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# Repeated amygdala-kindled seizures induce ictal rebound tachycardia in rats

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ABSTRACT

It is thought that cardiovascular changes may contribute to sudden death in patients with epilepsy. To examine cardiovascular alterations that occur during epileptogenesis, we measured the heart rate of rats submitted to the electrical amygdala kindling model. Heart rate was recorded before, during, and after the induced seizures. Resting heart rate was increased in stages 1, 3, and 5 as compared with the unstimulated control condition. In the initial one third of the seizures, we observed bradycardia, which increased in intensity with increasing stage and was blocked by injecting methyl atropine. During stage 5 seizures, a rebound tachycardia was observed that also increased in intensity with increasing number of seizures. This study demonstrated the influence of seizure frequency on cardiac autonomic modulation, providing a basis for discussion of potential mechanisms that cause patients with epilepsy to die suddenly.



## 1. Introduction

Epilepsy is the most common serious neurological condition, and its severity and seriousness in developing countries are well documented [1]. Furthermore, people with epilepsy are two- to threefold more likely to die prematurely as compared with people without epilepsy. The most common epilepsy-related category of death is sudden unexpected death in epilepsy (SUDEP) [2].

The exact pathophysiology of SUDEP remains unknown, but suspected mechanisms include cardiac arrhythmia, central and/or obstructive apnea, neurogenic pulmonary edema, and primary cessation of brain activity [3–6]. In fact, clinical seizures are often accompanied by intense autonomic changes, and there is evidence that sympathetic activity is increased even interictally in patients with temporal lobe epilepsy [6–8]. Ictal tachycardia is the more frequent arrhythmia [9,10], but ictal bradycardias, high-grade atrioventricular block, and asystole are also observed [6,11,12]. Curiously, ictal bradyarrhythmia appears to be associated with a higher risk for SUDEP [6]. Several case reports have described asystole or bradycardia during epileptic seizures and linked these conditions to a higher risk for SUDEP [13–16].

Animal models of epilepsy have provided a useful tool for studying the mechanisms underlying SUDEP [4,17–25]. Among these, the electric amygdala kindling model contains features that facilitate the study of cardiovascular adjustments associated with chronic epilepsies: the ictogenic zone is similar to that observed in temporal lobe epilepsy, development of seizure duration and intensity can be followed, and the number and time of occurrence of seizures can be tightly controlled; this model also has the advantage of enabling various procedures in unanesthetized, freely moving rats. In previous studies, Goodman et al. [21,22] described hypertension and a profound bradycardia in the amygdala kindling model. However, the impact of seizure frequency on these parameters and on resting heart rate was not evaluated.

We previously observed a correlation between number of seizures induced by electric kindling of the amygdala in rats and ictal tachycardia. When heart rate data during the seizure were analyzed, we observed a marked bradycardia and a rebound tachycardia. Interestingly, we found that the magnitude of the tachycardic component correlates positively with the number of seizures. In addition, resting heart rate was increased in rats with stage 5 seizures [26]. From this study, we sought to understand how the heart rate alteration occurs during the epileptogenetic process.

In the present study, we have examined the time course of peri-ictal and interictal features of heart rate modulation and tested whether peri-ictal heart rate regulation is related to the number of preceding seizures. Our results may help in understanding epilepsy-related alterations of cardiovascular properties and in developing a suitable animal model for SUDEP.

## 2. Methods

### 2.1. Animals

Adult male Wistar rats weighing 200–280 g were housed under environmentally controlled conditions (light/dark cycle with lights

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on from 07:00 to 19:00 hours, 22–24.8 °C) and permitted free access to food and water throughout the experiment. The procedures involving the animals and their care at the Experimental Neurology Laboratory of the Federal University of São Paulo (CEP Protocol 1873/09) conformed with the institution's guidelines, which comply with the International Ethical Guidelines for Biomedical Research [27].

### 2.2. Implantation of EEG and ECG electrodes and recording

Rats were anesthetized with ketamine (10 mg/kg, ip) and xylazine (4 mg/kg, ip) and placed in a stereotaxic instrument. A bipolar stimulating electrode of nichrome wire (100 μm) was implanted in the right amygdala, 2.5 mm posterior to bregma, 4.5 mm lateral to the midline, 8.5 mm below the surface of the brain. Two stainless-steel screws were implanted in the skull above the parietal region for EEG recording, and a third screw implanted in the frontal bone served as a ground.

The EEG recordings were obtained via an EEG analog recorder (Berger, TP.119; Brazil) connected to an amplifier (2K gain) and filtered (bandpass 0.03–40 Hz). Seizure duration was estimated from the time of stimulus delivery to cessation of ictal spikes on the EEG recording.

Electrocardiographic recordings were obtained with a pair of transcutaneous electrodes implanted under halothane anesthesia. However, often when animals reached stage 4 or 5, the intense muscle activity during seizures impaired ECG recordings. Therefore, rats were anesthetized with ketamine and xylazine, and stainless-steel spring electrodes (i.d. 0.25 mm, o.d. 0.5 mm), covered with polyurethane tubing except for the final 5–8 mm, were implanted just caudal to the diaphragm and in the mediastinum as described by Sgoifo et al. [28]. Electrodes were led subcutaneously to the back of the rat's neck and exteriorized.

