



Adapt or Die: Seizures Weaken Chemosensory Responses of Retrotrapezoid Nucleus Neurons to Hypercapnia

Epilepsy Currents

2024, Vol. 24(5) 367–369

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DOI: 10.1177/15357597241280478

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Neural Correlate of Reduced Respiratory Chemosensitivity During Chronic Epilepsy

Bhandare AM, Dale N. *Front Cell Neurosci.* 2023;17:1288600. PMID: 38193031

While central autonomic, cardiac, and/or respiratory dysfunction underlies sudden unexpected death in epilepsy (SUDEP), the specific neural mechanisms that lead to SUDEP remain to be determined. In this study, we took advantage of single-cell neuronal Ca^{2+} imaging and intrahippocampal kainic acid-induced chronic epilepsy in mice to investigate progressive changes in key cardiorespiratory brainstem circuits during chronic epilepsy. Weeks after induction of status epilepticus (SE), when mice were experiencing recurrent spontaneous seizures (chronic epilepsy), we observed that the adaptive ventilatory responses to hypercapnia were reduced for 5 weeks after SE induction with its partial recovery at week 7. These changes were paralleled by alterations in the chemosensory responses of neurons in the retrotrapezoid nucleus (RTN). Neurons that displayed adapting responses to hypercapnia were less prevalent and exhibited smaller responses over weeks 3–5, whereas neurons that displayed graded responses to hypercapnia became more prevalent by week 7. Over the same period, chemosensory responses of the pre-sympathetic rostral ventrolateral medullary neurons showed no change. Mice with chronic epilepsy showed enhanced sensitivity to seizures, which invade the RTN and possibly put the chemosensory circuits at further risk of impairment. Our findings establish a dysfunctional breathing phenotype with its RTN neuronal correlate in mice with chronic epilepsy and suggest that the assessment of respiratory chemosensitivity may have the potential for identifying people at risk of SUDEP.

Commentary

Upon entering the California Academy of Sciences, visitors are greeted by a large floor inscription that reads: “It is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is most adaptable to change.” Though a paraphrase of Darwin’s evolutionary theory, this quote could also apply to epilepsy. For instance, seizures can impair breathing, leading to altered levels of oxygen and carbon dioxide in the blood. If these changes are not properly sensed and adapted to with an appropriate ventilatory response, they can potentially lead to brain damage or even death.

While seizures have long been associated with changes in blood gases, it was the landmark MORTEMUS study that brought respiratory arrest into focus as a potential critical event leading to sudden unexpected death in epilepsy (SUDEP).¹ One possible explanation for fatal respiratory arrest following seizures is the failure of breathing to adapt to increases in blood CO_2 levels (PCO_2), the so-called hypercapnic ventilatory response (HCVR). Generalized convulsive seizures can evoke significant post-ictal increases in PCO_2 .^{2,3} Normally, central respiratory chemoreceptors located in the brainstem sense rising PCO_2 and trigger increases in breathing rate and lung tidal volume to restore normal CO_2 levels, thus averting life-threatening complications

due to acidosis. In people with epilepsy, HCVRs appear blunted, suggesting an inability to adapt to high PCO_2 levels, potentially increasing SUDEP risk.^{4,5} The elevated post-ictal PCO_2 levels can occur without dyspnea (shortness of breath) or air hunger, further exacerbating risk by preventing awareness of the need to breathe.^{5,6} Despite the importance of peri-ictal respiratory impairment as a potential SUDEP risk factor, the underlying neural circuitry and mechanisms remain elusive.

A new study by Bhandare and Dale⁷ offers valuable insights into how seizures impact chemosensory neural circuitry in the brainstem, leading to impaired adaptive respiratory responses. Using single-neuron Ca^{2+} imaging, they investigated whether mice with chemically induced epilepsy experience progressive changes in cardiorespiratory brainstem circuits that could impair HCVR, increasing vulnerability to SUDEP. Specifically, they monitored changes in neurons of the retrotrapezoid nucleus (RTN) and the rostral ventrolateral medullary (RVLM), brainstem regions that regulate chemosensory control of breathing and autonomic cardiovascular function, respectively.

To measure in vivo seizure-related changes in the RTN and RVLM, Bhandare and Dale conducted a series of complex surgical procedures and recordings in mice. First, they performed stereotaxic viral injections to transfect RTN or RVLM neurons with a



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genetically encoded Ca^{2+} indicator. Then, they implanted the mice with electroencephalography (EEG) electrodes, unilateral hippocampal cannulas, and microscopic gradient-index lenses for Ca^{2+} imaging. Finally, they installed cranial baseplates to hold miniature epifluorescence microscopes for visualizing Ca^{2+} levels in single RTN or RVLM neurons.

Once the mice were fully instrumented, they were anesthetized, “plugged” in to record EEG and image Ca^{2+} , and then administered unilateral intrahippocampal kainic acid (KA) through the attached cannula to induce status epilepticus (SE). After the KA injection, anesthesia was removed and the mice were placed into an open-field chamber to monitor behavior, EEG, and single-cell Ca^{2+} fluorescence in RTN or RVLM neurons in response to acute SE. Seven weeks later, when the mice had developed epilepsy, RTN and RVLM responses to KA-induced seizures were re-tested to assess the cumulative effects of chronic epilepsy on cardiorespiratory brainstem networks. Finally, recordings were also coupled with plethysmography to measure HCVRs to 3% and 6% CO_2 at the level of the whole animal and individual RTN and RVLM neurons. A notable strength of this study was its longitudinal design, which enabled imaging of the same neurons over several weeks, thereby tracking functional changes over time.

