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Non-linear Classification of Heart Rate Parameters as a Biomarker for Epileptogenesis

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Summary

Purpose—To characterize a biomarker for epileptogenesis based on cardiac interbeat interval characteristics.

Methods: Electrocardiograph (ECG) and electroencephalogram (EEG) signals were recorded from freely moving rats (n=23) before status epilepticus (SE) induced by i.p. pilocarpine (PILO) injection as baseline, and on days 1, 3 and 7 after SE. We assessed several features from cardiac interbeat intervals, including linear, non-linear and frequency parameters of interbeat intervals, and power spectra of interpolated intervals during epileptogenesis. After thresholding, the altered values were applied to a non-linear classifier. The non-linear classifier divided animals into two groups; with and without epilepsy, based on all collected data.

Results: We found that none of the single altered parameters in cardiac activity emerged as a sole biomarker for epileptogenesis. However, the non-linear classifier distinguished animals that later developed from those and did not develop epilepsy. The non-linear classification was performed on preliminary findings from 23 animals; six did not develop epilepsy and the rest did. The average positive predictive value (precision rate) was 78%. This was calculated based on the average sensitivity and specificity, which were 80.6% and 35.2% respectively, for the 100 classification passes. We also showed that these numbers would have increased as the number of subjects increased.

Conclusion: Changes to the brain caused by status epilepticus that lead to epileptogenesis have systemic effects, and alter cardiac activity. A non-linear classifier performed on several extracted features of cardiac interbeat intervals may be useful as a biomarker to identify animals with low and high probability of developing epilepsy after status epilepticus.

Keywords

Artificial Neural Network; Epileptogenesis Prediction; Animal Model of Epilepsy; Non-linear Classifier; Heart Rate

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Introduction

Early diagnosis and intervention are important for efficient treatment of any disease, including epilepsy (Stefan et al., 2006). Finding biomarkers that indicate the existence of unrecognized processes leading to the development of disease are critical for early intervention (Dichter, 2009; Li et al., 2008). If specific characteristics of heart electrical activity associated with epilepsy development after an initial precipitating injury (IPI) could be identified, such data would provide a biomarker for the identification of candidates for early therapy, and a means to compare the efficacy of a variety of interventions. Epileptogenesis after an IPI often progresses slowly, and in many cases, recurrent seizures appear after several years. In 60–80% of patients who develop epilepsy after an IPI, seizures are intractable to existing antiepileptic drugs (Kwan and Brodie, 2000; Pittau et al., 2009). Most efforts to find biomarkers of epileptogenesis have focused on brain indicators (Jacobs et al., 2001; Herman 2006; Dichter, 2009; Engel et al., 2009; Dube et al., 2010). The brain modifies cardiac rate and variation through sympathetic and parasympathetic components of the autonomic nervous system, and patterning of heart rate (HR, the time elapsing between two consecutive R waves in the electrocardiogram.) and its variability is extremely sensitive to seizure discharge (Nouri, 2009; Tigran et al., 2003; Goodman et al., 2008; Metcalf et al., 2009), with patterns changing during (Leutmezer et al., 2003; Hotta et al., 2009), a few minutes before, and after epileptic seizures (Delamont et al., 1999). Relationships between EEG and ECG have been studied for seizure detection (Greene et al., 2007; Bermudez et al., 2007), but all of these studies examined patterns after development of epilepsy.

Finding characteristics in ECG that can be affected during epileptogenesis could be helpful in predicting development of epilepsy. A predictor based on cardiac interval patterns possesses considerable practical benefits; an ECG recorder can be worn under clothing during normal daily activities, minimizing intrusive recording procedures, such as EEG acquisition. Determining how the cardiac rhythm is altered during epileptogenesis requires obtaining data from many subjects, data not readily available in humans, but possible from an animal model. In this study, we used the pilocarpine (PILO) model of chronic mesial temporal lobe epilepsy (Turski et al., 1983).

We investigated changes in different parameters of cardiac interbeat characteristics to determine if any of these measures could be a biomarker of epileptogenesis in the PILO model of chronic epilepsy.

