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Seizure-associated central apnea in a rat model: Evidence for resetting the respiratory rhythm and activation of the diving reflex

S.M. Villiere [a,e], K. Nakase [a], R. Kollmar [b,c], J. Silverman [c], K. Sundaram [c], M. Stewart [a,d,*]

[a] Department of Physiology & Pharmacology, State University of New York Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, United States
[b] Department of Cell Biology, State University of New York Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, United States
[c] Department of Otolaryngology, State University of New York Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, United States
[d] Department of Neurology, State University of New York Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, United States
[e] Research Initiative for Scientific Enhancement (RISE) Program, City University of New York Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225, United States

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ABSTRACT

Respiratory derangements, including irregular, tachypnic breathing and central or obstructive apnea can be consequences of seizure activity in epilepsy patients and animal models. Periods of seizure-associated central apnea, defined as periods >1 s with rapid onset and offset of no airflow during plethysmography, suggest that seizures spread to brainstem respiratory regions to disrupt breathing.

We sought to characterize seizure-associated central apneic episodes as an indicator of seizure impact on the respiratory rhythm in rats anesthetized with urethane and given parenteral kainic acid to induce recurring seizures. We measured central apneic period onsets and offsets to determine if onset-offset relations were a consequence of 1) a reset of the respiratory rhythm, 2) a transient pausing of the respiratory rhythm, resuming from the pause point at the end of the apneic period, 3) a transient suppression of respiratory behavior with apnea offset predicted by a continuation of the breathing pattern preceding apnea, or 4) a random re-entry into the respiratory cycle. Animals were monitored with continuous ECG, EEG, and plethysmography. One hundred ninety central apnea episodes (1.04 to 36.18 s, mean: 3.2 ± 3.7 s) were recorded during seizure activity from 7 rats with multiple apneic episodes. The majority of apneic period onsets occurred during expiration (125/161 apneic episodes, 78%). In either expiration or inspiration, apneic onsets tended to occur late in the cycle, i.e. between the time of the peak and end of expiration (82/125, 66%) or inspiration (34/36, 94%). Apneic period offsets were more uniformly distributed between early and late expiration (27%, 34%) and inspiration (16%, 23%). Differences between the respiratory phase at the onset of apnea and the corresponding offset phase varied widely, even within individual animals.

Each central apneic episode was associated with a high frequency event in EEG or ECG records at onset. High frequency events that were not associated with flatline plethysmographs revealed a constant plethysmograph pattern within each animal, suggesting a clear reset of the respiratory rhythm. The respiratory rhythm became highly variable after about 1 s, however, accounting for the unpredictability of the offset phase. The dissociation of respiratory rhythm reset from the cessation of airflow also suggested that central apneic periods involved activation of brainstem regions serving the diving reflex to eliminate the expression of respiratory movements. This conclusion was supported by the decreased heart rate as a function of apnea duration.

We conclude that seizure-associated central apnea episodes are associated with 1) a reset of the respiratory rhythm, and 2) activation of brainstem regions serving the diving reflex to suppress respiratory behavior. The significance of these conclusions is that these details of seizure impact on brainstem circuitry represent metrics for assessing seizure spread and potentially subclassifying seizure patterns.

1. Introduction

Cardiac and respiratory rhythm changes during most types of epileptic seizures indicate that seizure activity can spread to impact the autonomic nervous system by reaching medullary sympathetic premotor and parasympathetic motor neurons (e.g. (Goodman et al., 2008; Sakamoto et al., 2008)) and adjacent respiratory brainstem regions

* Corresponding author at: SUNY Downstate Medical Center, School of Graduate Studies, 450 Clarkson Avenue, MSC 41, Brooklyn, NY 11203, United States.
E-mail address: mark.stewart@downstate.edu (M. Stewart).
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(e.g. (Sowers et al., 2013) (Faingold et al., 2014; Faingold et al., 2010)). Respiratory changes during seizures can be significant (reviewed in (Devinsky et al., 2016; Massey et al., 2014; Sowers et al., 2013)). Reports of ictal tachypnea, bradypnea, and apnea (e.g. (Bateman et al., 2008; Bateman et al., 2010; Blum, 2009; Nakase et al., 2016; Nashef et al., 1996; Ryvlin et al., 2013; Seyal and Bateman, 2009; Seyal et al., 2010; Singh et al., 2013)) all point to an impact of seizure activity on respiratory rhythm generation and thereby a role in oxygen desaturation during seizures (Bateman et al., 2008; Seyal et al., 2010).

Animal studies involving rats (Nakase et al., 2016; Sakamoto et al., 2008; Stewart, 2011), mice (Faingold et al., 2010; Uteshev et al., 2010), cats (Paydarfar et al., 1991; Schraeder and Lathers, 1983), and sheep (Johnston et al., 1995; Johnston et al., 1997) have all contributed to a demonstration of the importance of ictal hypoxemia in seizure-induced death. Recently, we showed that seizures can cause episodes of central or obstructive apnea, and that the periods of obstructive apnea were lethal and due to severe laryngospasm caused by seizure spread to the recurrent laryngeal nerve, the principal motor nerve of the larynx (Nakase et al., 2016). By contrast, central apneic episodes, although potentially tens of seconds in duration, were associated with an open airway, modest decreases in heart rate, and no significant evidence of hypoxia in ECG records (Nakase et al., 2016).

