Decreased heart rate and enhanced sinus arrhythmia during interictal sleep demonstrate autonomic imbalance in generalized epilepsy

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ABSTRACT

We hypothesized that epilepsy affects the activity of the autonomic nervous system even in the absence of seizures, which should manifest as differences in heart rate variability (HRV) and cardiac cycle. To test this hypothesis, we investigated electrocardiogram (ECG) traces of 91 children and adolescents with generalized epilepsy and 25 neurologically normal controls during 30 minutes of stage 2 sleep with interictal or normal EEG. Mean heart rate (HR) and high-frequency HRV corresponding to respiratory sinus arrhythmia (RSA) were quantified and compared. Blood pressures (BP) from physical exams of all subjects were also collected and analyzed. RSA was on average significantly stronger in patients with epilepsy, while their mean HR was significantly lower after adjusting for age, body mass index, and gender, consistent with increased parasympathetic tone in these patients. In contrast, diastolic (and systolic) blood pressure at rest was not significantly different, indicating that the sympathetic tone is similar. Remarkably, five additional subjects initially diagnosed as neurologically normal, but with enhanced RSA and lower HR, eventually developed epilepsy, suggesting that increased parasympathetic tone precedes the onset of epilepsy in children. ECG waveforms in epilepsy also displayed significantly longer TP intervals (ventricular diastole) relative to the RR interval. The relative TP interval correlated positively with RSA and negatively with HR, suggesting that these parameters are linked through a common mechanism, which we discuss. Altogether, our results provide evidence for imbalanced autonomic function in generalized epilepsy, which may be a key contributing factor to sudden unexpected death in epilepsy (SUDEP).

Key words: SUDEP, heart rate variability, cardiac cycle, parasympathetic tone, children.
INTRODUCTION

Epileptic seizures are known to have profound effects on autonomic function (Wyllie 2011). To quantify these effects, many studies have focused on heart rate (Behbahani et al. 2015; Jeppesen et al. 2015; Varon et al. 2015a; Varon et al. 2015b; Zijlmans et al. 2002); cardiac abnormalities (Nei 2009; Smith and Delisle 2015; Varon et al. 2015a); respiration (Bulow and Ingvar 1963; Nashef et al. 1996); arterial blood pressure (Oztas and Turkel 2001); and oxygen saturation (Blum et al. 2000; Szurhaj et al. 2015), all during ictal periods. While these and other numerous studies have helped us understand autonomic imbalance associated with seizures, less is understood about autonomic function during the considerably longer interictal periods, especially during sleep (Varon et al. 2015b). Closing this critical gap in understanding has been suggested by several researchers (Kothare and Singh 2014) as essential to obtaining a complete picture of autonomic regulation in epilepsy. This in turn would: 1) better inform us on the physiological mechanisms of sudden unexpected death in epilepsy, SUDEP (Bozorgi and Lhatoo 2013; Lhatoo et al. 2015; Moghimi and Lhatoo 2013), and 2) improve the efficacy of risk factors used to provide clinical prognoses for both epilepsy and SUDEP (Lhatoo et al. 2015; Varon et al. 2015b).

Furthermore, although clinical observations have shown that parasympathetic activity is often decreased during ictal periods in refractory or medication-resistant epilepsy (Kolsal et al. 2014), there is conflicting evidence regarding parasympathetic tone in interictal periods. For instance, studies on patients with Rolandic epilepsy have shown that vagal tone is enhanced as compared to neurologically normal controls (Seri et al. 2012), while other studies have shown evidence of sympathetic overdrive and parasympathetic depression (Behbahani et al. 2015; Raju et al. 2012). In light of these opposing findings, several scientists have suggested that a more systematic investigation of sympatho-parasympathetic balance in the absence of seizures is necessary to develop current approaches to treating epilepsy and preventing SUDEP (Sarkis et al. 2015).

A recent longitudinal study, which followed children with epilepsy undergoing pharmacologic treatment over several years, concluded that “SUDEP is not a rare event in children” (Terra et al. 2009). As 1.4% of the subjects succumbed to SUDEP during the study, the authors noted that premature mortality rates associated with SUDEP could diminish if more pediatric research were conducted. Researchers investigating both focal and generalized epilepsy
have commented that data on children and young adults are scarce (El-Sayed et al. 2007; Seri et al. 2012), even though SUDEP makes up 14% of all reported cases of sudden unexpected death in children (Hesdorffer et al. 2015). Similarly, a Canadian pediatric surveillance study of children with epilepsy concluded that “risk factors for SUDEP in children are not well established,” and, citing the paucity of current research, called for more pediatric studies (Donner 2014).

“Most cases of SUDEP...occur while people are in bed, presumably sleeping” (Mostacci et al. 2015). In a clinical study, 58% of all reported SUDEP cases were found to have occurred during sleep; of these, 86% were unwitnessed but confirmed through autopsies (Lamberts et al. 2012). Systematic explorations of the linkages between sleep and SUDEP over the years have yielded interesting results: significant findings suggest that SUDEP most commonly occurs during the non-rapid eye movement (NREM) portion of sleep (Herman et al. 2001) and that epileptiform activity prone to triggering SUDEP is prevalent in shallower sleep stages (e.g. stage 2) or the transition to waking (Lamberts et al. 2012; Menezes Cordeiro et al. 2015). It has also been observed that when stage 2 sleep precedes REM sleep or wakefulness, it is accompanied by an activation of the adrenocorticotropic system that enhances sympathetic function; however, when stage 2 is followed by slow wave sleep (stage 3), sympathetic tone decreases in parallel with an activation of the renin-angiotensin system (Brandenberger et al. 2005). Because of this autonomic-endocrine duality, stage 2 sleep is of particular interest in the context of autonomic function in epilepsy, especially with regard to SUDEP. In addition, sleep is a consistent resting state and a good regime for study and comparison of autonomic function (Kanda et al. 2015).

