

Acid reflux induced laryngospasm as a potential mechanism of sudden death in epilepsy

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1. Introduction

There is growing evidence that laryngospasm may be linked to sudden unexpected death in epilepsy (SUDEP) (Lacuey et al., 2018; Nakase et al., 2016; Tavee and Morris, 2008).

There are an estimated 3.4 million Americans – including 470,000 children – living with epilepsy (Zack and Rosemarie, 2017). In approximately 30% of cases seizures cannot be controlled by medication (Brodie, 2013), and uncontrolled seizures are the primary risk factor for SUDEP (Devinsky, 2011). In the US, the annual mortality rate for SUDEP is approximately 4,000 unexpected deaths a year (Berg, 2001; Thurman David et al., 2014).

The exact cause of SUDEP is unknown, but respiratory and cardiac dysfunction are believed to play a crucial role (Devinsky, 2011; Massey et al., 2014; Ryvlin et al., 2013; Sowers et al., 2013), and laryngospasm has been proposed as a potential cause (Lacuey et al., 2018; Nakase et al., 2016; Tavee and Morris, 2008). The authors of Nakase et al. explored the potential link between laryngospasm and sudden death in an acute kainic acid (KA) model of seizure activity in rats and observed that obstructive apnea via laryngospasm is associated with cardiac dysfunction, SpO₂ decline, and death, while central apnea is not. Further, they observed that in every instance of obstructive apnea there was sudden death. Two clinical case reports support this mechanism as one possible cause of SUDEP in humans. We sought to explore the causes of this fatal laryngospasm.

Gastroesophageal reflux disease (GERD) is a common cause of laryngospasm (Vela et al., 2013). A reflex induces laryngospasm when stomach acid rises into the larynx, protecting the sensitive vocal tissue from damage. The exact cause of acid movement is unknown and may vary between patients: both the lower esophageal sphincter (LES) and upper esophageal sphincter (UES) may be insufficiently closed, allowing acid to move up from the stomach into the larynx (Postma and Halum, 2006); excess stomach acid may be generated; or both. Chemoreceptors innervated by both the superior laryngeal nerves (SLNs) and recurrent laryngeal nerves (RLNs) can induce laryngospasm, and SLN chemoreceptors will always trigger laryngospasm at low pH (Loughlin Christopher et al., 2009). GERD symptoms often occur at night, when the subject is prone or supine and acid can more easily move up the esophagus because gravity no longer draws it towards the stomach (Postma and Halum, 2006; Vela et al., 2013). Similarly, most SUDEP cases occur at night while the patient is asleep, and nighttime seizures increase the risk of SUDEP (Bateman Lisa et al., 2010; Bird et al., 2013; Lamberts Robert et al., 2011; Langan et al., 2000; McLean and Wimalaratna, 2007; Nobili et al., 2011; Ryvlin et al., 2013; Tao James et al., 2010). Epilepsy and GERD share a statistically significant comorbidity (Selassie, 2015), and uncontrolled epilepsy can result in chronic diffuse esophageal spasm (He et al., 2013). The authors of Nakase et al. demonstrated increased RLN firing during seizures, to which they attributed the sudden laryngospasm. We tested an alternative theory. The RLN, SLN, and other vagal tracts innervate both the esophageal sphincters. Vagus nerve activity can trigger the production of stomach acid (Debas and Carvajal, 1994), and overproduction of acid may contribute to acid movement. One possible explanation for the observed laryngospasm is that during seizure activity malfunctioning vagal tracts could increase the production of stomach acid, relax the esophageal sphincters, or both; allowing acid to move up from the stomach and

into the larynx, triggering laryngospasm. In GERD-induced laryngospasm during sleep, the patient normally wakes up and sits up, allowing gravity to move acid back down the esophagus, causing the larynx to relax. During a seizure, the patient remains unconscious, and the laryngospasm may continue until death.

To test this mechanism, we monitored the pH at two points inside the esophagus of rats during seizures, to determine if there was any acid movement, and the rate of that movement. We used the urethane-KA rat model from Nakase et al. and developed our own small-scale pH sensor using an antimony pH electrode and silver silver-chloride reference electrode. After discovering acid, we performed another set of experiments with the esophagus blocked, to confirm acid was the cause of fatal laryngospasm.

2. Materials and Methods

An expanded build guide is available in supplementary information.

2.1 Antimony Electrode:

We constructed the pH electrodes with antimony tips made from elemental antimony (Sigma Aldrich, Milwaukee) in a method similar to Caflisch et al. Electrodes were thin, at most 0.7 mm in diameter, and highly flexible (Figure 1a). We calibrated the pH electrodes in a Tris buffer system vs a traditional glass electrode (Orion, Thermo Fischer Scientific). After the first five experiments we used two pH electrodes spaced 1 cm apart to measure the progress of acid. Combined electrodes were less than 1 mm in diameter (Figure 1b).

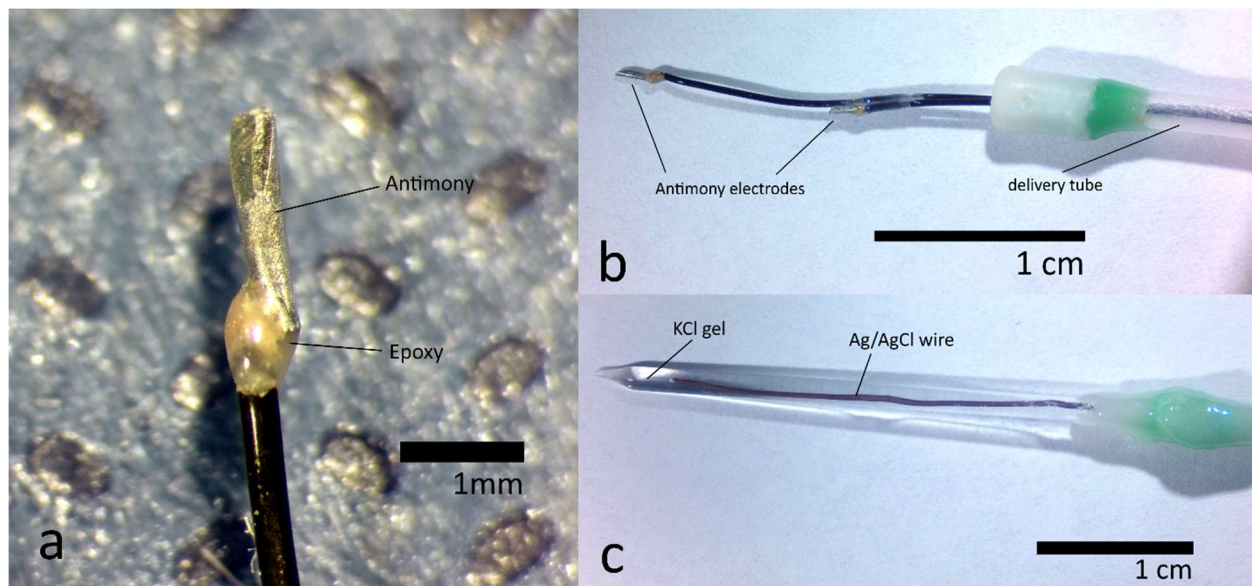


Figure 1: Custom devices. (a) Close-up image of the antimony pH electrode used in experiments. (b) Two pH electrodes together, inside the modified feeding tube used for delivery. These electrodes allowed monitoring of acid movement over time. (c) The silver silver-chloride reference electrode.