The ECG signal was amplified and filtered (0.5–100 Hz bandpass, Model 12C 16OS, Grass Technologies, Quincy, MA, USA). Signals were sampled at 1000 Hz (Power Lab/8SP; AD Instruments, Melbourne, Australia). Heart rate was measured from the interval between R peaks on the ECG, with Chart 5 software (AD Instruments).

The ECG was recorded for 30–40 minutes to analyze heart rate variability (HRV). HRV was analyzed in time (SDNN and RMSSD) and frequency (total power, LF, HF, LF nu, HF nu, LF/HF) using Kubios 2.0 HRV software (Kuopio University, Finland). Spectral analysis was performed using the fast Fourier transform algorithm, on 512 RR frames with 50% overlap. Values of the frequency domain were (LF) 0.2–0.75 Hz and (HF) 0.75–3 Hz.

### 2.3. Kindling process

Before the start of the kindling process, the afterdischarge threshold was measured in each rat. The amygdala was stimulated at 60 Hz for 2 seconds with biphasic square pulses of 1-ms duration at a current of 60 μA. If no afterdischarge was evoked, additional stimuli were administered 10 minutes apart, but the stimulus current was increased by 2 μA every second stimulus until an afterdischarge was observed. This current was used for the entire kindling procedure. The kindling procedure consisted of daily stimuli given at approximately the same time of the day.

Seizure behavior was graded according to Racine [29] as follows: 0 = behavioral arrest, 1 = score 0 symptoms with chewing and eye blinking, 2 = score 1 symptoms with head nodding, 3 = score 2 symptoms with forelimb clonus, 4 = score 3 symptoms with rearing, and 5 = score 4 symptoms with falling. Animals were considered fully kindled after three stage 5 seizures [30].

### 2.4. Design of the experiments

Electroencephalographic electrodes were implanted. Ten days later, the ECG was recorded for 30–40 minutes. That same day the kindling process was started. During each session, rats were placed in the stimulus cage, EEG and ECG recordings were started, and about 5 minutes later, the stimulus was administered. Baseline (control) heart rate was calculated from the ECG during the final 30 seconds before electrical stimulation. The first 30 seconds after the end of the seizure was considered the postictal seizure recording.

Some rats received methyl atropine nitrate (1 mg/kg, ip, $n = 3$) 15 minutes before stimulation to block parasympathetic activity.

The periods considered for HRV analysis were: before kindling (control period), at the 5th stage 5 seizure (5th period), and at the 10th stage 5 seizure (10th period).

Both ictal heart rate pattern (Figs. 1 and 5) and resting heart rate (Fig. 2) of each kindling stage were calculated from the first two seizures in each stage per rat, to ensure that rats were not in a transition period between kindling stages.

### 2.5. Data analysis

The parameters analyzed were: seizure duration, basal heart rate, maximal increase in heart rate from baseline during seizure; maximal reduction in heart rate from baseline during seizure; latency from the start of stimulation to the minimum and maximum heart rate; and duration of bradycardia and tachycardia. We also calculated the extent of bradycardia and tachycardia as areas under the curve.

Data are presented as means ± SE. Statistical analysis was done with Prism 5.0 software (GraphPad, San Diego, CA, USA). To test if the number of seizures was related to seizure duration and heart rate variation, we used Pearson's correlation. Changes in heart rate and HRV were analyzed with repeated-measures ANOVA when more than two groups were compared or with the *t* test, Tukey test, or Bonferroni test for multiple comparisons. $P<0.05$ was considered to indicate statistical significance.

## 3. Results

Rats took 12 ± 5 amygdala stimulations to reach stage 5 ($n = 8$) and remained, on average, 2–3 days in each stage. Not all rats passed through each stage: for example, rats often went directly from stage 3 to stage 5. Once in stage 5, the rats remained in that stage.

No changes in heart rate were observed during measurement of afterdischarge threshold (Fig. 1). During seizures, an intense bradycardia, starting immediately after the stimulations, was observed. In the initial stages of kindling, bradycardia lasted throughout the seizure, but in stages 4 and 5, heart rate returned to baseline levels before the end of the seizure (Fig. 1).

The intensity of the bradycardic responses tended to increase as the kindling process progressed. The peak bradycardia was maximal when animals reached stage 5. The same was observed when analyzed by the area under the curve (AUC). Bradycardia duration was longer in stage 3 and seizure duration was longer in stages 3 to 5. Latency to peak bradycardia was similar in all stages (Table 1). During stage 5 seizures, the initial intense bradycardic response was often followed by a rebound tachycardia. Occasionally, animals in stage 5 exhibited spontaneous seizures following seizures induced by amygdala stimulation. The heart rate responses during these spontaneous seizures closely resembled those observed during electric amygdala kindling, that is, a strong and marked bradyarrhythmia.

Administration of methyl atropine to rats in stage 5 (1 mg/kg, iv, 15 minutes before stimulation) abolished the bradycardic response (Fig. 2). In fact, heart rate increased during the seizure in atropine-treated rats, suggesting that both parasympathetic and sympathetic activity increased during the seizure.

