Transvenous Diaphragm Neurostimulation Mitigates Ventilation-associated Brain Injury

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Abstract

Rationale: Mechanical ventilation (MV) is associated with hippocampal apoptosis and inflammation, and it is important to study strategies to mitigate them.

Objectives: To explore whether temporary transvenous diaphragm neurostimulation (TTDN) in association with MV mitigates hippocampal apoptosis and inflammation after 50 hours of MV.

Methods: Normal-lung porcine study comparing apoptotic index, inflammatory markers, and neurological-damage serum markers between never-ventilated subjects, subjects undergoing 50 hours of MV plus either TTDN every other breath or every breath, and subjects undergoing 50 hours of MV (MV group). MV settings in volume control were VT of 8 ml/kg, and positive end-expiratory pressure of 5 cm H2O.

Measurements and Main Results: Apoptotic indices, microglia percentages, and reactive astrocyte percentages were greater in the MV group in comparison with the other groups (P < 0.05). Transpulmonary pressure at baseline and at study end were both lower in the group receiving TTDN every breath, but lung injury scores and systemic inflammatory markers were not different between the groups. Serum concentrations of four neurological-damage markers were lower in the group receiving TTDN every breath than in the MV group (P < 0.05). Heart rate variability declined significantly in the MV group and increased significantly in both TTDN groups over the course of the experiments.

Conclusions: Our study found that mechanical ventilation is associated with hippocampal apoptosis and inflammation, independent of lung injury and systemic inflammation. Also, in a porcine model, TTDN results in neuroprotection after 50 hours, and the degree of neuroprotection increases with greater exposure to TTDN.

Keywords: mechanical ventilators; brain injuries; post-ICU syndrome; apoptosis; ICU

At a Glance Commentary

Scientific Knowledge on the Subject: Mechanical ventilation is associated with hippocampal apoptosis and inflammation.

What This Study Adds to the Field: In a porcine model, mechanical ventilation is associated with hippocampal apoptosis and inflammation, independent of lung injury and systemic inflammation. Also, diaphragm neurostimulation results in neuroprotection after 50 hours, and the degree of neuroprotection increases with greater exposure to diaphragm neurostimulation.
Mechanical ventilation (MV) is a lifesaving technology that is the foundation of the modern intensive care unit (1, 2). However, many drawbacks of MV have been identified, such as ventilator-induced lung injury and ventilator-induced diaphragm dysfunction (1, 2). Recently a novel concept, ventilation-associated brain injury (VABI), has been proposed and studied (3–6).

VABI in neonatology has been associated with either the use of intermittent positive-pressure ventilation or hyperoxia (7). According to the proposed pathophysiology for VABI in neonates, intermittent positive-pressure ventilation or hyperoxia could lead to lung injury and consequently to systemic inflammation, triggering neuroinflammation and neuronal apoptosis (7). In adults, VABI has not been conclusively demonstrated yet. However, preclinical studies investigating VABI in fully grown subjects have shown that mechanically ventilated subjects have greater numbers of microglia, greater numbers of reactive astrocytes, and a higher incidence of cellular apoptosis compared with never-ventilated subjects (3, 5, 6, 8). Two recently published systematic reviews on MV associated with brain injury reported 13 preclinical papers that describe hippocampal apoptosis and neuroinflammation as experimental findings linked to VABI (9, 10).

In a porcine model, our group showed that even lung-protective MV settings were associated with brain injury after 50 hours of MV (5); moreover, in the same study, our group showed that serum concentrations of GFAP (gial fibrillary acid protein) and UCHL1 (ubiquitin carboxy-terminal hydrolase L1) were greater in the MV subjects than in the never-ventilated subjects, revealing an opportunity for the use of these markers for VABI (5, 6).

It has been hypothesized that either an inflammatory or a neural pathway might lead to the development of VABI (3, 4, 8, 11, 12). According to the systemic inflammatory pathway theory, ventilation-induced lung injury triggers systemic inflammation, which in turn could lead to brain injury (8, 11).

