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Impact of repeated kindled seizures on heart rate rhythms, heart rate variability, and locomotor activity in rats

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ABSTRACT

Although an impact of epilepsy on circadian rhythmicity is well-recognized, there are profound gaps in our understanding of the influence of seizures on diurnal rhythms. The effect on activity levels and heart rate is of particular interest as it might contribute to the disease burden. The kindling model with telemetric transmitter implants provides excellent opportunities to study the consequences of focal and generalized seizures under standardized conditions.

Data from kindled rats with generalized seizures revealed an increase in activity and heart rate during the resting phase. Total and short-term heart rate variabilities were not affected by electrode implantation or seizure induction.

Ictal alterations in heart rate associated with generalized seizures were characterized by a biphasic bradycardia with an immediate drop of heart rate followed by a transient normalization and a second more steady decrease.

In conclusion, the findings demonstrate that once daily generalized seizures can exert significant effects on heart rate rhythms. Respective alterations in patients would be of relevance for patient counselling and therapeutic management. Occurrence of biphasic bradycardia associated with seizure induction suggests that the kindling model is suitable to study the consequences and the prevention of ictal bradycardia, which may pose patients at risk for sudden unexpected death.

1. Introduction

It is well-accepted that a bidirectional relationship exists between circadian rhythmicity and epileptic seizures [1–4]. Whereas the 24-h rhythmicity of seizure activity has been studied in various epilepsy types and syndromes [5–8], there is still a paucity of knowledge about the impact of seizures on physiological circadian rhythms. Studies have indicated that specific epilepsy types can favor a selected chronotype in affected patients [9–12]. Moreover, evidence exists that sleep patterns can be tremendously altered in patients with epilepsy [13]. A reduction in circadian heart rate variability has also been reported in patients [14,15]. Clinical studies need to consider a bias by antiepileptic drug treatment [16]. Moreover, it is impossible to study the impact of single seizures in a standardized manner considering the uncontrolled occurrence of seizure activity. The same limitation holds true for animal models with spontaneous seizure activity. In respective models, hyperactivity with or without phase shifts has been reported in previous studies [17–19].

Further knowledge about the exact impact of seizures on circadian rhythmicity is of particular interest for several reasons. On one hand, alterations in diurnal rhythms can contribute to the burden of epilepsy affecting quality of life in patients [20,21]. In addition, respective alterations should be considered for disease management including therapy and patient counselling [16].

In experimental animals, the induction of seizures or epilepsy and its consequences must be considered for severity assessment as a presupposition for the ethical cost–benefit considerations by the authorities [22,23]. Alterations in circadian rhythmicity can affect the animals wellbeing [24]. On the other hand, model-associated stress can influence diurnal rhythms as indicated by data from exposure of laboratory rodents to stressful situations [25–29].

The kindling model represents a frequently used chronic model with a high predictive validity for the management of focal onset seizures [30,31]. Kindled seizures can be elicited in a controlled manner by stimulation via an implanted depth electrode. Characteristically, seizures evolve in duration and severity with repeated stimulations [32].

Kindling offers the opportunity to study the influence of single seizures on diurnal rhythms of activity and heart rate in a highly standardized manner. The progression associated with continued stimulations renders a basis for the separate study of the impact of focal seizures

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during the early kindling phase and of chronic seizures during the late kindling phase. In our study, we compared data from amygdala-kindled animals with focal or generalized seizures with those from control animals, which only received the telemetric transmitter implants, and with those from sham animals with telemetric transmitters, electrode implants, and handling procedures but no seizure induction. Based on this study design, we addressed the hypothesis that once daily kindled seizures exert a relevant impact on heart rate rhythms, heart rate variability, and on circadian patterns of locomotor activity. Datasets obtained provide information about an influence of depth electrode implantation with different duration as well as an influence of focal and generalized seizures.

2. Materials and methods

2.1. Animals

Investigations were in line with the German Animal Welfare act and the EU directive 2010/63/EU and carried out according to the Basel declaration as well as the replace, reduce, refine (3R) concept. Procedures and reporting comply with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. The study was approved by the Government of Upper Bavaria (reference number 55.2-1-54-2532-105-16). Female Sprague Dawley rats (Envigo, the Netherlands; n = 23; 200–224 g; corresponding to an age range of 10–11 weeks) were kept individually in macrolon cages type III (embedding: Lignocel® Select, J. RETTENMAIER & SÖHNE GmbH + Co KG, Germany; nesting material: 14 g; Enviro Dri®, Claus GmbH, Germany) under controlled environmental conditions (20–24 °C, 45–65% humidity, 12-h dark/light cycle [light from 6:00 a.m.–6:00 p.m.]) with freely available tap water and food (ssniff R/M Haltung, ssniff Spezialdiäten GmbH, Germany). New bedding and nesting material was provided once per week. Group classification was allocated randomly (https://www.randomizer.org/). Every attempt was made to minimize pain and discomfort and to reduce the number of animals used in the study. The Grimace Scale [33] and a modified version of the Irwin scale [34] were applied daily for health assessment. After an adaption phase of at least one week, twenty-three animals were implanted with a telemetric transmitter. Thirteen animals were additionally implanted with an electrode. Six of the electrode-implanted animals underwent the kindling procedure. Thus, the study design was based on three experimental groups all with telemetry transmitter implants: “Kindled”, animals undergoing the kindling procedure initially leading to focal seizures and eventually generalized seizures (n = 6), and two groups for comparison: “Sham” with a depth electrode implant (n = 6) and “Control” without any further intervention (n = 6). Two animals had to be euthanized because of the loss of their electrode assemblies; two animals died during the anesthesia, and one animal was excluded prior to data analysis based on a large number of artifacts in the electrocardiogram (ECG) signal. At the end of the experiments, animals were sacrificed by pentobarbital injection (600 mg/kg i.p., Narcoren®, Merial GmbH, Germany).