Resting heart rate tended to increase during the kindling process. Statistically significant (ANOVA, $F = 6.126$) increases were observed from 354 ± 8 beats/min (control) to 404 ± 17 beats/min (stage 1, $P<0.05$), 429 ± 19 beats/min (stage 3, $P<0.05$), and, maximally in

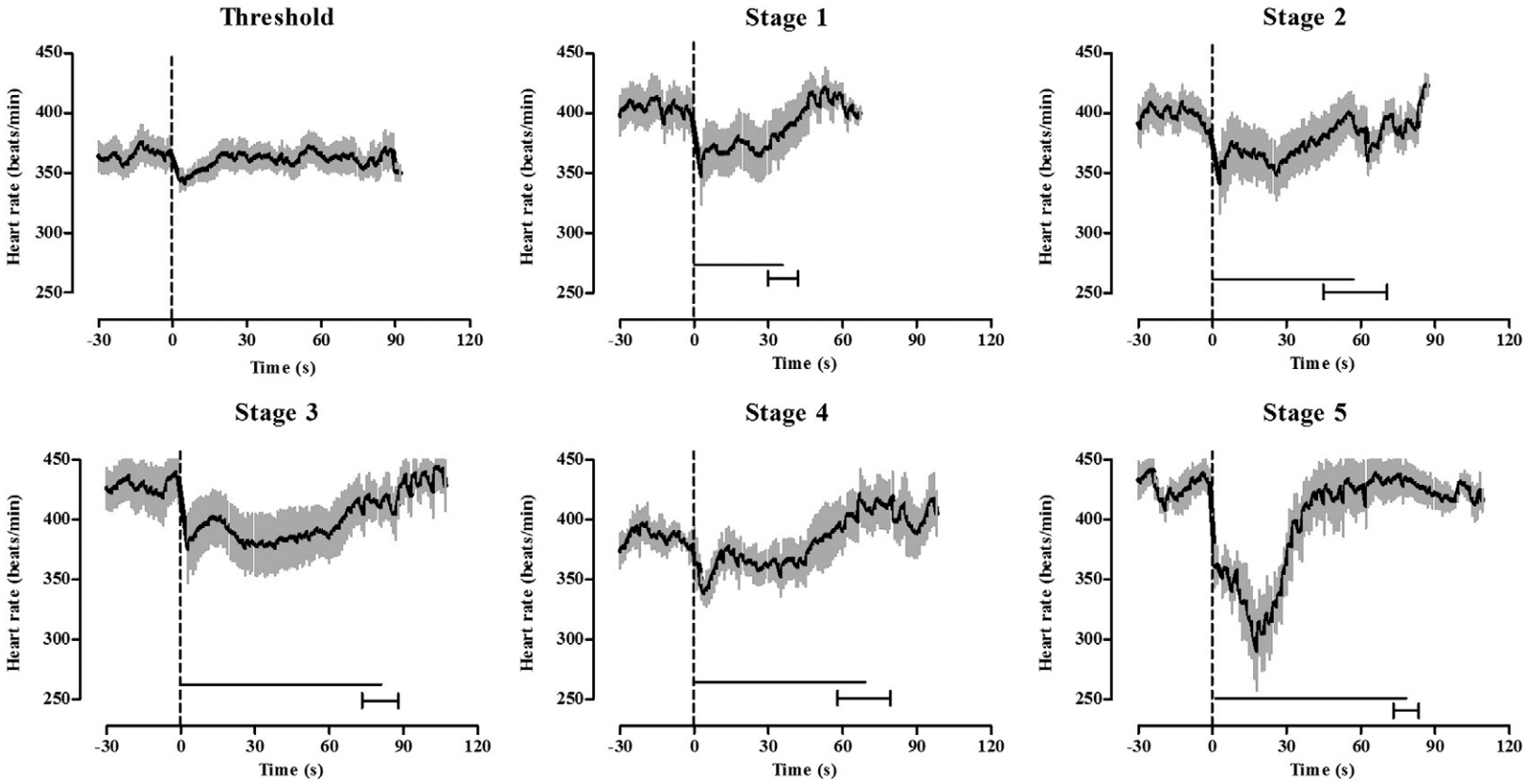


**Fig. 1.** Heart rate during afterdischarge threshold procedure and during seizures induced by stimulation of the amygdala throughout the process of kindling. The thick line represents the mean, and the shaded area, the SEM. The start of electrical stimulation is indicated by the vertical broken line. The horizontal line in the time axis represents the mean ± SEM of the seizure duration. Data were calculated from the first two seizures at each stage of each animal ($n = 16$ rats).

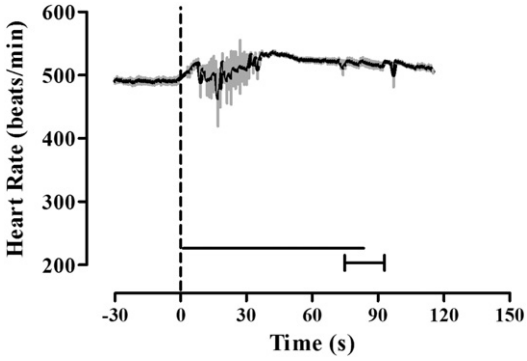


**Fig. 2.** Heart rate changes during stage 5 seizure after pretreatment with methyl atropine (1 mg/kg, ip, $n=3$). The thick line represents the mean, and the shaded area, the SEM. The start of electrical stimulation is indicated by the broken vertical line. The horizontal line in the time axis represents the mean ± SEM of seizure duration at each stage.

