



**Toward evidence-based severity assessment in rat models
with repeated seizures:
II. Chemical post-status epilepticus model**

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Key Words:	Behavior, Stress, Rodent, Pilocarpine, 3R

Toward evidence-based severity assessment in rat models with repeated seizures:

II. Chemical post-status epilepticus model

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Abstract

Objective:

Considering the complexity of neuronal circuits and their epilepsy-associated alterations epilepsy models cannot be completely replaced by in-vitro experimental approaches. Decisions about an ethical approval of in-vivo studies require a thorough weighing of the animal's burden and the benefit regarding the expected gain in knowledge.

Methods:

Based on combined behavioral, biochemical, and physiological analyses we assessed the impact on the animal's well-being and condition in different phases of the pilocarpine post-status epilepticus model in rats. Moreover, we addressed the hypothesis that telemetric seizure monitoring provides a refinement measure as compared to tethered recordings.

Results:

As a consequence of status epilepticus (SE) increased levels of impairment were evident in the early post-insult phase and late chronic phase, whereas only a mild impairment was observed in the interim phase. Parameters that stood out as sensitive indicators of animal's distress included burrowing, which proved to be affected throughout all experimental phases, saccharin preference, and fecal corticosterone metabolites (FCMs).

Relevant differences between animals with telemetric and tethered recordings included a decreased saccharin preference, increased FCM levels and increased adrenal gland weight in animals with tethered recordings.

Significance:

The cumulative burden with temporary but not long-lasting phases of a more pronounced impairment suggest a classification as severe as a basis for a laboratory-specific pro- and retrospective evaluation. Among the parameters analyzed, burrowing behavior and saccharin preference are standing out as candidate parameters, which seem to be well suited to obtain information about the animal's distress in epileptogenesis models. Based on selected

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parameters first evidence has been obtained that telemetric recordings may serve as a refine measure. The robustness of these findings requires further validation.

Keywords: Behavior, Stress, Rodent, Pilocarpine, 3R

Key Point Box:

- The data indicate that the assessment of burrowing behavior and saccharin preference can serve as indicators of severity.
- The findings suggest classification of the chemical post-status epilepticus model as severe.
- First evidence has been obtained that telemetric recordings may serve as a refinement measure.

1 Introduction

Considering the persistent major challenges in the clinical management of epilepsies, there is a continued need for animal experiments identifying and assessing strategies, which aim to overcome drug resistance, to interfere with pathophysiological mechanisms, and to prevent epilepsy.¹⁻⁴ As pointed out previously, experimental in vivo studies require a thorough ethical justification with a careful harm-benefit analysis.⁵ In many countries, along with the request for an animal experiment allowance scientists have to categorize the animal model according to severity classification schemes.⁶ This suggestion is then evaluated by regulative authorities and ethical committees providing the basis for the recommendation or decision to approve or reject. This procedure has to deal with the well-known uncertainties in judging and assessing the well-being of animals and the burden associated with interventions and disease models. Thus, there is an urgent need to replace subjective-opinion based decision-making by scientific approaches, and to develop evidence-based severity assessment schemes for epilepsy models. Respective efforts will even more importantly provide an improved basis to assess refinement measures and minimize severity.^{5; 7}

In a previous study, we have evaluated the impact of focal and generalized kindled seizures on the well-being of rats based on the analysis of various behavioral and biochemical parameters.⁵ Taken together the data suggested a categorization as a model with moderate severity based on a longer-lasting mild behavioral alteration.⁵

First described by Turski and colleagues (1983), the application of the pilocarpine model has increased over time so that the model represents one of the most frequently applied chronic epilepsy rodent models in the field of epileptology. The model is based on chemical induction of a SE by systemic administration of the direct parasympathomimetic drug.⁹⁻¹¹ As a consequence of the SE, rats develop spontaneous seizures following a latency phase.⁹ The

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model is characterized by extensive brain lesions, which are accompanied by pronounced behavioral alterations.⁹ Considering the wide-spread use of the pilocarpine model, it is of particular relevance to provide a scientific basis for the severity classification of the model.

In the past, efforts have been made to optimize the model by adjusting the pilocarpine administration protocol and by pretreatment.^{9; 12} However, respective approaches have mostly focused on a limitation of the mortality rates as a major issue in the application of the paradigm.

As emphasized by Lidster et al. (2016), further efforts should be made to optimize the protocols of post-SE models including the pilocarpine model considering animal welfare aspects. In this context, it has been hypothesized that telemetric recordings might serve as a valuable refinement measure replacing common tethered recordings.⁷ Along this line, we have not only comprehensively analyzed the impact of the rat pilocarpine post-SE model on behavioral patterns and biochemical parameters, but have also compared between groups with tethered versus telemetric recordings. In addition, analysis of telemetric data provided information about different experimental phases on circadian activity and heart rate patterns as well as heart rate variability as further distress-associated readout parameters.

One final note is that this study is part of a series in which the severity classification of three common rat epilepsy models (I. Kindling model published by Möller et al^{5; 13}, II. Chemical post-SE model in the present study and III. Electrical post-SE model submitted by Seiffert et al) is determined using identical behavioral and biochemical and physiological analyses.

2 Material and Methods

2.1 Animals and experimental groups

Sixty-two female Sprague Dawley rats (180-220 g, Envigo, Netherlands) were used for this study. Animals were housed individually and under controlled conditions (45-65 % humidity, 22-24 °C, 12h day/night cycle). Food and tap water was freely available. Every week the animals received a macrolon type III cage with new bedding material (Lignocel® Select, J. RETTENMAIER & SÖHNE GmbH + Co KG, Germany) and new nesting material (Enviro-Dri®, Claus GmbH, Germany).

