SUDDEN UNEXPECTED DEATH IN EPILEPSY: BIO-MARKERS, MECHANISMS, RISK IDENTIFICATION AND PREVENTION

EDITED BY: Christopher Michael DeGiorgio, Rainer Surges and Michael R. Sperling

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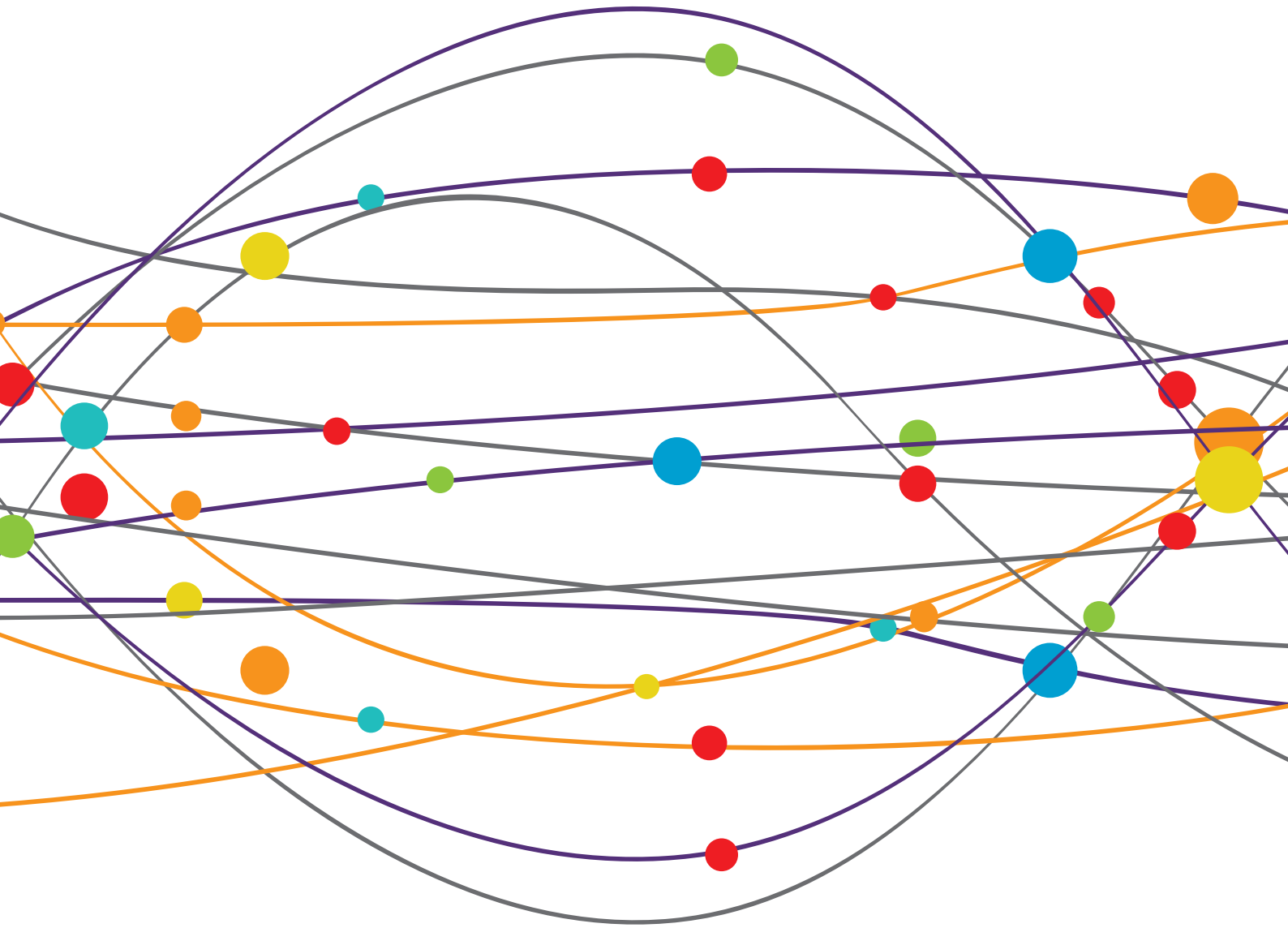

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SUDDEN UNEXPECTED DEATH IN EPILEPSY: BIO-MARKERS, MECHANISMS, RISK IDENTIFICATION AND PREVENTION

Topic Editors:
Christopher Michael DeGiorgio, University of California, Los Angeles, United States
Rainer Surges, Universitätsklinikum Bonn, Germany
Michael R. Sperling, Thomas Jefferson University, United States

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EDITORIAL
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Editorial: Sudden Unexpected Death in Epilepsy: Bio-markers, Mechanisms, Risk Identification and Prevention

Rainer Surges[1]*, *Michael R. Sperling*[2]* and *Christopher M. DeGiorgio*[3]*

[1] Department of Epileptology, University Hospital Bonn, Bonn, Germany, [2] Department of Neurology, Jefferson Comprehensive Epilepsy Center, Thomas Jefferson University, Philadelphia, PA, United States, [3] Department of Neurology, David Geffen-UCLA School of Medicine, Sylmar, CA, United States

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Campinas State University, Brazil

***Correspondence:**
Rainer Surges
rainer.surges@ukbonn.de
Michael R. Sperling
michael.sperling@jefferson.edu
Christopher M. DeGiorgio
cmd@mednet.ucla.edu

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Editorial on the Research Topic

Sudden Unexpected Death in Epilepsy: Bio-markers, Mechanisms, Risk Identification and Prevention

Sudden unexpected death in epilepsy (SUDEP) is a common cause of epilepsy-related mortality, with an incidence of 1.2 per 1,000 persons (1). SUDEP causes 15–17% of all epilepsy-related deaths and carries a lifetime risk of 4–8% (1, 2). Reliable biomarkers and predictors are still lacking, but the most important risk factors are nocturnal and frequent tonic-clonic seizures. Mechanisms include tachycardia and tachypnea followed by bradycardia, asystole, and apnea in SUDEP cases documented in epilepsy monitoring units (3). The underlying neuronal and molecular pathomechanisms that turn non-fatal into fatal seizures remain to be elucidated, but brainstem dysfunction and spreading depression as well as defects in serotonin-related signaling appear to be involved. Comprehensive information about SUDEP is recommended and desired by most patients, relatives, and caregivers. Improved seizure control by pharmacotherapy, epilepsy surgery, and neuromodulatory devices is key to SUDEP prevention. Nocturnal supervision is associated with reduced SUDEP risk, and clinically tested seizure detection systems are available.

CARDIORESPIRATORY MECHANISMS

Postictal breathing disturbances are a hallmark of monitored SUDEP cases (3). In this Research Topic, Vilella et al. investigated the occurrence and influencing factors of seizure-related central apnea in a prospective multicenter study. They found that postictal central apnea occurred in about 20% of tonic-clonic seizures (generalized convulsive seizures) in 20% of the patients, its recurrence risk amounted to about 50% in a given individual. Postictal central apnea was less frequent than ictal central apnea and seen in all epilepsy types, possibly indicating different pathomechanisms. In addition to respiratory dysfunction, recent studies described profound alterations of systemic arterial blood pressure and its regulation in association with seizures [e.g., (4, 5)], suggesting a

possible role in SUDEP (6). Nass et al. summarize the current knowledge on control of blood pressure in epilepsy, describe the relevant cerebral mechanisms and pathways and discuss how epilepsy- and seizure-related blood pressure alterations may contribute to premature mortality and SUDEP.

RISK FACTOR "NOCTURNAL SEIZURES" AND NUTRITION

Seizures arising from sleep or occurring during nighttime were identified as a risk factor for SUDEP (7). To date, it is still unresolved whether this is predominantly due to the fact that people are more often unsupervised or to distinct neuronal mechanisms and networks properties related to sleep. Purnell et al. review the possible factors contributing to the relationship between sleep and SUDEP and explain potential molecular and neuronal mechanisms. In a mouse model of Dravet syndrome with high mortality, Teran et al. observed that SUDEP rates and seizure frequency were higher in the early evening and nighttime, suggesting that specific circadian or sleep-related neuronal mechanisms increase the risk of SUDEP. They also noted that placing mice on a ketogenic diet led to a considerable reduction in mortality. Importantly, the mice fed with ketogenic diet did not have fewer seizures than control mice, indicating that nutritional effects on mortality and the SUDEP rate were independent of seizure frequency. In another experimental study of this Research Topic, Taha et al. tested the effects of dietary measures on seizure thresholds in rat models of kindling and acute seizures. They found that chronic dietary omega-3 polyunsaturated fatty acid deficiency did not alter seizure thresholds, but impaired the effects of acutely applied omega-3 docosahexaenoic acid (Taha et al.) Altogether, the results of these two experimental studies confirm that nutrition has an impact on brain excitability and underscore that more research should be performed on diet in the context of epilepsy and maybe SUDEP.

BIOMARKERS, RISK IDENTIFICATION, AND PREVENTION

The search for reliable biomarkers and predictors of an elevated SUDEP risk is of great importance for designing appropriate safety measures and interventional studies to prevent SUDEP. An increasing number of studies deals with the question whether brain regions involved in the regulation of autonomic function display functional and structural alterations that are linked to the SUDEP risk. If present, these alterations could serve as neuroimaging markers that would help to identify people at higher risk. In this Research Topic, Allen et al. have compiled recent findings on resting-state functional and structural MRI studies, strengthening the view that disturbed central autonomic and respiratory control contributes to the pathophysiology of SUDEP. For instance, studies on morphometry and cortical thickness revealed reduced volume and cortical thinning in the thalamus, frontal cortex and at brainstem sites in patients with SUDEP and in those with tonic-clonic seizures. Importantly, the authors point out that the MRI-based prediction of the individual SUDEP risk requires further studies. A personalized estimation of the danger, however, would be very helpful when counseling people with epilepsy and their relatives or caregivers. In this context, Shankar et al. ask if "the time has come to stratify and score SUDEP risk to inform people with epilepsy of their changes in safety". The authors present work which identifies the need to improve communication at a primary care level. They suggest that regular reviews using a structured risk factor checklist as a screening tool would help in earlier identification of people whose health is worsening and to justify referrals to specialists.

SUMMARY

This Research Topic compiles recent findings on seizure-related breathing disturbances and blood pressure regulation, reviews the impact of sleep and nutrition on brain excitability and SUDEP and summarizes our current knowledge on neuroimaging markers. The variety of the contributions illustrates that an increasing number of clinicians and researchers are committed to understand SUDEP, identify biomarkers and develop strategies and interventions to mitigate the SUDEP risk. These worldwide efforts are also the success of tireless activities of patients, families and bereaved to promote awareness for premature mortality in epilepsy and SUDEP (8).

AUTHOR CONTRIBUTIONS

RS drafted the editorial. MS and CD critically revised the draft.

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ORIGINAL RESEARCH
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Incidence, Recurrence, and Risk Factors for Peri-ictal Central Apnea and Sudden Unexpected Death in Epilepsy

Laura Vilella[1], Nuria Lacuey[2], Johnson P. Hampson[1], M. R. Sandhya Rani[3], Kenneth Loparo[4], Rup K. Sainju[5], Daniel Friedman[6], Maromi Nei[7], Kingman Strohl[8], Luke Allen[9], Catherine Scott[9], Brian K. Gehlbach[5], Bilal Zonjy[3], Norma J. Hupp[1], Anita Zaremba[3], Nassim Shafiabadi[2,3], Xiuhe Zhao[2], Victoria Reick-Mitrisin[2], Stephan Schuele[10], Jennifer Ogren[11], Ronald M. Harper[11], Beate Diehl[9], Lisa M. Bateman[12], Orrin Devinsky[6], George B. Richerson[5], Adriana Tanner[13], Curtis Tatsuoka[3] and Samden D. Lhatoo[1]*

[1] Department of Neurology, University of Texas Health Science Center at Houston, Houston, TX, United States, [2] Epilepsy Center, University Hospitals Cleveland Medical Center, Cleveland, OH, United States, [3] Department of Neurology, Case Western Reserve University, Cleveland, OH, United States, [4] Department of Electrical Engineering and Computer Science, Case Western Reserve University, Cleveland, OH, United States, [5] Department of Neurology, University of Iowa School of Medicine, Iowa City, IA, United States, [6] NYU Langone School of Medicine, New York, NY, United States, [7] Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, United States, [8] Division of Pulmonary, Critical Care and Sleep Medicine, University Hospitals Medical Center, Cleveland, OH, United States, [9] Institute of Neurology, University College London, London, United Kingdom, [10] Feinberg School of Medicine, Northwestern University, Chicago, IL, United States, [11] Department of Neurobiology and the Brain Research Institute, University of California, Los Angeles, Los Angeles, CA, United States, [12] Department of Neurology, Columbia University, New York, NY, United States, [13] Mercy Health St. Mary's Campus, Grand Rapids, MI, United States

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Edited by:
Andrea Romigi, Mediterranean Neurological Institute (IRCCS), Italy

Reviewed by:
Jose F. Tellez-Zenteno, University of Saskatchewan, Canada
Marino M. Bianchin, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil

***Correspondence:**
Laura Vilella
lvilellabertran@gmail.com

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Introduction: Peri-ictal breathing dysfunction was proposed as a potential mechanism for SUDEP. We examined the incidence and risk factors for both ictal (ICA) and post-convulsive central apnea (PCCA) and their relationship with potential seizure severity biomarkers (i. e., post-ictal generalized EEG suppression (PGES) and recurrence.

Methods: Prospective, multi-center seizure monitoring study of autonomic, and breathing biomarkers of SUDEP in adults with intractable epilepsy and monitored seizures. Video EEG, thoraco-abdominal excursions, capillary oxygen saturation, and electrocardiography were analyzed. A subgroup analysis determined the incidences of recurrent ICA and PCCA in patients with $\geq$2 recorded seizures. We excluded status epilepticus and obscured/unavailable video. Central apnea (absence of thoracic-abdominal breathing movements) was defined as $\geq$1 missed breath, and $\geq$5 s. ICA referred to apnea preceding or occurring along with non-convulsive seizures (NCS) or apnea before generalized convulsive seizures (GCS).

Results: We analyzed 558 seizures in 218 patients (130 female); 321 seizures were NCS and 237 were GCS. ICA occurred in 180/487 (36.9%) seizures in 83/192 (43.2%) patients, all with focal epilepsy. Sleep state was related to presence of ICA [RR 1.33, CI 95% (1.08–1.64), $p = 0.008$] whereas extratemporal epilepsy was related to lower incidence of ICA [RR 0.58, CI 95% (0.37–0.90), $p = 0.015$]. ICA recurred in 45/60 (75%) patients. PCCA occurred in 41/228 (18%) of GCS in 30/134 (22.4%) patients, regardless of epilepsy type. Female sex [RR 11.30, CI 95% (4.50–28.34), $p < 0.001$] and

ICA duration [RR 1.14 CI 95% (1.05–1.25), $p = 0.001$] were related to PCCA presence, whereas absence of PGES was related to absence of PCCA [0.27, CI 95% (0.16–0.47), $p < 0.001$]. PCCA duration was longer in males [HR 1.84, CI 95% (1.06–3.19), $p = 0.003$]. In 9/17 (52.9%) patients, PCCA was recurrent.

Conclusion: ICA incidence is almost twice the incidence of PCCA and is only seen in focal epilepsies, as opposed to PCCA, suggesting different pathophysiologies. ICA is likely to be a recurrent semiological phenomenon of cortical seizure discharge, whereas PCCA may be a reflection of brainstem dysfunction after GCS. Prolonged ICA or PCCA may, respectively, contribute to SUDEP, as evidenced by two cases we report. Further prospective cohort studies are needed to validate these hypotheses.

Keywords: apnea, breathing, epilepsy, ictal central apnea (ICA), seizures, sudden unexpected death in epilepsy (SUDEP), post-convulsive central apnea (PCCA)

INTRODUCTION

Sudden Unexpected Death in Epilepsy (SUDEP) is the leading cause of premature mortality in patients with intractable epilepsy (1). The main SUDEP phenotype comprises frequent generalized convulsive seizures in patients with early onset, longstanding epilepsy (2, 3). Both cardiac and respiratory mechanisms likely contribute to SUDEP pathophysiology (3, 4), although video electroencephalogram monitored (VEEG) deaths suggest that terminal cardiac arrest is almost always preceded by central apnea (5). Central, obstructive and mixed apneas have all been proposed as SUDEP mechanisms, and may occur during or after seizures (6–9). Whereas, ictal central apnea (ICA) is common, prolonged ICA with profound oxygen desaturation may pose SUDEP risk, as may post-convulsive central apnea (PCCA) (10–14). The latter, when combined with bradycardia/asystole, comprised the majority of observed deaths in the MORTEMUS series, and two near-SUDEP instances in one observational SUDEP risk study (14). Thus, it is evident that breathing dysfunction plays a major role in SUDEP, although the exact characteristics of respiratory compromise that contribute to death are unknown (15). Since central apnea (prolonged ictal or post-convulsive) seems a viable, agonal mechanism, we set out to determine incidence, recurrence, and characteristics of peri-ictal central apnea. We assessed its influence on potential seizure severity biomarkers, such as hypoxemia extent and post-ictal generalized electroencephalographic (EEG) suppression (PGES) in a prospective study of SUDEP risk biomarkers. Further, we describe two additional cases of near-SUDEP due to prolonged, exaggerated peri-ictal central apnea.

MATERIAL AND METHODS

Patient Selection

All patients were prospectively recruited participants in the NINDS Center for SUDEP Research's Autonomic and Imaging Biomarkers of SUDEP multi-center project (U01-NS090407), and its preliminary phase, the Prevention and Risk Identification of SUDEP Mortality (PRISM) Project (P20NS076965). This study was carried out in accordance with the recommendations of University Hospitals Case Medical Center Institutions Review Boards (UHIRB) and University of Iowa, School of Medicine, Iowa City (IA) Institutions Review Boards. The protocol was approved by UHIRB and University of Iowa, School of Medicine, Iowa City (IA) Institutions Review Boards. All subjects gave written informed consent in accordance with the Declaration of Helsinki. Informed written consent was obtained for publication of two clinical cases. Patients with intractable epilepsy (failure of adequate trials of two or more antiepileptic medications) (16) aged ≥18 years underwent video-electroencephalography (VEEG) evaluation in the adult epilepsy monitoring units (EMU) of participating centers from September 2011 until April 2018. We included patients with recorded electroencephalographic seizures with or without clinical correlate, who had complete polygraphic physiological recordings sufficient for analysis. Exclusion criteria were status epilepticus (SE), obscured or unavailable video, and insufficient multimodal physiological recording quality.

Demographic and clinical data were collected. Semiology was classified into two types (17). (1) Generalized convulsive seizures (GCS) which included generalized tonic-clonic seizures, focal to bilateral tonic-clonic seizures, and focal onset motor bilateral clonic seizures. (2) Non-convulsive seizures (NCS), which included seizures with focal onset without evolution to bilateral tonic-clonic seizures, myoclonic seizures, absence seizures, and electroencephalographic seizures without clinical correlate. We determined state (awake or asleep) at seizure onset. We defined the putative epileptogenic zone based on clinical history, seizure semiology, neuroimaging, and VEEG.

Data Collection

All patients underwent prolonged surface VEEG monitoring using the 10–20 International Electrode System. EEG and electrocardiogram (EKG) were acquired using Nihon Kohden (Tokyo, Japan), Micromed (Modigliani Veneto, Italy), and Xltek (Natus) acquisition platforms. Peripheral capillary oxygen saturation (SpO_2) was monitored using pulse oximetry (Nellcor OxiMax N-600x [Convidien], Masimo Radical-7 [Irvine], and SenTec Digital Monitoring System [Therwil BL]) and plethysmography (Ambu [Ballerup, Denmark] Sleepmate and

Perfect Fit 2 [Dymedix]). Chest wall and abdominal excursions were recorded using inductance plethysmography (Ambu, Ballerup, Denmark and Sleepmate or Perfect Fit 2, Dymedix, St Paul, MN, USA).

Breathing analysis for apnea utilized careful composite analysis of inductance plethysmography, EEG breathing artifact and visually inspected thoraco-abdominal excursions 2 min before seizure onset (clinical or electrographic, whichever that occurred first). Central apnea (cessation of thoraco-abdominal breathing movements) was defined as ≥1 missed breath without any other explanation (i.e., speech, movement, or intervention), with a minimal duration of 5 s. ICA referred to apnea in NCS, or apnea occurring in the pre-convulsive phase of GCS. PCCA referred to apnea after GCS; we preferred this term to post-ictal central apnea since it could occur after convulsions but before EEG seizure end. Incidences of ICA and PCCA were determined, as well as their durations. Apnea could not be, and was not assessed during tonic or clonic movements, because of invariable artifact presence in breathing channels. A subgroup analysis identified recurrences of ICA and PCCA (in ≥2 seizures).

Hypoxemia was defined as SpO_2 < 95% and where baseline SpO_2 was already <95% a >1% drop was considered significant. Oxygen desaturations were classified as mild (SpO2 90–94%), moderate (75–89%), and severe (<75%). For SpO_2 evaluation, several time points were considered. Firstly, SpO_2 was determined at baseline, 2 min pre-ictally as mean SpO_2 in a 15 s, artifact free epoch. For GCS and NCS, the overall desaturation nadir referred to the lowest SpO_2 value registered during and up to 3 min after the seizure. To evaluate respiration in the pre-convulsive phase of GCS, an additional desaturation nadir was considered during this phase in patients with ICA.

Presence and duration of PGES (18) after GCS was determined by a validated automated EEG suppression detection tool (19), and supplemented with visual analysis by two epilepsy neurophysiologists when the tool gave no solution. Presence and duration of post-ictal EEG burst suppression were also determined. Combined PGES and burst suppression comprised EEG "recovery" duration.

Statistical Analysis

Statistical analyses were conducted using SPSS (version 24; IBM Corp, Armol, NY, USA) and SAS for Windows 9.4 (SAS Institute Inc., NC, USA). Summary statistics were reported as mean ± standard deviation (SD; median, range). Relative risk (RR) for the primary outcome of ICA and PCCA at a seizure level was assessed by Generalized Estimating Equation (GEE) with same subject exchangeable correlation. All variables with a $p < 0.20$ in a univariate analysis were included in a multivariate Poisson GEE regression (20). Variables related to ICA and PCCA durations were determined using Cox Regression with robust sandwich covariance estimation (21). Lastly, recurrence of ICA and PCCA for each patient with at least two seizures in this data were categorized as binary outcomes and patient-level covariates were included in respective logistic regressions. Corresponding 95% CIs of risk and hazard ratios were generated from these models. The significance level for hypothesis testing was set at $p < 0.05$.

RESULTS

Demographic and Clinical Characteristics

Among 218 patients (130 female), 558 seizures met inclusion criteria. Four hundred and twenty-six seizures were previously reported in two different studies (13, 14).

Mean age at study was 40.2 years ± 14.7 (39; 18–77), mean epilepsy duration was 16.6 years ± 13.8 years (1 month−58 years) and mean age at epilepsy onset was 23.5 years ± 17.2 (20; 1 month-69 years). There were 321 NCS (in 128 patients) and 237 GCS (in 137 patients). State was sleep in 239 seizures and wakefulness in 318 seizures. One seizure arose from post-ictal coma in a patient with a seizure cluster.

There were 182 patients (493 seizures) with focal epilepsy and 33 patients (60 seizures) with generalized epilepsy. One patient had both focal and generalized epilepsy (2 seizures). Epilepsy type was unknown in 2 patients (3 seizures) (**Table 1**).

PGES could be determined in all but one GCS, where electrode artifact prevented accurate interpretation. PGES occurred in 165/236 (69.9%) GCS in 106/136 (77.9%) patients. Mean PGES duration was 38.9 s ± 21.2 (37; 1–169) and mean EEG recovery duration was 85 s ± 107.9 (54; 1–1,091).

Ictal Central Apnea (ICA) Incidence, Duration, and Recurrence

ICA could not be confidently ascertained in 71 seizures, all GCS, due to plethysmographic signal acquisition artifact. ICA occurred

TABLE 1 | Patient characteristics.

	Number of seizures (n = 558)	Number of patients (n = 218)
SEX		
Male	250	88
Female	308	130
EPILEPSY TYPE		
Generalized	60 (10.8%)	33 (15.1%)
Focal	493 (88.3%)	182 (83.5%)
Temporal	292 (52.3%)	115 (52.8%)
Frontal	90 (16.1)	33 (15.1%)
Multifocal	45 (8.1%)	15 (6.9%)
Lateralized	36 (6.5%)	11 (5%)
Occipital	11 (2%)	4 (1.8%)
Parietal	12 (2.2%)	2 (0.9%)
Insular	7 (1.3%)	2 (0.9%)
Focal and generalized	2 (0.4%)	1 (0.55)
Unknown	3 (0.5%)	2 (0.9%)
LATERALIZATION		
Right	169	70
Left	212	74
Bilateral	92	33
Generalized	60	33
Unknown	23	7
Focal and generalized	2	1

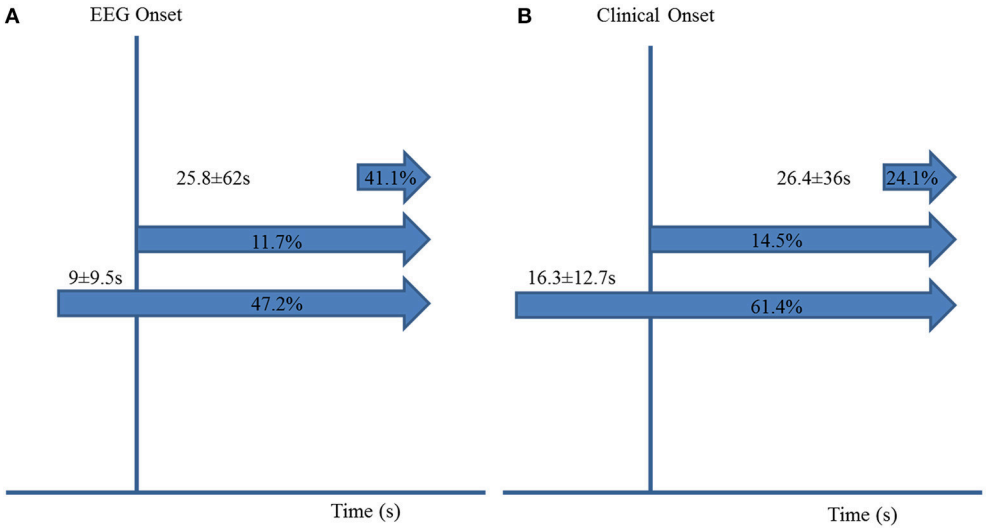

FIGURE 1 | Ictal central apnea (ICA) timing with respect to EEG onset **(A)** and clinical onset **(B)**.

in 180/487 (36.9%) seizures in 83/192 (43.2%) patients: 65/166 (39.2%) in GCS and 115/321 (35.8%) in NCS ($p = 0.960$).

ICA preceded EEG seizure onset in 85/180 (47.2%) seizures by 9 s ± 9.5 (7; 1–58). ICA occurred after EEG seizure onset in 74/180 (41.1%) seizures, with a delay of 25.8 s ± 62 (8; 1–436). ICA coincided with EEG seizure onset in the remaining 21/180 (11.7%) seizures (**Figure 1A**).

ICA was the sole manifestation in 14/180 (7.8%) seizures. It coincided with clinical onset in 24/166 (14.5%) seizures, started before clinical onset in 102/166 (61.4%), preceding it by 16.3 ± 12.7 s (12; 1–66 s), and started after clinical onset in 40/180 (24.1%) seizures, with a difference of 26.4 s ± 36 (14.5; 1–195) (**Figures 1B**, **2A,B**).

Information regarding nadir SpO_2 in NCS and the pre-convulsive phase of GCS was available in 141/180 (78.3%) seizures with ICA, with a mean value of 87.7% ± 9.4 (91; 46–99).

All 180 seizures with ICA were seen in focal epilepsies, and none in patients with generalized epilepsy. In patients with focal epilepsies ICA was more frequent in temporal lobe epilepsies than extratemporal ($p = 0.002$) but there was no association with laterality ($p = 0.215$). ICA incidence did not show any differences regarding sex ($p = 0.171$) or epilepsy duration ($p = 0.077$) but it was related to older age at study ($p = 0.004$) and older age at epilepsy onset ($p < 0.001$). ICA was more frequent in seizures arising from sleep than during wakefulness ($p = 0.013$). ICA was not related to PGES or PCCA and did not affect PGES, EEG recovery, hypoxemia or PCCA durations or SpO2 nadir in GCS ($p > 0.050$) (**Table 2**).

After multivariate analysis, sleep state was related to presence of ICA [RR 1.326, CI95% (1.075–1.637), $p = 0.008$] whereas extratemporal epilepsy was related to lower incidence of ICA [RR 0.579, CI 95% (0.373–0.900), $p = 0.015$].

Mean ICA duration was 20.9 s ± 17.5 (14; 5–97 s) and was longer in patients with NCS without subsequent GCS than those with subsequent GCS [HR 2.276; CI 95% (1.565–3.311), $p < 0.001$] and in temporal lobe epilepsy compared to extratemporal [HR 1.753, CI 95% (1.065–2.885), $p = 0.027$]. ICA duration did not correlate with awake/sleep state at seizure onset ($p > 0.050$). Longer ICA duration was associated with lower SpO_2 nadir during the NCS phase of seizures [HR 1.098, CI 95% (1.064–1.133), $p < 0.001$] and longer EEG recovery duration [HR 1.002, CI95% (1.001–1.003), $p = 0.003$]. ICA duration did not significantly correlate with hypoxemia duration, PGES duration, or SpO_2 nadir in GCS.

Mean number of seizures per patient was 2.6 ± 1.7 (2; 1–8). In the total sample, 92/218 (42.2%) patients either had only one seizure, or only one analyzable seizure for ICA. The remaining 126/218 (57.8%) patients had recurrent seizures and comment on ICA could be made. ICA occurred in 60/126 (47.6%) patients, and recurred in 45/60 (75%) of the patients. No clinical characteristics (age, age at epilepsy onset, epilepsy duration, sex, and epileptogenic zone) were related to ICA recurrence ($p > 0.05$).

Clinical Case 1-Prolonged ICA and near-SUDEP

A 36 year-old right handed man with intractable right temporal lobe epilepsy of unknown etiology since the age of nine was enrolled into the study. His seizure semiology consisted of psychic aura followed by auditory aura with impaired awareness, and rare secondarily generalization. The last generalized convulsion had occurred 4 years before the admission. He had co-morbid depression. Previous antiepileptic drugs (AEDs) were carbamazepine, phenytoin, valproic acid and zonisamide. At admission for presurgical evaluation he was on oxcarbazepine 1,800 mg/day. Physical and neurological examinations were normal. Brain MRI was normal and the interictal PET scan showed bilateral mesial temporal hypometabolism, more pronounced on the right. Interictal recordings showed right temporal sharp waves (maximum at T8>F8). Retrospective

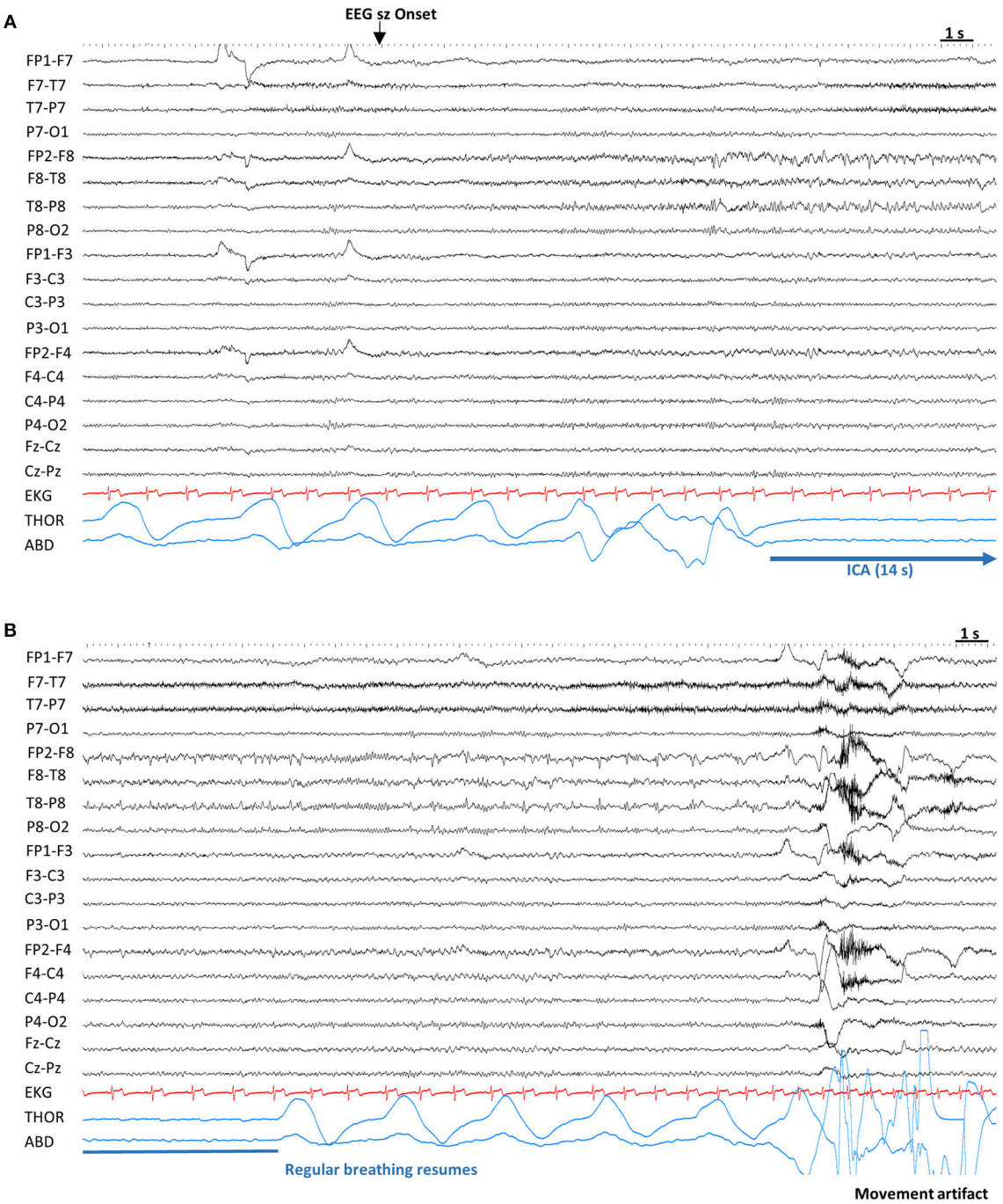

FIGURE 2 | Example of ictal central apnea (ICA). Sensitivity 7 μV, High Frequency Filter: 70 Hz, Time constant: 0.1 s. **(A)** Twelve seconds after the electrographic onset, ICA is noted without any other clinical signs. **(B)** Continuation of ICA, with a total duration of 14 s, followed by breathing resumption. ABD, abdominal; EEG sz onset, electrographic seizure onset; EKG, electrocardiogram; ICA, Ictal central apnea; s, seconds; THOR, thoracic.

review of older (non-study) VEEG records revealed a near-SUDEP incident (not included in the above analysis). The patient had 4 seizures during that admission. The first one, was a brief (<10 s duration auditory aura). The second and third seizures, were brief auras with rapid secondarily generalization, one arising from wakefulness and the other one arising from sleep. No comment about presence of ICA could be made on those seizures due to lack of plethysmography and rapid secondary generalization. No PCCA was noted in any of the GCS and regular breathing resumed immediately after clinical seizure end. These GCS occurred within 12 h of the fourth and last seizure. This was an apneic seizure with impaired

TABLE 2 | ICA incidence and seizure characteristics.

