.05$) compared to HL_{ETT}; no over-distension was observed. Tidal volumes were <5ml/kg throughout. Removing assist during expiration resulted in lung de-recruitment during ALI_{ETT}, but not during ALI_{NIV}. During $ALI_{ETT+VAG}$ the expiratory portion of EAdi disappeared, resulting in cyclic lung collapse and recruitment.

Conclusions: When using cNAVA in ALI, vagally mediated reflexes regulated lung recruitment preventing both lung over-distension and atelectasis. During non-invasive cNAVA the upper

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airway muscles play a role in preventing atelectasis. Future studies should be performed to compare these findings with conventional lung-protective approaches.

Abbreviation list

ABG	arterial blood gas
ALI	acute lung injury
ALI_{ETT}	acute lung injury with endotracheal tube
$ALI_{ETT+VAG}$	acute lung injury with endotracheal tube and vagotomy
ALI_{NIV}	acute lung injury with non-invasive ventilation
Bf	breathing frequency
cNAVA	continuous neurally adjusted ventilatory assist
$cNAVA_{AL}$	adequate level of cNAVA
CT	computed tomography
EAdi	electrical activity of the diaphragm
EELV	end-expiratory lung volume
HL_{CTRL}	healthy lung control
HL_{ETT}	healthy lung with endotracheal tube
PEEP	positive end-expiratory pressure
Pvent	ventilator pressure
VCV	volume controlled ventilation

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VILI ventilator induced lung injury

Vt tidal volume

Keywords: acute respiratory failure, mechanical ventilation, diaphragm electrical activity, neural control of breathing, CT scan

Part of the results have been presented in abstract format at the annual meetings of the American Thoracic Society (ATS) in San Francisco, USA, May 18-23, 2007 and of the Swiss Society of Intensive Care Medicine (SGI) in Interlaken, Switzerland, November 1-3, 2007.

Introduction

Continuous neurally adjusted ventilatory assist (cNAVA) is a new mode of mechanical ventilation. With cNAVA the electrical activity of the diaphragm (EAdi), reflecting the patient's neural respiratory drive, multiplied by an amplification factor (cNAVA level) is used to guide delivery of ventilatory pressure (Pvent) throughout the respiratory cycle, i.e. during inspiration *and* expiration. Hence, with cNAVA the patient's respiratory drive controls both, lung distension during inspiration and end-expiratory lung volume (EELV).

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In 1868, Hering and Breuer described two reflexes, which could potentially play a role in lung protection during cNAVA: the "inspiratory-inhibiting reflex" and the "deflation-sensitive reflex"[1]. These vagally-mediated reflexes sense lung stretch (and terminate inspiration), and lung deflation (and stimulate inspiration).[2] Experimental and clinical studies suggest that intrinsic lung-protective reflexes are involved when using ventilator modes for which the subject's neural respiratory drive controls timing and magnitude of assist.[3-11] Generally, the key findings pertain to a) down regulation of neural respiratory drive (reflected by EAdi) preventing increases in Pvent and tidal volume (Vt) when the proportionality constant (NAVA level) increases, b) presence of tonic EAdi activity throughout expiration in absence of positive end-expiratory pressure (PEEP), assumed to prevent lung-de-recruitment. However, no studies have specifically addressed if or how lung afferent reflexes may modulate neural respiratory drive to protect the lungs against over-distension and de-recruitment in acute lung injury (ALI).

We used a rabbit model with open thorax and bilateral pneumothoraces. This model removed the contribution of the respiratory muscles and chest wall to lung inflation and deflation and prevented receptors sensing changes in lung volumes associated with the magnitude of ventilatory assist. Thus, feedback control of neural respiratory drive relied mainly on vagally mediated reflexes from the lungs.

We hypothesized that systematically increasing the cNAVA level from zero to high levels and performing computed tomography (CT) would allow us to observe the reflex-regulation of lung recruitment. Specifically, we assessed the effect of the Hering-Breuer inflation and deflation

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reflexes on lung recruitment, distension and aeration with cNAVA before and after ALI. We further hypothesized that temporarily removing ventilator assist during expiration would allow evaluating whether the animals maintain lung recruitment using either so-called "tonic EAdi" when intubated or expiratory flow limitation generated by the upper airways when non-invasively ventilated. Vagotomy served to confirm that the involved reflexes were vagally mediated.

Some of the results have been previously reported in abstract form.[12]

Methods

The study was performed according to the National Institutes of Health guidelines for the use of experimental animals and was approved by the University of Uppsala. Seven adult male New Zealand white rabbits weighing 3.1–3.9kg were studied.

Sedation and instrumentation

Bilateral cannulation of the ear veins and arteries was performed. Continuous sedation (ketamine-hydrochloride 40mg/kg·h^{-1}, xylazine 4mg/kg·h^{-1}) and Ringer's lactate solution (5ml/kg·h^{-1}) was infused. Transcutaneous oxygen saturation, arterial blood pressure, body temperature, and heart rate were monitored. EAdi was assessed with an 8F oro-gastric catheter mounted with an array of 8 electrode pairs. Rabbits were ventilated in volume control ventilation (VCV) mode at 6 ml/kg with PEEP of 5 cmH_2O (Servo-I, Maquet Critical Care, Solna, Sweden).

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cNAVA

cNAVA was performed with a custom made device (NeuroVent R&D Inc., Toronto, Canada) that delivers pressure in proportion to the EAdi waveform throughout inspiration and expiration as previously described.[13,14] The proportionality between pressure delivered and EAdi was adjusted by multiplying the EAdi by a gain factor, i.e. the cNAVA level (in cm $H_2O/\mu V$).

cNAVA titration

For cNAVA titration, performed at various time-points (Figure 1), cNAVA level commenced at zero and was increased every 2 seconds by 0.08cm$H_2O/\mu V$ (during non-invasive cNAVA increments were 0.32cm$H_2O/\mu V$). The trend graphs for Pvent displayed a characteristic two-phased response, i.e. a transition from an initial steep increase to a less steep increase. Adequate cNAVA level ($cNAVA_{AL}$) was visually identified on Pvent trend graphs at the time-point where a plateau in peak ventilator pressure ($Pvent_{max}$) was reached.[7,15-17] On completion of titration, ventilation continued for 5 minutes at $cNAVA_{AL}$.

Computed tomography

Spiral CT images were acquired with a SOMATOM Sensation 16 (Siemens, Erlangen, Germany) with the following settings: 120kVp, 147mA, exposure time 500ms, 3mm slice collimination resulting in 33-39 single scans per spiral with a matrix of 512x512 voxels depending on lung dimensions.