For the study with tethered recordings, 44 animals were randomly divided in three experimental groups (randomizer.org), a naive (n=12 animals, without implant), sham (n=12 animals, with implant) and pilocarpine-treated tethered (n=20 animals, with implant and treated with pilocarpine) group. For the study with the telemetric recording 18 animals were divided in two groups in a randomized manner (randomizer.org): sham (n=6 animals, electrode and transmitter implanted) and pilocarpine-treated telemetric (n=12 animals, electrode and transmitter implanted and pilocarpine-treated). Animals were weighed regularly and controlled according to the severity assessment schemes. Additionally, the Grimace scale and the Irwin score were assessed. At the end of project, rats were euthanized by an overdose of pentobarbital (600 mg/kg i.p., Narcoren, Merial GmbH, Germany). Afterwards adrenal glands were sampled and weighed. This study was conducted following approval by the government of Upper Bavaria (reference number: AZ 55.2-1-54-2532-105-16) and was in line with the German Animal Welfare act, the ARRIVE guidelines concept and the Basel declaration including the 3R concept.

2.2 Electrode and transmitter implantation and status epilepticus induction

Electrodes were implanted in the right dentate gyrus of the hippocampus (AP - 3.9 mm, L + 1.7 mm, V + 4.1 mm relative to bregma).¹⁴ The surgical procedure was performed according to Di

Liberto et al. (2018). Following a recovery period of two weeks SE was induced as described by Di Liberto et al. (2018) , see supplementary S1 for a more detailed description of both the implantations and the induction of SE. A total of four out of 30 animals died after diazepam injections resulting in a final n of 15 pilocarpine treated animals in the tethered group and eleven treated animals in the telemetric group.

2.3 Tethered and telemetric recordings

To confirm the development of spontaneous recurrent seizures (SRSs) a video/EEG monitoring was performed. Monitoring and analysis in the tethered group was done as described by Walker et al. (2016). Monitoring in the telemetry group was done as described by Möller et al. (2019). In addition to the monitoring period during the chronic phase, recordings were made before induction of SE (baseline), seven days (early post-insult phase) and four weeks post-SE (latency phase) for investigating ECG.

Analysis of ECG was done using Ponemah® Software 6.41 (Data Sciences International, USA). In addition to the time domain parameters, for the frequency-domain parameter the ratio between low and high frequency bands (LF/HF with LF=0.1 – 1.0 Hz and HF=1.0 – 3.5 Hz) was calculated according to Thireau et al. (2008) . EEG recordings were analyzed using NeuroScore® Software 3.0 (Data Sciences International, USA).

2.4 Behavioral and biochemical parameters

The behavioral tests were performed according to previous studies conducted in our facility^{5; 15; 18}. Behavioral assessments were made at different time points throughout the study. Nest building activity, burrowing behavior, the Grimace scale and the Irwin score were analyzed repeatedly during different phases of the study. Social interaction test, burrowing paradigm, open field, black-white box, elevated-plus maze and saccharin preference test were assessed during the chronic phase. Throughout the study fecal samples were used to quantify

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3 corticosterone metabolites. At the end of the project hair samples were collected for the
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5 determination of corticosterone levels and serum for the investigation of brain-derived
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7 neurotrophic factor (BDNF) and corticosterone levels. Analyses of the biochemical parameters
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9 were done like described previously by Möller et al. (2018).
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13 **2.5 Statistics**

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15 GraphPad Prism (Version 5.04; GraphPad, USA) was used for the statistical analysis.
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17 Comparisons of naive versus sham and sham versus post-SE was tested using an unpaired t-
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19 test. For the analyses of data sets of the naive, sham and post-SE group a two-way repeated
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21 measure analysis of variance (ANOVA) was performed. Additionally, a Bonferroni post-hoc
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23 test was performed for individual comparisons. For the visualization of the telemetric
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25 recordings, the correlation matrix and the Principal Component Analysis (PCA) R version
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27 3.3.2¹⁹ with the R packages ggplot2²⁰, corrplot²¹ and made4²² were used. A Loess regression
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29 with a span of 0.15 was used to smooth the line graphs in Figure 4 and Figure S9.
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3 Results

3.1 Induction of status epilepticus and development of spontaneous recurrent seizures

SE in both groups was induced by fractionated injections of pilocarpine in 30 rats. Following the first injection of pilocarpine animals exhibited head nodding, tremor, facial clonus, immobility and chewing. After two to four injections all animals developed SE. During the video/EEG monitoring in the tethered group 13 out of 15 animals exhibited SRSs. One animal showed handling associated generalized seizures. Over the whole monitoring period the mean seizure frequency was 29.4 (Figure S3A, SD=46.2, median=11.5) and the mean total seizure duration was 2352.2 seconds (Figure S3B, SD=3034.6, median=938). All animals with telemetric recordings developed SRSs following induction of SE. The mean number of SRSs in this group amounted to 22.6 (Figure S3A, SD=39.5, median=3) and the mean seizure duration to 2072.3 seconds (Figure S3B, SD=2756.5, median=826).

3.2 Impact on nest building

In the early post-insult phase (five days following SE) and the latency phase (four weeks following SE) nest complexity scores proved to be in the control range (Figure S5). In the chronic phase following video/EEG monitoring with tethered or telemetric recordings, only those animals with telemetric recordings exhibited a significant reduction in nest scores (Figure 1B).

3.3 Impact on the Grimace scale, behavior in the burrowing paradigm and the open field

Analysis of the Grimace scale in the post-surgical phase revealed a difference in the recovery period regarding the electrode only versus combined electrode and transmitter implantation. The animals experiencing the longer lasting surgery showed an altered Grimace scale until day six (Figure S10B) whereas animals with single electrode implantation exhibited an increased Grimace scale until day four (Figure S10A) compared to baseline Grimace scores.