Investigation of autonomic balance has often been done through the use of heart rate variability (HRV), via a set of well-validated, non-invasive measures of baroreflex sensitivity and vagal tone (Shaffer et al. 2014; Tobaldini et al. 2013). In practice, the low-frequency/high-frequency (LF/HF) spectral power ratio of HRV, a relative measure of the amplitude of low-frequency (LF; 0.04-0.15 Hz) and high-frequency (HF; 0.15-0.4 Hz) modulations of heart rate (HR), is routinely used in clinical contexts to estimate sympatho-parasympathetic balance. These LF and HF ranges have been previously used to examine HRV in children (Akinci et al. 1993; Blood et al. 2015). However, recent studies have cast doubt on the accuracy of the LF component of HRV in assessing sympathetic tone (Goldstein et al. 2011), and consequently the accuracy of the LF/HF ratio in measuring autonomic balance (Billman 2013). In this study, we
will instead consider the non-normalized HF peak in the power spectral density of HR, widely regarded as the vagal effect of respiratory sinus arrhythmia, RSA (Shaffer et al. 2014; Yasuma and Hayano 2004). Use of the raw power of RSA affords inter-individual comparisons of vagal activity in the natural, absolute units of power density (Indic et al. 2008).

Vagal tone has also been effectively characterized using time-domain measures of HRV, including mean HR (Meghana et al. 2015) and the standard deviation of normal beat-to-beat (i.e., R-to-R, or RR) intervals, SDNN (Nayak et al. 2015). Decreased HR is often interpreted as sympathetic depression (Abukonna et al. 2013), but it may also be due to increased parasympathetic activity, and is closely linked with arterial blood pressure (henceforth referred to as BP) and baroreflex sensitivity (Taylor et al. 2015). Acute elevation of BP (unlike the chronic condition of hypertension) is considered to be primarily mediated by noradrenergic ganglionic pathways in the sympathetic nervous system (Guyenet 2006), and diastolic resting BP is a good measure of barosensitive sympathetic regulation (Joyner et al. 2010). In children, HRV-related parameters have been shown to correlate with age: specifically, systolic and diastolic BP increase with age in young children (Riley and Bluhm 2012), while resting HR decreases quickly after infancy and continues to decline steadily through adolescence (Fleming et al. 2011). Pediatric analyses of autonomic function using these measures should account for age dependence, and assess HR and BP’s contributions to sympathetic tone independently, due to interdependent regulation involving the arterial baroreflex (Swenne 2013).

To address the mentioned gaps in understanding of sympatho-parasympathetic balance in children with epilepsy and at risk of SUDEP, we analyzed and compared HRV and ECG waveforms as non-invasive parameters of the autonomic tone from patients with generalized pediatric epilepsy and neurologically normal controls during interictal and normal EEG periods in stage 2 sleep.

METHODS

Subject Cohort

Data collection from the EEG/ECG database of The Pediatric Epilepsy Unit in the Pediatric Neurology Department at University Hospitals in Cleveland, Ohio, was approved by the Institutional Review Board of University Hospitals and Case Western Reserve University. Subjects were selected based on availability of sleep ECG and EEG data, as well as diagnosis of
epilepsy (either generalized or no epilepsy diagnosis) from all subjects who had an overnight EEG conducted between January 1st, 2008 and November 1st, 2014 at the unit. Records were reviewed retrospectively. A total of 151 subjects were initially selected, 106 with generalized epilepsy and 45 neurologically normal (control group), with ages ranging from neonates to 22 years of age. Monitoring reports from those subjects included medical history, demographic information, and results from routine clinical tests performed on children in the Epilepsy Unit. Gender, height, weight, date of birth, date of physical exam and EEG/ECG evaluation, as well as resting systolic and diastolic blood pressures were obtained from these reports. The age of each subject was determined as the difference between the date of data acquisition and the date of birth.

Epochs of ECG and EEG data from overnight EEG sleep studies at University Hospitals were obtained for each subject. These clips contained 30 uninterrupted minutes of stage 2 sleep, as determined by a hospital technician, who also verified along with a pediatric neurologist that the EEG activity recorded was normal (or interictal, in epilepsy). All subjects with epilepsy who were selected had generalized epilepsy, with the majority of seizures being generalized tonic-clonic seizures. Other types of seizures included absence seizures, myoclonic seizures, and spasms. Subjects were grouped according to final diagnosis at the time of discharge.

Subjects from the control group were children who had visited the Epilepsy Unit due to sleeping problems, staring spells, recent head trauma, and/or who had family members (particularly siblings or parents) with epilepsy that indicated a potential inherited risk for developing epilepsy. It is common practice at the Epilepsy Unit that children coming in for a sleep evaluation also receive a routine EEG to confirm healthy brain activity. Subjects in the control group were advised to be monitored for a sleep study to rule out epilepsy as a diagnosis, and were diagnosed by a pediatric neurologist specialized in epilepsy not to have epilepsy according to their overnight EEG examination.

