experiments provide novel information for translational approaches in critical settings. Although further studies are required in human subjects, these findings can open a window for our knowledge to reduce neurological dysfunctions in critical patients, particularly those under long-term MV. ■

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References

1. Bassi TG, Rohrs EC, Fernandez KC, Ornoswska M, Nicholas M, Gani M, *et al.* Transvenous diaphragm neurostimulation mitigates ventilation-associated brain injury. *Am J Respir Crit Care Med* 2021;204: 1391–1402.
2. Grosmaitre X, Santarelli LC, Tan J, Luo M, Ma M. Dual functions of mammalian olfactory sensory neurons as odor detectors and mechanical sensors. *Nat Neurosci* 2007;10:348–354.
3. Zelano C, Jiang H, Zhou G, Arora N, Schuele S, Rosenow J, *et al.* Nasal respiration entrains human limbic oscillations and modulates cognitive function. *J Neurosci* 2016;36: 12448–12467.
4. Biskamp J, Bartos M, Sauer JF. Organization of prefrontal network activity by respiration-related oscillations. *Sci Rep* 2017;7: 45508.
5. Moberly AH, Schreck M, Bhattarai JP, Zweifel LS, Luo W, Ma M. Olfactory inputs modulate respiration-related rhythmic activity in the prefrontal cortex and freezing behavior. *Nat Commun* 2018;9: 1528.
6. Lockmann AL, Laplagne DA, Leão RN, Tort AB. A respiration-coupled rhythm in the rat hippocampus independent of theta and slow oscillations. *J Neurosci* 2016;36:5338–5352.
7. Almeida RFd, Ganzella M, Machado DG, Loureiro SO, Leffa D, Quincozes-Santos A, Pettenuzzo LF, *et al.* Olfactory bulbectomy in mice triggers transient and long-lasting behavioral impairments and biochemical hippocampal disturbances. *Prog Neuropsychopharmacol Biol Psychiatry* 2017;76:1–11.
8. Keilhoff G, Becker A, Grecksch G, Bernstein H-G, Wolf G. Cell proliferation is influenced by bulbectomy and normalized by imipramine treatment in a region-specific manner. *Neuropsychopharmacology* 2006;31: 1165–1176.
9. Ghazvineh S, Salimi M, Nazari M, Garousi M, Tabasi F, Dehdar K, *et al.* Rhythmic air-puff into nasal cavity modulates activity across multiple brain areas: a non-invasive brain stimulation method to reduce ventilator-induced memory impairment. *Respir Physiol Neurobiol* 2021; 287:103627.

Reply to Salimi *et al.*

From the Authors:

Our group greatly appreciated the comments from Salimi and colleagues in a recent letter on our article (1), proposing that the stimulation of neuropathways in conjunction with mechanical ventilation (MV) could result in better cognitive function after MV (1). Salimi and colleagues demonstrated that mice undergoing volume-control MV for 2 hours along with nasal puff synchronized to the breathing cycle had better working memory compared with mice undergoing volume-control MV alone (1). The authors reported that nasal puffs coupled with the respiratory cycle improved neural activity in many areas of the brain, especially the prefrontal cortex and ventral hippocampus (1). Furthermore, the authors stated that the resultant stimulation of the mechanoreceptors in the nasal cavity in synchrony with MV could promote neurogenesis and reduce neuroinflammation, and conversely, the inhibition of olfactory bulb activity has been associated with impaired neurogenesis and greater neuroinflammation (1). Although they concluded that the elimination of olfactory bulb activity might be associated with cognitive impairment after prolonged MV, the authors have not found statistical significance for the effect of nasal puffing on the protective theta and delta oscillations in the olfactory bulb and postulated that this was probably due to the inhibitory GABAergic circuits presented in this area (1). While the effects of nasal puffing on the theta and delta oscillations did not achieve statistical significance, the authors showed that nasal puffing considerably enhanced oscillatory activity in the prefrontal cortex and ventral hippocampus (1). The reported results supported the hypothesis that neural pathways might play an important role in ventilation-associated brain injury (VABI); moreover, VABI might be associated with cognitive impairment.

