

Peer Review File

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Reviewer A

Thank you for asking me to review this very interesting paper, I think it does merit publication however it does need major modifications. I have several major criticisms and numerous minor points which I believe warrant attention.

While this paper does achieve what it promises to a significant extent, the full potential of the paper is not realised as it is burdened to a significant extent by repetitive statements such as study design or excessive detail where it is not entirely necessary for example detailing the pathological sections of the first sheep's lung

I would ask in the first instance that the paper is reviewed by an English scholar in terms of the construct of sentences and its Fleisch-Kincaid readability noted.

This paper examines the feasibility and safety of placing a short-term phrenic pacing system in an ovine model. Its rationale being - were this to prove a successful strategy, prophylactic systems could be placed at the time of high-risk intrathoracic surgery to ameliorate the effects of iatrogenic phrenic nerve injury.

Reply 1: the paper was reviewed and corrected by a British medical writer, Andrew Lane. The Fleisch-Kincaid readability was noted and the score is 30.2, as shown in the picture attached.

Instructions: Cut-and-paste the text you want to test into the box below, then click "Calculate"; this will give you the text's readability scores.

Flesch-Kincaid Readability Calculator

Enter text here:

Calculate Reset

significant limitation was the displacement of the electrodes twice on the right phrenic nerve, due to anatomic reasons. Pathological analysis confirmed that the electrodes could safely be removed percutaneously at the end of the procedure without any damage to the phrenic nerve. This study paves the way for further clinical trials in cardiac and thoracic surgery settings where post-operative complications could be reduced by providing neurostimulation of the diaphragm .

Results

Flesh-Kincaid Grade Level: **13**

Flesch Reading Ease Score: **30.2**

Reading Level: **College** (Difficult to read)

Average Words per Sentence: 15.7

Average Syllables per Word: 1.9

Sentences: 180

Words: 2831

Specific Recommendations:

Title:

I wonder if the authors might consider an alternate title which might more clearly reflect the raison-d'etre for the study

Phrenic nerve stimulation in an ovine model with temporary removable pacing leads.

Reply 2: we have modified the title of the article as suggested by the reviewer.

Changes made:

Line 2: "Phrenic nerve stimulation in an ovine model with temporary removable pacing leads".

Abstract

This is an Ovine model – not a sheep model, the authors might consider revising this throughout the manuscript.

Reply 3: we have revised sheep model to ovine model throughout the manuscript.

Changes made: line 82, line 98, line 102, line 122, line 127, line 215, line 325, line 493.

Line 123:

The objective of this study was to.....

Reply 4: we have modified the the verb be from "is" to "was".

Changes made:

Line 78: "The objective of this study was to assess the feasibility and safety..."

Methods

Line 130-131

Perhaps A change in the beginning of the sentence

Temporary phrenic nerve pacing electrodes were placed into 4 anaesthetised female animals.

Reply 5: we have made the change to the beginning of the sentence as suggested by the reviewer.

Changes made:

Line 81-82: "Temporary phrenic nerve pacing electrodes were implanted surgically using an ovine model (4 animals)."

Line 132:

A significant number of sentences throughout the manuscript are difficult to understand at first read, for example

"Primary endpoint was per procedure minute ventilation induced by neurostimulation"

I wonder if this could be phrased differently

The primary endpoint of this study was the ability to successfully match the animal's minute ventilation at upon implantation of both phrenic nerve pacers on day 1.

Secondary endpoints were successful neurostimulation by both

Reply 6: we have rephrased the sentences from line 142 differently as suggested by the reviewer. We have also rephrased the sentences from line 333 to 342.

Changes made:

Line 82-84 and 187-188: "The primary endpoint was the ability to successfully match the animal's minute ventilation upon implantation of both phrenic nerve pacers on day 1. Secondary endpoints were successful phrenic neurostimulation by both electrodes 15..."

Results:

Line 140

Omit the line

4 female sheep were used (not a result)

Reply 7: as suggested by the reviewer, the line has been removed.

Changes made: on line 87, this sentence was deleted "4 female sheep were used".

Line 140

"Implanted" - rather than "implemented"

Reply 8: we have amended "implemented" to "implanted"

Changes made:

Line 87:" In 3 of 4 animals, electrodes were successfully implanted in both right and left..."

Line 143:

P= 0.4 - not 0.400

Reply 9: we have corrected as suggested.
Line 90: we have changes “0.400” to “0.4”.

Lines 144-145

In all left phrenic nerves- not nerve
Reply 10: we corrected the mistake.