Methods

All procedures described in this study were approved by the University of California at Los Angeles Institutional Animal Care and Use Committee. Experiments were performed on twenty three adult male (200–250g) Wistar rats.

Electrode implantation

Rats were anesthetized with isoflurane, 2%. Two pairs of tungsten microelectrodes (O.D. 50 μ m and 1mm vertical space separation between tips) were implanted in the right motor (AP=2.0, L=1.5; V=2.0) neocortex and in the hilus of right anterior hippocampus (AP=-3.0, L=2.0; V=3.5) areas of the (Paxinos and Watson, 1997). Reference and ground electrodes were placed above the cerebellum. Two subcutaneous electrodes (TWP50, Temporary Cardiac Racing Wire) were implanted on the right and left rib cage to record ECG. Leads from those electrodes were passed via flexible wires to the skull where they were cemented, together with EEG electrodes.

Data Acquisition

Baseline recordings of brain and heart electrical activity began one week after recovery from the electrode implantation procedure, and continued for 4–6 hours a day over 3–4 days. The data were recorded with Grass amplifiers in the frequency band from 0.1 Hz to 1.0 kHz (amplification $\times 1000$, sampling rate 10 kHz) using the Datapac 2K2 (RunTechnology, Mission Viejo, CA) data acquisition system, concurrently with video-monitoring. We examined the ECG one day prior to PILO administration, and on days 1, 3 and 7 following administration; the most dramatic changes in brain function related to epileptogenesis are expected during the first week after injection (Rakhade and Jensen, 2009; Vezzani and Granata, 2005; Oby and Janigro, 2006; Vezzani et al., 2008; Loscher and Brandt, 2010). We expected the most dramatic changes to occur in the ECG during the same time period.

Animal Model of Epilepsy

After a two-hour period of baseline activity recording, status epilepticus was evoked by PILO injection. SE can vary from stage 1 to 5 in different animals, however the development of epilepsy was independent of the severity of SE. Twenty hours before PILO injection, 3 mg/kg of LiCl (3 mmol/kg) were delivered intraperitoneally (i.p.) to each rat. Thirty minutes before PILO administration methyl scopolamine (1 mg/kg) was given subcutaneously (s.c.). PILO was given s.c. (25 mg/kg). Status epilepticus was terminated two hours after onset by i.p. injection of diazepam (5–10 mg/kg, i.m.). At the same time, 2 cc saline lactated Ringers and 5% Dextrose were injected s.c.

Seizure Monitoring

Beginning on the day following the PILO injection, animals were video monitored in their own cages for seizure detection 24 h/day for 4 months. The time of seizure occurrence and number of seizures were collected for each rat. In addition, EEG and heart electrical activity were recorded for 6–8 hours in each rat before and on days 1, 3 and 7 after status epilepticus. After the first week of status epilepticus animals were EEG/video monitored for 4 months. An animal would be considered as having epilepsy as soon as it had a seizure stage 3 or higher (Racine, 1972) during the observation time period. After 4 months, animals were injected with a lethal dose of Nembutal 60 mg/kg. When breathing stopped, they were perfused with 2.5% paraformaldehyde and brains were removed and sectioned for Nissl staining, to verify electrode placements.

Data Analysis

To obtain data under comparable conditions, we chose data during slow-wave sleep, a state readily identified by EEG recordings (Figure 1), and verified that state by concurrent video recording. In this state, epileptiform activity is more prominent (Bragin et al., 1999). Data recorded before status epilepticus, and on days 1, 3, and 7 post-status epilepticus were reviewed. Slow wave sleep states were selected from the periods when the animal was seen to be sleeping on the video file. Epochs with the highest amplitude of EEG signal were selected with a dominant of delta activity on the power spectrograms. Three samples of ECG with duration of 3 minutes were selected during these slow wave sleep periods for further analysis. In this experiment, periods of continuous slow-wave activity varied from 1 to 5 minutes. We used the average time periods of continuous sleep that could be found consistently in all files; these periods were sufficient to obtain features from heart rate.

