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Cardiac effects of repeated focal seizures in rats induced by intrahippocampal tetanus toxin: bradyarrhythmias, tachycardias and prolonged interictal QT interval

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Summary

Objective: To determine electrical changes in the heart in a chronic, non-status model of epilepsy.

Methods: Electrocorticogram (ECoG) and electrocardiogram (ECG) of 9 animals (5 made epileptic by intrahippocampal injection of tetanus neurotoxin (TeNT) and 4 control), are monitored continuously by radiotelemetry for up to 7 weeks.

Results: Epileptic animals develop a median of 168 seizures, with postictal tachycardias reaching a mean of 487 beats/min and lasting a mean of 661 seconds. Ictal changes in heartrate include tachycardia and in the case of convulsive seizures, bradyarrhythmias resembling Mobitz type 1 second degree atrioventricular block; notably the P-R interval increased before block. Postictally the amplitude of T wave increases. Interictally, QT dependence on RR is modest and conventional QT corrections prove ineffective. Interictal QT intervals, measured at a heartrate of 400 bpm, increased from 65 ms to 75 ms, an increase dependent on seizure incidence over the preceding 10-14 days.

Significance: Repeated seizures induce a sustained tachycardia and increase in QT interval of the ECG and evoke arrhythmias including periods of atrioventricular block during Racine Type 4 and 5 seizures. These changes in cardiac function may predispose to development in fatal arrhythmias and sudden death in humans with epilepsy.

Key Point Box:

- Repeated seizures are associated with increases in interictal QT interval, depending on seizure incidence over 10-14 days.

- Conventional QT correction methods prove counterproductive in our study.

- QT at heartrate 400 bpm (QT_{400}) are estimated either by selecting periods of ECG at that heartrate, or interpolating from the modest linear QT-RR relationship we find in our unanaesthetized rats.

- Seizures are associated with ictal and prolonged postictal tachycardia.

- Convulsive seizures are associated with arrhythmias including bradyarrhythmias, resembling Mobitz type 1 second degree AV block in humans.

Introduction

Cardiac, along with respiratory problems are the most likely causes of Sudden Unexpected Death in Epilepsy (SUDEP).[1] Repeated seizures have been associated with cardiac abnormalities, including prolongation of the QT interval of the ECG, in humans[2-5] and in experimental rodents.[6-9] QT interval reflects the duration of the cardiac ventricular action potential, and is important in cardiac pathophysiology, particularly when prolonged. Temporal lobe epilepsy features prominently in SUDEP series in adults.[1; 10] The main risk factor is the number of generalized tonic-clonic seizures;[11] arrhythmias during seizures also associate with risk of SUDEP.[10; 12] The pathophysiological changes underlying this effect remain unresolved, yet an understanding is fundamental to the development of therapeutic interventions to minimize the risk of SUDEP.

The most commonly-used animal models of chronic epilepsy rely on systemic injection of epileptogenic agents.[13] Direct actions on peripheral or central (e.g. brainstem) sites [7; 14-16] may induce cardiac and respiratory changes independently of seizures, complicating interpretation of results.[17] These models reveal prolongation of QTc (QT corrected to a standardized heartrate) following chronic epilepsy.[7;8] The hippocampal kindling model avoids the potential problem of subcortical or systemic effects of the epileptogenic agent, but does not induce spontaneous recurrent seizures; repeatedly evoked seizures in kindling do result in prolonged QTc.[9] Here, we use the tetanus neurotoxin (TeNT) model,[18; 19] in which toxin is microinjected directly into the hippocampus. This has the advantage of restricting the toxin's direct effects (on VAMP) to the vicinity of the injection site in the hippocampus,[18] inducing repeated spontaneous self-limiting seizures without inducing status epilepticus (SE). Thus, effects seen in peripheral organs must be due to central seizure-driven events.

The purpose of this study is to determine cardiac changes that occur during and between seizures, and their relationship with repeated seizures. Clinical measurements usually correct

for dependence on heartrate, but this process raises problems which we discuss below and

assess in our experimental evidence.[20; 21] Following a single injection of TeNT into the

hippocampus in rats, we record electrocorticograms (ECoG), electrocardiograms (ECG) and

seizure-related behaviors in freely-moving animals continuously for periods of weeks.

Materials and Methods

Ethics

Experimental procedures complied with the UK Animals (Scientific Procedures) Act 1986,

and were approved by ethics committees of the Universities of Birmingham and Oxford.

Animal housing

Rats are housed two per cage (one experimental animal, one "buddy") with free access to rat

chow and water. Lights turn on automatically from 07.00h -19.00h. Only authorized staff

have access to the room and are instructed to minimize disturbance to the rats. Data are

collected continuously via radiotelemetry and video recordings for analysis off-line.

Electrophysiology and induction of focus

Surgery. Under isoflurane anesthesia, male Wistar rats (220-320 g; Charles River) are

implanted with Telemetry Research TR50BB radiotelemeters (Millar Instruments, now Kaha

Sciences) into the abdomen following the manufacturer's protocol. During aseptic surgery

under isoflurane anaesthesia: wires are tunneled to the chest and head to make electrodes for

ECG and ECoG, respectively; ECG leads are sutured onto chest musculature near the right

shoulder and left caudal rib cage for lead 2 ECG; bur holes are drilled over the dorsal

hippocampus and superficial neocortex 3.5 mm caudal and ±3.0 mm lateral to bregma and

stainless steel ECoG electrodes cemented in place; a further bur hole is drilled over ventral

hippocampus 4.3 mm caudal and 4.4 mm lateral to bregma, and a guide cannula cemented in

place.[22] Incisions are repaired with absorbable sutures. After recording 6-14 days of baseline data, and after rats resumed normal growth (required as evidence of recovery from surgery), they are briefly anesthetized to inject 2.5 ng TeNT (1 µl in phosphate buffered saline with 2% bovine serum albumin; produced by List Biological Inc, supplied by Quadratech Diagnostics Ltd) into the ventral hippocampus 7.5 mm below the cortical surface[22] over 5 min. Recordings resume following recovery from anesthesia. Previously we showed that injection of TeNT into the hippocampus degrades its molecular target (VAMP) focally in the injected hippocampus,[18] showing that direct effects of TeNT are restricted to near the injection site.

