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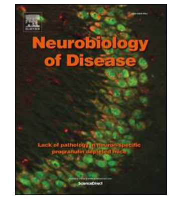

Diving responses elicited by nasopharyngeal irrigation mimic seizure-associated central apneic episodes in a rat model

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A B S T R A C T

The spread of epileptic seizure activity to brainstem respiratory and autonomic regions can elicit episodes of obstructive apnea and of central apnea with significant oxygen desaturation and bradycardia. Previously, we argued that central apneic events were not consequences of respiratory or autonomic activity failure, but rather an active brainstem behavior equivalent to the diving response resulting from seizure spread.

To test the similarities of spontaneous seizure-associated central apneic episodes to evoked diving responses, we used nasopharyngeal irrigation with either cold water or mist for 10 or 60 s to elicit the diving response in urethane-anesthetized animals with or without kainic acid-induced seizure activity.

Diving responses included larger cardiovascular changes during mist stimuli than during water stimuli. Apneic responses lasted longer than 10 s in response to 10 s stimuli or about 40 s in response to 60 s stimuli, and outlasted bradycardia. Repeated 10 s mist applications led to an uncoupling of the apneic episodes (which always occurred) from the bradycardia (which became less pronounced with repetition). These uncoupled events matched the features of observed spontaneous seizure-associated central apneic episodes. The duration of spontaneous central apneic episodes correlated with their frequency, i.e. longer events occurred when there were more events.

Based on our ability to replicate the properties of seizure-associated central apneic events with evoked diving responses during seizure activity, we conclude that seizure-associated central apnea and the diving response share a common neural basis and may reflect an attempt by brainstem networks to protect core physiology during seizure activity.

1. Introduction

It is estimated that between 2 and 3 million people living in the United States carry a diagnosis of epilepsy (e.g. (Delgado-Escueta et al., 1999; Dworetzky and Schuele, 2015; Sander, 2003)). Among this population, approximately 5000 people die annually from Sudden Unexpected Death in Epilepsy (SUDEP) (e.g. (Devinsky et al., 2016; Harden et al., 2017; Jehi and Schuele, 2015; Lamberts et al., 2015; Surges and Sander, 2012)). SUDEP is the most common cause of death in young people with epilepsy (e.g. (Devinsky et al., 2016; Dworetzky and Schuele, 2015; Friedman et al., 2018)). Though the mechanism of SUDEP remains obscure, human and animal data suggest that SUDEP occurrences are characterized by a sequence of events that include respiratory dysfunction, bradycardia, asystole, and death (e.g. (Devinsky et al., 2016; Kennedy and Seyal, 2015; Nakase et al., 2016; Ryvlin et al., 2013)).

Seizure spread to respiratory and autonomic nervous system brainstem structures can elicit episodes of central apnea and obstructive apnea in a rat model, which can be associated with significant oxygen desaturation and bradycardia (Nakase et al., 2016). Obstructive apnea in these animals was demonstrated to be caused by intense adduction of laryngeal muscles as a result of seizure spread to the recurrent laryngeal

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nerve, a branch of the vagus nerve that contains laryngeal motor axons for both abduction and adduction of laryngeal muscles (Nakase et al., 2016). In this animal model, episodes of obstructive apnea were characterized by a closed airway, intense non-productive inspiratory effort, pronounced hypoxemia, bradycardia, respiratory arrest, and eventually death (Nakase et al., 2016; Stewart et al., 2017) (see also (Lacuey et al., 2018)).

Central apneic events during seizure activity in these same animals were marked with sudden cessation of airflow and respiratory effort, a widely open airway due to active abduction of the vocal folds, and a relatively smaller decline in heart rate (Nakase et al., 2016; Villiere et al., 2017). These episodes included a respiratory rhythm reset and, we argued, resulted from activation of the diving response (reflex) by spread of seizure activity into respiratory brainstem regions (Villiere et al., 2017). Importantly, these episodes of central apnea involved considerable brainstem activity and contrast with the form of central apnea referred to as "terminal apnea" (Ryvlin et al., 2013), which was shown to occur late in the sequence of events from seizure to death and reflects, in our opinion, the period of respiratory arrest (Nakase et al., 2016; Stewart, 2018; Stewart et al., 2017).

The diving response or diving reflex is a highly conserved and integrative physiological response to cold-water stimulation of the face or nasal mucosa that includes a period of apnea coupled with the activation of both sympathetic and parasympathetic divisions of the autonomic nervous system (e.g. (Gooden, 1994; McCulloch, 2012)). The diving response conserves oxygen for the body core to maintain essential neural and cardiac functions by decreasing heart rate, and increasing peripheral vascular resistance (e.g. (McCulloch, 2012)); it also appears to be neuroprotective (Golanov et al., 2016).

To better evaluate our conclusion that seizure associated central apneic events resembled diving responses triggered by seizure spread to the brainstem, we evoked diving responses with nasopharyngeal irrigation in animals with or without ongoing seizure activity. Our objective was to (1) characterize diving reflex properties in our rat model and (2) compare diving responses during seizure activity with spontaneous seizure-associated central apneic events. Further, we sought to determine if patterns of central apnea during seizure activity (such as event duration and/or frequency of occurrence) might be positively or negatively associated with the tendency for obstructive apnea to occur, and thus serve as a predictor of obstructive apnea risk.

2. Materials and methods

All procedures were conducted at SUNY Downstate Medical Center and were approved by the Institutional Animal Care and Use Committee. Animal protocols complied with the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals. Adult male Sprague-Dawley albino rats were housed in AAALAC accredited facilities, which have a 12/12 h light/dark cycle and are maintained at 55% relative humidity and 23 °C. Animals were monitored daily and had ad libitum access to water and food.

