Influence of vigilance state on physiological consequences of seizures and seizure-induced death in mice

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Hajek MA, Buchanan GF. Influence of vigilance state on physiological consequences of seizures and seizure-induced death in mice. J Neurophysiol 115: 2286–2293, 2016. First published February 17, 2016; doi:10.1152/jn.00011.2016.—Sudden unexpected death in epilepsy (SUDEP) is the leading cause of death in patients with refractory epilepsy. SUDEP occurs more commonly during nighttime sleep. The details of why SUDEP occurs at night are not well understood. Understanding why SUDEP occurs at night during sleep might help to better understand why SUDEP occurs at all and hasten development of preventive strategies. Here we aimed to understand circumstances causing seizures that occur during sleep to result in death. Groups of 12 adult male mice were instrumented for EEG, EMG, and EKG recording and subjected to seizure induction via maximal electroshock (MES) during wakefulness, nonrapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep. Seizure inductions were performed with concomitant EEG, EMG, and EKG recording and breathing assessment via whole body plethysmography. Seizures induced via MES during sleep were associated with more profound respiratory suppression and were more likely to result in death. Despite REM sleep being a time when seizures do not typically occur spontaneously, when seizures were forced to occur during REM sleep, they were invariably fatal in this model. An examination of baseline breathing revealed that mice that died following a seizure had increased baseline respiratory rate variability compared with those that did not die. These data demonstrate that sleep, especially REM sleep, can be a dangerous time for a seizure to occur. These data also demonstrate that there may be baseline respiratory abnormalities that can predict which individuals have higher risk for seizure-induced death.

SUDEP may represent a convergence of untoward cardiac and respiratory failure. Increased respiratory and electrocerebral function and survival of preventive strategies. Here we aimed to understand circumstances causing seizures that occur during sleep to result in death. Groups of 12 adult male mice were instrumented for EEG, EMG, and EKG recording and subjected to seizure induction via maximal electroshock (MES) during wakefulness, nonrapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep. Seizure inductions were performed with concomitant EEG, EMG, and EKG recording and breathing assessment via whole body plethysmography. Seizures induced via MES during sleep were associated with more profound respiratory suppression and were more likely to result in death. Despite REM sleep being a time when seizures do not typically occur spontaneously, when seizures were forced to occur during REM sleep, they were invariably fatal in this model. An examination of baseline breathing revealed that mice that died following a seizure had increased baseline respiratory rate variability compared with those that did not die. These data demonstrate that sleep, especially REM sleep, can be a dangerous time for a seizure to occur. These data also demonstrate that there may be baseline respiratory abnormalities that can predict which individuals have higher risk for seizure-induced death.

Epilepsy affects millions of people worldwide (Banerjee et al. 2009). It is estimated that one in 26 people in the United States alone will develop epilepsy in their lifetime (Hesdorffer et al. 2011a). Upwards of 30% of patients with epilepsy develop refractory epilepsy (Schmidt 2009). Refractory epilepsy carries a high risk of death, especially sudden unexpected death in epilepsy (SUDEP), the leading cause of death in these patients (Hesdorffer et al. 2011b). Among neurological conditions, SUDEP is second only to stroke in terms of number of potential years of life lost (Thurman et al. 2014), reflecting the fact that SUDEP tends to occur in young individuals who otherwise would have been able to contribute to society for decades. Thus, SUDEP constitutes a major public health problem.

Retrospective analyses of SUDEP cases have revealed that seizure-induced respiratory and/or cardiac failure is a common etiology for SUDEP (Bateman et al. 2010; Nashef et al. 1996; Ryvlin et al. 2013). In the large multicenter MORTEMUS study of SUDEP cases from epilepsy monitoring units, apnea preceded terminal asystole in all definite SUDEP cases for which there was sufficient data (Ryvlin et al. 2013). It has been suggested that baseline reduction of heart rate variability (Degiorgio et al. 2010; Stein et al. 1994) or prolonged postictal suppression of cortical activity (Bozorgi and Lhatoo 2013; Lhatoo et al. 2010) may be biomarkers that herald an increased likelihood of SUDEP.

