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Cardiac changes in epileptic baboons with high-frequency microburst VNS therapy: A pilot study

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ABSTRACT

The epileptic baboon provides a natural model of idiopathic generalized epilepsy and sudden unexpected death in epilepsy (SUDEP). We sought to evaluate autonomic differences, including heart rate (HR), heart rate variability (HRV) and corrected QT-duration (QTc) between two epileptic (EB1, EB2) and one control (CB) baboon, and the autonomic effects of high-frequency (HF) microburst Vagal Nerve Stimulation (VNS) Therapy in the epileptic baboons. At baseline, EB2's HR was increased over both EB1 and CB, and EB1's HRV was decreased compared to the others. QTc-intervals were significantly prolonged in both epileptic baboons. EB1 became free of generalized tonic-clonic seizures (GTCS) with VNS therapy, whereas EB2's GTCS were reduced by a third. HR decreased in both epileptic baboons, but while HRV improved in EB1, it decreased in EB2. EB2 succumbed to SUDEP after 9 months. This pilot study demonstrates abnormalities in HR, HRV and QTc-intervals in epileptic baboons. HF VNS Therapy demonstrated different effects on HRV in the two epileptic baboons, which, in addition to persistent GTCS and elevated HR, may have contributed to SUDEP risk in EB2. Future studies are needed to establish normative values for HRV and determine variability of HR, HRV and QTc-intervals in epileptic baboons.

1. Introduction

Epilepsy can profoundly affect quality of life and increase mortality (Devinsky et al., 2016). Sudden unexpected death in epilepsy, or SUDEP, is a common cause of death in people with epilepsy, and there is a strong interest in developing biomarkers for identifying people at risk. Recently published AAN Practice Guidelines on SUDEP confirmed that frequent generalized tonic-clonic seizures (GTCS) are the main contributors to increased SUDEP risk (Harden et al., 2017). Several studies have looked at potential interictal autonomic biomarkers for SUDEP (Lotufo et al., 2012; DeGiorgio et al., 2010; DeGiorgio and DeGiorgio, 2016; Myers et al., 2018), including HRV and cardiac conduction abnormalities, particularly long QT syndrome (LQTS) (Goldman et al., 2016). HRV, which is controlled by parasympathetic and sympathetic outflow, is quantitated by analyzing changes in RR-intervals over time (square root of the mean of the sum of squares of their differences, or RMSSD) (Lotufo et al., 2012; DeGiorgio et al., 2010; DeGiorgio and DeGiorgio, 2016). Increased parasympathetic outflow leads to increased HRV, as is typically observed in sleep, while decreased parasympathetic outflow and/or increased sympathetic outflow decreases HRV. Decreased HRV, pathognomonic for chronic epilepsy and an adverse effect of most anti-seizure medications (Lotufo et al., 2012), is also associated with increased SUDEP risk (DeGiorgio et al., 2010; DeGiorgio and DeGiorgio, 2016; Myers et al., 2018).

Although one study in humans demonstrated that standard Low-Frequency (LF; 20–30 Hz) VNS Therapy can cause negative parasympathetic effects, such as decreases in HR in addition to respiratory changes (Mulders et al., 2015), another study failed to confirm autonomic changes (Garamendi et al., 2017). High-Frequency (HF; 250–300 Hz) microburst stimulation, which is a novel treatment paradigm did not demonstrate HRV changes in healthy Beagles (Martlé et al., 2014).

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This study primarily aimed to provide a proof-of-concept for utilizing a noninvasive platform to monitor cardiac and respiratory parameters, accelerometry and surface temperature, to evaluate the efficacy and safety of HF-VNS Therapy in a baboon model of idiopathic generalized epilepsy (IGE; Szabó et al., 2013). As the epileptic baboon is also a natural model for SUDEP (Szabó et al., 2009), we paid particular attention to HR, HRV and QT-interval changes after 15 weeks of HF-VNS Therapy (Szabó et al., 2017). We hypothesized that epileptic baboons would have altered HRV compared to a healthy, asymptomatic baboon, and that HF-VNS therapy, in addition to its anti-seizure effects, would reduce HR and normalize HRV.