As we will report in a separate manuscript (Talbot et al., in preparation) presenting and discussing a series of examples from various experimental paradigms with a focus on body weight development in the context of humane endpoint decisions, a transient drop of body weight characterized the very early phase following SE (data not shown).

The surgical procedure remained without consequences on burrowing behavior one week following the intervention, and at later time points during the experiment (Figure 2A, B, D, F). Following SE, the amount of gravel burrowed proved to be significantly reduced in all groups throughout the experiment (Figure 2B-G).

Whereas the time to onset of burrowing behavior remained unaffected in the group of animals with telemetry transmitters (Figure S2J, L, N), a persistent increase of the latency time became evident as a short- and long-term consequence of the SE in animals without transmitters (Figure 2I, K, M).

Except for the reduced immobility frequency, behavior in the open field was not altered in animals with electrode implantation or SE (Figure S4). The frequency of immobility proved to be lower in both groups of animals with SE as compared to respective controls groups (Figure 1C&D).

3.4 Impact on social interaction, anxiety-associated and anhedonia-associated behavior

Chronic electrode implantation did not exert relevant effects on the time spent in active social interaction (Figure 1E). In rats with SRSs, social interaction was affected in a negative manner resulting in a shorter total time spent with active interaction (Figure 1E&F).

In the elevated-plus maze paradigm behavioral patterns including the time spent in different areas and the distance moved did not differ between groups (Figure S6). However, for several parameters a higher level of inter-individual variance became evident in the group of animals with epilepsy. Moreover, the animals exposed to the tethered recordings exhibited a significantly lower number of head dips (Figure S6G).

In the black-white box, both groups of animals with epilepsy displayed reduced stretching behavior in comparison with the respective control groups (Figure 1G&H). In this paradigm none of the other parameters was affected as a long-term consequence of SE and epilepsy manifestation (Figure S7).

A reduced consumption of saccharin was revealed in animals with epilepsy, which were previously exposed to tethered recordings (Figure 1I). In contrast, animals with telemetric seizure monitoring did not confirm a significant alteration in saccharin preference (Figure 1J).

3.5 Impact on biochemical parameters

Analysis of samples from animals with electrode implants did not confirm alterations in adrenal gland weight or concentrations of BDNF and corticosterone (Figure 3A, C, Figure S8B).

In all rats with epilepsy, serum BDNF and hair corticosterone levels remained unaffected at the end of the projects (Figure S8). However, in rats with SRSs and a history of tethered recordings, the adrenal gland weight and FCM levels were increased (Figure 3A; Figure 3E). Whereas FCM concentrations were in the control range during the latency phase, an early increase was evident two days following SE. Surprisingly, serum corticosterone levels proved to be decreased in this group of animals at the end of the experiment (Figure 3C).

Considering this outcome, we additionally analyzed selected parameters reflecting activation of the hypothalamic-pituitary-adrenal gland axis in rats with previous telemetric recordings of spontaneous seizures. In these animals, adrenal gland weight as well as serum corticosterone and FCM levels proved to be in the control range except for a reduction of FCMs seven days following SE (Figure 3B, D&G).

3.6 Impact on home cage activity, heart rate and heart rate variability

Home cage activity levels monitored during different experimental phases were compared with baseline data recorded before induction of SE.

Recordings from the early post-insult phase and the latency phase demonstrated that the SE history resulted in higher levels of home cage activity during the dark phase (= activity phase). The short- and long-term impact of SE on dark phase activity is further reflected by group differences during the early post-insult and the chronic phase (Figure S9).

Both, day and night heart rate reached higher levels in the early post-insult phase and the latency phase (Figure 4A&B). Thereby, differences to implanted control animals were detected during all phases of epileptogenesis.

The total variability of heart rate was analyzed based on the standard deviation of NN intervals (SDNN). The analysis revealed a decreased SDNN during the early post-insult phase (Figure 4C&D). Throughout epileptogenesis, light phase (= resting phase) SDNN in implanted control animals exceeded that in animals with SE. During the dark phase SDNN proved to be decreased in rats with SE in the early post-insult and the latency phase.

None of the parameters of short-term variability (RMSSD = root mean square of successive differences; NN9 = percent of subsequent NN intervals, which deviate more than 9 ms; pNN9 = proportion derived by dividing NN9 by the total number of NN intervals) was altered.

In addition to the time domain analysis a frequency domain analysis was performed by analyzing the ratio between low and high frequency bands. No significant differences were observed as a consequence of SE (Figure S12).

3.7 Correlation matrix of all measured variables

Two correlation matrices illustrating the Spearman correlation coefficients between the majority of measured variables were calculated, one for animals prepared for tethered recordings (Figure 5) and one for animals prepared for telemetric recordings (Figure S11). As the number of significant correlations are too numerous to list only selected interesting findings are highlighted.

In animals with tethered recordings there is a set of behavioral parameters, which show a high number of correlations. These comprise variables measured in the burrowing, social interaction, and saccharin preference test as well as food intake and weight gain. These variables not only show strong correlations with each other but also with variables from other tests such as the black-white box, elevated-plus maze and the level of serum corticosterone and BDNF.

In animals with telemetric recordings, the inter-behavioral paradigm correlations are more scattered (Figure S11), with significant correlations found throughout more variables. When focusing on the relationship between heart rate variables and behavior a clearer pattern emerges. Heart rate measures correlate with a number of behavioral parameters, but perhaps most noteworthy is that strong correlations exist between behavioral and heart rate variables measured at different time points following SE. One example is saccharin preference, measured at twelve weeks post-insult which shows correlations with both heart rate and heart rate variability recorded only one week post-insult, while showing weaker correlations with the same parameters measured at four and nine weeks post-insult. The same tendency can be observed with performance in the black-white box and time spent in active social interaction. These parameters measured eleven weeks post-SE, exhibit stronger correlations with heart rate variables measured at four weeks post-SE as compared to heart rate variables measured at one or nine weeks post-insult.