All patients already diagnosed with epilepsy before the EEG/ECG evaluation were on a version of the ketogenic diet and had been taking anti-epileptic medications. For the overnight EEG/ECG evaluation itself, patients were asked not to take their medications during this period to minimize their effect on the recorded signals. Commonly taken anti-epileptic medications included enhancers of GABAergic signaling (clonazepam, tiagabine), modulators of sodium and calcium channels (carbamazepine, lamotrigine, zonisamide), antagonists of glutamate receptors...
(clobazam, phenobarbital), and medications that decrease blood pH (carbonic anhydrase inhibitors such as topiramate); more frequently, patients took combinations of these medications. Specific details regarding dosages (amount, frequency, timing, etc.) were not available to the data collection.

**Exclusion Criteria and Group Characteristics**

Subjects with unusable ECG data due to movement artifacts, interruptions, or technical issues were obviously excluded from the analyses. Subjects with monitoring reports but without corresponding EEG or ECG recordings on or immediately after the date of admission into the Pediatric Epileptic Unit were also excluded from the study. Subjects with multiple dates of admission and multiple sleep evaluations were identified, and only the most recently collected 30-minute clip from each of these patients was selected for analysis. Neither the nature of anti-epileptic medications, nor their possible combination or dose, were considered as exclusion criteria.

While finding subjects based on our selection criteria, we discovered five (5) subjects who were initially diagnosed as neurologically normal, but developed epilepsy after the first sleep study. Of these five subjects, two (2) had not been taking any medications regularly by the time of the overnight sleep study, and the other three were taking a sleep aid (melatonin), medication for attention deficit disorder (amphetamine), asthma medications (albuterol, montekulast, budesonide), and/or anxiety medications (risperidone, sertraline), but no anti-epileptic medications. They also had not been taking any of these medications at the time of EEG/ECG recording. None of these five subjects had reported experiencing any kind of seizure before the time of data acquisition, nor had they been previously diagnosed with epilepsy; additionally, the EEG data collected during the sleep study were deemed to be normal by a pediatric neurologist. However, hospital records more recent than the time frame of this retrospective study confirm that these subjects later developed and were diagnosed with epilepsy. We also observed that seven (7) neurologically normal subjects according to the EEG evaluation were taking anti-epileptic medication at the time of data acquisition as a preventive measure in order to treat psychogenic, non-epileptic seizures.

Both the group of five subjects who later developed epilepsy and the group of seven non-epileptic patients taking anti-epileptic medication were excluded from the main cohort, but were
analyzed separately to determine the potential of RSA and HR as prognostic biomarkers for epilepsy (see Results). The demographic composition of these two groups did not appear to be essentially different from the rest of the cohort. Importantly, no subjects from any group (including those listed above, controls, and patients with epilepsy) were taking medications of any kind while they were monitored for the sleep study and EEG/ECG data acquisition.

After applying all of the exclusionary criteria, 25 control subjects and 91 patients with generalized epilepsy remained in the main cohort for analysis. Subjects in the control group had a mean age of 7.5 years, with a standard deviation of 6.4 years, while patients with epilepsy had a mean age of 10.5 years, with a standard deviation of 5.0 years. Of the control subjects, 5 (20%) were male and 20 (80%) were female; while 64 patients (70%) were male and 27 (30%) were female in the epilepsy group. To minimize the effect of these asymmetric proportions on our results, we adjusted all measured parameters of interest for age, body mass index (BMI), and gender (see below).

Analysis of Heart Rate Variability

ECG data were imported into MATLAB (Mathworks, version 2015a) for analysis with custom written software (developed by SSS and RFG). ECG spike detection (R wave) was performed on ECG traces (with sampling frequency 200 Hz) and their time derivatives with a double threshold set at the 98th percentile of the mean-subtracted, rectified ECG and 98th percentile of the rectified, time derivative of the ECG, over a sliding time window of 10 seconds. Each signal fluctuation exceeding both thresholds within a margin of 0.2 seconds was registered as a single beat, marked at the maximum point of the ECG trace within that margin. Histograms of the inter-beat interval distribution were manually inspected for high variability and outliers due to missed or incorrectly detected beats. Errors in the automatic beat detection algorithm (less than 5%) were either due to sharp P or T waves that were erroneously identified as beats, or to blunt R waves that were not detected. These errors were manually corrected and blinded to the subject’s identity and diagnosis.

HR series were calculated as the reciprocal of each inter-beat interval and plotted against the time point of the initial beat in each interval. Cubic-spline interpolation was then employed to generate the instantaneous HR at regular time intervals with a sampling rate of 200 Hz over the
duration of the 30-minute recording. For each subject, the mean HR was calculated as the time
average of the instantaneous HR.

A zero-phase, digital, high-pass filter with a cutoff frequency of 0.1 Hz was applied to the
instantaneous HR in order to eliminate low-frequency variability uniformly across subjects.
MATLAB’s implementation of Thomson’s multitaper power spectral density (PSD) estimate
(Thomson 1982), was then used to obtain an estimate of the PSD of the HR for each subject. The
height of the peak in the PSD and its location were recorded for each subject, which correspond
to the power density and frequency of the respiratory sinus arrhythmia.

The power density unit of a signal measured in units of U is U^2/Hz; thus, the
instantaneous HR, measured in Hz, has power density measured in units of Hz^2/Hz = Hz, and
therefore both power density and frequency of RSA were measured in units of Hz. When
assessing correlations between power density of RSA and other parameters, power density was
instead expressed in units of decibels (dB) via the formula $\text{RSA}_{\text{db}} = 10 \cdot \log_{10} \left( \text{RSA}_{\text{Hz}} / 1 \text{ Hz} \right)$.

