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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, and M. Stewart^{a,d,*}

^aDepartment of Physiology & Pharmacology, State University of New York Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, United States

^bDepartment of Cell Biology, State University of New York Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, United States

^cDepartment of Otolaryngology, State University of New York Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, United States

^dDepartment of Neurology, State University of New York Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, United States

^eResearch Initiative for Scientific Enhancement (RISE) Program, City University of New York Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225, United States

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

*Corresponding author at: SUNY Downstate Medical Center, School of Graduate Studies, 450 Clarkson Avenue, MSC 41, Brooklyn, NY 11203, United States. mark.stewart@downstate.edu (M. Stewart).

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.

Keywords

Epilepsy; Respiration; Hypoxia; Larynx; Plethysmography

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 (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 and 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 larger than model breaths, or that had shapes unlike any models were grouped separately.

2.4. Data analysis and statistics

Analyses were done with Spike 2 software (Cambridge Electronic Design, Ltd., Cambridge, England). Data are reported as means $\pm$ standard deviation in the text and figures unless otherwise noted. All statistics were computed with GraphPad Prism 6 software or IBM SPSS Statistics (version 21). A $p < 0.05$ was predefined, after appropriate post-hoc correction for multiple comparisons where appropriate, to be statistically significant. Other details are given in the text and figure legends.

3. Results

One hundred ninety central apnea episodes (1.04 to 36.18 s, mean: 3.2 ± 3.7 s, median: 2.0 s) were recorded during kainic acid induced seizure activity in 7 rats, each of whom had 3 apneic episodes. All rats had a period of continuous seizure activity and the average number of discrete seizures in these animals was 7.7 ± 6.5 (range: 3 to 20 discrete seizures in a single animal). The number of apneic episodes per animal ranged from 3 to 53 (mean: 15.8 apneic episodes per animal, median: 9). Fig. 2B illustrates the distribution of apneic episode durations for the entire sample of apnea episodes and animals. No periods of central apneic activity were ever recorded in any of the pre-seizure baseline periods of 5 min.

The impact of seizure activity on basic respiration/ventilation parameters was an increase in respiratory rate, a decrease in tidal volume, with minimal impact on minute ventilation. The respiratory rate (breaths per minute) increased from 82.6 ± 7.2 at baseline to 400.2 ± 45.2 during seizure activity ($p < 0.0001$, $t = 19.30$, $df = 6$, paired, two-tailed t -test). The tidal volume (ml) decreased from 1.5 ± 0.4 at baseline to 0.4 ± 0.2 during seizure activity ($p = 0.0006$, $t = 6.56$, $df = 6$). The minute ventilation (ml/min) at baseline (121.0 ± 28.4) did not differ significantly from that during seizure activity (95.2 ± 41.0 ; $p = 0.214$, $t = 1.39$, $df = 6$).

3.1. Characteristics of central apnea onset

Central apneic episodes were characterized by an abrupt cessation of airflow as measured by plethysmography (Fig. 1). The majority of apneic period onsets occurred during expiration (125/161 apneic episodes, 78%; Fig. 2). 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%). With regard to our original question of whether central apneic episodes start with a preferred phase, the tendencies were clearly that expiration $>$ inspiration and that late cycle $>$ early cycle, but all phases were found (other details in the legend to Fig. 2).

3.2. Characteristics of central apnea offset

Apneic period offsets were more uniformly distributed between early and late expiration (27%, 34%) and inspiration (16%, 23%). It was also more common to end the apneic period with an irregularly shaped inspiratory or expiratory event ("unclassified").

To explore our original questions about whether apneic period offsets might indicate: 1) a reset of the respiratory rhythm, 2) a suspension or pausing of the respiratory rhythm with a continuation from the pause point at the end of the apneic period, 3) a transient suppression

of respiratory behavior with an uninterrupted “rhythm” in the background, or 4) a random re-entry into the respiratory cycle, we calculated the differences between onset and offset phases and between actual offset and predicted offset phases.

Differences between the respiratory phase at the onset of apnea and the corresponding offset phase varied over the full range of possible differences both within individual animals and across the set of animals (Fig. 3; additional statistical details appear in the legend).