Conversely, the neural pathway postulates that the cyclical alveolar stretch during MV alters the vagal pulmonary afferent signal, triggering VABI (3, 4). Although there is uncertainty as to the exact mechanism, preclinical studies have consistently reported hippocampal inflammation and apoptosis as a result of MV (3, 8, 11).

Temporary transvenous diaphragm neurostimulation (TTDN) is a hybrid ventilation strategy that combines bilateral phrenic nerve stimulation in synchrony with MV, aiming initially to rescue the diaphragm from atrophy secondary to MV (13, 14). In a preclinical study, our group has shown that TTDN also reduced atelectasis and preserved lung homogeneity during MV (15, 16). We hypothesize that by preserving lung homogeneity during MV, TTDN could either dampen the inflammatory process associated with MV or change the pulmonary afferent signal during MV. Consequently, reduced inflammation or a change in the vagal signal during MV could mitigate cellular apoptosis in the hippocampus. Some of the results of these studies have been previously reported in the form of abstracts (6, 14, 15).

**Methods**

**Animals**

Juvenile Yorkshire pigs (4–5 mo old) were procured, housed, maintained, and studied following the local animal care committee guidelines after UBC Ethics Committee and Animal Care Committee approvals.

**Experimental Protocol**

Subjects were assigned to four groups: lung-protective mechanical ventilation only (MV group), temporary transvenous diaphragm neurostimulation either other breath (TTDN50% + MV) or every breath (TTDN100% + MV) in synchrony with lung-protective MV, and those who were never ventilated (NV group). Temperature, mean arterial pressure, heart rate, glucose levels, and $\text{PaCO}_2$ were monitored during the experiment to ensure values stayed within normal ranges.

**Ventilation Settings**

Subjects were ventilated with Dräger Evita XL ventilators in volume control mode set to 5 cm H$_2$O positive end-expiratory pressure, 8 ml/kg Vt, and plateau pressure less than 30 cm H$_2$O. Vt values slightly over 6 ml/kg were necessary owing to greater dead space in our subjects but remained in the range of 6–8 ml/kg from the initial ARDSnet study (17). An airway sensor, placed between the ventilator “Y-piece” and the endotracheal tube, captured air flow and airway pressure. The sensor was connected to the neurostimulation system, which recorded the data during the experiments for later analysis. Esophageal pressure was measured at the end of inspiration. Transpulmonary plateau pressure was measured during the end-inspiratory plateau. Driving pressure was calculated as end-inspiratory plateau pressure – end-expiratory pressure.

**Diaphragm Contraction and Diaphragm Contribution During Lung-Protective MV**

A central venous catheter embedded with electrodes (LIVE Catheter; Lungpacer Medical Inc.) was used to stimulate the phrenic nerves bilaterally for diaphragm contractions, targeting a ventilator pressure–time product reduction of 15–20%, as previously described (2, 13, 14, 18). Ventilator pressure–time product was obtained by respiratory monitoring (FluxMed GrT; MBMed). Esophageal pressure, plateau pressure, driving pressure, and transpulmonary plateau pressure were measured at the beginning and at the end of the experiment.

**Hippocampal Sampling and Preparation**

Molecular, granular, and subgranular layers were randomly sampled in the dentate gyrus, and the pyramidal layer was randomly sampled in the CA1 and CA3 hippocampal areas (all in the same slide section). Equal-sized sample areas (200 μm by 200 μm) and equal numbers of areas (21 per subject per immunohistochemistry marker) were analyzed in all subjects. An independent laboratory (Wax-it Histology Services Inc.), blinded to sample group, performed immunohistochemical preparation and processing for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling, GFAP, and IBA-1 (ionizing calcium-binding adaptor molecule-1) markers.

**Cell Counting**

Hippocampal cells were classified (positive-stained, negative-stained, and extracellular matrix) and counted by machine-learning software (ImageJ).