3.8 Principal component analysis

Using data for parameters which were analyzed in all groups of animals with epilepsy from both the tethered as well as the telemetric recordings a principal component analysis (PCA) was performed. In this PCA only those data which were obtained immediately following the monitoring period were included, testing any lasting influence on subsequent behavior and other biochemical parameters. Overall the first two principal components capture 41% of the variance in the data (PC1: 29%, PC2:12%). The two groups are significantly different along

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3 PC2 ($F(1,24)=26.08$, $p<0.001$). The parameters contributing to this difference do not point
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5 towards a singular phenotype but rather comprise a mix of parameters from different
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7 experiments, i.e. the top five contributing factors include nest building, time spent in passive
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9 social interaction, the level of soiling, the level of BDNF and the level of corticosterone
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11 measured in serum.
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3 **4 Discussion**
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5 Several experimental influences need to be considered, when assessing the severity of chronic
6 epilepsy models.^{5; 7; 23} For post-SE models respective experimental procedures comprise the
7 surgical implantation of electrodes, the induction of the SE, and the video/EEG monitoring.
8 The impact of these procedures on the animals' wellbeing and condition during all phases of
9 the chronic model need to be taken into account for the overall severity classification of the
10 model.
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12 Stereotactic implantation can exert consequences on animal behavioral patterns also reflecting
13 putative detrimental effects on wellbeing.^{24; 25} The comparison between electrode-implanted
14 animals and naive control animals at various time points did not reveal relevant behavioral or
15 biochemical alterations. These data are rather in line with our previous findings, in which
16 animals with depth-electrode implantation exhibited only very minor behavioral alterations
17 nine weeks after surgery.⁵
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19 In order to assess the short- as well as long term consequences of the SE, selected parameters
20 were repeatedly analyzed allowing a comparison between experimental phases following SE.
21 In the chronic phase, several behavioral parameters showed alterations including reduced social
22 interaction and saccharin preference. These findings together with an increase in adrenal gland
23 weight at the end of the experiment, indicates that animals with epilepsy manifestation in the
24 chronic phase of the pilocarpine model show an elevated level of distress.
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26 Findings from assessment seven days and 28 days following SE with reduced burrowing
27 activity, but unaltered nest building, soiling, and normal or reduced FCM levels rather suggest
28 that the impact on the animals well-being is rather mild during this experimental phase. In this
29 context it is emphasized that in the pilocarpine model this phase is characterized by single
30 seizures, so that the definition of an actual 'latency' period is often difficult.
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32 Taken together our findings demonstrate an increased level of impairment in the very early
33 post-insult phase and the chronic phase, and mild impairment of wellbeing during the latency
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3 phase. As expected the impact of the pilocarpine-induced post-SE model on the animals'
4 wellbeing clearly exceeds that previously determined for the kindling paradigm.⁵ Considering
5 the duration of the total experimental procedure, the complete experiment needs to be classified
6 as severe according to the final report of a European expert working group in severity
7 classification (http://ec.europa.eu/environment/chemicals/lab_animals/pdf/report_ewg.pdf). In
8 this context, it is emphasized that a suggestion for a classification can only serve as a
9 recommendation, considering that the laboratory specific handling and experimental
10 procedures as well as further factors including origin, strain, age, sex of the animals can exert
11 a relevant influence, thus, requiring a laboratory-specific classification. Refinement measures
12 should be assessed that might help to minimize the severity conditions.⁷

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14 In this context, it is of relevance to identify sensitive and robust parameters that should be
15 included in severity-assessment schemes aiming to validate putative refinement measures and
16 to classify new epilepsy models. Both, nest building and burrowing have been discussed as non-
17 essential behaviors that can serve as easy-to-use indicators of wellbeing in laboratory rodents²⁶⁻
18 ³¹. In contrast to the persistent decrease of burrowing behavior throughout all experimental
19 phases, an influence on nest building was only observed in the chronic phase in animals with
20 preceding telemetric recordings. Thus, our findings suggest that burrowing behavior seems to
21 be a more sensitive indicator of wellbeing in chronic epilepsy models with induction of
22 epileptogenesis. In this context, it should also be considered that SRSs might cause a bias in the
23 chronic phase because complex nests may be destroyed during generalized tonic-clonic
24 seizures. When considering burrowing behavior as a parameter the effort for analysis needs to
25 be taken into account. While burrowing behavior in general can be easily assessed with a simple
26 experimental set-up, it requires a short training phase, baseline measurements, and for rats the
27 common procedure is based on analysis in a separate cage and not the home cage.^{27; 28; 30; 32}
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29 Among further paradigms saccharin preference is standing out as a low-input paradigm, which
30 can be applied in the home cage, thereby avoiding any procedures that might exert more
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pronounced effects on readout parameters. The paradigm represents a comprehensively validated test to detect anhedonia-associated behavior in laboratory rodents.³³⁻³⁵ Previous studies in epilepsy models already demonstrated a reduction in the consumption of sweet solutions³⁴⁻³⁶, a finding that we confirmed in the group of rats with tethered recordings. It is of interest, that saccharin preference remained unaffected by kindled generalized seizures⁵, indicating that the parameter might indeed help to distinguish between chronic epilepsy models with different severity.

Telemetric assessment of home cage activity, heart rate, and its variability has been suggested as an approach for assessment of distress in laboratory rodents.³⁷⁻⁴⁰ The findings from the present study partly confirm this suggestion with increased dark-phase activity evident during the early and late phase of epileptogenesis as well as increased heart rates throughout all day and experimental phases. However, only limited alterations of heart-rate variability parameters were observed with a decreased total variability. Thus, it remains questionable whether it is worthwhile to apply highly time-consuming and cost-intensive telemetry procedures in addition to comprehensive behavioral analysis in severity assessment studies.