Another consistent factor in SUDEP cases is that SUDEP tends to occur at night (Lamberts et al. 2012; Nobili et al. 2011). In the aforementioned MORTEMUS study, almost all of the definite SUDEP cases occurred at night. The majority of these occurred during sleep, with no clear association to one particular sleep state (Ryvlin et al. 2013). The specific mechanisms by which SUDEP occurs at night are unknown. It has been suggested that this could simply be a product of reduced monitoring during the nighttime when patients are supposed to be asleep, and thus resulting in delayed or absent resuscitation efforts (Langan et al. 2000; Nashef et al. 1998). It has also been suggested that this could be due to the patient ending up in the prone position following a nighttime seizure, leading to suffocation from airway obstruction (Tao et al. 2015). There is evidence to suggest that reduced supervision and delayed resuscitation can contribute (Seyal et al. 2013) and that many SUDEP victims are found in the prone position (Liebenthal et al. 2015; Tao et al. 2010); however, there may be other physiological reasons for sleep to be a prime time for SUDEP to occur (Sowers et al. 2013). For instance, there is state-dependent variability in cardiac and respiratory function (Buchanan 2013; Cajochen et al. 1994; Snyder et al. 1964), and sleep state can influence the frequency, severity, and duration of seizures (Bazil and Walczak 1997; Ng and Pavlova 2013). Thus, it follows that if a seizure were to occur during this time of cardio-respiratory instability, it could prove fatal. Therefore, SUDEP may represent a convergence of untoward cardiac and respiratory effects from both sleep and the seizure.

Here we examined whether seizures that occur during different sleep states have differential effect on electrocerebral, cardiac, and respiratory function and mortality. Seizures were induced via maximal electroshock (MES) in adult male mice during different vigilance states with concomitant measurement of electroencephalography (EEG), electromyography (EMG), electrocardiography (EKG), and breathing via whole-body plethysmography. We found that in this model the vigilance state during which the seizure was induced differentially affected respiratory and electrocerebral function and survival.
and that baseline respiratory rhythm irregularity predicted risk of seizure-induced death.

MATERIALS AND METHODS

Ethical approval. All procedures and protocols were approved by the Institutional Animal Care and Use Committee at Yale University School of Medicine.

Experimental animals. Adult male (24–32 g) mice from our colony were housed in standard cages in a 12 h light/12 h dark regimen with food and water available ad libitum. Breeding and genotyping of our mouse line, which are on a primarily C57BL/6J background, has been described previously (Buchanan et al. 2014; Zhao et al. 2006).

EEG/EMG headmount and EKG electrode implantation. EEG/EMG headmounts (8201; Pinnacle Technology, Lawrence, KS) were implanted as previously described (Buchanan and Richerson 2010). Briefly, under isoflurane (0.5–2% inh.) anesthesia, the skull was exposed and the headmount was attached to the skull with two 0.1 in. (anterior) and two 0.125 in. (posterior) stainless steel machine screws (000-120; Pinnacle Technology). EMG leads emanating from the posterior portion of the headmount were sutured into the bilateral nuchal muscles ~1 mm from the midline. Animals were concurrently implanted with EKG leads (MS303-76; Plastics One, Roanoke, VA) in the left chest wall and right axilla, and a subcutaneous temperature transponder (IPTT-300; Bio Medic Data Systems, Seaford, DE) was implanted over the scapulae. The base of the headmount, screw heads, and EMG leads were anchored with dental acrylic (Jet Acrylic; Lang Dental, Wheeling, IL), and the skin sutured closed leaving only the headmount socket exposed. Animals received pre- and postoperative analgesia with mecloxicam (0.3 mg/kg ip preoperatively; 0.05 mg/kg/day postoperatively in the drinking water for 7 days) and were allowed to recover for 7–10 days before being studied.