2. Methods

2.1. Animal selection

IGE has been characterized in the pedigreed baboon colony housed at the Southwest National Primate Research Center, belonging to the Texas Biomedical Research Institute (TBRI) in San Antonio, Texas (Szabó et al., 2013). Epileptic Baboon 1 (EB1) (16 year-old female) and EB2 (10 year-old female) were selected based on a history of GTCS and EEG documentation of generalized interictal epileptic discharges on scalp EEG (Szabó et al., 2017). Brain MRI in EB1 was normal, while EB2 exhibited left colpocephaly. The control baboon (CB) was a four year-old female without a history of seizures and a normal interictal EEG. Following VNS placement, treatment paradigms were tested over a 15-week period in EB1 and EB2. This study was approved by the IACUC at the TBRI, adhering to all rules and regulations governing the use of laboratory animals, as outlined in the United States Public Health Service's *Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal Research, 2011) and the Animal Welfare Act (Amended, 2009). Principles outlined in the ARRIVE guidelines and in the Basel declaration (http://www.basel.declaration.org) were also followed.

2.2. High-frequency microburst VNS therapy protocol

The baboons were housed in single cages and continuously monitored with Axis (Lund, Sweden) camera with infrared capabilities (Szabó et al., 2017). While CB was only monitored for one week, EB1 was monitored for 20 weeks (including a five-week baseline), whereas EB2 was monitored for 19 weeks (including a four-week baseline). Various VNS settings were tested over the 15-week period, including different current outputs (0.25 mA, 2 and 2.5 mA), numbers of pulses (4 vs 7), interburst intervals (IBI; 2.5, 1.5 or 0.5 s), stimulation intervals (1.8 vs 5 min) and overall duration (1 h, 2 h or 24 h per day). Signal frequency for burst stimulation remained at 300 Hz in all trials.

2.3. Data acquisition

All three animals were monitored noninvasively with the emkaPACK4G wireless system (emka TECHNOLOGIES, Inc., Falls Church, VA) for a mean 21 (range 20–22) hours (Table 1). Following sedation with intramuscular ketamine (10 mg/kg), the baboons' chest hair was shaved for electrode placement. Four electrodes were used to generate ECG, one resistance impedance plethysmography band was placed over the lower thorax, and one temperature sensor was placed midsternally, and maintained using a custom-fit vest. HR and HRV were assessed on a minute-to-minute basis. Six one-minute samples were evaluated every 4 h for QT-interval prolongation, and corrected for HR differences using the Bazett formula (QTcB; Ishizaka et al., 2009). Recordings were repeated in the epileptic baboons after 20 weeks of HF-VNS Therapy to evaluate treatment effects, whereas CB underwent only a single evaluation. During the first study in EB2, electrode artifact intermittently obscured some ECG channels, reducing the reliability of QT-interval measurements.

2.4. Analysis

Although the sample size limited statistical comparisons, two-sided Student *t*-tests were performed to compare baseline measures of HR and RMSSD between the epileptic baboons and CB, and the treatment effect in the two epileptic baboons (Table 1). Awake and sleep states were determined by light-dark cycles as well as accelerometry: the first 0 value on accelerometry (inertial measurement units) after lights were distinguished, and the last 0 value around the time they were turned on again, were used as markers.

3. Results

Table 1 demonstrates heart rate, RR-intervals, and HRV calculations for each study, and, in particular for awake and sleep states. Fig. 1 demonstrates pre- and post-treatment ECG measurements in EB1. EB2's HR was significantly increased overall compared to CB and EB1. Pre-treatment HRV was decreased for EB1 compared to CB and EB2, but QTcB-intervals were increased in both epileptic baboons.

HF-VNS Therapy reduced awake HRs for both epileptic baboons, but was increased nocturnally in EB2. While EB1's HRV improved with treatment, EB2's HRV decreased, but still aligned with EB1's post-treatment values. QTcB-intervals were significantly increased for EB1 and EB2 compared to CB, no treatment effect was noted in EB1.