Differences between the actual respiratory phase at offset and the phase predicted by extending the pre-apneic breathing pattern to the offset time were equally variable. The variability of difference values did not depend on the duration of the apneic episode. While there is no clear evidence of an offset phase predicted by the onset phase (chi-square not different from flat), there was a tendency for the offset phase to be end of expiration irrespective of onset phase (see Table 1).

3.3. Evidence for respiratory rhythm reset

As illustrated in Fig. 4 (first event in the record shown in A), episodes of central apnea were associated with a high frequency burst event that was pronounced in EEG records (and visible in ECG records) occurring at the onset of each central apneic episode and largely isolated by high pass filtering the EEG (see also (Nakase et al., 2016)). We excluded event detection during periods of obvious artifactual activity in recordings such as during manipulations of recording equipment. These high frequency EEG burst events marking apneic episode onsets also occurred in association with flatline plethysmography episodes that were <1 s in duration (i.e. below our threshold for inclusion) and during variable amplitude, but continuous airflow (second event in the record shown in A). The average frequency of central apneic episodes >1 s in duration was 0.5 ± 0.5 episodes/min (range: 0.1 to 1.8 episodes/min). The average frequency of high frequency bursts was much higher: 4.0 ± 2.6 bursts/min (range: 1.5 to 9.2 bursts/min). The average proportion of bursts marking apneic episodes >1 s in duration was $9.3 \pm 6.6\%$ (range: 1.1 to 23.4%). The burst events that occurred in the absence of an apneic episode >1 s in duration were associated with a highly stereotyped pattern of airflow within an animal (Fig. 4B). This stereotyped plethysmography pattern represents evidence that the respiratory rhythm was reset during each event. Fig. 4B also highlights how quickly the respiratory cycle variance caused the breathing pattern to individualize after each reset.

As these high frequency burst events occurred in association with expiration (Fig. 4), they may reflect an EMG signal in addition to a neural event, but these events are 1) associated with a minority of expiratory events, 2) not associated with other large expiratory events, and 3) clearly distinct from the EMG signals associated with intense effort to inspire during obstructive apnea that were previously described (Nakase et al., 2016).

3.4. Evidence for activation of the diving reflex

The evidence for a separate respiratory rhythm reset that is highly stereotyped combined with a cessation of airflow that is variable, suggested that the cessation of airflow may result from an independent process, e.g. abnormal activation of brainstem regions serving the diving reflex. The diving reflex is typically activated for many seconds to minutes and is

associated with co-activation of parasympathetic (slowing the heart) and sympathetic (raising systemic blood pressure) divisions of the autonomic nervous system.

We measured changes in RR intervals in ECG records and found a significant positive relation between the RR interval duration and the duration of the central apneic episode (Fig. 5), even with short apnea durations, consistent with the possibility that the diving reflex is activated. RR interval changes were, however, relatively small (<10% over the range of apnea durations tested). A correlation of EEG seizure activity amplitude with apnea duration (Fig. 6) indicates that while the systemic effects of these apneic episodes appear to be quite modest, there is clearly an impact on heart rate and EEG activity.

4. Discussion

Using a rat model that has been characterized with recordings during seizure activity of autonomic and laryngeal nerve activity, plethysmography, laryngoscopy, ECG, echocardiography, and blood pressure recordings, we studied periods of seizure-associated central apnea, defined as periods >1 s with no apparent respiratory effort and no airflow, to evaluate the impact of seizure spread on respiratory rhythm generation and breathing as inferred from changes in plethysmographs. Our main findings are: 1) the preferred phase for central apnea onset was the end of expiration, 2) onset phases did not predict offset phases, 3) events without cessation of airflow were detected that clearly indicated a respiratory rhythm reset, and 4) modest, but significant heart rate decreases occurred during apneic periods > 5 s. We conclude that: 1) the respiratory rhythm generator is occasionally reset during seizure events, 2) cessation of airflow during central apneic periods is likely due to the activation of brainstem regions serving the diving reflex, and 3) the variable offset phases are due to highly variable respiratory patterns after the first 1 s following respiratory rhythm reset. The reset and examples of continuous respiratory rhythm in some cases indicate that these central apneic episodes are not the result of slower mechanisms that might disable the respiratory rhythm generator. Finally, this is an excellent model system for studying seizure-related central apnea and the activation of the diving reflex.