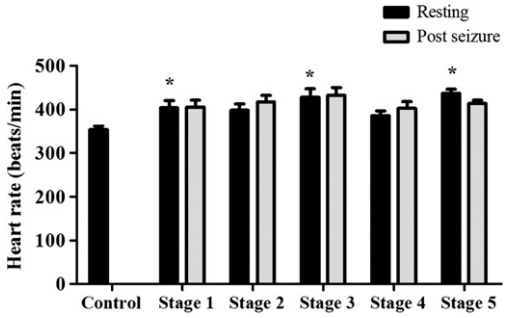


**Fig. 3.** Resting heart rate (mean ± SEM) before (black) and after (gray) seizures at various stages during electric kindling of the amygdala. *$P<0.05$ compared with control, Bonferroni's test ($n=8$ rats). Data were calculated from the first two seizures at each stage of each animal. Resting heart rate was the rate during the 30 seconds before stimulus delivery. Postseizure is the 30-second period after the end of the EEG seizure.

stage 5, 437 ± 9 beats/min ($P<0.05$) (Fig. 3). No significant changes in resting heart rate were observed after seizure cessation, as compared with the respective control period (Fig. 3).

Recently we reported that the rebound tachycardia is increased after repeated stage 5 seizures [26]. To further characterize the effects of repeated seizures on heart rate responses and baseline heart rate after full kindling, we compared data from initial and advanced stage 5 seizures (<10 seizures: $n=12$, 4 ± 2.4 seizures; ≥10 seizures: $n=10$, 15 ± 5 seizures).

Results obtained demonstrate that rats that had ≥10 stage 5 seizures had a lower resting heart rate (358 ± 10 beats/min, $P<0.05$) compared with rats that had <10 seizures (408 ± 13 beats/min). Additionally, as shown in Fig. 4, the HR pattern during seizures changed over the sequence of kindled stage 5 seizures. In animals with <10 seizures, HR reductions started immediately after the stimulations and the magnitude of bradycardia was maximal after 30 seconds of stimulus delivery. After that, a rapid return to resting levels was observed immediately after seizure cessation. On the other hand, in animals with ≥10 kindled stage 5 seizures, HR still decreased at the beginning of a seizure, reaching a minimum in the first 30 seconds, but immediately after that, an intense rebound tachycardia (~100 beats/min for 30 seconds) occurred that reached its maximum 60 seconds after stimulus delivery. HR still remained slightly above resting levels immediately after seizure cessation (387 ± 7 beats/min, $P<0.05$) compared with before the seizure. No statistical differences in seizure duration were noted between rats with <10 seizures and rats with ≥10 seizures (92 ± 6 and 96 ± 7 seconds, respectively).

This tachycardic response was measured as the maximal increase in heart rate and as the positive AUC. Results obtained demonstrated a positive correlation between number of seizures and maximal increase in heart rate (Pearson's $r=0.53$, $P<0.0001$) and between number of seizures and positive AUC (Pearson's $r=0.63$, $P<0.0001$). As expected, there was no correlation between number of seizures and seizure duration (Pearson's $r=0.21$, $P>0.05$).

Table 2 lists the values (means ± SEM) of HR parameters for the two groups. So, in accordance with Fig. 4, the group with ≥10 seizures had a significant increase in maximal positive HRV, AUC, and tachycardia duration. Indeed, no difference in the intensity of bradycardia was observed, although the bradycardic response was shorter and started earlier (20 seconds) in the group with ≥10 seizures.

Further, to confirm that these results were due to the recurrence of kindled stage 5 seizures and not to individual variability or differences during the kindling process, we compared the heart rate parameters during kindling progression (stages 1 to 5) in both groups (<10 seizures and ≥10 seizures). The results obtained demonstrated that the heart rate responses during kindling progression were similar in both groups, with an increasing bradycardic response from stage 1 to stage 5 (Fig. 5). Statistical analysis with two-way ANOVA demonstrated that, as described in the present study, kindling progression is associated with an increase in resting heart rate and increases in peak bradycardia and the bradycardic response (measured as the AUC) in both groups. However, there were no differences between the two groups with respect to the heart rate parameters analyzed (Table 3). These results indicate that the differences observed in the tachycardia components after recurrence (≥10) of stage 5 seizures are not due to differences in the kindling process.

There were no statistically significant differences in HRV time and frequency domains at the 5th and 10th stage 5 seizures, compared with the control period (Table 4).

## 4. Discussion

The main findings of this study are the following. Resting heart rate is increased by kindling of the amygdala. Bradycardic responses can be observed from the initial stages of the kindling process and increase progressively to a maximum in stage 5. Finally, once animals reach

**Table 1**
Heart rate changes during seizures induced by stimulation of the amygdala at various stages of the kindling process.

| Stage | $n$ | Number of seizures | Peak bradycardia (beats/min) | Latency to peak bradycardia (s) | Bradycardia AUC | Bradycardia duration (s) | Seizure duration (s) |
|---|---|---|---|---|---|---|---|
| 1 | 6 | 3 ± 1.8 | −86 ± 14 | 14 ± 4 | 1441 ± 450 | 30 ± 5 | 36 ± 5 |
| 2 | 7 | 2 ± 1.4 | −87 ± 10 | 19 ± 5 | 1870 ± 398 | 41 ± 7 | 51 ± 7 |
| 3 | 6 | 3 ± 2.4 | −105 ± 14 | 25 ± 6 | 3450 ± 875 | 66 ± 9[a] | 77 ± 6[a] |
| 4 | 5 | 3 ± 2.9 | −113 ± 27 | 22 ± 5 | 2808 ± 633 | 50 ± 3 | 72 ± 9[a] |
| 5 | 8 | 8 ± 3.1 | −253 ± 25[b] | 28 ± 3 | 4396 ± 446 [c] | 49 ± 4 | 80 ± 4[a] |

*Note*. Values are means ± SEM. ANOVA with post hoc Tukey test used for analysis.
[a] $P<0.05$ compared with stage 1.
[b] $P<0.05$ compared with stages 1–4.
[c] $P<0.05$ compared with stages 1 and 2.