Additionally, the two cross correlation matrices affirm both the burrowing and saccharin preference test as being robust behavioral paradigms, both of which show correlations with a number of costlier, more invasive and labor intensive measures such the telemetric measures, the elevated-plus maze and social interaction.

In this study, we also addressed the hypothesis that telemetric recordings can serve as a refinement measure minimizing the severity of the paradigm. This hypothesis has been proposed by Lidster and colleagues (2016), however, to our knowledge it has not yet been addressed by a scientific experimental approach. Whereas tethered recordings can despite the use of swivel systems be associated with restrictions in mobility related to the traction forces by the cable connection, telemetry setups require implantation of transmitters that despite

continued miniaturization of the devices might still represent some burden related to weight and size of the implanted transmitter.⁴¹ Regarding the impact of the surgical procedures comparison between animal prepared for telemetric vs. tethered recordings exhibited a longer lasting alteration in the Grimace scale.

Following the recording phase animals with preceding tethered vs. telemetric recordings exhibited a different level of behavioral and biochemical alterations. Reduced saccharin preference along with increased FCM levels and an increased adrenal gland weight may indicate that indeed tethered recordings can raise the burden for the animals in chronic models with seizure monitoring. Surprisingly, serum corticosterone proved to be reduced in animals with tethered recordings. However, this might reflect the fact that animals have adjusted to various experimental influences and thus, show less pronounced acute responses to handling before sampling of serum.

In conclusion, the cumulative burden with temporary but not long-lasting phases of a more pronounced impairment suggest a classification as severe as a basis for a laboratory-specific pro- and retrospective evaluation. Among the parameters analyzed, burrowing behavior and saccharin preference are standing out as candidate parameters, which seem to be well suited to obtain information about the animal's distress in chronic epileptogenesis models. Based on selected behavioral and biochemical parameters first evidence has been obtained that telemetric recordings may serve as a refine measure. The robustness of these findings and the candidate parameters requires further validation.

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Disclosure

The authors declare that they have no competing interest.

Ethical Publication Statement

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

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Figure legends

Figure 1: Nest building, social interaction, anxiety-associated and anhedonia-associated behavior. A, B, Nest complexity score in the chronic phase of the tethered (A) and telemetric (B) group. A significant reduction of nest complexity was observed in the animals with epilepsy in the telemetrically recorded animals ($P=0.158$) but not in the tethered group. C, D, Locomotor activity in the open field during the chronic phase. The animals of the tethered (C, $F(2,35)=20.65$, $P<0.0001$, epilepsy against both control groups $P<0.0001$) as well as the telemetric (D, $P=0.0221$) recorded epilepsy group showed a significant decrease of immobility. E, F, Time in active social interaction of the tethered (E, $F(2,16)=44.87$, $P=0.2580$, epilepsy against both control groups $P<0.0001$) and telemetric (F, $P<0.0001$) recorded epilepsy animals. In both groups animals which exhibited SRSs showed a significant reduction of active social interaction. G, H, Stretching postures in the black-white box. Both, the tethered (G, $F(2,35)=5.961$, $P=0.0509$, epilepsy against both control groups $P<0.05$) and telemetric (H, $P=0.0488$) recorded animals with SRSs showed a significant reduction of stretching postures in the black-white box compared to control groups. I, J, Saccharin consumption of the tethered epilepsy group (I, $F(2,34)=14.18$, $P=0.0003$, epilepsy against both control groups $P<0.001$) was significantly reduced whereas the telemetric epilepsy group (J) did not show a difference. Error bars indicate standard error of the mean. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$. Total n for the tethered group: naive $n=12$, sham $n=12$, epilepsy $n=13-14$. Total n for the telemetry group: sham $n=6$, epilepsy $n=10-11$.

Figure 2: Burrowing behavior in the post-surgical phase, during epileptogenesis and following epilepsy manifestation. A, post-surgical weight of burrowed gravel. No significant difference was observed between groups. B-G, Burrowed weight during the early post-insult phase (B, tethered ($F(2,35)=36.14$, $P=0.0008$, post-SE against both control groups $P<0.0001$) and C,

telemetric recorded animals ($P=0.0134$)), the latency phase (D, tethered ($F(2,35)=14.28$, $P=0.0008$, post-SE against both control groups $P<0.001$ and E, telemetric recorded animals ($P=0.0354$)) and chronic phase (F, tethered ($F(2,35)=11.06$, $P=0.0011$, epilepsy against both control groups $P<0.01$) and G, telemetric recorded animals ($P=0.0042$)). During all phases the animals with SE showed a significant reduction of burrowing behavior as compared to the control groups. H, time to onset of burrowing behavior after surgery. No significant difference was observed. I, K, M, Time to onset of burrowing behavior in early post-insult phase (I, $F(2,35)=17.33$, $P<0.0001$, post-SE against both control groups $P<0.0001$), latency (K, $F(2,35)=28.85$, $P=0.0004$, post-SE against both control groups $P<0.0001$)) and chronic phase (M, $F(2,35)=6.756$, $P<0.0001$, epilepsy against both control groups $P<0.05$). Animals with a history of SE exhibited a significant increase during all phases post-SE in the onset of burrowing behavior. J, L, N, Animals with telemetric recordings did not show a significant difference regarding the onset of burrowing behavior. Error bars indicate standard error of the mean. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$. Total n for the tethered group: naive $n=12$, sham $n=12$, epilepsy $n=14$. Total n for the telemetry group: sham $n=6$, epilepsy $n=11$.