Previously, we showed that seizure-associated obstructive apnea due to laryngospasm was able to cause rapid desaturation, dramatic cardiac evidence of hypoxia, respiratory arrest, and eventual death, but central apneic episodes caused comparatively little evidence of hypoxemia (Nakase et al., 2016). With regard to sudden death in epilepsy (SUDEP), we have argued that the obstructive apneic periods lead to death whereas central apneic episodes appear to be benign (Nakase et al., 2016; Stewart et al., 2016). That said, we believe that an understanding of the systemic impact of seizure activity depends upon an understanding of the mechanisms and consequences of seizure-associated autonomic, cardiac, and respiratory derangements. This study reinforces the findings of a modest impact of central apneic episodes and provides critical detail on the consequences of seizure spread into respiratory brainstem areas with a demonstration that the respiratory rhythm generator can be repeatedly reset and seizure activity can activate central airway reflex pathways.

4.1. Respiratory rhythm reset in central apneic episodes

Whereas central apneic period onset phases showed a strong tendency for late expiration, the scatter of offset phases and onset-offset differences pointed away from an actual resetting of the respiratory rhythm. Two key findings that, in fact, led to a demonstration of a rhythm reset were 1) every central apneic period onset was associated with a high frequency burst event detectable in the EEG, and 2) these EEG events also occurred during non-apneic periods in plethysmographs. Alignment of the non-apneic events by the EEG markers showed a very clear reset of the respiratory rhythm, which often included a full inspiration/expiration cycle that preceded the EEG event – indicating that the EEG marker occurs at a time that is after the actual reset.

The within- and across-animal variance of offset phases is also accounted for in the examination of identified non-apneic periods. The reset caused a highly stereotyped airflow pattern that lasted about 1 s, developing rapidly thereafter an unpredictable flow pattern typical of the seizure-associated irregular, tachypnic breathing. The central conclusion is that the respiratory rhythm is reset, but the absence of airflow in plethysmographs must be due to a separate process.

The high frequency burst events that were detected in EEG records in association with each central apneic episode and could be used to identify altered breathing without the absence of airflow suggest that a specific cortical event may trigger the brainstem responses observed. At this point, however, the origin of the high frequency burst events is unknown, and while these events helped to define the types of respiratory derangement caused by seizure activity, there is still much work to be done to define the mechanisms that determine when such events occur and how they spread from limbic cortical regions to brainstem.

4.2. The diving reflex

The mammalian diving response is an extremely powerful reflex response to nasopharyngeal stimulation with water that results in apnea, bradycardia, and increased systemic blood pressure (Golanov et al., 2016; Gooden, 1994; Panneton, 2013; Panneton et al., 2010; Panneton et al., 2012). The coordinated respiratory, parasympathetic, and sympathetic responses highlight the integration of these systems. Many areas involved with respiratory rhythm generation or motor behavior are also established as major contributors to sympathetic and parasympathetic outflow (see below). For example, pre-Bötzinger and Bötzinger complexes are respiratory structures closely associated with rostral ventrolateral medulla, the origin for premotor sympathetic neurons. Caudal VLM and nucleus ambiguus share connections with nucleus tractus solitarius (NTS), which has respiratory and autonomic roles, further blending cardiovascular and respiratory control.

Although all details of this complex pontine-medullary-spinal circuitry are not fully established, the core elements of the circuit include 1) activation of sensory inputs (via anterior ethmoid nerve) and chemoreceptors (e.g. carotid body and sinus) (Huang and Peng, 1976; McCulloch, 2012), which terminate in 2) the medullary dorsal horn (MDH; especially the ventral superficial parts), a structure that projects to 3) multiple targets including NTS, the ventrolateral medulla (caudal and rostral VLM and the Bötzinger complex), the

intertrigeminal region, lateral parabrachial nucleus, the Kölliker-Fuse nucleus, area A5, and the superior salivatory nucleus (Hollandsworth et al., 2009; Panneton et al., 2000; Rybka and McCulloch, 2006).