Seizure induction with MES. Animals were acclimated to the recording apparatus for 1 h per day on 3 consecutive days prior to being studied. On the trial day, baseline data were recorded for at least 30 min, and then each mouse received a single electroshock stimulation (50 mA; 0.2 s; 60 Hz sine wave pulses) via ear clip electrodes (modified, toothless, stainless steel alligator clips with saline moistened gauze) attached to a Rodent Shocker (Harvard Apparatus) during the vigilance state of interest. As seen previously, with these stimulus parameters many mice succumbed to the seizure (Buchanan et al. 2014). Seizure severity was assessed by determination of the extension-to-flexion ratio (E/F ratio): length of time the hindlimbs were extended beyond 90° divided by the length of time the hindlimbs were flexed (≤ 90°). Higher E/F ratios correlate with widespread propagation of epileptiform activity (Anderson et al. 1986). E/F ratio determinations were made off-line by post hoc video review. MES thresholds were determined for this mouse strain previously (Buchanan et al. 2014).

EEG/EMG/EKG data acquisition. EEG, EMG, and EKG data were acquired as described previously (Buchanan and Richerson 2010; Buchanan et al. 2014). Briefly, a preamplifier (8202-2L, Pinnacle Technology) was attached to the implanted headmount, and the animals were introduced to the recording chamber and allowed to acclimate as described. Preamplifier leads were then passed through a commutator (#8204, Pinnacle Technology) and into a conditioning amplifier (model 440 Instrumentation Amplifier; Brownlee Precision, San Jose, CA). EEG and EMG signals were amplified (50,000×), band-pass filtered (0.3–200 Hz for EEG; 10–300 Hz for EMG) and digitized (1,000 samples/s) with an analog-to-digital (A-D) converter (PCI-6221; National Instruments, Austin, TX) in a desktop computer (Dell) and acquired using software custom written in MATLAB (Mathworks, Natick, MA). EKG signals were passed through a separate conditioning amplifier (Grass LP511 AC; Astro-Med, West Warwick, RI) where they were amplified (20,000×) and band-pass filtered (0.3–300 Hz) and then digitized with the A-D converter as above. Body temperature signals from the implanted telemetry were sampled periodically with a telemetry reader wand (DAS-7007S, Bio Medica Systems).

Sleep-wake determination. Sleep state was assessed on-line in real time prior to delivery of the stimulation. A standard approach based on the EEG/EMG frequency characteristics was used to assign vigilance state (Franken et al. 1998) as follows: Wake - low amplitude, high frequency (7–13 Hz) EEG with high EMG power; Nonrapid eye movement (NREM) sleep - high amplitude, low frequency (0.5–4 Hz) EEG with moderate to low EMG power and lack of voluntary motor activity; Rapid eye movement (REM) sleep - moderate amplitude, moderate frequency (4.5–8 Hz) EEG with minimal EMG power except for brief bursts and minimal activity correlating with EMG bursts. Electroshocks were delivered when the animals were determined to be in the vigilance state of interest for at least 60 s. Vigilance states were verified off-line post hoc using custom software written in MATLAB. Fast Fourier transform (FFT) power spectra were created with MATLAB for each 10 s epoch of data and used along with EEG and EMG characteristics to verify scoring.

Breathing plethysmography. For quantification of ventilation the recording chamber was fit with an ultralow pressure/high-sensitivity pressure transducer (DC002NDRS; Honeywell International, Minneapols, MN). The analog output from the pressure transducer was digitized by the A-D converter (PCI-6221; National Instruments, Austin, TX), displayed on a computer monitor in real time using the acquisition program custom written in MATLAB. A mechanical ventilator (Mini-Vent, Harvard Apparatus) was used to deliver metered breaths (300 µl, 150 breaths/min) to the recording chamber to calibrate the breathing signal. Individual breaths were identified and measured using custom software written in MATLAB to aid in assessment of breathing parameters including respiratory rate (RR), tidal volume (VT), and minute ventilation (V̇E) as previously described (Hodges et al. 2008; Buchanan et al. 2014). Relative humidity, ambient temperature, body temperature, and atmospheric pressure (obtained from http://www.wunderground.com) were used to calculate V̇E assuming standard conditions (Buchanan et al. 2014; Drorbaugh and Fenn 1955).