Figure 3: Activation of HPA axis. A, Animals with tethered recordings exhibited a significant increase of adrenal gland weight in comparison to sham group ($F(2,35)=3.659$, $P=0.0361$, epilepsy against sham $P<0.05$). B, In the telemetric recorded group no difference to control groups was evident. Serum corticosterone levels in the tethered recorded epilepsy group were significantly reduced as compared to the naive group (C, $F(2,27)=3.561$, $P=0.0076$, epilepsy against naive $P<0.05$). This effect was not observed in the telemetric recorded group (D). E, In the tethered recorded epilepsy group a significant increase in FCM levels was observed two days ($F(2,35)=10.72$, $P=0.0002$, epilepsy against both control groups $P<0.001$) and thirteen weeks after SE induction ($F(2,35)=6.549$, $P<0.0001$, epilepsy against both control groups

P<0.05). G, In the telemetric recorded post-SE or epilepsy group a significant increase in FCM levels was observed one week after SE (P=0.0357). Error bars indicate standard error of the mean. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$. Total n for the tethered group: naive n=12, sham n=12, epilepsy n=13-14. Total n for the telemetry group: sham n=6, epilepsy n=10-11.

Figure 4: Heart rate and heart rate variability. A, C, Telemetric recordings of two days were performed at four different time points. Shown is the time based course of these parameters. B, D, Mean values of day and night were calculated and illustrated as boxplots for every time point. A, B Animals with a history of a SE exhibited increased heart rates in both dark and light phase during epileptogenesis (dark phase $P < 0.0001$, light phase $P < 0.0001$) and disease manifestation (dark phase $P = 0.0011$, light phase $P = 0.0023$). C, D, SDNN was significantly decreased in the dark and light phase in animals during epileptogenesis (dark phase $P = 0.0007$, light phase $P = 0.0031$). During the chronic phase differences were only evident in the dark phase ($P = 0.0187$). Error bars indicate standard error of the mean. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$. Total n is sham n=6, post-SE/epilepsy n=11.

Figure 5: Correlation matrix. Spearman correlations between biochemical and behavioral parameters are illustrated with a heat map.

Figure 6: Principal component analyses. Only data were considered for this analysis that were measured following the seizure monitoring in animals which experienced a SE. Thereby, this analysis aimed to identify parameters which separate the two groups with tethered versus telemetric recordings. A, Principal component one is shown on the x-axis capturing 29% of the variance in the data. The y-axis represents principal component two capturing 12% of the variance. Both groups are separated vertically on principal component two. B, Top parameters

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separating best between the groups include: nest building, time spent in passive social interaction, the level of soiling, serum BDNF and serum corticosterone concentrations.

For Review Only

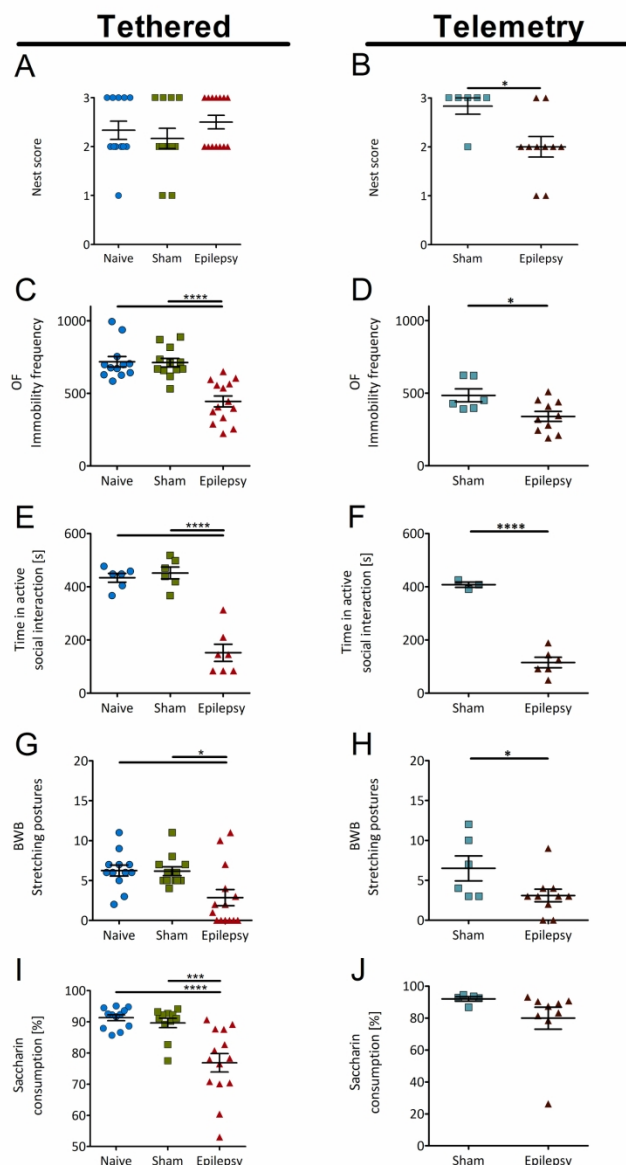


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against both control groups $P < 0.001$) was significantly reduced whereas the telemetric epilepsy group (J) did not show a difference, two animals were excluded due to leaking drinking bottles (one sham and one epilepsy). Error bars indicate standard error of the mean. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$. Total n for the tethered group: naive n=12, sham n=12, epilepsy n=14. Total n for the telemetry group: sham n=6, epilepsy n=10.