**Serum Samples**

All subjects had blood samples taken to determine serum concentration of biomarkers at the end of the experiment. Brain injury biomarkers were: $\text{SI00e}$, NSE (neuron specific enolase), GFAP, and UCHL1. Inflammatory biomarkers were: TNF-α, IL-1α, IL-1β, IL-6, IL-8, and IL-10. An independent laboratory, Eve
Technologies Corporation, blinded to study group allocation, analyzed the samples.

**Pao2/Fio2 Ratio**
Arterial blood gas samples were used to calculate the Pao2/Fio2 ratio. Mechanically ventilated subjects had samples taken at baseline, every six hours or when appropriate, and NV subjects had one sample taken before euthanasia.

**Lung Histology**
Lungs were harvested, and an independent laboratory, Wax-it Histology Services Inc., blinded to study group allocation, stained the lung slides with hematoxylin and eosin. Samples were then scored for lung injury by examiners who were blinded to the study groups, following a method adapted from Matute-Bello (19).

**Heart Rate Variability Analysis**
Root mean square of the standard deviation (RMSSD) of R–R intervals, a surrogate measure of autonomic nervous system activity, was calculated for the first 6 hours after experiment initiation and the last 6 hours before subject euthanasia.

**Statistical Analysis**
Statistical analyses used GraphPad Prism 8.4.2 software. Nonparametric tests were used and included either the Kruskal–Wallis test and Dunn’s multiple comparison test or the Wilcoxon signed-rank test, when appropriate. Data are expressed as median and interquartile range (IQR), unless otherwise stated. P values =<0.05 were considered statistically significant.

**Results**
Thirty-one female subjects were studied, with weights of 57 kg (43–66) in the MV group (n = 10), 55 kg (48–63) in the TTDN50% + MV group (n = 8), 69 kg (65–71) in the TTDN100% + MV group (n = 7), and 54 kg (50–56) in the NV group (n = 6), P = 0.0016. When appropriate, results were normalized to weight. Temperature, mean arterial pressure, heart rate, glucose levels, and Pao2 were within normal ranges for all subjects from all groups. All subjects that received TTDN had bilateral phrenic-nerve capture.

**Fluid Balance and Spontaneous Breathing Efforts**
Fluid balance to the ventilated subjects was within the target range of 0.1–2.0 ml/kg/hr (20). Spontaneous breathing activity during 50 hours of lung-protective MV was detected by direct examination of air flow and airway pressure recordings. The average number of breaths per subject per experiment was 36,917. The average number of episodes of spontaneous breathing activity (defined as subject-triggered breaths) was 8 per subject.

**Discussion**
Our study found that mechanical ventilation is associated with hippocampal apoptosis and inflammation, independent of lung injury and systemic inflammation. Moreover, our study also found that temporary transvenous diaphragm neurostimulation in synchrony with lung-protective MV considerably mitigates hippocampal apoptosis and neuroinflammation with lower microglia percentages and reactive astrocyte percentages after 50 hours of MV.

The association between MV and hippocampal apoptosis has been reported previously (3, 5, 12). The work presented in this study contributes to the growing body of evidence indicating that MV is associated with hippocampal apoptosis (3, 5, 12). Moreover, the brain insult is independent of lung injury and systemic inflammation (3, 5, 12). Previous experiments that investigated MV and its association with hippocampal apoptosis have not studied therapies to mitigate the brain injury associated with MV (3, 5, 12). Our study showed that TTDN mitigates hippocampal apoptosis associated with MV. Furthermore, the extent of hippocampal apoptosis mitigation increased with greater exposure to TTDN. Greater TTDN exposure resulted in lower hippocampal cell death, which is evidence that this intervention directly impacted hippocampal apoptosis. The TTDN100% + MV group showed degrees of hippocampal apoptosis after 50 hours that were statistically indistinguishable from the NV group, whereas the TTDN50% + MV group showed significant mitigation of apoptosis, but less so than the TTDN100% + MV group. Although our study did not evaluate the clinical impact of mitigating hippocampal apoptosis after MV, it establishes a foundation and biological plausibility that can be used as the basis of future studies.