EKG analysis. Heart rate (HR) and measurements of heart rate variability (HRV) were determined using custom software written in MATLAB and Kubios HRV 2.2 (University of Eastern Finland; http://kubios.uef.fi). HRV indices included the standard deviation of all R-R intervals (SDNN) and the root mean square of the standard deviation of the differences between R-R intervals (RMSSD). These are standard measurements that are good indicators of autonomic function (Stein et al. 1994) and are thought to be predictive of SUDEP risk (Degiorgio et al. 2010; Kalume et al. 2013). The coefficient of variance of the HR was determined with the aid of Microsoft Excel.

Statistics. Interactions between vigilance state and respiratory and cardiac measures were analyzed for all physiological variables using two-way analysis of variance, paired t-test, or two-tailed t-test assuming unequal variance as appropriate. Survival analyses were conducted with logistic regression. The significance threshold was P < 0.05 for all conditions. Analyses were accomplished using Microsoft Excel (Redmond, WA), OriginPro 9.0 (OriginLab, Northampton, MA), and Systat 11.0. Data expressed as x ± y represent means ± SE, unless stated otherwise. All error bars represent SE.

RESULTS

Seizures induced via MES during sleep were more likely to result in death. To determine whether the vigilance state during which a seizure occurred had any influence on survival, seizures were induced via MES in separate groups of mice during wakefulness, NREM sleep, and REM sleep (n = 12 per vigilance state). Strikingly, all mice that experienced a seizure that was induced during REM sleep died (Fig. 1). When seizures were induced during NREM sleep 67% of mice died,
and 50% died when seizures were induced during wakefulness (Fig. 1). Seizures induced during sleep were more severe compared with those induced during wakefulness with E/F ratios of 23.55 ± 2.23 when induced during NREM and 23.75 ± 2.98 when induced during wakefulness with 9.08 ± 2.98 when induced during wakefulness (n = 12 per group; P < 0.05 for NREM or REM compared with wakefulness; Fig. 2A). Seizures induced during sleep were also longer in duration compared with those induced during wakefulness lasting 32.89 ± 3.01 s when induced during NREM and 32.11 ± 3.98 s when induced during wakefulness (n = 12 per group; P < 0.05 for NREM or REM compared with wakefulness; Fig. 2B). There was no significant difference in severity (P = 0.435) or duration (P = 0.284) between those induced during NREM vs. REM sleep (Fig. 2). There was no significant difference in the severity [E/F ratios: wake (W), 9.47 ± 3.24 survive vs. 8.69 ± 2.72 died, P = 0.337; NREM (N), 22.38 ± 2.04 survive vs. 24.14 ± 3.22 died, P = 0.111] or duration (W, 16.13 ± 4.31 s survive vs. 18.04 ± 3.94 s die, P = 0.221; N, 32.47 ± 2.85 s survive vs. 33.10 ± 3.09 s die, P = 0.364) of seizures between those that survived compared with those that died when seizures were induced during wakefulness or NREM (Fig. 2).