Electrophysiology. Analogue outputs from the Telemetry Research system are recorded in contiguous 6-hour epochs for up to 9 weeks using a Power 1401 acquisition system and Spike2 software (Cambridge Electronic Design, Cambridge, UK (CED)), with time-locked video recording in three epileptic rats. Seizure detection is assisted by measuring power of the hippocampal recording in the 10-300 Hz band, which increases >4-fold during seizures. The Pan Tompkins method[23] identifies the peaks of R waves to measure instantaneous heartrate (reciprocal of R-R interval).

To relate cardiac pathophysiology to Racine score in the 3 rats with simultaneous video recording, we use the Random Sequence Generator at www.random.org to randomly select 3 seizures each Racine stage 2-5 from all 43 seizures in one rat and from first, middle and last thirds of seizures in two rats with 164 and 392 respectively. Measurements include ictal and postictal tachycardia, and depth and duration of any abrupt bradyarrhythmia.

Finding R wave peaks also allows for averaging ECG waveforms centered on the peak of the R wave. To measure ECG, we average 5-second epochs with steady heartrate, following a period of 60 seconds with as little change in heart rate as possible to minimize QT adaptation and hysteresis.[24] Adaptation of QT may occur in our data, but systematic study is impossible given our lack of control over rat behavior and hence heart rate. One rat has step changes in

heart rate both before and 18 days after TeNT injection (330 to 421 and 360 to 416 bpm),

which allow repeated measures of QT at the new higher heartrate. No progressive changes

occur over 30 seconds either before or 18 days after induction of epilepsy. While the data are

very limited, they suggest that adaptation is not a major factor under our conditions.

QT interval is measured, using averaged ECGs, from the onset of Q to the end of T identified

as the intersect between the isopotential line and a tangential line fitted to its descending

slope between 80% and 20% amplitude. This method is implemented in a semi-automated

script for Spike2 provided by CED (Figure 1*A*). QT corrections aim to make the resultant

QTc independent of RR and heartrate. The Bazett formula, used clinically, normalizes to a

heartbeat duration of 1 second which is inappropriate for rodents. Application of the Bazett

clinical correction to our data (Figure 1*B*) reveals that it exacerbates the impact of the RR

interval on QTc and increases QTc to unphysiologically high values, which may explain the

high values of QTc found in some previous reports using Bazett's correction on rat models.

A rat-specific version[20] normalizes to a standard heartrate of 400 bpm (RR=150 ms), at least

in rats under anaesthesia. The unanaesthetized rats in our study have QT with limited,

generally inverse, dependence on RR. This effect is greater for very low heartrates (high RR).

The result is that the Kmecova-Klimas correction[20] overcorrects QT (Figure 1*C*).

Superimposed ECGs confirm that QT is shorter for longer RR (Figure 1*D*).

The first QT measurements in the epilepsy syndrome examine the time course of changes

during interictal periods, avoiding corrections for heartrate by selecting periods when

heartrate is 400 beats/minute (5-s averages included 33 beats) to produce a measure "QT_{400}".

Measurements are restricted to the lights-on period 0700-1900 hours to avoid the risk of a

circadian variation in QT.[25; 26] Controls comprised of two vehicle-injected rats and two

injected with subthreshold doses of TeNT that failed to induce seizure activity. QT_{400} was

measured between 1100 and 1800 on 6 different days in each rat. There was no significant

difference in QT_{400} between these two groups (ANOVA, $F_{1,22}=2.27$, P=0.113), so they were pooled as a single set of seizure-free controls.

Although it is not possible, for safety reasons, to blind the experimenter to the toxin or vehicle at the time of surgery, files are renamed using numbers generated by the Random Sequence Generator at www.random.com before the relationship between QT and RR on epileptic rats is measured blind in second series of measurements. One-hour epochs are extracted from interictal periods 18 days after injection, and at least 30 min before or after the closest seizure, from both the light and dark parts of the circadian cycle. Internal controls are from similar epochs the day before injection in the same rats. QT, RR and mean RR over the previous 60 seconds are measured blind to the nature of the rat, day and time; graphs of QT against RR are made (Sigmaplot version 14) and QT_{400} estimated by interpolation. Statistical analyses use SPSS version 23 (IBM); values are presented as mean±SD unless otherwise stated.

Results

Cardiac electrophysiology

Spontaneous seizures start 3-9 days after injection of 2.5 ng TeNT, and the animals are recorded for several weeks. During seizures rats exhibit behavioral arrest, vibrissal twitching and/or purposeless chewing, which can progress to head nodding and to generalized seizures including forelimb clonus, rearing, and rearing and falling seizures ("Racine stage 5"). Seizures recur, often in clusters of 10-40 seizures over 2-5 days separated by one or more seizure-free days (Figure 2*A*). Seizure frequency usually decreases markedly 2-3 weeks after induction, but seizures are still seen in some rats at up to 7 weeks, before remission occurred. Total number of seizures experienced by each of the 5 rats ranges from 43 to 382. Individual

seizures last 83±37 seconds (mean±SD; range 19 to 317 seconds; Figure 2*A,B*), often

followed by postictal suppression of cortical activity, similar to postictal generalized EEG

suppression (PGES)[27] (Figure 2*C*, upper trace).

Heartrate changes during and after seizures

Resting heart rate (the slowest heart rate measured during 24-hour periods) increases

progressively during the epileptic syndrome, from 313.8±38.7 to 352.8±43.0 bpm by 3 weeks

after TeNT injection (paired *t*-test, p<0.014). By 4 weeks, heart rate decreases to 293.6±32.9

bpm, not significantly different from the starting value.