2.2 Reference electrode:

We constructed small silver/silver-chloride reference electrodes in a method similar to Hassel et al. (Figure 1c). References were compared to another silver/silver-chloride reference (Orion, Thermo Fischer Scientific) to verify accuracy.

2.3 Esophageal blocking:

We constructed a balloon catheter using latex from a condom tip and polyethylene tubing to fit in the rat esophagus. A pressure sensor allowed us to verify that the balloon remained inflated for the entire experiment. When deflated the balloon was <3 mm in diameter, and when fully inflated it could reach >7 mm.

2.4 Measurement of pH:

Female Long Evans rats (228g–327g, Envigo, Indianapolis, IN) were anesthetized with urethane (1.5 g/kg IP). We performed all of the animal work described in this paper in accordance with the regulations of the Institutional Animal Care and Use Committee (IACUC) using procedures approved by the Purdue Animal Care and Use Committee (PACUC) and comply with the National Institutes of Health guide for the care and use of Laboratory animals. Before and after all experiments, the pH sensor was calibrated in Tris buffers at pH 7.2 and 2.2 to verify accuracy. We used a modified feeding tube (Instech Laboratories) to deliver the pH electrodes (Figure 1b). The tube was inserted into the esophagus until met with resistance at the LES, then retracted approximately 3 mm to prevent the pH electrode from pushing through the LES. The pH electrode was pushed out of the feeding tube, and the feeding tube was removed. The reference electrode was placed in the subcutaneous space near the right shoulder and kept moist with saline. At the end of each experiment we advanced the pH electrode into the stomach to verify gastric pH.

In some experiments we used a balloon catheter to block acid movement up the esophagus. The balloon was inflated with saline until there was positive pressure. In these experiments we placed the pH electrode was positioned proximal to the balloon, as in trial experiments we observed that placing the pH electrode distal to the balloon allowed significant acid leakage past the balloon.

2.5 Other measures:

Animal respiration was monitored by measuring changes in air temperature with a nasal thermocouple similar to Marks et al. but placed just inside the nares. Heart rate and SpO₂ levels were monitored via a pulse oximeter on the animal's hindpaw. Animal temperature was maintained via a thermostatically controlled heating pad (Harvard Apparatus, 50-7212, Holliston, MA) or a hot water heating pad (Gaymar T/Pump, Kalamazoo, MI). In one animal outside the experimental groups we also monitored electrocorticography (ECoG) via bone screws, the measurement electrode placed at bregma AP –2.5 mm and lateral – 2.0 mm, and the reference electrode placed at bregma AP + 0.5 mm and lateral – 2.0 mm. We recorded ECoG data at 1.25 kHz, gain = 500, pass 3 Hz – 1.5 kHz. We defined seizure activity based on fast Fourier transform characteristics as described in Finnerty and Jefferys, however these always corresponded to an increase in amplitude of the ECoG signal, so for simplicity these analyses are not included. Other data processing information is available in supplemental information.

2.6 Experimental procedure:

Animals were prone for all experiments. Once all preparations were complete, baseline data for respiration and esophageal pH were collected for at least 5 min. Then we injected animals with KA (10 mg/kg IP), a dose shown to induce seizure activity, obstructive laryngospasm, and sudden death, starting 10-60 minutes after injections and lasting 3-4 hours (Naggar and Stewart, 2015; Nakase et al., 2016; Sakamoto et al., 2008). In two cases the initial dose of KA did not induce any observed changes, so these animals were given supplemental doses (2 mg/kg) at 60 min and 90 min after the primary injection. Control animals received injection of saline instead. Data was collected for 2 hours, unless a pH change was detected, in which case data was collected for 2.5 hours, to increase potential of observing sudden death.

2.7 Statistical analysis:

All group comparisons were done with Fisher's exact test. For quantitative data, normality is tested with the Shapiro-Wilks test, and homoscedasticity is tested with Bartlett's test. Values are calculated in R software. Data are reported as mean \pm standard deviation.

3. Results

We studied a total of 23 animals. Eleven animals were in the main experimental group, seven received esophageal blocking, and four were controls, two of which received esophageal blocking. One animal was used for ECoG verification of seizure pattern and correlating physical changes. As also observed in Nakase et al., we observed that KA can cause several different respiratory patterns and modes of death (example waveforms visible in Figure 2). The most common pattern was an increase in respiration rate from baseline (76.4 ± 15.5 breaths/min) to a rapid, irregular rate (180 ± 59.7 /min). This respiration pattern is consistent with previous examples of KA-induced seizure activity (Nakase et al., 2016) and the data from the animal with ECoG recording, suggesting that this respiration pattern is a result of seizure activity (Figure 3). Respiration waveform consisted of a pattern of one sharp peak followed by a series (2-20) of shallow breaths. We observed this pattern of respiration in 9/11 animals in the main group, 4/7 animals in the esophageal blocking group, and 0/4 animals in the control group. This respiration pattern may also present with transient apneas, which we defined as cessation of nasal airflow for at least 3 seconds. When present, transient apneas lasted 6.1 ± 2.6 s, occurred 6.25 ± 3.6 times per experiment in which they were present, with the first one occurring 80.8 ± 26.2 min after the primary KA injection. Transient apneas were observed in 4/11 animals in the main group, 3/7 animals in the esophageal blocking group, and 0/4 animals in the control group.