2.1. Recordings

Animals were anesthetized with urethane (1.5 mg/kg IP given as two injections separated by 20 min) and prepared for recordings of the electroencephalogram (EEG), electrocardiogram (ECG), pulse oximetry, echocardiography, tracheal pressure, and plethysmography as described in detail previously (Nakase et al., 2016; Sakamoto et al., 2008; Villiere et al., 2017). Briefly, (1) ECG was obtained from limb electrodes comprised of copper straps with conductive paste, (2) tracheal pressures were obtained from a force transducer attached to the sidearm of a tracheal t-tube implant (CyQ, Columbus Instruments, Columbus, OH), (3) pulse oximetry was continuously monitored from the thigh musculature (probe: TDR-43C, Med Associates, St Albans, VT; pulse oximeter: CANL-425SV-A,Med Assoc.), (4) echocardiography was performed with a 15 MHz probe attached to a Phillips 5500 echocardiography machine (Phillips, Andover, MA, USA), and (5) plethysmography was done with a head-out plethysmograph chamber with built-in pneumotach (PLY3013; Buxco, Wilmington, NC). Note that the tracheal pressures obtained from the t-tube sidearm do not represent the actual pressure developed during inspiration or expiration, but serve to indicate that tidal breathing is occurring and when the effort associated with inspiration or expiration is increased. Only when the system is closed, e.g. during controlled airway occlusion (Nakase et al., 2016; Villiere et al., 2018), does the pressure reflect the actual inspiratory or expiratory pressure. All signals were bandpass-filtered (1 Hz to ≤1 kHz) and amplified (Model 1800 AC amplifier with headstages; A-M Systems, Sequim, WA) before digitization at 2 kHz (Micro1401; Cambridge Electronic Design, Cambridge, UK) and processing with software (Spike2, Cambridge Electronic Design, Cambridge, England).

2.2. Diving responses

In diving response experiments, each animal received a tracheal t-tube implant through a ventral neck incision. After exposure of the trachea, the recurrent laryngeal nerves were separated from the tracheal sides before placing and tying the implant in place. The average time between the first dose of urethane and the first incision to prepare the animal was 82.8 ± 22.9 min.

The diving reflex was induced by application of ice-cold deionized water or ice-cold mist to the nasopharynx for 10 s or 60 s while the rat was supine (water was taken from an ice-water mixture at the start of each trial). The tracheal implant prevented aspiration. The average time between the first dose of urethane and the first application of mist as a diving response stimulus was 170.6 ± 36.8 min in animals not receiving kainic acid or prior to receiving kainic acid. In animals receiving kainic acid, the time between the injection of kainic acid and the first application of mist as a diving response stimulus during seizure activity was 55.2 ± 12.7 min. In figures, irrigation with water was used as a reference for the intensity of diving responses evoked by 10 s mist stimuli because water is a commonly used stimulus to elicit the diving response, and complete occlusion was used as a reference for the intensity and duration of the diving response components during 60 s mist stimuli.

For nasopharyngeal water application, polyethylene tubing (Clay Adams Intramedic tubing: inside diameter: 0.023″, outside diameter: 0.038) was inserted through one naris into the nasopharynx. A 12 ml syringe was used to apply water for 10 s or 60 s through a 23-gauge needle that connected the syringe to a 1-inch segment of polyethylene tubing. Water was free to fill and overflow the oral cavity and all water was suctioned from the nasopharynx at the end of each trial.

For nasopharyngeal mist application, an airbrush compressor (Central Pneumatic Oil-less Airbrush Compressor Model 60,329, Harbor Freight Tools) running at 40 PSI was used to aerosolize the ice-cold water for application via a 1-inch segment of the polyethylene tubing adapted to the end of the siphon airbrush (Model 95,810) with a 23-gauge needle.

The volumes that were applied averaged as follows: 3.94 ± 0.33 ml (n = 10 samples) during 10 s water stimulation; 0.22 ± 0.06 ml (n = 10 samples) during 10 s mist stimulation; and 1.32 ± 0.42 ml (n = 6 samples) during 60 s mist stimulation. We Stimulus onsets and offsets were recorded together with the other signals and used for offline analyses.

2.3. Seizure induction

Seizures were induced with kainic acid (KA; 10 mg/kg IP; Sigma-Aldrich, St. Louis, MO) as described previously (Nakase et al., 2016; Sakamoto et al., 2008) and evaluated from EEG recordings obtained from stainless steel screws placed epidurally over each hippocampus (through burr holes at skull coordinates of 3 mm lateral to the midline and 5 mm anterior to lambda; (Paxinos and Watson, 2007)) and referred to a stainless steel screw placed over cerebellum (in the midline). EEG signals were filtered (1 Hz −1 kHz), amplified, and digitized (2 kHz sampling rate) along with other signals as described above.

2.4. Data collection and processing

Statistical calculations were made in GraphPad Prism 6 software (La Jolla, CA) or with IBM SPSS Statistics (version 21 or 23; Armonk, NY) or R software (www.r-project.org). Data are presented in the text as means ± standard deviation and may be shown on graphs (specified in the figure legends) as means ± standard deviation, standard error of the mean, or with 95% confidence intervals. Significance was determined at $p < .05$. Additional details appear in the Results and figure legends.

3. Results

We evoked and analyzed diving responses in 24 rats, 10 of which had diving responses evoked during kainic acid-induced seizure activity. A separate set of 10 animals were treated with 60 s episodes of complete airway occlusion. An additional 12 animals were studied for spontaneous episodes of central apnea occurring during seizure activity and spontaneous episodes of obstructive apnea. Rat weights averaged 282.8 ± 58.2 g (range: 216–480 g) and did not differ between groups.

3.1. Basic properties of the diving response

As described in the Methods, the diving response was evoked with the application of ice-cold water (9 events in 7 rats) or ice-cold mist (9 events in 4 rats) applied to the nasopharynx for 10 s or ice-cold mist for 60 s (6 events in 5 rats). Examples of the diving responses to 10 s and 60 s stimuli are shown in Fig. 1.