2.2. Electrode and telemetric transmitter implantation

Implantation of the electrode (teflon-insulated bipolar stainless steel, diameter 0.45 mm) and the telemetric transmitter (ETA-F10 transmitters, Data Sciences International, USA) was performed under general anesthesia (chloral hydrate, 360 mg/kg, intraperitoneal). Pain management included the analgesic meloxicam (Metacam®, Boehringer-Ingelheim, Germany, 1 mg/kg, 30 min pre- and 24 h postsurgery, subcutaneous). Additionally, bupivacaine (Bupivacain, 0.5%, Jenapharm, Germany, subcutaneous) was locally administered at the implantation site of the telemetric transmitter and bupivacaine with epinephrine (Bupivacain, 0.5% with epinephrine 0.0005%; Jenapharm, Germany, subcutaneous) at the implantation site of the electrode-implant. Antibiotics (marbofloxacin, Marbocyl FD 1%, Vétoquinol, Germany, 1 mg/kg, subcutaneous) were administered twice per day for eight days starting one day before surgery. The implantation of the electrode into the right basolateral amygdala was performed as previously described by Walker et al. [35]. Stereotaxic coordinates were measured relative to bregma (anterior posterior (AP) - 2.2 mm, lateral (L) + 4.7 mm, and dorsoventral (DV) - 8.5 mm) using the atlas of Paxinos and Watson [36]. For recordings of ECG and locomotor activity, telemetric transmitters were implanted subcutaneously at the left ventral abdomen according to the recommendations of the supplier. The negative telemetry lead was tunneled subcutaneously and placed at the right thorax at the level of the shoulder blade. The positive telemetry lead was placed one-centimeter lateral to sternum at the level of the left rib bow. The leads were attached intramuscularly and fixed with nonabsorbable suture material (Daclon USP 5/0 EP 1, Monofilament black nylon, SMI AG, Belgium).

2.3. Kindling procedure

The kindling procedure was performed as previously described by Russmann et al. [37]. Prekindling afterdischarge threshold (ADT; spikes: frequency of at least 1 Hz, amplitude of at least twice the baseline activity) was determined after a postoperative recovery phase of two weeks. The average initial ADT was 192.5 (standard deviation [SD]: 143.8), which is within the expected range, indicating that the electrode was implanted in the correct location. The kindling procedure consisted of a fixed supra-threshold stimulation of 580 μA once daily (1:00 p.m.–3:00 p.m.) on five days per week. Racine's scale [38] was used to score seizure severity. In addition, seizure duration and afterdischarge duration were recorded.

2.4. Data acquisition

Cages were placed on receiver plates (RPC-1, Data Sciences International, USA) and measurements were processed using Ponemah® Software 5.20 (Data Sciences International, USA). Telemetric transmitters were turned on using a magnet (Data Sciences International, USA). Data were continuously collected and stored in segments of 5 min for activity and long-term ECG or every 1 s for short-term ECG with a sampling frequency of 1000 Hz. Telemetric recordings were performed at three time points throughout the experiments: two weeks after the implantation at two consecutive days prior to the start of the kindling phase (postsurgical phase), during the phase of the focal kindled seizures at three consecutive days, and during the phase of the generalized kindled seizures at three consecutive days. The three analyzed days during the phase of the focal kindled seizures comprised recordings during the first, until the third day of stimulation; the number of animals in this group decreased throughout these three days (stimulation 1 = 6, stimulation 2 = 5 and stimulation 3 = 3) as some animals already developed generalized (stage 5) seizures and therefore were excluded from the focal kindling group. Stimulation of animals that developed generalized seizures was interrupted and only resumed once all animals developed generalized seizures at the fifth stimulation day. Because of this, all animals experienced the same number of generalized seizures during the following stimulation days. The three analyzed days during the phase of the generalized kindled seizures comprised recordings during the 11th until the 13th day of stimulation. Until the 11th stimulation day, all animals had a total of six generalized seizures, meaning that during the recorded days animals experienced their seventh until ninth generalized seizure.

Additionally, ECG was recorded during the kindling process 5 min before and after the electric stimulus. Sham animals were continuously recorded over 10 min during this experimental phase. The ECG could not be evaluated in one sham animal because of connection problems of the telemetric transmitter. Video monitoring (Axis M1144-L Network camera, Axis Communications AB, Sweden) was performed once per hour to score the complexity of the nests. The shape of the nesting material was evaluated by a scoring system (0 = not touched/destroyed,

1 = flat, 2 = slightly curved, 3 = deep) according to Van Loo and Baumans [39].