			Univariate analysis	Multivariate analysis	
	ICA–	**ICA+**	***p***	**RR *(95% CI)***	***p***
Sex			0.171	0.87 (0.64–1.18)	0.383
Male	129	91			
Female	178	89			
Age at study (y.o)	38.2 ± 14.9	44.5 ± 15.3	0.004	–	–
Age at epilepsy onset (y)	18.7 ± 16.4	28 ± 17.1	<0.001	1.01 (1.00–1.02)	0.222
Epilepsy duration (y)	19.5 ± 14.1	16.6 ± 14.1	0.077	0.98 (0.97–1.00)	0.608
Epilepsy type			–	–	–
Generalized	51	0			
Focal	251	180			
Epileptogenic zone			0.002	0.58 (0.37–0.90)	0.015
Extratemporal	133	40			
Temporal	118	140			
Lateralization			0.215	–	–
Left	90	94			
Right	99	52			
State			0.013	1.33 (1.08–1.64)	0.008
Awake	191	92			
Asleep	116	87			
Semiology					
GCS	101	65	0.960	–	–
NCS	206	115			
PGES[a]			0.308	–	–
No	90	12			
Yes	70	53			
PGES duration[a] (s)	36.3 ± 15.8	43.3 ± 29.7	0.618	–	–
EEG recovery duration (s)	70.9 ± 61.8	106.2 ± 162.1	0.512	–	–
Recovery time to mild hypoxemia[a] (s)	41.8 ± 31.9	48.5 ± 46	0.903	–	–
Total hypoxemia duration[a] (s)	147.5 ± 70.4	149.9 ± 56.2	0.953	–	–
SpO_2 nadir[a] (%)	59.5 ± 14.4	58.3 ± 13.2	0.576	–	–
PCCA[a]			0.785	–	–
No	79	49			
Yes	18	14			
PCCA duration[a] (s)	8 ± 3.3	10.4 ± 6.7	0.509	–	–

GCS, generalized convulsive seizures; NCS, non-convulsive seizures; PCCA, post-convulsive central apnea; PGES, post-ictal generalized electroencephalographic suppression; SpO_2, peripheral capillary oxygen saturation; y, years y.o, years old.
[a]*Analyzed only in GCS.*

awareness, and respiratory arrest lasting for 285 s, as evidenced by video analysis and oxygen desaturation. After a period of several shallow breaths, breathing finally resumed normally 311 s after seizure onset. Ictal EEG showed rhythmic alpha activity arising over the right antero-mesial temporal lobe with bilateral spread. No alteration in heart rhythm was noted apart from tachycardia. The patient was repositioned, oxygen administered, and ventilated with a face mask. He later underwent invasive evaluation, had further seizures without apnea, and a right temporal lobectomy in 2016 which resulted in seizure freedom (Engel Class 1, [>2 years]) **Video 1**.

Post-convulsive Central Apnea (PCCA) Incidence, Duration, and Recurrence

Presence of PCCA could not be confidently ascertained in 9/237 (3.8%) GCS in 3/137 (2.2%) patients due to movement artifact. PCCA was present in 41/228 (18%) of GCS in 30/134 (22.4%) patients.

In 24/41 (58.65%) seizures (in 19 patients), PCCA was observed without EEG seizure. In 14/41 (34.1%) seizures (in 12 patients), PCCA occurred with ongoing EEG seizure activity. In 3 seizures (in 3 patients) PCCA recurred in the same seizure, occurring initially with EEG seizure discharges and

then after 1–2 breaths, recurring without accompanying seizure discharge(**Figures 3A,B**). In 13 seizures (in 11 patients), PCCA immediately followed clinical seizure end. In 25 seizures (in 20 patients), "delayed" PCCA occurred several breaths after clinical seizure end.

PCCA was more frequent in women than in men ($p = 0.004$) and occurred more often in generalized than focal epilepsies ($p = 0.016$). In focal epilepsy, it was more frequently seen in extratemporal than temporal ($p = 0.020$) patients, but there was no relationship with lateralization ($p = 0.148$). PCCA was unrelated to age at study or epilepsy onset, epilepsy duration and awake or sleep states ($p > 0.050$). Whereas, ICA presence was not related to PCCA occurrence, PCCA was significantly associated with longer ICA duration ($p = 0.001$).

Presence of PCCA was not related to PGES duration, and was not associated with EEG recovery duration and total hypoxemia duration ($p > 0.050$). However, PCCA was associated with longer SpO_2 recovery times to mild hypoxemia (>90%). [RR 1.01, CI95% (1.003–1.017), $p = 0.003$] (**Table 3**).

After multivariate regression analysis, female sex [RR 11.297, CI 95% (4.50–28.34), $p < 0.001$] and ICA duration [RR 1.149 CI 95% (1.053–1.254), $p = 0.001$] were related to PCCA, whereas absence of PGES was related to absence of PCCA [RR = 0.274, CI 95% (0.159–0.471), $p < 0.001$].

Mean PCCA duration was 8.9 s ± 4.9 (5–32). PCCA duration was longer in males [HR 1.844, CI 95% (1.06–3.19), $p = 0.003$]. Epilepsy type, awake/sleep state did not influence PCCA occurrence. PCCA duration did not correlate with age, epilepsy duration, PGES duration, EEG recovery, hypoxemia duration, or time to recovery to mild hypoxemia ($p > 0.050$).

Mean number of GCS per patient was 1.7 ± 1 (1; 1–5). In patients with GCS, 77/137 (56.2%) had only one GCS and the remaining 60/137 (47.8%) had two or more GCS. In the group of patients with repeated GCS, comment on PCCA could be made on 57/60 (95%) patients. PCCA was seen in 17/57 (29.8%). In 9/17 (52.9%) patients, PCCA was recurrent.

Clinical Case 2-Prolonged PCCA and near-SUDEP

A 15 year-old right handed girl with epilepsy since age 5 years was admitted for evaluation. She was not an enrolled study patient. Seizures occurred once or twice a month and lasted up to two with whole body sensory aura (tingling) followed by oral automatisms with impaired awareness. This was rarely followed by secondary generalized convulsions lasting 1–2 min. On several occasions, paramedics were summoned as an emergency because of cyanosis and unresponsiveness after generalized convulsions. On admission she was on lamotrigine 200 mg/day and levetiracetam 3,000 mg/day, having previously failed multiple other AEDS. She had no epilepsy risk factors and no family history of epilepsy. Her physical and neurological examinations were normal. Epilepsy protocol MRI brain scans were normal on two occasions. Inter-ictal brain FDG-PET showed focal hypometabolism in the anterior left temporal lobe tip. Non–invasive VEEG monitoring showed left temporal sharp waves, maximum at F7/T7/FT9. Four habitual clinical seizures were recorded without secondarily generalization. EEG onsets were left hemispheric but not further localizable.

She underwent invasive EEG monitoring for better localization of the epileptogenic zone. A left subdural grid (8 × 6) was implanted along with strips covering the left orbitofrontal, superior temporal, inferior temporal regions, as well as left anterior-anterior, anterior-middle and anterior-posterior temporal, left middle temporal, left middle-middle, and middle-posterior. A left anterior temporal seizure was recorded, with typical automatisms and impaired awareness, right face clonic movements, and a secondary generalized tonic clonic seizure. After clinical seizure end, the patient was immediately apneic (as evidenced by video analysis, cyanosis, and severe O_2 desaturation) for 126 s, followed by an isolated breath. A second period of apnea/hypopnea was then seen until regular breathing pattern resumed a total of 187 s after clinical seizure end. EEG seizure discharges were seen up to 25 s after clinical seizure end. Thirty nine seconds after clinical seizure end, there was concurrent progressive bradycardia followed by 10 s of asystole. Cardiac rhythm resumed, with a pattern of bradycardia and normal sinus rhythm, for 75 s, after which EKG signal was lost, but pulse artifact was evident on EEG. EEG suppression duration (all invasive electrodes), was ∼254 s. During the episode, there was repeated tactile nursing intervention. Further, her head was re-positioned and O2 administered. No active resuscitation measures were performed. Due to continuing seizures, the patient underwent responsive neurostimulation (RNS® System) and was temporarily seizure free for 3 years, until recent recurrence of focal seizures at last follow up **Video 2**.

DISCUSSION

In this study we summarize incidence and risk factors for both ICA and PCCA. Additionally, we describe two near-SUDEP instances of prolonged ICA and PCCA, respectively.

ICA incidence in our study (43.2%), on a larger number of patients, was similar to those previously reported (10, 13). Consistent with our previous reports, ICA was not observed in patients with generalized epilepsy, and was more frequent in patients with temporal rather than extratemporal epilepsy (13, 14). ICA preceded other clinical signs in the vast majority of seizures and in almost half of them, also preceded EEG seizure onset. The observation of ICA being an exclusive feature for focal epilepsies, and especially in those from the temporal lobe, is consistent with previous human stimulation studies pointing out the amygdala, hippocampus, and mesial temporal pole, regardless of lateralization, as the symptomatogenic zone for ICA (11, 13, 22). The absence of ICA in the GCS of generalized epilepsy is in large part due to immediate or rapid generalization where breathing compromise may be partly or wholly due to generalized muscle tonicity that includes respiratory musculature, rather than solely due to unequivocal central apnea. However, we cannot be sure that these patients truly do not have ICA.

Unlike previous publications (13), where no differences between awake/sleep states at seizure onset were found, in this more robustly powered study, ICA occurred more frequently in seizures arising from sleep. One possible explanation is that ICA is easier to detect in the sleep state, where acquisition

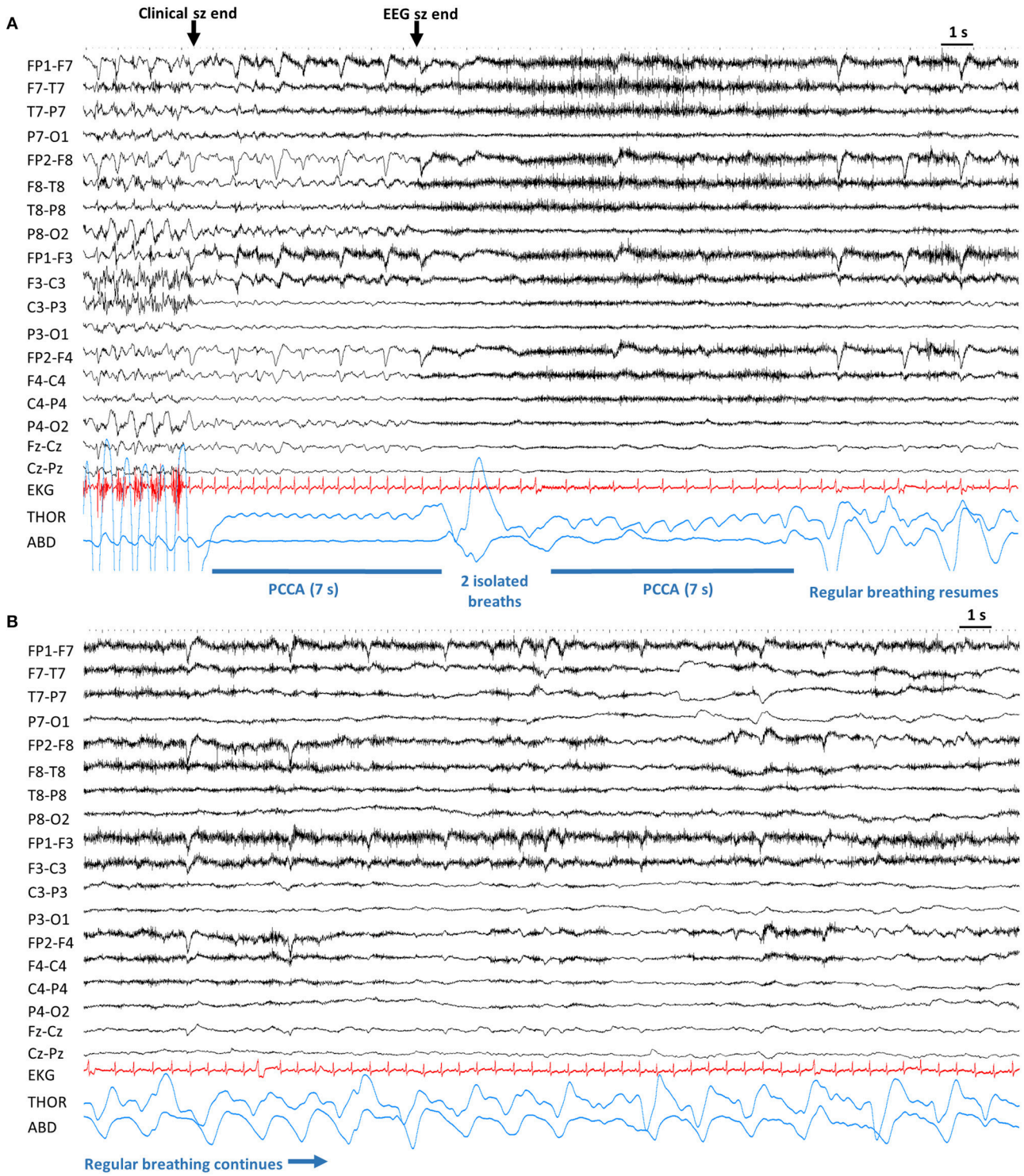

FIGURE 3 | Example of post-convulsive central apnea (PCCA). Sensitivity 20 μV, High Frequency Filter: 70 Hz, Time constant: 0.1 s. **(A)**. After the end of convulsive phase, the patient is apneic for 7 s. After the electrographic end, there are 2 noticeable breaths which are followed by another brief apnea. Lastly, regular breathing resumes. **(B)** Continuation from **(A)**, regular breathing continues. ABD, abdominal; Clinical sz end, end of clinical seizure. EEG sz end, electrographic seizure end; EKG, electrocardiogram; PCCA, post-convulsive central apnea; s, seconds; THOR, thoracic.

TABLE 3 | PCCA incidence and seizure characteristics.

			Univariate analysis	Multivariate analysis	
	PCCA –	**PCCA +**	***p***	**RR *(95% CI)***	***p***
Sex			0.004	11.29 (4.5–28.34)	<0.001
Male	103	10			
Female	84	31			
Age at study (yo)	37.7 ± 13.7	34.6 ± 14	0.267	–	–
Age at epilepsy onset	20.2 ± 17.2	19.5 ± 10.7	0.774	–	–
Epilepsy duration (y)	17.4 ± 12.1	14.9 ± 11.9	0.323	–	–
Epilepsy type			0.016	–	–
Generalized	25	11			
Focal	160	27			
Epileptogenic zone			0.020	4.48 (1.02–19.59)	0.046
Extratemporal	64	18			
Temporal	96	9			
Lateralization			0.148	–	–
Left	63	14			
Right	52	4			
State			0.738	–	–
Awake	97	20			
Asleep	90	20			
ICA duration	11.96 ± 5.75	18.6 ± 11.5	0.001	1.14 (1.05–1.25)	0.001
PGES[a]			0.091	0.27 (0.16–0.47)	<0.001
No	60	8			
Yes	126	33			
PGES duration[a](s)	38.7 ± 18.9	39.5 ± 27.8	0.804	–	–
EEG recovery duration[a] (s)	86.4 ± 80	83.5 ± 184.4	0.876	–	–
Recovery time to mild hypoxemia[a] (s)	36.2 ± 31.3	58.3 ± 42	0.003	–	–
Total hypoxemia duration[a] (s)	144.86 ± 70.3	139.1 ± 40.9	0.301	–	–
SpO_2 nadir[a]	60.76 ± 13.8	58.3 ± 16	0.555	–	–

GCS, generalized convulsive seizures; ICA, ictal central apnea; NCS, non-convulsive seizures; PCCA, post-convulsive central apnea; PGES, post-ictal generalized electroencephalographic suppression; SpO_2, peripheral capillary oxygen saturation; y, years; y.o, years old.
[a]*Analyzed only in GCS.*

artifact is less prevalent. However, awake recordings were not disproportionately excluded because of artifact and such disparities can be explained by physiological differences in breathing control during sleep and wakefulness (23). Breathing is under automatic control through multiple pontomedullary nuclei, the pre-Bötzinger complex (pre-BötC) comprising the main rhythm generator (24). Cortical and subcortical structures, such as thalamus, hypothalamus, amygdalo-hippocampal complex, cerebellum, and mesencephalic nuclei relay to pontomedullary respiratory centers and along with peripheral sensory feedback, modulate breathing output (25). There is increased evidence that serotoninergic neurons lying in the midline raphe nuclei play an active role in both arousal and chemoreception (26, 27). These neurons tonically excitate multiple components of the brainstem respiratory network, with interconnections with the pre-BötC, and also act as central chemosensors, detecting changes in tissue CO_2/H^+ modulating the aforementioned tonic excitatory drive to adjust ventilation accordingly (28). Mice with genetically deleted medullary serotoninergic neurons lack any arousal response to inhalation of CO_2 but have normal arousal responses to other stimuli such as hypoxia, sound and air puffs (26). Moreover, the activity of medullary raphe serotoninergic neurons is highest during wakefulness and absent during REM sleep (28). Most SUDEP cases occur at night, and in the MORTEMUS study this was true in the majority of monitored cases (5) animal studies reveal brainstem serotoninergic dysfunction during and after seizures, with decreased firing of the medullary raphe neurons during the ictal and post-ictal periods (29). Therefore, in the setting of a potentially dysfunctional serotoninergic network in epileptic patients, sleep would constitute a vulnerable period for breathing disturbances (30).

The near-SUDEP case with prolonged ICA, in whom breathing resumed after seizure end raises a number of interesting issues. First, it seems possible that unobserved and in the absence of active intervention, the outcome could have been fatal. This supports the contention that prolonged ICA is dangerous and potentially lethal. Second, the near-fatal seizure episode may be more akin to focal status epilepticus, and supports the view that the latter cannot be excluded in

at-home, unobserved deaths that are usually labeled SUDEP (31). Revisiting SUDEP definition (which mandates exclusion of status epilepticus) may be necessary. The episode mimics a sheep model of status epilepticus and apneic death (32). Third, seizure termination and resumption of breathing with seizure end, suggest that ICA is driven by seizure discharge (likely in the mesial temporal structures), rather than other mechanisms (13). Finally, why the majority of ICA instance are self-terminating and some become prolonged, is unresolved, but may reflect the consequences of damage caused by early onset, long-standing epilepsy, and frequent GCS to key breathing control sites (amygdala, hippocampus, dorsal thalamus, anterior cingulate, ventrolateral medulla etc.) (33–35), rendering greater susceptibility to exaggerated apneic responses.

PCCA incidence (22.4%) was almost half of ICA incidence, more frequently observed in female subjects (although more likely to be longer in male patients), and commoner in those with longer ICA duration. In contrast to ICA, PCCA was observed in both patients with focal, and generalized epilepsy. These differences suggest differing pathophysiologies (14). Whereas, ICA appears to be a semiological phenomenon most often resulting from seizure activity in the amygdalo-hippocampal complex, PCCA most likely results from seizure spread to the brainstem during GCS, regardless of epileptogenic zone (36). Breathing cessation may be derived either from active depolarization and activation of crucial breathing centers that generate apneic responses, such as the periaqueductal gray (37, 38), or disruption of the normal functioning of rhythm-generating neurons and its intricate network, leading to breathing cessation (39). This is consistent with animal models of SUDEP showing post-ictal depolarization in dorsal medulla (40), in which apnea and PGES precede cardiac arrest, and resemble the clinical phenotype of monitored SUDEP patients in the MORTEMUS study (5). Human neuroimaging and neuropathological studies have shown damage in key brainstem structures that modulate breathing, such as the medullary raphe and ventrolateral medulla, in SUDEP and high SUDEP-risk patients (33, 41, 42).

In our study, ICA duration conferred higher risk for PCCA and was related to lower SpO_2 nadirs in the pre-convulsive phase. This may indicate greater hypoxemia induced brainstem compromise, leading to PCCA. Seizure induced focal brainstem hypoxia, due to vasospasm, has been described in animal models and posited as a potential mechanism for SUDEP (43–45). Moreover, we found that the presence of PCCA was related to longer hypoxemia recovery times ($SpO_2 > 90\%$). Although causality could not be established, longer hypoxemia recovery times may be a consequence of PCCA. However, PCCA duration itself, was not related to hypoxemia severity or duration. PCCA durations were typically short, and hypoxemia severity may be more related to GCS severity, although PCCA impact on hypoxemia may become independently important in instances of prolonged PCCA.

Although PCCA occurred preferentially in women, its duration was longer in men, consistent with the SUDEP phenotype (2). Sex differences in breathing function and the protective role of estrogens in respiratory diseases may explain these findings (46–48), since differences in epilepsy phenotype do not explain these differences. As with ICA, duration rather than presence, may primarily influence SUDEP risk in PCCA.

PGES is a frequent finding in GCS, particularly in those arising from sleep and is related to the symmetric tonic phase, postictal immobility, lack of early oxygen administration, duration of oxygen desaturation and lower SpO_2 nadir values (49–51). PGES has been postulated as a SUDEP biomarker, especially if prolonged (>50 s) (18). Its relationship with ICA or PCCA has not been established, except indirectly through O_2 desaturation findings (50). In our study, PCCA was proportionally seen more frequently in seizures with PGES than in seizures without. The pathogenesis of PGES is not well-determined. Cortical neuronal exhaustion or a disruption of ascending inputs after a GCS, or a combination of both, are viable hypotheses. Disruption of ascending pathways such as from the reticular activating system may conceivably prolong the comatose post-ictal state, as well as modulate cortical neuronal activity, and thus, impair the protective behavioral effect of arousal to overcome PCCA (52).

ICA recurred in 75% of the cases, whereas PCCA recurred in 52.9%. This may further reinforce that ICA is a semiological, and therefore recurrent, phenomenon. However, PCCA seems only slightly less frequent, and may also be semiological rather than probabilistic, although our two case reports suggest that prolonged instances of either, are what potentially determine mortality risk. PCCA instances combined with bradycardia/asystole may be particularly dangerous. Our second clinical case of prolonged PCCA accompanied by asystole, resembles the clinical phenotype described in the MORTEMUS study and in a recent analysis of this cohort in a smaller number of patients (14). Invasive monitoring did not show ongoing seizure activity that was concurrent with apnea, reinforcing once again, the different pathophysiologies of ICA, and PCCA, with higher likelihood of involvement of subcortical structures, such as the brainstem, in PCCA (53, 54).

Our study has several limitations. First, it is an observational study in a select group of patients (i.e., primarily treatment resistant epilepsy) and does not necessarily reflect seizure phenomenology or SUDEP risk in a treatment responsive population. Detection of ICA was heavily dependent on extent of acquisition artifact, and hence we may have underestimated incidence. Alternatively, postictal immobility after GCS allowed PCCA identification in the majority of cases. Breathing analysis through polygraphic study was limited to thoraco-abdominal movement and pulse oximetry. Thus, additional information on the presence of mixed central/obstructive apneas, is unavailable. Our apnea definition differs from previous literature, which is based on the 10 s sleep-study criterion, and therefore, ICA and PCCA incidence may be overestimated in our study. However, based on our brain stimulation experiments on breathing modulation, where brief stimulation periods result in immediate and brief (<10 s) apneas, we believe our definition is both accurate and sensitive (12, 22). Our conclusions are based on a relatively small group of number seizures in the primary generalized epilepsy group compared to patients with focal epilepsy. Lastly, our study was based on surface EEG and persistence of intracranial seizure in deep, apnea causing

structures (12) cannot be completely excluded in patients with PCCA. However, Case 2 above, along with a previous case reported in literature, suggest that apnea in epileptic patients can occur in the absence of electrographic seizure (53).

CONCLUSIONS

Peri-ictal central apnea takes two main forms, as ICA or PCCA. ICA incidence is almost twice PCCA incidence and is only seen in focal epilepsies, suggesting different pathophysiologies. Both ICA and PCCA may be recurrent, but prolonged instances leading to SUDEP and near-SUDEP may be probabilistic instances. Prolonged ICA is related to presence of PCCA, possibly due to greater effect of ICA-induced hypoxemia on brainstem function. Absence of PCCA is associated with absence of PGES, suggesting that PCCA presence directly correlates with GCS severity. Alternatively, brainstem structures responsible for arousal and breathing may obviate PGES occurrence. Apnea preceding both EEG as well as clinical seizure onset in a substantial number of patients suggests that plethysmographic respiratory monitoring in regular clinical practice may have seizure detection value. Moreover, such monitoring may facilitate detection of prolonged ICA and PCCA, thus allowing SUDEP risk quantification although further evidence is required to confirm this. Both prolonged ICA and PCCA may contribute to SUDEP. Further prospective cohort studies are needed to validate these hypotheses.

DATA AVAILABILITY

The datasets analyzed in this study are available from the corresponding author on request.

AUTHOR CONTRIBUTIONS

LV had a major role in the acquisition and analysis of data, interpreted the data, drafted the manuscript for intellectual content. NL designed and conceptualized study, interpreted the data, revised the manuscript for intellectual content. JH analysed the data, performed statistical analysis, images and video editing. MR acted as a recruiter, revised the manuscript for intellectual content. KL analysed the data. RS, DF, MN, BG, SS, JO, RH, BD, LB, OD, and GR revised the manuscript for intellectual content. KS interpreted the data, revised the manuscript for intellectual content. LA, CS, BZ, NH, NS, XZ, VR-M, and AT performed data acquisition. AZ led and coordinated communication among sites; revised the manuscript for intellectual content. CT analysed the data and performed statistical analysis. SL designed and conceptualized study, performed analysis and interpretation of data, revised the manuscript for intellectual content.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://doi.org/10.6084/m9.figshare.7188998

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Blood Pressure in Seizures and Epilepsy

Robert D. Nass[1†], *Kevin G. Hampel*[2†], *Christian E. Elger*[1] *and Rainer Surges*[1*]

[1] *Department of Epileptology, University Hospital Bonn, Bonn, Germany,* [2] *Department of Neurology, University Hospital La Fe, Valencia, Spain*

In this narrative review, we summarize the current knowledge of neurally mediated blood pressure (BP) control and discuss how recently described epilepsy- and seizure-related BP alterations may contribute to premature mortality and sudden unexpected death in epilepsy (SUDEP). Although people with epilepsy display disturbed interictal autonomic function with a shift toward predominant sympathetic activity, prevalence of arterial hypertension is similar in people with and without epilepsy. BP is transiently increased in association with most types of epileptic seizures but may also decrease in some, illustrating that seizure activity can cause both a decrease and increase of BP, probably because of stimulation or inhibition of distinct central autonomic function by epileptic activity that propagates into different neuronal networks of the central autonomic nervous system. The principal regulatory neural loop for short-term BP control is termed baroreflex, mainly involving peripheral sensors and brain stem nuclei. The baroreflex sensitivity (BRS, expressed as change of interbeat interval per change in BP) is intact after focal seizures, whereas BRS is markedly impaired in the early postictal period following generalized convulsive seizures (GCS), possibly due to metabolically mediated muscular hyperemia in skeletal muscles, a massive release of catecholamines and compromised brain stem function. Whilst most SUDEP cases are probably caused by a cardiorespiratory failure during the early postictal period following GCS, a profoundly disturbed BRS may allow a life-threatening drop of systemic BP in the aftermath of GCS, as recently reported in a patient as a plausible cause of SUDEP in a few patients.

Keywords: hypotension, hypertension, seizure, epilepsy, autonomic nervous system, SUDEP

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***Correspondence:**
Rainer Surges
rainer.surges@ukbonn.de

[†] *These authors have contributed equally to this work*

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INTRODUCTION

People with epilepsy (PwE) have an elevated risk of acute myocardial infarctions and sudden cardiac death as compared to the general population (1–5). Multiple factors increase the likelihood of cardiovascular morbidity and mortality in PwE including detrimental effects of anti-seizure drugs on electrical properties of cardiomyocytes and on circulating blood components (e.g., lipids and related proteins as a major risk factor of coronary artery disease) as well as epilepsy-related negative effects on the autonomic nervous system (ANS) that lead to enhanced sympathetic tone, thereby facilitating cardiac arrhythmias and deregulated control of arterial blood pressure (BP) (6–8). In addition, seizure-related disturbances of cardiac function are frequently observed in

association with different seizure types and may be the cause of death in a significant proportion of people with epilepsy (7–9). In this article, we focus on systemic BP, its alterations in PwE and contribution to cardiovascular morbidity, mortality, and possibly sudden unexpected death in epilepsy (SUDEP).

NEURAL CONTROL OF BLOOD PRESSURE

The major determinant of oxygen and metabolite supply of tissues and organs is the blood perfusion which is regionally controlled by activity-dependent mechanisms. The overall perfusion of the body's organs is secured by the systemic BP. Arterial BP is defined as the pressure exerted by the blood on the artery walls (10). By convention it is measured in millimeters of mercury (mm Hg) above the surrounding atmospheric pressure. Mean arterial blood pressure (MAP) is determined by the product of cardiac output (CO) and total peripheral resistance (TPR) (MAP = CO×TPR) while CO is, in turn, the product of stroke volume (SV) and heart rate (HR) (CO = SV×HR) (11). The systemic BP shows a pulsatile profile due to the contraction of the cardiac cycle and the elastic behavior of the artery walls; the maximal pressure during heart contraction is named systolic arterial pressure (SAP), the minimal pressure between two heart contractions as the diastolic arterial pressure (DAP). To secure appropriate energy and oxygen supply, systemic BP is constantly maintained within given limits by regulatory pathways involving the autonomic nervous system. Our current knowledge on the neural control of blood pressure comes from decades of experimental research in animals, clinicopathological correlations in humans, mostly with stroke or epilepsy, electrical stimulation studies in humans with epilepsy and functional imaging studies in humans (12–17).

The peripheral part of ANS consists of the splanchnic nerves, autonomic ganglia and plexus with their adrenergic (sympathetic) or cholinergic (parasympathetic) nerve endings in most organs as well as afferent visceroceptor nerve endings such as baroreceptors in the aortic arch and carotid sinus (measuring BP), volume receptors in the pulmonary veins and atria (measuring blood volume) as well as chemoreceptors e.g., in the lungs' and kidneys' vascular systems (measuring pH, pCO_2, pO_2). The central ANS integrates visceromotor, neuroendocrine, pain, and behavioral responses (18). It comprises areas widely dispersed along the neuraxis such as the spinal cord (thoracic intermediolateral column, IML; sacral parasympathetic nuclei), medulla oblongata (nucleus tractus solitarii, NTS; dorsal vagal nucleus, DVN; nucleus ambiguous, NA; ventrolateral medulla oblongata, VLM), pons (parabrachial nucleus, pontine micturition center), mesencephalon (periaqueductal gray), diencephalon (various hypothalamic nuclei, bed nucleus of the stria terminalis, BST; thalamic nuclei), and telencephalon (insular cortex, amygdala, cingulate gyrus, medial prefrontal cortex). In this mini-review, we will focus on structures which are thought to be particularly important in BP control. Visceral afferent fibers reach the NTS in the medulla oblongata, which has multiple connections, most prominently with the neighboring ventrolateral medulla oblongata and other brain stem nuclei that relay to the IML in the thoracic spine as well as the NA and DVN. The caudal ventrolateral medulla oblongata and the rostral ventral medulla oblongata are chief modulators of the sympathetic, preganglionic intermediolateral column neurons in the thoracic spine, whilst the NA and DVN contain the preganglionic neurons of the vagal nerve which innervate the heart and is hence a major regulator of the parasympathetic systems (12, 14, 17).