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Protocol (Figure 1)

A spiral CT scan was obtained with animals in supine position, sedated and spontaneously breathing, before intubation and induction of acute lung injury (ALI), (healthy lung control, HL_{CTRL}).

Then animals were orally intubated (cuffed endotracheal tube (ETT), 3.5 I.D., Willy-Rusch GmbH, Germany), the EAdi catheter was inserted, midline sternotomy and distention of the sternum was performed and bilateral pneumothoraces were induced. Spiral CT scans were performed during cNAVA titration, and after ventilation at $cNAVA_{AL}$ for 5 minutes (HL_{ETT}).

Then, acute lung injury (ALI) was induced. After neuromuscular paralysis (pancuronium-bromide 0.02 mg/kg) in VCV mode (6 ml/kg, PEEP=5-7 cmH_2O), 2 mL/kg hydrochloric acid (pH 1.5) was instilled into the trachea, followed by an inspiratory hold at 25 cmH_2O for ten seconds. The procedure was repeated after five minutes. After another 10 minutes on VCV, an ABG was obtained. When spontaneous breathing was restored, spiral CT scans were performed during cNAVA titration, and after 5 minutes at $cNAVA_{AL}$ (ALI_{ETT}).

Then, the oral ETT was removed and assist was delivered non-invasively using a single nasal prong (3.0 or 3.5 I.D., Blue-line, Portex, Kent, UK). Spiral CT scans were performed during cNAVA titration, and after 5 minutes of $cNAVA_{AL}$ (ALI_{NIV}).

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Removing assist during expiration

In three animals, immediately following maintenance of $cNAVA_{AL}$ for 5 minutes we temporarily modulated assist, such that no pressure was applied during expiration (assist on inspiration only). This procedure was performed during both ALI_{ETT} and $ALI_{NIV.}$ Continuous CT scans of the same lung slice (static CT scans) were performed.

Vagotomy

In two animals, tracheostomy was performed (4.0 I.D., Mallinckrodt Inc., St. Louis, USA) followed by bilateral vagotomy (VAG) at the neck level to eliminate vagal reflexes.[15,18] Absence of the Hering-Breuer reflex was determined by distinct slowing of the respiratory rate.[18] This part of the protocol was performed to confirm previous work showing that removal of vagal feedback after ALI in rabbits is associated with abolishment of tonic EAdi during expiration and hence cyclic lung de-recruitment. CT scans were performed during cNAVA titration and after 5 minutes at $cNAVA_{AL}$ ($ALI_{ETT+VAG}$).

Data acquisition and analysis

A single investigator blinded to experimental conditions analyzed all CT images (Maluna 2.04, University of Göttingen, Germany) as previously described [19-21]. Using standard thresholds for Hounsfield units [20,22] we defined four degrees of aeration. Lung volume was calculated as the sum of gas volumes of all voxels that made up the lung volume in the spiral CT.

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EAdi was acquired at a sampling rate of 2kHz. Pvent and airflow were measured at the proximal end of either the ETT or the single nasal prong, and acquired at 100Hz. The EAdi and Pvent waveforms were characterized by the highest ($EAdi_{max}$ and $Pvent_{max}$) and lowest ($EAdi_{min}$ and $Pvent_{min}$) for each breath. Neural breathing frequency (Bf_{neural}) was calculated from the EAdi waveform. During the 5 minutes of $cNAVA_{AL}$, average values of these parameters were calculated in the final minute.

Statistical analysis

Statistical analysis was performed with SigmaStat™ (3.11, Systat Software Inc., San Jose, CA). We used Kolmogorov-Smirnov tests to assess data distribution. Paired data were compared with t-test or Wilcoxon Signed Rank test. HU distribution frequencies summed for the four attenuation compartments were compared between experimental conditions using Wilcoxon Signed Rank test. Level of significance was $p<0.05$.

Results

All animals survived the protocol. Heart rate remained stable, systolic and diastolic blood pressures at experiment completion were 77±5 and 55±5mmHg, respectively. ABG and breathing pattern values are given in Table 1. Ten minutes after induction of ALI, PaO_2/FiO_2 was reduced to 92±26 ($p<0.05$ vs HL_{ETT}). After 5 minutes at $cNAVA_{AL,}$ Vt was slightly different when comparing HL_{ETT} and ALI_{ETT} ($p=0.022$) (0.9ml/kg difference). Bf_{neural} increased after ALI ($p<0.001$).

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PaO_2/FiO_2 was lower during HL_{ETT} and ALI_{NIV} compared to ALI_{ETT} ($p<0.05$), and $PaCO_2$ was higher during all conditions compared to HL_{CTRL} ($p<0.05$).

Figure 2 shows the response of one intubated rabbit with ALI (ALI_{ETT} condition) to cNAVA titration. A video sequence of the same titration procedure is provided in the online supplement (Video 1).

Figure 3 shows group mean responses of $EAdi_{max}$ and $EAdi_{min}$ (Panel A) as well as $Pvent_{max}$ and $Pvent_{min}$ (Panel B) during cNAVA titration and after 5 minutes at $cNAVA_{AL}$. During HL_{ETT}, ALI_{ETT}, and ALI_{NIV}, increasing cNAVA levels reduced $EAdi_{max}$ and $EAdi_{min}$ whereas $Pvent_{max}$ and $Pvent_{min}$ increased initially and then plateaued. After five minutes at $cNAVA_{AL}$ there were further decreases in $EAdi_{max}$ and $EAdi_{min}$ as well as $Pvent_{max}$ and $Pvent_{min}$, especially during ALI_{NIV}.

As depicted in Figure 4 and Table 2, lung aeration distribution after 5 minutes of $cNAVA_{AL}$ was similar for HL_{CTRL} and HL_{ETT}, comprising poor aeration (about 60% of lung tissue), normal aeration (about 30%), and atelectatic lung tissue (about 10%). Lung volumes were not different during HL_{CTRL} and HL_{ETT} (47.1±10.5 ml and 38.2±6.5 ml, respectively) (Table 2).

Following ALI and regardless of interface (ALI_{ETT} or ALI_{NIV}), lung-regions classified as “normally aerated” increased to twice that observed during HL_{CTRL} ($p<0.05$) and HL_{ETT} ($p<0.05$) after 5 minutes of $cNAVA_{AL}$ (Figure 4). The contribution of lung regions classified as “poorly aerated”

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decreased to less than a third of the values observed during HL_{CTRL} ($p<0.05$) and HL_{ETT} ($p<0.05$). Lung regions classified as atelectatic were not different from HL_{CTRL} and HL_{ETT}. Lung volume increased significantly to 77.8±16.7ml and 65.3±17.5ml during ALI_{ETT} and ALI_{NIV} compared to both HL_{CTRL} ($p<0.001$, $p=0.02$) and HL_{ETT} ($p<0.001$, $p=0.014$).