Microglia and astrocytes are the primary cells that trigger and control the apoptotic process in the brain (21). This is important clinically as greater percentages of microglia and reactive astrocytes have been associated with acute cognitive dysfunction (22). For example, an analysis of hippocampal tissue harvested from deceased...
patients with acute respiratory distress syndrome (ARDS) and acute cognitive impairment before death showed increased numbers of activated microglia and reactive astrocytes when compared with patients with ARDS without acute cognitive impairment (22). In our study the microglia percentages in the hippocampus were considerably greater in the MV group than the other groups. Conversely, the TTDN100% + MV group showed microglia percentages and astrocyte percentages similar to the NV

Figure 1. Left: Dot plot of the hippocampal apoptotic indices (%) for all groups. Apoptotic indices found were 31.70 (29.79–43.76) for the mechanical ventilation (MV) group, 20.53 (10.85–26.46) for the TTDN50% + MV group, 6.57 (4.94–11.26) for the TTDN100% + MV group, and 0.96 (0.50–1.61) for the never-ventilated (NV) group. Post hoc analysis using Dunn’s multiple comparison test showed statistically significant differences between the MV and NV groups (31.70 vs. 0.96, \( P < 0.0001 \)), between the MV and TTDN100% + MV groups (31.70 vs. 6.57, \( P = 0.0041 \)), and between the TTDN50% + MV and NV groups (20.53 vs. 0.96, \( P = 0.0205 \)). Center and right: Examples of hippocampus slides for all groups, showing terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling–positive cells (brown). Scale bars, 100 μm. TTDN50% = temporary transvenous diaphragm neurostimulation every other breath; TTDN100% = temporary transvenous diaphragm neurostimulation every breath.

Figure 2. Left: Dot plot of percentages of IBA-1 (ionizing calcium-binding adaptor molecule-1)–positive hippocampal cells (%) for all groups. IBA-1–positive cell percentages found were 36.17 (30.71–48.27) for the mechanical ventilation (MV) group, 16.70 (10.82–22.42) for the TTDN50% + MV group, 9.80 (7.86–11.19) for the TTDN100% + MV group, and 10.12 (8.93–10.65) for the never-ventilated (NV) group. Post hoc analysis using Dunn’s multiple comparison test showed statistically significant differences between the MV and NV groups (36.17 vs. 10.12, \( P = 0.0006 \)), and between the MV and TTDN100% + MV groups (36.17 vs. 9.80, \( P = 0.0002 \)). Center and right: Examples of hippocampus slides for all groups, showing IBA-1–positive cells (brown). Scale bars, 100 μm. TTDN50% = temporary transvenous diaphragm neurostimulation every other breath; TTDN100% = temporary transvenous diaphragm neurostimulation every breath.
group. Furthermore, TTDN in synchrony with MV had a greater effect on microglia percentages when delivered every breath than when delivered every other breath; the TTDN100% + MV group had the lowest microglia percentages among the mechanically ventilated groups, and the TTDN50% + MV group had lower microglia percentages, but to a lesser degree than the TTDN100% + MV group. Not only are the lower proportions of microglia in the total cellular populations important, but groups receiving TTDN demonstrated a shift in the microglia cellular characteristics toward antiinflammatory predominance.

When found in the serum, GFAP is a protein known to correlate with astrocyte injury (23, 24). UCHL1 is another systemic marker commonly used to identify neuronal injury (23). Brain barrier integrity, it also reflects muscle activity (25–28). NSE is used as a marker for neuronal injury; however, NSE is also affected by the level of neuronal metabolism, because NSE is a protein responsible for neuronal glycolysis (25–28).