In addition to investigating a hybrid ventilatory strategy (temporary transvenous diaphragm neurostimulation [TTDN], synchronized to mechanical ventilation) to mitigate VABI, our group evaluated neurogenesis percentage (doublecortin-positive cells divided by doublecortin-positive cells plus doublecortin-negative cells) in the dentate gyrus in four groups, three of which were orally intubated: MV alone, TTDN every other breath plus MV (TTDN50% + MV), TTDN every breath plus MV (TTDN100% + MV), and never ventilated (NV, which were never intubated) (Figure 1). We found that the neurogenesis percentage was not statistically significantly different between the MV, TTDN100% + MV, and NV groups. The TTDN50% + MV group showed a statistically significant difference when compared with the NV group. However, the number of subjects analyzed was only four in the TTDN50% + MV group since we stored four of the eight brains from this group

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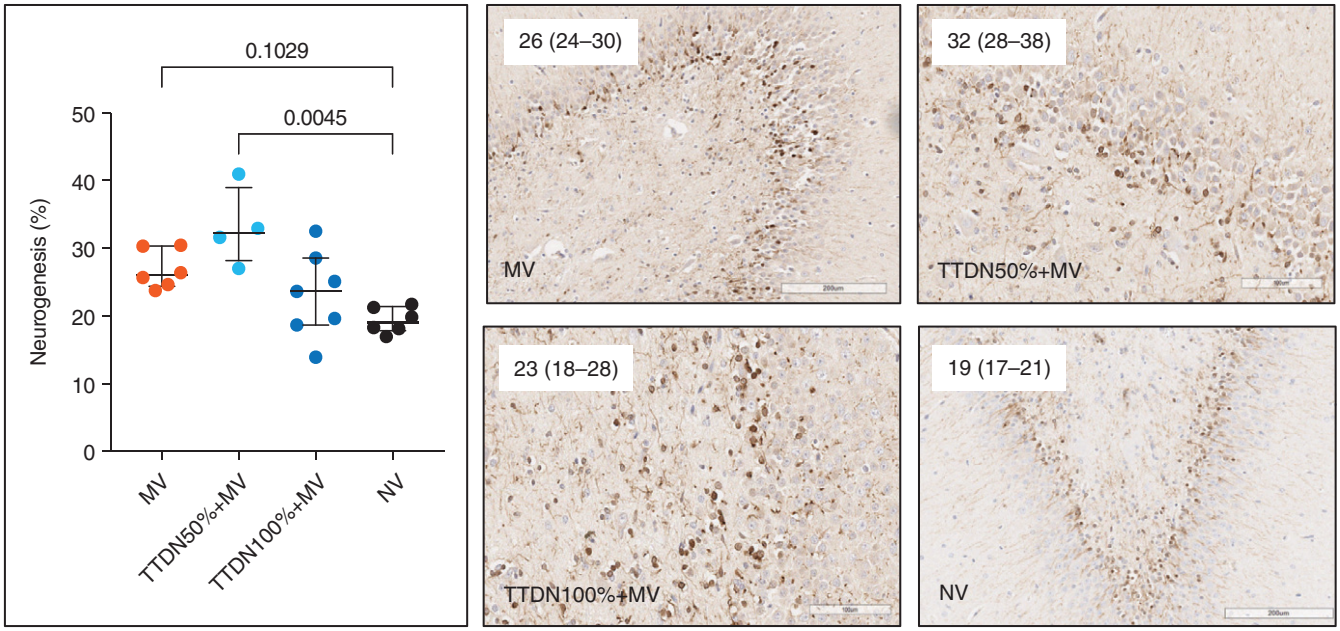