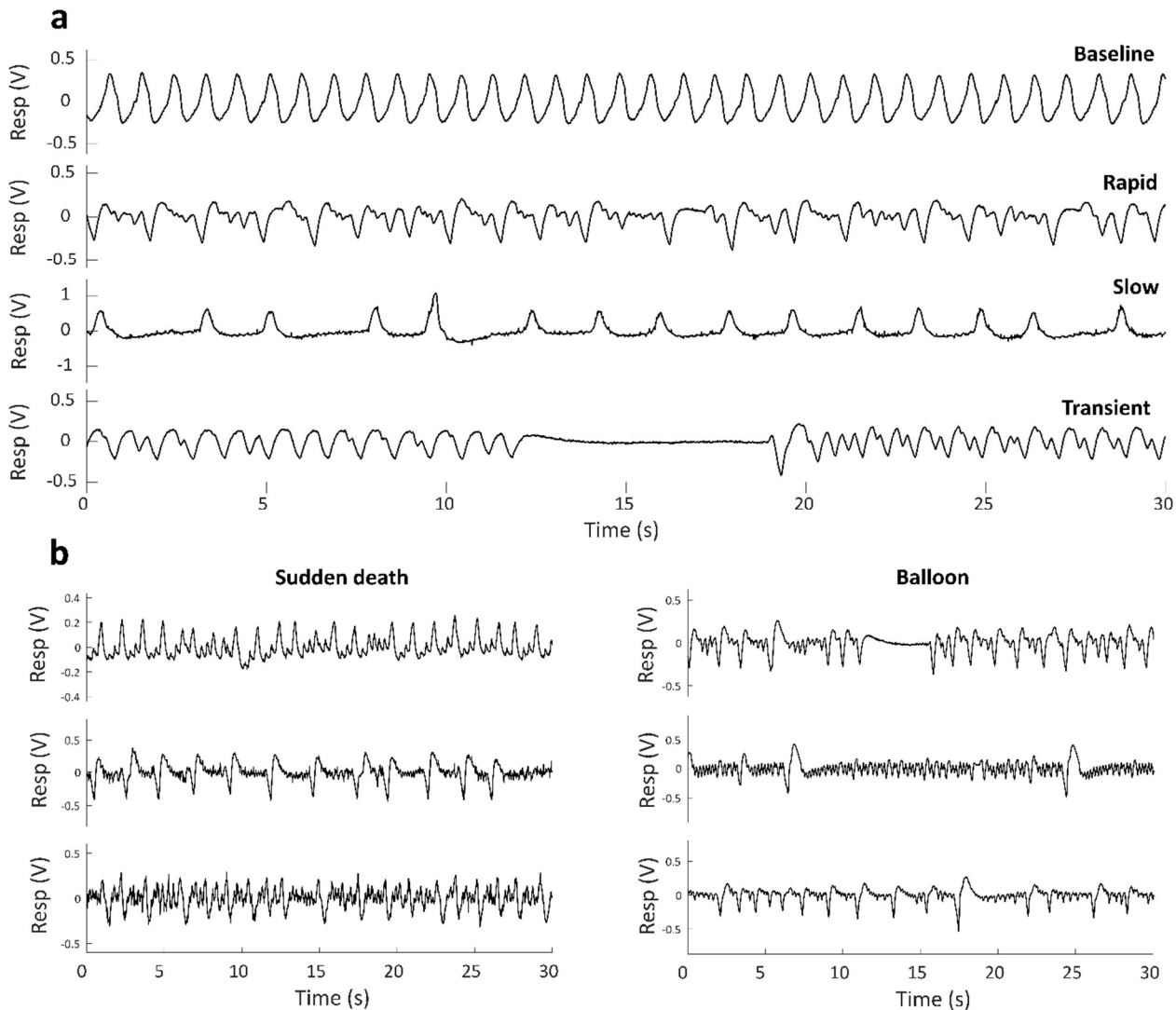


Figure 2: Respiratory waveforms. (a) example waveforms of animal respiration responses to KA. Baseline trace is before KA injection. Rapid trace is associated with acid movement, transient apnea, and sudden death. Slow trace is associated with no acid, no transient apnea, and gradual respiratory depression until death. Transient trace shows a transient apnea during KA response. (b) Comparison of waveforms between main group and balloon group. All animals on the left died of sudden laryngospasm. All of the animals on the right had balloon blocking and lived for the entire experiment duration. All animals had acid in the esophagus and all but one experienced transient apnea. The respiratory waveforms between the two groups are very similar, suggesting that the balloon may have prevented sudden death.

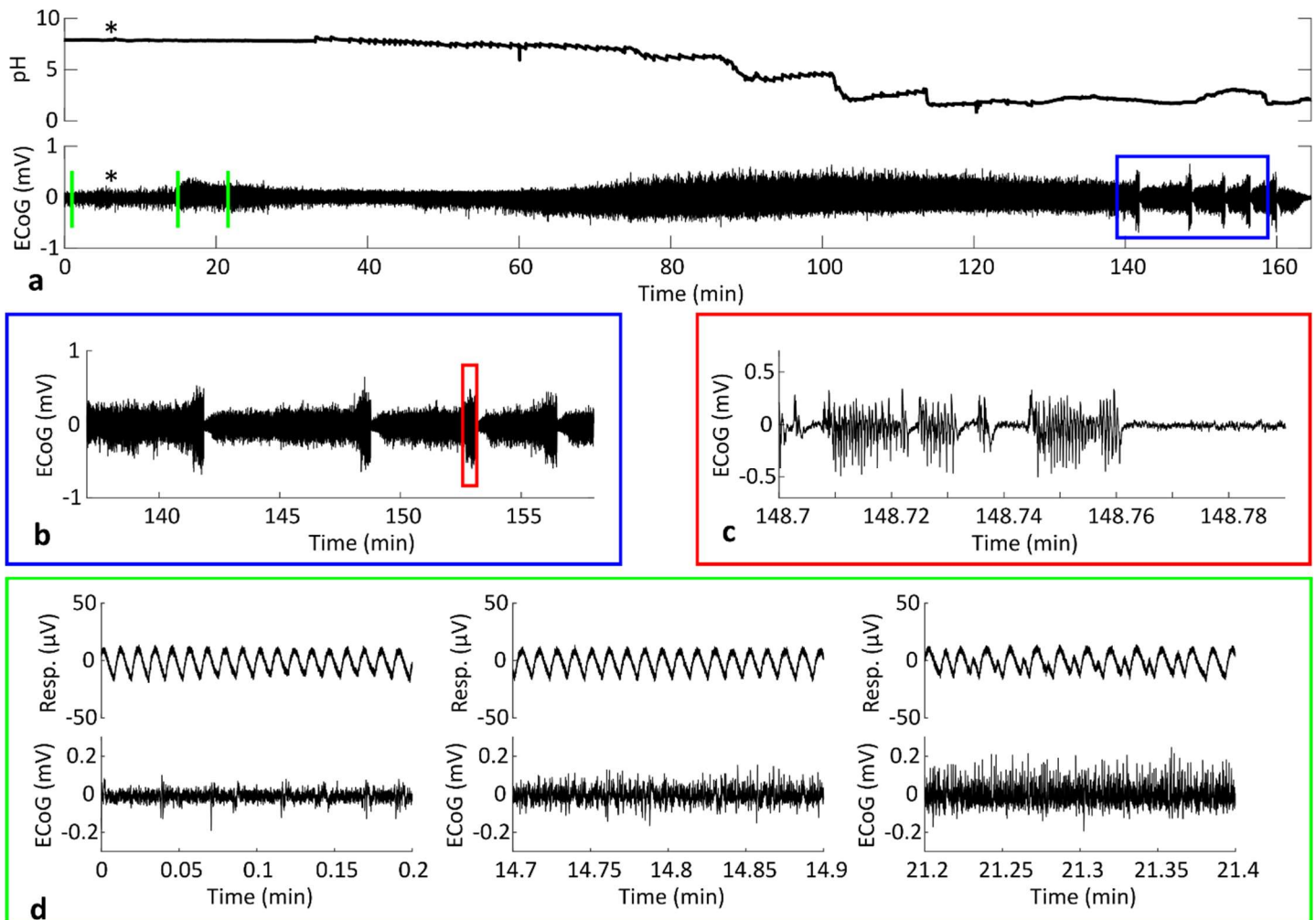


Figure 3: Results from experiment with KA induced seizures and electrocorticography (ECoG) recording. Colored boxes indicate excerpts. a) ECoG and pH measurement for the entire experiment. Asterisk denotes time of kainic acid injection. b) and c) Magnified views of discrete seizures with postictal ECoG depression. d) Progression of ECoG vs respiration rate and waveform. Left graphs are at baseline. Center graphs show the first increase in ECoG showing seizure activity. Right graphs show the first transition from baseline respiration to the pathological respiration pattern. The seizure activity precedes the change in respiration, suggesting the respiration pattern is a result of seizure activity. FFT analysis (not shown) verifies that changes in ECoG are due to high frequency seizure activity (30 Hz – 300 Hz).