The greater serum concentrations of S100β and NSE in the MV group than in the mechanically ventilated groups could be because of the shorter time between initiation of sedation and study termination (30 min vs. 50 h), reflecting more recent neuronal and metabolic activity in the MV group. Conclusions about the blood–brain barrier integrity between the groups are therefore difficult to draw (25–28). Nevertheless, elevated GFAP and UCHL1 serum concentrations are consistent with our histological findings of hippocampal apoptosis in the MV group. Similarly, there was both histological and serological evidence of mitigation of hippocampal injury in the subjects receiving TTDN.

Figure 3. Left: Dot plot of percentages of GFAP (glial fibrillary acid protein)–positive cells (%). GFAP-positive cell percentages found were 25.63 (21.21–28.66) for the mechanical ventilation (MV) group, 11.93 (5.81–15.78) for the TTDN50% + MV group, 10.41 (7.10–11.56) for the TTDN100% + MV group, and 10.69 (9.31–12.85) for the never-ventilated (NV) group. Post hoc analysis using Dunn’s multiple comparison test showed statistically significant differences between the MV and NV groups (25.63 vs. 10.69, \( P = 0.0037 \)), between the MV and TTDN100% + MV groups (25.63 vs. 10.41, \( P = 0.0004 \)), and between the MV and TTDN50% + MV group (25.63 vs. 11.93, \( P = 0.0221 \)). Center and right: Examples of hippocampus slides for all groups, showing GFAP-positive cells (brown). Scale bars, 100 μm. TTDN50% = temporary transvenous diaphragm neurostimulation every other breath; TTDN100% = temporary transvenous diaphragm neurostimulation every breath.
| Brain Injury and Neuroinflammation Outcomes | MV Group (n=10) | TTDN50% + MV Group (n=8) | TTDN100% + MV Group (n=7) | NV Group (n=6) | P Value (Kruskal-Wallis Test) | P Value (Dunn’s Multiple Comparison Test) |
|--------------------------------------------|----------------|--------------------------|---------------------------|----------------|-----------------------------|------------------------------------------|
| Hippocampal apoptotic index                | 31.70 (29.79–43.76) | 20.53 (10.85–26.46) | 6.57 (4.94–11.26) | 0.96 (0.50–1.61) | <0.0001 | MV vs. TTDN50% + MV ns MV vs. TTDN100% + MV 0.0041 MV vs. NV <0.0001 TTDN50% + MV vs. TTDN100% + MV ns TTDN50% + MV vs. NV 0.0205 TTDN100% + MV vs. NV ns |
| IBA-1-positive hippocampal cells*, %       | 36.17 (30.71–48.27) | 16.70 (10.82–22.42) | 9.80 (7.86–11.19) | 10.12 (8.93–10.65) | 0.0022 | MV vs. TTDN50% + MV ns MV vs. TTDN100% + MV 0.0002 MV vs. NV 0.0006 TTDN50% + MV vs. TTDN100% + MV ns TTDN50% + MV vs. NV ns |
| IBA-1-positive hippocampal cells with proinflammatory characteristics, % | 8.11 (6.74–9.69) | 2.50 (2.02–2.88) | 1.60 (1.10–1.77) | 1.63 (1.32–2.06) | 0.0004 | MV vs. TTDN50% + MV ns MV vs. TTDN100% + MV 0.0025 MV vs. NV 0.0011 TTDN50% + MV vs. TTDN100% + MV ns TTDN50% + MV vs. NV ns |
| IBA-1-positive hippocampal cells with antiinflammatory characteristics, % | 27.82 (23.63–32.00) | 12.23 (7.36–19.54) | 8.37 (6.86–9.41) | 8.20 (7.68–8.50) | 0.0004 | MV vs. TTDN50% + MV ns MV vs. TTDN100% + MV 0.0022 MV vs. NV 0.0018 TTDN50% + MV vs. TTDN100% + MV ns TTDN50% + MV vs. NV ns |
| GFAP-positive hippocampal cells, %         | 25.63 (21.21–28.66) | 11.93 (5.81–15.78) | 10.41 (7.10–11.56) | 10.69 (9.31–12.85) | 0.0009 | MV vs. TTDN50% + MV ns MV vs. TTDN100% + MV 0.0021 MV vs. NV 0.0037 TTDN50% + MV vs. TTDN100% + MV ns TTDN50% + MV vs. NV ns |
| GFAP serum concentration, ng/ml            | 0.40 (0.28–0.57) | 0.29 (0.25–0.32) | 0.04 (0.02–0.06) | 0.15 (0.07–0.23) | <0.0001 | MV vs. TTDN50% + MV ns MV vs. TTDN100% + MV <0.0001 MV vs. NV 0.0043 TTDN50% + MV vs. TTDN100% + MV 0.0015 TTDN50% + MV vs. NV ns TTDN100% + MV vs. NV ns |