2.5. Data analysis

Parameters were measured and calculated using Ponemah® Software 6.30 (Data Sciences International, USA). The activity was calculated in counts per minute (cpm). Activity counts are generated by the base plate containing the antennas recording the telemetric implant, as the animals move the signal strength varies in strength, when the signal strength changes by a certain amount an activity count is generated. The number of counts was generated dependent on both distance and speed movement. Additionally, heart rate, interbeat interval (NN-I), standard deviation of interbeat intervals (SDNN), square root of the mean squared differences of successive NN intervals (RMSSD), number of interval differences of successive NN intervals greater than 9 ms (NN9), and a proportion derived by dividing NN9 by the total number of NN intervals (pNN9) were calculated. The mean was calculated separately for the dark and the light phase. For the measurements in the postsurgical phase, the average value for each phase was calculated and used as a baseline for statistical analysis. Regarding statistical calculations, the light phase was shortened by approximately 2 h because of the absence of the animals for the daily kindling procedure. For the analysis of the short-term measurements, the heart rate was calculated.

2.6. Statistics

Statistical analyses were performed using GraphPad Prism (Version 5.04; GraphPad, USA). For the statistical analysis of the long-term measurements in the postsurgical phase, an unpaired *t*-test was performed to compare control, sham, and kindled animals. For the analysis of the phases of focal and generalized kindled seizures, a two-way repeated measures analysis of variance (ANOVA) with Bonferroni posthoc test was used to compare the groups. Statistics were performed separately for the dark and the light phase. R version 3.3.2 [40] and R package ggplot2 [41] were used to present the results. To prevent distortion of the boxplot graphs, the axes were fixed among all graphs within one figure, which results in a cutoff of extreme values in the boxplots in selected figures. Please note that the data are still present in the statistics and the calculation of the boxplots. Smoothing of line graphs in Figs. 1, 3, 4 and 5 are based on a Loess regression using a span of 0.15.

3. Results

3.1. Impact of repeated kindled seizures on activity

Telemetric recordings during different experimental phases provided information about home cage activity levels (Fig. 1) during the resting phase (lights on/day) and the activity phase (lights off/night). In the postsurgical phase, electrode-implanted rats (=sham rats and kindled rats before initial stimulation) exhibited increased activity levels during light and dark phases (Fig. 1a and b). During the dark phase this difference proved to be more pronounced with the mean activity of electrode-implanted rats exceeding that in control rats by 68%. In all groups, the dark phase proved to be characterized by at least two activity peaks: the first one occurring following lights off, and the second one toward the end of the dark phase (Fig. 1a). During the 2nd night an additional activity peak was observed in the middle of the dark phase.

Recordings during the next experimental phase did not reveal an impact of focal kindled seizures on home cage activity levels (Fig. 1c and d). Moreover, prolonged electrode implantation failed to affect activity. Mean activity levels proved to be in the same range in all groups.

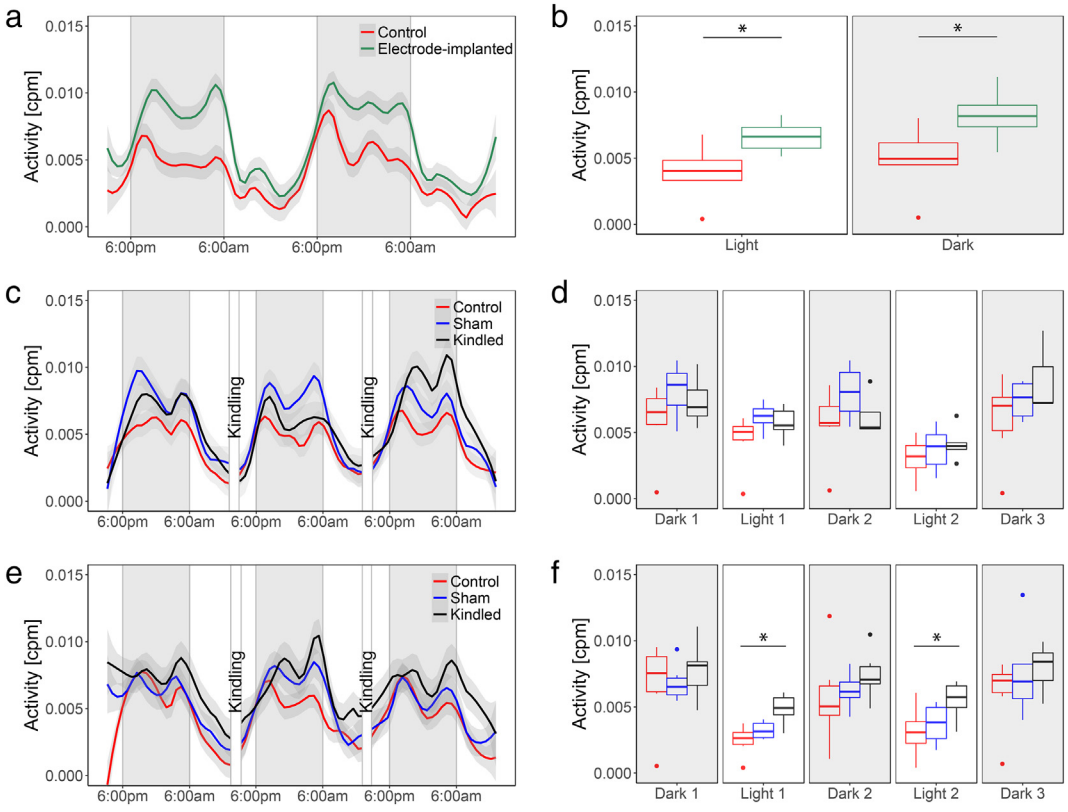

Fig. 1. Effect of surgery and kindling on locomotor activity. Locomotor activity (control and kindled animals: n = 6, sham animals: n = 6) is shown for the postsurgical phase (a and b), the phase of focal kindled seizures (c and d), and the phase of generalized kindled seizures (e and f). In the postsurgical phase, the activity of the electrode-implanted group exceeded the activity of the control group during the light and the dark phase (b). In the phase of generalized kindled seizures, the decrease in activity during the light phase proved to be less pronounced in kindled rats (f). Significant differences are indicated by asterisks ($P < 0.05$). The group size of the kindled animals differed at day two and day three of the phase of focal kindled seizures as some animals already exhibited generalized seizures: day two n = 5, day three n = 3. Activity is shown as counts per minute [cpm].

