Scientific Summary

Unexpected Death in Epilepsy (SUDEP) is defined as the sudden, unexpected, and unexplained death of a person with epilepsy. SUDEP accounts for between 8 and 17% of all epilepsy-related deaths[1], rising to 50% in patients for which current therapies are ineffective[2,3].

SUDEP is extremely challenging to study in patients, precisely because of its unexpected nature. I propose to utilize a transgenic mouse model that harbors an Scn8a mutation that was identified in a patient that suffered SUDEP, and reproduces many of the pathological hallmarks; including spontaneously occurring generalized tonic-clonic seizures and SUDEP-like deaths[4–6].

There is increasing evidence that central apnea is the primary cause of death in SUDEP. Patients commonly experience central, non-obstructive, apnea during and after seizures[7–9], and 12 out of 15 monitored cases of SUDEP presented with evidence of respiratory complications[10]. Our SUDEP model mice display a similar pathology. In both patients and our mouse model, I find that apnea occurs during the tonic phase of seizures, and recovers after the tonic phase ends (Figure 1, A and B). In the mouse model, seizure-induced death results when breathing does not recovery after the tonic phase (Figure 1C). *This preliminary data is currently part of a first author manuscript under review at* ***Science Translational Medicine****.*

Breathing requires the coordinated oscillation of inspiratory motor output. The neural circuitry that generates this oscillation (i.e. "inspiratory oscillator") resides in the brainstem. Central apnea occurs due to the absence of this oscillation. Absence of oscillation can be due to no muscle activity (e.g. severed spinal cord above C3) or constant tonic muscle activity (e.g. inspiratory breath hold). Considering the observed tonic muscle activity during seizure-induced apnea, **I hypothesize that seizure-induced apnea occurs because of tonic inspiratory activity (Aim 1), and recovery from apnea is possible once this tonic activity subsides (Aim 2; see Figure 2A for diagram).**

I propose to rescue breathing using channelrhodopsin2 (ChR2) to selectively photostimulate respiratory neurons of the Bötzinger Complex (BötC) and retrotrapezoid nucleus (RTN), that engage the inspiratory oscillator in distinct ways.

AIM 1: The tonic phase of a seizure causes tonic muscle contraction, possibly including inspiratory muscles (i.e. diaphragm), resulting in no oscillatory motor activity (Figure 2). The BötC is a group of mostly glycinergic neurons that provides powerful inhibition of the inspiratory oscillator[11–13]. To test whether tonic inspiratory activity causes apnea, I will photostimulate BötC neurons. I expect BötC stimulation to recover breathing during the tonic, but not postictal, phase.

AIM 2: After the tonic phase, muscle tone returns to normal, and it is possible to drive breathing via the inspiratory oscillator. The RTN contains neurons that potently activate the inspiratory oscillator[14–16]. To test when engagement of the inspiratory oscillator is possible, I will photostimulate the RTN. I expect RTN stimulation to recover breathing during the postictal, but not the tonic, phase.

Significance: This work will be the first to test the state of the inspiratory oscillator during seizure-induced apnea, which will lead to a better understanding of seizure-induced apnea and avenues of potential interventions for SUDEP. It therefore directly addresses ***CURE's priority research areas*** *of "Novel research that furthers our understanding of the causes and ultimate elimination of SUDEP"*, in addition to the ***2014 NINDS Benchmarks for Epilepsy Research*** *IV Part D. "Identify causes, risk factors, and potential preventive strategies for sudden unexpected death in epilepsy (SUDEP)".*

Future Directions

Data collected from both aims of this research proposal will provide the foundation for my first R01 application investigating mechanisms of SUDEP. The results will provide both a method to rescue apnea and reveal details about the state of inspiratory oscillator during and after seizures.

AIM 1: Once an apnea rescue-protocol is attained from the above proposed experiments; I will test the hypothesis that rescue of tonic phase apnea will prevent seizure-induced death (aka SUDEP). I will develop automated algorithms to detect spontaneous seizures and trigger optogenetic stimulation to rescue tonic phase apnea and prevent SUDEP. Automated seizure detection has already been achieved by others[17,18]. In my case, ECG signals will also be utilized, which will greatly improve detection of the tonic phase. I will then induce the apnea rescue protocol for spontaneous seizures to see if it prolongs life in our mouse model.