Removing assist during expiration

Removal of expiratory assist with ALI_{ETT}, resulted in lung de-recruitment, increased $EAdi_{min}$, and irregular $EAdi_{max}$ (Figure 5 and e-Figures 1 and 2). When expiratory assist was removed during ALI_{NIV}, two rabbits could maintain lungs fully recruited, with no change in $EAdi_{min}$ and a stable $EAdi_{max}$ and Bf_{neural} (Figure 5 and e-Figure 1). One rabbit maintained the same lung-recruitment during ALI_{NIV} as during ALI_{ETT} but with half the $EAdi_{min}$ and irregular $EAdi_{max}$ (e-Figure 2).

Vagotomy

Bilateral vagotomy ($ALI_{ETT+VAG}$) abolished $EAdi_{min}$ and consequently $Pvent_{min}$ compared to pre-vagotomy (ALI_{ETT}) (Figure 6 and e-Figure 3). When increasing cNAVA levels during $ALI_{ETT+VAG}$, the decrease of $EAdi_{max}$ was delayed (compared to ALI_{ETT}) causing $Pvent_{max}$ to over-shoot before reaching a plateau. (Figure 6, panel B, bottom right and e-Figure 3). After 5 minutes at $cNAVA_{AL}$ lungs were noticeably de-recruited (Panel C in Figure 6 and e-Figure 3). Lung volume decreased from 88.1ml and 79.9ml during ALI_{ETT} to 40.6ml and 45.8ml during $ALI_{ETT+VAG}$. Bf_{neural} decreased from 73bpm and 69bpm to 15bpm and 16bpm, respectively.

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Augmented neural inspiratory efforts (sighs) could be observed (Figure 6 and e-Figure 3). During cNAVA titration, sighs were observed in 4 of 7 rabbits during HL_{ETT} and in 6 of 7 rabbits during ALI_{ETT}. No sighs were observed in vagotomized rabbits.

Figure 7 depicts CT-images for inspiration and expiration in one animal during HL_{ETT}, ALI_{ETT}, ALI_{NIV}, and $ALI_{ETT+VAG}$. There is marked lung recruitment from HL_{ETT} to both, ALI_{ETT} and ALI_{NIV}. $ALI_{ETT+VAG}$ resulted in lung de-recruitment and induced cycling between lung-collapse and recruitment.

Discussion

Our study is unique and the first to demonstrate the functional impact of respiratory drive assessed as EAdi upon lung aeration, recruitment, and volume. We found that during ALI inherent, vagally-mediated reflexes favor a neural breathing pattern that promotes both lung aeration without over-distension and preservation of EELV.

More specifically, by using a ventilator that is neurally-controlled during inspiration *and* expiration, a model uncoupling the lungs from the respiratory muscles and chest wall, and sequential CT scans, we demonstrated that reflex responses to early experimental ALI involve: 1) an increase in diaphragm tonic activity which preserves EELV and prevents lung de-recruitment; and 2) inhibition of peak inspiratory EAdi, thus limiting lung-distending pressure and preventing over-distension. Interestingly, despite these reflex changes, Vt after ALI remained similar or lower than before ALI. Bilateral vagotomy abolished these responses,

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supporting that the above-described protective mechanisms were mediated by vagal afferents. We also demonstrated that during non-invasive ventilation, lung de-recruitment is prevented, presumably via expiratory flow limitation by upper airway muscles.

Preservation of EELV

HCL-induced ALI promotes heterogeneous injury and de-recruitment of the lungs.[23] During spontaneous breathing, maintenance of diaphragm activation during exhalation is a fundamental mechanism to prevent lung de-recruitment.[24,25] Previous work in both intubated rabbits with experimental ALI and in intubated children shows that tonic EAdi increases when PEEP is reduced.[3,26] In the present study, $EAdi_{min}$ markedly increased when lungs collapsed after ALI at the start of titration (cNAVA levels = zero). $EAdi_{min}$ then continuously decreased and lungs were recruited as $Pvent_{min}$ (i.e. minimal pressure during exhalation restoring EELV) increased following continuously increasing cNAVA levels.

Instantaneous appearance of tonic EAdi induced by lung de-recruitment after removing assist during “neural expiration” provided evidence that tonic EAdi is reflex governed. Furthermore, loss of tonic EAdi after bilateral vagotomy resulting in cyclic expiratory collapse and inspiratory recruitment (an extremely injurious pattern of ventilation[27]) support that the reflex maintaining EELV ~~is~~ was vagally mediated. The presence of tonic activity after ALI and its disappearance after bilateral vagotomy has been previously demonstrated in rabbits with ALI [3,15] and assumed to affect lung-recruitment.[26] The present study provides demonstrations in two rabbits how typical neural drive response to eliminated Hering-Breuer reflex influences lung-recruitment

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and breathing pattern. It should be noted that there was no possibility to control for non-lung effects of bilateral vagotomy at the neck.

Modulation of respiratory rate and expiratory time can also promote increases in EELV.[28] The significant increase of respiratory rate we observed after ALI may have contributed to lung recruitment. Additionally, the impact of sighs on lung recruitment observed in our study is in agreement with previous observations in ARDS and ALI.[29]

Another possible mechanism for preventing lung deflation is upper airway muscles involvement, which may be activated dynamically during exhalation to brake expiratory flow.[30] Beck et al previously demonstrated in rabbits with ALI that tonic EAdi disappeared when the endotracheal tube was removed.[31] In the present study we demonstrated that lungs remained fully recruited without appearance of tonic EAdi when expiratory assist was removed during ALI_{NIV}. We hypothesize that the deflation-sensitive Hering-Breuer reflex was activated when the upper airway muscles were "blocked" by intubation and inactivated when upper airway modulation was present.