In contrast, an impact of generalized kindled seizures on the resting phase became evident during the later phase of the kindling paradigm (Fig. 1e and f). Activity levels rapidly dropped in control and sham rats once lights were switched on. The decrease in activity proved to be less pronounced in generalized kindled rats. In this group, activity remained at significantly increased levels during the light phase. As the kindling procedure was performed once daily between 1:00 p.m. and 3:00 p.m., data were analyzed separately for the morning and the afternoon phase (Fig. 2). Interestingly, increased activity levels in generalized kindled rats were not only obvious following the kindling stimulation in the afternoon but also before daily seizure induction (Fig. 2a and b). Sham rats, which were transported to the laboratory and handled in the same way as kindled rats except for the stimulation, did neither differ from control nor kindled rats.

Rodent activity levels can be reflected by a diurnal course in nest complexity levels [42]. Thus, we have additionally analyzed nest complexity with 1-hour intervals from 3:00 p.m.–12:00 a.m. following surgery and in the phase with generalized kindled seizures (Suppl. Fig. 1). Nest complexity scores and their circadian rhythms did not differ between experimental groups.

3.2. Impact of repeated kindled seizures on heart rate

To assess the influence of once daily seizures on heart rate rhythms, we analyzed mean heart rates (Fig. 3) and the equivalent normal-to-normal interbeat interval (NN-I intervals; Suppl. Fig. 2). Following electrode implantations, mean heart rates (Fig. 3a and b) proved to be in a comparable range as in rats that only received a telemetric transmitter implant. During the dark phase two peaks in heart rates became evident in all groups: the first one was observed following lights off and the second one toward the end of the dark phase. Analysis of heart rates (Fig. 3c and d) during phases with daily kindled seizures did not confirm