AIM 2: Based on evidence from the proposed research above; I will investigate the upstream mechanisms that contribute to tonic phase apnea and the recovery. Depending on whether the inspiratory oscillator is inhibited or tonically activated will help direct future study into upstream neural centers that may be recruited during seizures and impact the inspiratory oscillator. One such region could be the amygdala, which has been show to promote apnea in patient and some experimental models. I will record relevant nuclei that may impact the oscillator during induced and spontaneous seizure. I will also perform in vitro patch clamp analysis of neurons that might impact the inspiratory oscillator. An example would be to study impairment of the RTN neurons. These neurons are an important CO_2-dependent drive to breath[19], and patients at risk for SUDEP have a reduced hypercapnic ventilatory reflex[8,20,21].

AIM 3: Because of the success of the above proposed experiments, I will also be able to propose an optogenetic approach to manipulate the autonomic nervous system. I also have preliminary data related to impairment of autonomic control during seizures (Figure 3). Maladapted autonomic control of the heart has been proposed by as a cause of SUDEP[22,23] and I have found that bradycardia, not tachycardia, follows a fatal seizure (Figure 3A). I am currently dissecting the autonomic control of the heart by pharmacologically blocking the sympathetic and parasympathetic arms separately to determine their role in seizures and recovery (Figure 3B and C). I will use a similar approach to test the hypothesis that altered autonomic control of the heart contributes to SUDEP, but optogenetically stimulating or inhibiting pre-sympathetic neurons of the rostroventrolateral medulla[24] and/or parasympathetic neurons of the nucleus ambiguous[25] during induced seizures.

Award Relevance

During the short time in Dr. Patel's laboratory, I have built my own system for simultaneous recording of breathing, cardiac activity, and seizures in mice. My knowledge of the brainstem circuitry in homeostatic control will determine which brainstem neurons to target for future *in vitro* and *in vivo* studies. Furthermore, I have trained and mentored undergraduate researchers and technical staff to participate in these research projects. Dr. Patel, and the other members of the Epilepsy Interest Group (EIG) here at UVA, have provided mentorship in the field of epilepsy research, including mechanisms involving ion channel physiology and recording chronic video/EEG. However, I plan to examine and manipulate the neural circuitries of the brainstem, spinal cord, and peripheral nervous system in epileptic mouse models. My knowledge of these systems gives me a unique perspective and enables me to ask novel questions concerning epilepsy and SUDEP. My research program will rely on my repertoire of brainstem physiological techniques for which I am an expert, including patch clamp, unit and field potential recordings, in addition to my training in viral vector injection, optogenetics and conscious cardiorespiratory recordings[4,6,8-12,14]. **In this way, I will not only distinguish myself from my mentor, but provide a new approach to epilepsy and SUDEP research.**

Figure 1 - Preliminary data (in review at Sci Trans Med)