We observed a second respiratory pattern where respiration decreased from baseline (81 ± 20 /min) to a much slower rate (33 ± 7.6 /min). Respiratory rate gradually decreased until death. The respiration pattern was even and regular, similar to baseline, but much slower. Transient apnea was never observed with this pattern.

Experiment outcome data is summarized in Figure 4.

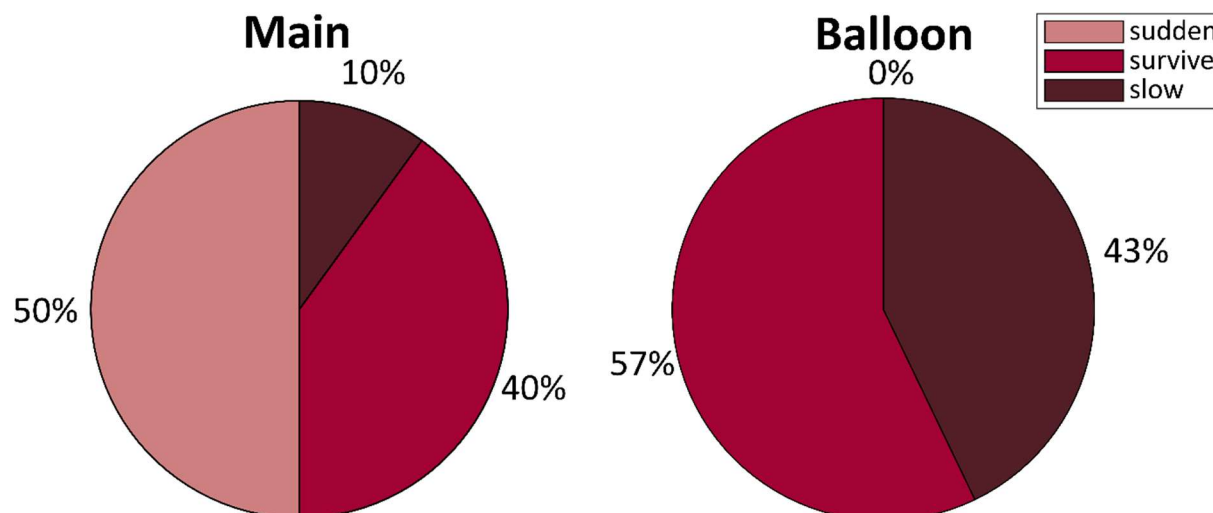


Figure 4: Summary of experiment results. In the non-esophageal blocking group five animals died suddenly, four survived, and one died of slow respiration. In the esophageal blocking group four animals survived, and three died of slow respiration.

3.1 Seizure recording

The animal with ECoG recording showed increased activity starting 10 min after KA injection (Figure 3), increased again approximately 50 min after injection, and continued until the end of the experiment. Around 50 min the animal also experienced a slow, steady pH change in the esophagus from pH 7.5 to 1.8. At approximately 140 min after injection the animal started having discrete seizures with pronounced postictal depression. These discrete seizures repeated until the end of the experiment. The animal displayed a change in respiration, as previously described, following the initial onset of seizure activity. Once the seizure activity increased at 50 min, the animal displayed moderate exophthalmos and twitching of the vibrissae. During discrete seizures the exophthalmos and vibrissae twitching became much more pronounced.

3.2 Uncategorized

One animal in the main experimental group displayed an atypical response to KA. The animal showed signs of strong seizure activity 30 seconds after KA injection, transient apneas 90 seconds after injection, and died four minutes after injection. The respiration rate and waveform were unstable and constantly changing. We believe this animal had an unusually strong response to KA, and clearly represents an outlier, so it is uncategorized. This animal is not included in further data presentation and statistical analysis.

3.3 Sudden death

We observed sudden death, as also observed by Nakase et al., which was characterized by the rapid respiration rate as defined above, followed by a sudden terminal apnea. In 4/5 cases we observed the abdomen of the animal still made apparent respiratory movements even after final apnea, suggesting effort to breathe and obstructive laryngospasm. In the fifth case the animal experienced convulsive movements of the dorsal thoracic musculature consistent with seizure activity during death, so we cannot be certain if there was respiratory effort. We observed sudden death in 5/10 animals in the main group, 0/7 animals in the balloon group, and 0/4 animals in the

control group. In all cases there was a large pH change in the esophagus from $\text{pH } 7.3 \pm 0.7$ to $\text{pH } 2.1 \pm 0.6$ prior to death (Figure 5). The initial pH change was detected 57.7 ± 19.7 min after primary KA injection. Death occurred 39.7 ± 28.8 min after the pH change and 97.4 ± 28.8 min after primary KA injection. Three of these animals experienced transient apneas. Detection of acid in the esophagus was statistically significantly related to sudden death – five of six animals with acid detected on all electrodes suddenly died, while none of the four without acid suddenly died ($p = 0.048$, Fisher's exact test, two-tailed).