The respiratory rhythm originates in the pre-Bötzinger complex of the ventrolateral medulla (e.g. (Forster et al., 2014; Marchenko et al., 2016; Ramirez et al., 2016; Ramirez and Richter, 1996; Rekling and Feldman, 1998; Smith et al., 2000)) and is projected into the spinal phrenic nucleus either monosynaptically or indirectly via projections that are relayed in the Kölliker-Fuse nucleus, the rostral ventral respiratory group, Böttinger complex, NTS, and several other areas (Dobbins and Feldman, 1994; Ellenberger and Feldman, 1988; Golanov et al., 2016; Huang et al., 1991; Monteau and Hilaire, 1991; Panneton, 2013; Panneton et al., 2012; Tan et al., 2010; Yokota et al., 2007; Yokota et al., 2008; Yokota et al., 2001; Yokota et al., 2004).

The apnea associated with the diving reflex appears to be a suppression of respiratory behavior as evidenced by experiments demonstrating that anterior ethmoid nerve stimulation was associated with the complete cessation of phrenic nerve bursting for the duration of stimulation (McCulloch, 2012). This interpretation is also supported by demonstrations that excitation of the Kölliker-Fuse nucleus can suppress output of the phrenic nucleus (Dutschmann and Herbert, 1996) (see also (Dutschmann and Dick, 2012; Dutschmann and Herbert, 2006; Song et al., 2010)). As we have already shown seizure spread into multiple autonomic medullary sites (Sakamoto et al., 2008), seizure spread into adjacent medullary respiratory centers seems certain, especially in view of ictal breathing abnormalities in these animals (Nakase et al., 2016). Our findings here suggest that respiratory rhythm generation can withstand or is protected from considerable overactivation, but seizure activity can reset the respiratory rhythm and occasionally silence the phrenic nucleus in a manner similar to activation of the diving reflex. This is a more likely explanation for the multiple brief central apneic episodes than a mechanism involving sensory activation by excessive ictal salivation, which would be expected to produce more frequent and longer duration apneic episodes as saliva accumulates, or other mechanisms that may lead to a prolonged disabling of the respiratory circuitry.

The strongest evidence that the diving reflex is not the mechanism for airflow cessation is the fact that the HR changed in our animals by <10%, whereas other studies have reported HR changes over 50% in rats (e.g. (Huang and Peng, 1976; Huang et al., 1991; Lin and Baker, 1975)). Application of glutamate to the NTS, however, was found to attenuate the HR response (Huang et al., 1991), and seizure spread to involve NTS may be expected to have a similar impact.

5. Conclusions

We have characterized episodes of seizure-associated central apnea and argued from the data for a separate respiratory rhythm reset and suppression of breathing behavior. The reset, but continuously active respiratory pattern generator, a diving reflex-mediated suppression of respiratory behavior, and the co-activity of autonomic subdivisions indicate that seizure-associated central apneic periods are active periods, not episodes where brainstem neurons

have failed. The main significance of this finding is that our growing understanding of the impact of seizure spread on cardiac and respiratory function enables a better understanding of which derangements are benign and which are malignant. Additionally, features of recordings may emerge as useful metrics for assessing seizure spread and potentially subclassifying seizure patterns. Unfortunately, our data do not permit us to identify the causes of apneic episode onsets and offsets, but they do indicate that the answer will have multiple parts, including: 1) the neuronal basis for resetting the respiratory rhythm generator, 2) the mechanism by which this reset signal also activates the neural circuitry to transiently suppress breathing, and 3) the neuronal determinants of the duration of the apneic episode. Clearly, many questions regarding the spread of seizure activity into respiratory centers remain unanswered, but this experimental preparation may be useful in approaching a number of them. A better understanding of apneic episode starts and stops could allow for improved control over oxygen status during seizures.

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Fig. 1.

Central apnea during seizure activity and strategy used for estimating onset and offset phases. A. Example recording to illustrate brief episode (about 2.5 s in duration) of central apnea defined as a period >1 s in duration with no evidence of airflow on plethysmography. Sweeps from top to bottom are high pass filtered EEG, plethysmograph, ECG, and two EEG recordings, one from each hemisphere over dorsal hippocampus. Segment of raw data is taken from a longer seizure episode; the onset and offset of the seizure itself are not illustrated. Calibrations are 0.025 mV filtered EEG, 0.2 ml plethysmograph, 0.05 mV ECG,

and 0.2 mV for both EEG channels. Time calibration is 4 s. B. Detail of apnea episode onset (top) and offset (bottom) with respective matched model breaths (shown in gray) used for calculation of onset and offset phase. Matched model breaths were taken from pre-apnea breathing (for the onset) and post-apnea breathing (for the offset) such that the breath closely matched the partial breaths at onset or offset. Time calibration is 1 s.