In every evoked diving response event, a period of apnea occurred in association with rapid decreases in heart rate (reported as an increase in RR interval) and oxygen saturation. Apneic periods associated with initial 10 s stimuli tended to outlast the stimuli (average apnea duration = 16.7 ± 5.6 s, N = 9; but see changes in apnea duration with repetition, below), whereas apneic periods associated with 60 s stimuli never lasted as long as the stimulus (average apnea duration = 24.8 ± 7.9 s, N = 6). The changes in oxygen saturation, RR interval duration, and inspiratory forces (airway pressure) are summarized for 10 s mist (blue) and water (orange) stimuli in Fig. 2. Also shown are diving response parameter summaries for 10 s mist stimuli given during the presence of seizure activity (shown in green and described in more detail in the next Results section). The greatest response magnitudes in all parameters were recorded during mist applications. The weakest responses were observed during water application. Mist application during seizure activity was intermediate between the other two conditions. Means are plotted with 95% confidence intervals at each point to identify significant changes from baseline. Significant differences between stimuli/conditions during post hoc comparisons at each point after two-way ANOVA are marked with asterisks or in the legends of Figs. 2 and 3.

Data summaries for 60 s stimuli are shown in Fig. 3, but the diving responses evoked with 60 s application of mist under baseline (blue) or seizure activity (green) are shown compared with 60 s episodes of controlled airway occlusion (obstructive apnea; shown in red). Respiratory frequency in the occluded animals is a measure of the frequency of attempted breaths and contrasts with the rapid cessation of inspiratory attempts in animals displaying the diving response. Inspiratory effort returned in all long-duration diving response animals, but the occluded animals typically displayed respiratory arrest (i.e. spontaneous breaths did not return after the occlusion was removed) and animals required resuscitation. The appearance of bradycardia was more rapid in the diving response compared to the longer period of development during obstructive apnea (Fig. 3 bottom left).

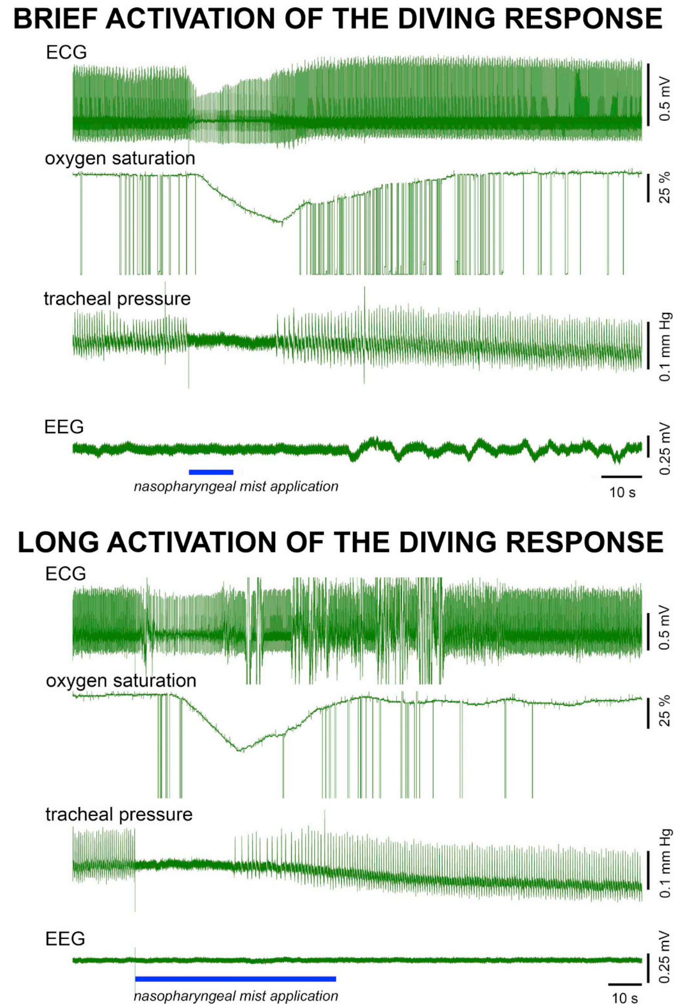

Fig. 1. Examples of diving responses evoked with nasopharyngeal mist application for 10 or 60 s.
Nasopharyngeal application of ice-cold mist for approximately 10 s (top panel) or 60 s (bottom panel) was associated with diving responses that included apnea (evident on the tracheal pressure traces), bradycardia (ECG is shown in the top trace of each panel), and a decrease in oxygen saturation (second trace of each panel). Note that the apnea duration outlasts the stimulus during short applications, but breathing returns before the long duration nasopharyngeal stimulus ends (bottom panel). Magnitude of the pulse oximetry change is comparable in both examples. Nasopharyngeal mist application time is indicated by the blue bars at the bottom of each panel. Calibrations on the figure. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Diving responses during seizure activity

The diving response was evoked with the application of ice-cold mist for 10 s (19 events in 5 rats) or 60 s (5 events in 5 rats) applied to the nasopharynx during kainic acid-induced seizure activity. As shown in Fig. 2, diving responses evoked by 10 s mist application were weaker during kainic acid-induced seizure activity (green plots) than during the pre-seizure or non-seizure states (blue plots). Changes in respiratory frequency, oxygen saturation, and RR interval duration were all smaller during seizure activity. During 60 s stimuli (Fig. 3), the pattern was similar, except that the changes in oxygen saturation during seizure activity were at least as large and sometimes greater than the pre-seizure/non-seizure events.