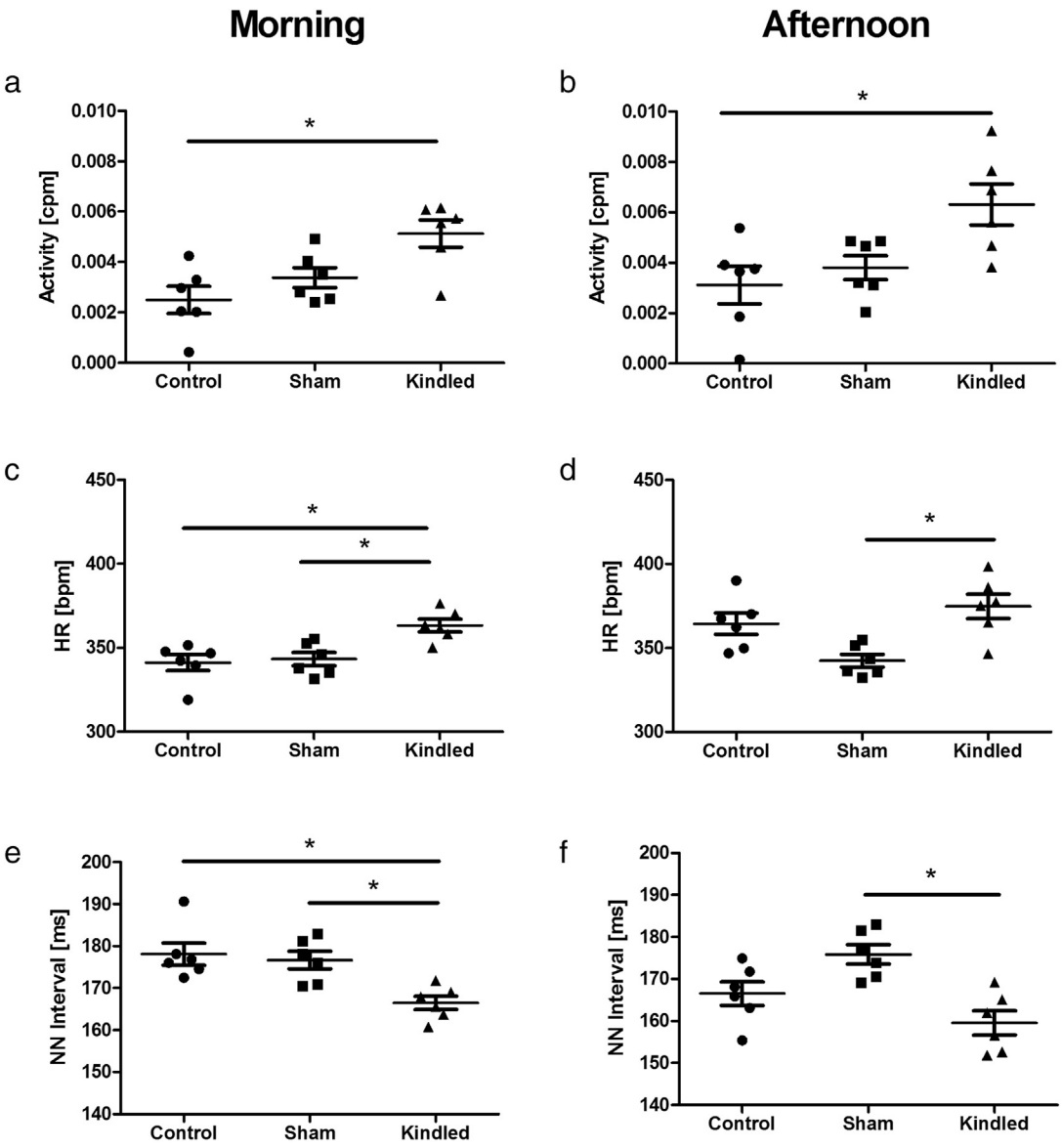

Fig. 2. Locomotor activity, heart rate, and NN interval for the morning and the afternoon phase. Mean values for locomotor activity, heart rate, and NN intervals are presented for the morning (6:00 a.m.–1:00 p.m., before daily seizure induction; a, c, e) and the afternoon (3:00 p.m.–6:00 p.m., following kindling stimulation; b, d, f) phases. Increased activity levels in kindled rats were obvious in the morning (a) and in the afternoon (b; control and kindled animals: n = 6, sham animals: n = 6). This also applies for the heart rate (c and d; control, kindled, and sham animals: n = 6) and the NN intervals (e and f; control, kindled and sham animals: n = 6), where group differences between sham and kindled and control rats were not only observed in the hours following kindling stimulation, but also before seizure induction. Data are given as mean ± S.E.M.. Significant differences are indicated by asterisks ($P < 0.05$).

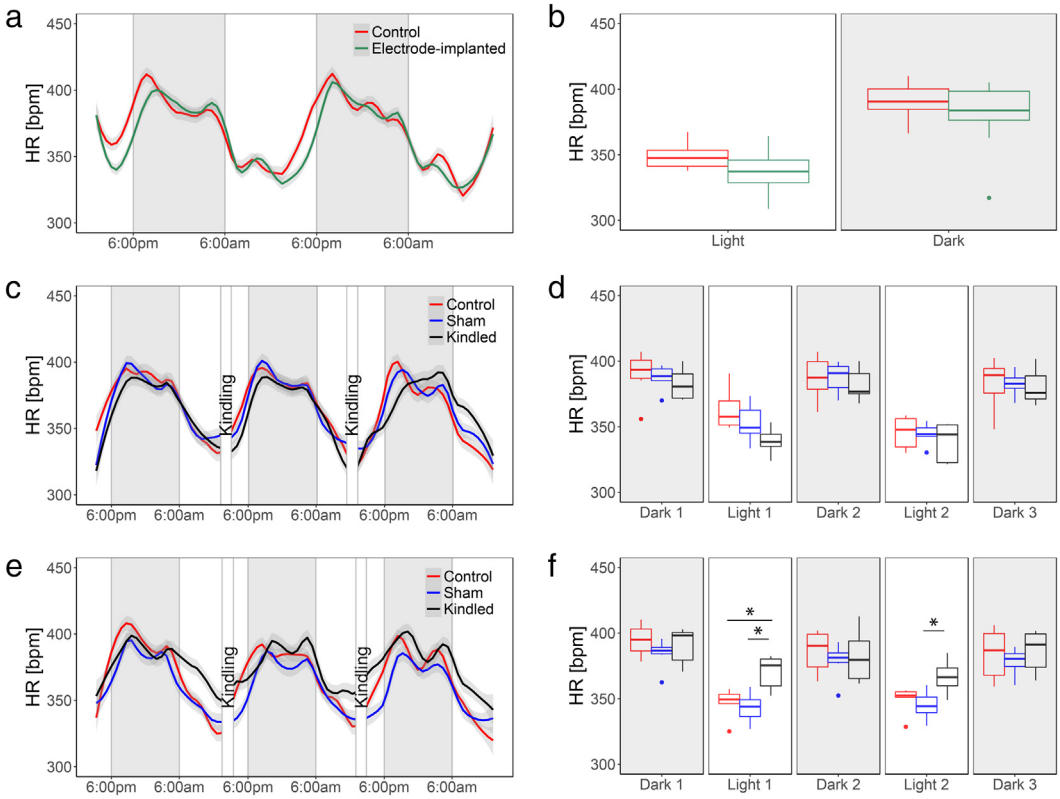

Fig. 3. Effect of surgery and kindling on heart rate. Heart rate (HR; control, kindled, and sham animals: n = 6) is shown for the postsurgical phase (a and b), the phase of focal kindled seizures (c and d), and the phase of generalized kindled seizures (e and f). Two peaks in heart rates became evident in all groups during the dark phase: the first one following lights off and the second one toward the end of the dark phase (a, c, e). Analysis of heart rates in the phase of generalized kindled seizures revealed an impact of seizures on mean heart rate during the light phase (f). Significant differences are indicated by asterisks ($P < 0.05$). The group size of the kindled animals differed at day two and day three of the phase of focal kindled seizures as some animals already exhibited generalized seizures: day two n = 5, day three n = 3.

an impact of focal seizure activity. However, following further kindling progression with induction of generalized seizures, an impact of seizures on mean heart rate (Fig. 3e and f) became evident during the light phase. Heart rate remained at higher levels in kindled rats with generalized seizures as compared with those of the sham and control rats on the day of the 11th stimulation and as compared with that of the sham rats on the day of the 12th stimulation (Fig. 3f).