We applied cubic-spline interpolation to extracted RR intervals (Daskalov and Christov, 1997), and applied statistical signal processing algorithms to detect changes in heart rate (HR) characteristics. In these experiments, we examined root mean square of successive differences (RMSSD), approximate entropy (ApEn) and power spectral density (PSD) of RR

intervals. RMSSD is a linear parameter to measure variation in successive heartbeats (Berntson et al., 1997). ApEn quantifies irregularity in individual cardiac interbeat intervals. The higher the ApEn, the more irregular the signal is (Fusheng et al., 2001; Richman and Moorman, 2000). PSD shows the power of RR intervals or different frequency bandwidths. We used auto-regressive procedures to calculate the power spectrum (Yamamoto et al., 1991).

In addition, we have used a non-linear classifier to divide the extracted features into different classes based on non-linear algorithms. Most of the approaches designed for solving classification problems presuppose the representation of patterns by a set of measurements, called features. To quantify changes in the extracted features, the differences were described as changes from before to each of the 3 days after SE; before to day 1, before to day 3, and before to day 7. Each input can have several features and all features should exist in all provided inputs. The classifier compares the inputs which are provided as vectors, and each component of the vector is one of the considered features. Being non-linear allows the classifier to assign different weights to each feature (component of the vector). In this way, the classifier will be more flexible than a linear classifier. A judicious selection of features for building classifiers is a crucial aspect of classifier design, and deserves careful consideration. On one hand, there is no penalty for using all available measurements in classifier design. On the other hand, too many features make the classifier increasingly complex, in fact, unnecessarily so, in case some of the measurements are redundant. We measured many characteristics from heart rate, such as RMSSD, ApEn, mean and standard deviation of RR intervals, power spectrum, Poincare plot, Shannon Entropy, Sample Entropy. We selected those that changed the most in the preliminary data that we gathered. The pattern recognition system was based on artificial neural networks (ANNs). ANNs are often useful for forming a model on the basis of a complex population of examples where no algorithm or descriptive rule exists (Bishop, 2008; Theodoridis and Koutroumbas, 2009).

Our data analysis regarding ANN can be explained briefly as the following. Before processing the data, the classifier divided the values randomly into three categories; training data, validating data and testing data. Data from all twenty three rats were provided to the classifier, and it randomly picked the rats to be used for training, validating or testing. In addition to the extracted features, another vector named “target” was provided to the classifier. The target vector defined expected output. In our target vector, ‘0’ represents with epilepsy and ‘1’ represents without epilepsy. The ANN arranged its internal structure consisting of “artificial neurons” (nodes) to give the expected outputs for the provided inputs. Sensitivity is defined as the percentage of animals with epilepsy that are diagnosed correctly in a group of all animals with epilepsy and specificity is the percentage of subjects without epilepsy that are diagnosed without epilepsy in a group of animals that do not have epilepsy. Finally, positive predictive value (PPV), or precision rate is the percentage of animals that their test results indicate they have epilepsy and they do have epilepsy. After reaching the PPV, the structure maintained the trained configuration for future extracted features from other animals.

Experimental Results

EEG records in this paper were used only for confirming the electrographic component of behavioral seizures and for determining slow wave sleep periods for selection of ECG samples for a further analysis. Investigation of relationship between EEG and ECG is a subject of other study.

Changes of individual parameters of heart rate activity after SE

After four months, seventeen rats developed epilepsy. They had stage 3–5 (Racine's scale) behavioral seizures occurring with a frequency 9–35 seizures per month. The earliest first seizure in this group was detected on day 9 and the latest first seizure was detected on day 46. In six rats no seizures were detected within a period of 4 months. No significant differences in mean heart rate emerged after status epilepticus in any animal. Three parameters of HR, however, changed more than other parameters. RMSSD coefficients were calculated at baseline, and for the first week after SE showed changes after SE. The RMSSD values on day 1, 3 and 7 after SE were normalized by the RMSSD before SE for a three-minute recording (Figure 2). However these changes were not consistent between animals either in the groups with and without epilepsy (the groups with and without epilepsy could not be separated by RMSSD values).