Protection from lung over-distension

We also found reflex-prevention of lung over-distension during cNAVA titration (with intact vagal nerves). $Pvent_{max}$ reached a plateau despite continued increase in cNAVA levels during both ALI_{ETT} and ALI_{NIV} cNAVA. Similar findings of EAdi down-regulation and lung-distending

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"pressure-ceiling" have previously been reported for transpulmonary pressures in rabbits with ALI[3] and during loaded breathing.[15] Several patient studies demonstrated plateaus in Vt, Pvent, or transpulmonary pressure during increasing NAVA levels associated with EAdi suppression.[7,9,10,16,17,33,34]

Our observation of a more favourable distribution of aeration with less atelectatic regions after ALI compared to normal lungs (i.e. HCL induced lung injury improved pattern of lung aeration in cNAVA ventilated rabbits) agree with findings that HCL-induced ALI induces heterogeneous injury and de-recruitment of the lungs.[23] This is in stark contrast to healthy homogenous lungs which are easily recruited.[35] Our findings of a major shift to normally aerated and not over-distended lung-regions after ALI is in agreement with lung aeration observed using current clinical standards e.g. so-called open-lung concept using assist-controlled ventilation at 6ml/kg predicted body weight with high PEEP (e.g. PEEP set at 2 cmH_2O above lower inflection point of pressure-volume curve).[36]

Method critique

To demonstrate lung-protective reflexes, muscle force, fatigue, and feedback should not be interfering factors. In our model, the respiratory muscles and chest wall mechanics played no role in ventilation, nor in lung recruitment. Using cNAVA we replaced the respiratory muscles with a "non-fatigable" neurally driven pressure delivery device that is efficient in generating pressure regardless of lung-volumes. Thus, any influence of respiratory muscle activity (e.g. weakness due to hyperinflation) was abolished. Additionally, while maintaining the efferent

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neural activation to the respiratory muscles, an open thorax and exposed lungs eliminated the passive outward recoil of the ribcage and diaphragm, which governs the FRC. EAdi allowed the diaphragm to contract, however its ability to generate pressure and distend the lungs was disabled. Also, the afferent feedback from the respiratory muscles was abolished due to inability to sense changes in ventilatory assist and lung volumes.

In our open chest model, pressure delivered with cNAVA closely reflected transpulmonary pressure (except when cNAVA was delivered non-invasively due to large pressure loss associated with leaks). Hence, the ventilator replaced the mechanical "pump-function" of the "disabled" respiratory muscles. cNAVA was controlled by EAdi, which responds to the same neural output as the respiratory muscles when functioning.

Our study has some limitations. Animals were unattended in the CT scanner, and thus may have received more sedation than usual. This may explain the higher $PaCO_2$ levels in our study compared to other studies[5,31] and the poorly aerated lungs before ALI.[37] It should be noted that the "open chest model" eliminates the "normal" intrapleural negative pressure recruitment of the lungs seen with an intact thorax, which could have influenced blood gases. However, despite the deep sedation level and open chest model, a strong lung-protective response could still be demonstrated after ALI.

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Conclusion

This is the first study using an in vivo model to describe and quantify reflex-mediated regulation of lung recruitment and Vt in response to experimental ALI. The reflex-mediated response was characterized by remarkable and homogenous lung-recruitment, prevention of atelectasis, and breathing with low Vt without over-distending the lungs. Our data also provide evidence that, after extubation, the upper airway muscles play a direct role in preventing atelectasis during non-invasive ventilation. Future studies should address if integration of ventilatory assist with neural reflexes may act to prevent VILI in a similar fashion to conventional lung-protective approaches, which aim to maintain an open lung and minimize Vt.

Authors contributions: Conception and design: LB, OM, GH, JB, AS, CS; Performing the experimental work: LB, OM, GH, CS; Analysis and interpretation: LB, OM, GH, JB, JT, AS, CS; Drafting the manuscript for important intellectual content: LB, OM, GH, JB, JT, AS, CS

Disclaimer

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Dr Hedenstierna: The Hedenstierna Research Laboratory has financial support by Maquet Critical Care AB, however, has no influence on the research being conducted at the laboratory.

Drs Beck and Sinderby have made inventions related to neural control of mechanical ventilation that are patented. The license for these patents belongs to Maquet Critical Care. Future commercial uses of this technology may provide financial benefit to Drs Beck and Sinderby through royalties. Drs Beck and Sinderby each own 50% of Neurovent Research Inc. Neurovent Research is a research and development company that builds the equipment and catheters for research studies. Neurovent Research has a consulting agreement with Maquet Critical Care.

Dr Slutsky consults for Maquet Critical Care, and is compensated for this work.

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Legends to Figures in the main text

Figure 1. Steps performed in the protocol

From top to bottom: Order of the protocol in seven sedated animals undergoing instrumentation, intubation, and thoracotomy (before and after acute lung injury), extubation and non-invasive ventilation. At each protocol step, animals underwent CT scans during cNAVA level titrations. HL_{CTRL} = healthy lung control; HL_{ETT} = healthy lung with endo-tracheal tube; ALI_{ETT} = after acute lung injury with endotracheal tube; ALI_{NIV} = after acute lung injury and non-invasive ventilation with nasal prong; $ALI_{ETT+VAG}$ = after acute lung injury with endotracheal tube and bilateral vagotomy. $cNAVA_{AL}$ = adequate cNAVA level (see Methods for description).

Figure 2. cNAVA level titration in an animal after acute lung injury (ALI_{ETT}).

Panel A: Top to bottom: Values for lung volume, electrical activity of the diaphragm (EAdi), and pressure delivered by the ventilator (Pvent) are plotted versus cNAVA level and time.

Panel B: Each CT-scan image in panel B corresponds to the times indicated by shaded columns 1-6 in panel A. The shaded columns denoted 4a-b and 5a-b in panel A and the corresponding CT-scans in panel B demonstrate the effect of spontaneous sighs on lung recruitment.

At cNAVA = zero, Pvent and volume were zero, EAdi was high with relatively small, breath–related swings that never returned to zero (so-called “tonic EAdi”) (Panel A) and the lungs were collapsed (Panel B, image 1). Linear increases of the cNAVA level initially increased Pvent and lung volumes as well as their swings. EAdi decreased and breath-related EAdi swings (so called “phasic EAdi”) appeared, resulting in lung recruitment (Panel B, images 2-4).

After about 40 seconds, both tonic and phasic EAdi decreased with increasing cNAVA level. Pvent and breath-related pressure swings plateaued and remained unchanged despite an almost threefold continued increase in the cNAVA level. Volume plateaued but increased in a stepwise fashion after spontaneous sighs by the animal. Spontaneous sighs produced significant increases in lung-recruitment (Panel B, images 4a-b and 5a-b) until the lungs occupied almost the entire thoracic cavity (Panel B, image 6).

A video sequence of the same titration procedure is provided in the online supplement (Video 1).

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Figure 3. Group mean responses of electrical activity of the diaphragm (EAdi), and pressure delivered by the ventilator (Pvent) during cNAVA titrations.

Responses of $EAdi_{max}$ (solid symbols) and $EAdi_{min}$ (empty symbols) (Panel A) as well as $Pvent_{max}$ (solid symbols) and $Pvent_{min}$ (empty symbols) (Panel B) during linearly increasing cNAVA levels and after 5 minutes of ventilation at $cNAVA_{AL}$.