(Continued)
Table 1. (Continued)

| Brain Injury and Neuroinflammation Outcomes | Median (IQR) | P Value (Kruskal-Wallis Test) | P Value (Dunn’s Multiple Comparison Test) |
|-------------------------------------------|--------------|------------------------------|------------------------------------------|
| **MV Group (n = 10)**                     | **TTDN50% + MV Group (n = 8)** | **TTDN100% + MV Group (n = 7)** | **NV Group (n = 6)**                     |
| UCHL1 serum concentration, pg/ml          | 96.96 (80.65–109.60) | 110.00 (97.59–200.40) | 44.68 (36.56–58.34) | 76.57 (42.48–90.26) | 0.0013 | MV vs. TTDN50% + MV ns | MV vs. TTDN100% + MV ns | MV vs. NV ns | TTDN50% + MV vs. TTDN100% + MV ns | TTDN50% + MV vs. NV ns | TTDN100% + MV vs. NV ns |
| S100β serum concentration, pg/ml          | 193.10 (129.20–223.30) | 230.50 (157.90–361.80) | 150.30 (110.30–200.10) | 360.90 (252.10–803.90) | <0.0001 | MV vs. TTDN50% + MV ns | MV vs. TTDN100% + MV ns | MV vs. NV ns | TTDN100% + MV vs. TTDN50% + MV ns | TTDN100% + MV vs. NV ns | TTDN100% + MV vs. NV ns |
| NSE serum concentration, ng/ml           | 16.84 (5.66–24.28) | 16.72 (8.29–27.74) | 4.17 (3.71–4.51) | 32.23 (6.86–38.25) | 0.0004 | MV vs. TTDN50% + MV ns | MV vs. TTDN100% + MV ns | MV vs. NV ns | TTDN100% + MV vs. TTDN50% + MV ns | TTDN100% + MV vs. NV ns | TTDN100% + MV vs. NV ns |

*Includes hippocampal cells with proinflammatory and antiinflammatory characteristics.

Definition of abbreviations: GFAP = glial fibrillary acid protein; IBA = ionizing calcium-binding adaptor molecule; IQR = interquartile range; MV = mechanical ventilation; ns = not significant; NSE = neuron specific enolase; NV = never ventilated; TTDN50% = temporary transvenous diaphragm neurostimulation every other breath; TTDN100% = temporary transvenous diaphragm neurostimulation every breath; UCHL1 = ubiquitin carboxy-terminal hydrolase L1.
parasympathetic predominance contributed to the observed neuroprotective effect, nor the mechanism of this increased parasympathetic tone. This will be important to elucidate in future studies.