We also assessed PSD of the interpolated RR interval series. Although changes in low frequency (LF, 0–0.04Hz) and high frequency (HF, 0.15–0.4 Hz) components of the power spectral estimates were observed (decreases and increases), the changes were not consistent (Figure 3). ApEn reflects complexity or irregularity of the heartbeat and also changed after SE. Since the cardiac pattern is controlled by autonomic regulatory structures in the brain, excessive activation through epileptic discharge or structural injury will be reflected in cardiac patterns. In Figure 4, the ApEn measurements are shown in twenty three rats.

Non-linear classification of heart rate parameters

Since we had four measurements for each characteristic, we needed three components to create the vector for each one. After processing the data, a three-component vector showed each extracted feature. After normalizing values by dividing them by the before-SE measurements for each animal, we created a new vector. We used the following thresholding algorithms which gave us the highest numbers for sensitivity, specificity and PPV. If the normalized value is more than `1', it is assigned `1', if between `0.5' and `1', it is assigned `-1' and if less than `0.5', it is assigned `0'. For each characteristic, we formed these vectors, which are shown in Figure 5. Some of the points overlaid each other (thus, the number of graph points is less than 23). As shown in Figure 5, a linear classifier cannot separate the animals with and without epilepsy from each other, especially since in all cases, some animals without epilepsy have the same coordinates as animals with epilepsy. However, a non-linear classifier is able to classify all features together. Twelve features were extracted from each rat (three measurements from four characteristics). We built a **12*23** matrix as an input to the ANN by the constructed vectors. Another input to the classifier is the expected outcome (target) and is a twenty three-component vector, because there are **twenty three** animals. Each component is either `0' (with epilepsy) or `1' (without epilepsy). We ran the classifier 100 times, to put randomly each animal in different categories (testing, validating and training). Specificity and sensitivity were calculated based on the average of test categories after running the non-linear classifier 100 times. For each run, the non-linear classifier randomly selected a group of animals for testing. The applied “feed-forward network” has 78% PPV, 80.6% sensitivity and 35.2% specificity.

Discussion

In this preliminary study, we investigated a new potential biomarker for epileptogenesis based on several heart rate variability (HRV) characteristics. We examined several linear, non-linear and frequency components of HRV after SE and the baseline before SE. We examined HRV one day prior to PILO administration and day 1, 3 and 7 following SE. This is the first study of a biomarker for epileptogenesis based on HRV parameters in freely moving animals.

Changes in heart activity in epileptic patients are described in several previous studies (Frysinger and Harper 1989). In TLE, the occurrence of epileptic discharge in the right insular cortex can result in tachycardia prior to seizure onset (Oppenheimer et al., 1992). Bradycardia can result from epileptic discharge spreading to the left insular cortex and amygdala (Healy and Peck, 1997). Onset and offset of tachycardia and bradycardia are accompanied by substantial changes in interbeat intervals, contributing to overall increased irregularity. These changes can be detected by ApEn, a non-linear statistical method to show irregularity in heart beats. Sample entropy was introduced to overcome the ApEn drawbacks, namely the bias and sensitivity to short-duration data (Richman and Moorman, 2000). In this study, we compared data over three days during the first week after status epilepticus, which will cancel the bias affect, since the data length is not short (we examined three-min of data, containing approximately 1000 heartbeats). With this duration of data, the sample entropy and ApEn show similar results (Richman and Moorman, 2000). RMSSD detects changes in successive heart beats. These changes will be more significant during disturbances in breathing (RR intervals shorten during inspiration, and increase during expiration (Jansen and Lagae, 2010)). Breathing changes typically appear after SE; discharge patterns in hippocampus and amygdala are correlated with respiratory period (Frysinger and Harper, 1989); such pathologic changes during epileptogenesis can alter respiratory function (Frysinger and Harper, 1990). Vagal activity influences the HF components of HRV in rats (Kuo et al., 2005) and it can be affected after SE. Status epilepticus induced by PILO can change breathing rhythm, especially during the first week after administration when the interictal epileptic discharges are most prominent. However, none of those parameters (RMSSD, ApEn and PSD) provided independent factors to determine epileptogenesis in animals. It is possible that further, more detailed non-linear analysis of several characteristics of heart activity may reveal changes related to epileptogenesis. There are several algorithms that can be used for classification. In this study, we are dealing with two groups; with and without epilepsy. The classification algorithms which can be applied to these kinds of problems with known outputs (with or without disease) are called supervised learning algorithms. Artificial Neural Network as a non-linear supervised learning algorithm can separate subjects that have different complex patterns in their extracted features. However, this algorithm like every other algorithm can show weakness to get the same results from a different datasets. In that case, we need to change our features and adjust them accordingly. We tried to cover this by randomly selecting different groups to test our algorithm and report the average specificity, sensitivity and PPV as the final performance. If we did not have done this, there were some cases with very much better performance than the reported one. We hope that by adding more subjects to this pool, we can verify or adjust our algorithm for other datasets.