From top to bottom, group mean values obtained with i) healthy lungs and orally inserted endotracheal tube (ETT) (HL_{ETT}), ii) ALI with orally inserted ET-tube (ALI_{ETT}), and iii) ALI with non-invasive ventilation (ALI_{NIV}).

Values presented are mean±SEM. All comparisons at 5 minutes versus inflection point are significant, $p<0.05$, unless indicated: NS = no significant difference from $cNAVA_{AL}$ compared to inflection point value.

Figure 4. Distribution of lung aeration after 5 minutes of ventilation at the adequate cNAVA level ($cNAVA_{AL}$)

Frequency distribution of lung aeration during: i) healthy lungs before intubation (HL_{CTRL}), ii) healthy lungs and orally inserted endotracheal tube (ETT) (HL_{ETT}), iii) ALI with orally inserted ET-tube (ALI_{ETT}), and iv) ALI with non-invasive ventilation (ALI_{NIV}). Aeration listed at top. Data presented for group mean with SD.

Figure 5. CT-images and continuous time plots of electrical activity of the diaphragm (EAdi), and pressure delivered by the ventilator (Pvent) in one animal when expiratory assist is removed

During intubated conditions (ALI_{ETT}), removal of expiratory assist (vertical arrow), resulted in elevated tonic EAdi, with minimally discernable phasic EAdi. Corresponding CT image indicates lung de-recruitment. When ventilated non-invasively (ALI_{NIV}), removal of expiratory assist did not result in lung de-recruitment, and tonic EAdi did not increase, indicating that the upper airway muscles maintained the lungs recruited. See also e-Figures 1 and 2.

Figure 6. Responses of electrical activity of the diaphragm (EAdi), and pressure delivered by the ventilator (Pvent) during cNAVA titration and lung aeration in one animal with bilateral vagotomy

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Responses of $EAdi_{max}$ and $EAdi_{min}$ (Panel A) as well as $Pvent_{max}$ and $Pvent_{min}$ (Panel B) in one rabbit during linearly increasing cNAVA level and after 5 minutes of ventilation at $cNAVA_{AL}$. Panel C shows distribution of lung aeration after 5 minutes of ventilation at $cNAVA_{AL.}$ Top panels show values obtained during ALI with orally inserted ET-tube (ALI_{ETT}) and bottom panels show values obtained during ALI and bilateral vagotomy with ET-tube via tracheostomy ($ALI_{ETT+VAG}$). See also e-Figure 3.

Figure 7. CT-images obtained in one animal under different experimental conditions

CT-images for inspiration and expiration in one animal during A) healthy lungs and orally inserted ET-tube (HL_{ETT}), B) ALI with orally inserted ET-tube (ALI_{ETT}), C) ALI with non-invasive ventilation (ALI_{NIV}) and D) ALI and bilateral vagotomy with ET-tube via tracheostomy ($ALI_{ETT+VAG}$).

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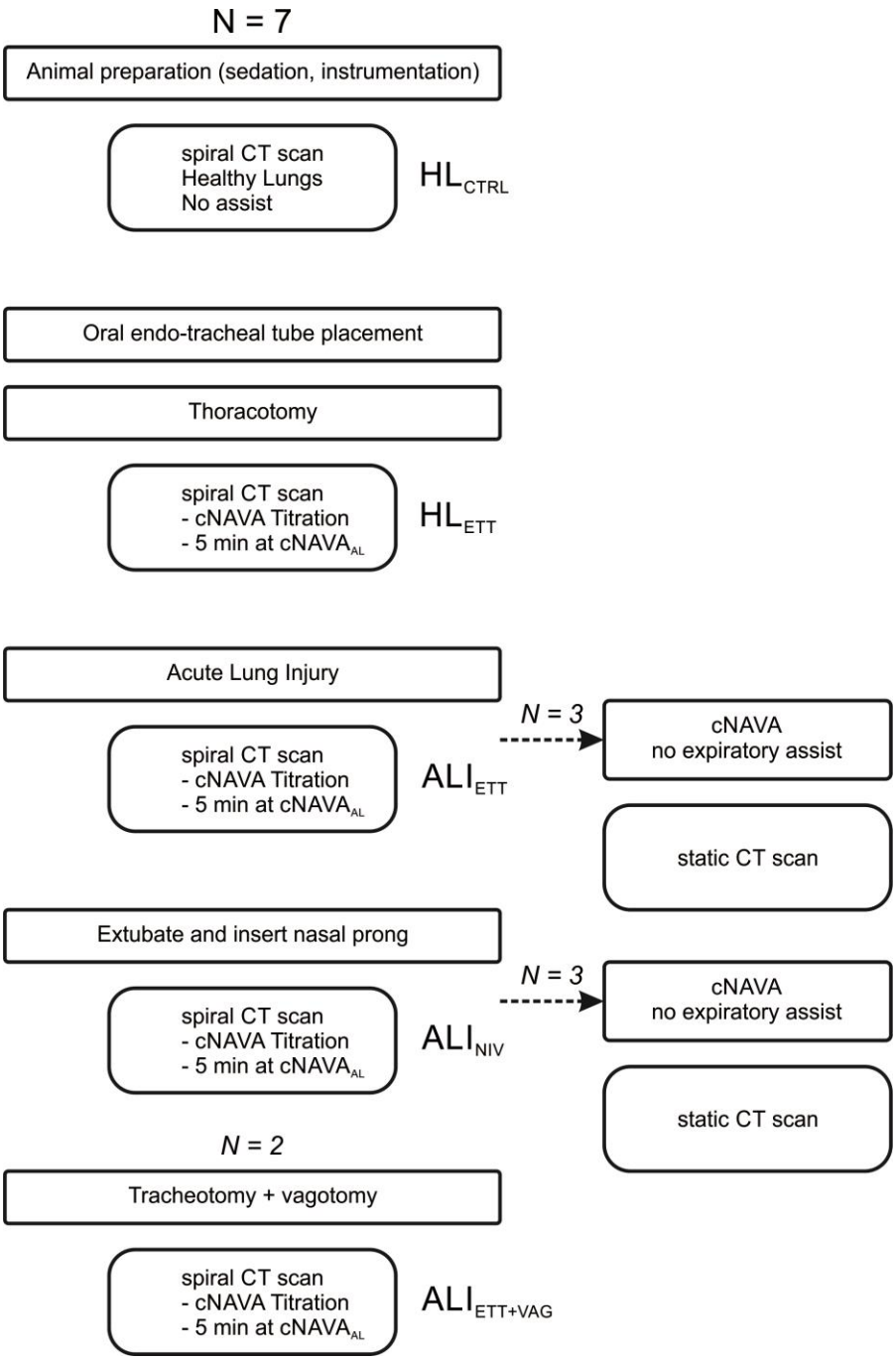
Fig.1