**ECG Waveform Analysis**

A cycle-triggered average ECG was computed for each subject as follows: the mean-subtracted
ECG waveform during each beat-to-beat interval was mapped onto a fixed interval of 360
linearly spaced samples, such that one sample corresponds to one degree in the cardiac cycle. We
then averaged these cycles degree by degree to obtain the average ECG waveform over the RR
interval.

**Adjustment of Measured Parameters for Age, Body Mass Index, and Gender**

When comparing parameters of HRV and ECG waveforms between groups, we investigated the
covariation of these parameters with age, BMI, and gender, with the intent to adjust for these
correlations if present. BMI was computed using the formula mass/height^2, with mass measured
in units of kg and height measured in units of m; height (in cm) and weight (in kg) measurements
were taken during the clinical check-up at the time of ECG data acquisition. Gender was coded
as a binary variable, with 0 representing male and 1 representing female.

A linear model of the form $y \sim \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3$, was then computed for each
parameter of interest, e.g. HR ($y$), as a function of age ($x_1$), BMI ($x_2$), and gender ($x_3$), using
MATLAB’s `fitlm` function. The validity of the model was assessed with three sequential tests whose outputs are reported in Tables 1-4: 1) An F-test for the null hypothesis that the regression coefficients $\beta_1, \beta_2, \beta_3$ are all equal to zero, or equivalently, that the model is constant, i.e. $y \sim \beta_0$. In Tables 1-4, we report the F-statistic, its critical value for significance, $F^*$, and its $p$-value. If $F < F^*$, parameter $y$ does not significantly covary with age, BMI, or gender, and does not require further adjustment. 2) If $F \geq F^*$, a $t$-test for each individual regression coefficient determines if the covariation of its associated variable with the parameter of interest, $y$, is significant. 3) The residuals of the model are then computed as the differences $y - (\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3)$ across subjects, and represent the adjustment of parameter $y$ for age, BMI and gender. If the adjustment is meaningful, the residuals must be normally distributed; otherwise the model does not capture relevant covariations in the dataset. We tested the null hypothesis of normality of the residuals with the Lilliefors test (Lilliefors 1967). In Tables 1-4, we provide the $D$-statistic of this test, its threshold value to reject the null hypothesis, $D^*$, and its $p$-value. The null hypothesis was not rejected for any of the parameters that required adjustment.

**Statistical Tests for Group Comparisons and Correlation Coefficients**

Statistical analyses for HRV and waveform measures between groups were conducted using MATLAB’s implementation of the Wilcoxon rank-sum test; a nonparametric test that compares the medians of two one-dimensional distributions through a ranking process (Wilcoxon 1950). Correlation coefficients, $r$, were computed according to Pearson’s formula (Press et al. 1992). The value of $r$ was then transformed as $t = r \sqrt{(N - 2) / (1 - r^2)}$ and compared to Student’s $t$-distribution with $N - 2$ degrees of freedom, where $N$ is the number of subjects, to determine the $p$-value (Press et al. 1992).

In the figures and tables, lack of statistical significance is indicated with “n.s.” (not significant), whereas statistical significance is indicated with a single asterisk for $p$-values between 0.05 and 0.01 (significant) and with two asterisks for $p$-values below 0.01 (highly significant).

**Sensitivity, Specificity, Accuracy and ROC**
Sensitivity (SE) and specificity (SP) measure the performance of a binary classification test (i.e. does the subject have epilepsy or not?). Sensitivity is defined as the proportion of positive cases that are correctly classified as such (i.e., fraction of subjects with epilepsy that are classified as epileptic). Similarly, specificity is defined as the proportion of negative cases that are correctly classified as such (i.e., fraction of subjects who are not epileptic and are classified as controls). Accuracy (AC) is a “compromise” between SE and SP, defined as the ratio of correctly labeled cases out of the total number of cases. The receiver-operating characteristic (ROC) was obtained by plotting the SE and SP at increasing values of the parameter for binary classification (Florkowski 2008). The optimal discriminating threshold was determined as the value of the parameter that maximized the sum of SE and SP.

RESULTS
Heart Rate Variability in Epilepsy
Clear differences in both mean HR and HRV are readily observed in patients with epilepsy as compared to control subjects. Figures 1A and 1B show two representative ECG traces during sleep in a control subject and a patient with epilepsy, respectively. It is apparent that the mean HR is lower in the patient with epilepsy, as evidenced by the increased beat-to-beat (i.e. RR) intervals. In addition, the instantaneous HR displays a stronger oscillation, corresponding to respiratory sinus arrhythmia (RSA) over the short 30-second window depicted (Fig. 1, C and D). The amplitude of the oscillation is quantified by the high-frequency peak in the power spectrum of instantaneous HR computed over the 30-minute epoch; the amplitude is much higher in the subject with epilepsy (Fig. 1, E and F). The combination of lower HR and stronger RSA points to an enhancement of parasympathetic activity in epilepsy during interictal periods in stage 2 sleep.

These observations are consistent across subjects. Figures 2A and 2B respectively demonstrate that the median power of RSA is increased by 97% in epilepsy (medians: 0.028 Hz for control, 0.055 Hz for epilepsy; $p = 3e-4$, Wilcoxon rank-sum test), whereas the frequency of RSA, a measure of the respiratory frequency, is not significantly different between groups (medians: 0.313 Hz for control, 0.87 for epilepsy; $p = 0.06$, Wilcoxon rank-sum test). In contrast, Figure 2C
shows that the HR is reduced by 21% (medians: 1.64 Hz for control, 1.29 Hz for epilepsy; \( p = 4e-5 \), Wilcoxon rank-sum test) during interictal periods in stage 2 sleep.