The main regulatory loop for short-term BP control is termed baroreflex. It sets HR and SV by an interplay of the sympathetic and parasympathetic system, whereas TPR is predominantly set by the sympathetic activity (**Figure 1**) (12, 14, 17). If BP drops, for instance in the orthostatic reaction or in the second phase of a Valsalva maneuver, the firing rate of the baroreceptor afferents to the NTS decreases, which will in turn lead to a disinhibition of cardio-acceleratory neurons in the VLM and to an inhibition of cardio-inhibitory neurons in the NA and DVN, thereby increasing HR, SV, and TPR to re-increase BP. If BP rises, for instance during intense muscular effort or the last phase of a Valsalva maneuver, the firing rate of the baroreceptor afferents to the NTS increases, which will in turn inhibit activity of cardio-acceleratory neurons in the VLM and their projections to the IML, which innervate the arterial blood vessels and in turn enhance activity of cardio-inhibitory neurons in the NA and DVN, thereby decreasing HR, SV, and TPR to lower BP again (17). Diencephalic connections of the NTS include thalamic nuclei as well as the hypothalamus and its endocrine regulatory centers. Among the telencephalic connections of NTS, the amygdalar-hippocampal complex and the insular cortex, anterior cingulate gyrus and medial prefrontal area are of particular importance (12–17, 19). These supratentorial centers regulate the "desired" levels of sympathetic and parasympathetic output according to behavioral tasks and emotional states and adjust a neural set point of BP (12–14, 16, 17). The most recognized examples are the activation of the sympathetic nervous system at the same time as the beginning of muscular exercise or in fight or flight situations (16). The central ANS is an integrated, reciprocal, interconnected network, in which isolated parts cover specific, in part lateralized aspects. In **Box 1**, we give a brief overview of the most widely accepted substrates of the telencephalic autonomic control centers in humans.

Abbreviations: ANS, autonomic nervous system; BP, blood pressure; BRS, baroreflex sensitivity; BST, bed nucleus of the stria terminalis; CO, cardiac output; DAP, diastolic arterial pressure; DVN, dorsal vagal nucleus; FS, focal seizure(s); FBTCS, focal to bilateral tonic-clonic seizure(s); GCS, generalized convulsive seizure(s); HR, heart rate; IML, intermediolateral column; MAP, mean arterial blood pressure; mm Hg, millimeters of mercury; mPFC, medial prefrontal cortex; mPOA, medial preoptic area; NA, nucleus ambiguous; NTS, nucleus tractus solitarii; pCO_2, partial pressure of carbon dioxide; PGES, postictal generalized electroencephalographic suppression; pO_2, partial pressure of oxygen; PwE, people with epilepsy; SAP, systolic arterial pressure; SUDEP, sudden unexpected death in epilepsy; SV, stroke volume; TPR, total peripheral resistance; VLM, ventrolateral medulla oblongata.

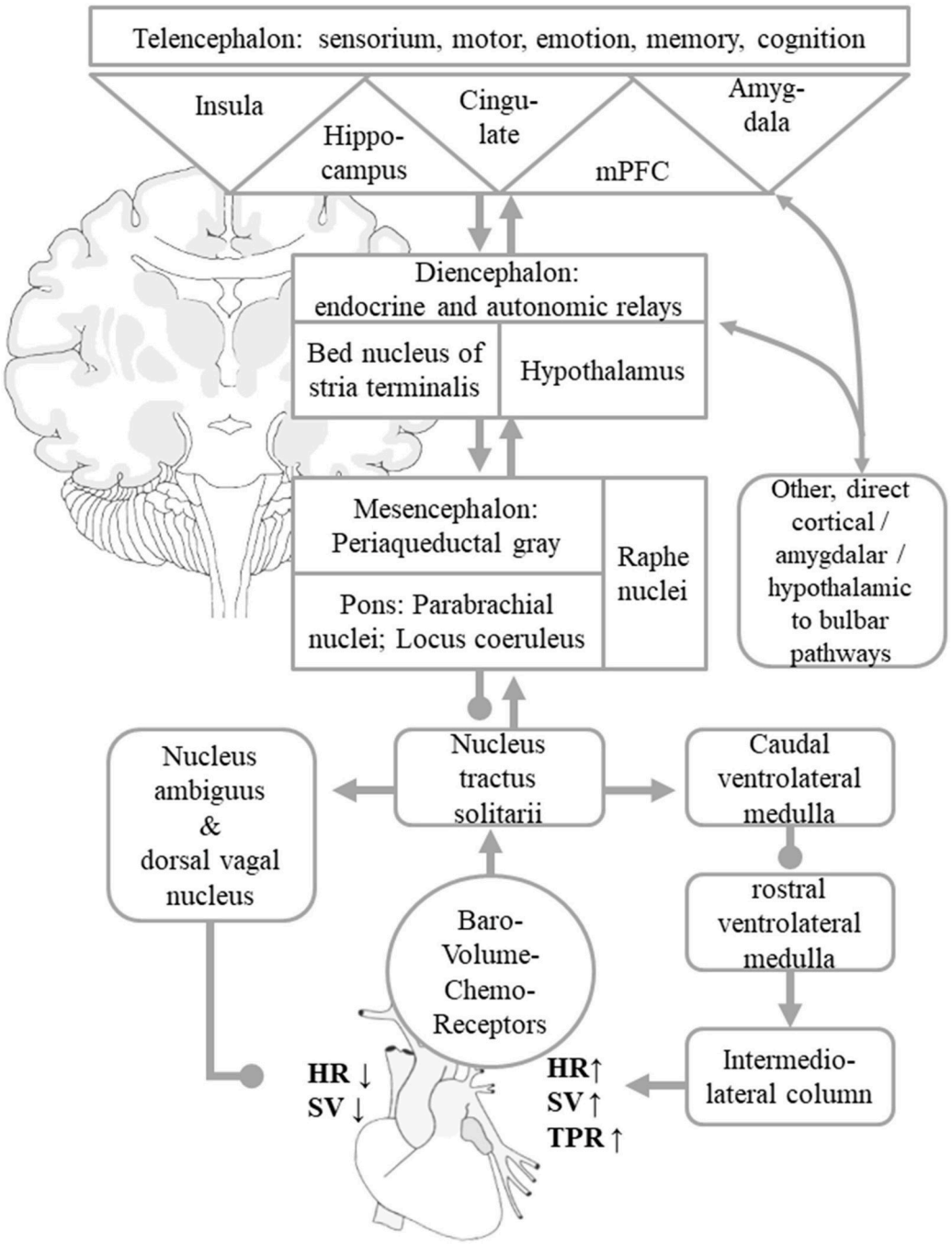

FIGURE 1 | Simplified scheme of central autonomic control of BP homeostasis. Visceral afferents from baro-, volume-, and chemoreceptors reach the NTS, which works as a comparator of information from peripheral visceroaffents and information on behavioral tasks and emotional states signaled by the central autonomic network. These are mediated by indirect and direct connections to the hypothalamus, thalamus, insular cortex, amygdala, cingulate gyrus, and other mPFC areas. These allow adaptations of the neural BP setpoint to given behavioral and emotional states. The NTS asserts control over the NA and DVN, from where parasympathetic efferents mediate a reduction of HR and SV. The NTS regulates sympathetic efferents that originate in the intermediolateral column of the spinal cord by adjusting the caudal and rostral ventrolateral medulla nuclei. The sympathetic system increases total peripheral resistance, HR, and SV. For the sake of clarity, the diagram only shows the indirect "hierarchical" pathways, even though multiple direct pathways from telencephalic and diencephalic visceral control areas to parasympathetic and sympathetic nuclear areas that bypass diencephalic relay centers and the NTS itself exist as well. Arrows indicate predominantly excitatory pathway, dots predominantly inhibitory connections. The figure is adapted from Myers (15), Zanutto et al. (16), and Gianaros and Sheu (19).

Box 1 | Selected Supratentorial Centers of BP Control

Insular Cortex

Situated deeply within the lateral sulcus of the brain and covered by the frontal, temporal, and parietal opercula, the insular cortex has wide ranging, bidirectional connections, e.g., to the dorsal thalamus, frontal, temporal, parietal, cingular, and olfactory cortices as well as the hippocampus and amygdala (20). In the context of BP control, it integrates viscerosensory information from the dorsal thalamus with interoceptive and exteroceptive, memory and cognitive stimuli, including signals of taste, olfaction, temperature perception, auditory processing, vestibular function, pain, emotional experience, empathy, and social cognition. The insular cortex thereby contributes to maintenance of emotional and physiological homeostasis (15, 21). Much of our knowledge on insular function is derived from studies on intracerebral electrical stimulation in PwE (22–24). Electrical stimulation of the insular cortex can have excitatory or inhibitory effects on heart rate, depending on the stimulated insular part and possibly the hemisphere (i.e., the right insula is described to exert more sympathetic and the left insula more parasympathetic activity in some studies) (13, 22, 25–27). Seizures rarely originate in the insula but spread of ictal activity to the insula from adjacent regions is very common. Features of insular seizures include somatosensory, visceral and motor symptoms. They can also mimic frontal lobe, temporal lobe, and parietal lobe seizures (22).

Medial Prefrontal Cortex

The medial prefrontal cortex (mPFC) in humans comprises the Brodman areas 32 and 24 in the anterior cingulate region, area 14 in the gyrus rectus and area 25 in the subcallosal area (28, 29). Corresponding regions in rodents, in which much more is known about autonomic mPFC functions are referred to as a infralimbic cortex (area 25) and prelimbic cortex (area 32) (15). They have substantial connections to stria terminalis and raphe nuclei as well as the NTS and posterior hypothalamus, which act as intermediaries to the DVN, NA and IML (15). The mPFC takes part in the integration of visceral sensory and visceral motor signals as well as in the guidance of emotional behavior. The prefrontal cortex receives input from all sensory modalities and uses this information to make the most rewarding decision. Patients with lesions in this area develop either disinhibited or apathetic, dysfunctional behavior and lack the physiological, visceral response to emotional stimuli (29, 30). Seizures originating in the mPFC lead to fearful behavior (31). Decreased mPFC activity in fMRI studies were associated with baroreceptor unloading (32), increased mPFC activity with enhanced HR variability (33). A recent study in PwE undergoing diagnostic video-EEG monitoring using intracranial electrodes revealed that electrical stimulation of the Brodman 25 area led to almost immediate decrease of SAP without affecting DAP or HR, suggesting that this telencephalic region contributes to the BP regulation by selective modulation of cardiac output (34). The corresponding infralimbic region in rodents is considered to drive sympathetic activation (35). The mPFC interacts with the ventral hippocampus in adjusting the HR to exercise (36).

Amygdala

The amygdala comprises multiple interconnected nuclei deeply nested in the temporal lobe (37). The amygdala plays an important role in emotional processing, especially in fear and anxiety but also learning and social behavior. The lateral amygdala receives sensory information regarding the external environment from sensory thalamic and sensory cortical afferents. It projects them to the medial, central and basolateral amygdala, which is reciprocally connected with the prefontal cortices, hippocampus and sensory areas. As demonstrated mostly in animal studies, the basolateral amygdala also has indirect connections with the DVN and NA via the bed nucleus of the striatum (BST). The medial amygdala is indirectly connected to the same parasympathetic nuclei via the BST and medial preoptic area (mPOA) of the hypothalamus as well as with the IML neurons in the spinal cord via the posterior hypothalamus. The central amygdala is directly connected to the NTS and VLM and has indirect connection with the NA and DMV through the NTS, BST, mPOA, and parabrachial nuclei, as well as indirect connections with the IML via the lateral hypothalamus, VLM, locus coeruleus, and raphe nuclei (15). In humans, the activation, volume and functional connectivity of the amygdala appear to covary with stressor-evoked BP reactivity and even atherosclerosis. PwE frequently have an increased volume or altered functional connectivity of the amygdala (38).

As outlined above, the short-term BP regulation is secured by the baroreflex; the baroreflex sensitivity (expressed as change of interbeat interval per change in BP) is set to accommodate different states of arousal, stress or physical exertion by the central ANS. Apart from baroreceptor sensitivity, the central ANS is also implicated in the long-term BP control, with the NTS acting as a comparator between peripheral afferents and a setpoint determined by ventral autonomic afferents (16). Dysregulation of this setpoint has an impact on long-term BP control and other effects mediated by the sympathetic system. Furthermore, chronic emotional and psychosocial stress can perpetuate cardiovascular diseases and is a cardiovascular risk factor of similar importance and magnitude as smoking or diabetes (39). This may be partially linked to an increased amygdalar resting state activity, as recently shown in a functional MRI study in apparently healthy adults (40). Apart from chronic stress, acute stress can lead to cardiovascular complications in cases of exaggerated autonomic reactivity during emotional or neurological crisis, the best known of which is the "broken heart syndrome" also known as Takotsubo- or stress-cardiomyopathy (41). Acute stress is a hallmark of seizures and status epilepticus, whilst chronic emotional distress is common in epilepsy (42, 43). Dysfunction of the amygdalar-hippocampal complex itself is one of the most frequent causes of temporal lobe epilepsy, possibly contributing to the elevated rate of cardiovascular diseases in PwE (44). Previous clinical studies have shown an association between epilepsy and an elevated risk of myocardial infarction. In a European study, PwE had an almost 5-fold increased risk for myocardial infarction and poorer prognosis thereafter, independent of age, sex, location, and classic cardiovascular risk factors (3). These findings were largely replicated in a Chinese study showing that people with newly diagnosed epilepsy were 4–5 times more likely to acquire or die of a heart disease and stroke than age-matched controls, especially if enzyme inducing anti-seizure drugs were used (45). Another recent US-American study confirmed the elevated risk of myocardial infarction in PwE (46). In this context, we want to stress that a few cases labeled as SUDEP and in whom further diagnostics or subsequent postmortem were not performed, myocardial infarction may underlie the sudden death. However, if cardiac diagnostics in the acute phase or postmortem are done and display signs of acute myocardial infarction, the death is—by definition—not due to SUDEP (47).

SEIZURE-RELATED CHANGES IN CARDIAC AUTONOMIC FUNCTION

Besides the above-mentioned chronically abnormal activity in brain regions involved in the regulation of BP control, seizures themselves often exert acute effects on various functions of the autonomic nervous system. These include gastrointestinal (spitting, nausea, vomiting, defecation) and other vegetative reactions (piloerection, urination, skin flush etc.) as well as alterations of respiratory (tachypnea, hypopnea, apnea) and cardiac function (e.g., tachycardia, bradycardia) (48).

Sympathetic outflow is commonly enhanced during seizures, as shown by elevated levels of circulating catecholamines (49, 50), increased HR, QT-shortening, elevated electrodermal activity or reduced HR variability (51, 52). About 80% of focal seizures go along with ictal sinus tachycardia (which increases CO), whilst cardiac arrhythmias such as atrial fibrillation or ventricular tachycardias are very rare (53). Ictal bradycardia is less common than tachycardia and occurs mostly in temporal lobe seizures. Ictal asystole was detected in about 0.3% of focal seizures (FS) recorded in video-EEG monitoring units, notably with a recurrence risk of ∼40% (54, 55). The mechanisms underlying ictal bradycardia and asystole may include an acute, directly seizure-related dysregulation of parasympathetic networks in the amygdala, cingulate gyrus, and insular cortex or the activation of the physiologic vagal reflex pathway (44, 55). Ictal asystole shortens and terminates the seizure activity because of global cerebral hypoperfusion, possibly preventing the evolution to generalized convulsive seizures (GCS) (56, 57). The related BP drop, however, may also cause syncope with loss of muscle tone and risk of falls and injuries (57–59). All reported episodes with ictal asystole and bradycardias were self-limited, suggesting that ictal asystole is usually not linked to SUDEP (54). In view of the high recurrence risk and the associated danger of falls and injuries, however, the implantation of a cardiac pacemaker should be considered in affected patients in whom full seizure control cannot be achieved (55, 59–61). In contrast to ictal asystole, postictal asystole appears to be less frequent and was exclusively reported to occur in the early phase after GCS [including focal to bilateral tonic-clonic seizures (FBTCS) and generalized tonic-clonic seizures]. Postictal asystole is commonly secondary and caused by severe hypoxemia (which suppresses heart activity) which, in turn, is due to postictal central apnea (62). The mechanisms leading to postictal apnea are not well understood but may be related to postictal generalized suppression of brain activity and a direct depression or increased inhibition of respiratory centers in the brainstem. The fatal cascade consisting of GCS → postictal apnea → hypoxemia → terminal asystole is likely to be the commonest cause of SUDEP and may be reversed by immediate cardiopulmonary resuscitation (54, 62).

SEIZURE-RELATED CHANGES IN BLOOD PRESSURE

While the effects of seizures on HR were extensively studied and recently reviewed (44), seizure-related alterations of BP are less well investigated, mainly because of methodical issues. For instance, studies with intermittent BP monitoring using conventional cuffs attached to the upper arm do not allow capturing rapid changes and the peri-ictal time course of BP (63, 64). Intraarterial BP recordings were anecdotally reported during epileptic seizures but are not suited for systematic larger scale studies (65). Non-invasive methods to continuously measure beat-to-beat BP are available nowadays and allow recording of the time course of peri-ictal BP changes but may be compromised by movement-related artifacts (34, 66–70).

Blood Pressure During Focal Seizures

In our recent study with continuous non-invasive BP recordings in 37 patients with focal epilepsy undergoing video-EEG monitoring, MAP, SAP, and DAP increased by 20–30% on average during 35 FS and returned to baseline within 10 min after seizures cessation (**Figure 2A**) (70). Peri-ictal alterations of BP had a similar time course as the concomitant increase in HR and did not depend on oxygen saturation. FS with impaired awareness showed a stronger increase in BP than those without impaired awareness (70). Notably, peri-ictal BP modulation was stereotypic in those patients with recordings of more than one seizure of the same type. The most frequent pattern was a concomitant increase of BP and HR, which is in line with previously published case reports (**Figure 2C**) (69, 71). In some patients with FS, however, BP decreased whilst HR increased (**Figure 2D**) (70). Jaychandran and colleagues also found, on average, a seizure-related increase in BP in 42 patients with 57 FS (72). They reported that ictal hypertension (defined as SAP>140 mm Hg and/or DAP>90 mm Hg) was observed in 26.3% of the patients, whereas ictal hypotension (defined as SAP<90 mm Hg and/or DAP<60 mm Hg) occurred in 8.7%.

The mechanisms leading to seizure-related BP alterations are unclear, but probably involve stimulation or inhibition of central autonomic function by epileptic activity that propagates into neuronal networks of the central ANS. For example, electrical stimulation of insular and thalamic areas as well as basal ganglia in humans can increase both BP and HR (27, 73). In addition, FS may also increase HR and BP through a release of catecholamines via stimulation of adrenergic receptors in heart and blood vessels (49, 50, 71). Surprisingly, a seizure-related decrease of BP was accompanied by an increase in HR in a subgroup of patients (**Figure 2D**) (70). This pattern suggests that the pathways modulating BP and HR involve distinct brain regions. This assumption is further supported by the finding that 3 patients with implanted depth electrodes showed a significant drop of SAP upon electrical stimulation of the mPFCs Brodmann area 25 without apparent changes in HR or DAP (35). Thus, Brodmann area 25 is possibly a symptomatogenic zone of cardiac contractility (and SV) leading to ictal hypotension.

Blood Pressure During Generalized Convulsive Seizures

Data on BP during GCS are scarce because seizure-related movements usually prevent reliable BP measurements throughout the tonic–clonic phase (70, 72). According to

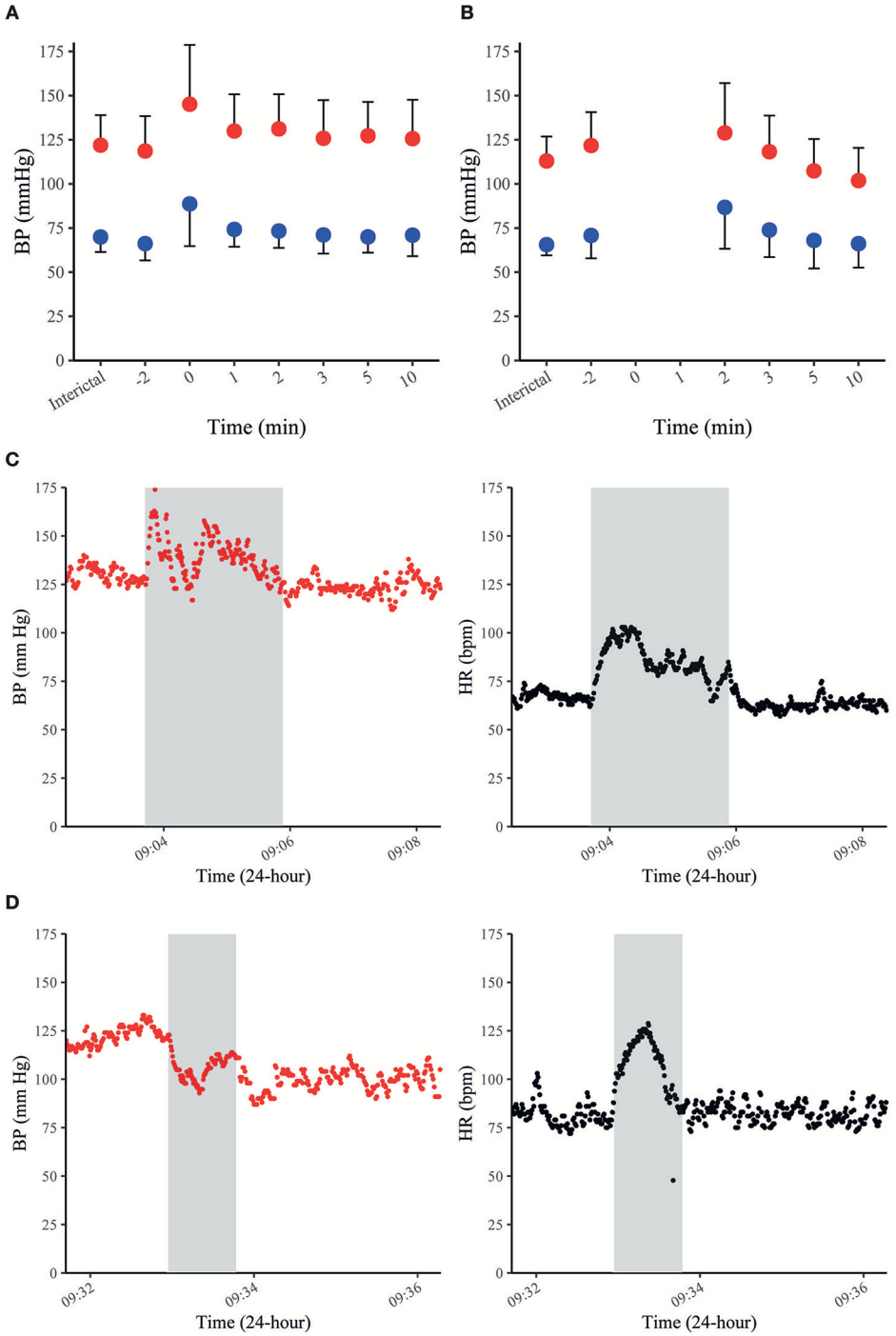

FIGURE 2 | BP in FS and FBTCS. Summary graphs of seizure-related SAP (in red) and DAP (in blue) at different time points in 35 FS of 28 patients **(A)**. Summary graphs of seizure-related SAP (in red) and DAP (in blue) at different time points in 10 FBTCS of 9 patients **(B)**. The x-axis represents different peri-ictal time points (e.g., −2 equals two min prior to the seizure-onset, 0 indicates the time of the seizure). The point charts represent mean ± SD. Examples of time-course of SAP and HR during FS in a patient with concomitant increase of SAP and HR **(C)** and a decrease of SAP and increase of HR in another patient **(D)**. The gray boxes indicate the duration of individual seizures in each figure. Data were previously published in Hampel et al. (70).

anecdotal reports, BP appears to have two distinct patterns during the *ictal* phase of GCS. Pattern 1 was observed in two GCS of two patients and consisted of a concomitant increase of BP and HR (65, 68). Pattern 2 was documented in two GCS of two other patients and displayed an initial BP increase which was rapidly followed by a considerable

drop in BP (65). Pattern 1 could also be explained by a seizure-induced stimulation of the sympathetic branch of the central nervous network and catecholamine release (49). Pattern 2 with an early drop of BP may be due to central autonomic effects rather than a Valsalva-mediated reflex, as it has also been described in muscle-relaxed and highly oxygenated depressive patients during electroconvulsive therapy (74). In the *postictal* phase, BP returns to baseline within a few minutes after seizure termination or, if BP has decreased during the ictal phase, it increases again and then returns to baseline within a few minutes (65, 70). In our study, postictal MAP, SAP, and DAP were slightly elevated and then dropped to baseline or even below pre-ictal values 2 min after seizure cessation (**Figure 2B**) (70). In the early postictal phase, SAP was less elevated than MAP and DAP, but decreased even stronger within some minutes after seizure cessation. In contrast to BP, HR was strongly elevated 2 min after seizures termination and remained elevated 10 min after seizure cessation. This opposite time course of BP and HR is possibly caused by an immediate muscular hyperemia (that frequently follows exercise of skeletal muscles) finally leading to a decreased TPR and a subsequent drop in systemic BP (75). An alternative explanation is that cardiac contractility is impaired postictally, causing a relatively selective decrease in SV and SAP. Although systematic studies on heart function in the early postictal period are lacking to confirm this assumption, cases of seizure-related stress cardiomyopathy and even frank Takotsubo cardiomyopathy with ventricular fibrillation have been reported, suggesting that especially GCS may alter cardiac contractility (76, 77).

A drop in systemic BP should be counteracted by an increase of HR via the arterial baroreflex (**Figure 1**) which, in turn, may be compromised by seizure-related alterations of the reflex loop. Indeed, the baroreflex sensitivity (BRS) was recently shown to be markedly impaired in the early postictal period following 7 FBTCS in 7 patients, whereas BRS was intact after 19 FS in 19 patients (78). These findings were largely replicated in a study by Esmaeili et al. including 9 FBTCS and 14 FS of 18 patients (79). The apparent impairment of BRS in the aftermaths of FBTCS is possibly due to metabolically mediated muscular hyperemia in skeletal muscles following the generalized tonic-clonic convulsions (which overdrives neutrally-mediated sympathetic effects) on the one hand and the massive release of catecholamines with subsequent acceleration of HR on the other hand. Alternatively, exhaustion or suppression of neuronal activity after FBTCS may compromise brain stem function including the networks in the caudal VLM, the rostral ventral medulla, and the NA (68, 80). For instance, FBTCS are commonly followed by a postictal generalized electroencephalographic suppression (PGES) (81, 82) and in one patient, postictal hypotension was observed in association with PGES (68). Opposite to this assumption, however, postictal changes of BP and BRS were not related to occurrence or duration of PGES (70, 78, 79). Altogether, these results must be taken with caution and larger-scale studies are needed to confirm this hypothesis.

Clinical Implications of Seizure-Related Alterations of Blood Pressure and Baroreflex Sensitivity

Most SUDEP cases are probably caused by a cardiorespiratory failure during the early postictal period following GCS (62). Current data suggest that BP changes in association with FS are moderate and BRS is not significantly altered (70, 72). In GCS, however, BRS is markedly impaired postictally on the one hand and postictal BP appears to return rapidly to or below baseline levels (70, 78, 79). A recent case report even described a life-threatening drop of systemic BP with a MAP of 40 mm Hg in the aftermath of a GCS (68). Such a dramatic hypotension in combination with an impaired BRS is likely to favor the fatal SUDEP cascade, as an insufficient compensatory baroreflex response to decreased BP may compromise systemic or cerebral blood supply and cause significant hypoxemia of the organs (83). When exceeding given thresholds, the deprivation of oxygen and metabolites could result in irreversible tissue damage or dysfunction, facilitating in turn mechanisms ultimately leading to SUDEP.

INTERICTAL ALTERATIONS OF BLOOD PRESSURE AND BAROREFLEX IN EPILEPSY

According to previous surveys, the prevalence of arterial hypertension is similar in PwE as compared to the general population (84). This is in line with smaller scope studies reporting similar interictal BP values in people with FBTCS and healthy controls (85) as well as in individuals with epilepsy who later died of SUDEP and two matched control groups with and without epilepsy (86). The authors of the latter study found, however, that DAP tended to be higher in SUDEP patients, suggesting that the sympathetic tone is elevated in this patient group (86). In fact, subtle signs of cardiovascular autonomic dysfunction such as altered HR variability (HRV) at rest (87) or in response to orthostasis and other autonomic tests are common in PwE, possibly augmented by anti-seizure drugs such as carbamazepine (88). For instance, attenuated HRV, which is an established risk factor for cardiovascular morbidity and mortality, is significantly decreased in PwE, indicating a shift of autonomic function toward a predominant sympathetic activity and lower vagal activity. This sympathovagal imbalance may be further reinforced due to the effect of anti-seizure drugs (88, 89) and during phases of sleep related apnea, both in people with focal and generalized epilepsy syndromes (90, 91).

Furthermore, interictal BRS was shown to be impaired in people with temporal lobe epilepsy (92) and reflex epilepsy (93), adding to the notion that PwE may be more vulnerable in regard to cardiovascular diseases due to autonomic imbalances such as alterations of baroreflex function, which might in fact be more common than assumed.

In PwE, interictal alterations of autonomic function and BP homeostasis may be at least partially due to acute or chronic side-effects of anti-seizure drugs. For instance, rapid intravenous application of phenytoin and sedatives such as barbiturates, benzodiazepines, and anesthetic agents are known to lower BP or to induce hypotension (94). These acute effects are likely to be induced by inhibition of voltage-gated sodium and calcium channels with subsequent decrease of cardiac contractility and SV [for review see e.g., (95)]. Probably the most common effects of anti-seizure agents on BP are of indirect nature and related to weight gain [e.g., upon intake of valproic acid, gabapentin, and pregabalin (6)] and detrimental effects on circulating cardiovascular risk factors such as dyslipidemia and hyperhomocysteinemia mostly caused by enzyme-inducing anti-seizure drugs (e.g., carbamazepine, phenobarbital, phenytoin) which in turn may lead to atherosclerosis with decreased blood vessel flexibility and reactivity (96–98). A minor subgroup of cases with sudden death may be explainable due to genetic overlaps with genetically caused cardiac arrhythmias and epilepsy, e.g., by mutations in potassium channel genes KCNQ1 and KCNH2 or sodium channel genes SCN5A (99).

CONCLUSIONS

Systemic BP is permanently monitored and maintained within given limits by the baroreflex loop which, in turn, is modulated by supratentorial and cortical neuronal networks involved in the central ANS. Although PwE display altered interictal autonomic function with a shift toward predominant sympathetic activity and impaired BRS, prevalence of arterial hypertension appears to be similar in PwE as compared to the general population. BP is transiently increased in association with most types of epileptic seizures but may also decrease in some. Postictal arterial hypotension is facilitated by metabolically mediated muscular hyperemia in skeletal muscles and an impaired BRS following GCS, facilitating insufficient blood supply and possibly life-threatening hypoperfusion of body organs as a plausible cause of SUDEP in some cases.

AUTHOR CONTRIBUTIONS

RN drafted the manuscript and created Figure 1. KH has contributed to the writing of the manuscript and created Figure 2. CE has critically revised the manuscript for important intellectual content. RS has conceived and revised the manuscript.