3.3. Neither electrode implantation nor repeated kindled seizures affect heart rate variability

The total variability of the heart rate was assessed based on an analysis of the standard deviation of NN intervals (SDNN). Neither electrode implantation nor focal or generalized kindled seizures affected SDNN in a significant manner (Fig. 4). In order to determine short-term variability and spontaneous alterations in heart rate, we additionally analyzed the square root of the mean squared differences of successive NN intervals (RMSDD; Suppl. Fig. 3), the number of pairs of subsequent NN intervals as well as the percent of subsequent NN intervals, which deviate more than 9 ms (NN9), and the proportion derived by dividing NN9 by the total number of NN intervals (pNN9; Suppl. Figs. 4 and 5). None of these parameters proved to be altered as a consequence of electrode implantation 15–18 days before (Fig. 4a and b; Suppl. Figs. 3–5a and b). Moreover, prolonged electrode implantation of 21–23 days (Fig. 4c and d; Suppl. Figs. 3–5c and d) and 34–36 days (Fig. 4e and f; Suppl. Figs. 3–5e and f) did not reveal any impact on these heart rate variability parameters.

Surprisingly, our data did not confirm an impact of focal or generalized kindled seizures on either of these short-term heart rate variability parameters.

3.4. Immediate effect of seizure activity on heart rate

In addition to the analysis of circadian rhythms and heart rate variability, we determined the immediate impact of the stimulation and seizure induction on the heart rate data (Fig. 5 and Suppl. Fig. 6).

Focal seizure activity remained without a relevant immediate effect on heart rates (Suppl. Fig. 6). Associated with the elicitation of a generalized seizure, we observed a drop in the mean heart rate of the kindling group, reaching a mean trough level of 194 bpm. This initial phase of stimulation- and seizure-associated bradycardia lasted about 36–41 s followed by a transient return to control levels. Interestingly, the response to the 11th and the 13th stimulation proved to be biphasic with a second decrease in heart rates occurring 117–121 s after the stimulation (Fig. 5a and c). The mean duration of motor and electrographic seizure activity amounted to 77 and 77.5 s at the 11th stimulation, to 69 and 64 s at the 12th stimulation, and to 86.5 and 79.5 s at the 13th stimulation.

The stimulus-associated acute alterations in heart rate were reflected by biphasic increases in NN intervals as well as a transient rise in SDNN (data not shown).

4. Discussion

Telemetric recordings in amygdala-kindled rats revealed a relevant impact of generalized kindled seizures on diurnal activity and heart rate patterns. However, heart rate variability remained unaffected by daily kindled seizures. With regard to immediate ictal alterations, analysis of the electrocardiographic data during seizures and in the early postictal phase revealed a biphasic bradycardia occurring in response to generalized seizure induction.

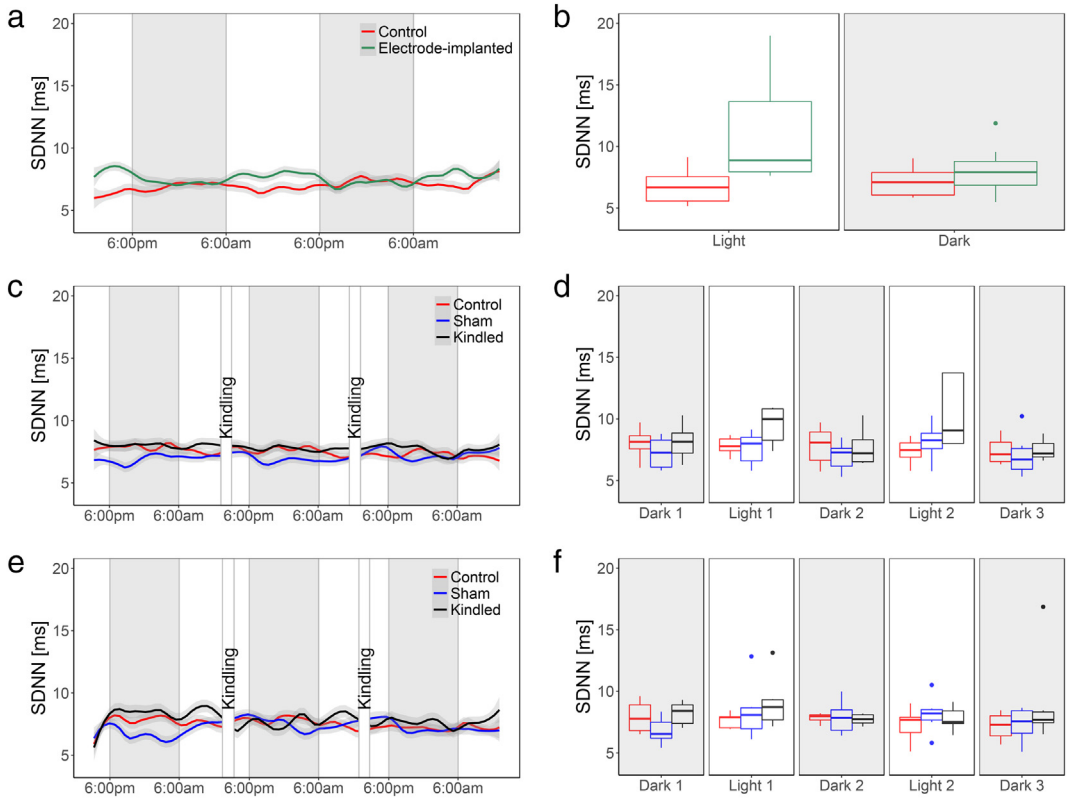

Fig. 4. Effect of surgery and kindling on SDNN. SDNN (control, kindled, and sham animals: n = 6) is shown for the postsurgical phase (a and b), the phase of focal kindled seizures (c and d), and the phase of generalized kindled seizures (e and f). Neither electrode implantation nor focal or generalized kindled seizures had an impact on SDNN. The group size of the kindled animals differed at day two and day three of the phase of focal kindled seizures as some animals already exhibited generalized seizures: day two n = 5, day three n = 3.