These three HRV parameters (power of RSA, frequency of RSA, and HR) were regressed on age, BMI, and gender to correct for possible effects of these variables, as described in Methods. Table 1 displays the \( F \)-test for the linear model, the regression parameters, their significance based on \( t \)-tests, and Lilliefors test values for the regression model for RSA (see Methods). The regression models for power of RSA (\( F = 1.09, p = 0.355, F \)-test for non-constant model) and frequency of RSA (\( F = 1.87, p = 0.139, F \)-test for non-constant model) were both not significant. However, the model outlined in Table 2 reveals that HR significantly covaries with age and gender (\( F = 28.8, p = 7e-14, F \)-test for non-constant model).

[Tables 1 & 2 around here]

Importantly, the effect of decreased HR in epilepsy is still highly significant after adjusting for age, BMI, and gender (\( p = 2e-3 \), Wilcoxon rank-sum test; Fig. 2D). Since these covariates do not affect the significance of the difference in HR between groups, for the sake of clarity in interpretation, we used the raw HR for most of the adjusted HR. In addition, we show that power of RSA and adjusted mean HR are negatively correlated (\( r = -0.50, p = 1e-8 \), Pearson’s correlation; Fig. 2E), consistent with their being simultaneously modulated by parasympathetic activity. This correlation is mostly accounted for by the subjects with epilepsy, as it loses significance when considering the controls only (\( r = -0.11, p = 0.62 \), Pearson’s correlation). Finally, frequency of RSA and HR share a significant positive correlation (\( r = 0.26, p = 4e-3 \), Pearson’s correlation; Fig. 2F), a well-documented trend (Fleming et al. 2011; Pitzalis et al. 1998; Song and Lehrer 2003).

[Figure 2 around here]

Heart Rate Variability as Biomarker for Epilepsy

Can RSA power and mean HR during interictal sleep serve as sensitive clinical metrics? To test this possibility, we computed the sensitivity, specificity and accuracy of these measures (see Methods) to identifying individuals at risk for developing epilepsy and, potentially, experiencing
SUDEP. To this end, we plotted the receiver-operating characteristic (ROC) curves for each parameter (Fig. 3, A and B) and determined their optimal discriminating thresholds (see Methods). We note that the optimal threshold for RSA power (0.05 Hz) labels more than half of the subjects with epilepsy in our cohort as epileptic (SE = 57%), but correctly labels all of the neurologically normal controls (SP = 100%). The optimal threshold for mean HR (1.40 Hz) is more sensitive (SE = 73%) but less specific (SP = 76%). Both measures have a moderately high accuracy (AC = 66% for power of RSA; AC = 73% for mean HR). A combination of both RSA power and mean HR appears even more promising in the context of classification. In particular, we found the ratio of power of RSA to mean HR (conveniently unitless) to be also significantly different between groups ($p = 2e^{-5}$, Wilcoxon rank-sum test), with ROC curve as shown in Fig. 3C. Its optimal discriminating threshold (0.03) was 63% sensitive, 100% specific, and 71% accurate. Figure 3D displays for comparison the SE, SP, and AC for RSA, HR and RSA/HR.

To test the classification performance of RSA, HR and their ratio as prognostic biomarkers, we used the optimal thresholds for the three parameters to attempt classification of two groups of subjects previously excluded from analysis (see Exclusion Criteria in Methods): 1) five subjects that developed epilepsy at a later point, and 2) seven subjects that were taking anti-epileptic medication at the time of data acquisition to prevent non-epileptic, psychogenic seizures, along with other abnormalities including tics, spells, and fainting. We determined that the optimal thresholds for both power of RSA and RSA/HR independently identified 10 out of 12 (83%) of these subjects as those with epilepsy, whereas the optimal threshold for mean HR classified 7 out of 12 (58%) of these subjects as those with epilepsy.

**Normal Blood Pressure and Sympathetic Tone at Rest**

Up to this point, our results are consistent with an enhancement of parasympathetic tone in generalized epilepsy, but the decrease in HR is also consistent with withdrawal of sympathetic tone. To test this, we analyzed the resting diastolic blood pressure (BP) and, for the sake of completeness, the systolic BP. These measurements were taken at rest before sleep trials, but serve as a control for comparing sympathetic tone in a non-sleep resting state, as is routinely done in clinical settings (Joyner et al. 2010). We found that neither systolic BP (medians: 112 mmHg for control, 109 mmHg for epilepsy; $p = 0.97$, Wilcoxon rank-sum test) nor diastolic BP (medians: 60.5 mmHg for control, 65.5 mmHg for epilepsy; $p = 0.14$, Wilcoxon rank-sum test)
were significantly different between groups (Fig. 3, E and F). In the process of adjusting for natural covariates, both BP measures were found to be positively correlated with age; systolic BP was additionally correlated with BMI ($F = 19.8$, $p = 2e-10$, $F$-test for non-constant model), and diastolic BP was additionally correlated with gender ($F = 11.0$, $p = 2e-6$, $F$-test for non-constant model). Neither adjusted systolic BP ($p = 0.15$, Wilcoxon rank-sum test) nor adjusted diastolic BP ($p = 0.75$, Wilcoxon rank-sum test) were found to be significantly different between groups. These results suggest that sympathetic tone in pediatric epilepsy during interictal periods, at least during wakefulness at rest, is comparable to normal sympathetic tone.