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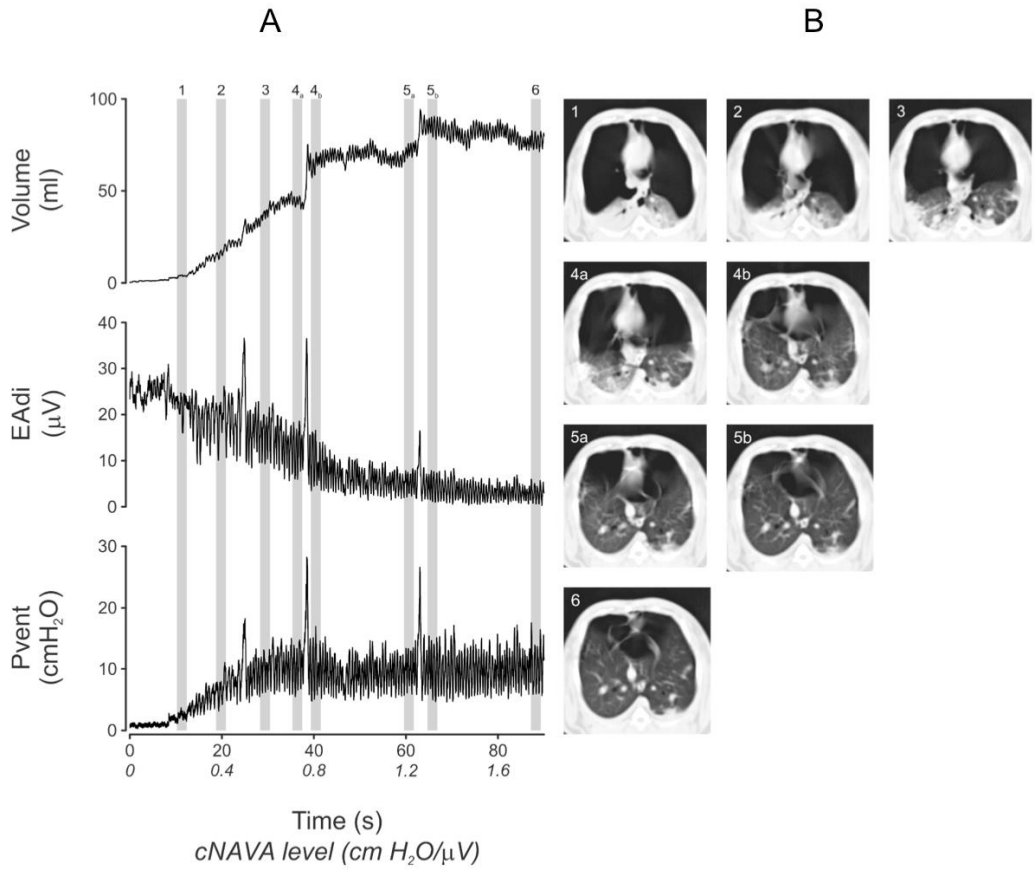

Figure 2

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Fig.3

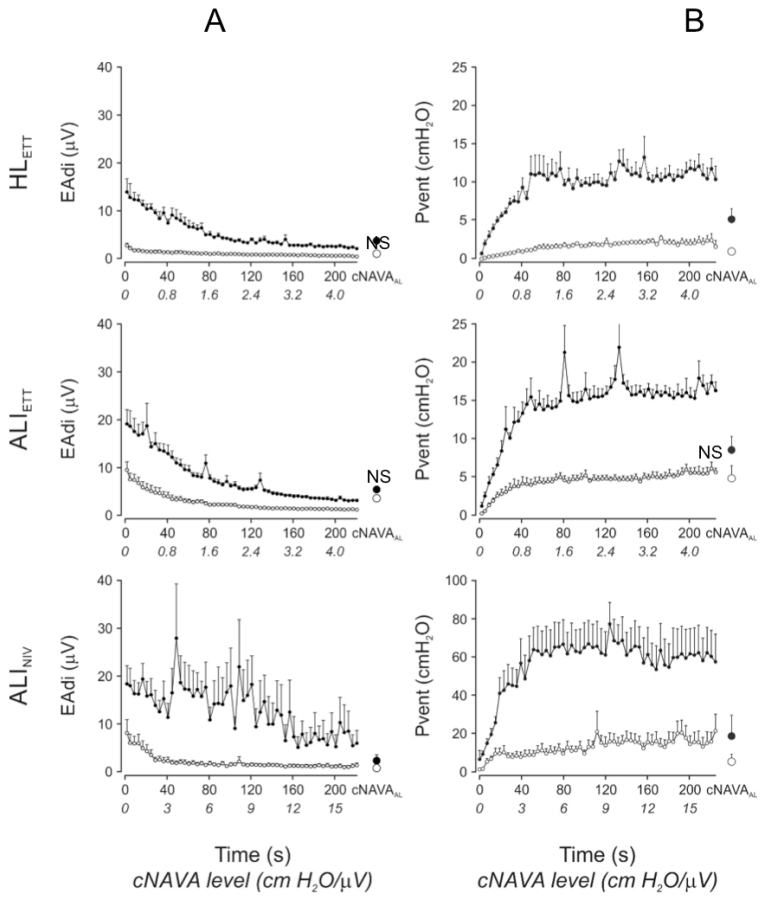

Figure 3

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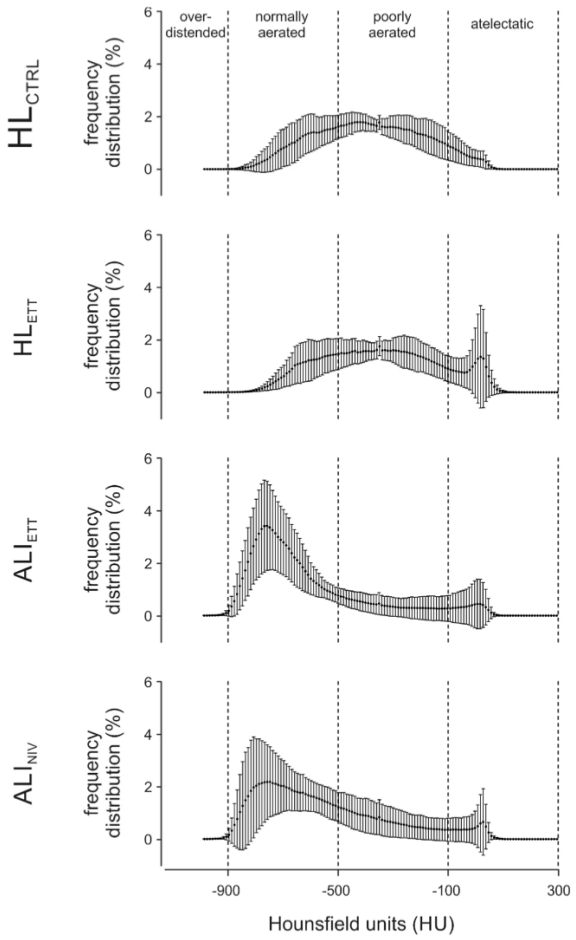