Seizures induced via MES during sleep were more likely to be associated with respiratory suppression. To determine whether the vigilance state during which a seizure occurred had an effect on the respiratory and/or cardiac sequelae of the seizure, respiratory and cardiac function were assessed before, during, and after seizures induced with MES during each vigilance state. At baseline, as expected, there were state-dependent differences in breathing during the different vigilance states, with increased RR (W, 174.80 ± 16.65 breaths/min; N, 189.21 ± 14.53 breaths/min; R, 223.58 ± 39.33 breaths/min; n = 12 per group; P < 0.05 for all comparisons), reduced $V_T$ (W, 20.28 ± 2.14 μl/g; N, 17.58 ± 1.36 μl/g; R, 14.66 ± 2.61 μl/g; n = 12 per group; P < 0.05 for all comparisons), and $V_E$ (W, 3.55 ± 0.36 ml·min$^{-1}$·g$^{-1}$; N, 3.09 ± 0.22 ml·min$^{-1}$·g$^{-1}$; R, 3.28 ± 0.20 ml·min$^{-1}$·g$^{-1}$; n = 12 per group; P < 0.05 for all comparisons) during NREM and REM sleep compared with wakefulness (Fig. 3A). Similarly, as expected, there was state-dependent variation in cardiac activity at baseline (Fig. 4A).

Consistent with what was demonstrated previously for seizures induced via MES during wakefulness (Buchanan et al. 2014), all seizures were associated with respiratory arrest during the seizure regardless of the vigilance state during which it was induced. Among survivors, seizures induced during NREM sleep caused a longer duration of postictal apnea compared with those induced during wakefulness (W, 2.16 ± 1.08 s, n = 6; N, 5.94 ± 1.47 s, n = 4; P < 0.05; Fig. 3B). When breathing resumed following the postictal respiratory arrest, there was a reduced rate (W, 148.11 ± 21.58 breaths/min, n = 6; N, 111.89 ± 19.69 breaths/min, n = 4; P < 0.05), reduced $V_T$ (W, 19.24 ± 1.22 μl/g, n = 6; N, 13.42 ± 1.89 μl/g, n = 4; P < 0.05), and consequently reduced $V_E$ (W, 2.85 ± 0.29 ml·min$^{-1}$·g$^{-1}$, n = 6; N, 1.76 ± 0.28 ml·min$^{-1}$·g$^{-1}$, n = 4; P < 0.05) following seizures induced during NREM sleep compared with those induced during wakefulness (Fig. 3B). There were no significant differences in cardiac measures among the different conditions (Fig. 4B). It should be noted that this assessment could not be performed in mice that died, because breathing did not recover to allow postictal assessment. Thus, this assessment could not be performed for seizures induced during REM sleep since all mice died in this condition.

**Mice that died following a seizure had increased baseline RR variability.** To determine whether there were any physiological indicators that correlate with survival from a seizure, breathing and cardiac activity were assessed at baseline in all animals, and the data were sorted by survival status. Among seizures induced during a given vigilance state, there was increased respiratory rhythm irregularity in those mice that went on to die from the seizure compared with those that survived the seizure (Fig. 5A). There was a nonsignificant trend toward reduction in HRV in mice that died compared with those that survived when seizures were induced during different vigilance states (Fig. 5B).

**Prolonged EEG suppression following seizures induced during NREM sleep compared with wakefulness.** We previously observed reversible suppression of EEG activity following non-
fatal seizures induced during wakefulness (Buchanan et al. 2014). To determine whether the vigilance state during which a seizure occurred influenced the duration of the postictal EEG suppression total EEG power was determined with FFT before, during, and after seizures induced via MES during each vigilance state. In all mice that died from the seizure there was immediate and complete suppression of EEG power following the seizure irrespective of the vigilance state during which it was induced (Fig. 6A). Following nonfatal seizures, there was a reduction of EEG power that returned to baseline over some minutes after the end of the seizure. The duration of the suppression was longer for seizures that were induced during NREM sleep compared with those induced during wakefulness (5.19 ± 0.72 min vs. 3.04 ± 0.65 min; \( P < 0.05 \); Fig. 6B). The magnitude of the suppression was similar for seizures induced during wakefulness and NREM sleep (Fig. 6B). Since this
suppression is seen in mice that survived, it does not seem to contribute to the mechanism of death. However, such alterations in EEG may correlate with reduction in sensitivity to stimulation, and thus could prove to be relevant as postictal recovery strategies are developed.