As an approach to understanding the impact of seizure activity on respiratory rhythm generation, we studied episodes of central apnea during seizure activity to determine if the abrupt central apneic period onsets and offsets occurred with a particular pattern, and if patterns indicated 1) a reset of the respiratory rhythm, 2) a transient suspension of the respiratory rhythm, where the rhythm is "paused," resuming at the end of the apneic period, 3) a transient suppression of respiratory behavior with apnea offset predicted by a continuation of the breathing pattern preceding apnea, or 4) a random re-entry into the respiratory cycle. The results would contribute to an understanding of the sensitivity or durability of respiratory rhythm generation as seizure activity spreads into the brainstem.

2. Materials and methods

All procedures were approved by an Animal Care and Use Committee and conducted in accordance with the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals. Adult male Sprague-Dawley albino rats (180–340 g; Harlan, Chicago, IL) were housed in AAALAC-accredited facilities and maintained on a 12 h light:dark cycle with a temperature of 23 °C and humidity of 55%, monitored daily, and had unrestricted access to water and food. Urethane (1.5 g/kg ip) was used for anesthesia. Urethane limits seizure spread into neocortical regions as evidenced by the absence of convulsive movement (Saito et al., 2006), but does not limit seizure spread caudally into hypothalamic brainstem areas, nor does it impair spontaneous breathing (e.g. Sakamoto et al., 2008; Nakase et al., 2016). The methods are described in detail in Nakase et al. (2016).

2.1. Recordings

2.1.1. EEG recordings

Epidural EEG electrodes were placed after the scalp over dorsal surface of skull was incised, the skin retracted, and the periosteum scraped from skull surface. Burr holes were drilled for placement of stainless steel screw electrodes bilaterally over dorsal CA3 (5.8 mm anterior to lambda, 3.5 mm lateral to midline; (Paxinos and Watson, 1998)), and over cerebellum as a recording reference. Signals were amplified, filtered to pass 1 Hz to 1 kHz, and digitized at 2 kHz. Seizure activity was recognized as an average peak-to-peak or RMS (root mean square: square root of the mean of squared voltage values in a sample) amplitude that was ≥3 times the baseline peak-to-peak amplitude, typically associated with frequent spiking. Seizure onset and offset times were estimated from the EEG raw data and the rate of change of the amplitude measure. A high pass filter was applied in Spike 2 (367 Hz, −3 dB/octave rolloff) to one of the EEG records in each file to isolate an artifact that occurred at the beginning of each apnea episode. This method is similar to our method for detecting inspiratory effort during airway occlusion (Stewart et al., 2016).

2.1.2. ECG recordings

Limb-lead ECG was recorded using copper strips coated with conductive gel wrapped around each forelimb and the tail for limb-lead ECGs. Signals were amplified and filtered to pass 1 Hz to 1 kHz and digitized at 2 kHz. The presence of abnormally shaped QRS complexes indicated ectopic beats. Rate was calculated from the number of beats per unit time. Rhythm was assessed by reviewing P waves and associated QRS complexes for variations in wave shape, beat-to-beat intervals, and atrial-ventricular coupling.

2.1.3. Plethysmography

Tidal-breathing flow-volume loops were recorded using head-out plethysmography (Renninger, 2006). Analyses were taken of records that were digitized together with other signals. Episodes of central apnea were defined as periods >1 s with no evidence airflow during plethysmography with rapid onset and offset (Nakase et al., 2016).

2.2. Seizure induction

Rats received kainic acid (KA; 10 mg/kg; Sigma-Aldrich, St. Louis, MO) given intraperitoneally. As described in more detail previously (Saito et al., 2006; Sakamoto et al., 2008; Stewart, 2011), spontaneous seizure activity without motor convulsions began within 10–60 min and consisted of an initial long episode of nearly continuous seizure activity (>5 min) that resembled status epilepticus (SE) followed by much briefer (tens of seconds) recurring discrete seizures. Measurements were taken from both the periods of continuous seizure activity and discrete seizures. The main advantage of this preparation is that the absence of motor convulsions 1) leaves cardiovascular and respiratory function intact and accessible for monitoring methods during seizure activity that would be much more difficult or impossible in a convulsing animal, and 2) eliminates the potential for systemic metabolic consequences of prolonged or intense skeletal muscle contractions (Stewart, 2011). Some measurements, however, for example laryngoscopy (Nakase et al., 2016), echocardiography (Sakamoto et al., 2008), and laryngeal nerve activity (Nakase et al., 2016) have only been measured during seizure activity in this preparation.

2.3. Estimation of onset and offset phases for apneic episodes

The "onset phase" is the term used to describe the point in the respiratory cycle at which breathing ceased. To estimate this point, the existing features of the full or partial cycle at the beginning of an apnea episode were used to identify from the preceding breaths a "model" breath that most closely matched the partial breath (Fig. 1). After identifying a model breath, the area of the partial breath relative to the area of the model breath was used to estimate the fraction represented by the partial breath. Similarly, a different fraction was determined using the total times for the partial breath and the model breath. Fractions were binned in quarter cycles where any phase of expiration up to and including the peak of expiration was one quarter ("early expiration"), all phases after the peak up to an including the completion of expiration were grouped in quarter 2 ("late expiration"), inspiratory phases up to and including the peak of an inspiratory cycle were grouped in quarter 3 ("early inspiration"), and inspiratory phases after the peak up to and including the completion of inspiration were quarter 4 ("late inspiration;" Fig. 2). The same approach was used to classify the offset phases, but differences between the onset phase and offset phase of each apneic episode were calculated from the fractional values, not the binned values. Breaths at the onset or offset that were