Pronounced changes in heart rate also occur during and following seizures. The most

consistent is tachycardia, both ictal and postictal. Ictal tachycardia starts within 2-3 seconds

of electrographic seizure onset and can be interrupted by bradyarrhythmias. In 19 of the 20

Racine stage 5 seizures in the random sample of videoed rats (see Methods), the seizure starts

with behavioural immobility lasting 19.5 ±5.7 seconds associated with an increase in heart

rate of 38 ±21 bpm. In the example shown in Figure 2*C*, this increase terminates with the

onset of rearing (bradycardia at red arrow in Figure 2*C*) before falling and rearing repeatedly

(tonic-clonic convulsions) from 20 to 45 seconds after seizure onset (greyed section on plot

of heartrate corresponds to bradyarrhythmia, discussed below). The seizure in Figure 2*C*

stops at 65 seconds. At the start of the postictal period, the rat experiences tachycardia while

immobile in a prone position for 1 min until the dashed arrow on Figure 2*C*, when it starts

repeated jaw movements. At the solid arrow, about 260 seconds after seizure onset, it

becomes active and explores its cage for 5 min 42 seconds. This example shows that

tachycardia occurs independently of postictal active behaviors (exploration and grooming are

common), when postictal motor activity occurs it prolongs postictal tachycardia, reflecting

physiological modulation of heart rate. Postictal tachycardia with behavioural inactivity

occurs in 17 of the random sample of 20 convulsive Racine stage 5 seizures from 3 rats,

where the postictal rat was visible in the video (see Methods), peaking at 424 ±46 bpm and lasting 75 ±55 seconds.

In a sample of three rats (including 2 without video), postictal heartrate for all 322 seizures increases from 314±39 bpm to reach a mean of 487±34 bpm, ranging from 409 to 578 bpm (paired *t*-test p<0.001). It persists for 661±484 seconds (range 28 to 3948 seconds). Only two of a series of 127 seizures in three rats with continuous video recording lack postictal tachycardia. In one of these seizures, the rat fights with its cage-mate before the seizure and its heartrate is already at a high average of 505 bpm over the 5 minutes preceding the seizure, which may mask postictal tachycardia. In the other case, heartrate does not change from the pre-ictal value of 430 bpm; this seizure has brief and modest motor symptoms (mainly slow rearing).

More detailed statistical analysis of the relationship between postictal tachycardia and Racine stage is performed on a random sample of 84 seizures from the 3 rats with video recording (see Methods). The peak heart rate is significantly related to Racine stage of the corresponding seizure (Figure 2*D*; ANOVA $F_{3,80}$=5.0; P=0.003; Dunnett post hoc tests: Racine stage 2 differs from stage 5 P<0.002, and from stage 4 P=0.008). For each Racine stage maximal postictal heart rate is significantly greater than preictal (paired *t*-tests; P<0.001 in each case). The increase in postictal heart rate from preictal does not depend on Racine stage (ANOVA $F_{3,80}$=0.64; P=0.59; Supplemental Figure 2*A*), most likely due to variation in preictal rat activity and heart rate.

Blinded measurements from a random sample of 23 Racine 5 seizures reveal that the T wave amplitude expressed as a fraction of the QRS wave is significantly larger during postictal tachycardia following tonic-clonic seizures (ratios: preictal, 0.34, early ictal 0.36, after tonic-clonic seizures 0.57; ANOVA $F_{2,21}$=6.1, P=0.008; Dunnett post hoc test against preictal, ictal P=0.91, after tonic-clonic seizures p<0.01); QT is unaffected (P=0.59)(Figure 2*E,F*).

Bradyarrhythmias

Bradyarrhythmias occur during tonic-clonic components of motor seizures. (Figure 3*A*). They are absent in non-convulsive seizures (Figure 3*B,* note different heart rate scale from 3*A*). In each of the 3 rats in which we have video recording time-locked to the electrophysiological data, we randomly select seizures from each Racine stage 2-5, to assess ictal tachycardia and bradyarrhythmia (see Methods). Ictal tachycardia increases significantly with Racine stage (Figure 3*C* left; ANOVA $F_{3,80}$=5.07, P=0.003; Dunnett post hoc test, Racine stage 2 differs from stages 4 and 5 P<0.001). Peak ictal heart rate is significantly greater than preictal for each Racine stage (paired *t*-tests, P<0.005). The dependence of peak ictal heart rate on Racine stage could be related to the corresponding motor behaviours (Racine 2 head nodding, 3 to 5 progressively adding: forelimb clonus, rearing, and rearing and falling); it could also result from the neural circuits recruited during different degrees of seizure generalization. The increase of peak ictal from preictal heart rate was not significantly related to Racine stage (Figure 3*C* second panel from left; $F_{3,80}$=1.6, P=0.2). None of the 20 non-convulsive Racine 2 seizures exhibit bradyarrhythmias, nor do all but one of the 16 Racine 3 seizures (Figure *3B)*; the exception is an anomalous seizure where the rat makes 4 vertical jumps over a 10-second period starting 18 seconds after the end of a bradyarrhythmia which lacks clear motor signs (Supplemental Figure S1). Fifteen of 27 Racine 4 seizures and 18 of the 21 Racine 5 seizures are associated with substantial drops in heartrate from 367.6±45.9 to 150±111.7 bpm (related-samples Wilcoxon Signed Rank test, p<0.001), lasting 17.0±5.3 seconds (Figure 3*C*, centre and right). Both bradyarrhythmia duration and heart rate decrease from preceding ictal tachycardia depend on Racine stages (Figure 3*C* centre and right; independent samples Median Test P<0.001 in both cases); these measures differ between Racine 5 seizures and each of Racine 2, 3 and 4 (Mann-Witney U test significance shown on boxplots in Figure

3*C*). The minimum heartrate reached during bradyarrhythmias is 118.7±47.6 bpm, and is independent of Racine scale and rat (data not shown; ANOVA P=0.72 and 0.83 respectively).

Close inspection of the ECG record during the bradyarrhythmias reveals that the heart misses one beat in two, or two in three, consistent with development of ==Mobitz type-1 second degree atrio-ventricular (AV)== block in humans (Figure 4*A-C*).[28] ==Note the stable instantaneous frequency of the ictal heartbeat (lower graphs Figure 4*A,B*), before bradyarrhythmia causes dispersion with lower values settling on half or one third the preceding frequency.== Expansion of the dotted box in Figure 4*B* reveals periods of missed beats often start with an isolated P wave (arrows, Figure 4*C*). The PR interval increases progressively shortly before the onset of arrhythmias (Figure 4*D*). The illustrated case ends at the onset of bradyarrhythmia shown in the central panel. ==Data from a total of 10 Racine stage 5 seizures from the 3 rats with video show significantly increased PR intervals just before bradyarrhythmia onsets (paired *t*-test, P<0.001; Figure 4*D*, right).== In 3/17 seizures periods of asystole up to 3s duration occur (Figure 4*B*).