DISCUSSION

SUDEP occurs more commonly during sleep, but the specific reason for this has not previously been elucidated. It has been proposed that environmental factors, such as reduced supervision and prone sleeping position, may be the primary cause; however, whether sleep state could be an independent risk factor has not been explored. Here we provide evidence in one animal model that the sleep state during which a seizure occurs can differentially affect respiratory and electrocerebral function following seizures and contribute to mortality. We further show that baseline respiratory rhythm irregularity may predict which animals are more likely to die from a seizure. Finally, we demonstrate that if a seizure occurs during REM sleep, a time when seizure typically will not occur spontaneously, it has a high likelihood of being fatal.

Sleep state differentially influences respiratory consequences of seizures. It is well known that sleep state influences breathing. Changes in breathing during sleep are due in part to reduction in activity of respiratory motor neurons.

**Fig. 5.** Increased baseline respiratory rhythm variability in mice that ultimately died from an MES-induced seizure. Coefficient of variance of the interbreath interval (IBI CV, A), coefficient of variance of the interheart-beat interval (HR CV, B), and measures of HRV (SDNN, C; RMSSD, D) for mice that survived (white; \( n = 6, 4, \) and 0, respectively) and mice that died (black; \( n = 6, 8, \) and 12, respectively) from seizures induced by MES during Wake, NREM, or REM. *\( P < 0.05. \) Abbreviations as in Fig. 4. IBI CV: W, \( 11.79 \pm 2.72\% \) survive vs. \( 22.20 \pm 7.34\% \) die; N, \( 9.92 \pm 2.44\% \) survive vs. \( 20.96 \pm 6.87\% \) die; R, \( 24.33 \pm 6.77\% \) die; HR CV: W, \( 11.79 \pm 2.72\% \) survive vs. \( 9.45 \pm 2.65\% \) die; N, \( 14.92 \pm 5.06\% \) survive vs. \( 11.79 \pm 3.08\% \) die; R, \( 14.61 \pm 5.69\% \) die; SDNN: W, \( 107.68 \pm 25.19\) ms survive vs. \( 90.03 \pm 1.65\) ms die; N, \( 76.06 \pm 24.33\) ms survive vs. \( 72.99 \pm 22.52\) ms die; R, \( 97.40 \pm 46.69\) ms die; RMSSD: W, \( 10.91 \pm 2.11\) ms survive vs. \( 9.43 \pm 1.58\) ms die; N, \( 7.89 \pm 1.22\) ms survive vs. \( 6.46 \pm 1.32\) ms die; R, \( 9.70 \pm 2.16\) ms die.

**Fig. 6.** Prolonged suppression of electroencephalography (EEG) activity following nonfatal seizures induced during NREM sleep. Total EEG power between 0.5 and 20 Hz plotted vs. time relative to seizure onset (*time 0*) in 10 s epochs. Fast Fourier transform (FFT) power is plotted relative to 1 min of baseline EEG during wakefulness prior to seizure induction. Mean data are presented for mice that died (A) following seizures induced during wakefulness (black; \( n = 6 \)), NREM (gray; \( n = 8 \)), and REM (dotted; \( n = 12 \)), and those that survived (B) following seizures induced during wakefulness (black; \( n = 6 \)) and NREM (gray; \( n = 4 \)).
that are more purely respiratory, such as those controlling the diaphragm, are least affected by sleep states changes. Motor neurons that are typically more susceptible to non-respiratory influences, such as those involved with regulating upper airway tone and those controlling abdominal and intercostal accessory respiratory muscles, reduce their activity during sleep (Orem et al. 1977; Orem et al. 2002; Orem et al. 1974). Additionally, primary respiratory neurons in the dorsal and ventral respiratory groups governing inspiration and expiration, respectively; the pontine respiratory groups dictating the shape of the respiratory pattern; and the pre-Bötzinger complex, which houses the respiratory pattern generator (Smith et al. 1991) all alter their activity in a sleep-state dependent manner (Douglas 1984; Montandon and Horner 2013; Orem 1980; Orem et al. 1985; Orem et al. 1974). Subtle differences in the integrity of these systems between individuals (Shea and Guz 1992; Shea et al. 1990) may explain why some might be more susceptible to the additional effects of seizure. Whether systematic differences in the function of these systems exist in patients with epilepsy is not known.