Changes made:

Line 91: the “s” was added to “nerves”.

Begin explanation about Right phrenic nerves as a separate sentence

One the right side stimulation was possible at all times in one animal, however each of the other animals could not be stimulated on the right side during at least one timepoint possibly due to lead displacement.

Reply 11: we have modified the sentence as suggested by the reviewer.

Changes made:

Line 91: “On the right side, stimulation was possible at all times in 1 animal but not in the remaining 3 animals for at least one time point, possibly due to lead displacement.”

Introduction:

Line 204

Is a risk factor “for” rather than “of”

Reply 12: we have made the correction.

Changes made:

Line 107: we have replaced “of” by “for”.

Line 210

Since Decades, phrenic nerve neuro..... this should be rephrased into a sentence with a simpler construct.

Reply 13: this sentence as well as the one that followed have been rephrased for clarity.

Line 111-117: “It has been proposed that electrical pacing of the diaphragm could prevent or alleviate ventilation-induced diaphragm dysfunction (10, 11, 12) and phrenic nerve neurostimulation is used in quadriplegic patients for partial or complete weaning from mechanical ventilation (13, 14). Critically ill patients may benefit from temporary phrenic nerve neurostimulation to mitigate ventilator-induced diaphragm dysfunction and to anticipate and prevent weaning failure (15), which may be of particular interest in thoracic/cardiac surgery patients (16, 17).”

Line 225

Enables, perhaps I would continue this sentence

.... would enable a similar minute ventilation

Reply 14: This was amended as suggested by the reviewer.

Line 123-124: “We investigated the hypothesis that this technique would enable similar minute ventilation compare to mechanical ventilation.”

Methods:

I would amend first line: I think several words are redundant in this sentence for example prospective, interventional or experimentation but not both

I would rephrase

This interventional animal study was carried out at the Carpentier... in accordance with the guidelines for the care and use.....

I would omit the sentence lines 235 – 237 completely:

The authors are ... resolved.

Lines 237 -238

A large (not “Big”) animal model was chosen as it was most likely to approximate the size in a clinical setting.

Line 240

Our team has “big” experience – perhaps our team has “significant” experience...

Reply 15: as suggested by the reviewer, we omitted the following sentences in order to improve the manuscript: “We chose a big animal model as it fits best to test surgical medical devices which are destined to be use in humans. Moreover, phrenic nerves are easily accessible in ovine model and our team has a big experience with this model as it was used in previous studies on diaphragm pacing.”

Changes made:

Line 127-130:” This interventional animal study used an adult female ovine model and was carried out at the Carpentier laboratory (Georges Pompidou European Hospital, Paris, France) in accordance with the guidelines for the care and use of laboratory animals. The authors are accountable for the accuracy and integrity of the work.”

Animal Preparation:

Line 254:

A three leads electrocardiogram allowed to monitor....

Perhaps

Intraoperative monitors included ECG and transcutaneous Oxygen saturations

Reply 16: the sentence has been modified.

Changes made:

Line 140: “Continuous fluid infusion was provided during the procedure, and ECG and transcutaneous oxygen saturation were monitored.”

Line 265:

A new prototype

I would amend

A prototype temporary phrenic pacer lead....

Reply 17: The expression “a new prototype” has been amended to “a prototype temporary phrenic pacer lead”.

Changes made:

Line 151: “A prototype temporary phrenic pacer lead was positioned and maintained on the distal portion of the phrenic nerves using absorbable thread (polyglactine 910 3/0, Ethicon, New Jersey, USA).”

There should be greater detail on the electrode here , how many pacing points and

which combinations were used as anodes and cathodes.

Reply 18: more detail about the electrode have been added.

Changes made:

Line 151-160: “The electrode was 16 mm long with a diameter of 1.35 mm and comprised 4 contact rings, each separated by 4 mm. The 4 contact rings allowed sequential multipolar stimulation. Only a portion of motor units in the diaphragm muscle were activated by a single stimulus pulse. Most of the motor units were at rest during successive stimulus pulses, allowing an extended recovery time after the contractile phase.”

Line 278:

Pulse impulsion - do the authors mean pulse “duration” of 200microseconds

Reply 19: we have replaced “impulsion” with “duration”.

Changes made:

Line 165: “...pulse duration 200 μ s...”