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REVIEW
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Dead in the Night: Sleep-Wake and Time-Of-Day Influences on Sudden Unexpected Death in Epilepsy

Benton S. Purnell[1,2,3], *Roland D. Thijs*[4,5,6] *and Gordon F. Buchanan*[1,2,3]*

[1] Department of Neurology, Carver College of Medicine, University of Iowa, Iowa City, IA, United States, [2] Neuroscience Program, University of Iowa, Iowa City, IA, United States, [3] Iowa Neuroscience Institute, Carver College of Medicine, University of Iowa, Iowa City, IA, United States, [4] Stichting Epilepsie Instellingen Nederland (SEIN), Heemstede, Netherlands, [5] NIHR University College London Hospitals Biomedical Research Centre, UCL Institute of Neurology, London, United Kingdom, [6] Department of Neurology, LUMC Leiden University Medical Center, Leiden, Netherlands

Sudden unexpected death in epilepsy (SUDEP) is the leading cause of epilepsy-related death in patients with refractory epilepsy. Convergent lines of evidence suggest that SUDEP occurs due to seizure induced perturbation of respiratory, cardiac, and electrocerebral function as well as potential predisposing factors. It is consistently observed that SUDEP happens more during the night and the early hours of the morning. The aim of this review is to discuss evidence from patient cases, clinical studies, and animal research which is pertinent to the nocturnality of SUDEP. There are a number of factors which might contribute to the nighttime predilection of SUDEP. These factors fall into four categories: influences of (1) being unwitnessed, (2) lying prone in bed, (3) sleep-wake state, and (4) circadian rhythms. During the night, seizures are more likely to be unwitnessed; therefore, it is less likely that another person would be able to administer a lifesaving intervention. Patients are more likely to be prone on a bed following a nocturnal seizure. Being prone in the accouterments of a bed during the postictal period might impair breathing and increase SUDEP risk. Sleep typically happens at night and seizures which emerge from sleep might be more dangerous. Lastly, there are circadian changes to physiology during the night which might facilitate SUDEP. These possible explanations for the nocturnality of SUDEP are not mutually exclusive. The increased rate of SUDEP during the night is likely multifactorial involving both situational factors, such as being without a witness and prone, and physiological changes due to the influence of sleep and circadian rhythms. Understanding the causal elements in the nocturnality of SUDEP may be critical to the development of effective preventive countermeasures.

Keywords: SUDEP, sleep, epilepsy, circadian rhythms, breathing

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***Correspondence:**
Gordon F. Buchanan
gordon-buchanan@uiowa.edu

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INTRODUCTION

The leading cause of epilepsy-related death in patients with refractory epilepsy is sudden unexpected death in epilepsy [SUDEP; (1–5)]. Among neurological conditions, SUDEP is second only to stroke in terms of years of potential life lost to disease (4). There are no effective ways to reliably predict or prevent SUDEP (3, 5–8). SUDEP is hypothesized to be the result of predisposing factors in the patient and seizure induced perturbation of respiratory, cardiac, and electrocerebral

function (3, 5, 7–9). In cases of SUDEP which have been recorded in an epilepsy monitoring unit, respiratory arrest appears to be the primary cause of death as terminal apnea precedes terminal asystole in each case (10).

It is consistently observed that SUDEP happens more during the night and the early hours of the morning (2, 10–14). Lamberts et al. observed that 62% of SUDEP cases happened between midnight and noon and that 58% of SUDEP cases were sleep-related (12). In SUDEP cases occurring in epilepsy monitoring units, 87.5% of deaths were observed to happen during the night (10). In a meta-analysis of definite, probable, and possible SUDEP, Ali et al. observed that 69.3% of SUDEP cases were presumed to have happened during sleep (13). Furthermore, patients who die of SUDEP are about twice as likely to have nocturnal seizures than those who did not die of SUDEP (12, 14). The increased nocturnal incidence of SUDEP is often attributed to an increased risk of SUDEP during sleep; however, there are a number of factors which might contribute to the nighttime predilection of SUDEP. These factors fall into four categories: influences of (1) being in the absence of a witness, (2) lying prone in bed, (3) sleep-wake state, and (4) circadian rhythms. A consistent issue for determining the cause of the nocturnality of SUDEP is disentangling the potential effect of sleep from the effect of circadian rhythms, not to mention complicating factors such as being without a witness and prone in bed. In humans, sleep typically happens during the night. Consequently, circadian rhythms and homeostatic sleep processes are often considered together; nevertheless, these are distinct processes. Indeed, sleep and circadian rhythmicity alter physiologic processes, such as cardiac and respiratory function, independent of one another (15–20). For a comprehensive meta-analysis of SUDEP cases which consider sleep state or time-of-day as a potential risk factor, please see (13) and (21). The aim of this review is to discuss evidence from patient cases, clinical studies, and animal research which is pertinent to the nocturnality of SUDEP and to consider the implications for clinicians, patients, and the development of preventative strategies. The definition of SUDEP established by Nashef et al. is used for the purposes of this review unless otherwise specified (22).

BEING IN THE ABSENCE OF A WITNESS

Most SUDEP cases are unwitnessed (12, 23). This suggests that the presence of someone who could intervene after a seizure may be protective against SUDEP (2, 12). Seizures which happen during the hours of the day usually occupied by sleep are more likely to be unwitnessed than those occurring during wakefulness (24, 25). Increasing nocturnal supervision by the use of monitoring devices, regular checks or having someone else sleep in the same room is associated with a decreased risk of SUDEP (2, 6, 14). The mechanism by which the presence of another person might differentiate survival from SUDEP is not clear; however, nursing interventions such as repositioning and supplemental oxygen administration are associated with shorter seizures, a reduction in postictal EEG suppression and improved respiratory function (23, 26).

Given the potential for life saving interventions in the time after a severe seizure, the development and distribution of devices capable of predicting seizures and/or detecting seizures and alerting others holds great promise for reducing the rate of nighttime SUDEP. Accurate seizure forecasting would potentially allow for preventative measures to be taken to reduce the chance that an approaching seizure results in SUDEP. Unfortunately, seizure forecasting has proved quite challenging (27).

Conversely, automated seizure detection devices have the potential to detect convulsive seizures with some degree of reliability (28, 29). While EEG is still the most reliable modality for seizure detection, an EEG apparatus is likely not realistic in the home setting. Additionally, while over-night video monitoring improves the detection of nocturnal seizures in a clinical setting it may not be reasonable to expect someone to monitor patients in this way in the home setting (30). The development of automated seizure detection algorithms which use video data to trigger an alarm in response to seizures have considerable promise for reducing the rate of nocturnal SUDEP (31). Unfortunately, there is a scarcity of long-term home-based data to support the efficacy of nocturnal monitoring and seizure detection devices (32, 33). Furthermore, reliable alarms only have the potential to prevent death if there is another person who is able to quickly intervene in response to the alarm. Lastly, increased monitoring of at risk patients by caregivers or devices is unlikely to be successful in all cases as SUDEP has been known to occur even in the presence of medical professionals after the patient announced "I'm going to have a seizure" (34, 35).

LYING PRONE IN BED

In the majority of SUDEP cases, the victim is found in the prone position regardless of the supposed vigilance state of seizure origin (36–38); however, possible, probable and definite SUDEP cases which are inferred to have happened during sleep are more likely to be found prone than those which are inferred to have happened during wakefulness [**Figure 1B**, (13)]. Furthermore, non-fatal convulsive seizures infrequently result in a patient inverting into the prone position (39). It is generally agreed that ending a convulsive seizure in the prone position may contribute to SUDEP (39, 40). The most plausible explanation for this is that breathing during the postictal period is more likely to be impaired while prone consequent to upper airway occlusion or asphyxiation against the substrate on which the body is positioned (40–42).

The nose and mouth being pressed against pillows or the other accouterments of beds may impair postictal respiration by increasing inspiratory resistance and by causing the patient to rebreathe the trapped air (23, 43). Under normal circumstances, this obstruction of the airway would arouse the person and cause them to reposition, this response may not be possible in the time following a seizure (39, 44, 45). "Anti-suffocation" pillows are currently available; unfortunately, there is a paucity of evidence as to their effectiveness (23, 46).

Another sudden death condition, sudden infant death syndrome (SIDS) shares common features with SUDEP,

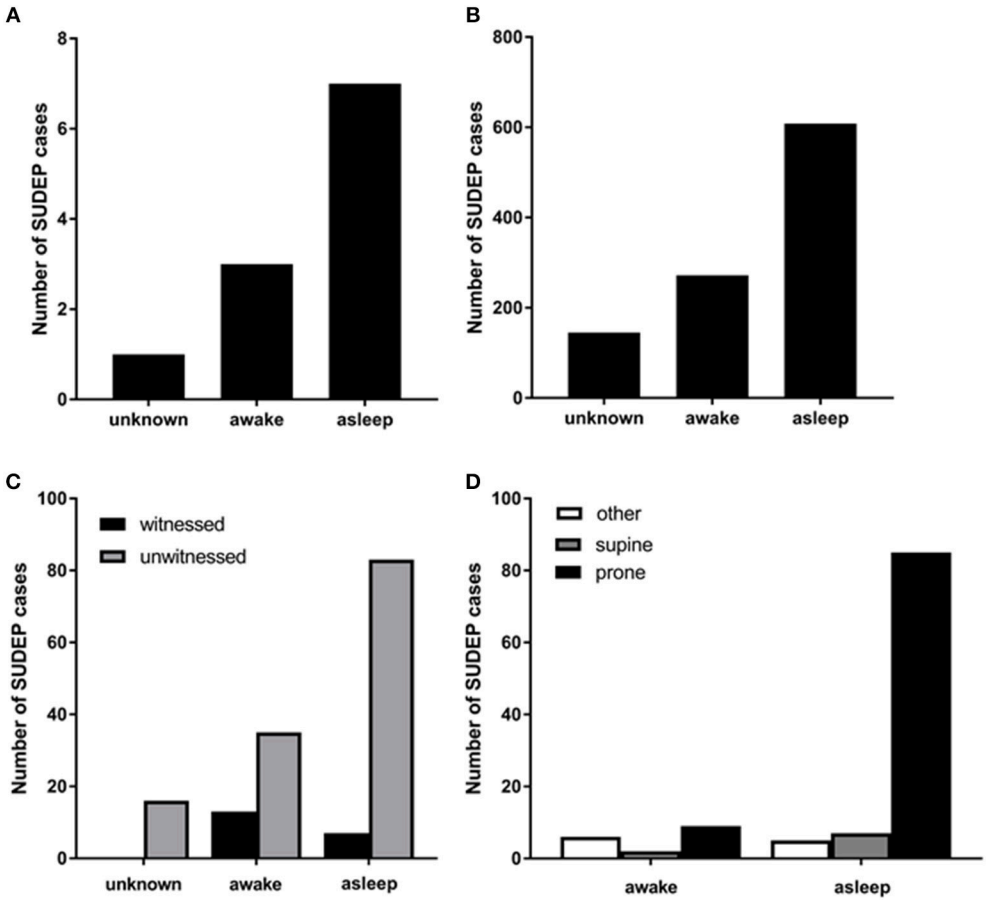

FIGURE 1 | (A) Numbers of SUDEP cases in different vigilance states captured by EEG in the mortality in epilepsy monitoring units study (MORTEMUS: redrawn with permission from Ryvlin et al. (10). **(B)** numbers of sleep-related definite, possible, and probable SUDEP cases [redrawn with permission from Ali et al. (13)]; **(C)** numbers of witnessed and unwitnessed SUDEP cases in sleep and wakefulness [redrawn with permission from Lamberts et al. (12)]. **(D)** numbers of definite, possible, and probable SUDEP cases in sleep and wakefulness and in different body positions [redrawn with permission from Ali et al. (13)].

including that patients are often found prone immediately following nighttime rest. SIDS rates were reduced significantly by the "Back to Sleep" campaign, which encourages care givers to place infants supine to sleep (47). A similar initiative has been proposed to reduce SUDEP rates (48); however, it is not clear whether sleeping in a supine position would be meaningfully protective against SUDEP as body position may change following a convulsive seizure (39).

SLEEP

Sleep alters respiratory, cardiac, and electrocerebral physiology in ways that may be relevant to SUDEP. During sleep, airway patency is decreased thereby increasing airway resistance and increasing the likelihood of airway occlusion (49, 50). Inspiratory drive is lower during non-rapid eye movement (NREM) sleep and lowest during rapid eye movement (REM) sleep (51). Chemical stimuli potently regulate breathing. Failure of the respiratory system to respond to rising CO_2 and falling O_2 levels consequent to seizure induced respiratory dysregulation is theorized to be important in SUDEP etiology. CO_2 levels are higher during sleep (17, 51).The hypercapnic ventilatory response is attenuated in NREM sleep in comparison to wakefulness (51–55). The respiratory response to hypoxia is decreased in both NREM and REM sleep (56). Interestingly, the hypoxic ventilatory response of women is less affected by sleep than men (57). This difference in responsiveness to O_2 depletion during sleep may be responsible for the decreased risk of definite, possible, and probable SUDEP in females (58, 59). Seizures which occur during sleep are associated with lower periictal blood oxygenation (60).

Cardiac and autonomic activity is also modulated by sleep in ways which are potentially relevant to SUDEP (61, 62). QT interval is longer during sleep than it is during wakefulness (15). It is hypothesized that a dramatic shift from parasympathetic to sympathetic drive may have a role in the dysregulation of cardiorespiratory function in SUDEP (63).

Sleep disordered breathing may also play a meaningful role in the nocturnality of SUDEP. Refractory epilepsy patients are at an increased risk of sleep disordered breathing, particularly

obstructive sleep apnea [OSA; (64, 65)]. OSA increases a patient's risk of sudden cardiac death (66). Interestingly, sudden cardiac death in patients with obstructive sleep apnea happens more during the night which is similar to the temporal distribution of SUDEP but unlike that of sudden cardiac death in the general population which tends to happen more during the morning (66, 67). Obstructive sleep apnea is associated with autonomic dysfunction and lower resting oxygen saturation which might increase a patient's vulnerability to SUDEP (68–70). Whether OSA is increased in SUDEP cases has not been studied yet.

It is generally agreed that NREM sleep facilitates the occurrence of seizures and that seizures rarely occur during REM sleep (71, 72). Seizures which occur during sleep are longer and more likely to evolve into focal to bilateral tonic-clonic seizures (73). It is unclear whether the incidence of focal to bilateral tonic-clonic seizures among those with nocturnal seizures can be explained by the lower seizure threshold during sleep as this could also attributed to differences in epilepsy etiology (e.g., nocturnal seizures are more common in frontal lobe epilepsy) (74, 75). Regardless, the increased risk of a sleep-related seizure generalizing may confer an increased risk of SUDEP. Seizures which originate from sleep have more severe perturbation of cardiac activity (76). In an analysis of non-fatal seizures in patients who went on to die of SUDEP it was found that SUDEP victims had a larger surge in heart rate following seizures which happen during sleep in comparison to the seizures of patients who did not die of definite or probable SUDEP (63). It is not clear whether postictal generalized EEG suppression, a state which might facilitate SUDEP, is meaningfully altered by vigilance state of seizure origin. Some studies have observed that sleep increases the probability and duration of postictal generalized EEG suppression (60, 77–80). Conversely, other studies have not seen any association between sleep and postictal EEG suppression (81–83). In summary, there is some evidence to suggest that seizures which originate during sleep have different physiologic consequences than wake seizures in ways that are potentially meaningful to SUDEP.

The inherently unpredictable nature of SUDEP makes it difficult to study in humans; however, evoked seizures in animal models allow the physiological sequelae of seizures to be studied at any permutation of circadian phase and sleep state. Seizures which are induced during NREM sleep using maximal electroshock (MES) are longer, more severe, and more likely to result in death by seizure induced respiratory arrest than seizures induced during wakefulness (84). Non-fatal MES-induced seizures during NREM sleep also result in longer PGES, a greater degree of respiratory suppression, and longer apnea than seizures induced during wakefulness (84). Seizures induced in REM sleep in this model are universally fatal (84). The increased mortality seen after seizures induced during REM sleep is interesting given that seizures are less common during REM sleep (72, 85); however, this may not be true in some rodent models where REM sleep and the associated hippocampal theta rhythm might make seizures more likely (86). In one genetic mouse model with spontaneous seizures escalating sleep deficits preceded the fatal seizure suggesting that chronic sleep disturbances might play a role in SUDEP pathophysiology (87).

Because SUDEP is so frequently unobserved and rarely captured on EEG, it is not possible to determine the sleep state of origin for the fatal seizure in most cases. Patients who died of SUDEP are more likely to have had a nocturnal pattern of seizures and to have a history of seizures originating during sleep (12, 30, 63). As discussed above, meta-analyses of unwitnessed SUDEP cases classify a SUDEP case as being "sleep-related" if it happened at night and in the general vicinity of a bed. These criteria are suboptimal; notwithstanding, using these criteria, a majority of SUDEP cases are "sleep-related" [**Figures 1A–D**, (12, 13, 37)]. Due to the presence of EEG at the onset of the fatal seizure, the insights provided by the mortality in epilepsy monitoring units study (MORTEMUS) are crucial to teasing apart the role of sleep in SUDEP. In this study, seven of the 10 cases for which sleep state could be determined occurred during sleep (1 during REM, 1 during stage 1, 2 in stage 2, and 3 in sleep stages 3 or 4; **Figure 1A**, (10)).

CIRCADIAN RHYTHMS

Circadian rhythmicity affects breathing independently of sleep state (17–20). Humans that are subjected to a constant routine paradigm, which spreads sleep and activity through the 24 h day, exhibit alterations in breathing at different times of day regardless of their sleep-wake state (16, 88, 89). Animal studies also demonstrate circadian differences in breathing (90, 91). Diurnal organisms, such as humans, are more active during the day and have greater ventilation during the day (88, 92). Conversely, nocturnal organisms such as rodents, which are more active during the night, display increased ventilation during the night (17, 91).

In humans, the hypercapnic ventilatory response is higher during the morning and afternoon but decreases substantially during the night (16, 88). There are also circadian differences in sensitivity to CO_2 in rodents with a decrease in sensitivity during the day (90). The hypoxic ventilatory response is regulated in a circadian fashion in humans with greater sensitivity during the day (88, 92, 93); however, in rodents the response to hypoxic conditions is coupled to metabolism which changes in a circadian fashion in such a way that there are no net differences in the response to hypoxia at different times of day (91). The respiratory changes associated with seizures alter blood gas levels (94–97). Differences in how breathing responds to changes in blood gas levels at different times of day may alter a patient's ability to respond to seizure-induced respiratory changes. Lastly, respiratory tissues such as the larynx, trachea, and lung have peripheral circadian oscillators which operate under the purview of the central oscillator in the suprachiasmatic nuclei (SCN; 97). Bilateral SCN lesion disables the peripheral oscillators in these tissues as does genetic deletion of the clock genes cryptochrome 1 and 2 (98).

Circadian oscillations in baseline breathing, respiratory response to challenges, and clock gene expression in peripheral tissues are meaningful for a variety of disease states. The airway occlusion which is seen in sleep apnea is exacerbated by circadian changes in airway patency (99). Asthma is often worsened

at night and respiratory irritants and allergens cause worse respiratory distress at this time (100–102). Chronic obstructive pulmonary disease symptoms are altered by circadian phase and these patients are more likely to require intubation in the morning (103). SIDS is thought to result, in part, from respiratory failure and occurs predominantly at night (104, 105).

The SCN is thought to play a role in autonomic regulation and thus explain why circadian changes may also impact cardiovascular control (106, 107); however, patients with impaired function of the SCN appeared to have similar cardiac function during sleep in comparison to healthy controls (108). Reduced heart rate variability (HRV) is an established risk factor for sudden cardiac death (109) and has been implicated in SUDEP risk although the few case-controls studies that have been published have conflicting findings (110–112). HRV is subject to circadian regulation in addition to the modulating effect of sleep state (113, 114). Day-night HRV dynamics appear to be altered in epilepsy patients; however, without identifying the role of sleep state or employing a forced desynchrony paradigm to isolate the influences of sleep state from circadian ones, it is difficult to state categorically whether this effect is mediated by sleep state or due to an independent circadian effect (115, 116). Cardiac responses to stimuli which are known to elicit a vagal response, such as compression of the eye, are regulated in a circadian fashion with the largest responses coming in late night and the early hours of the morning (117). QT lengthening or shortening may lower the threshold for ventricular fibrillation. Seizure-induced ventricular fibrillation may be seen in a minority of (near) SUDEP cases (118, 119). QT interval is modulated by both sleep state and circadian phase with QT intervals being longer during sleep and later in part of the night (15, 120).

It is well appreciated that seizures and interictal epileptiform discharges are regulated in a circadian manner (121–126). Analysis of seizure type, seizure timing, and sleep state of seizure origin indicates that sleep state and time-of-day independently affect seizures (124, 127). The influences of sleep state and circadian rhythms are also dependent on the site of seizure origin (128). It is unclear why the location of the seizure onset zone would alter the circadian distribution of seizures; however, it is known that different brain areas respond differently to the progression of circadian time (129).

Recently, infradian patterns in seizures have been identified which were previously underappreciated (130). These multidian rhythms have an influence on the occurrence of seizures which is comparable in strength to that of circadian phase. It is not clear whether seizures that happen at different points in these infradian oscillations are more likely to cause cardiorespiratory complications.

There are day-night differences in seizure severity and susceptibility consequent to DBA/2 audiogenic seizures and electrically induced seizures (131). It is postulated that these differences are causally related to day-night variations in serotonin and norepinephrine levels in different brain areas (131, 132). It is unclear on the basis of this study if there are any day-night differences in seizure induced death and whether these differences are independent of seizure severity. Furthermore, whether these differences are endogenously circadian, as opposed to being due to differential lighting conditions, was not investigated (131).

The only published data on the time-of-day of spontaneous death in an animal model of seizure induced death is from Kv1.1 null mice (133). These mice exhibit spontaneous seizures originating in the temporal lobe and typically die consequent to a seizure before 10 weeks of age. Kv1.1 null mice have an attenuated circadian rhythm in cardiac activity and the majority of their deaths occur during the night with peaks in mortality at the light/dark transition points [**Figure 2**, (133)]. This study did not monitor the vigilance state of the animal at the time of the fatal seizure, so it is impossible to determine whether this is an effect of sleep, circadian time, or both.

Seizures induced by MES during the day, the rodent inactive phase, are similar to seizures induced during the night in terms of duration and severity; however, MES seizures induced in the day during sleep resulted in a greater degree of postictal respiratory suppression (**Figure 3**, (134)). Seizures induced during this time also resulted in prolonged EEG suppression. This effect was even greater when seizures were also induced during sleep (134). Two caveats to this data are that only two time points were compared, and these experiments were conducted with the animals in a light-dark cycle environment. A broader sampling of time points throughout the 24 h day and conducting experiments in constant darkness, i.e., in the absence of circadian entraining light cues, may reveal a different temporal pattern.

SUDEP victims are more likely to have a nocturnal pattern of seizures (12, 14). Presently, it is impossible to determine whether this effect is driven by circadian rhythms or homeostatic sleep processes. Epilepsy surgery candidates, the population at the highest risk of SUDEP, have decreased HRV during the night compared to healthy controls (116). SUDEP is not uniformly distributed throughout the 24 h day (12). SUDEP presumed to have happened during sleep most commonly occurred between 0400 and 0800. SUDEP which is presumed to have happened during wakefulness most commonly occurred between the 0800

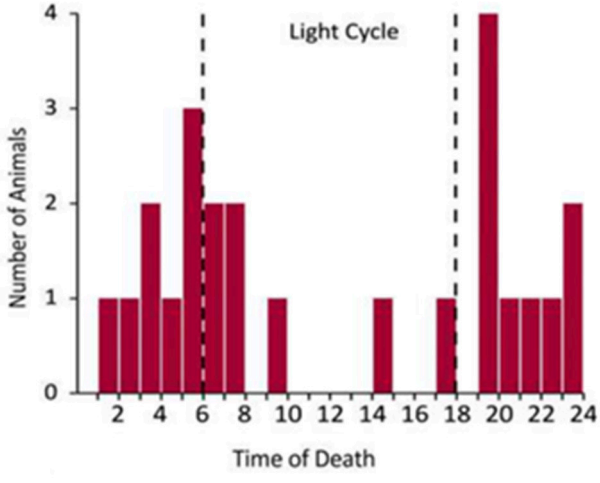

FIGURE 2 | Temporal distribution of spontaneous seizure induced death in Kv1.1 knockout mice [redrawn with permission from Moore et al. (133)].

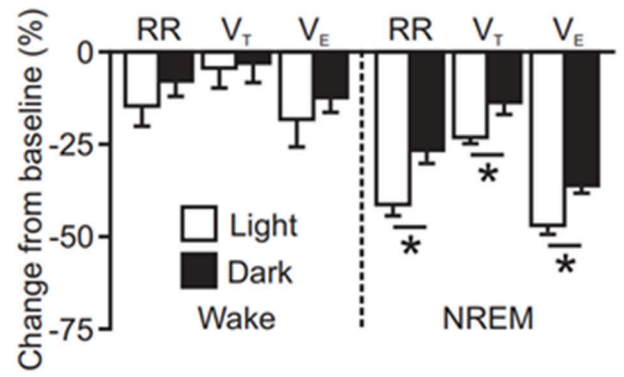

FIGURE 3 | Quantification of seizure induced suppression of breathing (RR; respiratory rate, V_T; tidal volume, V_E; minute ventilation) following maximal electroshock seizures induced during wakefulness or non-rapid eye movement sleep (NREM) at different times of day [redrawn with permission from Purnell et al. (134)]. $^*P < 0.05$.

and 1200.The increased risk of seizures between 0400 and 1,200 is interesting as it suggests that there may be a circadian component to SUDEP which occurs independent of sleep state. If the nocturnality of SUDEP was attributable to sleep factors alone it would be expected that SUDEP frequency would decrease dramatically after 0800 when most people are no longer sleeping. In the MORTEMUS of SUDEP occurring in epilepsy monitoring units, most deaths occurred during the night. Conversely, most cases of near-SUDEP occurred during the day (10).

POTENTIAL MECHANISMS

Serotonin

In the central nervous system, serotonergic neurons are found in the raphe nuclei along the midline of the brainstem (135). Serotonergic neurotransmission modulates breathing, sleep-wake regulation, circadian rhythmicity, and seizures (136–139). Neuronal activity in the raphe nuclei is highest during wakefulness, reduced during NREM sleep, and almost entirely silent during REM sleep (140). Serotonin levels vary depending on circadian phase in areas such as the dorsal raphe, locus coeruleus, and hippocampus [**Figure 4**, (132, 141, 142, 149)]. Seizures suppress serotonergic neurotransmission in the ictal and the postictal period (150). Increases in serotonergic neurotransmission is a critical component of the arousal response to inspired CO_2 (151–153). Stable breathing requires serotonergic neurotransmission (138). Seizure induced disruption of normal serotonergic arousal mechanisms may prevent the normal arousal response to CO_2 in the postictal period and facilitate death (105).

In epilepsy patients, selective serotonin reuptake inhibitors (SSRI) reduce seizure associated hypoxemia (95). Larger seizure induced changes in serum serotonin are associated with a reduction in the tonic phase of a convulsive seizure (154). Furthermore, interictal serum serotonin levels are associated with shorter PGES (154). In the DBA/1 mouse model of seizure induced respiratory arrest, administration of the SSRIs fluoxetine, fluvoxamine, paroxetine, sertraline, and fluoxetine prevent respiratory arrest and death (155–158). The likelihood of seizure induced respiratory arrest is also reduced by administration of 5-hydroxytryptophan, a molecule required in serotonin synthesis (159). Conversely, serotonin antagonism increases the likelihood of seizure induced respiratory arrest consequent to audiogenic seizures (157). Seizures are more severe and more likely to be fatal in mice with a genetic deletion of serotonin neurons in the central nervous system ($Lmx1b^{f/f/p}$) mice vs. wild type counterparts. Seizure induced death is reduced following MES by SSRIs and 5-HT_{2A} receptor agonists, but not a 5-HT_{2C} agonist (160). Times in which serotonergic activity is lower, such as the during the night, may lower seizure threshold and make seizures which do occur more dangerous (149, 161, 162)

Adenosine

Adenosine is a purinergic transmitter which is found in many brain areas and known for its role in sleep-wake regulation, breathing, epilepsy, and a variety of other diseases (163–166). Adenosine accumulation and clearance is regulated in a circadian fashion in a variety of brain areas [**Figure 4**, (146–148, 167)]. Adenosine levels increase during wakefulness and are depleted during sleep (163, 168). The sleep disturbances often associated with epilepsy may be explained by alterations in adenosine signaling (169). Adenosine is an endogenous anticonvulsant and adenosine levels increase during seizures (170, 171). Furthermore, manipulations to adenosine or its clearance modulate epileptogenesis (171–173). Having recurrent seizures may, in turn, decrease, or increase adenosine levels in different brain areas (169). Adenosine analogs applied to the brainstem of rats cause prolonged suppression of breathing (174, 175). Adenosine analogs administered intracerebroventricularly decrease respiration and elicits apnea in cats (176). Inhibition of adenosine clearance initially prevents the escalating severity of motor seizures following kainate injection; however, the adenosine kinase inhibited animals quickly progress to more severe motor seizures and invariably die, whereas animals not subjected to adenosine kinase inhibition do not die. Treating with caffeine following seizure onset prolongs survival in mice subjected to inhibition of adenosine clearance prior to seizure induction with kainate (177). These results suggest that an unchecked surge in adenosine consequent to a seizure may result in precariously increased levels of neuronal inhibition and thereby facilitate death (177).

Norepinephrine

Norepinephrine, a catecholaminergic neurotransmitter found in the rostral brainstem including in the locus coeruleus, modulates seizure activity, (178) breathing, (179), and is subject to circadian regulation in an array of different brain areas [**Figure 4**, (132, 143–145)]. Like serotonin, norepinephrine promotes wakefulness and is an important part of the ascending arousal system (180). In DBA/1 mice, the selective norepinephrine reuptake inhibitor venlafaxine and the SSRIs fluoxetine and fluvoxamine, which also potentiate noradrenergic activity, are more effective in preventing seizure induced respiratory arrest

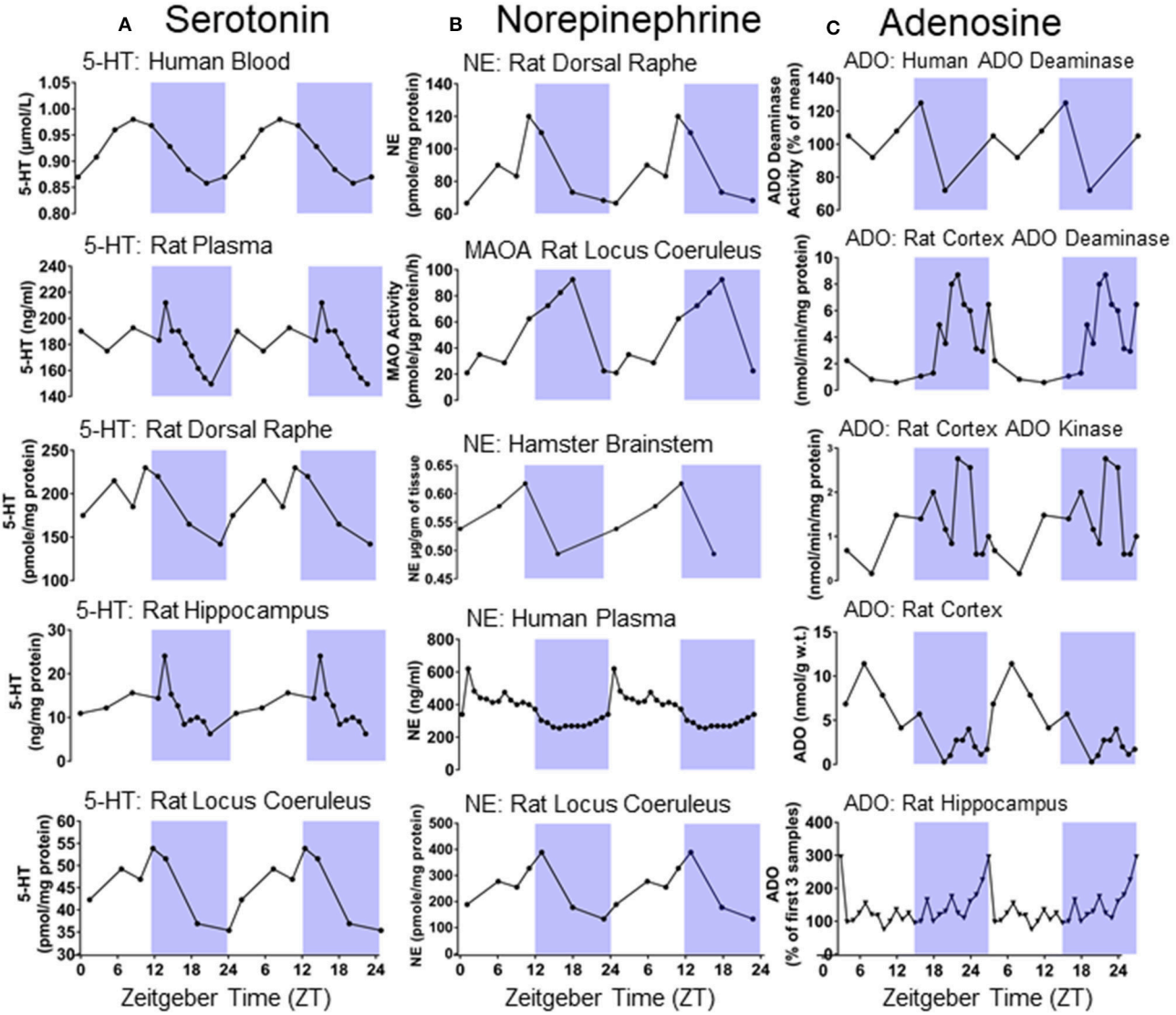

FIGURE 4 | (A) Double plotted day/night differences in serotonin (5-HT) levels in different tissues of humans and rodents [redrawn with permission from Rao et al. (141); Mateos et al. (142); Agren et al. (132)]. **(B)** Double plotted day/night differences in norepinephrine (NE) and Monoamine oxidase A (MAOA) in different tissues of humans and rodents [redrawn with permission from (132, 143–145)]. **(C)** Double plotted day/night differences in adenosine (ADO) and its metabolizing agents in different tissues of humans and rodents [redrawn with permission from (146–148)].

than the selective SSRI paroxetine (155, 157). Respiratory arrest is also reduced in DBA/1 mice with the norepinephrine reuptake inhibitor atomoxetine (181, 182). In light of this evidence, times at which noradrenergic tone is low might make seizure induced respiratory arrest more likely.

