CALS HONORS THESIS

By Daniel Cordiner (dcordiner@ufl.edu)

THE EFFECTS OF INTERNAL DESYNCHRONIZATION ON SPONTANEOUSLY SEIZING RATS

Advisor: Dr. Sachin Talathi (talathi@ufl.edu)
Abstract

The aim of this research is to use internal desynchronization protocol to determine whether endogenous circadian activity in epileptic rats is altered. If there is an observable change in circadian activity as a result of epilepsy, this may provide evidence that the occurrence of seizure follows a circadian pattern, allowing the predication of future seizures based on other circadian biomarkers.

Core body temperature (CBT) was used as a biomarker for endogenous circadian activity in these experiments. The research uses an RF transducer implanted in the abdominal cavity of a rat to monitor body temperature and EEG is used to simultaneously monitor brainwave activity. It was determined that there was a difference in circadian rhythms in a healthy rat when compared to an epileptic rat. These results suggest that endogenous circadian rhythms are altered in an epileptic rat, which could have implications for seizure occurrence.

Introduction

Circadian rhythms are biological variations that repeat with cycles close to 24 hours. The timing of this near 24-hour period is controlled by the suprachiasmatic nucleus (SCN), a part of the brain located in the hypothalamus. It is also known that these rhythms are subject to entrainment by light cycles. This entrainment is associated with clock genes in the ventrolateral (VL) SCN, which interprets information received by the retina, whereas rhythms produced endogenously are associated with clock genes in the dorsomedial (DM) SCN. “Internal desynchrony” is created when the period of the circadian rhythm is different from that of the imposed light cycle. If this is the case, the
un-entrained circadian rhythm is called endogenous, because it is maintained entirely from within the organism and not by the light cycle.

This research was motivated by a study performed at the University of Barcelona. This study showed that it was possible to uncouple the endogenous circadian rhythm from the light induced circadian rhythm in an animal model (Cambras, 2007). Although this has previously been accomplished in humans by the experimenter modifying his own rest-activity behavior, it has proven difficult to accomplish the same results in animals. This research is focused on the observation of the core body temperature (CBT) circadian rhythms in a spontaneously seizing rat. CBT is known to be a reliable biomarker for circadian rhythms and understanding how circadian rhythms are decoupled in an epileptic brain may provide valuable insight into the nature of seizure-inducing disorders such as epilepsy.

**Material and Methods**

**Animals and Surgery.**

All experiments were approved by IACUC. Sprague Dawley rats were used for the experiments. The rats were housed individually in plastic containers as shown in Figure 4. All surgeries and analysis of CBT and electroencephalography (EEG) recordings were performed at the University of Florida.

All rats were housed in a room with an automatic light timer, which was set to 12 hours of light and 12 hours of dark during the control protocol (24-hr LD cycle) and 11 hours of light and 11 hours of dark during the internal desynchronization protocol (22-hr LD cycle) developed by Cambras et al (Cambras, 2007). During the dark hours, red light
was used to visually inspect the rats so as not to interfere with the experiments (rats cannot detect red light).

The rats were anesthetized and an RF transducer chip that records CBT and locomotor activity was surgically inserted into the abdomen. Screw electrodes were placed into the frontal and parietal lobes of the brain in addition to ground and reference screws. A bipolar stimulating electrode was placed into the CA1 hippocampus. After surgery, the rats were allowed to recover for one week.

**Inducing seizure**

The implanted bipolar electrodes were connected to a stimulator and the ventral hippocampus was electrically stimulated to induce status epilepticus. Stimulation continued until regular grade 5 seizures were observed. Grade 5 seizures are defined as an observation of the rat losing balance due to the intensity of the seizure. If two of these Grade 5 seizures are seen within the space of ten minutes then stimulation is ended. After stimulation, the rats were allowed to recover for 1-2 weeks. After this time, the rats were connected to an EEG for brain wave analysis. The EEG recordings, such as those seen in Figure 3 were then closely monitored for the appearance of spontaneous seizure. Video recordings were also used to confirm the EEG recordings. Once a seizure was confirmed, CBT recordings were initiated.

**Analysis of CBT**

The cages were placed onto an RF receiver, which recorded the signal coming from the RF transducer chip. The signal was sent to a computer using the VitaView software. Later the information was converted to actograms by MATLAB. The
actograms were then converted to periodograms, allowing circadian patterns to be viewed (see Figure 4).

**Timeline**

*Figure 6* shows the specific stages of the experiments. Following implantation of the headstage electrodes and an RF transducer, the rat was allowed to recover for one week and then control recordings (on an un-stimulated rat) began. The control 24-hr LD cycle lasts for 2 weeks and the control 22-hr LD cycle lasts for 3 weeks. (Empirically, it was determined that the circadian pattern was more consistent for the 22-hr protocol if allowed to continue for an extra week). When the control experiments were complete, the rat was stimulated and a latent period of roughly 2 weeks was observed. After this time, the rats began to spontaneously seize and the previous experiments were repeated on the now epileptic rats.

**Results**

**Control experiments**

*Figure 1* shows periodograms revealing the rhythmicity of CBT. The graph on the left shows the results of the 24-hr LD cycle. We can see a single CBT peak at 24 hours. Also looking at *Figure 1*, the graph on the right shows the effect of adjusting to the 22-hr LD cycle. We now see two peaks, one at 22 hours and another at approximately 25 hours.