Lengthened Ventricular Diastole Relative to Cardiac Cycle

All significant effects reported thus far are chronotropic; i.e., they refer to the cardiac rhythm. We then wanted to investigate whether inotropic effects (related to cardiac contraction), and dromotropic effects (related to conduction of electrical activity in the heart) may be affected by epilepsy. To this end, we analyzed canonical features of the ECG waveforms. Two representative cycle-triggered ECG traces (Fig. 4A) show that the relevant waves are similar in amplitude in epilepsy and control, but that cardiac intervals of interest seem to be different. We thus analyzed these amplitudes and durations across subjects.

We focused first on the QT interval, a measure of the duration of ventricular systole, using standard methodology to identify its start and end (van Noord et al. 2010), and normalized it relative to the RR interval (see Methods). When comparing between groups, the percentage of the RR interval that is QT appeared to be significantly shorter in epilepsy (medians: 39.4% for control, 36.7% for epilepsy; $p = 3e-3$, Wilcoxon rank-sum test; Fig. 4B). However, regression of the QT interval on age, BMI, and gender revealed a negative correlation with age and a strong correlation with gender (Table 3), both of which have been previously reported in the literature (Rautaharju et al. 1992). After adjusting the QT/RR parameter (see Methods), we found that this shortening was no longer significant between groups ($p = 0.36$, Wilcoxon rank-sum test; Fig. 4C).

[Tables 3 & 4 around here]
We also analyzed the percentage of the RR interval that is PR, or the duration of atrial systole. This interval was significantly shorter in epilepsy (medians: 21% for control, 17% for epilepsy; \( p = 6e-5 \), Wilcoxon rank-sum test; Fig. 4D) and did not produce a significant regression with age, BMI, or gender (\( F = 2.4, \ p = 0.07 \), F-test for non-constant model), and, consequently, does not appear to be due to covariations with those three variables. This result indicates that in addition to chronotropic affects, generalized epilepsy is associated with dromotropic effects, at least during stage 2 sleep.

A shortening in atrial (PR) but not ventricular (QT) systole relative to the whole cardiac cycle must occur at the expense of lengthening the diastole. We thus investigated the ventricular diastole via the relative duration of the TP interval, and indeed, the TP/RR parameter was found to be significantly longer in epilepsy (medians: 43% for control, 51% for epilepsy; \( p = 4e-6 \), Wilcoxon rank-sum test; Fig. 4E). After adjustment for significant correlations with age and gender (Table 4), the relative TP interval was still significantly longer in epilepsy (\( p = 5e-3 \), Wilcoxon rank-sum test; Fig. 4F). Importantly, the lengthening of the relative TP interval was positively correlated with RSA power (\( r = 0.41, \ p = 4e-4 \), Pearson’s correlation; Fig 4G) and negatively correlated with adjusted HR (\( r = -0.70, \ p = 1e-18 \), Pearson’s correlation; Fig. 4H), suggesting that these three parameters are mechanistically linked.

Finally, we analyzed the relative amplitude between R and S waves (the largest voltage difference between the two peaks), and found that this quantity is not significantly different between groups (medians: 558 μV for control, 699 μV for epilepsy; \( p = 0.49 \), Wilcoxon rank-sum test; Fig. 4I). Other relative amplitudes displayed similar non-significance and are omitted for simplicity. These results were maintained after adjusting for age, BMI, and gender, suggesting that inotropic effects are negligible in generalized epilepsy.

DISCUSSION

Summary

In light of the relatively sparse literature on interictal autonomic control in epilepsy (Sarkis et al. 2015) and inconsistent results regarding epileptic parasympathetic activity during these periods
(Behbahani et al. 2015; Raju et al. 2012; Seri et al. 2012), we analyzed HRV of children and adolescents during sleep in the absence of seizures and found two key indicators of increased vagal tone: enhanced RSA and decreased HR. These results are consistent with previous findings (Seri et al. 2012) and extend them to pediatric patients with generalized forms of epilepsy other than focal benign Rolandic epilepsy. We also investigated parameters of the ECG waveforms and found that the ventricular diastole normalized by the RR interval was significantly longer in generalized epilepsy at the expense of shorter atrial systole, demonstrating abnormal conduction of electrical activity in the heart. Other physiological parameters, including frequency of RSA (corresponding to respiratory frequency), systolic and diastolic BP, and the amplitudes of the Q, R, S and T waveforms of the ECG were similar between controls and patients with generalized epilepsy.

Possible Physiological Mechanisms
The three main findings of this paper—enhanced RSA, decreased HR, and lengthened ventricular diastole—can be readily explained by increased parasympathetic tone, leading to enhanced cholinergic neuromodulation of the cardiac cycle. The question of why the parasympathetic tone is enhanced demands further and deeper investigation. It may be due to a homeostatic mechanism, secondary to the primary cause of epilepsy; or, perhaps more intriguingly, increased parasympathetic tone may be a leading factor in the etiology and development of the disease. The fact that enhanced RSA and lower HR preceded epilepsy in five subjects in our cohort that were originally diagnosed as neurologically normal lends support to the latter interpretation.

As for the homeostatic interpretation, we note that the increased parasympathetic tone during sleep in the absence of epileptiform activity contrasts to the sympathetic overexpression frequently observed during and after ictal events (Behbahani et al. 2015; Kolsal et al. 2014; Nagai 2015; Poh et al. 2012). These discrepant observations suggest that the function of the autonomic nervous system is fundamentally different during ictal and interictal periods in children with generalized epilepsy, and that assessing the risk of multifactorial conditions like SUDEP requires a more nuanced understanding of the mechanisms of autonomic activity both during and in the absence of seizures.
Is Abnormal HRV in Epilepsy Iatrogenic?