Interictal ECG waveform – QT segment

QT interval is an important measure of cardiac performance; changes are described in both clinical and experimental epilepsies.[2; 3; 6] Conventions exist for correcting QT for its dependence on heartrate,[20; 25; 26; 29] but have known limitations. During the present study we find no correction method that satisfactorily succeeds in removing dependence on the R-R interval ("RR", inverse heartrate) in freely-moving rats (Figure 1), and therefore either restrict measurements to periods when heart rate is stable at 400 bpm (QT_{400}; Figure 5); or interpolate QT_{400} from measurements at a range of heart rates (Figure 6).

First we examine the time course of any changes in interictal QT over the course of the epilepsy syndrome. We avoid the risk of complications from circadian variation, by limiting

measurements to the lights-on period (0700-1900 hours, as close to midday as possible).

QT_{400} difference from the initial measurement removes inter-animal variation (Figure 5*B*).

The consistent finding in 5 epileptic rats is that interictal QT_{400} increases progressively during

the seizure syndrome, from a pre-injection mean of 63.9±7.7 ms to 71.1±7.3 ms 14 days after

injection (p<0.001, paired *t*-test), and to 71.0±9.9 ms 21 days after injection (p=0.022, paired

t-test); means at 7 and 28 days do not differ from pre-injection values, but some individual

rats do, reflecting variations in the timing of the seizure syndrome (Figure 5*B*: symbols with

black outlines indicate >1.96*SD estimated from 5 pre-injection measurements for each rat).

QT_{400} relaxes back to baseline 7 days after the last seizure, or as the seizure incidence

declines. QT_{400} does not change significantly over 14 days in 4 control rats (71.0±6.6 ms *v.*

69.1±8.1 ms; p=0.27, paired *t*-test). Typical averaged ECG waveforms (selected as nearest

case to their respective group means; Figure 5*C*) show the prolongation of QT_{400} at 14 days in

an epileptic rat. The increase in QT_{400} correlates significantly with the number of seizures

over the preceding 3-14 days, but not with the cumulative total of seizures (Supplemental

Table 1). The strongest correlation is for a 10-day seizure history (Figure 5*D*; Pearson

correlation coefficient *r*=0.58, P=0.008 for all seizures and *r*=0.54, P=0.01 for convulsive

Racine 4/5). This suggests QT prolongation depends on seizure incidence over the previous

~10 days.

To determine whether the epilepsy-related change in QT depends on heart rate we plot

interictal QT against RR measured blind during the light and dark periods of the day, before

and 18 days after injection of TeNT (see Methods; Figure 5, Supplemental Figure S2).

Vectors to each point indicate mean RR during the preceding minute to indicate risk of QT

adaptation. Interpolation of the QT-RR plots confirm that QT_{400} increases following repeated

seizures, from 62.5±8.7 ms to 68.4±2.9 ms 18 days after injection (paired *t*-test P<.004). QT

is longer during the light part of the day, 69.6±7.2 ms vs 60.8±9.8 ms in the dark (paired *t*-

test P<0.001). These plots also reveal that the relationship between QT and RR does not change qualitatively after the repeated seizures of the chronic epilepsy model; the increase in QT with repeated seizures occurs across the range of heartrates measured (Figure 6; Supplemental Figure S2). Note that since the range of heart rates available for measurement is determined by the spontaneous activity of individual rats, complete datasets across a wide range of heart rates are not possible. Very low levels of activity during the light period before induction, for example, mean that moderate and high heart rates are rarely achieved.

Discussion

Application of QT corrections for rat

In humans the QT interval is accepted to be influenced by heart rate, requiring correction methods to standard heart rate.[29] In the present study in rats, which shows tachycardia and increased QT interval develop with progression of the seizure syndrome, we find that the Bazett correction, which is widely applied to human ECG, is unsuitable for the high heart rates of rats. A refinement of Bazett's formula, the Kmecova-Klimas correction, aims to overcome this limitation.[20] Surprisingly, this correction exacerbates the relationship between QT and RR in our data, mainly because the relationship between the raw measurements was weak and, if anything, inverse, in marked contrast to the earlier work.[20] The earlier study differs from ours in using anaesthetized rats, and controlling heart rate using drugs, contrasting with the unanaesthetized, freely-moving rats with endogenously-controlled heart rate in the present work. A study in unanaesthetized mice, also reports the relationship between QT and RR to be relatively flat.[30] Another study in rats anaesthetized with barbiturate, shows a modified measure of QT (to the peak of the T wave, "QaT") also is independent of heart rate.[31] Neither tribromoethanol[20] nor barbiturate[31] are commonly used as anaesthetics in studies of cardiovascular function in small rodents. The impact of anaesthesia on the relationship between QT and RR needs further investigation, including for more

commonly used anaesthetics. We conclude that at present, most available correction methods

may be inappropriate in unanaesthetised rats. In the light of these findings we use the raw

data for the analysis of QT either measuring at, or interpolating to, 400 bpm.

Changes in cardiac function during the seizure syndrome

We use intrahippocampal TeNT to induce a seizure profile characterized by repeated self-

limiting seizures over a period of several weeks without SE. We are confident that

cardiovascular effects are due to locally induced seizure activity rather than direct actions on

remote cardiovascular control areas, because the loss of VAMP (the direct effect of TeNT) is

restricted to the injected hippocampus.[18] The spontaneous self-limiting seizures of the TeNT

model also avoid potential complications from long-term consequences of SE or systemic

effects of the epileptogenic agents used in other models. The rat TeNT model resembles

temporal lobe epilepsy in humans, in that is associated with development of similar cardiac

abnormalities such as baseline (interictal) QT interval prolongation,[3; 7; 32] increased heart

rate,[4] ictal tachycardia,[2; 33] and arrhythmias during generalized tonic-clonic seizures.[10; 12]

None of the rats die unexpectedly in the present study, so we do not have evidence for a

model of SUDEP, but it does provide a useful means to study the manner in which repeated

self-limiting seizures can impact on cardiac function. In acute rat models that induce SE,

animals do die, but the brain is challenged excessively.[17; 34] In the present study only a

proportion of seizures were convulsive (Racine stages 4-5) and, apart from experiencing

seizures, the animals were in good health. In human epilepsy some individuals are at much

higher risk of SUDEP than others,[35] which may be linked to their inability to overcome the

challenge of the intense autonomic disturbances associated with exposure to repeated

seizures.[36]