EB1 responded extremely well to HF-VNS therapy with complete seizure control, whereas EB2's seizures were reduced by 33% (Szabó et al., 2017). EB1 continued treatment for two years, whereas EB2 succumbed to SUDEP 9 months after initiation of VNS Therapy. Her necropsy report was unremarkable except for pulmonary congestion and left ventricular hypertrophy.

4. Discussion

In this small pilot study evaluating autonomic measures in epileptic baboons, before and after HF microburst VNS Therapy, we observed differences of HR and HRV between studies performed at baseline and after a short treatment period. This is the first study collecting autonomic data, HRV and QT-duration in particular, in epileptic baboons. Some shortcomings of this pilot study included the obscuration of QT-interval measurements in EB2's baseline study, the need to sedate the baboons in order to place telemetry equipment, which may have affected early measurements, and the age difference between the epileptic and control baboons. Nonetheless, long-term monitoring eliminates the effects of short-acting sedatives, such as ketamine, and while a larger number of age-matched healthy controls would have been ideal for baseline comparisons, HR does not vary significantly with age in baboons (Yeung at al., 2016). Overall, noninvasive monitoring of cardiac functions was effective and well-tolerated by the baboons.

In all three baboons, baseline cardiac measures were in line with previous studies (Ishizaka et al., 2009; Vice and Rodriguez, 1965). While there is limited normative data even in healthy, control baboons, one study including both sexes and mixed ages demonstrated similar heart rates (115 +/- 27 beats per minute) (Vice and Rodriguez, 1965) and QT-intervals (270 ms) (Herrmann and Williams, 1965). The epileptic baboons demonstrated increased HRs, as well as QTcB-interval measurements, at baseline. As age effects on HR are negligible (Yeung at al., 2016), the significant increase in HR in both baboons may reflect an altered autonomic output in epileptic baboons. QTcB-intervals, on the other hand, are expected to decline with age, as demonstrated both in cynomolgus monkeys and humans (Ishizaka et al., 2009). Hence, significant QT-interval prolongation in both epileptic baboons, raises the suspicion of underlying genetic mutations or acquired cardiac conduction defects. HRV measurements also differed between the epileptic baboons. The magnitude of these HRV differences, in addition to QT-interval prolongation, needs to be studied in a larger cohort, and their potential clinical significance validated in baboons eventually

Table 1
Cardiorespiratory Measurements during Awake and Sleep States.

Baboon		Epochs	Heart Rate	RR-intervals	RMSSD	QTcB
CB		Overall	113 +/− 23‡	551 +/− 109*‡	32 +/− 30*‡	352 +/− 6*‡
		Awake	136 +/− 17‡	449 +/− 54*‡	12 +/− 8*	(QT 258)
		Sleep	98 +/− 13‡	620 +/− 78*‡	46 +/− 31*‡	
EB1	Pre-Tx	Overall	114 +/− 12$^{∫#}$	530 +/− 54$^{*∫#}$	11 +/− 7$^{*∫#}$	380 +/− 7*
		Awake	124 +/− 9$^{∫#}$	486 +/− 37$^{*∫#}$	6+/−5$^{*∫#}$	(QT 281)
	(1 GTCS/wk)[13]	Sleep	106 +/− 7$^{∫#}$	566 +/− 38$^{*∫#}$	14 +/− 7$^{*∫#}$	
	Post-Tx	Overall	111 +/− 12$^{∫#}$	549 +/− 59$^{∫#}$	19 +/− 17$^{∫#}$	382 +/− 16$^{#}$
		Awake	118 +/− 11$^{∫#}$	511 +/− 48$^{∫#}$	11 +/− 7$^{∫}$	(QT 277)
	(no GTCS)[13]	Sleep	105 +/− 10$^{∫#}$	578 +/− 49$^{∫#}$	25 +/− 11$^{∫#}$	
EB2	Pre-Tx	Overall	132 +/− 22$^{‡∫#}$	468 +/− 78$^{‡∫#}$	29 +/− 29$^{∫#}$	NA
		Awake	150 +/− 16$^{‡∫#}$	405 +/− 51$^{‡∫#}$	12 +/− 12$^{#}$	
	(3 GTCS/wk)[13]	Sleep	114 +/− 9$^{‡∫#}$	532 +/− 40$^{‡∫#}$	46 +/− 31$^{∫#}$	
	Post-Tx	Overall	128 +/− 16$^{∫#}$	475 +/− 57$^{∫#}$	20 +/− 14$^{∫#}$	370 +/− 4$^{‡#}$
		Awake	138 +/− 14$^{∫#}$	437 +/− 48$^{∫#}$	12 +/− 9	(QT 250)
	(2 GTCS/wk)[13]	Sleep	119 +/− 11$^{∫#}$	507 +/− 44$^{∫#}$	27 +/− 14$^{∫#}$	