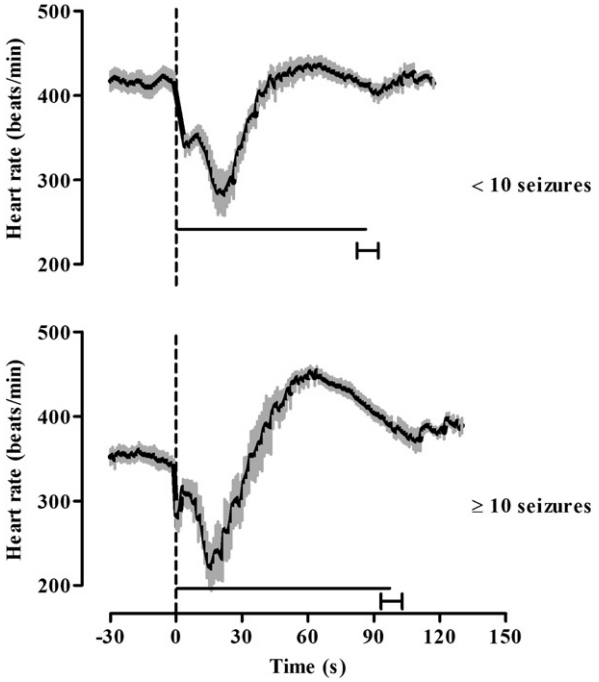


**Fig. 4.** Heart rate responses after <10 stage 5 (top) and ≥10 (bottom) stage 5 seizures. The thick line represents the mean, and the shaded area, the SEM. The start of electrical stimulation is indicated by the vertical broken line. The horizontal line in the time axis represents the mean ± SEM of seizure duration. Group with <10 stage 5 seizures: $n = 12$, 4 ± 2.4 seizures); group with ≥10 stage 5 seizures: $n = 10$, 15 ± 5 seizures).

full-blown stage 5 seizures, the repetition of these seizures seems to alter heart rate responses. In animals with more than 10 complete seizures, an initial bradycardia was followed by a sustained increase in heart rate until the end of the seizure.

We had already observed bradycardia in the first stage 1 seizures, unlike Goodman et al. [21], who reported bradycardia only from seizure 6 on, independent of generalization of the seizure. We found, like Goodman et al. [22], that peak bradycardia occurred during the first 30 seconds after stimulation and that the bradycardia was of parasympathetic origin, as it was suppressed with methyl atropine. In Goodman and colleagues' studies, like ours, parasympathetic activation during the seizure became more intense during the kindling process. A marked ictal bradycardia is a common finding in the kindling model and in other experimental models of epilepsy, including seizures induced with kainic acid [4] and pentylenetetrazole [31]. A similar response was observed in spontaneous seizures occurring in fully kindled stage 5 rats.

**Table 2**
Effect of repeated stage 5 seizures on ictal changes in heart rate.

| | Group with <10 seizures ($n = 12$, 4 ± 2.4 seizures) | Group with ≥10 seizures (n = 10, 15 ± 5 seizures) |
|---|---|---|
| Resting heart rate | 408 ± 13 | 358 ± 10[a] |
| Peak bradycardia (beats/min) | −247 ± 18 | −245 ± 13 |
| Peak tachycardia (beats/min) | 72 ± 7 | 130 ± 16[a] |
| Bradycardia AUC | 3928 ± 261 | 3091 ± 260 |
| Tachycardia AUC | 1905 ± 348 | 5765 ± 809[a] |
| Latency to peak bradycardia (s) | 29 ± 3 | 19 ± 2[a] |
| Latency to peak tachycardia (s) | 55 ± 5 | 49 ± 5 |
| Bradycardia duration (s) | 47 ± 3 | 30 ± 3[a] |
| Tachycardia duration (s) | 40 ± 5 | 64 ± 9[a] |
| Seizure duration (s) | 92 ± 6 | 96 ± 7 |

*Note*. Values are means ± SEM.
[a] $P<0.05$, Student's $t$ test.