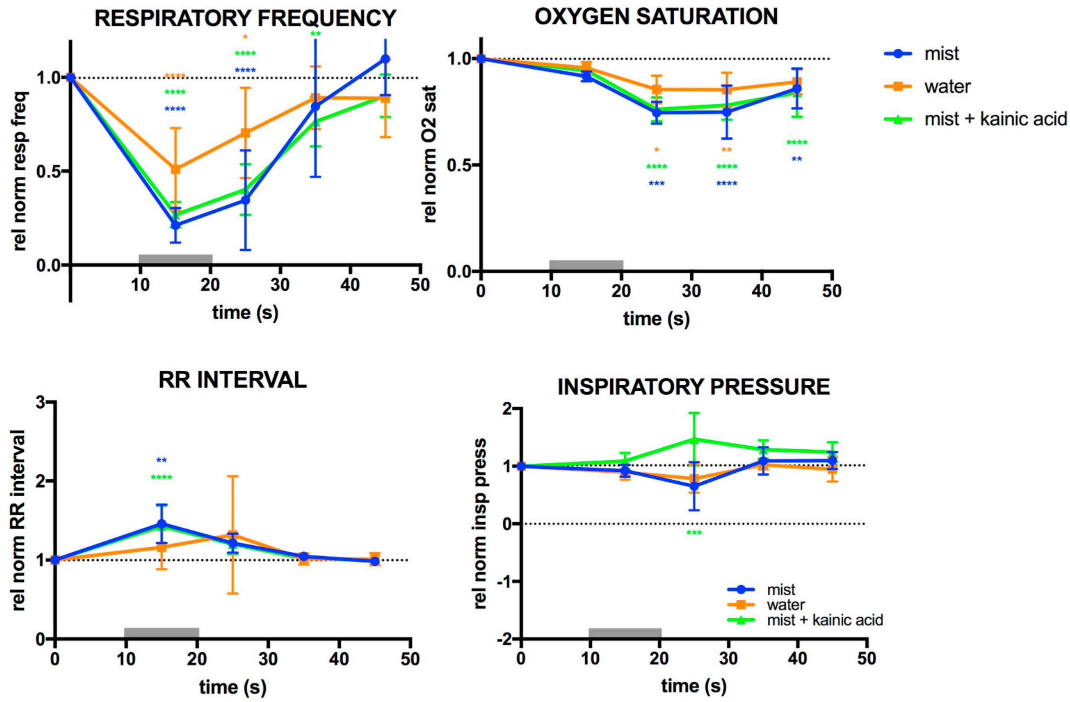

Fig. 2. Summary of cardiovascular and respiratory impact of short duration diving response activation.
Summaries of changes in respiratory frequency (upper left), oxygen saturation (upper right), RR interval duration (lower left), and tracheal pressure (lower right) during nasopharyngeal application of ice-cold mist (blue) or ice-cold water (orange) for periods of 10 s. Apnea and bradycardia are characteristic of the diving response. Also shown are the changes associated with nasopharyngeal mist application during seizure activity (green). Numbers of evoked diving responses analyzed: mist = 9, water = 9, mist + KA = 19. Plotted are means +/− 95% confidence limits. Repeated measures two way ANOVA: overall $F(34, 136) = 4.300$, $p < .0001$ upper left; 2.384, $p < .0001$ lower left; 3.736, $p < .0001$ upper right; 2.663, $p < .0001$ lower right; time (row factor) $F(4, 136) = p < .0001$ upper left, 0.0002 lower left, < 0.0001 upper right, and NS lower right. Asterisks on the plot indicate significant differences of points relative to the value at time 0 after post hoc correction with Sidak's multiple comparisons test. Post-hoc comparisons between groups showed differences as follows: Respiratory frequency (during mist application): mist vs water $p = .029$, mist + KA vs water $p = .042$; (1st point after the end of the mist or water application): mist vs water $p = .006$, mist + KA vs water $p = .007$; RR Interval and Oxygen saturation (during mist or water application): NS; Inspiratory pressure (1st point after the end of the mist or water application): mist vs mist + KA $p < .0001$, water vs mist + KA $p = .0001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In addition to the response set that included apnea, an RR interval increase, and a decrease in oxygen saturation, the peak-to-peak amplitude of the EEG (a crude measure of seizure intensity) decreased. Changes in EEG were evaluated as integrals of 10 s bins before, during, and after diving response stimuli or controlled airway occlusion. EEG integrals showed the largest drop during 60 s controlled airway occlusion (integral declined to 30.2 ± 8.1% of pre-occlusion value; n = 4) versus 60 s ice-cold mist application (54.8 ± 20.0% of pre-occlusion value; n = 4) and 10 s mist application (61.1 ± 24.3% of pre-occlusion integral; n = 19). Changes in EEG amplitude were tightly correlated with RR interval changes ($R^2 = 0.88$; $p = .0178$, two-tailed).

Evoked apnea onset (10 s mist only) occurred at the end of a complete or aborted expiration in 86% of events and at the end of an aborted inspiration in the remainder. Breathing resumed with an inspiratory effort in 95% of evoked events. The preferred onset phase for evoked events matched the preferred onset phase for spontaneous seizure-associated central apneic events (Villiere et al., 2017). Offset phases for spontaneous seizure-associated central apneic events were more variable.

A critical feature of spontaneous seizure-associated central apneic episodes was their relatively smaller impact on cardiovascular parameters compared with obstructive apneic episodes (Nakase et al., 2016; Villiere et al., 2017), which may be due to glutamatergic excitation in brainstem areas such as the nucleus tractus solitarius (Huang et al., 1991). We found that we could achieve a replica of the seizure-associated central apneic behavior by inducing the diving response multiple times. As shown in Fig. 4, the cardiac and EEG changes associated with evoked diving responses were initially substantial, but the magnitude of cardiac and EEG changes decreased with response replication. This behavior is summarized in Fig. 5. Although the magnitudes of multiple parameters, including apneic duration, decreased in response to equivalent stimuli, the magnitude of oxygen desaturation remained similar across replicates (i.e. the rate of desaturation increased). The average time between repeated episodes was 4.2 ± 2.1 min (N = 12 episodes in 7 rats; range: 1.6 to 9.1 min; range in the number of replicates: 2–5). The late replicates closely resembled the spontaneous seizure-associated central apneic events (see Fig. 6).

The response parameter changes as a function of replication were not due to a trend toward different starting oxygen saturations or RR intervals as a function of time or event number, i.e. the slopes of these starting values as a function of replicate number were flat. The slope for the relation of starting oxygen saturation against event number was −0.0045 ($R^2 = 0.020$, n = 19 mist + KA events; slope = −0.0067, $R^2 = 0.045$, n = 21 mist events including KA and non-KA) and the slope for the relation of RR interval against event number was 0.0005 ($R^2 = 0.005$, n = 19 mist + KA events; slope = 0.0001, $R^2 = 0.0002$, n = 21 mist events including KA and non-KA).

3.3. Spontaneous episodes of seizure-associated central apnea

We sought to compare spontaneous seizure-associated central apneic episodes with evoked diving responses and to determine if the occurrence of spontaneous seizure-associated apneic events predicted the occurrence of obstructive apnea.