Two pathways for VABI have been discussed previously in the literature: the inflammatory and the neural pathways (3, 4, 8, 12, 37). Several preclinical studies have shown that the inflammatory pathway may trigger VABI (8, 11). According to this hypothesis, inflammatory proteins and inflammatory cells are released into the bloodstream because of ventilation-induced lung injury (30, 38). These inflammatory proteins and inflammatory cells reach the hippocampus through the circumventricular organs, such as the plexus choroid, without having to cross the blood–brain barrier, thereby triggering VABI (30, 38). For instance, one preclinical study showed that when inflammation was blocked in mechanically ventilated subjects, brain injury was mitigated (8); when the authors knocked out Toll-like receptor 4 (an inflammatory sensing protein) to block inflammation, mice showed less hippocampal injury than wild-type subjects after MV (8). However, in our experiment, the systemic inflammatory markers and lung injury scores were similar between the groups, indicating that the experimental conditions did not result in appreciably different degrees of inflammation and lung injury. The similar lung injury scores and the absence of significant differences in systemic inflammation between the mechanically ventilated groups provide evidence against the inflammatory pathway triggering VABI. Another preclinical study provided supporting evidence against the inflammatory pathway triggering VABI, demonstrating that the brain insult associated with MV was similar between pigs with healthy lungs mechanically ventilated for 12 hours and pigs whose lungs were injured by oleic acid mechanically ventilated for 12 hours (12). The authors concluded that the MV itself triggered the brain injury and not the lung injury induced by oleic acid injection (12).

In other preclinical studies, the neural pathway has been shown to trigger hippocampal apoptosis by abnormal activation of pulmonary stretch receptors, such as pulmonary TRPV4 (transient receptor potential vanilloid channel 4) (3, 4). Pulmonary TRPV4 is a cation-selective protein acting as a polymodal signal integrator that responds to pulmonary stretch in addition to a variety of other stimuli, such as mechanical force, products of lipid peroxidation, and prostaglandins (4). When TRPV4 is activated, it releases adenosine triphosphate, stimulating the purinergic receptors, which in turn contributes to the pulmonary vagal afferent signal (4). The vagal afferent signal reaches the hippocampus through the nucleus tractus solitarius–locus coeruleus–hippocampus pathway, releasing dopamine in the hippocampus (39). It has been shown that injurious MV settings (20–30 ml/kg) result in a hyperdopaminergic state, initiating hippocampal apoptosis by the dephosphorylation of the protein kinase B/cytokine synthesis kinase-3β (3). To confirm that the vagus nerve played an important role in VABI, one preclinical study showed that either chemical or surgical vagotomy resulted in mitigation.

Table 2. Serum Inflammatory Marker Results for the Mechanically Ventilated Groups

| Serum Inflammatory Markers (End of Study) | Concentration (pg/ml) [Median (IQR)] | P Value (Kruskal-Wallis Test) |
|------------------------------------------|--------------------------------------|------------------------------|
|                                           | MV (n = 10)                          | TTDN50% + MV (n = 8)         | TTDN100% + MV (n = 7) |
| IL-1α                                    | 12.89 (5.10–45.74)                   | 9.15 (5.10–18.33)           | 13.78 (2.21–48.26)   | 0.7832 |
| IL-1β                                    | 153.70 (66.61–458.00)                | 100.30 (18.23–136.10)       | 182.30 (115.40–263.60) | 0.1378 |
| IL-6                                     | 45.27 (24.29–215.90)                | 17.90 (0.33–215.00)         | 126.80 (77.52–179.70) | 0.1056 |
| IL-8                                     | 12.99 (0.00–55.06)                   | 9.48 (6.35–15.83)           | 3.67 (0.00–9.41)     | 0.4473 |
| IL-10                                    | 195.00 (115.60–937.20)               | 83.64 (52.38–256.20)        | 169.10 (63.74–5501.00) | 0.3155 |
| TNFα                                     | 3.58 (0.00–25.82)                    | 7.17 (0.20–19.48)           | 0.00 (0.00–0.00)     | 0.2896 |