Applying a “feed forward artificial neural network” to the gathered data, we have shown that this classifier can be promising for this application (Bishop, 2008; Theodoridis and Koutroumbas, 2009). This study showed that this classifier was able to categorize subjects into groups with and without epilepsy with 78% PPV. Sensitivity was 80.6% and specificity was 35.2%. As mentioned earlier in the experimental results section, the non-linear classifier performed on twelve extracted features. It has been shown that the non-linear classifier needs at least the same number of subjects as the number of features (twelve) in each category (with and without epilepsy) to reach to its highest performance (Bishop, 2008). Having less than twelve subjects without epilepsy caused a weak performance in classification of animals without epilepsy and consequently a low specificity. We measured specificity, sensitivity and PPV for the first 12 and 17 animals (Figure 6). As seen in Figure 6, by increasing the number of subjects PPV, sensitivity and specificity increase monotonically as well. We anticipate that we will reach higher specificity in future by having more subjects without epilepsy.

We acknowledge that the change in the heart rate variability could be related to the amount of the brain damage, which we did not examine in this study and it can be investigated in future studies.

Conclusion

These findings demonstrate that epileptogenesis might be predicted using heart rate characteristics applied to a non-linear classifier. However more subjects are needed to confirm these preliminary results, and to obtain higher PPV from a non-linear classifier.

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Figure 1.
Example of simultaneous recording of EEG from the neocortex (neo), hippocampus (hip) and heart rate in a freely moving rat. Arrow indicates the transition between slow wave sleep and awake state.

Figure 2.

RMSSD of RR intervals measured in twenty three animals before and after SE. The post- SE values were normalized by the pre-SE value for each animal during a three-minute recorded file during sleep (Animals with epilepsy are shown in blue and without epilepsy in red.)

Figure 3.

Power spectrum analysis of interpolated RR intervals for twenty three rats after SE (day 1, 3 and 7) normalized by the before SE value for each animal. Low frequency (LF, 0-0.04Hz) upper panel, and high frequency (HF, 0.15-0.4Hz) in lower panel. (Animals with epilepsy are shown in blue and without epilepsy in red.)

Figure 4.

ApEn of RR intervals measured in twenty three animals pre- and post-SE. The post-SE values were normalized by the pre-SE value for each animal (Animals with epilepsy are shown in blue and without epilepsy in red.)

Figure 5.

Extracted features after thresholding in a 3D graph for each characteristic. Some of the points overlaid each other (thus, the number of graph points is less than 23), we put the number of overlaid points next to that point, when two or more points from different groups overlaid each other the number from each group has shown with its own color. We classified those points in common between two different groups as without epilepsy. As seen, even a non-linear classification on each component only is unable to classify animals into two groups. (Animals without epilepsy in red, and with epilepsy in blue)

Figure 6.

PPV, sensitivity and specificity were measured in three groups of animals, 12 animals, 3 without epilepsy and 9 with epilepsy, 17 animals by adding 5 more animals to the previous group, in which 1 of them had epilepsy and 4 did not have epilepsy (in total, 4 without epilepsy and 8 with epilepsy), and finally adding 6 more animals, in which 2 of them were without epilepsy and 4 of them were with epilepsy (in total, 6 without epilepsy and 17 with epilepsy). As seen, all numbers increased monotonically as the number of subjects increased.