Legend: RMSSD square root of the mean of the sum of squares of the RR-interval differences, QTcB refers to corrected QT-interval using the Bazett formula (milliseconds) as well as mean QT in parentheses, CB control baboon, EB1&2 epileptic baboons, Tx (VNS therapy), GTCS generalized tonic-clonic seizures, wk week, NA not applicable, statistically significant (two-tailed T-Test, $p < 0.05$) differences between * CB and EB1 baseline, ‡ CB - EB2 and EB2 baseline (except for QTcB), $^{∫}$ pre- and post-treatment in either EB1 or EB2, and $^{#}$ EB1 or EB2 both pre- and post-treatment.

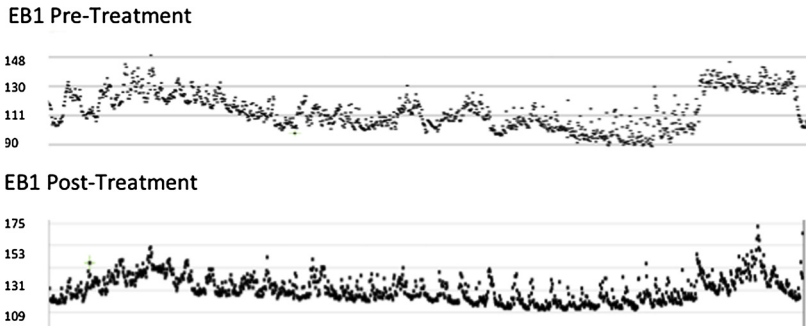

Fig. 1. Pre- and Post-treatment Heart Rates for EB1.
Legend: Heart Rates (beats per minute) vs time (one-minute samples over 21 h); heart rate was extracted at each time point using the emka's. txt/EXCEL analysis package. Note that there was more dispersion pre-treatment in EB1, and return of physiologically cycling heart rates with high-frequency, microburst VNS Therapy.

succumbing to SUDEP (Szabó et al., 2009).

The autonomic effects of HF-VNS Therapy have not been evaluated in animal models of epilepsy nor in humans. As reported in people with epilepsy undergoing standard VNS Therapy, HR decreased in our epileptic baboons (Mulders et al., 2015). HF-VNS Therapy did not affect QT-intervals in one baboon, also consistent with human studies (Garamendi et al., 2017). The change of HRV seen in this study (increasing in the baboon with decreased baseline values, while decreasing in the baboon with a normal baseline HRV), was not expected. It is unclear whether this finding reflects a regression to the mean, which would still suggest that HRV is abnormal in epileptic baboons, the variable effect of VNS Therapy in two baboons related to different seizure outcomes vs an adverse effect of VNS therapy in one of them, or the natural history of chronic epilepsy in this animal model. Persistent GTCS (Devinsky et al., 2016; Harden et al., 2017) and decreased HRV are both risk factors for SUDEP in humans (DeGiorgio et al., 2010; DeGiorgio and DeGiorgio, 2016; Myers et al., 2018), and may have contributed to SUDEP in EB2. More epileptic baboons need to be studied to validate the role of any of these factors (seizure control, changes in HR, HRV, or QT-prolongation) as potential causes for SUDEP in the baboon.

Author contributions

MD, DP and CAS designed and performed the study; MD, DP, RS, FS and CAS interpreted the data; MD, DP and CAS generated the figure and table; MD, AS and FS drafted the manuscript. MD, AS, FS, RS and DP edited and approved the manuscript.

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Declaration of Competing Interest

The authors have nothing to declare.

Ethical publication 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 these guidelines.

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