An alternative, mechanistic interpretation of our results is that abnormal HRV in generalized epilepsy is caused by the antiepileptic medications themselves. We believe that this possibility is unlikely because patients with higher values of RSA power (>0.1 Hz, i.e. greater than twice the optimal discriminating threshold) took anti-epileptic medications that were also taken by patients with RSA values comparable to the controls. In fact, there were only three epileptic patients with high values of RSA power who took medications that no other patient took: cefdinir, azithromycin, and midazolam (each patient took only one of these medications, so there was no overlap); the first two are non-penicillin antibiotics, and the third is a sleep aid of the benzodiazepine class, which is not primarily used to treat epilepsy.

But perhaps the strongest argument against the interpretation that our findings are iatrogenic is that the 5 subjects who were neurologically normal at the time of the ECG recordings, and who did not take any antiepileptic medications at that time, already presented with abnormally higher RSA and lower HR before developing epilepsy at a later point. Two of them did not take any medications at all and the other three took medications for attention deficits (e.g. amphetamine), asthma (e.g. albuterol), and anxiety (e.g. sertraline).

We also note that as a standard clinical procedure, patients that come to the Epilepsy Unit for overnight EEG/ECG monitoring are told to not take medications before monitoring or while they are being monitored in order to obtain an uncontaminated EEG/ECG.

Cautionary Remarks on Vagal Nerve Stimulation

A more detailed understanding of autonomic function in epilepsy is crucial to effectively administering therapies for epilepsy such as vagus nerve stimulation (VNS), which has more recently become an established method of treating drug-resistant epilepsy (Connor et al. 2012). It is well-known that VNS affects chronotropic and inotropic regulation of cardiac activity, and that controlling specific parameters of vagal stimulation (such as current and frequency) can elicit different changes to cardiorespiratory function (Rousselet et al. 2014). However, numerous authors have questioned whether VNS in its current form is an appropriate treatment for epilepsy, as its mechanism of action is still unclear, and as it does not appear to lower the mortality rate due to complications including SUDEP (Granbichler et al. 2015). In fact, VNS may even increase the risk of SUDEP (Annegers et al. 1998). VNS has been shown to regulate...
sympathetic activation of the sinoatrial node (Zhou et al. 2015) and reduce resting HR (Mulders et al. 2015), and is believed to enhance parasympathetic activity (Kampusch et al. 2015). Additionally, VNS is hypothesized to worsen sleep breathing disorders via altered laryngeal motility (Zambrelli et al. 2015), and has been shown to trigger obstructive sleep apnea, a condition often coupled with an overexpression of parasympathetic activity (Ebben et al. 2008; Parhizgar et al. 2011; Vollono et al. 2015).

In the context of our findings, VNS may further increase the abnormally enhanced vagal tone we observe in patients with epilepsy, thereby enhancing the respiratory modulation of HR and incidence of apnea during sleep, when VNS is often activated (Ebben et al. 2008), and decreasing the HR which potentially increases the risk of SUDEP. Our results suggest that a more thorough investigation into the autonomic effects of vagal neurostimulation during sleep is necessary to better understand and appropriately use therapies like VNS, especially in pediatric epilepsy, where consequences of autonomic perturbation may be more severe and perhaps fatal (Annegers et al. 1998).

Alternative Therapeutic Targets for Epilepsy

In considering alternative treatments for epilepsy, the use of parasympathetic modulators appears to have been overlooked as well. Common anti-epileptic medications in use are known to impact sympathetic regulation, but the short- and long-term effects of these medications on parasympathetic function are poorly understood. For instance, benzodiazepines are an often-prescribed family of drugs that allosterically enhance GABAergic function to enhance inhibition and hence manage epileptic conditions (Riss et al. 2008). They ostensibly inhibit sympathetic neuronal activity, but a mechanistic understanding remains elusive (Zahner et al. 2007), and the discussion regarding the impact of benzodiazepines on parasympathetic function is meager.

Further research into parasympathetic routes to controlling seizures and preventing SUDEP may be fruitful provided that 1) the mechanisms of action of drugs that affect autonomic function are better understood, and that 2) care is taken to appropriately regulate parasympathetic tone in epilepsy during interictal periods.

Heart Rate Variability as Prognostic Biomarker
The consideration of RSA and HR as potential biomarkers for the development of epilepsy is a natural ramification of the significant alterations to autonomic balance observed in our study. We find that the use of power of RSA and mean HR (either before or after correcting for age effects) as predictors for epilepsy is moderately accurate, while the ratio of power of RSA to mean HR is highly specific and more accurate than either parameter independently. Most remarkably, all three measures classified more than half of subjects not diagnosed with epilepsy, but with a history of seizures, as having epilepsy, while power of RSA and the RSA/HR ratio both correctly identified five subjects as having epilepsy before clinical diagnosis. This demonstrates the potential of HRV as a non-invasive, prognostic biomarker for epilepsy, which has been previously overlooked.