Whereas Fig. 5 shows the effects of diving response replication in a setting where we can precisely know the event number because every

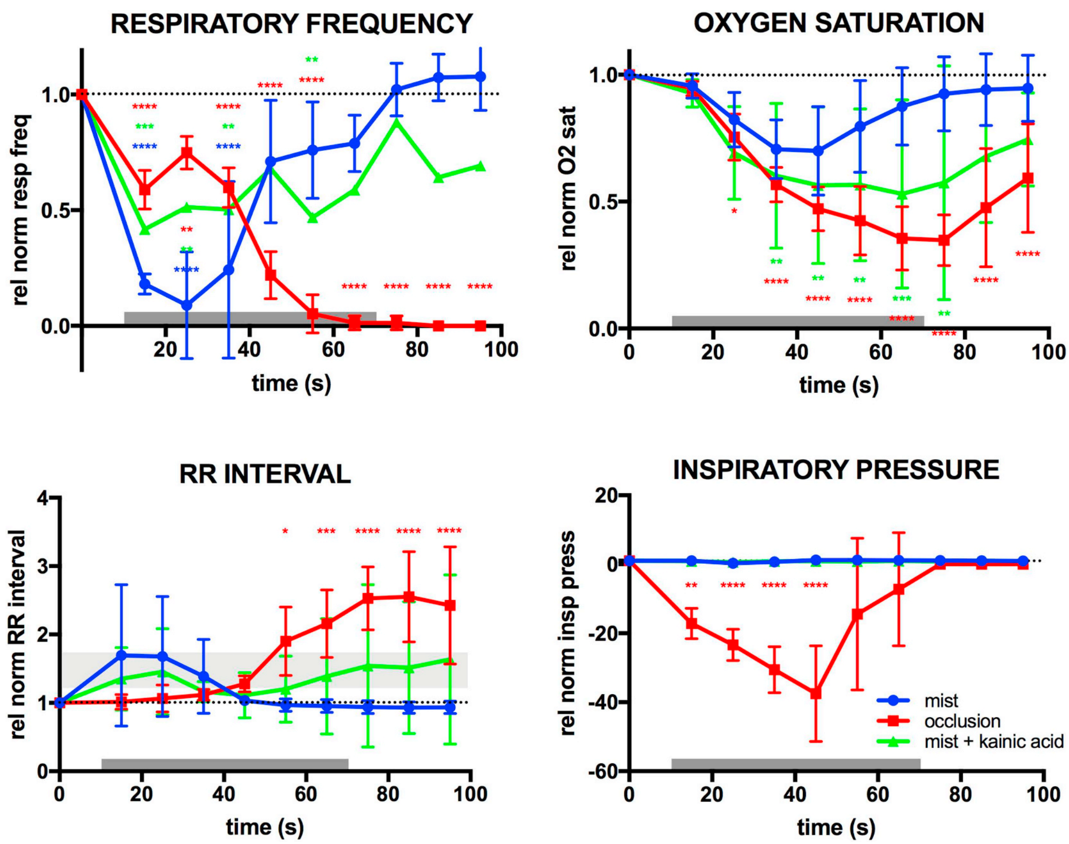

Fig. 3. Summary of cardiovascular and respiratory impact of long duration diving response activation.

Summaries of changes in respiratory frequency (upper left), oxygen saturation (upper right), RR interval duration (lower left), and tracheal pressure (lower right) during nasopharyngeal application of ice-cold mist (blue) for 60 s in comparison to controlled airway occlusion (red) for 60 s and diving response activation by mist application for 60 s during seizure-activity (green).

Plotted are means ± 95% confidence limits. The gray horizontal bar shown in the RR interval plot is a reference for comparison with Fig. 5. The y-axis range of the bar is equal to the range between the peak and offset RR interval change for the plot of events ≥3rd replicate. Ordinary two way ANOVA: time (row factor) $F(9, 160) = 19.01$, $p < .0001$ upper left; $F(9,170) = 3.403$, $p = .0007$ lower left; $F(9,169) = 16.58$, $p < .0001$ upper right; and $F(9,160) = 2.93$, $p = .0030$ lower right. Asterisks on the plot indicate significant differences of points relative to the value at time 0 after post hoc correction with Sidak's multiple comparisons test. In post-hoc analyses, mist (blue) differed from occlusion (red) at all time points after the baseline point in respiratory frequency ($p < .0001$), all but the 35 and 45 s time points in RR interval (p = .0042, 0.0001, < 0.0001, < 0.0001, < 0.0001), the last 6 time points in oxygen saturation (p = .0193, < 0.0001, < 0.0001, < 0.0001, < 0.0001), and the 15–55 s times in inspiratory pressure (p = .0035, < 0.0001, < 0.0001, < 0.0001, 0.0152). Mist (blue) differed from mist + KA (green) at 25, 55, 85, and 95 s for respiratory frequency (p = .0006, 0.0282, 0.0004, 0.0019); at 65, 75, and 85 s for oxygen saturation (p = .0033, 0.0027, 0.0221); and did not differ for inspiratory pressure or RR interval. Mist + KA (green) differed from occlusion (red) at all times except 15, 25, and 35 s for respiratory frequency (p = .0002 at 55 s, < 0.0001 at all other times); at 65–95 s for RR interval (p = .0373, 0.0043, 0.0024, 0.0308); at 15–45 s for inspiratory pressure (p = .0327, 0.0024, < 0.0001, < 0.0001); and did not differ for oxygen saturation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

event is evoked, Fig. 6 shows how the durations of spontaneous events vary with seizure activity, but we have no control over the timing of spontaneous events, the timing between such events, or the event durations. There is a clear pattern of diminished cardiovascular impact associated with evoked event replication that may explain the appearance of spontaneous events.

As illustrated in the event example depicted in Fig. 6, the impact of spontaneous seizure-associated central apneic episodes on ECG or EEG activity was minimal (see also (Nakase et al., 2016; Villiere et al., 2017)). Both RR interval increases and EEG amplitude decreases have been previously reported to be small, but statistically significant (Villiere et al., 2017).