SUMMARY

The reason that SUDEP happens more during the night is likely multifactorial involving both situational factors, such as being unattended, and physiological changes due to the influence of sleep and circadian rhythms. Human studies suggest that being without a witness and prone following a seizure, which is more likely during the night, might increase risk for nocturnal SUDEP. At the same time, experimentation in animal models and observation of human seizures indicate that both sleep and circadian phase may adversely affect postictal cardiovascular recovery. Sleep and circadian phase have additive effects on breathing which may compound in some way to produce a hazardous postictal state. Similarly, it may be that sleep, and circadian phase have additive effects on vulnerability to seizure induced respiratory arrest. When the factors associated with being without a witness and prone are added to the mix along with the potential effects of sleep and circadian phase SUDEP might be more likely (**Figure 5**).

Altering the circumstances in which a seizure occurs is currently the best way for reducing the probability of nocturnal

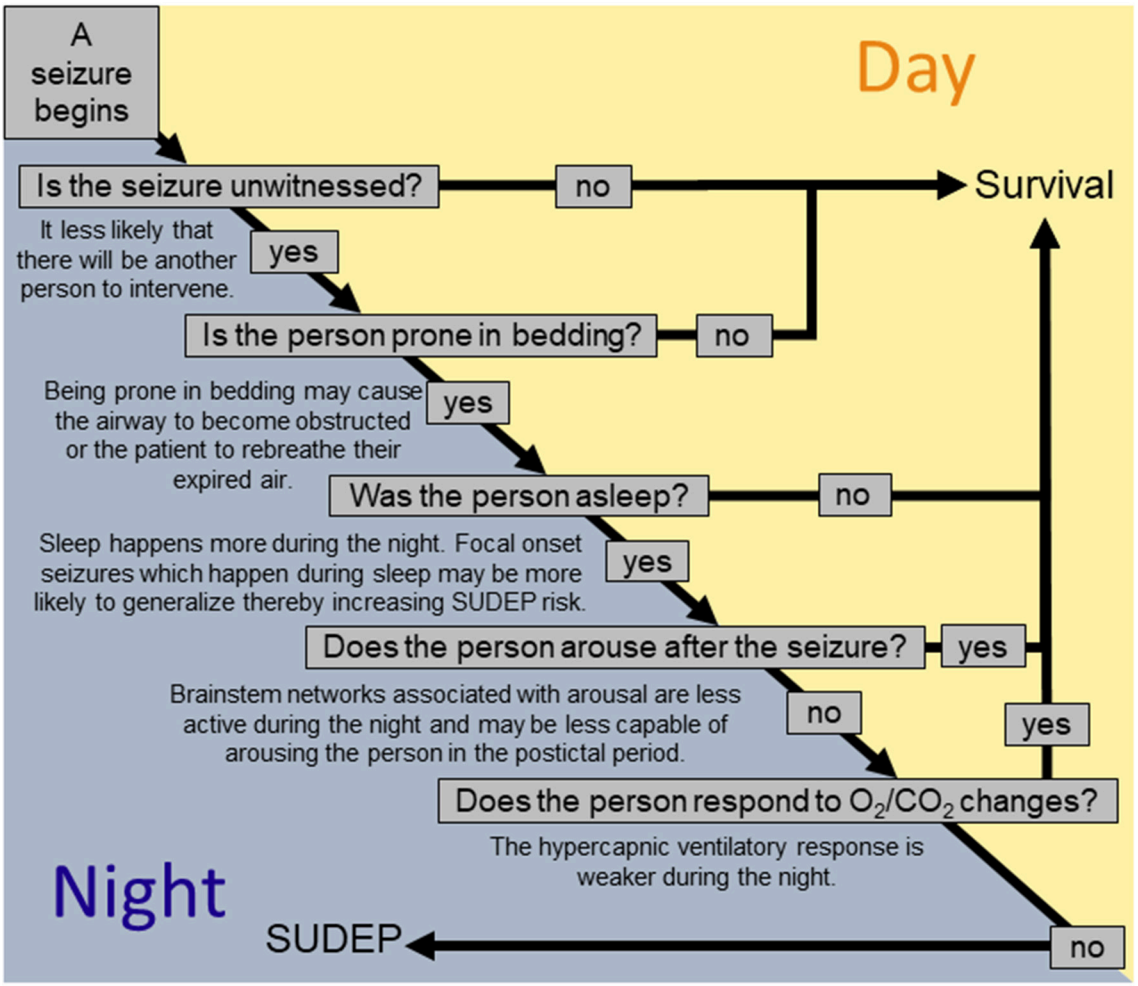

FIGURE 5 | A schematic representation of how different factors relevant to the night might alter the likelihood that a seizure results in SUDEP.

SUDEP, but it is not enough. Patients who do not sleep alone or are being monitored by the use of a device seem to be somewhat protected against SUDEP; however, numerous SUDEP cases have occurred in the direct presence of medical professionals and none of their interventions were sufficient to prevent death. Families and caregivers should be educated about SUDEP and given instruction in basic seizure first aid; however, it should be made abundantly clear that such interventions might be sufficient to prevent death, but it might not and those who have lost someone due to SUDEP are in no way at fault. The risk of SUDEP, nocturnal and otherwise, should be taken into account by patients considering any choice which might alter their likelihood of having a seizure such as adherence, titrating off their medications, switching medication, or pursuing surgical interventions or other non-pharmacological measures.

AUTHOR CONTRIBUTIONS

BP drafted the initial document which was edited by RT, GB, and BP.

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Time of Day and a Ketogenic Diet Influence Susceptibility to SUDEP in *Scn1a*$^{R1407X/+}$ Mice

Frida A. Teran[1,2,3*†], *YuJaung Kim*[1,4†], *Megan S. Crotts*[1], *Eduardo Bravo*[1,3], *Katlynn J. Emaus*[1] *and George B. Richerson*[1,3,5,6]

[1] Department of Neurology, University of Iowa, Iowa City, IA, United States, [2] Medical Scientist Training Program, University of Iowa, Iowa City, IA, United States, [3] Iowa Neuroscience Institute, University of Iowa, Iowa City, IA, United States, [4] Department of Biomedical Engineering, University of Iowa, Iowa City, IA, United States, [5] Department of Molecular Physiology & Biophysics, University of Iowa, Iowa City, IA, United States, [6] Neurology, Veterans Affairs Medical Center, Iowa City, IA, United States

Sudden unexpected death in epilepsy (SUDEP) is a major cause of mortality in patients with drug-resistant epilepsy. Most SUDEP cases occur in bed at night and are preceded by a generalized tonic-clonic seizure (GTCS). Dravet syndrome (DS) is a severe childhood-onset epilepsy commonly caused by mutations in the *SCN1A* gene. Affected individuals suffer from refractory seizures and an increased risk of SUDEP. Here, we demonstrate that mice with the *Scn1a*$^{R1407X/+}$ loss-of-function mutation (DS) experience more spontaneous seizures and SUDEP during the early night. We also evaluate effects of long-term ketogenic diet (KD) treatment on mortality and seizure frequency. DS mice showed high premature mortality (44% survival by P60) that was associated with increased spontaneous GTCSs 1–2 days prior to SUDEP. KD treated mice had a significant reduction in mortality (86% survival by P60) compared to mice fed a control diet. Interestingly, increased survival was not associated with a decrease in seizure frequency. Further studies are needed to determine how KD confers protection from SUDEP. Moreover, our findings implicate time of day as a factor influencing the occurrence of seizures and SUDEP. DS mice, though nocturnal, are more likely to have SUDEP at night, suggesting that the increased incidence of SUDEP at night in may not be solely due to sleep.

Keywords: epilepsy, seizure, ketogenic diet, SUDEP, breathing, sleep, circadian

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***Correspondence:**
Frida A. Teran
frida-teran@uiowa.edu

†These authors have contributed equally to this work

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INTRODUCTION

Sudden unexpected death in epilepsy (SUDEP) is estimated to occur in approximately 27% of patients with epilepsy (1). This number can increase to 50% in patients with poorly controlled and severe epilepsy (2). Although the mechanisms underlying SUDEP are not fully understood, an increasing body of evidence suggests SUDEP is due to seizure-induced cardiorespiratory arrest (3, 4). However, little is known about the circumstances leading up to SUDEP. A strong association with sleep has been documented in a number of studies (3, 5). Although a significant majority of patients are found in bed in the prone position at the time of death (6–8), the occurrence of SUDEP during sleep varies widely among published case studies (9).

This suggests that circadian or other factors may be involved rather than time of day having an effect strictly due to sleep stage.

Many types of epilepsy have a substantial genetic component. Channelopathies involving the neuronal voltage-gated sodium channel *SCN1A* result in a wide spectrum of epilepsy phenotypes ranging from febrile seizures to Dravet Syndrome (DS) (10, 11). DS is a devastating epileptic encephalopathy of childhood-onset that typically manifests as febrile seizures in the first year of life and progresses to refractory epilepsy (12). In patients with DS, the risk of SUDEP is estimated to be 15 times higher than in other pediatric epilepsies (13). Premature death occurs in 21% of DS patients, with SUDEP accounting for nearly half of these deaths (14). Children with DS develop several comorbidities, such as ataxia, cognitive impairments, and sleep disturbances (11). Murine models of DS have proven to be an effective research tool for understanding the pathophysiology of SUDEP as they recapitulate many aspects of the clinical condition: they have heat-induced seizures, spontaneous seizures and a high incidence of premature mortality due to SUDEP (15). Notably, these mice also display impaired sleep architecture homeostasis (16).

A recent study found that time of day can have an independent influence on physiological changes associated with a seizure, particularly breathing (17). This is important as seizure-induced changes in respiratory physiology contribute to SUDEP in patients (3, 18–24) and in DS mice (15). In the present study, we aimed to determine in DS mice whether: (1) spontaneous seizures and SUDEP are more likely to occur in the light or dark phase; (2) seizure frequency changes in the days prior to SUDEP; and (3) treatment with a high-fat, low-carbohydrate ketogenic diet (KD), which has been proven to be protective in other seizure models (25, 26), results in fewer spontaneous seizures and SUDEP.

MATERIALS AND METHODS

Mouse Husbandry and Genotyping

A pair of *Scn1a*$^{R1407X/+}$ heterozygous male mice on a C3HFeB/HeJ background were provided by Miriam Meisler (University of Michigan, Ann Arbor, Michigan, USA), and were bred with C3HFeB/HeJ female mice (Jackson Laboratory) to establish a breeding colony. *Scn1a*$^{R1407X/+}$ mice are referred to as "DS mice" for the entirety of this manuscript. Breeding and genotyping of these mice have been previously described (27). Briefly, DS mice were genotyped by PCR amplification with the primers DS-F (5′ CAATGATTCCTAGGGGGATGTC 3′) and DS-R (5′ GTTCTGTGCACTTATCTGGATTCAC 3′). Genomic DNA was PCR amplified, digested with HpaII, and separated on 2% agarose gels containing 0.15 μg/ml ethidium bromide. Digestion of the PCR product with HpaII generated 2 fragments (295 bp and 223 bp) from the WT allele and an uncut fragment (518 bp) from the mutant allele. DS mice were housed in a 12:12 h light-dark regimen (lights on 7:00 a.m. to 7:00 p.m.) in standard cages with food and water available *ad libitum*. Body weight was monitored weekly from the time of weaning (P21) until P60.

Diet Groups

DS mice were randomly weaned onto either a control diet consisting of standard chow (7013, Teklad Diets, Madison, WI, U.S.A) or a KD (Bio-Serv F3666, Frenchtown, NJ, U.S.A.) (see **Table 1**).

TABLE 1 | Composition of Diets.

	Control (TD 7013)		KD (F3666)	
	% by weight	% kcal from	% by weight	% kcal from
Protein	20	23	11.4	4.8
Carbohydrate	64.8	59	10.8	1.8
Fat	8.2	18	77.8	93.4
Kcal/g		3.1		7.24
F:P+C		0.1:1		6.3:1
Animals (*N*)		124		66

Monitoring of Spontaneous Seizures and Deaths in Mice

DS mice were housed in their home cages under continuous video surveillance to monitor for spontaneous seizures and deaths from P16-P60 as previously described (15). Briefly, video recordings were made at 30 frames per second using web cameras with night vision (FL8910W; Foscam Digital Technologies). Up to 32 cameras were connected to a single computer, and video recordings were saved in 8-h segments and stored on an external hard drive using commercial video webcam software (Blue Iris 4; Foscam Digital Technologies). When a mouse was found dead in a cage, the video was reviewed to determine time of death and whether death was preceded by a behavioral seizure. In the light-dark cycle, light phase was defined as the period between 07:01 and 19:00 h, whereas the dark phase refers to the period between 00:01–07:00 and 19:01–24:00 h.

Seizure Semiology

Animal seizure activity and deaths were assessed by video review by an observer blind to the diet groups. Seizures detected during video review were scored using a modified Racine scale (28). To ensure consistency of seizure classification, only spontaneous seizures scoring 4 (rearing with forelimb clonus and loss of postural control, bilateral myoclonus, and/or wild running and jumping) or 5 (tonic hindlimb extension) were documented.

β-HB Measurement

To determine whether the ketogenic diet increased circulating levels of ketone bodies in mice, blood samples were collected from P35-40 DS mice randomly selected from each diet group to test for the ketone body beta-hydroxybutyrate (β-HB). Animals were anesthetized with a Ketamine/Xylazine cocktail (87.5 mg/kg Ketamine/12.5 mg/kg Xylazine, IP). Blood samples were collected via cardiac puncture into an EDTA pre-coated syringe to prevent coagulation and centrifuged at 3,500 rpm, 4°C, for 5 min to obtain plasma. β-HB levels were determined in duplicate using a commercially available enzyme colorimetric β-HB Assay kit (BioVision, Mountain View, CA). OD450 readings were

determined using plate spectrophotometry (BioTek Synergy 4, Winooski, VT).

Statistical Analysis

Statistical analysis was performed with Prism 8 (GraphPad Software, Inc., La Jolla, CA, U.S.A.). Comparisons across groups were done with unpaired two-tailed *t*-tests and differences across time points were determined using repeated measures one-way analysis of variance (ANOVA) with appropriate *post hoc* test if indicated as noted. A two-way ANOVA was used to evaluate seizure frequencies between and within light/dark phases and diet groups. Survival curves were constructed using the Kaplan-Meier method and comparisons made with the Log-rank test. All data points are presented as averages ± standard error of the mean (SEM) unless noted. Significance was set at $P < 0.05$.

RESULTS

DS Mice Had a Higher Incidence of SUDEP From Late Evening to the Middle of the Night

To determine the time of day at which spontaneous deaths are most likely to occur, long-term video surveillance was maintained starting between P16 & 21 for all mice and continued until P60 or until death to monitor for spontaneous seizures and sudden deaths. SUDEPs in 61 DS mice on a control diet were captured on video. Review of video recordings revealed that all deaths occurred after a GTCS with hindlimb extension (Racine scale 5), similar to previous observations (15, 29). The time of death was determined and a histogram of number of deaths vs. time of day (**Figure 1A**) revealed that SUDEP predominantly occurred in late evening (18:00–19:00) or in the first part of the dark phase between 19:00 and 05:00. The total number of deaths that occurred in the dark phase was 1.65-fold greater than the number in the light phase (38 vs. 23) (**Figure 1B**). However, the number of deaths peaked just before the lights went out and remained high predominantly during the early part of the night. Taking this into account, when the day was divided into 8-h segments, the number of SUDEPs between 18:00 and 02:00 (36) was 3.6-fold greater than those between 10:00 and 18:00 (10) and 2.4-fold greater than between 02:00 and 10:00 (15) (**Figure 1C**).

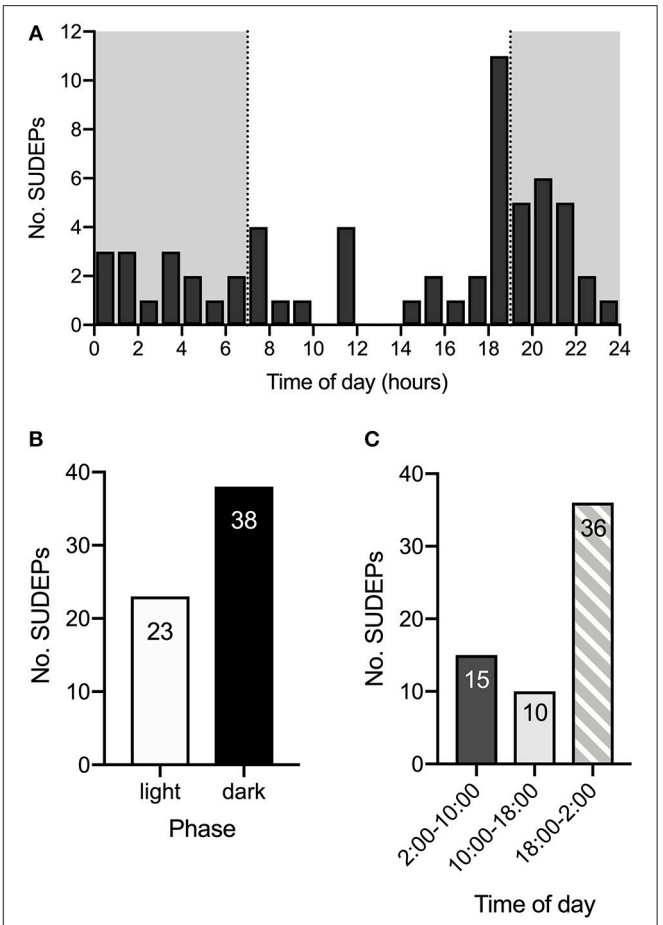

FIGURE 1 | SUDEP is more common during late evening and early night. **(A)** Histogram of time of SUDEP ($n = 61$) across the 24-h day for *Scn1a*$^{R1407X/+}$ mice. The dark phase is denoted by the gray background. **(B)** SUDEP was more common during the dark phase than the light phase in *Scn1a*$^{R1407X/+}$ mice. **(C)** When the 24-h day was divided into 8-h segments, there were more SUDEPs that occurred between 18:00 and 02:00 than the other two segments. Data were obtained from DS mice fed a control diet.

DS Mice Had a Higher Incidence of Spontaneous Seizures in Late Evening and Early Night

To determine the time of day during which spontaneous seizures are most likely to occur, video recordings from 21 DS mice randomly selected from the control diet group in 13 cages (1.62 ± 0.87 mice per cage, mean ± SD) were reviewed starting at an age of P20 and continuing for 6.38 ± 2.96 days (mean ± SD). A total of 121 spontaneous non-fatal seizures were captured on video. Since some cages had up to three mice and individuals could not always be identified consistently throughout the time of recording, the number of seizures recorded from each cage was divided by the number of mice per cage and then by the number of days recorded, to obtain an estimate of the average number of seizures per day for each mouse. Non-fatal seizures also occurred in late evening (18:00–19:00) and during the early part of the night (19:00–01:00) (**Figure 2A**), similar to the distribution of SUDEP. There were more than three times as many seizures per mouse in the dark phase (1.51 ± 0.42) than in the light phase (0.41 ± 0.09) ($p = 0.005$, Wilcoxon matched-pairs signed rank test) (**Figure 2B**). Similar to what was found for SUDEP, the difference was even greater when the number of seizures was determined between 18:00 and 01:00 compared to between 01:00 and 18:00.

Seizure Frequency Increased the Day Prior to Death

To determine the relationship between seizure frequency and death, 16 DS mice that died of SUDEP (at any age) while on a control diet were randomly selected and video recordings of the last 5 days prior to death were reviewed. For each mouse, days before death were defined as 24-h consecutive periods prior to the time of death. Fatal seizures in DS mice followed

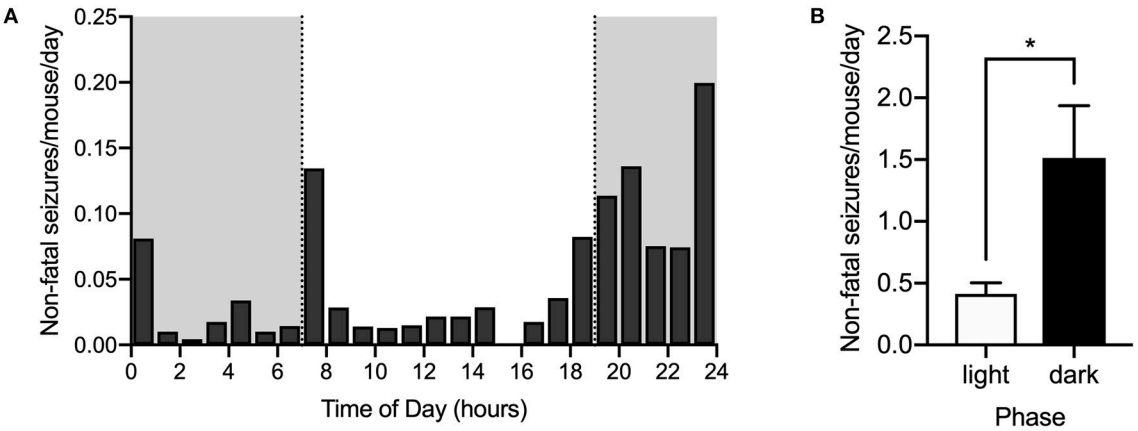

FIGURE 2 | Seizures are also more common during late evening and early night. The number of non-fatal seizures per day for each mouse was calculated as described in the text based on monitoring video recordings. **(A)** Histogram of spontaneous convulsive non-fatal seizures (Racine 4 & 5) per day per mouse distributed across the 24-h day. Dark phase is denoted by the gray rectangles ($n = 21$ mice from 13 cages). **(B)** Seizures were more frequent in the dark phase than in the light phase (*$p = 0.005$, Wilcoxon matched-pairs signed rank test). Data were obtained from DS mice fed a control diet.

a stereotypical progression: seizures began with forelimb clonus (Racine 3) followed by rearing and falling to the side (Racine 4), eventually leading to a GTCS ending with tonic hindlimb extension (Racine 5). All deaths occurred immediately following Racine 5 seizures, which we previously reported to be due to terminal apnea followed by bradycardia (15). Most mice had few or no spontaneous seizures until the last 1–2 days prior to death [$F_{(5, 90)} = 13.51$, $p < 0.0001$] (**Figure 3**). A run-up of seizures prior to death has been reported previously but was only examined during the last day before death (29).

KD Treatment Reduced Mortality in DS Mice

To test the effect of the KD on survival and seizures, a separate group of DS mice ($n = 66$) was fed a KD in parallel with control-fed mice. Blood was sampled from a randomly selected subset of mice (P35-40) from each diet group (KD, $n = 7$; control, $n = 6$) to measure levels of β-HB. As expected, KD-treated mice had significantly higher circulating levels of β-HB compared to control ($p = 0.001$, Mann-Whitney U test) (**Figure 4A**). Long-term video surveillance was maintained as described above. During that time, 56% of DS mice on a control diet ($n = 124$) died of SUDEP (**Figure 4B**). As we have previously reported, there was no difference in mortality between male and female mice (15). KD treatment ($n = 66$) significantly increased survival by approximately 42% (from 44 to 86%, $p < 0.0001$, Log-rank test) when compared to mice fed a control diet (**Figure 4B**). Since only 7 deaths were recorded in the KD group, we were not able to determine whether deaths in KD-treated mice occurred more often at night (4 deaths) than during the day (3 deaths).

We next assessed the effect of the KD on seizure frequency. To this end, video recordings from 9 randomly selected mice in 4 cages (2.25 ± 0.96 mice per cage, mean ± SD) from the KD group were reviewed starting at an age of P20 and continuing for 5 days per mice. A total of 40 spontaneous, non-fatal seizures in KD-treated mice were captured on video. The average number of seizures per day for each mouse was calculated as described above. KD-treated mice had an average of 1.867 ± 0.409 seizures per day, but when compared to control mice (1.925 ± 0.465), the difference was not statistically significant ($p = 0.2766$, Mann-Whitney U test) (**Figure 4C**). We then determined the time of day during which spontaneous seizures were most likely to occur. KD-treated mice experienced more seizures in the dark phase (1.44 ± 0.34) than in the light phase (0.42 ± 0.14) ($p = 0.0430$, Wilcoxon matched-pairs signed rank test) Taken together, both diet groups had more seizures in the dark phase than in the light phase [$F_{(1, 28)} = 10.95, p = 0.0026$], but no differences within each phase were found between the KD and control group ($p = 0.939$) (**Figure 4D**).

DISCUSSION

In the current study, we demonstrated that *Scn1a*$^{R1407X/+}$ mice had a high incidence of premature mortality that was associated with an increased incidence of spontaneous GTCSs 1 day prior to death. We also made the novel observation that DS mice treated with a KD had a significant reduction in mortality compared to mice fed a control diet, but surprisingly this was not associated with a decreased incidence of spontaneous seizures. Spontaneous seizures and SUDEP in DS mice occurred more frequently at night, suggesting that time of day influences seizures and their outcome, but unlike epilepsy patients SUDEP was more common in mice during the time of day when they are more likely to be awake and active.

Spontaneous Seizures and SUDEP Were Influenced by Time of Day in *Scn1a*$^{R1407X/+}$ Mice

A consistent factor in human SUDEP cases is that they occur more frequently at night (5, 9, 30), and it is widely assumed

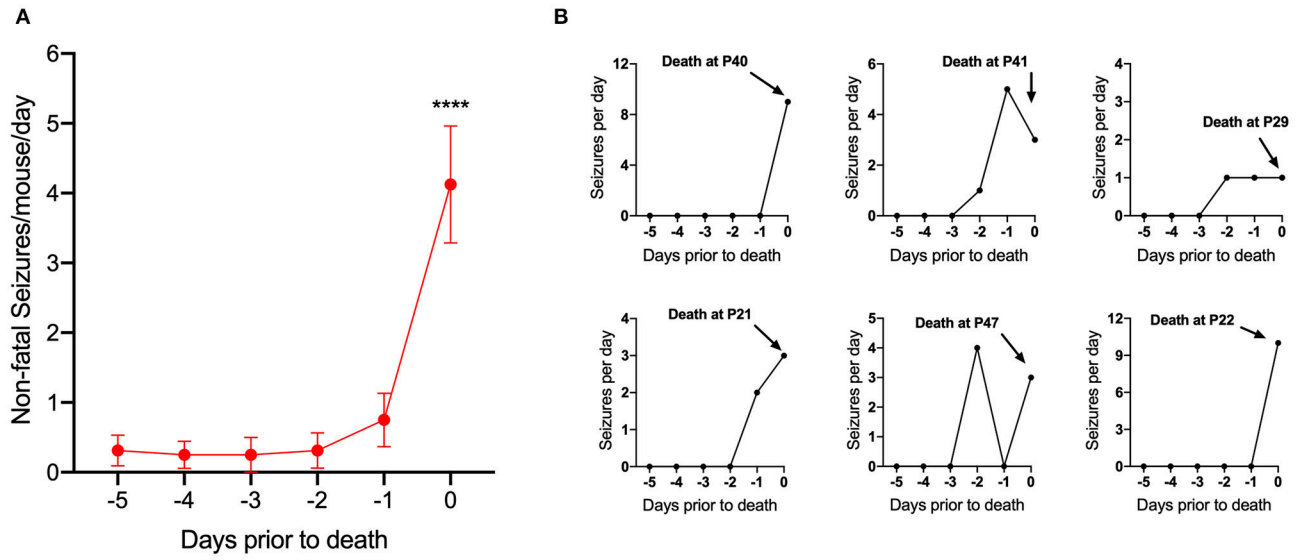

FIGURE 3 | Seizure frequency increased 1–2 days prior to death. **(A)** Number of non-fatal convulsive seizures per mouse per day plotted as a function of days prior to death ($d = -1$ was 24 h prior to death). Seizures significantly increased during the last 1–2 days prior to death [$F_{(5, 90)} = 13.51$, $p < 0.0001$] ($n = 16$ mice). ****$p <$ 0.0001. **(B)** Representative examples of spontaneous seizure progression for 5 days prior to SUDEP in 6 *Scn1a*$^{R1407X/+}$ mice.

that death occurs during the sleep state. However, the specific mechanisms that cause SUDEP to occur at night are unknown, and it is possible that nighttime prevalence is due to circadian effects, the physical environment of being in bed, lack of supervision, or some other factor rather than being due to sleep. Here we made the novel observation that spontaneous seizures and SUDEP in *Scn1a*$^{R1407X/+}$ mice occur more often at night, but since mice are nocturnal they are more likely to be awake at that time.

It still remains possible that sleep state is an independent risk factor for seizure occurrence and mortality. Although mice are nocturnal, their sleep is highly fragmented, and they have frequent short sleep bouts even at night. There is state-dependent variability in cardiac and respiratory function (31–33), which is important as SUDEP in *Scn1a*$^{R1407X/+}$ mice is due to seizure-induced respiratory arrest (15). Sleep state can also influence the frequency and severity of seizures (34). Previous studies found that seizures induced via maximal electroshock (MES) during sleep were more likely to be fatal (5, 17). Although the vigilance state during which non-fatal and fatal seizures occurred was not determined in our study, seizures and SUDEP in our DS mice predominantly happened at night when mice are mostly awake. That SUDEP occurs mostly at night could also implicate a circadian influence, changes in motor activity, effects of light, or other entrained variables such as body temperature.

Interestingly, our data indicate that the peak incidence of seizures and SUDEP occurred in late evening before the lights went out (18:00–19:00) and ended before transitioning back to light phase between 01:00 and 05:00, suggesting it was not strictly the light/dark cycle that dictated risk of death. Instead, mice were more likely to die during the transition from the period of sleep (during the day for mice) to the period of increased motor activity (during early night). Nevertheless, major circadian abnormalities including a longer circadian period and severely impaired circadian photoresponsiveness have been identified in *Scn1a*$^{+/-}$ mice (35). Whether intrinsic circadian deficits in DS mice play a role in seizure occurrence or SUDEP is yet to be explored. One way to address this could involve manipulating light-dark cues or maintaining animals in constant darkness to assess the relationship between seizures and SUDEP and their intrinsic free-running circadian clock.

There Was a Large Increase in Seizure Frequency in the 24 h Prior to SUDEP

The circumstances surrounding SUDEP cases remain elusive, but it is believed that most deaths are preceded by a GTCS (18). A previous study reported a progression of increasingly severe spontaneous seizures preceding death in *Scn1a*$^{+/-}$ mice, but seizures were monitored for only 24 h prior to death (29). In the present study, the last 5 days prior to death were reviewed. Most mice had few or no spontaneous seizures 3–6 days before SUDEP, but there was a surge in the frequency of seizures in the last 1–2 days prior to death. Interestingly, we did not observe a consistent pattern. For instance, one mouse had one GTCS that was fatal, whereas another had 10 non-fatal seizures prior to the final one that resulted in death. The reasons underlying this variability in the number of GTCS leading up to death are unknown.