A new finding of the present study is the demonstration that the profile of heart rate variations is changed by the number of stage 5 seizures in animals submitted to the kindling model, suggesting that repeated generalized seizures change the autonomic response. With recurring seizures, the bradycardic phase shortened, although peak bradycardia did not change. However, bradycardia was followed by a rebound tachycardia that subsided with the end of the seizure. To further characterize this effect, heart rate responses observed after ≥10 stage 5 seizures were grouped and compared with those observed with <10 stage 5 seizures. This division was based on a previous study by our research group [26] that showed correlation between tachycardia and number of stage 5 seizures. As reported preliminarily, we observed that the tachycardic component of the response was increased with recurrence of stage 5 seizures, and that the intensity of the preceding bradycardia did not change, although the duration of this bradycardia decreased. While the number of seizures used to divide the groups was somewhat arbitrary, it allows a more detailed analysis of the heart rate responses after repeated stage 5 seizures, and it shows that distinctive changes in heart rate responses can occur relatively rapidly. Analysis of the kindling process in both (≥10 and <10 stage 5 seizures) groups indicated a similar progression of seizures and heart rate responses until stage 5 was reached. Therefore, the increase in the tachycardic response observed after repeated seizures may be attributed to the recurrence of the seizures and not to individual differences and/or different progression of the kindling process.

It is conceivable that with extension of the number of seizures in the kindling model, the heart rate pattern would continue to change, with a further decrease in the duration of bradycardia and an increase in the duration and/or magnitude of tachycardia, a response more similar to the response typically seen in patients with epilepsy. Of note, individuals analyzed in clinical studies on SUDEP typically had a long history of epilepsy or had refractory epilepsy and, therefore, had experienced a large number of seizures.

It seems unlikely that this tachycardia was due to more intense motor activation-initial stage 5 seizures and later stage 5 seizures were similar in duration, and motor activity did not obviously increase with repeated seizures once the rats were in stage 5. In addition, Sakamoto et al. [4] showed that tachycardia can also be observed in seizures not accompanied by motor activity. These researchers observed tachycardia and bradycardia during seizures induced with kainic acid in anesthetized rats. Therefore, it seems that the changes in heart rate response are a consequence of changes in the central mechanisms that control the autonomic nervous system. It seems that sympathetic activation during the seizure became more intense with increasing chronicity of the epileptic condition.

Interestingly, our data indicate that both groups are similar with respect to the maximum heart rate reached during rebound tachycardia. Indeed, although peak tachycardia is stronger in the group with ≥10 seizures, because this group had a lower resting heart rate, during seizures both groups reached a similar maximal heart rate (≈450 beats/min), suggesting that perhaps maximal heart rate is endogenously limited. Nevertheless, it is noteworthy that the duration and the amplitude of ictal tachycardia (measured as the AUC) were also increased in the group with ≥10 seizures, suggesting that the increased tachycardic response was not only due to a lower basal heart rate.

Accordingly, patients who died of SUDEP had cardiac microlesions, and it has been suggested that recurrent seizures may induce coronary vasospasm and myocardial damage as a result of sympathetic activation [32]; it is noteworthy that these patients had intractable epilepsy. Also, patients with refractory temporal lobe epilepsy who presented with episodes of asystole had signs of degeneration of postganglionic cardiac sympathetic fibers, and this