Figure 1. Left: dot plot of neurogenesis percentage (doublecortin-positive cell percentage) found for all groups, reported as median (interquartile range): 26 (24–30) for the mechanical ventilation (MV) group (red dots, *n* = 6), 32 (28–38) for the temporary transvenous diaphragm neurostimulation (TTDN)50% + MV group (light blue dots, *n* = 4), 23 (18–28) for the TTDN100% + MV group (dark blue dots, *n* = 7), and 19 (17–21) for the never ventilated (NV) group (black dots, *n* = 6). Kruskal-Wallis test showed a statistically significant difference between groups, *P* = 0.0050. *Post hoc* analysis using Dunn's multiple comparison test showed a statistically significant difference between the TTDN50% + MV and NV groups (32 versus 19, *P* = 0.0045). There was a tendency to significance in the difference between the MV and NV groups (26 versus 19, *P* = 0.1029). All other *P* values are greater than 0.11. Four brains from the TTDN50% + MV group were stored frozen and therefore were not stained with doublecortin assay. Center and right: examples of hippocampus slides for all groups, showing doublecortin-positive cells (brown) and doublecortin-negative cells (light blue). Scale bars: MV and NV, 200 μm; TTDN50% + MV and TTDV100% + MV, 100 μm.

frozen, affecting the binding between the assay and the target protein and therefore did not include the frozen samples in the analysis. The robust neurogenesis percentage observed in all groups was probably due to the age of the subjects investigated (2). These results indicate either that MV in orally intubated subjects does not affect the neurogenesis percentage in the hippocampus, contrary to what was proposed by Salimi and colleagues, or that our study (3) was underpowered to observe the effect on neurogenesis rate in orally intubated subjects undergoing MV for 50 hours, compared with the NV group. Nevertheless, the MV group tended to have an increased neurogenesis rate (rather than a reduced neurogenesis rate as proposed by Salimi and colleagues) compared with the NV group. To answer the question of whether MV affects neurogenesis in our model, we ran a *post hoc* analysis including only the MV and NV groups, which showed a statistically significant difference between these two groups (Figure 2). The same set of experiments that investigated neurogenesis also showed that the TTDN groups had lower neuroinflammation and cellular death compared with the MV group (3) and that the levels of neuroinflammation and cellular death in the TTDN-every-breath group were not statistically significantly different compared with the NV group (3). Our findings suggest that although the olfactory bulb was not stimulated by a nasal airflow, MV might have affected neurogenesis due to increased levels of inflammation and cellular death in the hippocampus, which we hypothesize triggers a "defensive" response to cellular loss, stimulating neurogenesis. Our data support this hypothesis, showing no statistically significant difference between the TTDN100% + MV and NV groups. As we believe that a different pathway has affected hippocampal neurogenesis in our experiments, it is impossible to show whether nasal puffs would provide further neural protection beyond TTDN because these two therapies might act via two distinct pathways.

Our studies differ in a variety of variables, including, but not limited to, animal model, animal age, duration of experiment, medications used, mock ICU environment, and ventilation strategy. However, it is intriguing to consider that nasal puffs could be added as a supplementary strategy to TTDN for preventing VABI. This would require a larger study size to evaluate, as TTDN in synchrony with lung-protective MV for 50 hours also resulted in improved lung homogeneity, reduced atelectasis formation, and attenuated diaphragm atrophy in addition to the mitigation of VABI (3–7). Nevertheless, the noninvasive nature of providing nasal puffs synchronized to MV makes it an appealing intervention to study.