A recent report of three SUDEP cases showed that no convulsive seizures or abnormal electroencephalographic (EEG) activity were observed prior to death (36). However, the absence of cortical EEG activity does not exclude hidden seizures that may have contributed to the fatal cascade. The cardiorespiratory collapse observed in these and other witnessed SUDEP cases implicates a brainstem mechanism, such as the spreading

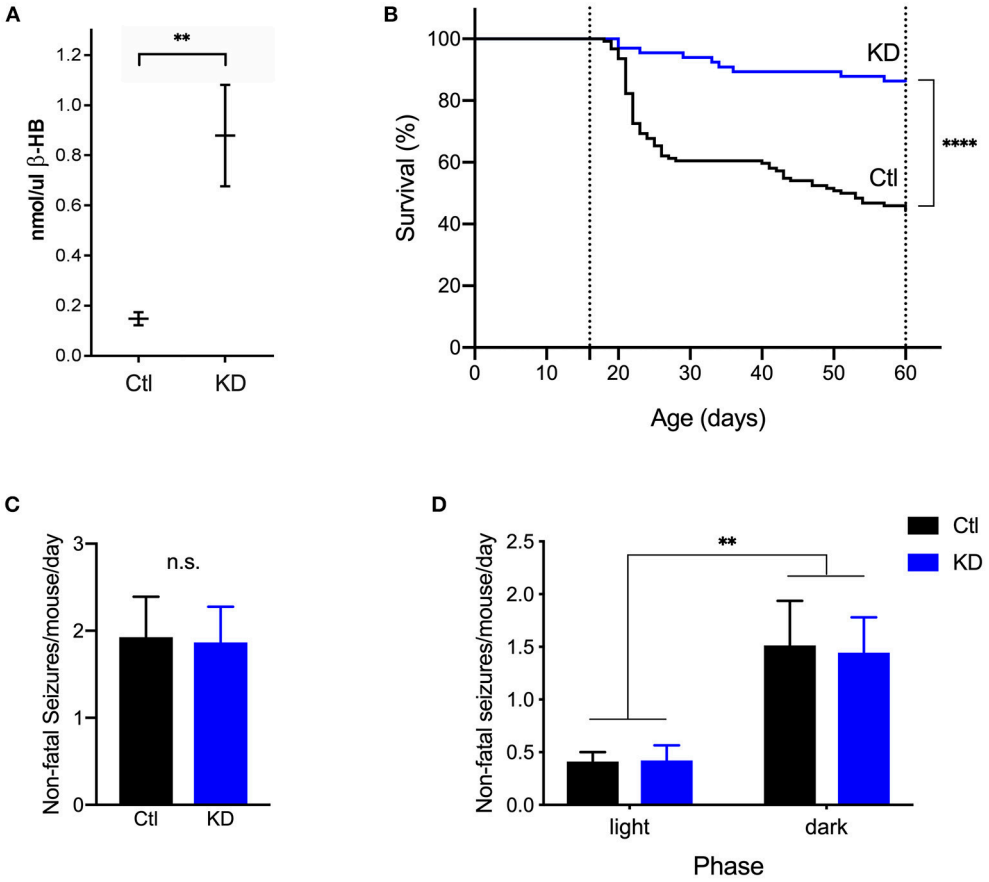

FIGURE 4 | The ketogenic diet reduces mortality in *Scn1a*$^{R1407X/+}$ mice. **(A)** As expected, beta-hydroxybutyrate (β-HB) levels were significantly increased in DS mice treated with a KD ($n = 7$) compared to control (Ctl, $n = 6$) (**$p = 0.0012$, Mann-Whitney U test). **(B)** Video surveillance revealed that 55% ($n = 69$ of 124) of DS mice fed a control diet (Ctl) spontaneously died after a generalized seizure with tonic hindlimb extension between P16 and P60. Treatment with a KD significantly increased survival of DS mice (KD, $n = 57$ of 66). ****$p < 0.0001$. **(C)** There was no difference in the frequency of seizures between diet groups. **(D)** Both diet groups experienced more seizures in the dark phase than in the light phase [$F_{(1, 28)} = 10.95, p = 0.0026$]. ($n = 9$ mice from 4 cages for KD, $n = 21$ mice from 12 cages for Ctl).

depolarization initiated by high potassium or tetanic neuronal stimulation in mice (37). Since our mice were not instrumented, EEG activity was not assessed. This limited our detection threshold to convulsive seizures. Whether non-seizure SUDEP ever occurs in DS mice is not known. Nevertheless, all sudden deaths we documented were preceded by behavioral seizures.

Effect of a Ketogenic Diet on SUDEP and Seizures in *Scn1a*$^{R1407X/+}$ Mice

Previous studies have shown that a KD increases seizure threshold in multiple models of inducible seizures (38), including flurothyl-induced seizures in heterozygous *Scn1a* knockout (*Scn1a*$^{+/-}$) mice (39). A KD has also been shown to reduce spontaneous seizures and extend the lifespan of *Kcna1*-null mice, a model of early onset epilepsy and SUDEP (25, 40). To our knowledge, the present study is the first to assess the effect of chronic treatment with a KD on spontaneous seizures and sudden death in *Scn1a*$^{R1407X/+}$ mice. Herein, we report that a KD markedly increases survival in DS mice to P60 from 44 to 86%. Since previous work has shown anticonvulsant effects of a KD, we hypothesized that reduced mortality in KD-treated mice was associated with a decreased incidence of spontaneous seizures. To our surprise, no significant differences in seizure frequency or severity were observed between diets. This suggests that the protection conferred by the KD on mortality in our DS mice is not due to an antiseizure effect. One possibility is that the KD prevents the propagation of seizures from the forebrain to brainstem nuclei that are critical for cardiorespiratory control (21). This could be explained by recent findings showing that KD-treatment in rodents rescued cytological and molecular correlates of chronic epilepsy, such as cortical gliosis and cell loss in the CA1 and CA3 hippocampal areas seen in chronic temporal lobe epilepsy (41). However, the effects of the KD on brain cytology were not explored in this study.

What Is the Link Between Seizures and SUDEP?

An important question is, what are the circumstances that lead to a seizure becoming fatal? In the present study, KD treatment prevented death without affecting the frequency of seizures, which challenges the notion that uncontrolled GTCS are the

strongest risk factor for SUDEP. One possibility is that KD-treatment may stabilize seizure-induced respiratory changes by indirect mechanisms, thus preventing fatal apnea. How dietary manipulations such as the KD influence respiratory physiology has not been explored.

A related question is why are seizures and SUDEP both tied to time of day if seizures do not always lead to death? The answer to this question will require a better understanding of what factors link seizures and SUDEP to time of day.

Limitations

A limitation to our study is the breadth of days and ages analyzed, as for most of our experiments we only reviewed video recordings from P20 to ∼P26. We chose this age range because that is when many spontaneous deaths occur (**Figure 4B**), so that it might be expected that there would be a greater likelihood of cardiorespiratory dysregulation. In addition, studying a cohort of older mice would exclude all mice that had died at an earlier age—a group that may have more cardiorespiratory abnormalities. It is possible that seizure frequency is affected by the KD at a later age, which would be consistent with previous observations from *Kcna1*-null mice (25).

The mechanism by which a KD protects DS mice from SUDEP is unclear. Since seizure frequency was determined visually in uninstrumented animals, only seizures scoring 4 and above in a modified Racine scale were documented. Very few KD-treated DS mice died of SUDEP, therefore we have not recorded cardiorespiratory parameters during SUDEP in any of that group. Further studies with EEG monitoring and plethysmography will be necessary to determine whether terminal events, such as GTCS and apnea, are altered by the KD.

Clinical Relevance

To this day, about twenty-seven FDA-approved AEDs are available, yet more than one third of epilepsy patients have inadequate control of seizures. As a result, the use of KDs has resurfaced in past decades to treat refractory epilepsy. Because administration and adherence to the strict KD are difficult for both patients and caregivers, elucidating the protective mechanism of the KD has increasingly become a pursuit of great interest for clinical and basic research.

CONCLUSION

The unpredictable and often unwitnessed occurrence of SUDEP presents a challenge to investigators and clinicians in research studies on patients. DS mouse models provide an efficient research tool for understanding the pathophysiology of SUDEP and developing effective therapies as they recapitulate human DS. Here we use a murine DS model to show the relationship between time of day and SUDEP, and an unexpected lack of effect of a KD on seizures despite protection against SUDEP.

DATA AVAILABILITY

The datasets generated for this study are available on request from the corresponding author.

ETHICS STATEMENT

All procedures and experiments involving mice were carried out with approval of the University of Iowa Institutional Animal Care and Use Committee, and in strict accordance with the recommendations of the ACP Guide for the Care and Use of Laboratory Animals (2011).

AUTHOR CONTRIBUTIONS

FT, YK, MC, KE, and GR were responsible for the collection and analysis of data. FT, YK, and GR were responsible for the conception and design of the experiments. FT drafted the manuscript. All authors were responsible for interpretation of the data and revised the manuscript critically for important intellectual content. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Dietary Omega-3 Polyunsaturated Fatty Acid Deprivation Does Not Alter Seizure Thresholds but May Prevent the Anti-seizure Effects of Injected Docosahexaenoic Acid in Rats

Ameer Y. Taha[1,2], Marc-Olivier Trepanier[2,3,4], Flaviu A. Coibanu[4], Anjali Saxena[4], Melanie A. Jeffrey[2,4], Nadeen M. Y. Taha[3], W. McIntyre Burnham[2,4] and Richard P. Bazinet[2,3]*

[1] Department of Food Science and Technology, College of Agriculture and Environmental Sciences, University of California, Davis, Davis, CA, United States, [2] EpLink, the Epilepsy Research Program of the Ontario Brain Institute, Toronto, ON, Canada, [3] Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, ON, Canada, [4] Department of Pharmacology and Toxicology, Faculty of Medicine, University of Toronto, Toronto, ON, Canada

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Christopher Michael DeGiorgio, University of California, Los Angeles, United States

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***Correspondence:**
Ameer Y. Taha
ataha@ucdavis.edu

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Background: Brain concentrations of omega-3 docosahexaenoic acid (DHA, 22:6n-3) have been reported to positively correlate with seizure thresholds in rodent seizure models. It is not known whether brain DHA depletion, achieved by chronic dietary omega-3 polyunsaturated fatty acid (PUFA) deficiency, lowers seizure thresholds in rats.

Objective: The present study tested the hypothesis that lowering brain DHA concentration with chronic dietary n-3 PUFA deprivation in rats will reduce seizure thresholds, and that compared to injected oleic acid (OA), injected DHA will raise seizure thresholds in rats maintained on n-3 PUFA adequate and deficient diets.

Methods: Rats (60 days old) were surgically implanted with electrodes in the amygdala, and subsequently randomized to the AIN-93G diet containing adequate levels of n-3 PUFA derived from soybean oil or an n-3 PUFA-deficient diet derived from coconut and safflower oil. The rats were maintained on the diets for 37 weeks. Afterdischarge seizure thresholds (ADTs) were measured every 4–6 weeks by electrically stimulating the amygdala. Between weeks 35 and 37, ADTs were assessed within 1 h of subcutaneous OA or DHA injection (600 mg/kg). Seizure thresholds were also measured in a parallel group of non-implanted rats subjected to the maximal pentylenetetrazol (PTZ, 110 mg/kg) seizure test. PUFA composition was measured in the pyriform-amygdala complex of another group of non-implanted rats sacrificed at 16 and 32 weeks.

Results: Dietary n-3 PUFA deprivation did not significantly alter amygdaloid seizure thresholds or latency to PTZ-induced seizures. Acute injection of OA did not alter amygdaloid ADTs of rats on the n-3 PUFA adequate or deficient diets, whereas acute injection of DHA significantly increased amygdaloid ADTs in rats on the n-3 PUFA adequate control diet as compared to rats on the n-3 PUFA deficient diet ($P < 0.05$). Pyriform-amygdala DHA percent composition did not significantly differ

between the groups, while n-6 docosapentaenoic acid, a marker of n-3 PUFA deficiency, was significantly increased by 2.9-fold at 32 weeks.

Conclusion: Chronic dietary n-3 PUFA deficiency does not alter seizure thresholds in rats, but may prevent the anti-seizure effects of DHA.

Keywords: after-discharge seizure threshold, amygdala, pentylenetetrazol, omega-3 deficiency, DHA

INTRODUCTION

Epilepsy is a progressive neurological disorder characterized by self-sustained periods of neuronal hyperexcitability (1, 2). Approximately one third of people with epilepsy have uncontrolled and persistent seizures despite being treated with anti-seizure medications (3). These individuals are particularly vulnerable to seizure-related psychiatric co-morbidities such as depression and anxiety, and sudden unexplained death in epilepsy (SUDEP) (4–6).

The main problem in people with epilepsy is that they have a low seizure threshold in one or more parts of the brain (1, 7). While mutations in several genes (e.g., sodium channel subunit mutations) may underlie epileptic seizures (8), environmental factors such as light or sound intensity may play a role in provoking a seizure episode in seizure-prone individuals (9, 10). Understanding factors that lower seizure thresholds may help inform on strategies that enable better seizure control in people with drug-resistant epilepsy.

Dietary lipids may also play a role in regulating seizure thresholds in epileptic patients. In particular, omega-3 polyunsaturated fatty acids (n-3 PUFAs) derived from plants (11) or seafood (12), were reported to raise seizure thresholds in rodents (13–17). The main n-3 PUFA found in the brain is docosahexaenoic acid (DHA, 22:6n-3). DHA regulates multiple processes within the brain, including gene transcription, neurotransmission, and the production of anti-inflammatory lipid mediators involved in resolving neuroinflammation (18–22).

DHA can be obtained preformed from the diet, or through liver elongation and desaturation of dietary alpha-linolenic acid (ALA, 18:3n-3) (23). ALA is thought to compete for elongation-desaturation with omega-6 linoleic acid (LA, 18:2n-6), which can be elongated-desaturated into arachidonic acid (AA, 20:4n-6) and docosapentaenoic acid (22:5n-6; DPA n-6) (24). Rats chronically fed an n-3 PUFA deficient diet show significant reductions in brain DHA concentration and increases in n-6 DPA (but not AA) concentration (25).

Mice fed an n-3 PUFA deficient diet for 30–34 days were reported to have greater susceptibility to magnesium-dependent audiogenic seizures than mice fed an n-3 PUFA adequate diet (15, 26). Consistent with these rodent studies, one epidemiological study reported a higher incidence of seizures in children born to mothers consuming low (117 mg/day) or high (817 mg/day) long-chain n-3 PUFAs during pregnancy as compared to children of mothers consuming intermediate levels of n-3 PUFAs (400–600 mg/day) (27). Another study reported reduced seizure incidence in infants born to mothers who received 800 mg/day of DHA during the second and third trimesters of pregnancy, as compared to mothers not supplemented with DHA (i.e., given a vegetable oil placebo) (28). Collectively, these studies suggest that low intake of n-3 PUFAs may reduce seizure threshold and increase the risk of seizure occurrence.

The present study tested the hypothesis that chronic dietary n-3 PUFA deprivation will lower seizure thresholds in the amygdala, a focus involved in the etiology of drug-resistant complex-partial seizures (29, 30). Seizure thresholds were measured over a period of 9 months, because we expected brain DHA levels to decrease within several months of initiating the n-PUFA deficient diets, due to the 4–12 weeks half-life of DHA in the adult rat brain (31–33). Amygdaloid seizure thresholds were also measured following acute oleic acid (OA, 18:1n-9) or DHA injection to n-3 PUFA adequate and deficient rats, to test whether seizure thresholds would increase by DHA administration. The present study also tested the effects of n-3 PUFA deficiency in the pentyleneterazol (PTZ) model of generalized tonic-clonic seizures in rats (34). Brain DHA and n-6 DPA composition was measured in a parallel group of non-seizure tested rats.

MATERIALS AND METHODS

Subjects and Treatments

Experimental procedures followed the Canadian Council on Animal Care guidelines, and were approved by the Animal Care Committee of the Faculty of Medicine of the University of Toronto.

Male Wistar rats (Charles River, La Prairie, QC, Canada), aged 53–60 days, were housed individually in transparent plastic cages with corn-cob bedding in a 12 h light-dark cycle vivarium maintained at 21°C. Food (Teklad Global, 2018 18% Protein Rodent Diet) and water were available *ad libitum*. All subjects were handled for a period of 7 days, following arrival from the breeding farm.

Two parallel experiments were then initiated as outlined in **Figures 1A,B** and described in detail below. The first experiment involved electrode implantations into the basolateral amygdala of 20 rats, followed by repeated seizure threshold measurements over a 34 week period during which the subjects were fed an n-3 PUFA adequate or deficient diet (**Figure 1A**). These rats were also treated with OA or DHA to test their effects on seizure threshold under the two dietary conditions. The second experiment involved a parallel group of non-implanted rats ($n = 42$) that were randomized to the n-3 PUFA adequate and deficient diets. These subjects were sacrificed after 16 or 32 weeks of dietary treatment to measure the effects of diet on brain DHA levels,

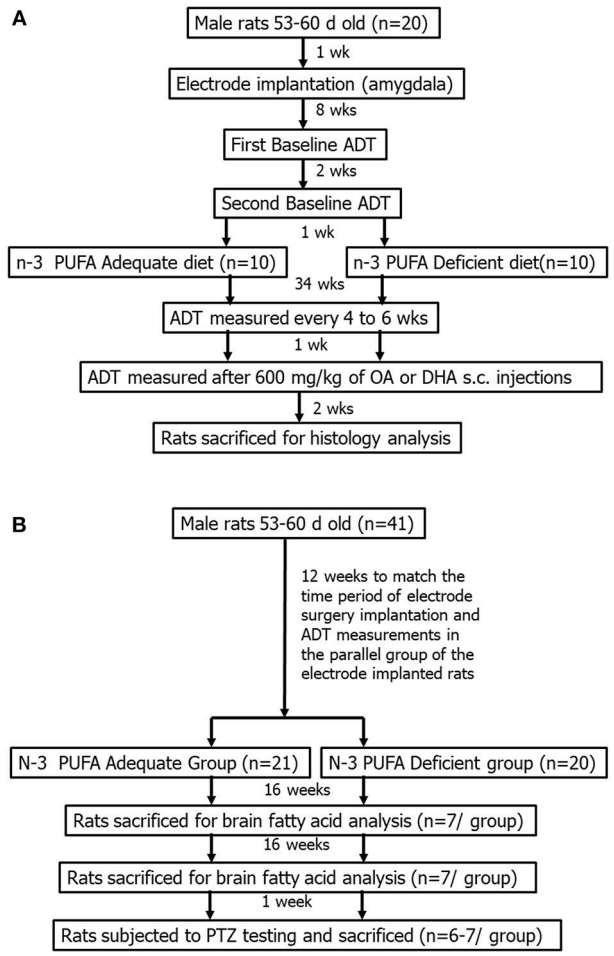

FIGURE 1 | General experimental design for implanted **(A)** and non-implanted **(B)** rats. The amygdala-implanted rats **(B)** were randomized to an n-3 PUFA adequate or deficient diet for a total of 37 weeks. They were subjected to repeated ADT measurements, once every 4–6 weeks, for 34 weeks. ADTs were measured between weeks 35–37, following i.p., injection with 600 mg/kg of oleic acid (OA) or docosahexaenoic acid (DHA). The non-implanted rats **(B)** were randomized to the same diets for 33 weeks. Two-thirds of the animals were sacrificed following 16 and 32 weeks of dietary treatment, and one-third was subjected to pentylenetetrazol-induced seizures after 33 weeks of dietary treatment.

or subjected to PTZ seizure testing after 33 weeks of treatment (**Figure 1B**).

Procedure for Electrode Implantation

Twenty rats were surgically implanted with stainless steel bipolar electrodes (MS303/1, Plastics One, Roanoke, VA, USA) aimed at the right basolateral amygdala ($n = 20$), under isoflorane anesthesia. The amygdala coordinates were as follows: anterior-posterior, −2.8; medial-lateral (bregma), 4.6 (bregma); and dorsal-ventral, −8.6 (skull surface at bregma). The incisor bar was set and maintained in the horizontal position by aligning bregma and lambda to the horizontal plane. The electrodes were fixed to the skull with 3–4 stainless steel anchor screws and acrylic dental cement (Nuweld, LD, Caulk). All subjects were subcutaneously injected with buprenorphine analgesic (0.05 mg/kg) and physiological saline (1 ml/kg) for rehydration following surgery.

Afterdischarge Threshold (ADT) Measurements

Baseline afterdischarge thresholds (ADTs) in the amygdala were measured 8 weeks following surgery, using the ascending series method (**Figure 1A**). Subjects were placed in a corn-bedded open field chamber and connected to a Grass model S-88 stimulator (Grass Instruments, Quincy, MA, USA), which delivered pulses through the recording electrode. The subjects received a one-second train of stimulation pulses at a frequency of 60 Hz, composed of a 1 ms positive and 1 ms negative phase separated by 0.5 ms. The initial stimulus intensity was 40 μA. The current was increased in steps of 20 μA up to 400 μA, and then in steps of 40 μA from 400 μA onwards, until an afterdischarge was evoked. The interval between stimulations was 5 min. The same electrodes were used for stimulating and recording focal electroencephalographic (EEG) activity.

Baseline ADT was measured again, 2 weeks later, after which, the animals were randomized to the n-3 PUFA adequate or deficient diets (see next section). ADTs were measured once every 4–6 weeks thereafter for 34 weeks. The second baseline ADT measurement was used as the reference point of comparison for assessing subsequent changes in seizure thresholds because the first baseline threshold measurements are known to drop drastically (but plateau to some extent) following the first stimulation (16).

Diets Administration and ADT Measurements

Amygdaloid subjects were started on the control n-3 PUFA adequate diet or the experimental n-3 PUFA deficient diet, 1 week after the second baseline ADT measurement. The diets were mixed every 2–3 days in our laboratory, and stored at 4°C. The cornstarch and sucrose components of the diets were obtained from Disley Food Services (Scarborough, ON, Canada). The oils were obtained from Loblaws Supermarkets (Toronto, ON, Canada). Other ingredients (protein, fiber, vitamins, minerals and antioxidant) were obtained from Dyets Inc. (Bethlehem, PA, USA).

The control AIN-93G diet contained (g/kg): casein (200), cornstarch (530), sucrose (100), soybean oil (70), cellulose (50), vitamin mix (10), mineral mix (35), L-cysteine (3), choline bitartrate (2.5) and tertbutyl hydroquinone (0.014). The n-3 PUFA deficient diet contained identical macronutrient composition, but the source of fat was derived from 24 g/kg of safflower oil and 46 g/kg of coconut oil in lieu of the soybean oil. The fatty acid composition of the AIN-93G control and n-3 PUFA deficient diets is presented in **Table 1**.

ADT Measurement Following DHA or Oleic Acid Administration

ADTs were measured between weeks 35–37 in the amygdaloid implanted subjects following OA or DHA injection (**Figure 1A**).

TABLE 1 | Fatty acid composition (% of total fatty acids) of the n-3 PUFA adequate and n-3 PUFA deficient diets.

	n-3 PUFA adequate	n-3 PUFA deficient
6:0	1.04 ± 1.80	0.72 ± 1.25
7:0	0.20 ± 0.22	0.16 ± 0.08
8:0	0 ± 0	7.96 ± 0.25
10:0	0.09 ± 0.08	5.56 ± 0.13
12:0	0.10 ± 0.09	35.04 ± 0.56
14:0	0.23 ± 0.01	11.71 ± 0.12
15:0	0.15 ± 0.01	0.11 ± 0.003
16:0	12.34 ± 0.23	8.23 ± 0.10
18:0	3.71 ± 0.12	2.24 ± 0.01
20:0	0.29 ± 0.01	0.12 ± 0.01
22:0	0.57 ± 0.32	0.18 ± 0.07
24:0	0.05 ± 0.08	0 ± 0
Total SFAs	18.77 ± 1.40	72.04 ± 0.36
18:1 t9	0 ± 0	0.092 ± 0.003
18:1 c9	16.28 ± 0.20	7.22 ± 0.08
18:1 c11	1.22 ± 0.02	0.31 ± 0.006
19:1 c7	0.297 ± 0.003	0 ± 0
22:1 n9	0 ± 0	0.07 ± 0.06
Total MUFAs	17.84 ± 0.25	7.70 ± 0.11
18:2 n6	53.58 ± 0.91	19.54 ± 0.26
18:3 n6	0.09 ± 0.08	0.12 ± 0.002
20:2 n6	0.18 ± 0.04	0 ± 0
Total n-6 PUFAs	53.85 ± 0.96	19.66 ± 0.27
18:3 n3	9.53 ± 0.21	0.60 ± 0.02
Total n-3 PUFAs	9.53 ± 0.21	0.60 ± 0.02

Data are mean ± SD of n = 3 representative samples per diet. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

The rats received a subcutaneous injection of DHA (600 mg/kg) or an equivalent dose of OA control 1 week after the last ADT was measured on week 34. A week later, treatments were switched, meaning that rats that received OA, received DHA. ADTs were measured following injection as described above. ADT measurements were initiated within 15 min post-injection. This ensured that each subject reached its expected ADT by ∼1 h post-injection, in view of a study showing that it takes 1 h for DHA to increase seizure threshold (35).

The rationale for the 600 mg/kg dose is based on body weight. We had previously reported that DHA raises seizure thresholds in the PTZ seizure model 1 h following injection at a dose of 300–400 mg/kg in rats weighing 200–300 g (35–37). The rats in the present study weighed ∼834 g at the time of seizure testing. Because unesterified DHA has a short plasma half-life and high volume of distribution associated with increased adiposity in heavier rats, the higher DHA dose of 600 mg/kg was selected to account for the greater body weight that likely increases the volume of distribution.

To compare the effects of OA and DHA on seizure thresholds, we subtracted the ADT measured on weeks 35 and 36, following OA or DHA injection, from the previous ADT on week 34, and the ADT following OA or DHA injection on week 37 from the ADT measured on weeks 35 and 36. In other words, the change in ADT was measured by subtracting the ADT following OA or DHA injection, from the prior ADT level.

Dietary Treatment to Rats Used for Determining Brain Fatty Acid Composition

A parallel group of subjects were obtained from the breeding farm and placed on the n-3 PUFA adequate ($n = 21$) or deficient diets ($n = 20$) at the same time as the implanted animals (**Figure 1B**). They were handled in a similar manner upon arrival and throughout the course of the experiment, being placed in an open field for 30 min once a month. Two-thirds of these subjects were sacrificed at 16 and 32 weeks ($n = 7$ per group per time-point) post diet initiation with CO_2 asphyxiation. The remaining one-third ($n = 6$–7 per group) was sacrificed at 33 weeks as described in the following section. The rationale for sacrificing the rats after 16 or 32 weeks of dietary treatment is based on the 4–12 weeks half-life of brain DHA, which led us to predict that a duration of 3–4 half-lives would be required to observe measurable effects of diet on brain DHA levels (31–33). The brains were excised immediately after CO_2 asphyxiation, and dissected to separate piriform-amygdala from a 1 mm coronal section of the left hemisphere. The dissected pyriform-amygdala samples were stored in a minus 80°C freezer until they were subjected to fatty acid analysis as described below.

PTZ Seizure Testing in Rats on the n-3 PUFA Adequate and Deficient Diets

The PTZ seizure test was performed on the remaining group of subjects with no implanted electrodes following 33 weeks of dietary treatment (**Figure 1B**; $n = 14$). Subjects were injected intraperitoneally with 110 mg/kg of PTZ, and observed in an open field for 10 min. This dose was chosen because it reliably induced tonic-clonic seizures in a separate group of subjects that were of the same age. The latency to the onset of myoclonic jerks and tonic-clonic seizures was determined by two observers, of whom one was blinded and the other was handling the animals. Subjects were euthanized with sodium pentobarbital (100 mg/kg) upon visibly showing tonic-clonic convulsions.

Sacrifice and Tissue Fixation

Electrode-implanted subjects were deeply anesthetized with sodium pentobarbital (100 mg/kg), and subjected to a direct current of 100 μA for 30 s in order to lesion the site of the electrode implant for subsequent histological evaluation of the position of the electrode tip. The subjects were then decapitated, and the brains were dissected quickly and stored in formalin for a few weeks to ensure complete fixation of the tissue. The brains were then transferred to 20% sucrose solution containing 0.1% sodium azide and stored at 4°C for a few weeks until they were histologically examined.

Histological Confirmation of Electrode Placement

The right hemisphere that contained the implanted electrode was used for histological confirmation as previously described. In brief, the hemispheres were chilled in isopentane on dry ice

and sectioned using a cryostat (Leica Instruments, Willowdale, Ontario, Canada) maintained at −25°C. Coronal sections were obtained at a thickness of 40 μm and mounted onto gelatin coated glass slides. The electrode tract was visible to the naked eye, so sections were collected close to where the tract ended, and subsequently confirmed under light microscopy (Research Analysis System Model 421251; Amersham, MI). Subjects with misplaced electrodes were excluded from subsequent data analysis.

Fatty Acid Analysis of the Pyriform-Amygdala

Total lipids were extracted from pyriform-amygdala by the method of Folch et al. (38) after being weighed to the nearest tenth of a milligram. The weighed samples were grinded in 6.5 ml of 0.9% KCl using a glass-grinder, and washed twice with 5 ml methanol, and twice with 10 ml of chloroform. Diheptadecanoyl L-α-phosphatidylcholine (Sigma, St. Louis, MO) in chloroform was added as an internal standard to the total lipid extracts, which were then dried under nitrogen and reconstituted in 2 ml of chloroform.

Total lipids directly methylated in 14% methanolic BF_3 (2 mL) and hexane (2 ml) at 100°C for 1 h. The samples were allowed to cool at room temperature for 10 min and centrifuged at 1,200 g following the addition of deionized water (2 ml). The upper hexane layer containing fatty acid metyl esters (FAMEs) was reconstituted in 50 μl hexane and analyzed by gas-chromatography as described in the following section.

Fatty Acid Methyl Ester Analysis by Gas-Chromatography

FAMEs were analyzed on a Varian-430 gas chromatograph (Varian, Lake Forest, CA, USA) equipped with a Varian FactorFour capillary column (VF-23 ms; 30 m × 0.25 mm i.d. × 0.25 μm film thickness) and a flame-ionization detector. FAMEs were injected in splitless mode. The carrier gas was helium, set to a constant flow rate of 0.7 ml/min. The injector and detector ports were set at 250°C. FAMEs were eluted using a temperature program set initially at 50°C for 2 min, increased at 20°C/min to 170°C held at 170°C for 1 min, increased at 3°C/min to 212°C and held at 212°C for 5 min. Peaks were identified by retention times of authentic FAME standards of known composition (Nu-Chek-Prep, Elysian, MN).

Dietary Fatty Acid Analysis

The fatty acid composition of the n-3 PUFA adequate and deficient diets was also determined by gas-chromatography. Total lipids were first extracted from ∼0.5 g of powdered diet in chloroform/methanol (2:1 v/v) after adding 2 mg of unesterified heptadecanoic acid as an internal standard (Sigma, St. Louis, MO). Saline (0.9% w/v, 2 ml) was added to separate polar compounds. The bottom layer containing total lipids was transferred to test-tubes. A portion of the extract was dried under nitrogen, reconstituted in 2 ml of hexane, and directly methylated with 2 ml of 14% boron trifluoride in methanol at 100°C for 1 h. The hexane layer was extracted and FAMEs were analyzed by gas-chromatography as described above.

Data Presentation and Statistical Analysis

The data are presented as means ± SD. Data analysis was performed using Sigma Stat v.3.2 (Jandel Corporation) or Graphpad Prism v 5.0 (La Jolla, CA). A two-way repeated measures analysis of variance (ANOVA) was used to determine the effects of diet and time on body weight, ADT, seizure duration and seizure score. A two-way ANOVA was used to test the effect of diet and time on pyriform-amygdala DHA and n-6 DPA composition. Due to the small sample size, the Mann-Whitney *U*-test was used to assess differences in the latency to PTZ-induced seizures between the n-3 PUFA adequate and deficient groups. Statistical significance was accepted at $P < 0.05$.

RESULTS

Electrode Placements

Although, the surgeries were aimed at placing the electrodes within the basolateral amygdala, subjects with electrodes falling within the amygdaloid complex were included in the analysis. Electrodes were successfully implanted within the amygdala in 5 out of 8 n-3 PUFA adequate control rats and 4 out of 8 n-3 PUFA deficient rats. Subjects with electrodes misimplanted outside the amygdaloid complex were, therefore, excluded from subsequent analyses.

In the successfully implanted subjects, electrodes were within the basolateral amygdala for 3 (out of 5) and 2 (out of 4) subjects within the n-3 PUFA adequate and deficient groups, respectively. The electrodes for the remaining subjects were within the amygdala, but slightly medial or anterior to the basolateral amygdala. We were not able to compare differences in ADTs within the different amygdala foci due to the small sample size, which is why we accepted subjects with successful implants anywhere within the amygdala. This is also consistent with our primary hypothesis, which aimed to test the effects of diet on amygdaloid seizure thresholds.

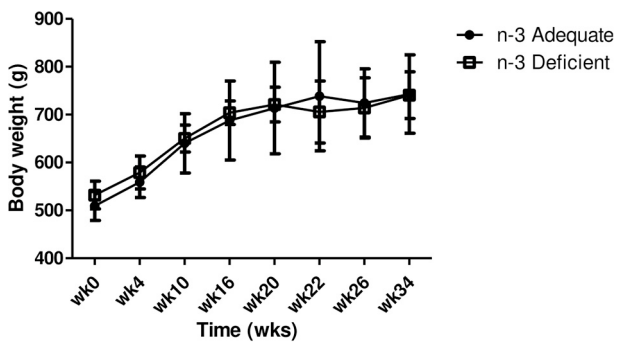

FIGURE 2 | Effects of chronic n-3 PUFA deficiency on body weight over time. Rats were placed on a n-3 PUFA adequate (control) or n-3 PUFA deficient diet. Data are mean ± SD of $n = 5$ n-3 PUFA adequate controls and 4 n-3 PUFA deficient rats. Two-way repeated measures ANOVA showed a significant effect of time on body weight ($P < 0.0001$). There was no significant effect of diet on body weight ($P > 0.05$). Subjects' weights increased over time, regardless of diet.

Body Weight

The data related to body weight of implanted rats from the start of the baseline ADT measurements and throughout the 34 weeks period of threshold measurements is shown in **Figure 2**. A two-way repeated measured ANOVA showed a significant effect of time ($P < 0.0001$) but not of dietary treatment ($P > 0.05$) on body weight. As shown in **Figure 2**, both n-3 PUFA adequate and deficient rats gained weight over time. There were no significant differences in body weight between the groups at any time point.

Effect of Dietary n-3 PUFA on Amygdaloid ADT and Seizure Duration

Seizures were successfully recorded at baseline ("week 0") and at 4, 10, 16, 20, 22, 26 and 34 weeks thereafter. Recordings were obtained for all subjects with amygdala implants, except for one n-3 PUFA adequate control rat on weeks 22, 26, and 34 and one n-3 deficient rat on weeks 10, 16, and 20. These two subjects did not show an afterdischarge when measured during these time periods, probably due to a transient (but unconfirmed) infection. We were not able to retrieve raw ADT files for one control rat on week 16 and one n-3 deficient rat on week 20. ADTs that were not successfully obtained for these 4 rats during the 1–3 time-points were not included in the statistical analysis. Thus, ADTs successfully recorded for these 4 rats during other weeks, as well as the rest of the amygdala-implanted subjects, were included in the statistical analysis.