Experimental protocol

**This section does need a full rewrite

It seems repetitive and the reader easily becomes lost, for example

Lines 289 into 290 “Cautiously concealed under the skin” and Line 305 into 306, “hidden under the sheeps skin”. Seem to be making the same point about the same point within the experimental protocol

Line 291:

At day fifteen half of the animals underwent (not “undergone”)

I would suggest a rephrase

At day fifteen two of the animals were anaesthetised and neurostimulation sessions commenced...

At day 30 the second two animals were

Reply 20: the experimental protocol section has been rewritten to reduce repetition and make it easier to read.

Changes made:

The expression “hidden under the sheeps skin” (line 176) has been omitted because it was making the same point within the experimental protocol. We have replaced “undergone” by “underwent” (line 183). “At day fifteen half of the animals underwent” (line 177-180) was also rephrased: “On day 15, two of the animals were anaesthetized and a neurostimulation session was conducted using each electrode separately (second session). Bilateral thoracotomy was then performed to allow visualization of the percutaneous removal of the electrodes.”

Statistical Analysis

I would omit the first line (341 -342 In....performed) and just begin this with Categorical ...

Reply 21: the first sentence of the statistical analysis has been omitted.

Changes made:

Line 205: we started this section with “Categorical variables...”

Electrode implantation and phrenic Nerves Neurostimulation.

Rephrase 360 – 363. Possibly

Six of eight leads were successfully implanted into three of the four animals and neurostimulation was considered effective for each of these animals

Reply 22: The sentence has been rephrased according to the reviewer's suggestion.

Changes made:

Line 213-214: "Six of eight leads were successfully implanted into three of the four animals and neurostimulation was considered effective for each of these three animals (Table 1)."

The reader is overburdened with detail about the first sheep, I would limit the explanation to dense pleural adhesions prevented the attachment of the right phrenic lead in the first animal.

I would delete from

accessing (Line 364)... parenchyma (end line 368) I would also leave out figure 2, it adds very little to the study

Reply 23: We made the modification according to the reviewer's suggestion.

Changes made:

We have simplified the data on the first animal by deleting from "accessing..." (line 214) to "...parenchyma" and adding the following sentence: "no specific diagnosis of the right lung could be determined after sacrifice and pathologic analysis" (line 216).

We also left out Figure 2.

Discussion

I do not agree with the statements from lines 439 – 441

I am not sure that such a limited period of phrenic stimulation represents proof of concept such that the next step might be implantation of Cardiothoracic patients, Significant problems with lead displacement were encountered with this study and certainly modifications in lead design, the method by which the lead is fixed could be considered for future work in the area.

Reply 24: we have been more moderate with our statement and modified it.

Changes made:

Line 275-278: "However, following the significant displacement of the leads on the right side, further research is needed to improve the design of the leads and the method of fixation before starting clinical studies in patients undergoing cardiothoracic surgery."

Please rephrase lines 463-464

Perhaps

Diaphragmatic stimulation whether via stimulation via the motor points within the diaphragm itself (Synapse Biomedical Raymond Onders et al) or via phrenic stimulation (Atrotech FInalnd, Avery Medical US) has been successfully used for decades in the context of high spinal injuries

Reply 25: we have rephrased the sentences according to the reviewer's suggestion.

Changes made:

Line 298-300: "Diaphragmatic stimulation, whether via the motor points within the diaphragm itself via phrenic stimulation, has been used successfully for decades in the context of high spinal cord injuries".

Line 480

Main “setback” rephrase

One significant limitation of our study was the secondary displacement of two electrodes....

Reply 26: “Main setback” has been rephrased.

Changes made:

Line 315-316: “One significant limitation was the secondary displacement of two electrodes...”.

Line 486

Once the sheep were awoken

Rephrase

Lead displacement of the two right leads occurred following emergence from the first surgical procedure....

Reply 27: we replaced the sentence “once the sheep were awoken...” with a more appropriate sentence.

Changes made:

Line 324: “As a result, although the initial stimulation was efficient, on the right side two lead displacements occurred following the first surgical procedure”.

Figures

I would omit completely figure 2, I think it adds little to the overall discussion.

Reply 28: Figure 2 has been omitted.

Table 1.

I would change the title of this table.

Perhaps

Electrode placement and efficacy at each study interval

Reply 29: the title of Table 1 was changed.

Changes made:

Line 512: “Electrode placement and efficacy at first session, day 15, and day 30”.

Reviewer B

The authors present pilot studies outlining an approach to stimulating the diaphragm for trophic purposes, and demonstrate that intraabdominal leads are well tolerated and that stimulating thresholds and minute ventilation are well preserved over a 30 day period. Lead stability appears greater on the left than right side. The results suggest the viability of their approach in the management of patients with diaphragmatic deconditioning from a variety of causes.