Over the course of the epilepsy syndrome evoked by TeNT in rats, the interictal heart rate

increases progressively and QT interval of the ECG becomes prolonged. The increase

depends on seizure incidence over the preceding 10 days (3-14 day periods all show significant correlations with QT). As seizures remit or their incidence wanes, interictal heart rate and QT recover over a period of a week; this suggests that seizure clusters may present additional risk of repolarization abnormality. Previous reports of QT prolongation following two weeks of convulsive seizures induced in a kindling model show increased susceptibility to arrhythmias.[9] A pharmacological investigation into the origin of seizure-related cardiac changes is outside the scope of our study. However, others using a systemic kainic acid-induced epilepsy model, which also induces increased QT, show that it could be prevented by beta adrenergic blockade indicating a sympathetically-mediated effect.[16] These authors[9] also show QT prolongation increases susceptibility to arrhythmias in rats following two weeks of kindling-induced Racine 4 and 5 convulsive seizures The sustained interictal tachycardia that develops in the present study is consistent with prolonged sympathoactivation and, with the prolongation of interictal QT, suggests that exposure to multiple short, intense seizures (seizure clusters) could lead to cardiac dysfunction.

A striking finding in the present study is that convulsive (Racine stage 5) seizures, and to a lesser extent stage 4 seizures, are associated with arrhythmias that include the development of ictal bradyarrhythmia consistent with development of Mobitz type 1 second degree AV heart block.

Several reports suggest this effect is parasympathetically mediated. In urethane-anaesthetized rats, vagal stimulation induces frequency-dependent cardiac slowing and AV block.[37] At the highest frequencies employed (50Hz), the heart may stop completely.[37] Increases in vagal nerve activity during acute SE induced by systemic administration of KA, can also lead to bradyarrhythmias and death due to AV block.[34] The onset of arrhythmias induced by vagal activation is preceded by lengthening of the PR interval consistent with slowed conduction though the AV node,[38] as it is in our animals. Thus it seems likely that the bradyarrhythmia

and periods of asystole seen during the periodic convulsive seizure activity in the present study are a consequence of intense vagal activity.

The combination of sympathetic and parasympathetic over-activation of the heart is particularly dangerous. The sympathetically-mediated increase in QT may increase susceptibility of arrhythmia whilst at the same time vagal activation can evoke AV block. This pattern of response: ictal tachycardia with periods of bradyarrhythmia and even asystole, has been reported in a minority of seizures in individuals judged to be at high risk of SUDEP.[12] Whilst this may not pose a grave risk to life in patients with healthy hearts, it could be especially dangerous in those in which cardiac function is already compromised.

In addition to its effect on the heart, vagal activation during increased sympathetic drive has been shown to predispose to periods of apnoea.[37] Although we did not measure respiration in the chronic model in this study, it is interesting that acute SE induced by systemic or intrahippocampal injection of kainic acid, also induces intense sympathoactivation punctuated by apnoeas, which were often lethal in these models.[17; 34; 39]

The mechanism via which focal seizure activity initiated in the hippocampus produces life-threatening disturbances in cardiac and respiratory function is intriguing. Hippocampal seizure activity is known to propagate to the amygdala,[40] a key forebrain region for controlling cardiorespiratory function through its descending connection to key medullary cardiovascular control centres.[41; 42] Studies in patients at high risk for SUDEP show higher than normal resting functional interconnectivity between the bilateral amygdalae and bilateral hippocampi,[42;43] which may pose a risk of exaggerated and abnormal descending influences on both cardiac and respiratory control centres. If the seizure syndrome continues, pathophysiological changes in cardiac function may develop, which would represent a significant risk for development of heart failure or fatal arrhythmias, especially in individuals with pre-existing heart disease.

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Conflicts of Interest

Authors declare that they have no conflicts of interest.

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Supplemental Figure Legends

Supplemental Figure S1.

Anomalous heart rate changes during a Racine 3 seizure. Rat lacks any evidence of clonic

or tonic seizures through the period of the Mobitz-type arrhythmia, here dropping 3 in 4

cycles of ECG, with heart rate dropping from 400 to 100 bpm. Later in seizure the rat makes

4 jumps from immobility, which trigger further missed beats**.**

Supplemental Table S1

Dependence of interictal QT400 on seizure history. Pearson correlation coefficients and

corresponding significance values between interictal QT_{400} and the number of seizures during

the preceding number of days shown in the row "History". Seizure counts are divided into

Racine 4/5 seizures, or those with ictal bradyarrhythmias where video recording is absent,

and all seizures as shown in row "Type". $P<0.01$ shown in bold red font; $P<0.05$ shown in

brown font.

Supplemental Figure S2.

QT-RR plots for 4 epileptic rats (separated by thick dashed lines). Each plot presents data

for 1-hour epochs during interictal periods 18 days after injection ("epi"), or a corresponding

period before injection of TeNT. Epileptic epochs start at least 30 minutes after previous

seizure and end at least 30 minutes before the next. "Dark" and "light" indicate the lighting

conditions in the monitoring room. Linear regression for the dark pre-injection condition is

reproduced in grey in the remaining 3 panels for each rat. Reference lines mark RR = 150 ms
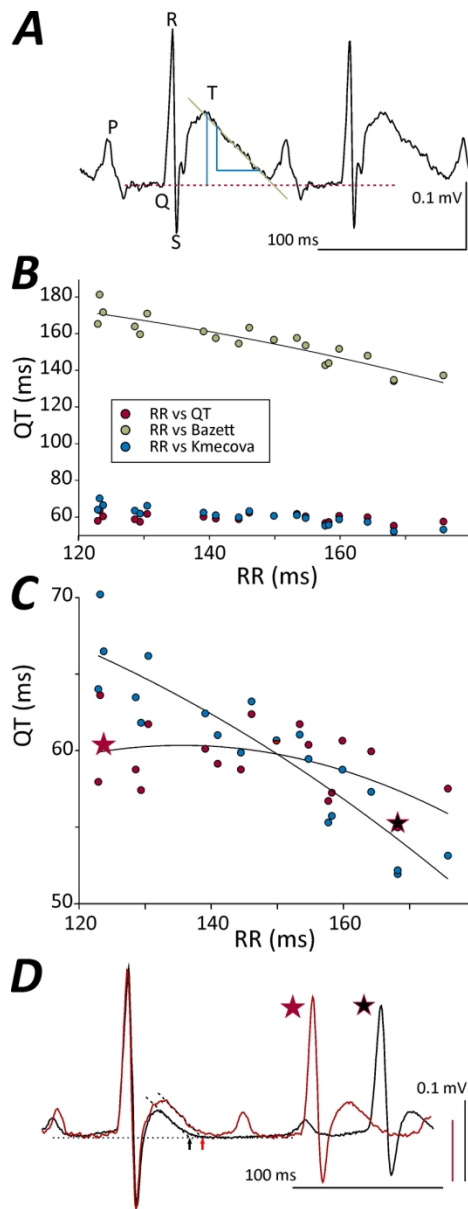
and QT for the dark pre-injection case.