Spontaneous central apneic episodes from the same rats used for diving response studies were observed in 3 of 10 animals. In these animals, 50 events were detected with durations > 1 s, but < 1.5 s, and 22 events with durations ≥1.5 s were counted. These were all too brief for an analysis of corresponding changes in HR or other systemic variables.

A key finding was that spontaneous seizure-associated central apneic episodes tended to be longer in duration when many of them occurred (Fig. 6 summary plots). The longer durations of spontaneous replicates contrast with the shorter durations of evoked replicates (Fig. 5), suggesting that the "stimulus signal" for the spontaneous replicates gets longer – most probably longer than each period of apnea, given the patterns in apnea duration with replication.

We evaluated the potential for central apneic episodes serving as predictors (positive or negative) of obstructive apnea risk. In a sample of 12 rats, at least 1 episode of central apnea (≥ 3 s in duration) was detected in 6/12 rats and obstructive apnea was detected in 7/12 rats. Obstructive apnea occurred in 1/7 rats without any prior episodes of central apnea. Central apneic episodes did not predict later obstructive apneic episodes, nor did they predict safety from such episodes. The presence of ≥1 episode, ≥2 episodes, or ≥3 episodes of central apnea did not predict whether animals would experience obstructive apnea (Fisher exact test, p = 1, 1, 1). There were no statistically significant differences in central apneic episode duration (two-tailed t-test, $p = .5$, $t = 0.6962$, $df = 7$) or number of recorded episodes ($p = .7$, $t = 0.3445$, $df = 7$; crude frequency $p = .7$, $t = 0.3864$, $df = 7$) in

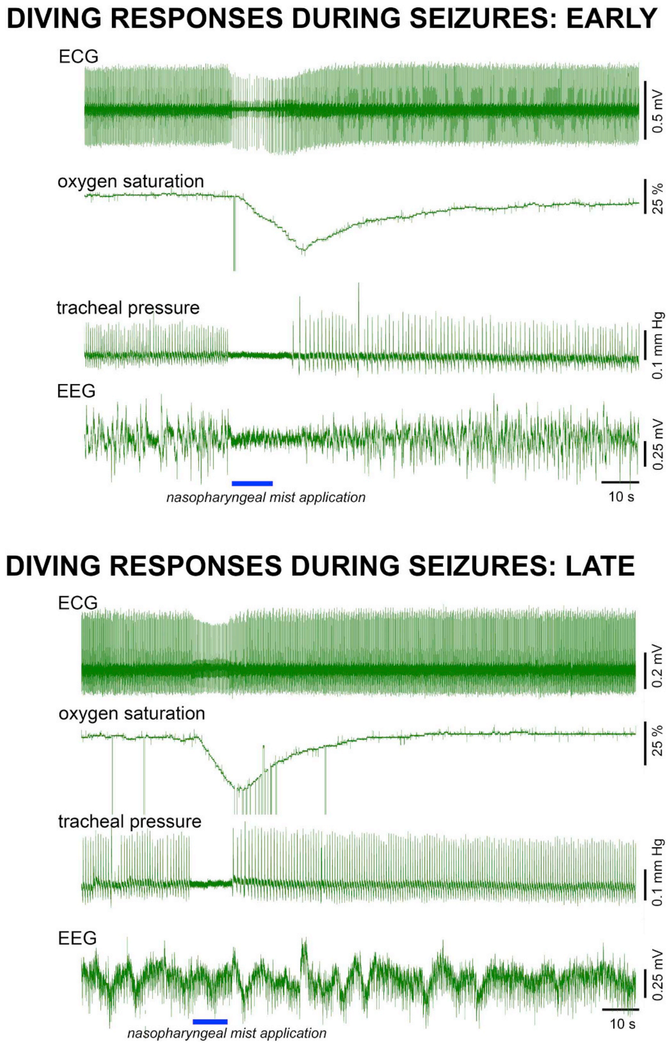

Fig. 4. Examples of diving responses evoked with repeated nasopharyngeal mist application for 10 s.
Nasopharyngeal application of ice-cold mist for approximately 10 s during seizure activity induced with systemic kainic acid was associated with diving responses that included apnea (evident on the tracheal pressure traces), bradycardia (ECG is shown in the top trace of each panel), and a decrease in oxygen saturation (second trace in each panel). The cardiac and respiratory consequences of the diving response stimulus were significantly more pronounced in the earliest events and got progressively weaker with repeated stimulus presentations. Note also that the apnea duration outlasts the stimulus, but gets shorter in response to later stimuli. See Fig. 5 for summary presentations. Calibrations on the figure.

animals that eventually showed obstructive apnea compared with animals that never showed obstructive apnea. No spontaneous apneic episodes were observed in any animals in the absence of kainic acid-induced seizure activity. This includes the time after which kainic acid was given systemically, but before any EEG evidence of seizure activity.

4. Discussion

Seizures can be associated with episodes of obstructive or central apnea. We argued previously that central apneic episodes resembled breath-holding (Nakase et al., 2016) or the diving response (Villiere et al., 2017) because of seizure spread to brainstem areas (e.g. (Sakamoto et al., 2008)). In this study, we sought to (1) characterize the properties of diving responses in urethane-anesthetized rats with or without kainic acid-induced seizure activity and (2) use the results as a basis for comparison with spontaneous seizure-associated central apneic events. We found that nasopharyngeal irrigation with cold mist was a more potent stimulus than irrigation with water and irrigation for 10 or 60 s elicited the typical diving response with apnea and significant bradycardia, but 60 s stimuli could not induce apnea for the entire time, i.e. breathing resumed in spite of the continuous