**Seizure experiments**

*Figure 2* shows the same experiments as the controls except that the rats were spontaneously seizing (i.e. the rats were epileptic). The periodogram on the right shows
what happens to CBT in an epileptic rat under the 24-hr LD protocol. We see a single peak at 24 hours as in the control rats. When we adjusted to the 22-hr LD cycle, we now see three peaks at 22 hours, 25 hours and approximately 29 hours.

**Discussion**

This research can be broken down into two sets of results. This first set are the control experiments. These experiments were performed on rats with healthy brains and only the light cycle was manipulated. Looking at *Figure 1* we can see that with a 24-hr LD cycle (12 hours of light and 12 hours of dark) the rats CBT became fully entrained to the light cycle with the period almost exactly running at 24 hours. This would suggest that the length of day is somewhat responsible for maintaining the circadian rhythm of CBT. *Figure 1* also shows what happens when the length of day is changed to 22 hours (i.e. 11 hours of light and 11 hours of dark). We now see two distinct peaks of CBT. One of these peaks is at almost exactly 22 hours and corresponds to the light cycle. The other peak is at approximately 25 hours. This peak does not correspond to any exogenous influences such as light and seems to be generated endogenously, i.e. from within the rat’s brain. Why does shortening the light cycle by just two hours create two distinct rhythms of CBT? Free running endogenous circadian rhythms exhibits periodicity of about 25 hours. In the presence of a 24 LD cycle, the heterogeneity in the period of the driver oscillator (LD cycle) and the driven oscillator (endogenous circadian rhythm) is not large and thus the LD cycle is able to entrain the endogenous circadian rhythm to oscillate with period of about 24 hours. However as the heterogeneity in the
period of driver oscillator is increased, the entrainment between the driver and driven oscillator is weakened. As a result the we now see a peak in CBT rhythm at about 25 hours which is primarily due to its regulation by the un-entrained endogenous circadian pacemaker and another peak at 22 hours corresponding to the light induced periodicity in the CBT rhythm. This is evidence of internal desynchrony. The significance of this result is that we can isolate the endogenous rhythm and examine the effects of an epileptic brain on this rhythm.

The next set of results can be summarized by Figure 2. Here we see the effects on CBT of rats that are spontaneously seizing. The periodogram on the right corresponds with CBT for the 24-hr LD cycle. The graph looks very similar to the healthy control rat. We can see a period of 24 hours, which corresponds to the light cycle, indicating that as before the endogenous rhythm has been fully entrained to the period of the light cycle. It is only when we change the light cycle to 22 hours that we notice an interesting difference. Looking at Figure 2, we now see two peaks just as we did in the healthy control rats, one peak at 22 hours and another at 25 hours. However, this time there is a third peak at around 29 hours. It is possible that this change in circadian rhythms is responsible for the occurrence of seizure in epileptic rats, which would strengthen the hypothesized connection between epilepsy and circadian rhythms.

During the course of these experiments, several problems were encountered that hindered the data collection process. The first problem arose from the need to keep the rats connected to EEG monitor at all times via an implanted head stage. It occurred on more than one occasion that a seizing rat would dislodge the head stage, meaning we could no longer use that rat for additional data. That meant repeating the processes of
surgery, control experiments and stimulation to get back to where we were. As the control experiments took 4-6 weeks, this was quite a time consuming process. As a consequence of this, more time than was initially budgeted was required to repeat these experiments and confirm the results. Another confounding effect was the learning curve involved with brain and abdominal surgery. In addition to the technical aspects of surgery, there was also the procedural side outlined by Animal Care Services (ACS) involving the maintenance of maximum care and comfort of the animals.

Overall, the implications of this project suggest that internal desynchronization unmasks a change in the circadian rhythms of an epileptic rat. This could have implications on seizure occurrence, possibly allowing prediction of when seizures will occur based on other indicators such as body temperature. It has been suggested by previous researchers that there is a possible link between epilepsy and circadian rhythms (Talathi, 2009). This research strengthens that possibility by suggesting that the occurrence of seizure follows a circadian pattern.

**Citations**


Figures

**Figure 1: CBT recordings of control rat.**
This figure shows the CBT recordings for a control rat. 24-hr LD cycle (left) showing a single peak at 24 hours where the CBT is fully entrained by light. The 22-hr LD cycle (right) shows two peaks, the one at 22 hours corresponds to the LD cycle and the other at 25 hours is produced internally.

**Figure 2: CBT recordings of seizing rat.**
This figure shows the CBT recordings for a seizing rat. 24-hr LD cycle (right). There is still a single light-entrained peak at 24 hours. However under the 22-hr LD cycle (left), we now see three significant peaks, suggesting internal desynchronization alters the endogenous circadian rhythm of CBT.
Figure 3: A screen shot of electroencephalography (EEG) recordings from two rats. The first rat occupies channels 1-3. The channels correspond respectively to readings from the frontal lobe, electromyography (EMG) and the parietal lobe. Channels 4-6 are recordings from a different rat and correspond respectively to readings from the frontal lobe, electromyography (EMG) and the parietal lobe.

Figure 4: Experimental Setup. The experimental setup showing two rats connected through implanted headstages to comutators and finally to a PC to interpret the neural signal. CBT is also monitored via the RF receiver (top picture) and the signal is transmitted to a different computer (bottom picture) where the signal is interpreted. A CCD camera was used in conjunction with EEG to provide supportive video data for seizure confirmation.
Figure 6: Timeline for the experiments.
This shows a sample timeline for a complete control (naïve) and epileptic rat. It includes the recovery times from surgery and the “latent period” which is the time between electrical stimulation and when the rat begins to spontaneously seize.