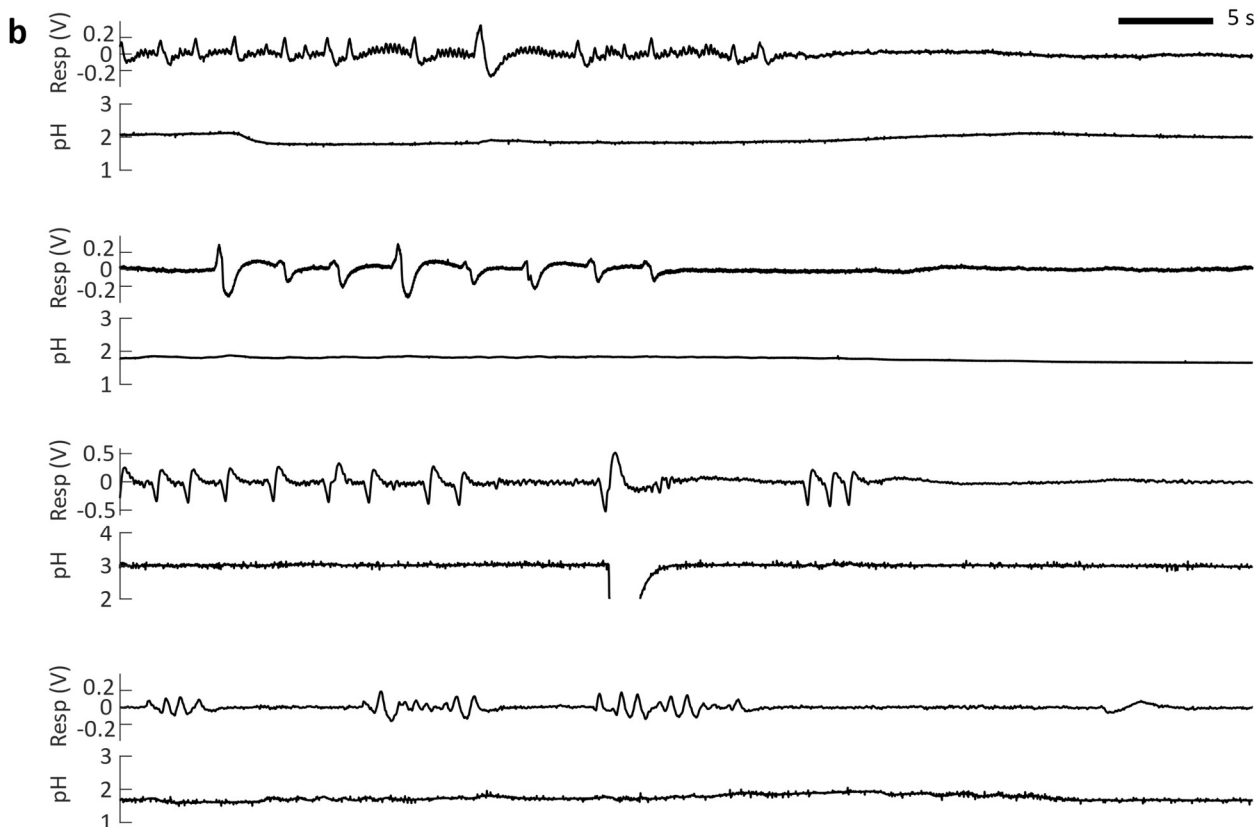
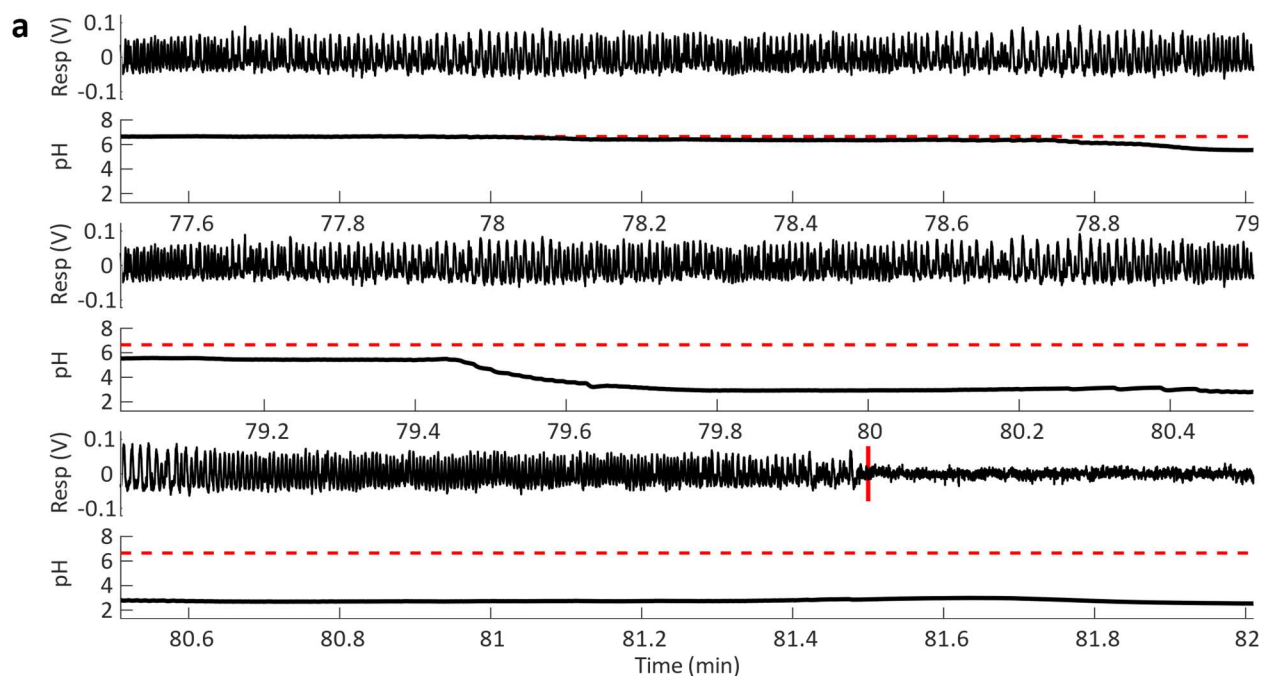


Figure 5: a) Thermocouple (top) and pH data (bottom) from an animal just before sudden death. Horizontal red dotted line indicates baseline pH. Vertical red line indicates time of death. pH drops slightly starting at 78.8 minutes and drops again sharply at 79.5 minutes. Time from pH drop to death is ~90 seconds. This animal was still moving its chest after airflow cessation, suggesting obstructive laryngospasm as the cause of death. b) Four additional respiration and pH

traces from the other animals that died suddenly. Each trace shows the 60s preceding death. The respiration trace for the second animal (third trace from the top) does not show the rapid shallow breaths like the other traces, however this was an issue with the recording. We can confirm that this animal had rapid shallow breathing in between the larger breaths, like the other animals.

3.4 Respiratory depression

We observed death from gradual respiratory depression in 1/10 animals in the main group, 3/7 animals in the balloon group, and 0/4 animals in the control group. Respiration slowed soon after KA injection and never recovered, gradually slowing until the animal died. There was no pH change, no transient apnea, and no evidence of effort to breathe during final apnea. Death occurred 46 ± 20.7 min after primary KA injection. This is faster than sudden death, which occurred 97.4 ± 28.8 min after KA injection. Both groups are approximately normally distributed ($p = 0.9, 0.2$, respectively, Shapiro-Wilk test), homoscedastic ($p = 0.6$, Bartlett's test), and statistically significantly different from one another, ($p = 0.045$, t-test, two-tailed).

3.5 Survival

Survival until end of data recording session occurred in 4/10 animals in the main group, 4/7 animals in the balloon group, and 4/4 animals in the control group. Sudden death occurred statistically significantly more frequently in the main experimental group (5/10) than in the esophageal blocking group (0/7) ($p = 0.044$, Fisher's exact test, two tailed).