Although there is increasingly persuasive evidence that VABI exists, there is a need for more translational and functional studies to confirm the clinical impact of this injury. Mechanisms for mitigation of VABI, such as the one we report herein and the one Salimi and colleagues reported, are vitally important to evaluate new avenues to help patients (1, 3). For instance, studies aiming to investigate VABI mitigation in an injured-lung model should be conducted, increasing the generalizability of the findings. While they may be challenging to conduct, new studies could reveal new therapeutic modalities for mechanically ventilated patients since many complications secondary

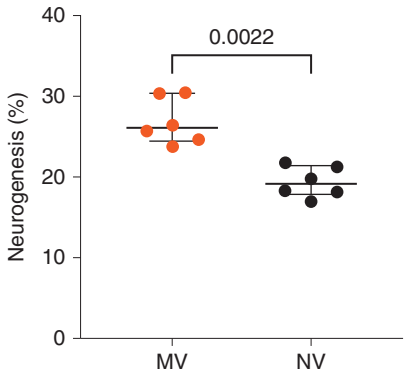

Figure 2. *Post hoc* analysis using the Mann-Whitney test showed a statistically significant difference in neurogenesis percentage between the mechanical ventilation (MV) group (red dots, $n = 6$) and the never ventilated (NV) group (black dots, $n = 6$) (26 versus 19, respectively, $P = 0.0022$).

to MV have been identified in recent years (3–7). Among these complications are ventilation-induced lung injury and ventilation-induced diaphragm dysfunction, and more recently, VABI (3–5, 8, 9). Thus, any new therapy intended to protect patients would also ideally mitigate ventilation-induced lung injury and ventilation-induced diaphragm dysfunction while mitigating VABI. Nevertheless, it is exciting to conceive of a future where ventilation strategies could be not only lung protective but also diaphragm and brain protective.

Despite the many questions that remain to be answered, the investigation of VABI and its mitigation should always be encouraged and congratulated, improving the knowledge in this emerging field. ■

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References

1. Ghazvineh S, Salimi M, Nazari M, Garousi M, Tabasi F, Dehdar K, *et al.* Rhythmic air-puff into nasal cavity modulates activity across multiple brain areas: A non-invasive brain stimulation method to reduce ventilator-induced memory impairment. *Respir Physiol Neurobiol* 2021; 287:103627.
2. Steiner E, Tata M, Frisén J. A fresh look at adult neurogenesis. *Nat Med* 2019;25:542–543.
3. Bassi TG, Rohrs EC, Fernandez KC, Ornoswska M, Nicholas M, Gani M, *et al.* Transvenous diaphragm neurostimulation mitigates ventilation-associated brain injury. *Am J Respir Crit Care Med* 2021;204:1391–1402.
4. Rohrs E, Ornowska M, Bassi T, Fernandez K, Reynolds S. Transvenous phrenic-nerve-stimulated diaphragm contraction during mechanical ventilation decreases loss of end-expiratory lung volume in non-injured lungs. *Am J Respir Crit Care Med* 2019;199:A5802.
5. Rohrs E, Bassi T, Fernandez K, Ornowska M, Nicholas M, Wittmann J, *et al.* Diaphragm neurostimulation during mechanical ventilation reduces atelectasis and transpulmonary plateau pressure, preserving lung homogeneity and PaO2/FiO2. *J Appl Physiol* 2021; 131:290–301.
6. Dres M, Gama De Abreu M, Similowski T. Late breaking abstract - temporary transvenous diaphragm neurostimulation in difficult-to-wean mechanically ventilated patients - results of the RESCUE 2 randomized controlled trial. *Eur Respir J* 2020;56:4352.
7. Ataya A, Silverman EP, Bagchi A, Sarwal A, Criner GJ, McDonagh DL. Temporary transvenous diaphragmatic neurostimulation in prolonged mechanically ventilated patients: a feasibility trial (RESCUE 1). *Crit Care Explor* 2020;2:e0106.
8. Goligher EC, Ferguson ND, Brochard LJ. Clinical challenges in mechanical ventilation. *Lancet* 2016;387:1856–1866.
9. Bassi TG, Rohrs EC, Fernandez KC, Ornowska M, Nicholas M, Gani M, *et al.* Brain injury after 50 h of lung-protective mechanical ventilation in a preclinical model. *Sci Rep* 2021;11:5105.