Sensitivity of the respiratory system to hypcapnia and hypoxia is reduced during sleep and becomes progressively more decreased as one progresses into deeper stages of NREM sleep and into REM sleep (Bulow 1963; Douglas et al. 1982; Phillipson et al. 1978; Santiago et al. 1984). Thus, if breathing is halted by a seizure during sleep, the accumulated CO₂ may not mount a sufficient respiratory response to adequately stimulate breathing and sustain life, owing to the reduced sensitivity to CO₂. It may also be true that the accumulation of CO₂ may be too large and outside the range to maintain respiratory drive. Similarly, independent of the respiratory effects, the CO₂ stimulus may be insufficient to stimulate arousal and restore airway patency as typically occurs with arousal, such as in sleep apnea. This is likely to be exacerbated by reduction of activity and sensitivity of nuclei involved in breathing and sleep-wake regulation such as serotoninergic nuclei of the medullary and dorsal raphe (Buchanan and Richerson 2010), respectively, and noradrenergic neurons of the locus coeruleus, among others.

Seizures cause varying degrees of respiratory dysregulation depending on the seizure type and where they originate (Bate-man et al. 2008; Blum 2009). Generalized tonic-clonic seizures cause muscles, including those involved in breathing, to contract during the seizure, thus contributing to seizure-induced apnea. There is evidence that seizures can have far reaching effects on brain loci involved in arousal and sleep-wake regulation, largely through inhibition of network connections to these sites (Blumenfeld 2012; Blumenfeld et al. 2004; Englot et al. 2009; Englot et al. 2008; Furman et al. 2015; Gummadavelli et al. 2015; Motelow et al. 2015; Sedigh-Sarvestani et al. 2014a), but may also include brain-stem structures involved in regulation of breathing (Blumenfeld et al. 2009) including 5-HT neurons (Zhan et al. 2016). Recently, it has been shown that seizures initiate spreading depolarization that influences cardiac and respiratory function (Aiba and Noebels 2015). It follows that since breathing patterns are set by vigilance state, a seizure occurring during different vigilance states would differentially affect respiratory function depending on the starting point. Here we found that vigilance state does indeed influence the effect of a seizure on respiratory function.

Implications of abnormal baseline breathing in mice that ultimately died from seizures. It was somewhat surprising to find that the mice that died from seizures had irregular baseline respiratory rhythms. Though this was a subtle difference, it was consistent and statistically significant. Inspection of the baseline respiratory data from the entire population of animals used in this study revealed that this population of mice displayed a similar mean respiratory rate with similar standard deviation compared with published mouse populations (Friedman et al. 2004; Hodges et al. 2008). However, separating the mice that died from the ones that survived revealed the subtle variation in the coefficient of variance of the respiratory rhythm. This was considered a surprising finding because these mice are not a specific model of hyperexcitability. Therefore, it does not necessarily speak to respiratory instability caused by epilepsy but speaks to a possible naturally occurring respiratory variation that could make a subject more likely to die should they experience a seizure. Given that by definition patients with epilepsy have increased proclivity to having seizures, then identifying respiratory rhythm instability in epilepsy patients could help identify who is at risk for dying from a seizure, or at risk for SUDEP. It will be informative moving forward to determine whether there are similar variances in respiratory rhythms in seizure- and/or “SUDEP-prone” animals models, such as DBA mice (Faingold et al. 2010; Tupal and Faingold 2006) or mouse models of Dravet syndrome (Auerbach et al. 2013; Kalume et al. 2013). Interestingly, at least one of the Dravet models has abnormal sleep architecture (Kalume et al. 2015), but whether there is any association between sleep state and seizure-related death in this model is not known.