Figure 1. QT corrections in rats. A. Averaged ECG waveform illustrates the method for estimating the QT interval. Red dashed line shows isopotential, blue lines indicate T wave amplitude and 80% and 20% heights used for fitting line (light green) to the descending phase of T. B. QT corrections exacerbate the impact of heartbeat duration (RR) on QT. Raw QT is plotted in red. The Bazett correction $QT_c=QT/(RR)^{1/2}$ (green, on extended y axis) is widely used in clinical practice, but is ill-suited to the faster rat heart rate. Kmecova & Klimas adapt Bazett's correction for the rat (blue): $QT_c=QT/(RR/150)^{1/2}$. All variables in milliseconds. Both corrections increase the dependence of QT on heartrate. C. Raw and Kmecova & Klimas correction in more detail. D. Averaged ECGs with short (red) and long (black) RR; data points shown in C by stars. Raw QT is little affected by RR until it slows below 160 ms, where it is prolonged. Broken lines show linear fit to T-wave intersecting the isopotential line at points marked by black and red arrows. Voltage scales were adjusted to match R wave amplitudes.

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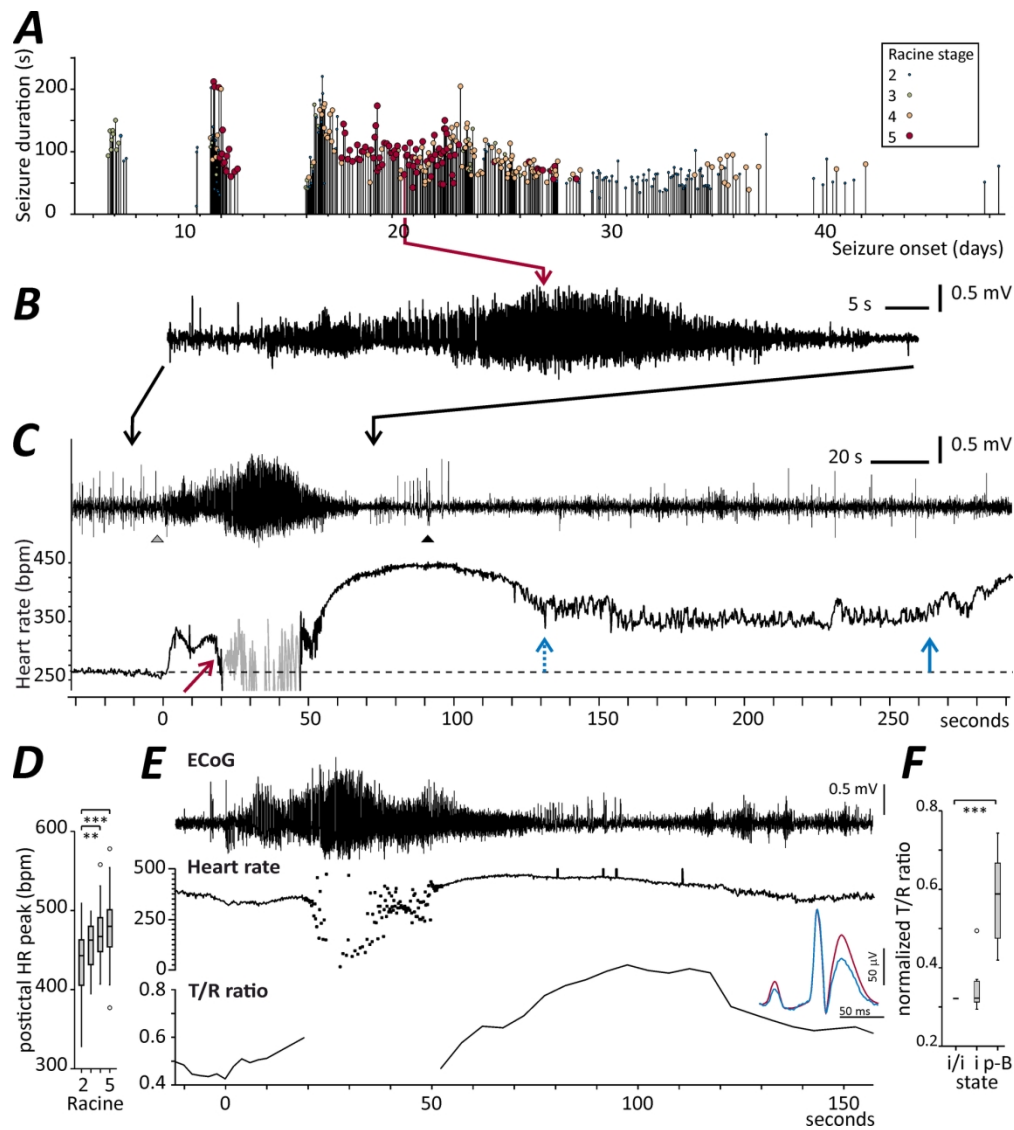41
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Figure 2. Seizures and ECGs during chronic epilepsy induced by intrahippocampal tetanus neurotoxin. A. Seizure profile for one rat, shows seizure duration (y-axis), Racine stage (circles, see key), and time after injection (x-axis). B. Expansion of seizure marked red in (A), 21 days after injection; triangles indicate start and end of seizure. C. Same seizure as (B) on slower timescale, shows electrographic activity (upper trace) and corresponding heart rate (lower trace). Heart rate increase at seizure onset coincides with raising head, red arrow indicates sinus bradycardia related to loss of muscle tone. Arrhythmia during convulsive stage is in grey lines (see also Figures 3, 4). Rat remained prone and immobile after seizure (~50 seconds), until the dashed blue arrow when it started making repeated jaw movements. At the solid blue arrow, it explores its cage. D. Boxplot shows postictal tachycardia increases with Racine stage (median is horizontal bar, interquartile range is grey box and 10-90% are vertical bars). E. Seizure ECoG recording aligned with heart rate and ratio of T to R waves measured from ECG averaged over 2 seconds. T to QRS ratio increases after bradyarrhythmia. In this case T waves remain elevated for approximately 200 seconds after seizure onset. E, insert. Averages of 5-second epochs of ECG aligned by R wave peaks, from before (blue) and after (red) bradyarrhythmia. F. Boxplot of T/QRS ratio for 3 randomly selected Racine 5 seizures from each of the 3 rats with video recording. Values normalized to the preictal T/QRS ratio, and scaled to the grand mean of the preictal ratios. Significant differences indicated by horizontal links: ** P<0.008, *** P<0.002.