Respiration for all non-control animals was consistent with the rapid irregular pattern displayed by animals that died suddenly. In the main group, 2/4 animals experienced a large pH change from 7.4 ± 0.2 to 2.4 ± 0.6 , however in one of these animals this pH change was detected only on the most distal electrode, while the pH on the proximal electrode did not change. One of these animals experienced transient apneas. In the balloon group, 3/4 animals experienced transient apneas lasting as long as 20 seconds and had respiration waveforms similar to sudden death animals (Figure 2b). All three of these animals also had acid detected in the esophagus. In two cases the acid leaked past the balloon, and in one case the acid was measured in the esophagus after death. In one case of acid leakage the acid was detected quickly by both of the electrodes, and there was a corresponding spike in pressure in the balloon, suggesting that acid pushed forcefully past the balloon. Acid leakages occurred near the end of the experiment and did not cause laryngospasm. The control animals had baseline respiration for the entire experiment and experienced no transient apneas or pH changes. There was no difference between balloon and non-balloon controls. All animals that experienced acid reflux or sudden death had received a KA injection and displayed signs of seizure activity. No control animal ever experienced reflux or sudden death.

3.6 Acid kinetics:

Initial pH changes were detected 63.8 ± 20.5 min after primary KA injection. The average time it took for the pH to change from the high value to the low value was 6.5 ± 13.7 min. In two cases the pH change occurred in < 10 s. In six animals in which there was a pH change and two recording electrodes, the average delay between the pH change on the electrodes was 15.2 ± 18 min. In one case the lag between electrodes was < 5 s.

3.7 Other observations

Three times after death we observed a pool of liquid at the animal's mouth, which was never present in control animals (Figure 6a). We measured the pH of this fluid to be 2.7 ± 1.2 . In all cases the pool of liquid was present after death, but before any of the electrodes had been pulled out of the esophagus. In one case, after removing electrodes, a large amount of stomach contents came out of the animal's mouth, forming a large stain on the surgical surface. Food contents were clearly visible in this fluid (Figure 6b). All animals with acid exiting the mouth had a previous pH change in the esophagus and had also displayed the rapid, irregular respiration pattern. In most animals with the rapid, irregular respiration rate we observed signs of seizure activity including exophthalmos, twitching of vibrissae, and in some cases minor motor convulsions. Urethane suppresses motor activity, so these motor convulsions are likely reduced compared to similar experiments using different anesthetics (Saito et al., 2006). We can confirm that these behaviors correspond to seizure activity based on other, currently unpublished, experiments from our laboratory with electrocorticography recording during acute KA-induced seizure activity (see Figure 3). We observed that transient apneas tended to cluster, occurring several times within 1-3 minutes, and then not occurring for many minutes. Seizure activity began 10-60 minutes after primary KA injection. Seizure activity continued until the end of the experiment, as KA induces continuous seizure activity for at least three hours (Saito et al., 2006). We noticed no difference in seizure onset or duration between sudden death animals and animals that survived.

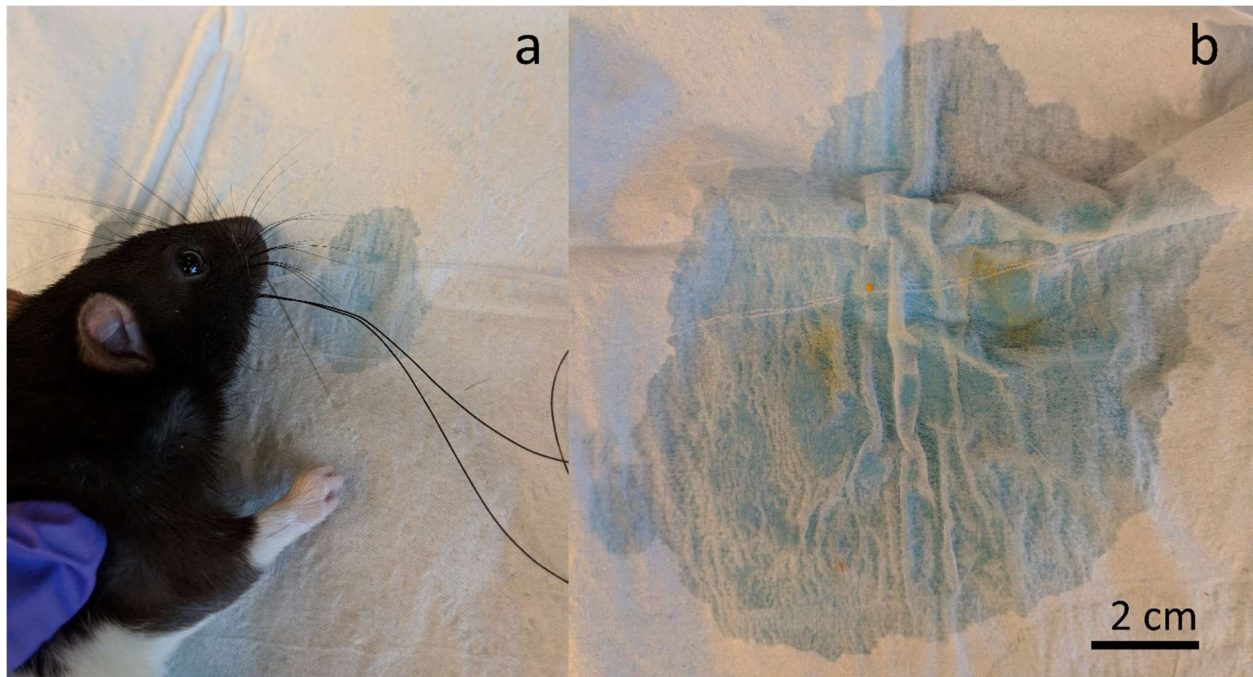


Figure 6: Evidence of acid movement out of mouth. Colors and contrast enhanced to better visualize the wet spot. (a) An animal with fluid at the mouth before any electrodes have been removed. (b) Fluid after electrodes are removed. Yellow food particles are visible. The pH of this fluid was measured to be very low, about pH 2.

4. Discussion:

We have observed that the presence of acid in the esophagus is 100% correlated with sudden death from laryngospasm in acute KA-induced seizures. Further, when acid is prevented from moving up the esophagus, sudden deaths do not occur, indicating causation. We have also observed that acidic contents can move all the way into the mouth and out of the animal. We believe these observations are strong evidence that acid is the primary trigger of laryngospasm and subsequent death in the acute KA model. We have also observed that transient apneas occur in most cases prior to sudden death and may be a useful predictor. We observed that animals had varying behavior in response to KA, suggesting that there may be multiple mechanisms involved. In Nakase et al. and Sakamoto et al. the authors found that initial response to KA (10-60 minutes after injection) is associated with continuous seizure activity (*status epilepticus*), followed by shorter recurring seizures of varying length that may or may not qualify as *status*. We hypothesize that gradual respiratory depression, which occurred within 46 ± 20.7 min of induction of seizure activity may occur during the initial period of *status*, while sudden death via laryngospasm, which occurred later in experiments (97.4 ± 28.8 min after induction of seizure activity) may occur during the shorter recurring seizures that follow.

The presence of acid in the esophagus and mouth is extraordinary because rats cannot normally vomit. They lack the musculature to produce retching, and acid movement does not occur, even when given emetic agents (Horn et al., 2013). In humans there is constantly acid in the esophagus, and even regularly appearing in the larynx in some cases. Normal patients will have esophageal pH < 4 approximately 5% of the time (Lutsi and Hirano, 2006). This is not the case for rats, in which the presence of any acid at all is abnormal.