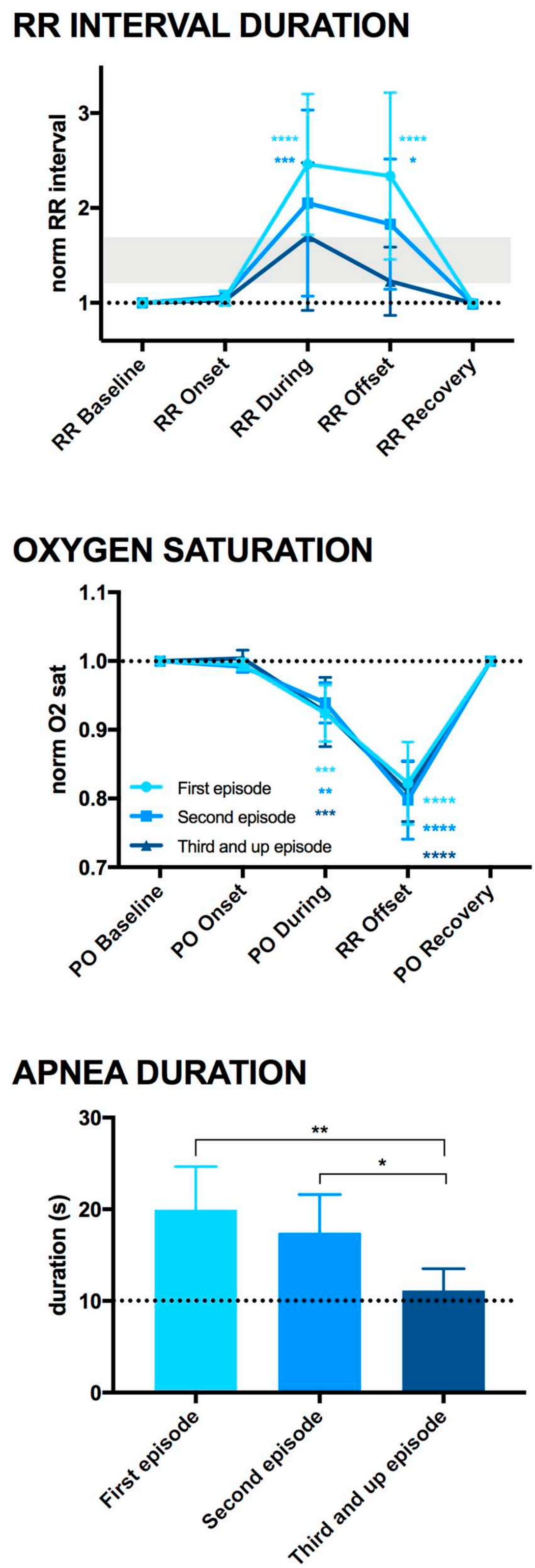

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Fig. 5. Summary of impact of repeated activation of the diving response on systemic variables.
Repeated applications of nasopharyngeal mist to induce the diving response resulted in progressively less bradycardia and shorter apnea durations during stimuli. Plots show changes in the development of bradycardia (RR intervals, top) and apnea duration (bottom), but the degree of change in oxygen saturation was constant across repeated stimuli (middle). RR interval changes associated with spontaneous seizure-associated central apneic events fell within the range of values shown for the ≥3 events (dark blue) between the peak (RR During) and end of the episode (RR Offset). Equivalent desaturations over shorter evoked apneic periods meant higher rates of desaturation and suggested a limit to the degree of desaturation before breathing restarted. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

stimulation. Repeated 10 s mist applications led to an uncoupling of the apneic episodes (which always occurred) from the bradycardia (which became less pronounced with repetition). These later uncoupled events matched the features of observed spontaneous seizure-associated central apneic episodes where heart rate changes were significant, but small (this paper, (Nakase et al., 2016; Villiere et al., 2017)). Our ability to replicate the properties of seizure-associated central apneic events with evoked diving responses during seizure activity supports the idea that seizure-associated central apnea and the diving response share a common neural basis. Given that seizure-associated central apneic episodes result from an active brain state (Nakase et al., 2016; Villiere et al., 2017), we suggest that the pattern of increasing event duration when there are multiple events indicates that central apneic episodes are not due to an aberrant activation or an inactivation of brainstem circuitry, but rather an attempt to protect core blood flow during seizure activity and in the face of response properties weakening with repetition.

4.1. Uncoupling diving response elements

The diving response is a remarkable example of co-activation of both divisions of the autonomic nervous system. In mammals, the diving response (reflex) is an extremely powerful reflex response to facial or nasopharyngeal stimulation with water (as one example) 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). Muskrats and rats have been frequently used as animal models to study the diving response (McCulloch, 2012). Whereas the complete mechanistic details of this complex response are still being elucidated, the coordinated respiratory, parasympathetic, and sympathetic responses highlight the integration of these systems.

The core elements of the circuit include (1) activation of sensory inputs (via ophthalmic branches of the trigeminal nerve, the trigeminal ganglion, and anterior ethmoid nerve) and chemoreceptors (e.g. carotid body and sinus) (Golanov et al., 2016; Huang and Peng, 1976; McCulloch, 2012; Panneton, 2013), which terminate in (2) the medullary dorsal horn, a structure that projects to (3) multiple targets including nucleus tractus solitaries (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).

Bradycardia is a consequence of increased parasympathetic outflow to the atria and conducting fibers, whereas blood pressure increases result from α-adrenergic innervation of the vasculature. These autonomic pathways are engaged as sensory inputs reach NTS and the VLM. Heart rate and blood pressure changes are significant and depend somewhat on the details of the animal's condition and the particular stimulus used for diving response activation (Huang and Peng, 1976; Huang et al., 1991; Lin and Baker, 1975; McCulloch, 2012). In humans, bradycardia associated with diving varies from person to person, with