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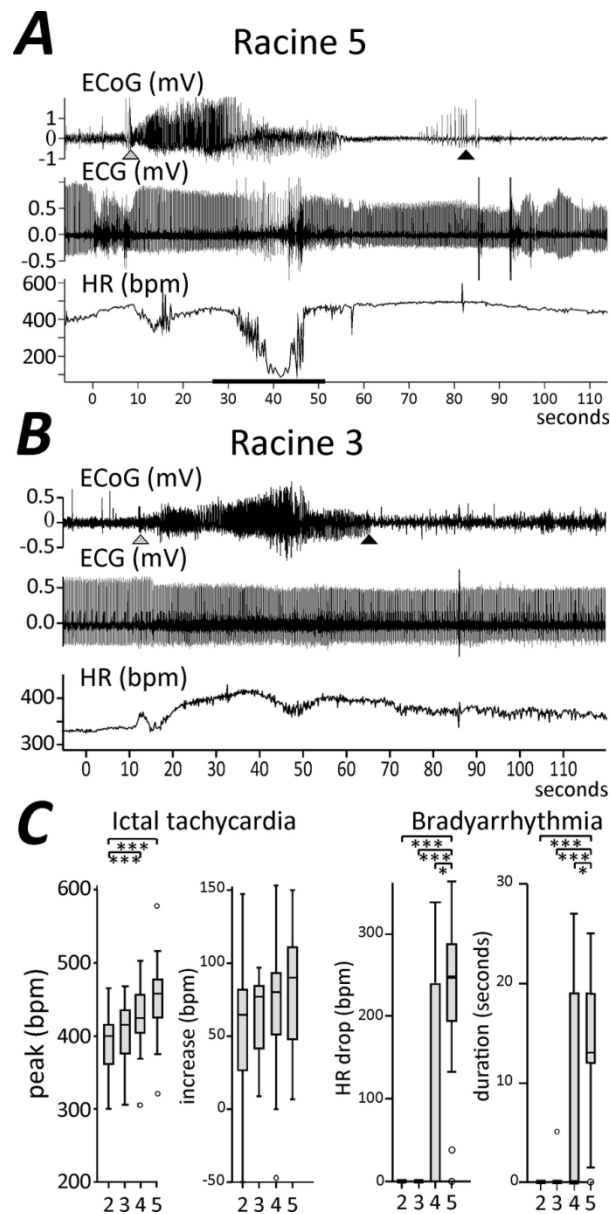
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Figure 3. Ictal tachycardia and bradyarrhythmia during seizures induced by intrahippocampal TeNT. A. ECoG, ECG and heartrate during a Racine stage 5 convulsive seizure. Note prominent bradyarrhythmia, superimposed on ictal tachycardia, between 31 and 47 seconds on the timescale. B. Similar recording during a Racine stage 3 non-convulsive seizure. A modest bradycardia superimposed on the ictal tachycardia but heart remains in sinus rhythm. C. For a random sample of seizures classified on the Racine scale (x-axis, 2-5), quantification of, from left: peak ictal tachycardia, increase of postictal tachycardia from preictal heart rate, heart rate decrease during bradyarrhythmia from preceding ictal tachycardia peak, and duration of bradyarrhythmia. Box and whisker plots show median (thick horizontal bar, which appears on the x-axes for Racine scores 1-4), interquartile range (grey box) and 10-90% (whiskers or vertical bars). Outliers are marked by circles or stars, but are included in medians. Significant differences indicated by horizontal links: peak tachycardia by ANOVA with Dunnett post hoc test using Racine 2 as control; bradyarrhythmia heart rate drop and duration by Mann-Witney U Tests comparing Racine 5 with each of 2, 3, 4.