Fig. 2.

Distributions of apnea durations and onset or offset phases. A. Model sine wave used to illustrate the classification of partial breaths as early or late expiration (positive phases) or inspiration (negative phases). Early expiration was defined by the range $0 < X < 90$ (blue). Late expiration was defined by the range $90 < X < 180$ (dark gray). Early inspiration was defined as $-180 < X < -90$ (red). Late inspiration was defined as $-90 < X < 0$ (light gray). B. Frequency distribution of apnea durations. Range was 1.04 to 36.18 s (mean $\pm$ std. dev. were 3.2 ± 3.7 s, median = 2.0 s). C. Distribution of apnea onsets by phase (colors match those used in top left panel). D. Distribution of apnea offsets. Very unusual breath shapes were grouped as “unclassified” and breaths that were larger than possible pre-or post-apnea model breaths were grouped as “oversized” (black bars). Taking only the 4 quarter cycles, the onset and offset distributions were both significantly different from flat (onset: chi square = 69.34, df = 3, $p < 0.0001$; offset: chi square = 30.44, $p < 0.0001$). Note the preference for late expiration at event onset, and the overall tendency for expiration over

inspiration and the preference for late in either cycle over early. At event offset, the number of unclassified breaths was increased, but the preferences for late expiration and late cycle remain.

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Fig. 3. Predicting offset phase by onset phase or calculation from respiratory rate/rhythm. Top: plot of the difference between the onset phase and the offset phase as a function of the duration of the apnea period. Phase differences are expressed in degrees with zero degrees indicating no difference and phase differences are expressed between zero and ± 180 degrees. An offset phase occurring earlier than its onset phase is plotted to the left and offset phases occurring later than their corresponding onset phases are plotted to the right. The abscissa (apnea duration) is plotted on the vertical and the ordinate (phase lag/lead) is plotted on the

horizontal for easier viewing. Two animals are highlighted to illustrate that the full range of phase differences occurred within single animals. One animal (orange) has 31 events plotted, and the other (purple) has 10. Inset to the upper right plots the frequencies of the phase differences in 45 degree bins. This distribution was not different from flat (chi square = 6.8, $df = 7$, $p = 0.45$). Bottom: plot of the difference between the actual offset phase and the phase predicted by continuing the pre-apnea breathing pattern through the time of the apnea event. Other features similar to plots in the top half of the figure. Frequency distribution (inset) did not differ from flat (chi square = 6.3, $p = 0.50$).

Fig. 4.

Phase reset of respiratory rhythm revealed by events that are not masked by the diving reflex. A. Example record of two events, one event that includes complete cessation of breathing effort as evidenced by flatline plethysmograph, and a later event where respiratory effort did not stop. Records from top to bottom are high pass filtered EEG (top EEG channel was filtered), plethysmograph, ECG, and two EEG recordings, one from each hemisphere over dorsal hippocampus. The pronounced artifacts evident on the filtered EEG trace are associated with the central apneic episode lasting about 1.5 s (onset indicated by arrow), and

a later event that does not include cessation of airflow. High frequency events are evident in the full bandpass EEG records. Segment of raw data is taken from a longer seizure episode; the onset and offset of the seizure itself are not illustrated. Calibration are 0.025 mV filtered EEG, 0.2 ml plethysmograph, 0.05 mV ECG, and 0.2 mV for both EEG channels. Time calibration is 2 s. B. Shows 20 superimposed sequential non-apneic events from a single animal to highlight the complete alignment of the pre- and post-artifact plethysmograph records. This alignment, given the broader range of phases leading up to the event onset, indicates a resetting of the respiratory rhythm, but the rhythm after about 1 to 1.5 s becomes highly variable. Calibrations are 0.05 mV filtered EEG and 0.2 ml plethysmograph. Time calibration is 1 s.