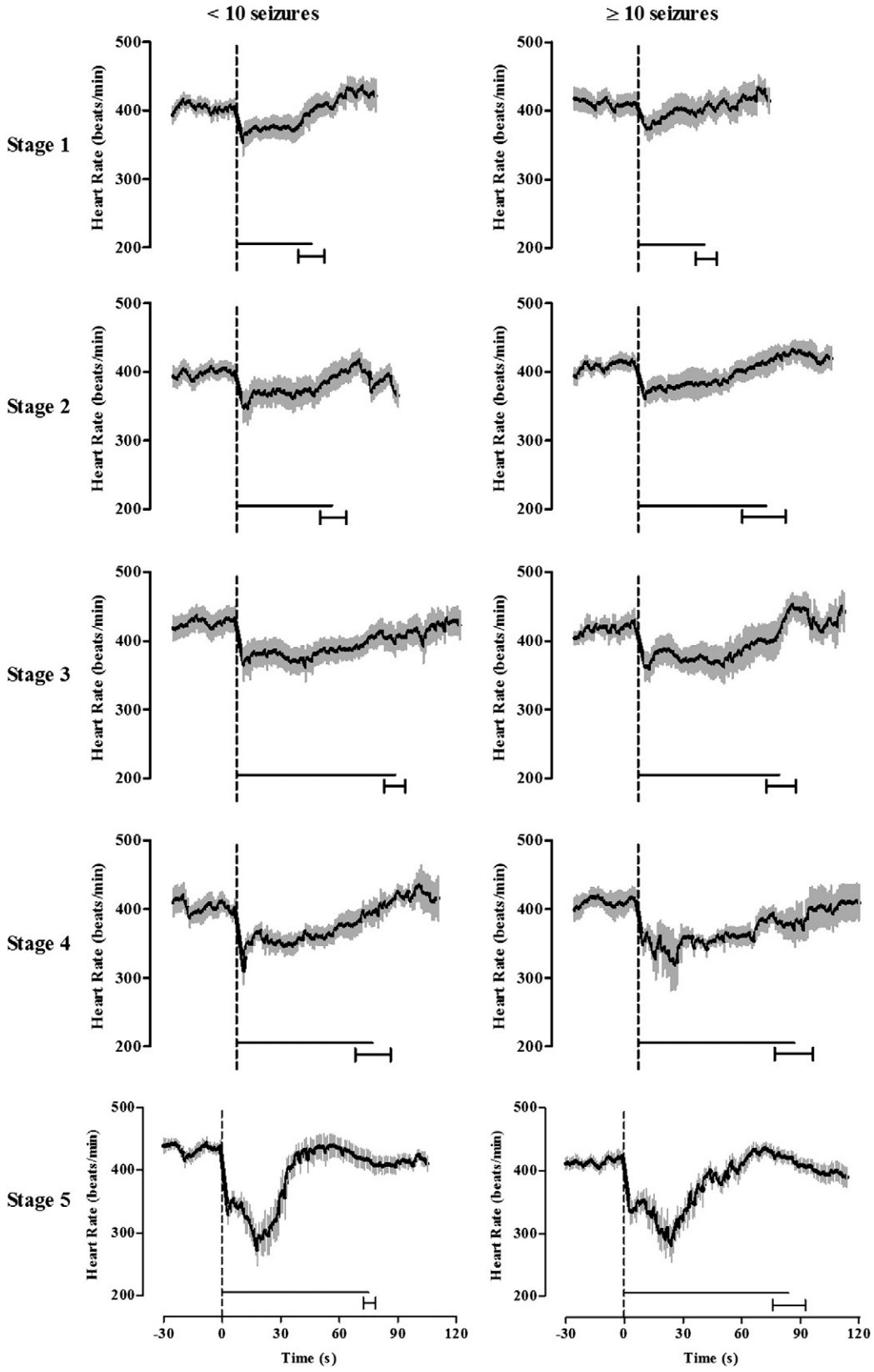


**Fig. 5.** Heart rate during seizures induced by amygdala kindling progression in both groups: <10 stage 5 seizures (left) and ≥10 (right) stage 5 seizures. The thick line represents the mean, and the shaded area, the SEM. The start of electrical stimulation is indicated by the vertical broken line. The horizontal line in the time axis represents the mean ± SEM of seizure duration. Data were calculated from the first two seizures at each stage of each animal. Group with <10 stage 5 seizures: $n = 12$; group with ≥10 stage 5 seizures: $n = 10$.

degeneration may be due to excessive sympathetic activation during seizures [6]. It was suggested that cardiac sympathetic damage may potentiate asystole and may lead to SUDEP [6]. However, it is also possible that sympathetic overactivity itself may be the cause of SUDEP. Prolonged studies with animal models of epilepsy, such as the amygdala kindling model, may elucidate this important aspect.

**Table 3**
Heart rate during kindling stages in groups with different number of seizures.

| | Group | Control | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 5 |
|---|---|---|---|---|---|---|---|
| Resting heart rate (beats/min) | <10 seizures | 342 ± 6 | 415 ± 13[a] | 399 ± 22 | 418 ± 22[a] | 406 ± 23[a] | 435 ± 9[a] |
| | ≥10 seizures | 351 ± 7 | 417 ± 12[a] | 411 ± 10[a] | 422 ± 13[a] | 416 ± 13[a] | 406 ± 8[a] |
| Peak bradycardia (beats/min) | <10 seizures | | −59 ± 16 | −75 ± 8 | −106 ± 14 | −99 ± 16 | −214 ± 22[b] |
| | ≥10 seizures | | −51 ± 9 | −60 ± 9 | −77 ± 16 | −150 ± 40[b] | −193 ± 17[b] |
| Peak tachycardia (beats/min) | <10 seizures | | 17 ± 6 | 57 ± 10 | 32 ± 5 | 34 ± 16 | 40 ± 13 |
| | ≥10 seizures | | 32 ± 4 | 46 ± 6 | 24 ± 5 | 15 ± 2 | 35 ± 9 |
| Bradycardia AUC | <10 seizures | | 1042 ± 406 | 1551 ± 386 | 3014 ± 592 | 2676 ± 948 | 4180 ± 337[b] |
| | ≥10 seizures | | 864 ± 464 | 1545 ± 408 | 2579 ± 1001 | 3650 ± 703[b] | 3793 ± 494[b] |
| Tachycardia AUC | <10 seizures | | 55 ± 45 | 159 ± 79 | 105 ± 59 | 335 ± 229 | 713 ± 445 |
| | ≥10 seizures | | 201 ± 116 | 419 ± 246 | 27 ± 13 | 17 ± 13 | 430 ± 184 |
| Latency to peak bradycardia (s) | <10 seizures | | 9 ± 4 | 12 ± 4 | 15 ± 5 | 18 ± 4 | 23 ± 3 |
| | ≥10 seizures | | 10 ± 3 | 26 ± 11 | 18 ± 9 | 30 ± 7 | 24 ± 2 |
| Latency to peak tachycardia (s) | <10 seizures | | 24 ± 14 | 37 ± 15 | 63 ± 15 | 51 ± 15 | 53 ± 8 |
| | ≥10 seizures | | 21 ± 2 | 72 ± 16[b] | 63 ± 9 | 69 ± 3 | 61 ± 7[b] |
| Bradycardia duration (s) | <10 seizures | | 29 ± 6 | 38 ± 9 | 59 ± 9[b] | 38 ± 9 | 54 ± 6 |
| | ≥10 seizures | | 23 ± 6 | 42 ± 8 | 60 ± 7[b] | 67 ± 9[b] | 62 ± 8 [b] |
| Tachycardia duration (s) | <10 seizures | | 5 ± 3 | 5 ± 2 | 6 ± 2 | 14 ± 9 | 17 ± 6 |
| | ≥10 seizures | | 10 ± 5 | 16 ± 7 | 4 ± 2 | 4 ± 3 | 21 ± 4 |
| Seizure duration (s) | <10 seizures | | 35 ± 6 | 45 ± 6 | 74 ± 5[b] | 64 ± 8[b] | 75 ± 3[b] |
| | ≥10 seizures | | 31 ± 5 | 60 ± 10 | 66 ± 7 | 73 ± 9[b] | 84 ± 8[b] |