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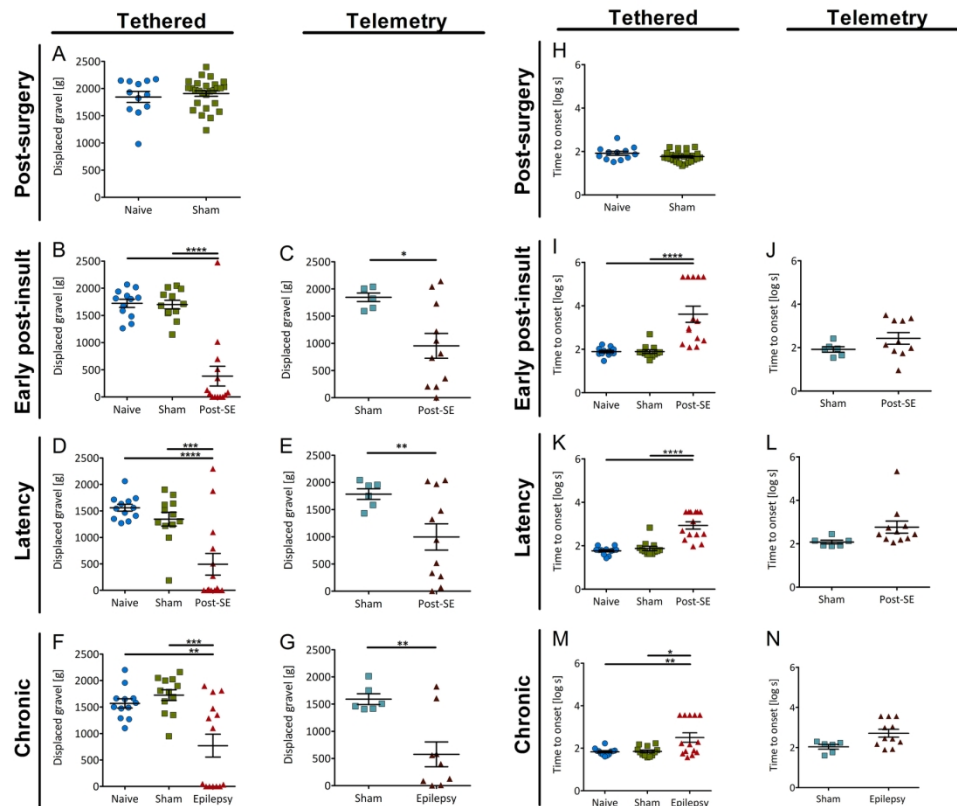


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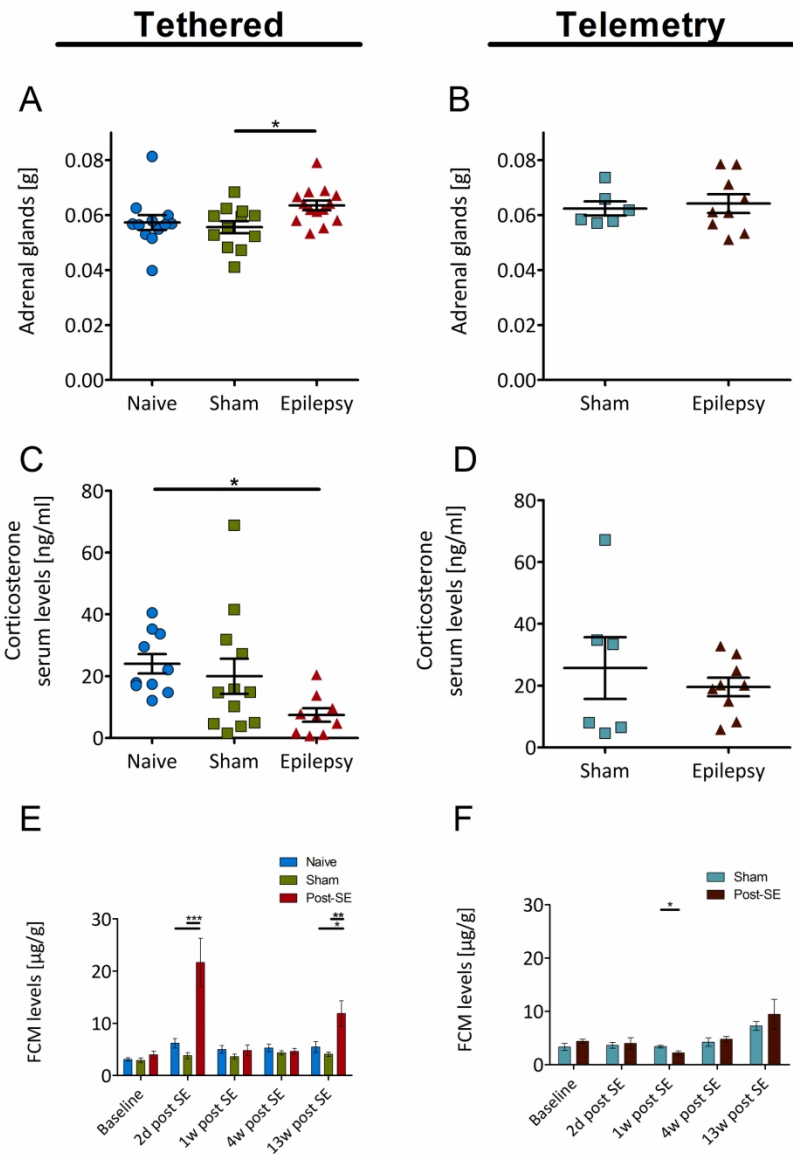


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epilepsy n=9.