Bhandare and Dale’s work reveals several key findings with potential implications for SUDEP. They demonstrated that during SE, seizures spread to the brainstem, causing significant increases in the activity of RTN and RVLM neurons. However, by seven weeks later, during chronic epilepsy, seizure induction failed to significantly increase the activity of RTN or RVLM neurons for reasons that were not apparent. Terminal cardiorespiratory failure in SUDEP is thought to initiate minutes after a seizure, but no post-ictal measurements were obtained.

During chronic epilepsy, mice exhibited altered baseline breathing phenotypes, including reductions in lung tidal volume and minute ventilation. Furthermore, when epileptic mice were exposed to hypercapnia, they exhibited depressed tidal volumes and minute ventilation for 3 to 5 weeks after SE, indicating an attenuated HCVR and impaired respiratory chemosensitivity. However, by 7 weeks post-SE, the HCVR in epileptic mice had recovered to healthy levels, prompting questions about the potential importance of this mechanism in SUDEP pathophysiology.

A main strength of this study was the ability to categorize chemosensitive RTN and RVLM neurons into five functional subtypes based on their firing patterns. Bhandare and Dale focused on two main types: excited adapting (E_A) and excited graded (E_G) neurons. E_A neurons are silent at rest but exhibit initial transient bursts of Ca^{2+} activity with exposure to 3% CO_2 . In contrast, E_G neurons are silent at rest but exhibit increased Ca^{2+} activity with exposure to 3% CO_2 , increasing further at 6% CO_2 .

In the RTN, at 3 weeks post-SE during chronic epilepsy, the proportion of E_A neurons significantly decreased, but later normalized. This decrease in the fraction of E_A

neurons correlated with a decrease in baseline neuronal activity that subsequently returned to normal levels at later time points. Additionally, E_A neurons exhibited greatly attenuated responses to hypercapnia during chronic epilepsy. In contrast to E_A neurons, E_G neurons significantly increased in proportion by 7 weeks post-SE, possibly accounting for the recovery of the HCVR at this time. In the RVLM, analogous measurements revealed no significant changes in the function of E_A neurons. However, by 7 weeks post-SE, the proportion of E_A neurons had significantly decreased, and the fraction of E_G neurons had significantly increased. Whether the observed changes in the proportion and firing patterns of neuronal subtypes were due to plasticity or differential vulnerability to seizure-induced cell death among different types of neurons was not tested. Regardless, these results demonstrate that seizures alter the neuronal composition and chemosensory responses of brainstem networks, specifically in the RTN where adapting E_A neurons appear particularly affected.

This study possessed some nuances that limit its translatability for SUDEP. No SUDEP events were recorded or reported among the mice, weakening the model’s relevance to SUDEP. Technical limitations prevented the measurement of neuronal activity during spontaneous seizures, which are more clinically relevant. Experiments utilized only male mice, leaving unclear how including females might have affected the results. Finally, the brainstem contains several important chemosensory regions that were not investigated here, such as the serotonergic raphe nuclei.⁸ Thus, the RTN may be one of several brainstem regions underlying abnormal adaptive ventilatory responses in epilepsy.

The discovery by Bhandare and Dale that dysfunctional RTN neurons, specifically adapting E_A neurons, contribute to seizure-related respiratory chemosensitivity deficits represents a significant advance. These findings are important for SUDEP because they pinpoint a specific brainstem neuron population that could lessen the baseline drive to breathe after a seizure. The findings also reinforce the potential value of assessing respiratory chemosensitivity as a biomarker of SUDEP risk. As evidence accumulates that seizures can impair the ability of respiratory chemosensory neurons to adapt breathing to changing PCO_2 levels, it is becoming clearer that this failure to adapt could have deadly consequences.

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Declaration of Conflicting Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.



Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Ryvlin P, Nashef L, Lhatoo SD, et al. Incidence and mechanisms of cardiorespiratory arrests in epilepsy monitoring units (MORTEMUS): a retrospective study. *Lancet Neurol*. 2013;12(10):966-977.
2. Seyal M, Bateman LM, Albertson TE, Lin T-C, Li C-S. Respiratory changes with seizures in localization-related epilepsy: analysis of periictal hypercapnia and airflow patterns. *Epilepsia*. 2010;51(8):1359-1364.
3. Kim Y, Bravo E, Thimbeck CK, et al. Severe peri-ictal respiratory dysfunction is common in Dravet syndrome. *J Clin Invest*. 2018;128(3):1141-1153.
4. Teran FA, Sainju RK, Bravo E, et al. Seizures cause prolonged impairment of ventilation, CO₂ chemoreception and thermoregulation. *J Neurosci*. 2023;43(27):4959-4971 .
5. Sainju RK, Dragon DN, Winnike HB, et al. Ventilatory response to CO₂ in patients with epilepsy. *Epilepsia*. 2019;60(3):508-517.
6. Harmata GI, Rhone AE, Kovach CK, et al. Failure to breathe persists without air hunger or alarm following amygdala seizures. *JCI Insight*. 2023;8(22):e172423.
7. Bhandare AM, Dale N. Neural correlate of reduced respiratory chemosensitivity during chronic epilepsy. *Front Cell Neurosci*. 2023;17:1288600.
8. Teran FA, Massey CA, Richerson GB. Serotonin neurons and central respiratory chemoreception: where are we now? *Prog Brain Res*. 2014;209:207-233.