The data related to ADT (μA), percent change in ADT from baseline and seizure duration over the 34 week measuring period are presented in **Figures 3A–C**. Two-way repeated measures ANOVA showed a significant effect of time but no effect of treatment or interaction between time and treatment for ADT, % change in ADT and seizure duration. As shown in **Figure 3**, ADT (**Figure 3A**) and the % change in ADT (**Figure 3B**) decreased gradually over time in both n-3 adequate and deficient rats, whereas seizure duration increased over time (**Figure 3C**). A peculiar observation is that absolute ADT values appeared to decrease more for the n-3 PUFA adequate group at 26 and 34 weeks (**Figure 3A**), but after correcting for the small but insignificant differences in baseline ADT between the two groups, this effect was no longer seen (**Figure 3B**).

ADT Following OA or DHA Injection

OA or DHA (600 mg/kg) were injected subcutaneously to n-3 PUFA adequate and deficient rats between weeks 35 and 37, to test whether DHA raises ADT. In particular, we wanted to address whether ADTs increased in n-3 deficient rats following DHA injection.

Figure 4 shows the change in ADT and seizure duration following s.c., injection of n-3 PUFA adequate and deficient rats with OA or DHA. Two-way ANOVA revealed a significant interaction between diet and fatty acid injection ($P = 0.048$), but no significant main effect of diet or injection on ADT (**Figure 4A**). *Post-hoc* analysis with Bonferroni's *post-hoc* test indicated that the change in ADT following DHA treatment was significantly higher in rats on the n-3 adequate group as compared to rats on the n-3 deficient group ($P < 0.05$). The change in ADT following OA injection was not significant between n-3 adequate or deficient rats. Also, no significant differences between OA and DHA injection were observed.

Two-way ANOVA showed no significant effect of diet or fatty acid (OA or DHA) injection on seizure duration (**Figure 4B**). Also, no significant interaction was detected.

Latency to PTZ-Induced Seizure Onset Following 33 Weeks of Treatment With an n-3 PUFA Adequate or Deficient Diet

Body weights measured at the time of seizure testing were not significantly different between the two groups (n-3 PUFA

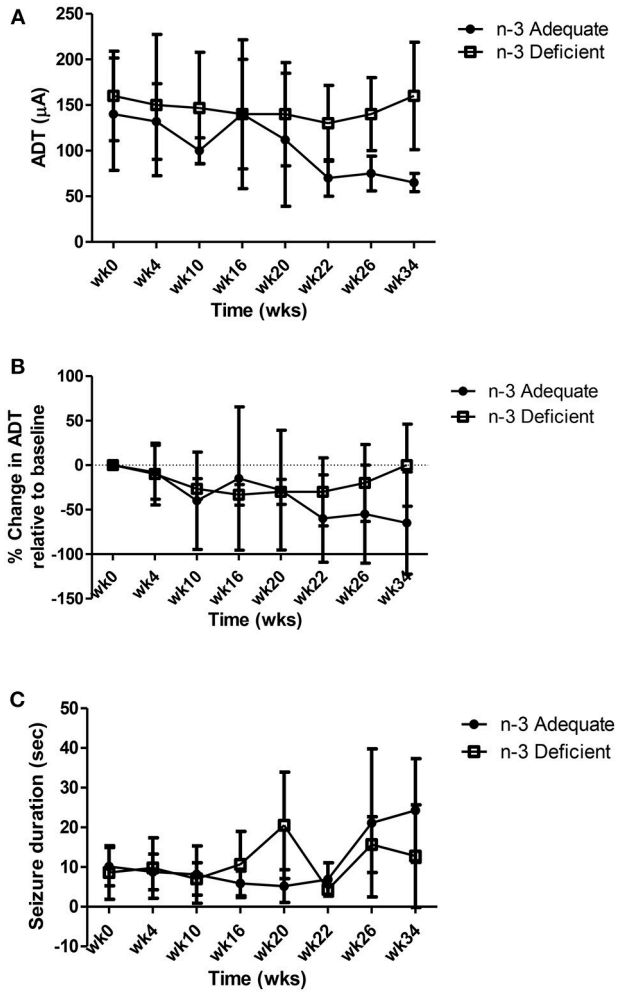

FIGURE 3 | Effect of chronic n-3 PUFA deficiency on **(A)** ADT (μA), **(B)** percent change in ADT over time and **(C)** seizure duration. ADTs and seizure duration were recorded for 34 weeks from rats maintained on the n-3 PUFA adequate diet ($n = 5$) or n-3 PUFA deficient diet ($n = 4$). Two-way repeated measures ANOVA showed a significant effect of time but no effect of treatment or interaction between time and treatment on ADT, % change in ADT and seizure duration. ADT **(A)** and the % change in ADT **(B)** decreased gradually over time in both n-3 adequate and deficient rats, whereas seizure duration increased over time **(C)**.

adequate, 873 ± 125 g, $n = 7$; n-3 PUFA deficient 789 ± 116 g, $n = 6$, $P = 0.29$ Mann-Whitney U-test).

The data related to the onset of myoclonic jerks and tonic-clonic seizures following PTZ administration to non-implanted rats maintained on the n-3 PUFA adequate or deficient diet are presented in **Figure 5**. One rat from the PUFA n-3 deficient group did not seize within the 10 min observation period so it was not included in the statistical analysis. As shown in **Figure 5A,** the latency to mycolonic jerks in the rats that seized did not differ significantly between the groups ($P = 0.15$ by Mann-Whitney U-test). The latency to tonic-clonic seizures (**Figure 5B**) was lower by 57% in the n-3 PUFA deficient group as compared to controls, but the difference was not statistically significant ($P = 0.06$).

Piriform-Amygdala DHA and N-6 DPA Composition

Piriform-amygdala DHA and n-6 DPA were measured in a parallel group of non-electrode implanted subjects administered the n-3 PUFA adequate or deficient diets for 16 and 32 weeks. **Figure 6** shows piriform-amygdala DHA (6-A) and n-6 DPA (6-B) composition, expressed as percentage of total fatty, of rats fed an n-3 PUFA adequate or deficient diet for 16 and 32 weeks. A two-way ANOVA followed by Benferroni's *post-hoc* was used to assess the effect of diet and time on pyriform-amygdala DHA and n-6 DPA% composition.

There was no significant effect of diet, time or interaction between diet and time on DHA percent composition. There

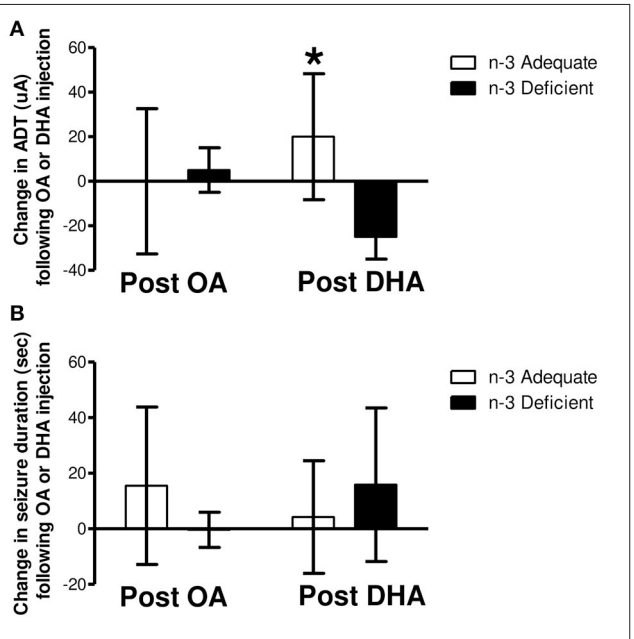

FIGURE 4 | Change in ADT 1 h post s.c. injection of OA or DHA (600 mg/kg) to rats fed an n-3 PUFA adequate or deficient for 37 weeks. Rats of each dietary group randomly received 600 mg/kg of oleic acid (OA) or docosahexaenoic acid (DHA) approximately 1 week after the last ADT was taken on week 34. ADTs and seizure duration were measured within 1 h of fatty acid treatment. One week later, the treatments were switched (i.e. rats that received OA now received DHA), and ADTs were measured within 1 h of fatty acid treatment. The difference in ADT following OA and DHA treatment of each subject, per diet, was determined (i.e., DHA ADT—OA ADT) using the prior ADT as a reference point. As shown in **(A)**, two-way ANOVA revealed no main effect of diet or fatty acid treatment on the change in ADT. A significant diet and fatty acid treatment interaction was detected ($P < 0.05$). *Post hoc* analysis of the means with Bonferroni's *post-hoc* test indicated that the change in ADT following DHA treatment was significantly greater in rats on the n-3 adequate diet as compared to rats on the n-3 PUFA deficient diet (*$P < 0.05$). No significant main effects or interaction were detected for seizure duration **(B)**.

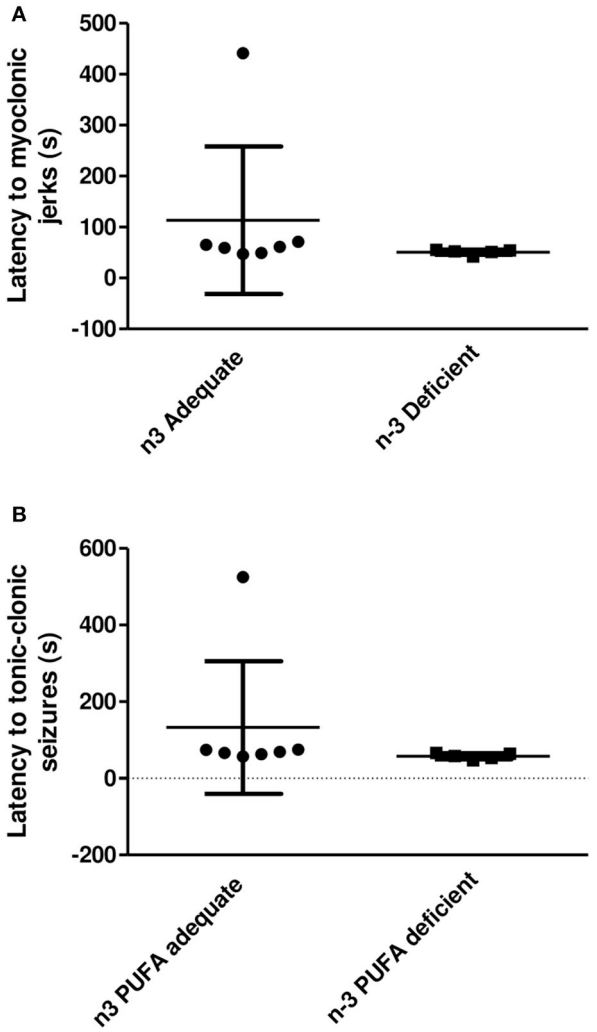

FIGURE 5 | Latency to the onset of myoclonic jerks and tonic clonic seizures following PTZ administration (110 mg/kg, i.p.) to rats treated with an n-3 PUFA adequate or deficient diet for 33 weeks. One rat from the n-3 PUFA deficient group was excluded because it did not seize within the 10 min observation period. Data are mean ± SD of $n = 7$ n-3 PUFA adequate and $n = 5$ n-3 PUFA deficient subjects. **(A)** Latency to the onset of myoclonic jerks. A Mann-Whitney U was used to compare latencies. The latency to the onset of myoclonic jerks did not differ significantly between the groups ($P = 0.15$). **(B)** Latency to the onset of tonic-clonic seizures. A Mann-Whitney U was used to compare latencies. The latency to the onset of tonic-clonic seizures was lower in the n-3 deficient group as compared to the adequate group. This difference, however, was not statistically significant ($p = 0.06$).

was a significant effect of time ($P = 0.0261$) and diet ($P = 0.0006$) on n-6 DPA percent composition, but no significant interaction was detected ($P = 0.0997$). *Post-hoc* comparison of the means indicated that the difference between the n-3 adequate and deficient groups at 32 months was statistically significant. N-6 DPA was 2.9-fold higher in the n-3 PUFA deficient group as compared to the n-3 PUFA adequate group.

DISCUSSION

The present study showed that chronic dietary n-3 PUFA deficiency, achieved by removing ALA from the diet, did not significantly alter amygdaloid seizure thresholds or latency to PTZ-induced seizures. Acute OA injection did not alter ADTs, whereas DHA increased amygdaloid ADTs in the n-3 PUFA adequate group relative to the n-3 PUFA deficient group. Chronic PUFA n-3 deficiency increased pyriform-amygdala n-6 DPA percent composition without altering DHA composition.

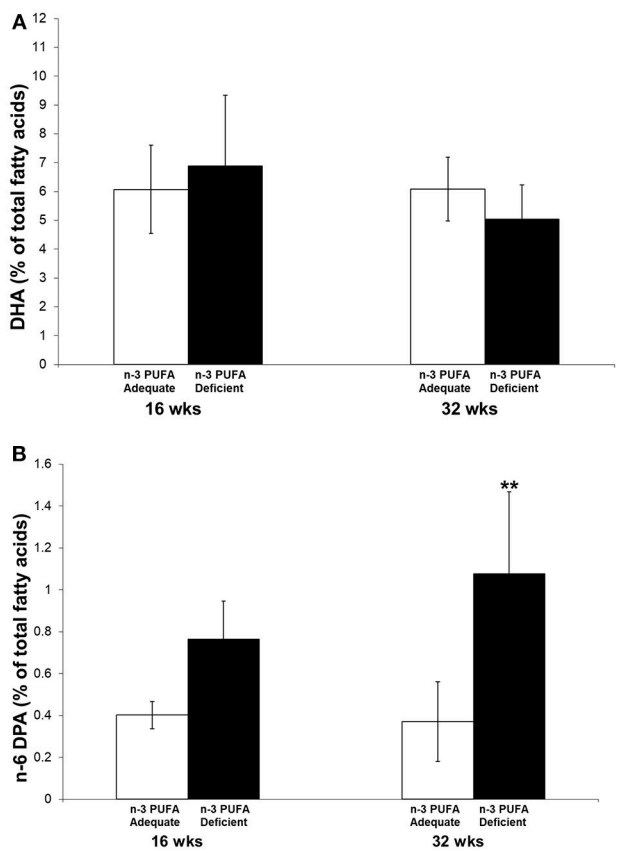

FIGURE 6 | DHA **(A)** and n-6 DPA **(B)** % of total fatty acids in piriform-amygdala of rats fed an n-3 PUFA adequate or deficient diet for 16 and 32 weeks. Data are mean ± SD of $n = 7$ per diet group per time-point for each fatty acid, except n-6 DPA, for which $n = 6$ in control rats at 32 weeks because it was not detected in one sample. A two-way ANOVA followed by Benferroni's *post-hoc* was used to assess the effect of diet and time on pyriform-amygdala DHA and n-6 DPA % composition. There was no significant effect of diet, time or interaction between diet and time on DHA percent composition. There was a significant effect of time ($P = 0.0261$) and diet ($P = 0.0006$) on n-6 DPA percent composition, but no significant interaction was detected ($P = 0.0997$). *Post-hoc* comparison of the means indicated that the difference between the n-3 adequate and deficient group was significant at 32 weeks (**$P < 0.01$).

Our findings do not support the hypothesis that n-3 PUFA deficiency lowers seizure thresholds in adult rats. This may be related to the fact that the amygdala-pyriform DHA composition was not reduced following dietary n-3 PUFA deprivation.

Previous studies reported a significant reduction in cortical and whole brain DHA concentrations and percent composition following chronic n-3 PUFA deficiency (39–41). The lack of changes in DHA composition in the pyriform-amygdala suggests that this brain region may be less sensitive to the effects of dietary n-3 PUFA manipulation as compared to other brain regions. Consistent with this suggestion, we reported that chronic fish oil supplementation to rats for 6 months did not significantly increase piriform-amygdala DHA concentration (16).

Another possibility accounting for the lack of change in piriform-amygdala DHA composition is that the n-3 PUFA deficient diet was initiated during adulthood (at 5 months of age). Other studies initiated n-3 PUFA deficiency during development or at weaning (∼21 days post-partum) (39–41). In rats, DHA accretes in the brain during the first 29 days of life, and early n-3 PUFA deficiency interferes with brain DHA accretion and concentration (42). The extent of brain DHA depletion when dietary n-3 PUFA deficiency is initiated during adulthood, and after DHA accretion rate has reached steady-state, is not known. It is possible that adipose tissue contributes to maintaining pyriform-amygdala DHA status throughout adulthood when n-3 fatty acids are absent from the diet (43). An alternative but unconfirmed hypothesis is that DHA turnover within the pyriform-amygdala complex is slow compared to other brain regions such as the cortex. Regional differences in brain DHA turnover in relation to concentration should be further examined in future studies.

N-6 DPA, a marker of n-3 PUFA deficiency was significantly increased 32 weeks after the rats were started on the n-3 PUFA deficient diet. It is unlikely that n-6 DPA altered ADTs, because it was reported to have no effect on excitatory hippocampal sharp waves *ex vivo* (44). Future studies should confirm these findings *in vivo*, however.

Pages et al. reported that mice fed an n-3 PUFA deficient diet for 30–34 days were more susceptible to magnesium-dependent audiogenic seizures than mice fed an n-3 PUFA adequate diet (15, 26). We did not observe significant changes in amydgaloid afterdischarge or PTZ seizure thresholds following n-3 PUFA deprivation in the present study. Differences in study outcomes are likely due to the seizure model used. The studies by Pages et al. used a magnesium deficient diet, which may have lowered seizure thresholds sufficiently for audiogenic provocation (15, 26).

Acute DHA injection increased amygdaloid seizure thresholds in rats on the n-3 PUFA adequate diet, but not in rats on the n-3 PUFA deficient diet, while OA had no significant effect on ADTs. The increase in amygdaloid seizure thresholds following DHA injection in the n-3 adequate group is consistent with previous studies which showed that injected or dietary DHA raises seizure

thresholds in rats on an n-3 adequate diet (16, 17, 36, 37, 45, 46).

In our previous studies, the increase in seizure latency following acute DHA injection was attributed to the increase in plasma unesterified DHA concentration (37), the form available for brain uptake (32, 47, 48). The lack of significant effect of unesterified DHA on amygdaloid ADTs in the n-3 PUFA deficient group, is likely because injected DHA did not increase plasma unesterified DHA concentrations to therapeutic levels. Plasma unesterified DHA before and after acute DHA administration to n-3 PUFA adequate and deficient rats was not measured in the present study, a limitation which should be addressed in future studies. Unesterified DHA is known to reduce neuronal excitability by acting on GABA or voltage gated ion channels (44, 49–51), or through its oxygenated metabolites such as neuroprotectin D1 (21), which were reported to reduce electrically induced hippocampal excitability in rodents (52).

The North American diet may be low in DHA (53–55), but it is not omega-3 deficient *per se*. Although extreme n-3 deficiency as modeled in the present study is not likely to be clinically prevalent (53–55), this study demonstrates the importance of dietary n-3 PUFA status as a potential modifier of the anti-seizure effects of DHA. It is not clear, however, as to whether people with epilepsy have low or deficient n-3 fatty acid intake or circulating DHA levels.

The main limitation of this study is the low sample size. While the repeated stimulations over time confirm no changes in ADT between the diets, the PTZ and acute OA and DHA injection experiments were only performed once. These studies should be reproduced with a larger number of subjects. Another limitation is that not all electrodes were within the intended basolateral amygdala target; some were medial or lateral but were within the amygdala. Thus, our findings cannot be generalized to the basolateral amygdala or specific structures within.

In conclusion, dietary n-3 PUFA deprivation for 8–9 months did not alter amygdaloid seizure thresholds or the latency to PTZ-induced seizures. Injected DHA, however, raised amygdaloid seizure thresholds in rats on the n-3 PUFA adequate diet, but had no effect in rats on the n-3 PUFA deficient diet, suggesting that dietary n-3 PUFA status may modulate the anti-seizure effects of DHA. Clinical assessment of dietary n-3 fatty acid background and circulating DHA status is warranted in intervention studies addressing the role of DHA supplementation in people with epilepsy. Understanding the role of DHA in raising seizure thresholds may reduce seizure incidence and the risk of SUDEP in at-risk individuals (56–58).

AUTHOR CONTRIBUTIONS

AT, RB, and WB designed the study. AT and M-OT contributed to the data and statistical analysis. AT, M-OT, AS, NT, FC, and MJ performed the experiments.

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Neuroimaging of Sudden Unexpected Death in Epilepsy (SUDEP): Insights From Structural and Resting-State Functional MRI Studies

Luke A. Allen[1,2,3]*, *Ronald M. Harper*[3,4,5], *Samden Lhatoo*[3,6], *Louis Lemieux*[1,2] and *Beate Diehl*[1,2,3]

[1] Department of Clinical and Experimental Epilepsy, UCL Queen Square Institute of Neurology, London, United Kingdom, [2] Epilepsy Society MRI Unit, Chalfont St Peter, London, United Kingdom, [3] The Center for SUDEP Research, National Institute of Neurological Disorders and Stroke, Bethesda, MD, United States, [4] Department of Neurobiology, David Geffen School of Medicine at UCLA, University of California, Los Angeles, Los Angeles, CA, United States, [5] Brain Research Institute, University of California, Los Angeles, Los Angeles, CA, United States, [6] Department of Neurology, University of Texas Health Sciences Center at Houston, Houston, TX, United States

The elusive nature of sudden unexpected death in epilepsy (SUDEP) has led to investigations of mechanisms and identification of biomarkers of this fatal scenario that constitutes the leading cause of premature death in epilepsy. In this short review, we compile evidence from structural and functional neuroimaging that demonstrates alterations to brain structures and networks involved in central autonomic and respiratory control in SUDEP and those at elevated risk. These findings suggest that compromised central control of vital regulatory processes may contribute to SUDEP. Both structural changes and dysfunctional interactions indicate potential mechanisms underlying the fatal event; contributions to individual risk prediction will require further study. The nature and sites of functional disruptions suggest potential non-invasive interventions to overcome failing processes.

Keywords: biomarkers, SUDEP, MRI, functional connectivity, structural imaging biomarkers

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***Correspondence:**
Luke A. Allen
luke.allen.15@acl.ac.uk

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INTRODUCTION

Sudden unexpected death in epilepsy (SUDEP) is the leading cause of untimely death in epilepsy (1), with a 20-fold increase in incidence over that of sudden death in the general population (2, 3). SUDEP is largely sleep-bound (around 60% of events) and occurs unwitnessed in nearly 90% of cases (4). With no structural, or toxicological indicators of the cause of death, precise underlying SUDEP mechanisms remain elusive.

Observational studies within epilepsy monitoring units (EMUs) show autonomic and respiratory dysfunction preceding SUDEP. A comprehensive assessment of the incidence and mechanisms of cardiorespiratory arrests in EMUs (5) revealed severe alterations to cardiac and respiratory function in the post-ictal period of generalized tonic-clonic seizures (GTCS) which led to SUDEP ($n = 10$ cases). Specifically, transient cessations in breathing preceded terminal apnoea, and ultimately terminal asystole. Prolonged peri-ictal apnea (6), with or without bradycardia and asystole, and post-convulsive central apnea (7) may play a role in SUDEP risk. Cortical and sub-cortical structures that modulate autonomic and breathing processes are of great interest to pre-mortem risk identification through imaging (8), particularly since electrical stimulation studies confirm the role of brain regions often involved in epileptic seizures (9). Sustained post-ictal hypotension is also associated with GTCS (10), further indicating alterations to

central autonomic control processes following GTCS. Overall, this key evidence demonstrates centrally-mediated disruption to autonomic and breathing regulatory processes following GTCS (5, 10) and cases of observed SUDEP (5).

GTCS are the leading SUDEP risk-factor (11); experiencing three or more seizures of this type per year is associated with the largest increase in risk (12). The possibility that seizures, especially GTCS, propagate to, and rapidly involve, central autonomic and respiratory brain sites, leading to dysfunction, has been previously hypothesized (13, 14); yet, this central issue to SUDEP remains unresolved.

Neuroimaging is a powerful tool to explore structural and functional brain alterations within distinct sites and networks crucial for autonomic and respiratory regulatory processes in patients who (after being scanned) succumb to SUDEP. Such assessments allow the investigation of structural (tissue) abnormalities or disrupted networks related to SUDEP, and have the potential to shed light on underlying mechanisms and provide biomarkers to prospectively identify living patients at heightened risk. In the following sections, evidence from structural and functional magnetic resonance imaging (MRI) investigations into SUDEP will be discussed, together with potential interventions to overcome deficient processes.

EVIDENCE FROM STRUCTURAL MRI

Structural MRI enables the identification and characterization of brain tissue abnormalities, regional alterations in brain volume, cortical thickness and morphometry, and abnormal structural connections (fiber tracts). Such techniques have been widely applied to epilepsy (15–17), and have the potential to improve understanding of underlying brain physiology and highlight quantifiable disease biomarkers (18, 19). Although the precise pathological mechanisms of SUDEP are not known, some imaging studies have highlighted structural changes to cortical, sub-cortical, and brainstem structures in those who subsequently succumbed to SUDEP and those at greatest risk, indicating morphological disturbances among sites involved in central autonomic and respiratory regulation. In the remainder of this section we provide an overview of the main relevant imaging findings, and interpret them in relation to other, independent work.

Tissue Loss in Thalamic, Brainstem, and Frontal Sites

Voxel-based morphometry (VBM) has been used to investigate regional gray matter changes in subjects who later died from SUDEP ($n = 12$) and comparable high-risk, low-risk, and healthy controls (20). Gray matter volume of the bilateral posterior thalamus (pulvinar nuclei) was found to be reduced in SUDEP cases and those at high-risk, compared with healthy, and low-risk controls. Although correction for multiple comparisons was not employed in this study, more recent work (21) confirmed posterior thalamic loss (though confined to the left only) in a larger cohort ($n = 25$ SUDEP cases) which employed family-wise-error rate (FWER) correction of p-values. Thalamic volume loss in patients who experience GTCS, and therefore who are at greatest risk of SUDEP, has been widely demonstrated (22–24), including loss specifically within the pulvinar (22). We note that in congenital central hypoventilation syndrome, a condition involving breathing and cardiovascular dysfunction, blood flow responses to hypoxia and hypercapnia were found to be altered in the posterior thalamus (25, 26), further supporting its role in mediating control of breathing (27, 28). In other conditions involving impaired autonomic and respiratory function, such as obstructive sleep apnoea (29) and heart failure (30), posterior thalamic volume loss also appears. Posterior thalamic loss raises the possibility that strategic control of low oxygen and CO_2 is at risk, a serious handicap during ictal events where recovery from low oxygen and high CO_2 necessitates appropriate responses to such ventilatory conditions.

A recent investigation into neocortical morphometry in patients with GTCS ($n = 53$) revealed widespread thinning, most prominently within the frontal lobe, including orbitofrontal sites, which are involved in cardiovascular regulation (31), and in temporal and parietal cortices (32). The results of volumetric studies are consistent with these findings, revealing tissue loss within the frontal cortex (23), including medial and lateral orbitofrontal regions (22) in patients with GTCS. Those cortical changes should be viewed in the context of volume changes in thalamic sites, since sensory information classically synapses in the thalamus before projecting to cortical sites, with reticular thalamic sites providing an aspect of focus to afferent input. Many of these projections are reciprocal, providing a basis to induce structural alterations in subcortical areas following changes in cortical thickness.

In addition to changes among cortical and sub-cortical structures, more-caudal brain alterations have also been identified in cases of SUDEP. VBM revealed reduced volume of the periaqueductal gray [PAG; (21, 33)]. Volume loss also appears in the medulla oblongata, which becomes progressively more extensive the closer to SUDEP from MRI (34). Portions of the medulla form the final common pathway for cardiovascular and respiratory control. The PAG plays a significant role in cardiorespiratory patterning and recovery; deficient post-ictal PAG-driven compensatory mechanisms have been linked to SUDEP in a mouse model (35). That role stems from projections from forebrain areas, including the amygdala, and its own projections to parabrachial and ventrolateral regions for breathing control (36); concerns of PAG contributions to breathing partially stem from susceptibility of its neurons to opiates (37), with their well-known depression of breathing. PAG neurons show time-locked relationships to both the respiratory (38), and cardiac (39) cycles, as revealed by animal studies, and these relationships are *sleep-state dependent*. In this context, the fact that SUDEP appears preferentially during sleep emphasizes the need to study any functional connectivity changes with the PAG in the context of state change.

Overall, there is accumulating evidence of widespread structural loss, particularly within anatomic regions related to cardiorespiratory functions such as thalamic, frontal lobe (including medial and orbital divisions) as well as brainstem sites in people who suffered SUDEP and in those at greatest risk.

Tissue Gain in Limbic, Insula, and Sensory Sites

In addition to regional reductions, regional increased volume, and cortical thickness in key autonomic, breathing, and sensory sites have been observed in SUDEP cases and those at high risk. Compared with low-risk and healthy subjects, cases of SUDEP and those at high risk show increased gray matter volume of the right amygdala and anterior hippocampus (20), which are known to be involved in breathing regulation (40). More recent imaging work demonstrates enlargements to additional anterior limbic structures, including the bilateral amygdala, parahippocampal gyrus and entorhinal cortex (21) in SUDEP cases and high-risk subjects. The subcallosal cortex, a region involved in blood pressure regulation (8), is also enlarged—but only in those who subsequently died (21). Bilateral increased mesial temporal structure volumes, including the amygdala, appear in a sub-type of mesial temporal lobe epilepsy (m-TLE) who also had poor post-surgical outcome (41). We note that patients in whom surgery has failed to reduce seizure frequency encompass the group at greatest risk of SUDEP risk, when compared with population-based incidence cohorts, prevalence cohorts, populations from epilepsy clinics, and even refractory epilepsy cohorts (1). Increased volume may reflect gliosis or inflammation, potentially resulting from ongoing hypoxic damage (42) occurring following seizures (43), although this must be confirmed in human epilepsy studies. Uncontrolled GTCS, often seen in subjects who die and those at high-risk, could accelerate such processes, although further work is required to confirm this process.

Patients who experience GTCS also show cortical thickening across a number of sites (32): The post-central gyri, anterior insulae and the subgenual, anterior, posterior, and isthmus cingulate exhibited cortical thickening in GTCS patients ($n = 53$) compared with healthy controls ($n = 530$). While patients who experience GTCS are at highest risk of SUDEP, assessments of cortical thickness are needed in patients who died from SUDEP, since studies including only at-risk populations are complicated by limited interpretability. Elevated volume and cortical thickness are traditionally considered as being linked to improved function or compensatory mechanisms, such as the increased volume within visual cortex observed in deaf vs. hearing individuals (44), and elevated peripheral V1 volume in those with macular degeneration (45). In the context of seizures, however, the explanation for increased volume and thickening is poorly developed, and further investigation is required. For SUDEP, elevated volumes in selected areas, e.g., the amygdala and subcallosal regions, raise concerns; if the increased volumes indeed reflect enhanced function, then the potential for those structures to induce apnea (amygdala) or hypotension (subcallosal region) may place the patient at risk.

Summary of Structural Imaging Findings

Evidence from morphometry and cortical thickness studies in SUDEP and at-risk groups (i.e., patients with GTCS) demonstrates reduced volume and cortical thinning in thalamic (primarily within posterior portions), frontal (medial and orbital cortex), and midbrain/cerebellar/brainstem sites (**Figure 1**). Increased volume and regional cortical thickness appear in limbic regions, primarily anterior mesial temporal, especially the amygdala, and cingulate structures, the insula, and sensory areas (**Figure 1**). Overall, the highlighted volumetric alterations indicate structural injury to key autonomic and respiratory control pathways, including cortical, sub-cortical, and caudal structures; therefore, a possible interpretation is that these abnormalities reflect a mechanism that increases the risk for dysfunction, particularly in circumstances under which autonomic and respiratory processes are challenged, such as during and after GTCS (5). However, a causal link between volume changes and autonomic and respiratory dysfunction is yet to be established in the SUDEP literature and further work is required to elucidate the relationship between volumetric changes and the extent of autonomic and respiratory dysfunction. Further studies which utilize segmentations of regional structures to validate differences in volume are required to overcome the constraints of the typically limited sample size of SUDEP studies.

EVIDENCE FROM RESTING-STATE FMRI

Resting-state (RS) fMRI is a brain imaging technique in which subjects undergo fMRI scanning while lying "at rest" in the sense that they are not subjected to any experimental stimulus or task; they are usually asked to lie quietly and stay awake, with their eyes closed. Although it has been argued that the "rest state" in question lacks specificity, this technique has the advantage of being applicable to a wide range of subjects, such as those incapable of performing a specific task [such as in comatose individuals, i.e., (46)] and thus has become an important tool in the study of the patterns of functional connectivity [FC; (47)]. FC describes the connectivity between spatially distant neurophysiological events which share functional properties (48, 49). FC is based on the temporal correlations of spontaneous (i.e., resting state) BOLD (blood oxygen level dependent) fMRI signal fluctuations between regions. From these measures, resting brain functional connectivity can be explored in multiple ways, the extent of which will not be covered in this short article [for a comprehensive review, see (50)]. In the following we review the main findings of this type of study in relation to SUDEP.