Data from the phase with generalized kindled seizures indicated a disturbance of the resting phase of the animals. Subsequent to higher activity levels toward the end of the dark phase, activity remained at significantly higher levels during the light phase. Hyperactivity in experimental models of epilepsy has been previously reported from monitoring in behavioral paradigms such as the Open-field test [43–46]. However, with testing in these paradigms the course of the rhythm may be affected by removal from the home cage, transfer to the behavioral laboratory and associated handling. This assumption is indirectly supported by findings revealing differences in behavioral paradigm outcomes depending on the time of the day [47,48].

In this context, it is emphasized that we observed a difference in telemetric home cage activity data between kindled rats and the control and sham groups not only following the induction of a generalized kindled seizure in the afternoon (between 3:00 p.m.–6:00 p.m.) but also before seizure elicitation. These findings indicate that generalized seizures can affect circadian activity rhythms with an impact on resting phases [49,50].

This might reflect disturbances of sleep patterns, which have been reported in patients [13,51,52]. The occurrence of sleep disturbances mostly affects patients with insufficient seizure control [53]. It can have a significant impact on their quality of life [21]. In this context, future analysis of sleep patterns and circadian rhythm in kindled rats including electroencephalogram and electromyographic analysis would be of additional interest.

Our results from the focal seizure phase indicate that focal seizures do not exert relevant effects on heart rate rhythms and variability. This finding is supported by the lack of any influence of focal kindled

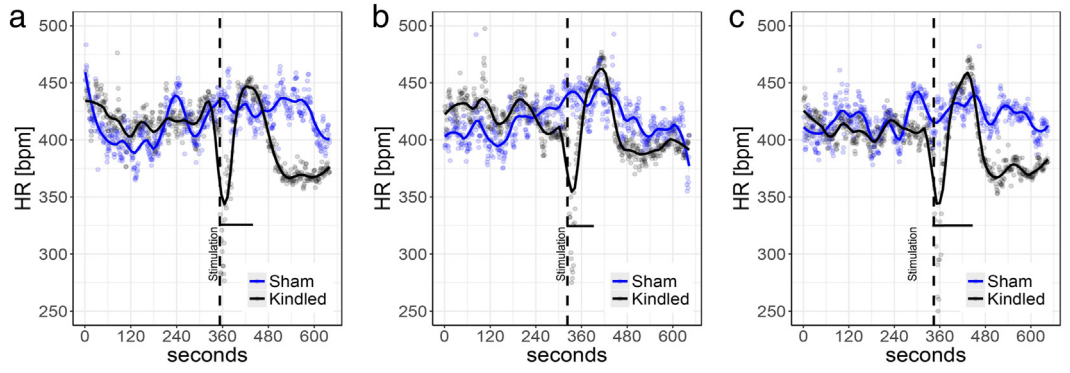

Fig. 5. Ictal heart rate in the phase of generalized seizures. Mean heart rate per group during the 11th (a; kindled and sham animals: n = 6), the 12th (b; kindled and sham animals: n = 6), and the 13th (c, kindled and sham animals: n = 6) stimulation in the phase of generalized kindled seizures. A drop in heart rate, which was associated with the elicitation of the seizure, was observed in the phase of generalized seizures (c and d). On the days of the 11th and 13th stimulation, a significant reduction of the mean heart rate in the kindled animals was observed after the stimulation. The time point of stimulation is indicated by the dashed line. The horizontal line represents the mean motoric seizure duration (a: 78 s; b: 69 s; c: 87 s).

seizures on heart rate and NN intervals. In apparent contrast, daily induction of generalized seizures resulted in significant alterations of heart rates and NN intervals, which remained at elevated levels during the resting phase of the animals. The fact that we confirmed a difference between sham and kindled rats suggests that the heart rate alterations observed in kindled animals are dominated by a significant impact of seizures.

To our knowledge, the influence of kindled seizures on resting heart rate has only been analyzed during short intervals in earlier experimental studies [54,55]. Healy et al. [56] described bradycardia 24 h and seven days following the last generalized kindled seizure. The measurements were based on two-minute recordings with connection of the animal to a recording device [56]. These experimental conditions may have contributed to the outcome, which is in contrast with our present findings. An increased resting heart rate has been reported in a study based on short-term recordings initiated 5 min before kindling stimulations [54]. In patients with epilepsy, evidence for an imbalanced autonomic nervous function is intensely discussed based on various studies assessing heart rates [57,58]. In line with our experimental results, an increased interictal heart rate has been reported by various clinical studies [59–61].