The manuscript has some merit, even as a pilot study, subject to the following main concerns:

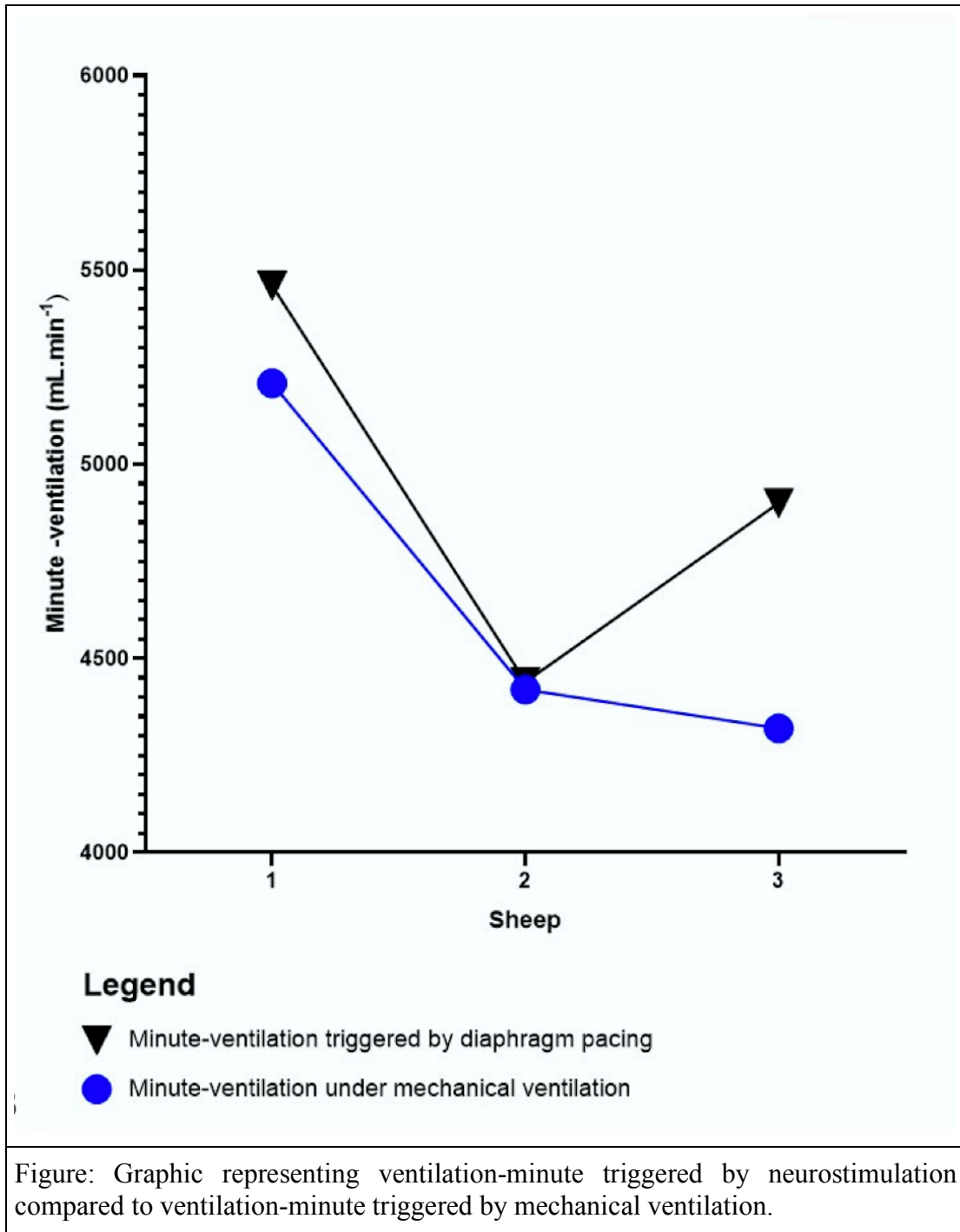
1. Lead stability: The authors state that differences in lead stability are related to “anatomic factors”, but do not seem to elaborate. What are the anatomic differences that seem to favor stabilization of the leads on the left vs. the right? Please discuss.