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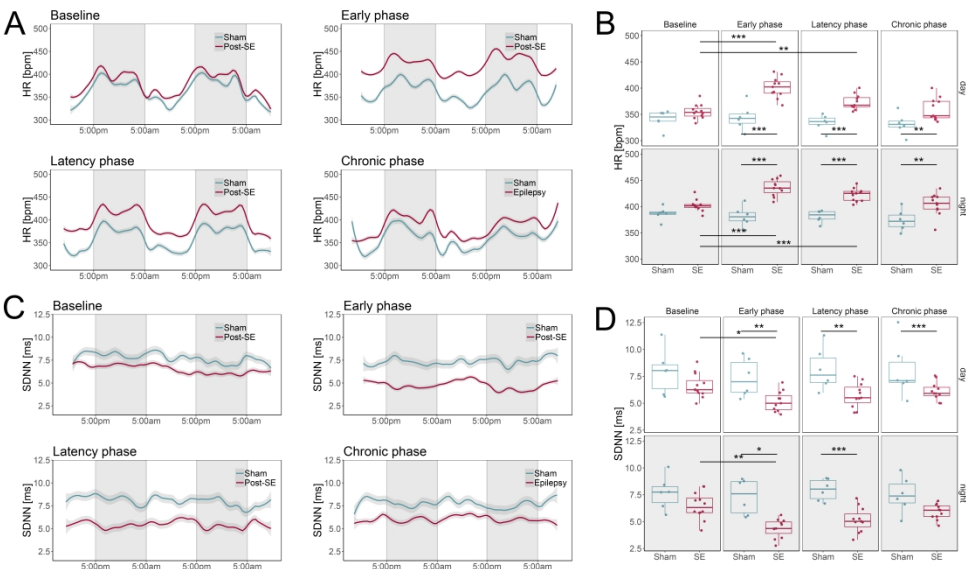


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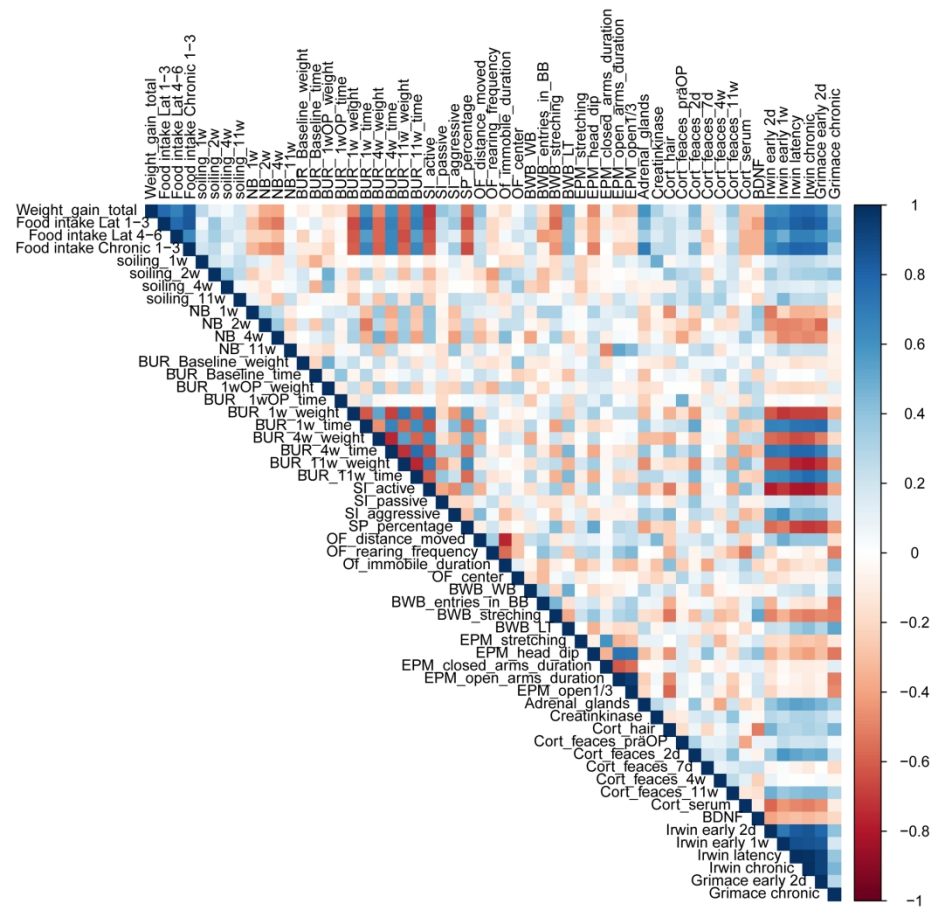


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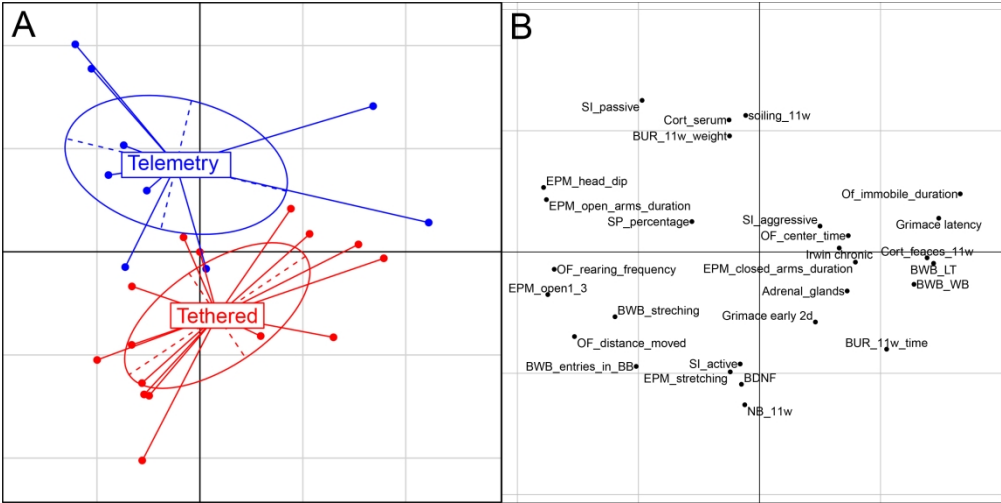


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