Even though few studies investigate the direct effect of focal seizures on heart rate alterations, one possible explanation for the discrepancy between the observed effects of focal versus generalized seizures can be the datasets showing a correlation between the number of seizures an animal experienced or the strength of their ADTs and the resulting heart rate alterations. The more stimulations an animal receive, the stronger the immediate bradycardia effects following a stimulation become, and the more apparent overall heart rate alterations develop [54,55,62,63]. The stimulated basolateral amygdala shares direct excitatory connections with structures in the brainstem that in turn exert direct control over cardiovascular functioning [64,65]. The changes that we observe in heart rate only appear after animals experienced generalized seizures, indicating that either generalized seizures spread to the brain stem where they can affect structures involved in cardiovascular function or repeated seizures are needed to achieve structural changes higher up in the pathway in the amygdala. Studies in patients with temporal lobe epilepsy (TLE) suggest the former. Depending on the patient's history, TLE is associated in varying degree to structural damage in brain stem structures involved in autonomic control [66,67].

A vast literature exists on clinical epilepsy-associated changes in heart rate variability (e.g., reviewed by [57,58,68]). Whereas chronic TLE proved to be associated with lower heart rate variability (e.g., [14,69–71]), a study in patients with new onset untreated epilepsy did not reveal disease-associated alterations in heart rate variability [72]. The lack of changes in heart rate variability in kindled rats might actually reflect these data, as a short kindling paradigm might rather model the early phase following epilepsy manifestation in patients.

Thus, autonomic imbalance with an increased parasympathetic influence resulting in suppressed heart rate variability might only develop in a delayed manner following prolonged duration of epilepsy. Therefore, it might be of interest to further study circadian rhythms and heart rate variability at later time points following continued kindling stimulations.

Taking into account that suppressed heart rate variability might reflect an inability to adjust to acute demands based on immediate cardiac responses, epilepsy-associated decreases in variability are discussed as one risk factor of sudden unexpected death in epilepsy (SUDEP) [57,58,70]. Our data along with clinical findings suggest that this risk factor might only become relevant with further disease progression.

In the context of SUDEP, ictal alterations in heart rate as a matter of course receive even more attention than those preceding ictogenesis. Interestingly, both, ictal tachycardia and bradycardia have been described clinically (reviewed by [57,58]). Thereby bradycardia seems to be less frequent than ictal tachycardia [58]. As bradycardia can progress to asystole usually lasting 10–30 s [58,73,74], it can be an important factor for SUDEP risk [75]. In kindled rats, we observed an immediate and rapid drop in heart rates in response to induction of a generalized seizure. This course reflects a sudden heart rate reduction, which seems to occur in some patients as described by Vaughn et al. [76].

In the kindling paradigm, recent findings suggest a short bradycardic phase in the initial one-third of the seizure followed by rebound tachycardia [54]. In contrast, we observed a biphasic occurrence of ictal bradycardia with a short initial bradycardic phase lasting 36–41 s followed by a short normalization of heart rates and a second more delayed decline of heart rates resulting in a longer lasting bradycardic phase. This 2nd phase might cause a prolonged postictal risk predisposing to asystole and cardiac failure. Respective alterations were only observed following generalized seizures, and they were only evident at two out of three stimulation days. In some patients, oscillatory patterns of heart rate have been described following initial ictal bradycardia [77,78].

According to our findings, amygdala kindling seems to provide an experimental model situation allowing to further study the consequences of ictal bradycardia and its therapeutic modulation. While the kindling paradigm is used widely and is considered a valuable tool for epilepsy research, there are limitations with this model. One limitation is that with a standard protocol the seizures are not spontaneous but are induced directly by an electrical stimulation [79]. Future studies are planned to compare heart rate alterations between different models of epilepsy. In addition, one caveat is that the successful kindling procedure has been confirmed by investigating the ADT values and not by histological analysis of the amygdala.

The outcome of this study is not only of relevance with regard to a general study of seizure-induced alterations in circadian rhythms and ictal heart rate alterations, but also provides information about the burden of experimental animals. The data indicate that once daily generalized seizure can significantly impact home cage activity and heart rate with a disturbance of resting phases, suggesting an overall impact on circadian rhythmicity This consequence of generalized kindled seizure activity needs to be considered as a factor influencing the wellbeing of laboratory rodents exposed to seizure elicitation. This assumption is also supported by the fact that it is known that sleep disturbances in patients with epilepsy can significantly impact their quality of life [21]. In addition to this conclusion, our data revealed an early impact of electrode implantation 15–18 days following surgery. Surprisingly, animals that received a depth electrode implantation in addition to the peripheral telemetric transmitter implant exhibited hyperactivity during light and dark phases. Considering that surgical interventions rather result in hypoactivity of affected animals [80–82], the observed increase in activity levels might be related to local effects of the electrode implant in the target brain region. These effects proved to be transient as no difference to control rats was evident at later time points of analysis.

In conclusion, our study demonstrates that once daily generalized seizures can exert significant effects on circadian activity and heart rate rhythms. Provided that these findings reflect a respective impact of seizure activity in patients, these alterations should be considered for patient counselling and therapeutic management. Moreover, they should be considered for evidence-based severity assessment as the disturbance of resting patterns is likely to cause distress in affected animals.

Biphasic bradycardia associated with generalized kindled seizures suggests that the model is suitable to study the circumstances, the consequences, and the therapeutic prevention of ictal bradycardia. This is of particular interest as ictal bradycardia can progress to asystole and may predispose patients to a risk for cardiac arrest and SUDEP.

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Author contributions

C.M., F.W., and M.K. conducted the experiment. C.M., M.D., and K.S. analyzed the data and drafted the figures. C.M., V.B., and H.P. designed the study, interpreted the data, and drafted the manuscript. All authors revised the manuscript and figures and gave their final approval.

Declaration of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yebeh.2018.11.034.

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