Fig. 5.

Changes in RR interval duration with apnea duration. Plot of the percent change in RR interval duration pre-apnea compared to the end of an apnea episode against the duration of the apnea episode. RR interval changes for apnea episodes were binned into three categories: between 1 and 5 s ($n = 137$), between 5 and 10 s ($n = 22$), and >10 s ($n = 8$). RR interval changes were significantly positive for the 5–10 s ($p < 0.001$, ##) and >10 s groups ($p < 0.01$, #). Comparing groups with an ANOVA, the 5–10 s and >10 s groups were significantly larger than the 1–5 s group ($F[2, 164] = 17.37$, $p < 0.0001$; $p < 0.001$, **; $p < 0.0001$, ***). Shown are the means $\pm$ SEM.

Fig. 6.

Changes in EEG amplitude with relatively long duration apnea episodes. Although there were no significant changes in seizure intensity measured by changes in seizure RMS amplitude due to periods of central apnea, an examination of the longest duration apnea episodes suggests a trend toward reduced seizure intensity.

Table 1
Frequency data for onsets, offsets, and conditions using area or time for estimations of onset and offset phases.

Onset and offset phases for periods of central apnea												
	PHASE											
	Early expiration			Late expiration			Early inspiration			Late inspiration		
	N	%		N	%		N	%		N	%	
Calculations based on area (all events)												
Phase at onset	43	22.6	82	10	23.3	43.2	13	6.8	23	12.1	21	11.1
Phase at offset	11	5.8	46	24.2			16	8.4	37	19.5	68	35.8
Offset given onset												
Early expiration onset	4	9.3		23.3	3	7.0	4	9.3	21	48.8	1	2.3
Late expiration onset	2	2.4	22	26.8	12	14.6	11	13.4	27	32.9	8	9.8
Early inspiration onset	1	7.7			1	7.7	8	61.5	3	23.1		13
Late inspiration onset	1	4.3	10	43.5	1	4.3	7	30.4	4	17.4		23
Unclassified onset phase	3	14.3	2	9.5	1	4.8	2	9.5	11	52.4	2	9.5
Onset with oversized breath			2	25.0			2	25.0	2	25.0	2	25.0
Calculations based on time (all events)												
Phase at onset	25	13.2	96	50.5	8	4.2	27	14.2	21	11.1	13	6.8
Phase at offset	20	10.5	35	18.4	30	15.8	29	15.3	68	35.8	8	4.2
Offset given onset												
Early expiration onset	1	4.0	6	24.0	4	16.0	2	8.0	11	44.0	1	4.0
Late expiration onset	10	10.4	21	21.9	20	20.8	9	9.4	32	33.3	4	4.2
Early inspiration onset							6	75.0	2	25.0		8
Late inspiration onset	6	22.2	5	18.5	4	14.8	6	22.2	6	22.2		27
Unclassified onset phase	3	14.3	2	9.5	1	4.8	2	9.5	11	52.4	2	9.5
Onset with oversized breath			4	30.8	1	7.7	2	15.4	6	46.2		13
Largest N, %												
Largest N, % with defined phase												

Summary of onset and offset phases calculated by using area under the onset breath and its matched model breath or the offset breath and its model breath. Calculations using the area of the aborted breath and a matched model breath are in the top half of the table; calculations using the time of the aborted breath and a matched model breath are in the bottom half of the table. The number (N) and fraction (%) of the total of apneic episodes of each onset or offset phase is given. In the second set of data for each section, the distribution of offset phases was determined for each type of onset phase and reported separately. The highest number in each row is highlighted with bold/italic type. The highest number within the set of 4 identifiable phases (i.e. excluding the undefined and oversized breaths) is highlighted with gray shading. Note that the late expiration phase was the most common phase for apnea offset, regardless of the onset phase, but this is a very weak pattern in many cases. Chi square analyses to

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compare the distribution of events within the 4 identifiable phases were significantly different from flat for onsets (area: $n = 161$, $p < 0.0001$; time: $n = 156$, $p < 0.0001$) and offsets based on area ($n = 110$, $p < 0.0001$) but not time ($n = 114$, $p = 0.25$).