Altered Connectivity Between Central Autonomic and Respiratory Sites

To date, two studies using RS-fMRI have focused on the FC between brain regions related to central mediation of autonomic and respiratory processes in patients with epilepsy (a summary of results is illustrated in **Figure 2**). Tang et al. (51) compared FC between 13 brain structures (medulla, midbrain, pons, and bilateral amygdala, hypothalamus, thalamus, insula, and anterior cingulate) in relation to SUDEP risk in $n = 25$ patients. High-risk patients exhibited reduced FC between the pons and right thalamus, midbrain and right thalamus, bilateral anterior cingulate and right thalamus, and between the left and right thalamus. In another study Allen et al. (52) in $n =$

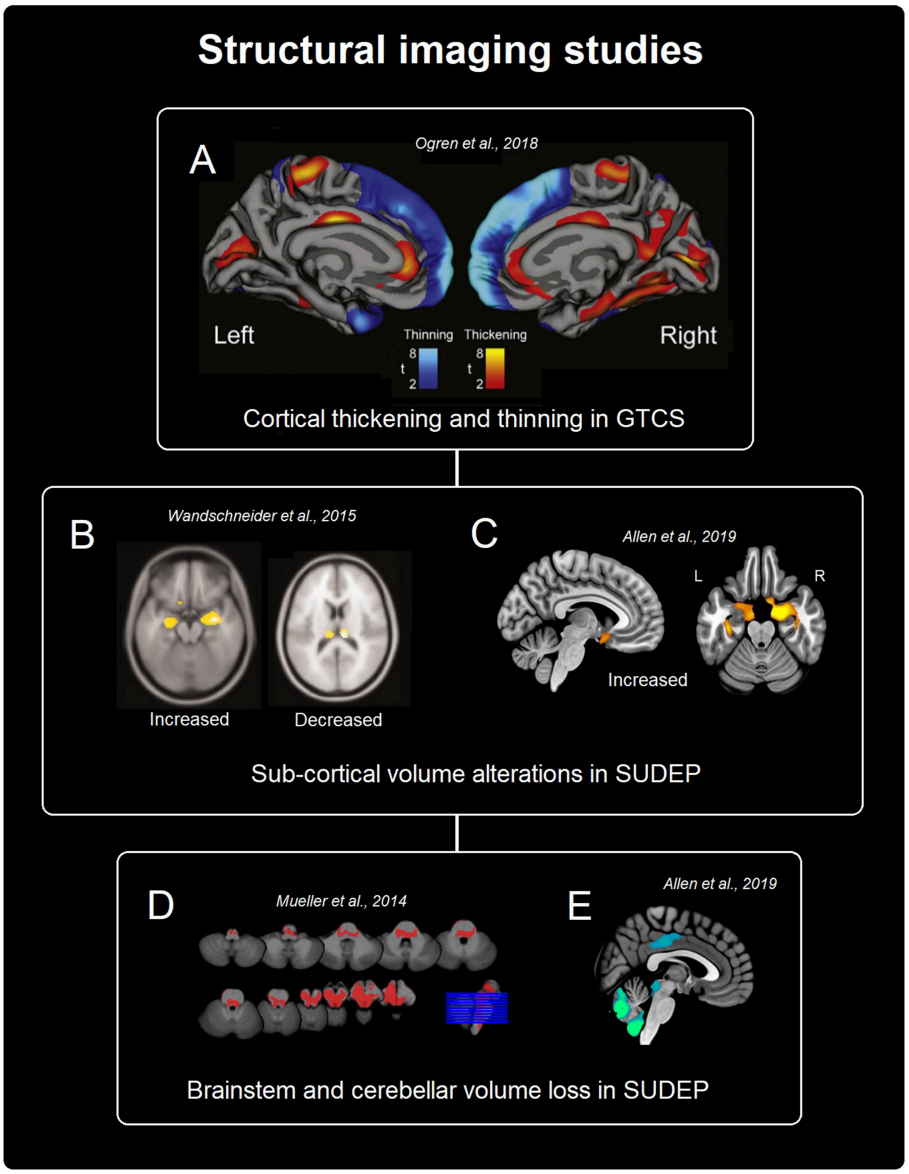

FIGURE 1 | Summary of structural findings from imaging studies in SUDEP and populations at high-risk of SUDEP. **(A)** Shows cortical thickness changes in patients with GTCS (32). **(B,C)** Show sub-cortical gray matter alterations in SUDEP [(20), **B** and (21), **C**]. **(D,E)** Depict brainstem and cerebellar volume loss related to SUDEP [(33), **D** and (21), **E**].

32 patients with TLE demonstrated reduced FC between the brainstem and thalamus, and thalamus and anterior cingulate, as well as reductions between the right anterior cingulate and bilateral putamen in high-risk subjects, relative to low-risk subjects. In addition, elevated FC was shown, primarily involving connections between the bilateral medial/orbital frontal cortices and bilateral mesial temporal structures (hippocampus and amygdala), as well as between bilateral medial/orbital frontal cortices and bilateral insula cortex.

Both of the above-discussed studies investigated patients at high and low risk for SUDEP, but no resting-state fMRI studies to date have included cases of actual SUDEP. Thus, a major limitation of both studies, is that the imaging correlates of risk factors associated with SUDEP are in fact reported, not necessarily the correlates of SUDEP itself. This issue remains a critical and inherent concern of all imaging studies into SUDEP, since cases of SUDEP are scarce, leading studies to rely on risk stratification of living subject datasets.

Despite their pitfalls, both experiments demonstrate altered networking among autonomic and breathing-related brain areas in those at high-risk for SUDEP. Larger studies, and investigations involving cases of SUDEP, may offer confirmation of disturbed connectivity and insights into the pathogenesis of SUDEP, which is still largely undefined. Overall, RS-fMRI has provided insights into connectivity changes in patients at high-risk of SUDEP which indicate altered communication among

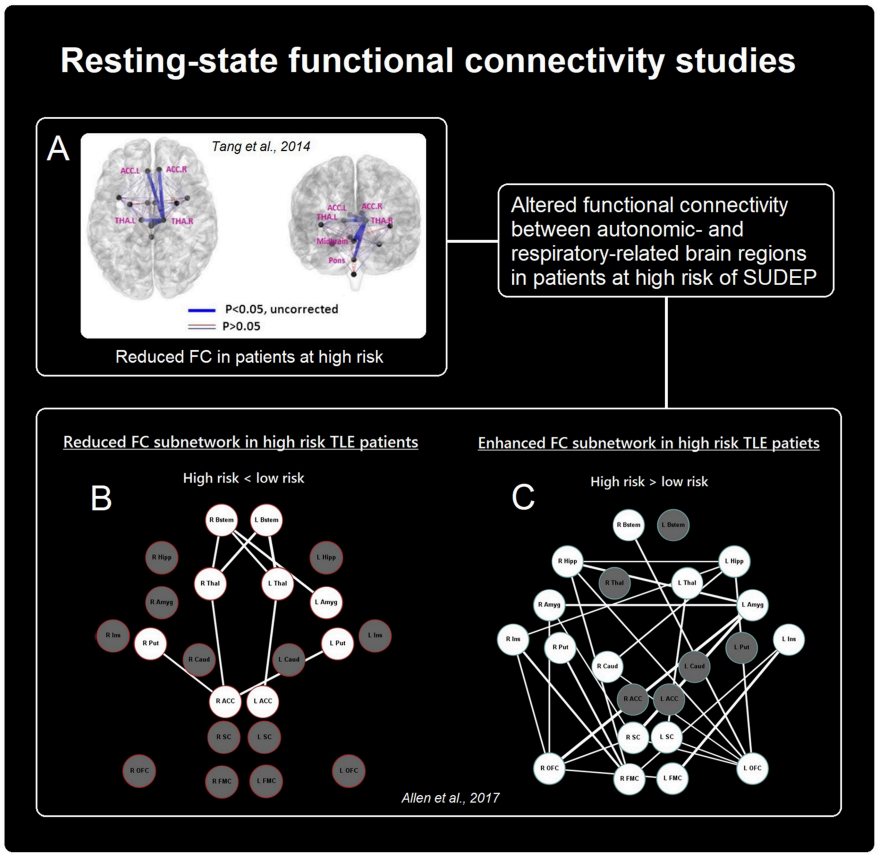

FIGURE 2 | Summary of rs-FC findings in patients at risk of SUDEP. Altered connectivity between cortical and sub-cortical autonomic- and breathing-related sites. **(A,B)** Show reduced functional connectivity in patients at high risk [Adapted from (51), **A** and (52), **B**], while **(C)** Shows increased connectivity between primarily frontal and limbic sites in those at high-risk [Adapted from (52)].

key brain regions contributing to autonomic and breathing regulatory processes. However, given the currently small body of literature, further work is required involving larger cohorts, healthy subjects, and victims of SUDEP to confirm initial work and characterize FC changes linked to SUDEP and other relevant clinical factors.

RELATIONSHIP BETWEEN STRUCTURAL CHANGES AND FUNCTIONAL CONNECTIVITY DISRUPTIONS

Some of the observed brain volume changes in SUDEP, and those at high risk for SUDEP, align with changes highlighted in the functional connectivity studies. For example, reduced volume within the thalamus observed in SUDEP and high-risk patients (20), as well as those with GTCS (22–24), appears to relate to the reduced connectivity of the thalamus (51, 52). Additionally, elevated volume and cortical thickening found in limbic structures such as the amygdala (20), bear resemblance to the increased FC of the bilateral mesial temporal structures in TLE patients at high risk (52).

Despite some homologous findings across structural and FC studies, further work is required for example to elucidate the link between volumetric changes and connectivity disruptions. In this respect, future studies should focus on combined volumetric and connectivity-based experiments [i.e., (22)] on the same cohorts of individuals and in larger datasets involving a diverse range of epilepsy sub-types.

FURTHER CONSIDERATIONS AND FUTURE DIRECTIONS

Relationships Between Regional Brain Volume and Clinical Epilepsy Variables

The volume of some brain structures has been found to correlate with clinical epilepsy-related variables, particularly in the thalamus. Disease duration, for example, correlates negatively with thalamic volume, as has been demonstrated extensively (22, 53–56), including gray matter within the pulvinar nuclei (20)—volume loss here is also associated with greater seizure frequency (57). Additionally, GTCS frequency correlates with cortical thickness of the cingulate and insula (32). However, both disease duration and seizure frequency are also major

SUDEP risk factors (58); thus, a central objective for the field lies within disentangling the effects of the former from what is believed to be sequalae of the fatal event—representing a major challenge, since it is likely that they both contribute to the underlying mechanisms of SUDEP. This issue brings to light an overarching concern for all studies into SUDEP, namely the problem of defining imaging correlates of such under defined pathology. Modeling and controlling for clinical factors (e.g., disease duration, medications, and seizure frequency) in relation to regional brain volume changes are important aspects of SUDEP research, and should be carried out when considering brain alterations, since the observed volumetric changes may be related to presence of GTCS or epilepsy duration. Long-term prospective studies are needed to investigate all contributory factors of volume loss and connectivity alterations, including sex-specific alterations, as highlighted previously in a cortical thickness study of patients with GTCS (32).

Future Studies

Given the relative rarity of SUDEP, multi-center collaborations, including such consortia as the Center for SUDEP Research (a center without walls initiative, funded by the National Institute of Neurological Disorders and Stroke), which bring together investigators from institutions across the US and UK and utilize open data sharing, will be crucial. Also, the integration of multi-modal imaging data, acquired prospectively seems essential for improved characterization of the relationship between structural and functional brain alterations: diffusion MRI, RS-fMRI, and T1- and T2-weighted MR images to investigate how volume changes and structural and functional connectivity alterations among regulatory structures arise and change in relation to clinical manifestations such as seizure frequency and disease duration. Additionally, the availability of ever larger retrospective datasets (including genetic data) for the wider research community would benefit efforts to better characterize SUDEP (including potential sub-types) and establish biomarkers using data-driven approaches.

Volumetric and morphological structural changes within the brainstem are a crucial aspect of research into SUDEP mechanisms, since the region contains many of the final common autonomic and respiratory pathways. Some anatomical properties of the brainstem and MR resolution limitations have restricted imaging studies to volumetric evaluation, either by gray and white matter segmentation, or amount of warping required to match a common template. Both techniques, which rely on T1-weighted contrast, may be insufficiently sensitive to detect underlying tissue changes within critical structures, particularly since many are small nuclei which lie on the border of white and gray matter. However, other newer procedures, such as quantitative MR T1/T2 ratio scans will enable assessment of myelin integrity, providing insights into allowing necessary evaluation of supportive tissue for neuronal processes in the brainstem and elsewhere.

Combined Resting-State and Autonomic fMRI Studies

The observed disruptions of resting-state patterns in SUDEP patients mandate the assessment of failed vital functions, namely studies which incorporate concurrent recordings of autonomic and breathing patterns during fMRI scanning, enabling characterization of associations between resting FC and resting cardiovascular and breathing processes. In addition, conventional correlations of "evoked" fMRI changes to breathing and cardiovascular changes to triggered challenges, e.g., CO_2 or hypoxia provocations, Valsalva maneuvers, cold pressor, or hand grip challenges may be useful to show magnitude of responses, timing delays or advancements between linked respiratory and cardiovascular areas. In other pathologic conditions, such as heart failure or congenital central hypoventilation syndrome, both distortions in timing and amplitude of linked structures appear (59, 60). Such "triggered" fMRI signal/physiological change correlations have the potential to show how dependencies between any given cortical or subcortical areas influence other areas; how time-delayed interactions can contribute to inappropriate timing of upper airway activation relative to diaphragmatic descent, leading to airway obstruction, or result in inappropriate or untimely compensatory blood pressure changes to challenges. Both scenarios can lead to physiologically-compromised circumstances, but the risk can be revealed by triggered fMRI studies.

Relevance to the Identification of Preventative Interventions

The observed alterations in FC between brain structures which have the potential to elicit a cardiovascular or breathing crisis leading to SUDEP raise the issue of how 1 day we might be able to intervene in those dysfunctional pathways to avoid or overcome such crises. Potential targets for intervention are the neurotransmitters in the affected pathways or the enhancement of pathways for protective recovery circuitry. In addition, advances in neuromodulation procedures offer a means to intervene directly in disrupted functional pathways, which is of particular use here, since 31% of epilepsy patients are drug resistant (61). Neuromodulatory techniques, such as invasive stimulation of the vagus, has been effective for the decrease of seizure incidence [for a review, see (62)]; Furthermore non-invasive vagal stimulation can both reduce seizure incidence, and modify breathing and cardiovascular patterns (63–68). Therefore, the combination of identifying disrupted cardiovascular/respiratory functional pathways, and implementation of inputs from cranial nerves that will influence those pathways through non-invasive or invasive neuromodulatory techniques have the potential impact disrupted vital functions that lead to the fatal scenario in SUDEP.

SUMMARY AND CONCLUSIONS

People who succumb to SUDEP, and those at risk, undergo regional brain structural changes and resting-state fMRI alterations between essential areas regulating cardiovascular and breathing control, indicating a structural and functional basis for impaired communication between areas necessary for recovery from compromised vital circumstances. The

findings, although limited in sample sizes, are sufficiently apparent that indications of structural and functional changes may signal risk for SUDEP and shed light on underlying mechanisms. Moreover, both the structural and functional outcomes suggest means for potential interventions with specialized pharmacologic or neuromodulatory procedures. The proper characterization of the respective roles of the known risk factors, such as GTCS and disease duration, in relation to imaging findings can contribute to understanding SUDEP mechanisms, and warrant further investigation to disentangle clinical factors from what may be related to SUDEP.

AUTHOR CONTRIBUTIONS

LA and RH designed and prepared the manuscript. SL, LL, and BD contributed to preparation, critical review, and editing of the manuscript.

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Has the Time Come to Stratify and Score SUDEP Risk to Inform People With Epilepsy of Their Changes in Safety?

Rohit Shankar[1,2], Craig Newman[3], Alistair Gales[4], Brendan N. McLean[5], Jane Hanna[6], Samantha Ashby[6], Matthew C. Walker[7] and Josemir W. Sander[7,8,9]*

[1] Cornwall Partnership NHS Foundation Trust, Truro, United Kingdom, [2] Exeter Medical School, Knowledge Spa, Royal Cornwall Hospital, Truro, United Kingdom, [3] Plymouth University Peninsula Schools of Medicine and Dentistry, Plymouth, United Kingdom, [4] Wairau Hospital, Blenheim, New Zealand, [5] Royal Cornwall Hospital, Truro, United Kingdom, [6] SUDEP Action, Wantage, United Kingdom, [7] NIHR University College London, Hospitals Biomedical Research Centre, UCL Institute of Neurology, London, United Kingdom, [8] Chalfont Centre for Epilepsy, Buckinghamshire, United Kingdom, [9] Stichting Epilepsie Instellingen Nederland (SEIN), Heemstede, Netherlands

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Rainer Surges, Uniklinik RWTH Aachen, Germany

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Adam Strzelczyk, Universitätsklinikum Frankfurt, Germany
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****Correspondence:***
Rohit Shankar
rohit.shankar@nhs.net

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Recent publication of the American Academy of Neurology SUDEP guidance highlighted the importance to American clinicians of making people with epilepsy aware of SUDEP risk. It is the first guideline to do this in the United States. It follows precedent set out in the UK by National Institute of Clinical Excellence in 2004. While a significant achievement, the lack of clarity of how to deliver this guidance in an enduring and person-centered manner, raises concerns on how its long-term effectiveness in risk mitigation. Shared decision-making with an emphasis on delivering person-centered communication to foster self-management strategies is increasingly recognized as the ideal model of patient–clinician communication in chronic diseases such as epilepsy. The tension between delivering evidence-based risk information, yet, tailoring it to the individual is complex. It needs to incorporate the potential for change not only in seizure factors but also other health and social factors. Safety advice needs to be dynamic and situation sensitive as opposed to a "one off" discussion. As a significant minority of people with epilepsy have drug-resistant seizures, the importance of keeping the advice contextual at different intervals of the person's life cannot be overstated as many of them are managed in primary care. We present some exploratory work, which identifies the need to improve communication at a primary care level and to review risks regularly. Regular reviews using a structured risk factor checklist as a screening tool could identify, sooner, people who's health issues are worsening and justify referrals to specialists.

Keywords: risk factors, communication, classify, risk assessment, SUDEP

Living is a risky business. Risk is the chance that an activity or action could lead to harm. Currently, there is no such option as zero risk. People will generally modify behavior and change lifestyle if they feel that there is a person-centered advantage or benefit. To bring about such a change, people need to know and comprehensively understand specific risks. Health risks are conveyed to individuals by clinicians in a myriad of ways of variable quality and effectiveness.

As there are no specific "rules," a host of clinician and individual factors play a role in person-centered communication (1). The lack of a clear structure to capture systemically this quality of communication could influence outcomes. The current level of evidence and the availability of a structure to deliver the information needed for individuals to be able to understand and be more aware of their distinct risk underpin the strength of individual risk avoidance.

New guidance from The American Academy of Neurology (AAN) recognizes the importance of communicating the risk of Sudden Unexpected Death in Epilepsy (SUDEP) to people with epilepsy (2). It is a welcome document, the first of its kind for American clinicians, which was critically developed and which provides clarity on the current level of evidence of the various risk factors highlighted to date in the literature. It also establishes the importance of a discussion of these risks with individuals mirroring the position taken by the UK's National Institute of Clinical Excellence (NICE) (3) guidance for epilepsy since 2004. While this is a major step forward, similar to NICE, the AAN guidance lacks elaboration on how to deliver person-centered risk assessment. It stops short of providing guidance or support to empower clinicians in having this critical conversation in clinic. Another concern with such guidance is that the discussion of SUDEP risk is expected to occur once at the time or near the time of diagnosis. There seems to be a lack of recognition of the importance to keep updating risk assessment and feedback based on the course an individual's epilepsy takes and how this may change. It has been shown in chronic conditions, epilepsy in particular that risk can change over time and is heavily influenced by varying life factors (4). People with epilepsy, often to their detriment, are rarely aware of changing risk. Thus, what can start as "low" risk can over time switch to a "higher" risk without the individual or the care giver or even the clinician being fully aware.

It is important that risk assessments in epilepsy are person-centered, contextual, and focused on the "here and now," and reviewed at regular intervals to ensure that there is a full picture of individual risk status. It is worth noting that most people with epilepsy, especially in countries where there are public health systems, as in the UK, are not usually supported by specialist epilepsy services but mainly by primary care where knowledge of up-to-date epilepsy and associated risk issues may be lacking and vary significantly. This can also be case when neurologists who are not epilepsy specialists (5) are involved.

The extent of the problem is highlighted by the fact that in 2013 in the UK around 1,200 people died due to epilepsy, which was roughly the same number who died from asthma in the same year (6). This is despite the number of people with asthma being 10 times larger (6). The data from National Statistics suggested that up to 60% of the epilepsy deaths could have been avoidable while only a quarter of asthma deaths were identified as preventible (6). This suggests that there is significant room for improvement in the way risk identification and management of people with epilepsy happen in the community.

In primary care (the General Practice system), epilepsy remains a common and regular presentation with Public Health expenditure in the UK for neurological conditions, second only to stroke (7, 8). Around 600 people with epilepsy in the UK die of SUDEP each year (7, 9). It is likely that these statistics underrepresent the true number of epilepsy deaths each year in the UK.

In 2004, an incentivizing scheme for the provision of quality care and to standardize improvement in the delivery of primary care was introduced in England called Quality Outcome Framework (QOF). The focus was to encourage primary care to manage common chronic conditions and enable the implementation of preventative strategies (10). Epilepsy had four QOF outcome indicators, one of which included an annual monitoring of people aged 18 and over on drug treatment. Epilepsy drug treatment monitoring was withdrawn from the scheme in 2014, raising concerns as there are now less opportunities to review individual risk changes as a result of the abandonment of the annual reviews. No clear evidence has, however, emerged as yet to the impact of this (11).

The SUDEP and Seizure Safety Checklist ("Checklist")[1] is a free, practical, evidence-based tool available in the UK for regular clinical use (12, 13). Its aim is to help person-centered communication to empower individuals and families with epilepsy to take shared responsibility with clinicians to make meaningful changes to improve their seizure risk outcomes. It also enables clinicians to identify change and compare with baseline in a structured manner. Description of the Checklist is provided in Appendix 1.

The Checklist has 19 modifiable and non-modifiable factors, providing an outline for clinician's discussions with individuals, which can be repeated annually or when a person with unstable epilepsy is reviewed urgently or routinely. Clinicians in secondary care have found the tool practical and time efficient (10 min), but it has not yet been systematically used in primary care though anecdotal feedback is that it is used significantly by clinicians working in primary care or out in the community.

The concept has been tested as a telehealth project for a year in a single site large primary care practice in mid-Cornwall having 16,000 people registered to it to risk assess "high risk" individuals with epilepsy in the catchment (14). "High risk" was defined as over 10 years of treatment-resistant seizures but "stable" in the community. The telehealth team called on a three monthly basis and ran the Checklist with the registered users. All results were communicated back to the GP. Of the 46 people with epilepsy in the practice who received the telehealth screening, 17 were referred for several interventions during the year that would not have happened without the on-going screening. However, a problem of this study was that it identified "high risk" based on a single factor of 10 years treatment resistance and not on a holistic risk issue. Thus, another study was set up to look at all registered people with epilepsy in a different primary care practice.

The setting was a medium sized primary care facility covering a mixed urban and large rural area in SW England covering around 12,000 people. A database identifying all people with

[1]https://www.sudep.org/checklist (Accessed: October 28, 2017).

epilepsy in the practice with a baseline risk score was proposed using the Checklist factors. The purpose was to allow for rapid revaluation of risk status during an annual review or consultation to help identify seizure risk change using the Checklist to help recognize, categorize, and stratify seizure risk. Person-centered advice could then be provided based on the changed findings. Using the facilities, standard digital clinical management system, EMIS Web (EmisHealth, Leeds, UK), a search was created to identify anyone with epilepsy, epileptiform conditions, or seizures. Each risk factor of the Checklist was then identified and relating codes were then automatically searched in the database. This was done for each individual, thus allowing individual risk score analysis as well as analysis of the population.

A total of 107 target individuals were identified. All 19 risk factors were applied. The mean score was 4.1. The range was from 1 to 9 though only two people scored 9 and three had scores of 8. There were no data coded or any documented evidence for four of the risk factors (nocturnal surveillance, pregnancy, prone sleeping position, and nocturnal seizure presence) in any individual.

Undertaking this evaluation resulted in each of the individuals at the primary care facility undergoing a baseline screen and s risk factors scoring. It has also identified common risk factors and individual modifiable factors. The perceived lack of awareness for risk among primary care clinicians and the lack of clinical codes to identify the presence of major established risk factors is of concern. Given the overall high standards followed in this facility, it could be expected that similar shortcomings would be seen in other practices leading to concerns of the gap in knowledge and awareness.

These data were presented to the facility clinical practitioners (physicians and nurses) and the realization that some risk factors may have been missed in certain individuals' stimulated discussion. This was highlighted by the discussion around the risk factor of nocturnal seizures where no coding data were present. Facility practitioners acknowledged that it was not a practice to ask of nocturnal events during reviews. As this is a modifiable high risk factor, this warrants full incorporation into records as it may help reduce risk in certain patient-groups. Indeed, as a result of these findings, the annual epilepsy review structure of the facility was changed to include the Checklist risk factors.

The strength of the Checklist was its ability to motivate risk considerations of SUDEP with people with epilepsy and their families. All 107 identified when reviewed and given feedback felt the conversation was useful again confirming the importance of person-centered discussion of risk. It showcased the importance of sharing risk knowledge in giving ownership to people with epilepsy and their carers. This supports findings of another recent study where the structured use of the Checklist in specialist epilepsy clinics led to the reduction of risk scores. It has helped possibly reduce the burden of SUDEP in the long term. It should be stressed that for most risk factors, it is not clear if a modification of these factors changes the actual SUDEP risk and this is an area which needs further exploring (2).

This exercise has also allowed positive discussions and learning among physicians, nurses, and individuals within the facility about SUDEP and risk factors and has acted as a catalyst to hopefully improve care, monitoring and outcomes in the longer term. It also highlights the value of risk identification and coding in epilepsy community care, through the use of the clinical Checklist. The value of education and empowerment is intuitive in all areas of clinical risk reduction and is particularly relevant in epilepsy.

This intervention was well received and is easily translatable to most primary care settings, so the following recommendations would be reasonable:

1. People with epilepsy should have an annual seizure safety risk assessment at primary care.
2. An earlier interim assessment needs to be triggered if any person with epilepsy presents with: decline in seizure control, alteration to AEDs or relevant medications, change in comorbidities in particular use of alcohol or other substances or with psychiatric issues.

It is appreciated that the postulated move to stratify risk with the current level of evidence might be a controversial one. It could be argued that such an attempt could confound and cause fright. An example was the 1995 "pill scare" when third generation Oral contraceptive pills were proposed to double venous thromboembolism risk compared with older alternatives; however, this was later established not to be the case (15). In the interim, there was a noted increase in unintended pregnancies and increased rates of abortions as a result of the "pill scare." This example highlights an extreme situation especially where new data or research have been used. For chronic conditions, given the diversity and cumulative effects of numerous factors over a lifetime, a perfect risk assessment is unlikely to be delivered. In the case of epilepsy, people will still continue to prematurely die if steps are not put in place to reduce their risks. Those bereaved by the condition and those who have supported interventions are united in their determination to stop any unnecessarily deaths. In such situations, evidence-based pragmatism with a focus on improving individual wellbeing and safety is the way forward. Risk stratification, while not ideal in current day practice, could be the lowest common acceptable denominator to structure current evidence into small bite-size packages of information to improve knowledge of clinicians, measure and map risk, and empower individuals as part of a holistic approach to epilepsy risk management as highlighted by this small study.

It is important to note that creating such baseline scores and risk stratification for known risk factors is still only a first step in improving awareness among clinicians and people in such settings. Enabling them to then have risk discussions using tools such as the Checklist, and work together to reduce these risks where possible must follow to help tackle premature mortality. There have been other attempts to provide collective risk factors toward SUDEP most notably the SUDEP-7 Inventory (16). The SUDEP-7 Inventory similar to the Checklist has undergone a range of testing. Commonalities with the Checklist include the use of similar background literature to evidence the risk factors (17). The differences are in the focus of the inventory,

target group, and its purpose. The SUDEP-7 risk factor items are primarily concentrated on seizures and are correlated to electro-physiological variations (18). Its principal role is to provide a screening inventory focused on biomarkers (18). This is different from the ambitions of the Checklist, which looks to communicate person-centered risk to individuals with a view to empowering them to make necessary adaptations in their day-to-day life (19). As the two tools work differently, there could be a case made to use them symbiotically. This concept would require further testing.

The manner of discussing SUDEP is not without challenges. These challenges are diverse and include personal, professional, cultural, institutional, and resource issues (20, 21). Personal and professional beliefs of whether it makes a difference to discuss are still an ongoing debate (20, 21). Cultural attitudes may also play a role (21). Availability of trained physicians itself is a concern in many parts of the world. Where there are services available, often there is no time or space to have such sensitive conversations as about SUDEP. Thus, while SUDEP discussion and continued risk mitigation may be a "step too far" in many areas, it would still be the practice to aspire to.

ETHICS STATEMENT

We confirm that we have read the journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. No ethical approval was needed as this was a service improvement project.

AUTHOR CONTRIBUTIONS

All authors contributed to the concept, development of the paper, editing, and finalizing of it. RS wrote the initial draft with CN. AG collected data. BM, LS, and MW gave supervision and oversight.

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Conflict of Interest Statement: RS, JH, and SA are the main stakeholders of the "SUDEP and Seizure Safety Checklist." RS, JH, SA, BM, and CN are developers and key stakeholders of EpSMon. MW and JS are members of the SUDEP and Seizure Safety Checklist review panel. JS has received departmental research support from GSK, Eisai, and UCB Pharma and has been consulted by and received fees for lectures from Bial, Eisai, and UCB Pharma outside the submitted work. MW has received departmental research support from Vitaflo and has been consulted by and received fees for lectures from GSK, Pfizer, Eisai, and UCB outside the submitted work. RS has received institutional and research support and personal fees from LivaNova, UCB, Eisai, Special Products, Bial, and Desitin outside the submitted work. BM has received research support and personal fees from Eisai, UCB, and Desitin outside the submitted work.

APPENDIX 1

The SUDEP and Seizure Safety Checklist ("Checklist") and the EpSMon (22, 23) (As Described by SUDEP Action & Plymouth University)

The Checklist is a free, award-winning risk assessment tool for clinicians, which encompasses known modifiable and non-modifiable risk factors of SUDEP and associated concerns with a view to:

- Assist clinicians to open a positive discussion with people about epilepsy and risk assessment;
- Support a person-centered discussion of risk, focusing on whether known risk factors apply to a particular individual;
- Help clinicians educate people with epilepsy about their personal risk and possible lifestyle changes, which might reduce those risks;
- Promote the safety goal by identifying modifiable risk factors which may guide management;
- Create documentary evidence for clinicians on the impact of the treatment plan over time and demonstrate effective clinical governance while enhancing individual safety;
- Provide some assurance to bereaved families that every effort was made to reduce risk and prevent a fatality when a death occurs.

An example on how to administer is provided[1]; clinicians can also register for the tool, or find out more *via* www.sudep.org/checklist. The Checklist is managed by SUDEP Action (Secretariat and PPI Leads) and Cornwall Partnership NHS Foundation Trust (Clinical Leads).

The Checklist also underpins the content of a mobile app for people with epilepsy, EpSMon, which has been recognized as one of eight innovations selected for the current NHS Innovation Accelerator Programme.[2] The App brings lifesaving information to the fingertips of adults with epilepsy, enabling them to monitor their own seizures and well-being between medical appointments. EpSMon also shows whether risk factors have improved or worsened enabling people with epilepsy to seek medical help sooner if required. It is free to download for iPhone and Android devices in the UK; further information can be found at www.epsmon.com. EpSMon is a partnership between Cornwall Partnership NHS Foundation Trust, SUDEP Action, Plymouth University, and Royal Cornwall Hospitals Trust. The Checklist and EpSMon are also part of the UK Epilepsy Commissioning Toolkit.[3]

The introduction of an app delivering education and risk assessment is innovative in respect to current practice, but easily perceived as an efficient route to providing best practice. The app's ability to prompt timely assessments, assess current understanding, track engagement, deliver bespoke education, and recommend clinical support could be invaluable to the care for epilepsy in the community. Future identified developments will include medication adherence tools, the development of manualized approaches for services to implement the Checklist and EpSMon app alongside each other and will look to explore the potential of automatic flagging of at risk individuals to health teams. Use of it to reduce potential harm has been strongly supported by a recent Cochrane review on SUDEP prevention (24) and a National Institute of Health Research UK Systematic appraisal of emerging technologies for the diagnosis, treatment, and management of epilepsy (25).

[1] https://www.youtube.com/watch?v=Z9KHQvsapAc.

[2] https://www.youtube.com/watch?v=e3mECsSVgHI.

[3] http://www.epilepsytoolkit.org.uk/.

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