Reply 1: mediastinal pleura in sheep are thinner than mediastinal pleural in humans and

tear down when manipulated. Moreover, on the right side, in ovine models, the phrenic nerve runs along the superior and inferior vena cava while contact with the right atrium is very limited. When fixing the electrode on the left side, we can make a large knot from one side to the other of the electrode. However, this is not the case on the right side where we could damage the inferior vena cava which can be lethal to the animal. Changes made (lines 317-328): Mediastinal pleura in sheep are thinner than in humans and tear more easily when manipulated. Moreover, in the right thoracic cavity of the ovine model, the phrenic nerve is in close proximity to both the superior and inferior vena cavae, which complicates the attachment of the electrode compared to the left thoracic cavity. Since anatomic relations of the right phrenic nerve with the right atrium in ovine models are limited, we were aware of possible vascular injury and the electrodes on the right side may have been attached less tightly than on the left side. As a result, although the initial stimulation was efficient, on the right side two lead displacements occurred following the first surgical procedure. Such difficulties would not be expected in humans as the right phrenic nerve in humans descends along the right atrium before terminating in the diaphragm, in front of the inferior vena cava. Furthermore, it is accompanied by fatty tissue that is not present in the ovine model.

2. Respiratory physiology: Recording examples showing the effect of stimulation on respiratory signals would be a welcome addition to the manuscript. Specifically, please include 2 or 3 figures showing the effect of stimulation on tidal airflow and esophageal (intra-pleural) pressure compared to off stimulation. Also indicate whether both hemidiaphragms or simply a single hemidiaphragm need to be stimulated to stabilize minute ventilation.

Reply 2: we focused on minute-ventilation with bilateral phrenic nerve stimulation or with mechanical ventilation under general anesthesia and were able to provide a specific figure for this.



We did not study the effect of stimulation on tidal volume or esophageal pressure. We intend to focus on these parameters in future studies of the tPNA electrode.

Changes made:

Line 307: “We only studied bilateral diaphragm stimulation to stabilize minute-ventilation. We cannot confirm that we can stabilize minute-ventilation with only a single hemidiaphragm stimulation. Further investigations are necessary to assess this possibility as well as effect of stimulation on esophageal pressure and tidal volume.”

3. Entrainment: If you stimulate a single hemidiaphragm phasically, do you see the

unstimulated hemidiaphragm contract simultaneously? Is the unstimulated hemidiaphragm entrained to the respiratory rhythm drive through the stimulated hemidiaphragm?

Reply 3: we evaluated the main criteria only through bilateral diaphragm stimulation. On day 15 and day 30, we sought right and left hemi-diaphragm contraction by stimulating separately each electrode. However, we did not investigate this during the periods when unstimulated as this was not part of the study objectives. This question is an interesting area to investigate in future studies concerning Atrotech tPNA electrode, and we are grateful to the reviewer for the suggestion.

Changes made: no changes could have been made in this sense.

4. Diaphragmatic muscle histology and function:

a. Did you examine the muscle fiber histology after stimulation at the end of the 15 or 30 days? If so, did the muscle fiber histology change?

Reply 4: we did not examine the muscle fiber histology at the end of the 15 or 30 days. We did not consider the duration of stimulation (15 minutes) to be long enough to significantly impact muscle fiber histology. Muscle fiber changes by stimulation or lack of stimulation was the object of a previous study we published (doi: 10.1183/09031936.00045613) in which animals were stimulated for 72 hours.

Changes made: We mention this in the discussion.

Line 284-289:

“We previously published a pilot study of unilateral phrenic nerve neurostimulation in an ovine model and showed reduced muscle atrophy and muscle fiber injury in the neurostimulated hemi-diaphragm (12). These results have been corroborated by other experimental studies, eg, pigs under mechanical ventilation that received transvenous phrenic nerve stimulation had less diaphragm atrophy (11).”

b. Can you provide measurements of diaphragmatic function? Specifically, can you measure the transdiaphragmatic pressure swings generated by stimulating a single hemidiaphragm and by stimulating both hemidiaphragms, and compare these responses to unstimulated tidal inspirations?

Reply 4: Unfortunately, we cannot provide measurements of transdiaphragmatic pressure by stimulating a single or both hemi-diaphragms as this was not done during this study. We considered that the inclusion of ventilation-minute as a main criteria would allow us to evaluate the efficacy of this new set of electrodes without mechanical ventilation.

Changes made: no changes were made as those information were not available.

5. English language: The written form of manuscript is rather clear, yet there are many idiomatic expressions that should be corrected. Perhaps final editing by a native English speaker would solve the problem?

Reply 5: a thorough review of the manuscript has been completed by a native English speaker and medical writer by the name of Andrew Lane. He has corrected the mistakes which were present.