Figure 4

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Fig.5

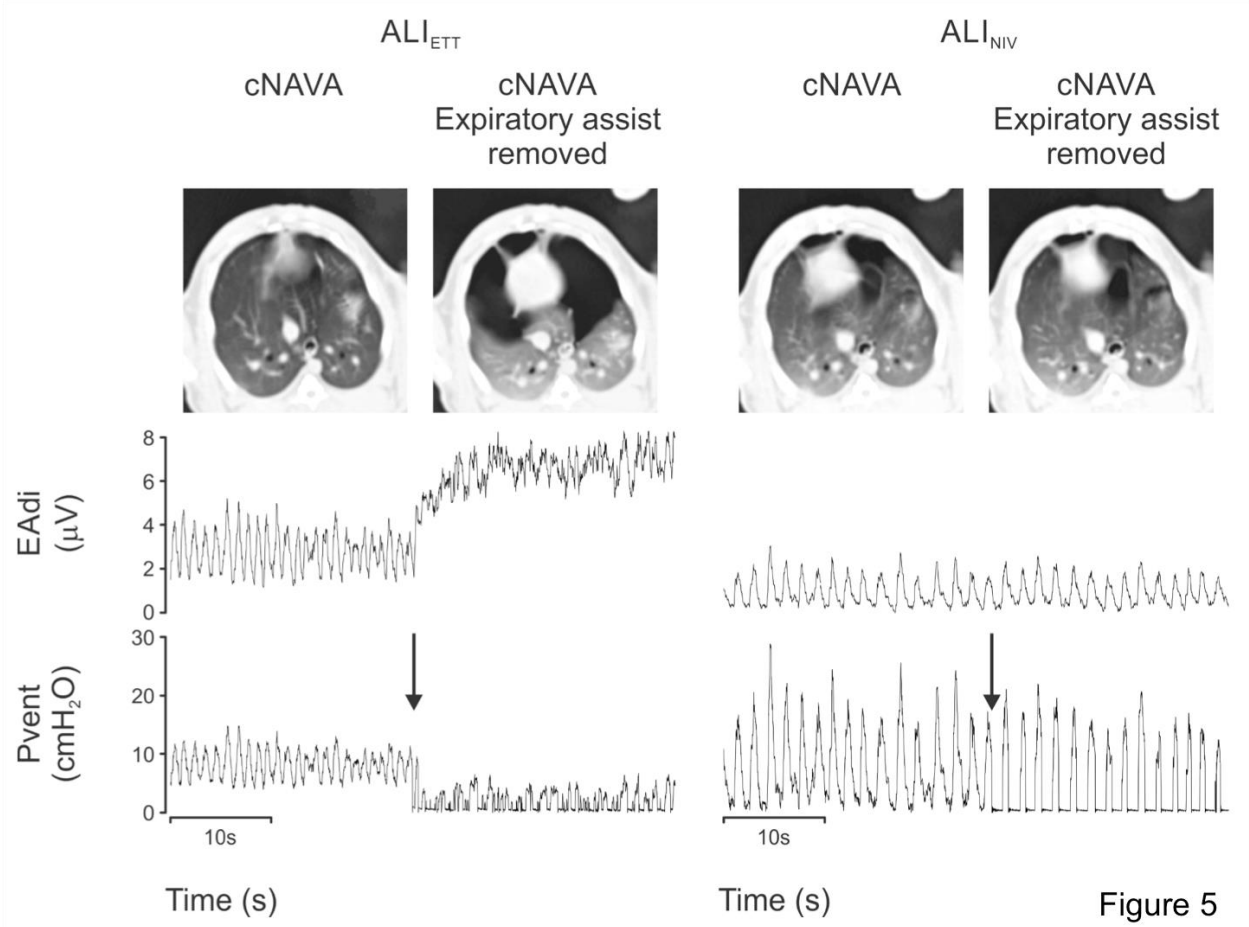

Figure 5

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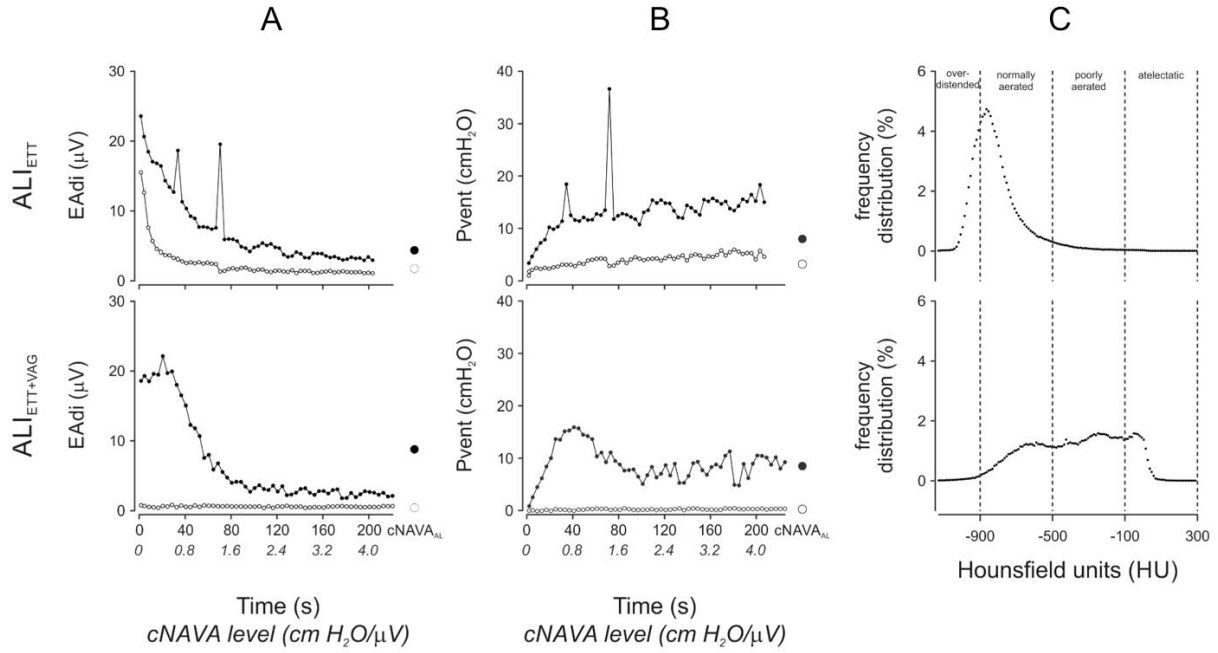