Definition of abbreviations: IQR = interquartile range; MV = mechanical ventilation; TTDN50% = temporary transvenous diaphragm neurostimulation every other breath; TTDN100% = temporary transvenous diaphragm neurostimulation every breath. Blood samples were taken at the end of the experiment (50 h).
Mechanical ventilation is associated with hippocampal apoptosis and inflammation, independent of lung injury and systemic inflammation. In a porcine model, temporary transvenous diaphragm neurostimulation results in neuroprotection when applied in synchrony with lung-protective MV for 50 hours. The

### Definition of abbreviations

- IQR = interquartile range
- MV = mechanical ventilation
- NV = never ventilated
- TTDN50% = temporary transvenous diaphragm neurostimulation every other breath
- TTDN100% = temporary transvenous diaphragm neurostimulation every breath

### Conclusions

Mechanical ventilation is associated with hippocampal apoptosis and inflammation, independent of lung injury and systemic inflammation. In a porcine model, temporary transvenous diaphragm neurostimulation results in neuroprotection when applied in synchrony with lung-protective MV for 50 hours. The
Table 4. Lung Physiology Results for the Mechanically Ventilated Groups

| Time         | Measurement                               | Median (IQR) | MV Group \((n = 10)\) | TTDN50% + MV Group \((n = 8)\) | TTDN100% + MV Group \((n = 7)\) | P Value (Kruskal-Wallis Test) | P Value (Dunn’s Multiple Comparison Test) |
|--------------|-------------------------------------------|--------------|------------------------|-------------------------------|-------------------------------|-------------------------------|-----------------------------------|
| Baseline     | Esophageal pressure, cm H₂O               | 9 (6–14)     | 9 (6–13)               | 10 (8–12)                     | ns                            | MV vs. TTDN50% + MV             | —                                 |
|              | Plateau pressure, cm H₂O                  | 16 (14–16)   | 16 (15–18)             | 16 (15–17)                    | ns                            | MV vs. TTDN50% + MV             | —                                 |
|              | Driving pressure, cm H₂O                  | 11 (9–11)    | 11 (11–13)             | 12 (11–12)                    | ns                            | MV vs. TTDN50% + MV             | —                                 |
|              | Transpulmonary plateau pressure, cm H₂O   | 9 (8–10)     | 10 (8–13)              | 6 (5–7)                       | 0.0015                        | MV vs. TTDN50% + MV             | ns                                 |
| Study end    | Esophageal pressure, cm H₂O               | 7 (6–10)     | 7 (6–10)               | 10 (6–12)                     | ns                            | MV vs. TTDN50% + MV             | —                                 |
|              | Plateau pressure, cm H₂O                  | 19 (18–21)   | 20 (18–22)             | 18 (16–18)                    | 0.0258                        | MV vs. TTDN50% + MV             | ns                                 |
|              | Driving pressure, cm H₂O                  | 14 (13–16)   | 15 (13–17)             | 13 (12–14)                    | 0.0258                        | MV vs. TTDN50% + MV             | ns                                 |
|              | Transpulmonary plateau pressure, cm H₂O   | 14 (13–16)   | 14 (12–16)             | 7 (5–13)                      | 0.0018                        | MV vs. TTDN50% + MV             | ns                                 |

Definition of abbreviations: IQR = interquartile range; MV = mechanical ventilation; ns = not significant; TTDN50% = temporary transvenous diaphragm neurostimulation every other breath; TTDN100% = temporary transvenous diaphragm neurostimulation every breath. Esophageal pressure, plateau pressure, driving pressure, and transpulmonary plateau pressure were measured at the beginning and the end of the study. Esophageal pressure was measured at the end of inspiration. Transpulmonary plateau pressure was measured during the end-inspiratory plateau. Driving pressure was calculated as end-inspiratory plateau pressure – end-expiratory pressure.
neuroprotection observed was characterized by levels of hippocampal apoptosis, hippocampal inflammation, and neurological injury markers in the serum similar to those in the never-ventilated group. In addition, the degree of neuroprotection increases with greater exposure to TTDN. This is an important finding that supports further translational research.

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