*Note.* Group with <10 stage 5 seizures: $n = 12, 4 \pm 2.4$ seizures); group with ≥10 stage 5 seizures: $n = 10, 15 \pm 5$ seizures. Control: before kindling process for each group. Values are means ± SEM. Two-way ANOVA, followed by Bonferroni post hoc test was used for analysis.
[a] $P<0.05$, compared with its control.
[b] $P<0.05$, compared with stage 1.

We have previously reported evidence of autonomic disturbance in rats with epilepsy induced with pilocarpine [33]. In these rats, as in the current study, resting heart rate was elevated. However, in Langendorf preparations with isolated hearts of epileptic and control rats, heart rates were similar, suggesting the increased heart rate in intact epileptic rats was due to a change in autonomic cardiac control [33]. Such changes may increase the probability of SUDEP.

In a previous study with a kindling model, we also observed an increase in resting heart rate in kindled rats, compared with the period before beginning stimulation. Similarly, we showed that the implantation of amygdala electrodes did not alter resting heart rate [26].

An interesting finding of the present study was that resting heart rate was lower in the group with repeated (≥10) stage 5 seizures. This is somewhat unexpected as we observed an increase in resting heart rate through stages 1 to 5 during the kindling process, and once the animals were submitted to recurrent seizures, the tachycardic component of the seizure increased. Previous studies have found that repeated seizures in temporal lobe epilepsy cause signs of increased sympathetic activity during the interictal period, but rarely have changes in resting heart rate been reported [6–8]. Mukherjee et al. [34] described evidence of higher sympathetic tone and lower parasympathetic tone in patients with refractory epilepsy compared with those with well-controlled epilepsy, but did not find any differences in resting heart rate. Previous studies using kindling-induced seizures also did not report changes in resting heart rate in stage 5 [21,22].

Therefore, both increased resting heart rate during kindling stages and reduced resting heart rate after repeated stage 5 seizures suggest that alterations in resting heart rate occur only during epileptogenesis. If, and how, such changes predispose to SUDEP remains unknown.

Although our data indicate changes in resting HR, we did not find significant differences in HRV at the 5th and 10th stage 5 seizures, compared with the control period. However, one limitation of our study was the small sample size ($n = 5$ rats), a significant factor in HRV studies. It is possible that analysis of a larger population may indicate changes in autonomic modulation of the HR. It should also be pointed out that the epilepsy history of the rats used in our study is much shorter and the number of seizures lower than in patients with epilepsy evaluated in clinical studies of HRV. In patients too, the duration of the epilepsy condition may influence autonomic activity, as Persson et al. [35] failed to find changes in HRV in patients with newly diagnosed epilepsy.

In summary, our experimental study suggests that the tachycardia occurring in stage 5 may potentially reflect autonomic changes that could predispose to SUDEP. That is, although no differences were found in interictal HRV, we do not reject that this tachycardia is a consequence of sympathetic activation, which could predispose to abnormal cardiac repolarization, and may be one of the causes of SUDEP [36]. The study of the cardiovascular consequences of chronic epilepsy with animal models of chronic epilepsy, including kindling, may help identify the mechanisms that underlie SUDEP.

**Table 4**
Heart rate variability at different time points of stage 5 kindling seizure.

| | *P* value | Control (before kindling) | 5th stage 5 seizure | 10th stage 5 seizure |
|---|---|---|---|---|
| SDNN ($ms^2$) | 0.9 | 6.4 ± 0.7 | 5.9 ± 0.5 | 6.4 ± 1.1 |
| RMSSD ($ms^2$) | 0.9 | 7.7 ± 1.1 | 7.4 ± 0.8 | 8.3 ± 1.8 |
| Total power ($ms^2$) | 0.7 | 63.6 ± 25.1 | 42.0 ± 11.2 | 50.8 ± 28.6 |
| LF ($ms^2$) | 0.6 | 20.8 ± 8.2 | 13.9 ± 5.0 | 11.8 ± 5.7 |
| HF ($ms^2$) | 0.7 | 33.9 ± 15.4 | 21.4 ± 5.9 | 33.7 ± 22 |
| LFnu (%) | 0.7 | 36.4 ± 3.5 | 35.0 ± 2.0 | 31.4 ± 5.6 |
| HFnu (%) | 0.7 | 63.7 ± 3.5 | 65.0 ± 2.0 | 68.6 ± 5.6 |
| LF/HF | 0.4 | 0.64 ± 0.1 | 0.57 ± 0.1 | 0.51 ± 0.1 |

*Note.* $n = 5$ for all groups. Values are means ± SEM. Repeated-measures ANOVA was used for analysis. SDNN, standard deviation of all RR intervals; RMSSD, square root of the mean squared differences of successive RR intervals; LF, low frequency; HF, high frequency.

## Ethical approval

We have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

## Conflict of interest statement

None of the authors has any conflict of interest to disclose.

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