Figure 6

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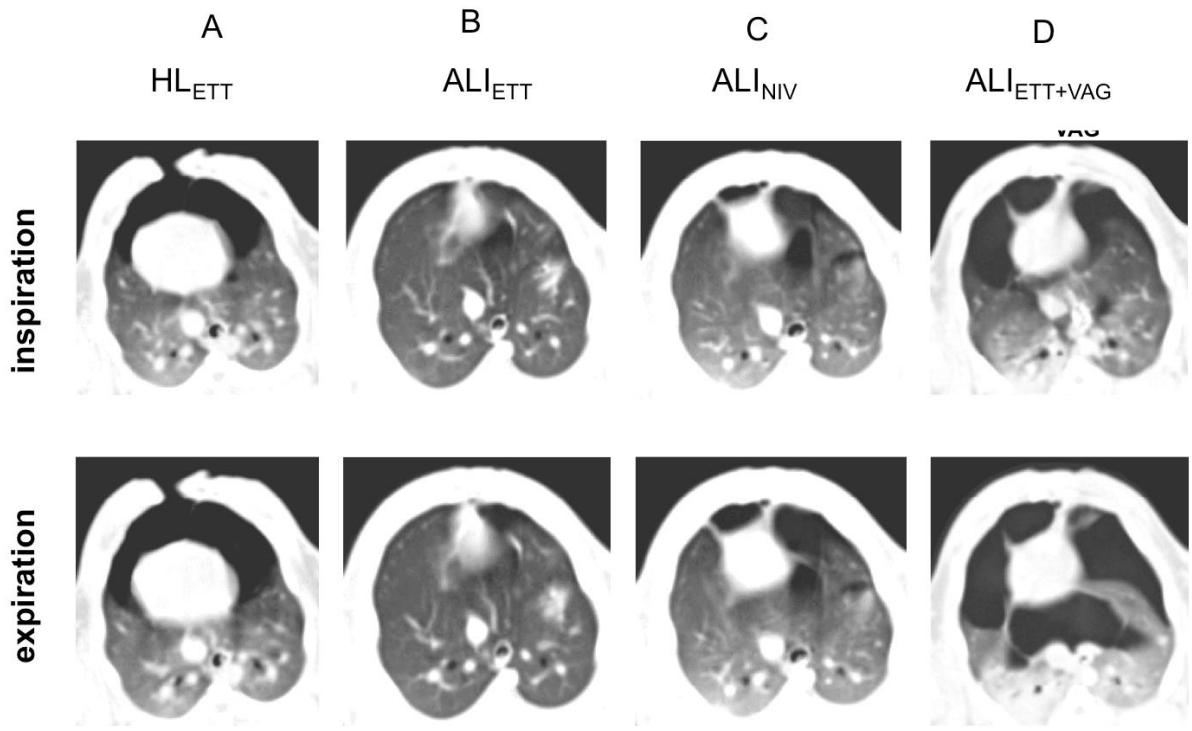

FIGURE 7

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Table 1: Group mean breathing pattern, vital signs and blood gas values assessed after 5 minutes on $cNAVA_{AL}$ during healthy lung conditions and after acute-lung injury conditions. HL_{CTRL} = healthy lung control; HL_{ETT} = healthy lung with endo-tracheal tube; ALI_{ETT} = after acute lung injury with endotracheal tube; ALI_{NIV} = after acute lung injury and non-invasive ventilation with nasal prong. V_T = tidal volume; Bf_{neural} = neural breathing frequency; HR = heart rate.

	HL_{CTRL}	HL_{ETT}	ALI_{ETT}	ALI_{NIV}
V_T (ml/kg)		3.8 ± 0.6	4.7 ± 0.9[b]	
Bf_{neural} (bpm)		42 ± 9	72 ± 12[b]	52 ± 10[c]
SaO_2 (%)	95.0 ± 7.0	92.5 ± 4.2	98.1 ± 1.3	96.6 ± 3.9
PaO_2/FiO_2	420 ± 129	254 ± 101 [a]	342 ± 137	245 ± 132 [a]
$PaCO_2$ (mm Hg)	50.7 ± 11.0	72.3 ± 15.2 [a]	75.3 ± 10.0 [a]	79.4 ± 12.2 [a]
pH	7.36 ± 0.08	7.28 ± 0.06 [a]	7.26 ± 0.05 [a]	7.23 ± 0.06 [a]
HR (per min)	177 ± 19	173 ± 10	178 ± 11	176 ± 8

Values presented are mean ± SD. [a] indicates $p<0.05$ vs HL_{CTRL}; [b] indicates $p<0.05$ HL_{ETT} vs ALI_{ETT} ; [c] indicates $p<0.05$ ALI_{ETT} vs ALI_{NIV}

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Table 2: Group mean distribution of lung aeration and lung volume for the different conditions after five minutes of ventilation at $cNANA_{AL}$. HL_{CTRL} = healthy lung control; HL_{ETT} = healthy lung with endo-tracheal tube; ALI_{ETT} = after acute lung injury with endotracheal tube; ALI_{NIV} = after acute lung injury and non-invasive ventilation with nasal prong. V_L = lung volume. Values are mean ± SD.

	HL_{CTRL}	HL_{ETT}	ALI_{ETT}	ALI_{NIV}
Over-distended lung tissue %	0	0	0	0
Normally aerated %	32 ± 16	28 ± 16	78 ± 26[a,b]	66 ± 27[a,b]
Poorly aerated %	60 ± 13	57 ± 11	16 ± 15 [a,b]	27 ± 20 [a,b]
Atelectatic %	8 ± 6	15 ± 15	5 ± 11	7 ± 9
V_L (ml)	47 ± 10.5	38.2 ± 6.5	77.8 ± 16.7 [a,b]	65.3 ± 17.5 [a,b]

[a] $p<0.05$ vs HL_{CTRL}

[b] $p<0.05$ vs HL_{ETT}