Possible mechanisms for REM sleep-related seizure mortality. Seizures rarely occur during REM sleep in human patients with epilepsy (Ng and Pavlova 2013) or in most animal seizure models (Shouse et al. 2004). However, it has been shown that if there is an alteration in hippocampal theta rhythm, as is seen in the rat tetanus toxic model of epilepsy, seizures occur more commonly during REM sleep (Sedigh-Sarvestani et al. 2014b), though, in the initial report of this phenomenon, increased mortality was not observed (Sedigh-Sarvestani et al. 2014b). In the MES model, a generalized seizure is forced to occur. In our hands this invariably resulted in death when the seizure was forced to occur during REM sleep. Evaluation of the preictal cardiac and respiratory parameters revealed that, as typically occurs during REM in humans and most rodent models (Campen et al. 2002; Friedman et al. 2004; Shea and Guz 1992), there was less regularity of both the respiratory and cardiac rhythms during REM sleep. This suggests that the additional insult of a seizure during this “unstable” period from a cardio-respiratory standpoint overwhelms the system and the animal succumbs to the seizure.

Here we observed similar baseline respiratory rhythm variability in mice that died when seizures were induced during REM sleep as we did for mice that died when seizures were induced during other vigilance states. To the extent that we could assess it in this study, we did not observe a substantial contribution to death from cardiac dysregulation. However, in rats, seizures induced by MES are associated with cardiac instability that recovers over time (Darbin et al. 2003; Darbin et al. 2002; Nari-toku et al. 2003). Differential effects of sleep state were not examined in those studies. It is certainly reasonable to consider that the additive physiological insult of
sleeve and REM sleep pushes the physiological instability of the cardiac as well as respiratory systems above a threshold and leads to mortality. In every instance the immediate cause of death was respiratory arrest, consistent with what has been seen for seizure induced during wakefulness during the daytime in this model (Buchanan et al. 2014).

Limitations of the MES model. The MES model is a model of seizure induction in an otherwise seizure-naïve brain. Therefore, it is not a model of epilepsy, per se, but it is a good model with which to examine the effects of a single seizure. An advantage of this model is that seizures can be induced at a time, or vigilance state, of the investigator’s choosing. In this case, it allowed seizures to be induced in REM sleep, a time when they do not often occur normally, and allowed us to learn that if a seizure occurs during REM sleep it has a high likelihood of being fatal (100% in this study). We have previously used this model to demonstrate differences in the physiological consequences of seizures between different genetic mouse models and now used it to explore sleep state-dependent effects. Given that a single generalized seizure can cause death, data from the MES model are relevant to SUDEP.

Conclusions

Based on population estimates, nearly 1% of Americans live with refractory epilepsy. This figure is likely higher for the world-wide population. These individuals are at high risk for SUDEP. It is widely appreciated that SUDEP occurs more commonly during the nighttime and most likely during sleep. This study demonstrates that sleep state can be an independent risk factor for seizure-associated death in certain populations of mice. Data from this study suggest that the small subset of patients that might have seizures during REM sleep would be at increased risk for SUDEP. These data also suggest that individuals with epilepsy who have somewhat irregular breathing at baseline may be at increased risk for SUDEP. It will be important moving forward to continue to understand factors that ordinarily prevent seizures from occurring during REM sleep and to look for possible respiratory biomarkers, such as irregular respiratory rhythm, in human patients with epilepsy.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.A.H. and G.F.B. performed experiments; M.A.H. and G.F.B. analyzed data; M.A.H. and G.F.B. interpreted results of experiments; M.A.H. and G.F.B. prepared figures; M.A.H. and G.F.B. drafted manuscript; M.A.H. and G.F.B. edited and revised manuscript; M.A.H. and G.F.B. approved final version of manuscript; G.F.B. conception and design of research.
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