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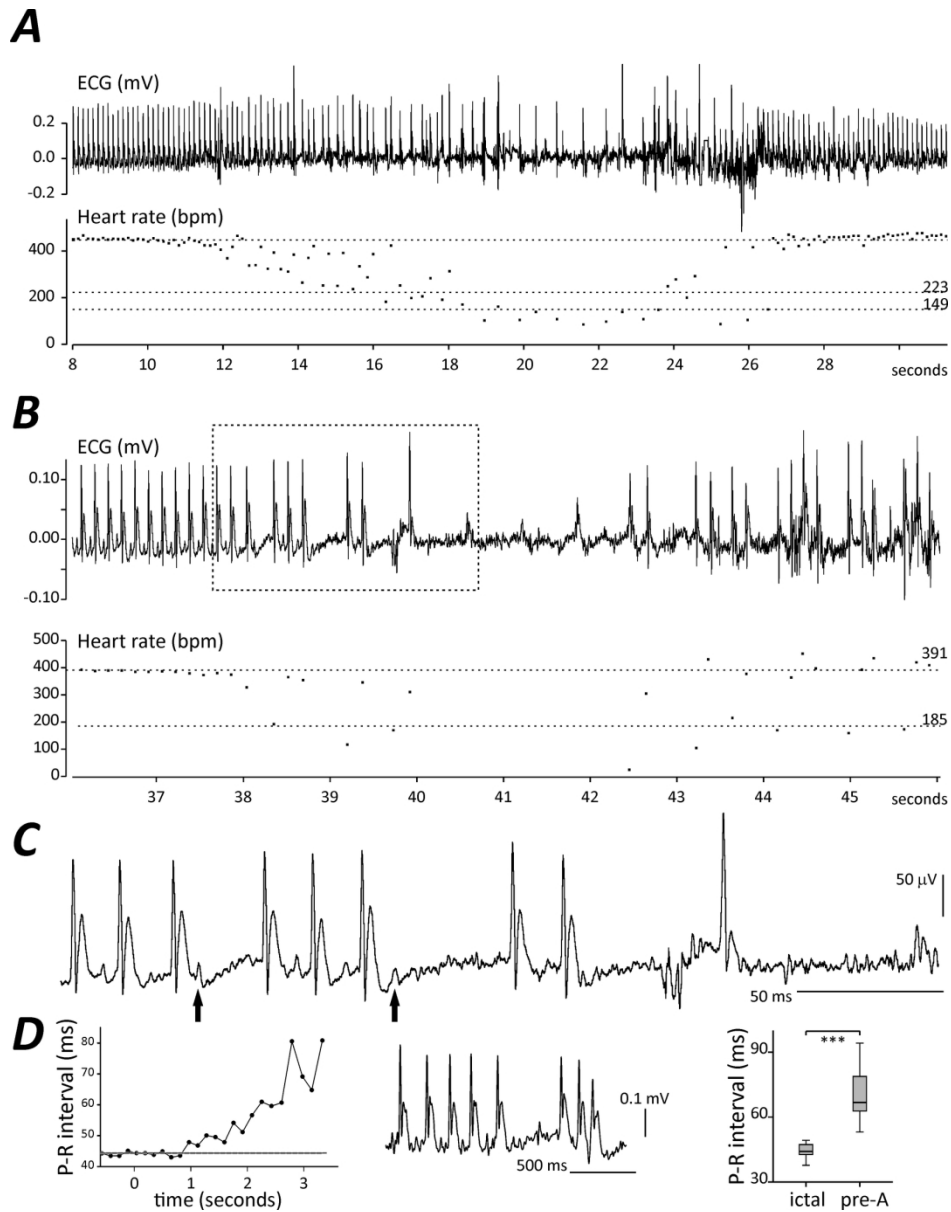48
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Figure 4. Ictal bradyarrhythmias. A. Detail of ECG and heartrate during period of Racine 5 seizure marked by thick line on time axis of Fig 3A. ECG shows deviation from its normal highly regular pattern. Instantaneous frequency (reciprocal of previous R-R interval) reveals intermittent loss of 1 beat in 2 (alternating 400 and 200 bpm) between 14 and 16 seconds on the timescale, and then loss of 2 beats in 3 (149 bpm) until 24 seconds. B. ECG from another rat experiencing a Racine stage 5 convulsive seizure reveals intermittent loss of single beats (alternating 391 and 185 bpm) before an asystole starts at 40 seconds. C. Section of ECG marked by dotted box in B expanded to show waveform. Note P waves at start of section of missed beats (black arrows). D. Increase in P-R interval preceding missed beats: left graph shows an example leading to the onset of the arrhythmia shown in the centre (similar to that shown in C). Dashed grey horizontal lines show 95% confidence intervals, and solid black line shows mean, for P-R during the preceding interictal period. Boxplot shows P-R intervals during ictal sinus rhythm (ictal) and during the beat preceding bradyarrhythmia (pre-A), for 10 Racine stage 5 seizures from 3 rats.

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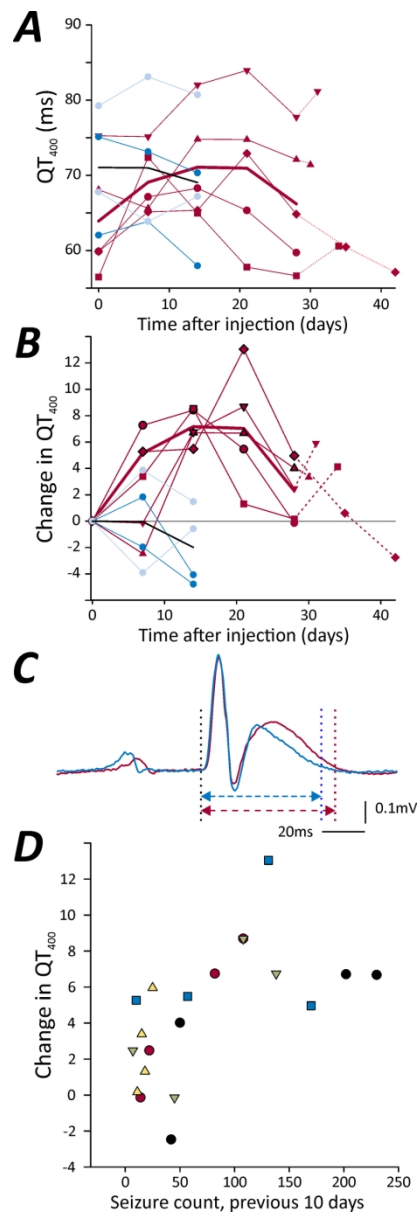48
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Figure 5. Changes in QT duration in epileptic and control rats. A. The QT segment of the ECG, measured from averages centred on R wave peaks during 5-second epochs when heartrate was 400 bpm, shows prolongation during the weeks following TeNT injection (red symbols and dashed red lines represent individual animals; continuous thick orange line, group mean). One rat (inverted triangles) stops seizing after 3 weeks. Local regulations prevented our collecting data for more than a few days after the seizures ended (dashed lines). The remaining 4 continue seizing for more than 4 weeks. Control rats (blue symbols and lines) and non-epileptic rats injected with TeNT (pale blue symbols and lines) failed to show an increase over 2 weeks (controls and non-epileptic rats did not differ significantly and are pooled to a single group mean shown as a black line). B. Same data as A, subtracting initial values to reduce inter-rat variability. Symbols for values more than 1.96 SD from the pre-injection mean QT400 have black outlines. C. Averages of ECGs at 2 weeks for the rat most closely matching the median QT400 for all epileptic rats (red), aligned by R wave with control rat (blue); arrows show duration of QT. D. Change in QT400 plotted against total seizures during 10 days preceding each measurement. Individual rats have their own symbol shapes and colours. Overall these measurements are correlated significantly (see Results and Supplemental Table 1).

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