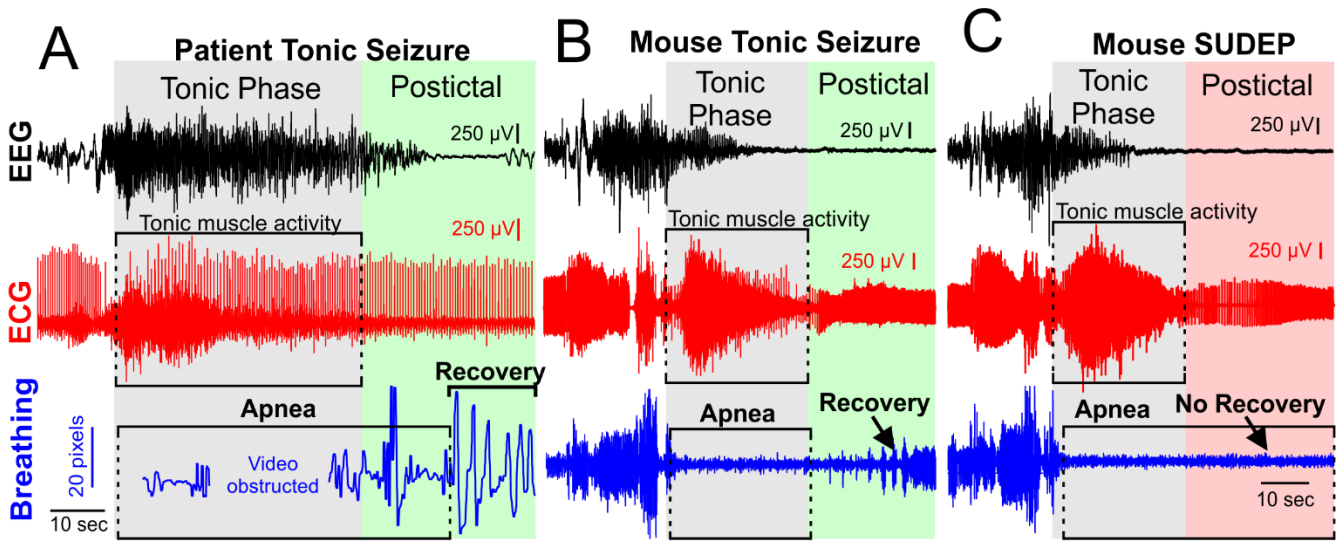


Figure 2 - Aims 1 and 2

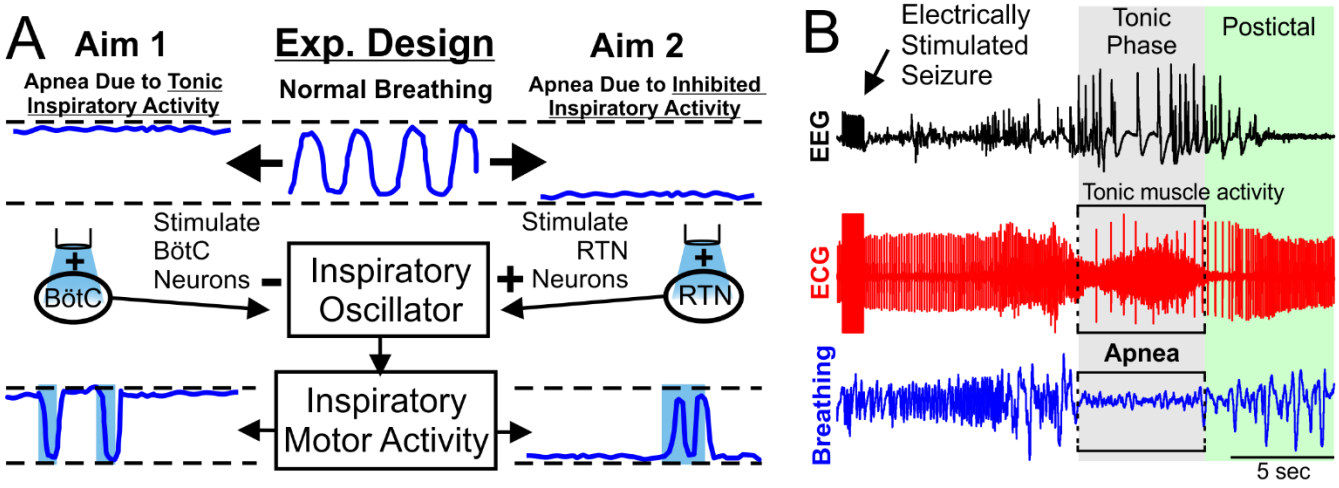
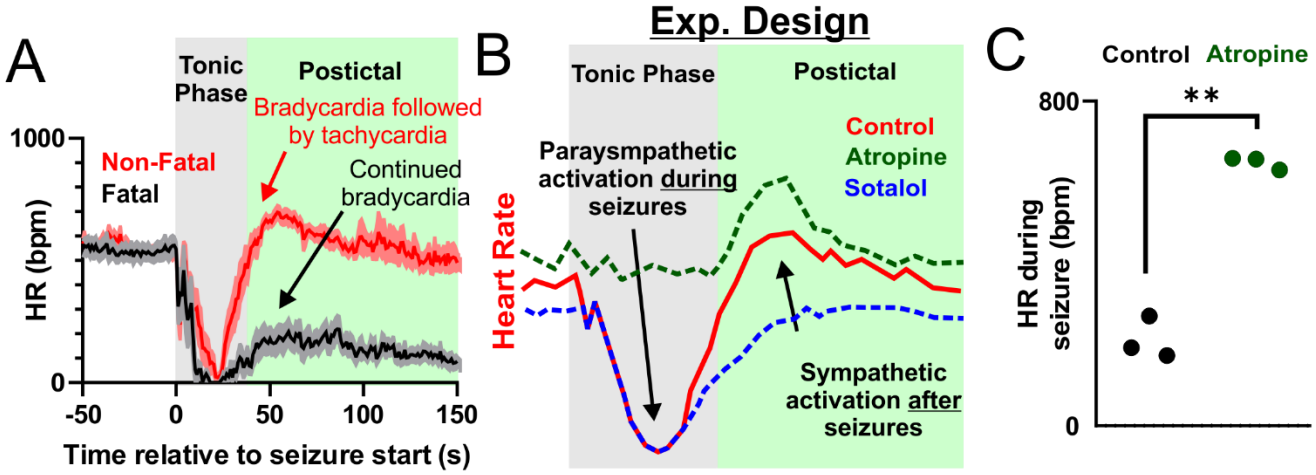


Figure 3 - Future Directions



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