Limitations
We retrospectively analyzed data from a single pediatric unit. As is the case for any study with a geographically/temporally constrained dataset, it will be important for future studies to expand the analyses to an even more diverse set of subjects, such as those with focal epilepsy. Our study is also constrained to stage 2 sleep (the only one systematically available for subjects in the database) as the regime for comparison. While studies have shown that stage 2 sleep and its transition between slow wave sleep and REM or wakefulness are of high interest in the context of autonomic function in SUDEP (Kanda et al. 2015; Menezes Cordeiro et al. 2015), we did not have control either over the selected portion of stage 2 sleep available for each subject, nor over which epoch of stage 2 sleep was selected over the course of the overnight observation period at the Pediatric Epilepsy Unit. Consequently, we could not determine whether the stage 2 sleep EEG and ECG data were taken immediately following or preceding stage 1 sleep, stage 3 sleep, REM sleep, or awakening. This information would be useful in light of a previous study demonstrating autonomic-endocrine profiles in stage 2 depending on its occurrence relative to contiguous stages of sleep (Brandenberger et al. 2005). Further studies may address all of these concerns by collecting and maintaining ECG and EEG data throughout the sleep period, and analyzing each epoch of interest with respect to the total elapsed time of sleep.

Conclusions
Our results provide novel evidence that parasympathetic tone is enhanced during interictal periods in stage 2 NREM sleep in children with generalized epilepsy. We show that certain measures of HRV during interictal activity in sleep, including RSA and mean HR, may be overlooked risk factors for epilepsy, and that the RSA/HR ratio may be a potential prognostic biomarker for epilepsy itself. These results set the stage for developing a more detailed understanding of autonomic dysfunction in patients with epilepsy by highlighting the dissimilarity between sympatho-vagal imbalance during ictal and interictal periods. Our findings also shed light onto a possible autonomic mechanism for SUDEP, and promote investigation into parasympathetic modulators as potential treatments for epilepsy, particularly in children and adolescents.

**AUTHOR CONTRIBUTIONS**

RFG conceived the project. AGN and IET reviewed medical records and selected the subjects. AGN, SSS and RFG collected and preprocessed the data. SSS and RFG analyzed the data, rendered the figures, and wrote the manuscript. SJL provided early feedback that led to the analyses in Figs. 3 and 4. All authors reviewed and edited the manuscript draft.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**ETHICAL CONSIDERATIONS**

This research project was conducted in accordance with the ethical standards guidelines and regulations set by the Institutional Review Board at University Hospitals and Case Western Reserve University.

**ACKNOWLEDGMENTS**

We thank Dr. Ahmad Zrik for his assistance with clinical EEG reading and interpretation. We also thank Prof. Hillel J. Chiel for helpful suggestions on the manuscript. This work has been supported by a Biomedical Researcher Award of The Hartwell Foundation (RFG).
FIGURE CAPTIONS

**Fig. 1:** HRV in representative control (left) and epilepsy (right) subjects. **A** and **B:** Raw ECG traces over periods of 30 seconds; decreased HR is readily apparent in the subject with epilepsy. **C** and **D:** Instantaneous HR over the same 30-second periods as in **A** and **B:** lower baseline and increased variability are visible in the patient with epilepsy. **E** and **F:** Power spectral densities of HR over a 30-minute period, filtered above 0.1 Hz; high-frequency modulation of HR (RSA peak around 0.3 Hz) is enhanced in epilepsy.

**Fig. 2:** Analysis of HRV across control (circles) and epilepsy (diamonds) subjects. **A:** RSA tends to be increased in patients with epilepsy. **B:** Frequency of RSA (a measure of the respiratory frequency) is not different in epilepsy compared to the control group. **C:** Mean HR tends to be lower in epilepsy. **D:** After adjusting for age, BMI, and gender, HR is still significantly lower in epilepsy. **E:** RSA and HR are negatively correlated, consistent with their being mediated by parasympathetic activity. **F:** Frequency of RSA (respiration) and HR are positively correlated, as expected.

**Fig. 3:** Sensitivity and specificity of HRV parameters, and comparison of blood pressures. **A,** **B,** and **C:** ROC curves for power of RSA, mean HR, and RSA/HR ratio, respectively. Solid diagonal lines mark the optimal thresholds for classification, and their numerical values are shown. **D:** Comparison of sensitivity, specificity, and accuracy for RSA, HR, and RSA/HR ratio. **E** and **F:** Systolic and diastolic blood pressures at rest are similar in both control (circles) and epilepsy (diamonds) groups.

**Fig. 4:** ECG waveform analyses. **A:** Comparison of mean-subtracted, cycle-triggered average ECG traces centered at R wave and plotted over two beat-to-beat periods. **B** and **C:** The QT interval relative to the RR interval appears to be shortened in epilepsy (diamonds) as compared to controls (circles); however, after adjusting for age, BMI, and gender, there is no significant difference in QT interval between groups. **D:** In contrast, the relative PR interval (atrial systole) is significantly shorter, and does not depend on age, BMI or gender. **E** and **F:** This relative shortening occurs at the expense of a longer relative ventricular diastole (TP/RR). **G** and **H:** The
adjusted relative ventricular diastole correlates positively with the RSA power, and negatively with the adjusted HR. I: The RS relative amplitude is not significantly different between groups.

TABLES

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Table 1: Regression analyses for power of RSA. RSA power is independent of age, BMI, and gender.

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Table 2: Regression analyses for HR. The HR significantly covaries with age and gender but not BMI.
Table 3: Regression analyses for QT/RR. The relative QT interval significantly covaries with age and gender but not BMI.

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Lilliefors test for non-normality of residuals

| Lilliefors test | D = 0.0798 | D# = 0.0826 | p = 0.068 |

Table 4: Regression analyses for TP/RR. The relative TP interval significantly covaries with age and gender but not BMI.

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Lilliefors test for non-normality of residuals

| Lilliefors test | D = 0.0581 | D# = 0.0827 | p = 0.436 |
REFERENCES


Goldstein DS, Bento O, Park MY, and Sharabi Y. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Experimental physiology* 96: 1255-1261, 2011.