4.1 Time delay between acid reflux and laryngospasm:

There was a wide time range from pH change to fatal laryngospasm. In a healthy animal, laryngospasm should occur instantaneously once acid has reached the larynx. We could not place electrodes at the top of the esophagus, so we cannot determine when the acid reached the larynx. We spaced the pH electrodes to measure the speed of acid movement, but there was no correlation between speed and time until fatal laryngospasm, possibly because the velocity of acid movement was observed to be variable between stomach and first electrode, first and second electrodes, and from there to the larynx. In some cases, the pH change on a single electrode was very slow, and there was a lag of several minutes between the two electrodes, suggesting acid is slowly leaking up the esophagus. In other cases, the pH change was rapid, and the lag between the two electrodes was less than five seconds, suggesting a forceful burst of acid up the esophagus.

4.2 The kainic acid model:

The KA model is useful because it can produce temporal lobe epilepsy-like seizures in an acute setting (Naggar and Stewart, 2015; Nakase et al., 2016; Sakamoto et al., 2008). However, the KA model does not, by definition, produce SUDEP like seizures, as it induces seizures in an otherwise healthy brain and induces *status epilepticus*. The KA model also has some variability in the mechanisms of death. We observed significant variability between animals with the same dosing. Some animals showed little seizure activity, some showed transient apnea, some respiratory depression, etc. We cannot be certain which of these behaviors is SUDEP like, and which are not. The KA model is useful because it can replicate deaths from seizures in an acute

setting, which no true model of SUDEP can, but these results must still be proven in a chronic model of epilepsy.

4.3 Respiratory depression:

In several experiments we observed responses with continuous respiratory depression eventually resulting in death. Nakase et al. similarly report animals with slow, gasping respiration as a response to seizure activity. As the timeline and physical observations of this response are different from the sudden deaths, we believe that the two responses are distinct, but we cannot speculate as to the mechanism causing respiratory depression.

4.4 Limitations:

This study has several limitations including a small sample size, lack of electrocardiography recording, lack of electrocorticography recording for most animals, and potential confounds by systemic KA administration.

4.5 Mechanism:

We believe that acid induced laryngospasm may be a potential mechanism for SUDEP. We hypothesize that seizure activity causes relaxation of the LES and UES, overproduction of stomach acid, or both, which combine to force acid up the esophagus and into the larynx. Once in the larynx, the acid triggers an obstructive laryngospasm, from which the patient is unable to recover.

4.6 A broader mechanism?

We speculate that these experiments may provide insight into other mechanisms of sudden death. Sudden infant death syndrome (SIDS), kills approximately 2,000 infants every year in the US (Murphy et al., 2013). There is evidence that SIDS is preceded by laryngeal inflammation (Scadding et al., 2014), which may be caused by stomach acid. The risk for SIDS can be reduced by placing a baby supine, not prone, to sleep (Athanasakis et al., 2011). The supine position places pressure on the esophagus and may impede acid movement. Like SUDEP, SIDS often occurs at night, and infants would not be able to sit up and clear acid if it entered their larynx in the way that an adult would. It is possible that the mechanism for SIDS is related to acid induced laryngospasm as well.

4.7 A note on previous work:

In Nakase et al., the authors severed the SLN in order to prevent reflexive laryngospasm and subsequently observed obstructive laryngospasm and death. Reflexive laryngospasm could be triggered by stomach acid entering the larynx, following the reflux we report here. From these results they determined that the cause of obstructive laryngospasm is most likely seizure activity spreading to the laryngeal motor neurons. Our findings question this hypothesis. One possible explanation may be found in Loughlin et al., in which the authors demonstrated that reflexive laryngospasm is still possible even with the SLN cut, but only under certain circumstances. We do not dispute other recent proposals for mechanisms of SUDEP (Aiba and Noebels, 2015; Ryvlin et al., 2013), but instead suggest that there may be multiple mechanisms, or multiple components to particular mechanisms.

To date we have early evidence to support and no evidence to contradict the hypothesis that some sudden deaths from seizures are caused by stomach acid-induced laryngospasm. This mechanism is important to explore because several anti-epileptic drugs, such as Acetazolamide, Carbamazepine, Clobazam, Ethosuximide, Gabapentin, Levetiracetam (WebMD, 2018a, b, c, d, e, f), and more have side effects that increase stomach acid movement into the esophagus.

5. Conclusions

We have demonstrated that sudden death due to laryngospasm in the kainic acid model of epilepsy is always preceded by a significant pH drop in the esophagus. Further, we have shown that eliminating this pH drop also eliminates sudden death. This knowledge may inform future research into sudden death in epilepsy and may contribute to a hypothesis that we have presented. This mechanism may be relevant in other mechanisms of sudden death, such as SIDS.

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Disclosure of Conflicts of Interest:

None of the authors has any conflict of interest to disclose.

References:

- Aiba, I., Noebels, J.L., 2015. Spreading depolarization in the brainstem mediates sudden cardiorespiratory arrest in mouse SUDEP models. *Science Translational Medicine* 7, 282ra246.
- Athanasakis, E., Karavasiliadou, S., Styliadis, I., 2011. The factors contributing to the risk of sudden infant death syndrome. *Hippokratia* 15, 127-131.
- Bateman Lisa, M., Spitz, M., Seyal, M., 2010. Ictal hypoventilation contributes to cardiac arrhythmia and SUDEP: Report on two deaths in video-EEG-monitored patients. *Epilepsia* 51, 916-920.
- Berg, A., 2001. Mortality in Epilepsy. *Epilepsy Currents* 1, 28-28.
- Bird, J.M., Dembny, K.A.T., Sandeman, D., Butler, S., 2013. Sudden Unexplained Death in Epilepsy: An Intracranially Monitored Case. *Epilepsia* 38, S52-S56.
- Brodie, M.J., 2013. Road to refractory epilepsy: The Glasgow story. *Epilepsia* 54, 5-8.
- Caflisch, C.R., Pucacco, L.R., Carter, N.W., 1978. Manufacture and utilization of antimony pH electrodes. *Kidney International* 14, 126-141.
- Debas, H.T., Carvajal, S.H., 1994. Vagal regulation of acid secretion and gastrin release. *The Yale Journal of Biology and Medicine* 67, 145-151.
- Devinsky, O., 2011. Sudden, Unexpected Death in Epilepsy. *New England Journal of Medicine* 365, 1801-1811.
- Finnerty, G.T., Jefferys, J.G.R., 2000. 9–16 Hz Oscillation Precedes Secondary Generalization of Seizures in the Rat Tetanus Toxin Model of Epilepsy. *Journal of Neurophysiology* 83, 2217-2226.
- Hassel, A.W., Fushimi, K., Seo, M., 1999. An agar-based silver|silver chloride reference electrode for use in micro-electrochemistry. *Electrochemistry Communications* 1, 180-183.
- He, Y.Q., Sheng, J.Q., Wang, J.H., An, H.J., Wang, X., Li, A.Q., Wang, X.W., Gyawali, C.P., 2013. Symptomatic diffuse esophageal spasm as a major ictal manifestation of post-traumatic epilepsy: a case report. *Diseases of the Esophagus* 26, 327-330.
- Horn, C.C., Kimball, B.A., Wang, H., Kaus, J., Dienel, S., Nagy, A., Gathright, G.R., Yates, B.J., Andrews, P.L.R., 2013. Why Can't Rodents Vomit? A Comparative Behavioral, Anatomical, and Physiological Study. *PLoS ONE* 8, e60537.
- Lacuey, N., Vilella, L., Hampson, J.P., Sahadevan, J., Lhatoo, S.D., 2018. Ictal laryngospasm monitored by video-EEG and polygraphy: a potential SUDEP mechanism, *Epileptic Disorders*.
- Lamberts Robert, J., Thijs Roland, D., Laffan, A., Langan, Y., Sander Josemir, W., 2011. Sudden unexpected death in epilepsy: People with nocturnal seizures may be at highest risk. *Epilepsia* 53, 253-257.
- Langan, Y., Nashef, L., Sander, J.W.A.S., 2000. Sudden unexpected death in epilepsy: a series of witnessed deaths. *Journal of Neurology, Neurosurgery & Psychiatry* 68, 211.
- Loughlin Christopher, J., Koufman James, A., Averill David, B., Cummins Michelle, M., Kim, Y.J., Little John, P., Miller Inglis, J., Meredith, J.W., 2009. Acid-Induced Laryngospasm in a Canine Model. *The Laryngoscope* 106, 1506-1509.
- Lutsi, B., Hirano, I., 2006. Ambulatory pH Monitoring: New Advances and Indications. *Gastroenterology & Hepatology* 2, 835-842.
- Marks, M., South, M., Carter, B., 1995. Measurement of respiratory rate and timing using a nasal thermocouple. *Journal of Clinical Monitoring* 11, 159-164.
- Massey, C.A., Sowers, L.P., Dlouhy, B.J., Richerson, G.B., 2014. Mechanisms of sudden unexpected death in epilepsy: the pathway to prevention. *Nature Reviews Neurology* 10, 271+.

- McLean, B.N., Wimalaratna, S., 2007. Sudden death in epilepsy recorded in ambulatory EEG. *Journal of Neurology, Neurosurgery & Psychiatry* 78, 1395.
- Murphy, S.L., Xu, J., Kochanek, K.D., 2013. Deaths: Final Data for 2010. NIH, National Vital Statistics Reports.
- Naggar, I., Stewart, M., 2015. A Rat Model for Exploring the Contributions of Ventricular Arrhythmias to Sudden Death in Epilepsy, pp. 241-250.
- Nakase, K., Kollmar, R., Lazar, J., Arjomandi, H., Sundaram, K., Silverman, J., Orman, R., Weedon, J., Stefanov, D., Savoca, E., Tordjman, L., Stiles, K., Ihsan, M., Nunez, A., Guzman, L., Stewart, M., 2016. Laryngospasm, central and obstructive apnea during seizures: Defining pathophysiology for sudden death in a rat model. *Epilepsy Research* 128, 126-139.
- Nobili, L., Proserpio, P., Rubboli, G., Montano, N., Didato, G., Tassinari, C.A., 2011. Sudden unexpected death in epilepsy (SUDEP) and sleep. *Sleep Medicine Reviews* 15, 237-246.
- Postma, G.N., Halum, S.L., 2006. Laryngeal and pharyngeal complications of gastroesophageal reflux disease. *GI Motility Online*.
- Ryvlin, P., Nashef, L., Lhatoo, S.D., Bateman, L.M., Bird, J., Bleasel, A., Boon, P., Crespel, A., Dworetzky, B.A., Høgenhaven, H., Lerche, H., Maillard, L., Malter, M.P., Marchal, C., Murthy, J.M.K., Nitsche, M., Patariaia, E., Rabben, T., Rheims, S., Sadzot, B., Schulze-Bonhage, A., Seyal, M., So, E.L., Spitz, M., Szucs, A., Tan, M., Tao, J.X., Tomson, T., 2013. Incidence and mechanisms of cardiorespiratory arrests in epilepsy monitoring units (MORTEMUS): a retrospective study. *The Lancet Neurology* 12, 966-977.
- Saito, T., Sakamoto, K., Koizumi, K., Stewart, M., 2006. Repeatable focal seizure suppression: A rat preparation to study consequences of seizure activity based on urethane anesthesia and reversible carotid artery occlusion. *Journal of Neuroscience Methods* 155, 241-250.
- Sakamoto, K., Saito, T., Orman, R., Koizumi, K., Lazar, J., Saliccioli, L., Stewart, M., 2008. Autonomic consequences of kainic acid-induced limbic cortical seizures in rats: Peripheral autonomic nerve activity, acute cardiovascular changes, and death. *Epilepsia* 49, 982-996.
- Scadding, G.K., Brock, C., Chouiali, F., Hamid, Q., 2014. Laryngeal Inflammation in the Sudden Infant Death Syndrome. *Current Pediatric Reviews* 10, 309-313.
- Selassie, A., 2015. Risk Factors of Epilepsy Outcomes: Comorbidities in Population with Epilepsy in South Carolina.
- Sowers, L.P., Massey, C.A., Gehlbach, B.K., Granner, M.A., Richerson, G.B., 2013. Sudden unexpected death in epilepsy: Fatal post-ictal respiratory and arousal mechanisms. *Respiratory Physiology & Neurobiology* 189, 315-323.
- Tao James, X., Qian, S., Baldwin, M., Chen, X.J., Rose, S., Ebersole, S.H., Ebersole John, S., 2010. SUDEP, suspected positional airway obstruction, and hypoventilation in postictal coma. *Epilepsia* 51, 2344-2347.
- Tavee, J., Morris, H.I., 2008. Severe postictal laryngospasm as a potential mechanism for sudden unexpected death in epilepsy: a near-miss in an EMU. *Epilepsia* 49, 2113-2117.
- Thurman David, J., Hesdorffer Dale, C., French Jacqueline, A., 2014. Sudden unexpected death in epilepsy: Assessing the public health burden. *Epilepsia* 55, 1479-1485.
- Vela, M.F., Richter, J.E., Pandolfino, J.E., 2013. *Laryngopharyngeal Reflux, Practical Manual of Gastroesophageal Reflux Disease*, 1st ed. John Wiley & Sons, Ltd, pp. 154-160.
- WebMD, 2018a. Acetazolamide side effects.
- WebMD, 2018b. Carbamazepine side effects.
- WebMD, 2018c. Clobazam side effects.
- WebMD, 2018d. Ethosuximide side effects.

WebMD, 2018e. Gabapentin side effects.

WebMD, 2018f. Levetiracetam side effects

Zack, M.M., Rosemarie, K., 2017. National and State Estimates of the Numbers of Adults and Children with Active Epilepsy — United States, 2015, MMWR Morb Mortal Wkly, pp. 821-825.