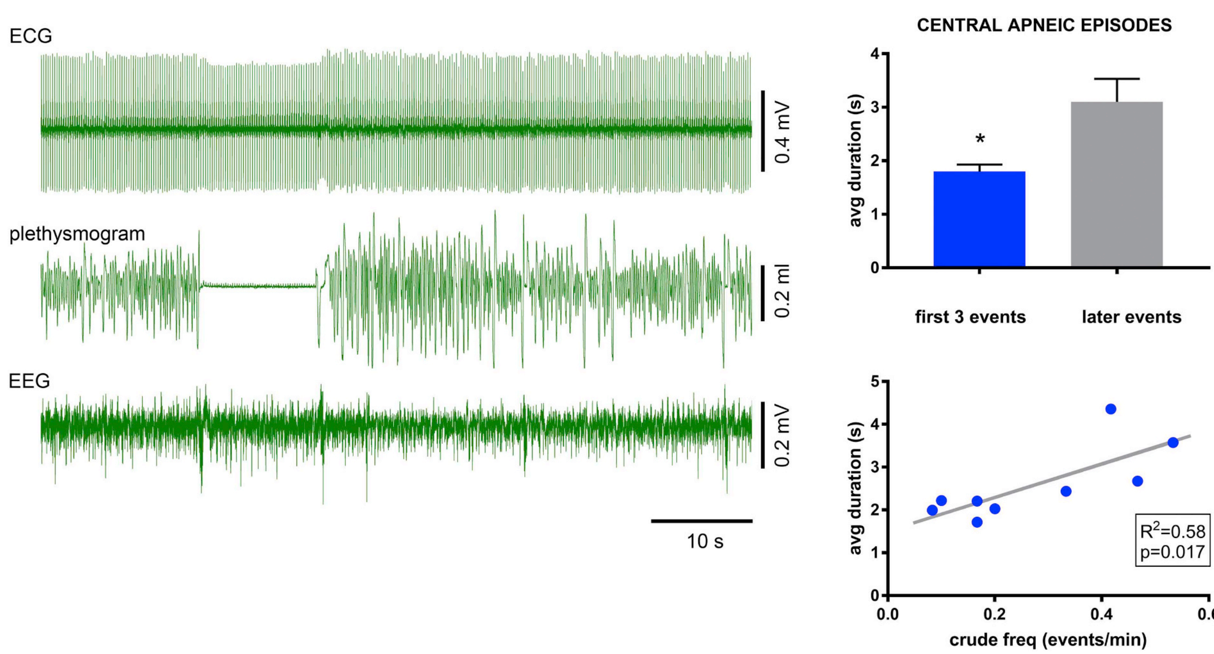

Fig. 6. Spontaneous central apneic episode duration correlates with frequency of occurrence.
Example spontaneous seizure-associated central apneic episode whose duration was about 10 s and summaries of apneic episode properties when multiple episodes are recorded in individual animals. The example shown was the 10th recorded and well-discriminated event of 12 total measured events from this animal. Note the minimal impact of the apneic episode on the ECG or EEG (similar to what has been reported previously in (Nakase et al., 2016; Villiere et al., 2017)). Calibrations on the figure. In the summary plots to the right, the top panel shows that apneic episodes early in a period of seizure activity were, on average, significantly shorter than later episodes (average of first 3 episodes vs average of later episodes: two-tailed *t*-test, $p = .0202$, $t = 2.362$, $df = 96$; shown in top figure as means ± SEM; F test to compare variances: $F(59, 37) = 17.05$, $p < .0001$. There was also a clear positive correlation of the average apneic episode duration with the number of apneic episodes recorded ($R^2 = 0.54$; $p = .025$; not plotted) and their crude frequency of occurrence (events/min of recording; $R^2 = 0.58$, $p = .017$; bottom plot shows data plus linear regression).

declines ranging from 15%–40%, but can much more severe (Alboni et al., 2011; Hiebert and Burch, 2003; Zbrozyna and Westwood, 1992).

Systematic changes in specific diving response elements have been linked to lung volume, intrathoracic pressure, and heart rate at the onset of the diving response. Repeated forced diving in ducks showed smaller heart rate changes (Gabrielsen, 1985). Studies in dogs, seals, and humans indicate that bradycardia is most severe when the lung volume is smaller (Foster and Sheel, 2005). A possible explanation is that when lung volumes are smaller pulmonary stretch receptors are less active (Foster and Sheel, 2005). A second explanation is that when respiration ceases with smaller lung volumes, hypoxia results in larger chemoreceptor stimulation which causes greater diving-related bradycardia (Foster and Sheel, 2005). Increased intrathoracic pressure was shown to be important for the "full" diving response (Zbrozyna and Westwood, 1992).

As seizure activity spread to brainstem is a potent activator of both divisions of the autonomic nervous system (Goodman et al., 1999; Sakamoto et al., 2008; Stewart, 2018), smaller changes in the differential activation of autonomic areas by the diving response stimulus are expected, and this outcome is evident in Figs. 2 and 3 of this paper. In this context, glutamate application to the NTS attenuated the heart rate change in during the diving response (Huang et al., 1991), as one would expect seizure activity to do. A cumulative persistence of autonomic firing (seen during seizure activity, e.g. (Sakamoto et al., 2008)) could also contribute to the heart rate changes due apparently to repetition. Although the overall data show no tendency for progressive changes in the starting heart rate, one animal that was tested with 9 replicates, showed a heart rate decrease of about 10% over the last 5 trials. Additionally, not all diving response stimuli produce the same results (e.g. water and mist in this manuscript), and if seizures are driving the efferent diving response circuitry as part of central apneic episodes, some partial activation of elements is plausible. Clearly, more specific information about brainstem, autonomic, and respiratory systems needs to be obtained to fully elucidate the overlap between the diving response and seizure-associated central apneic episodes and the contributions of cumulative autonomic activity. Having a model where events can be reproducibly evoked is an important step toward accomplishing such studies.

5. Conclusion

Are seizure-associated central apneic episodes themselves detrimental? The cardiovascular impact of these episodes is typically small (see e.g. (DelRosso and Hoque, 2013; Nadkarni et al., 2012) and, thus far, there is no evidence for harm. In our rat model, the occurrence, frequency, or duration of central apneic episodes are also not predictive of more serious conditions, such as obstructive apnea. On the contrary, the finding of longer duration central apneic events when (1) there are many central apneic events, and (2) later in long duration seizures suggests that central apneic episodes may reflect an attempt by brainstem networks to protect core physiology. A deeper understanding of seizure spread into brainstem regions will be necessary to further define this possibility and may permit additional biomarkers (see e.g. (Stewart et al., 2017)) to be established for identifying risk of severe systemic consequences of epileptic seizures.

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