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FULL-LENGTH ORIGINAL RESEARCH

Epilepsia®

Toward evidence-based severity assessment in rat models with repeated seizures: III. Electrical post-status epilepticus model

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Abstract

Objective: Ethical approval of experiments in chronic epilepsy models requires a careful balancing of the expected gain-in-knowledge with the level of distress. Thus recommendations for evidence-based severity assessment and classification are urgently needed for preclinical epilepsy research.

Methods: Therefore, we have completed a comprehensive analysis of alterations in behavioral, biochemical, and physiological parameters in a rat electrical post-status epilepticus model. Selected parameters were repeatedly analyzed during different experimental phases to obtain information about the level of distress throughout the course of the model.

Results: Behavioral patterns comprised an increase in activity along with a reduction in risk assessment behavior, active social interaction, saccharin preference as well as nonessential, but evolutionary-determined behavior such as nest building and burrowing. Among the biochemical parameters, fecal corticosterone metabolites proved to be increased in different phases of the experiment. In the early post-insult phase, this increase was reflected by elevated serum corticosterone concentrations. Telemetric recordings demonstrated increases in home cage activity and heart rate in selected experimental phases but argued against relevant changes in heart rate variability. Comparison between animals with tethered or telemetric recordings including a principal component analysis revealed differences between both groups.

Significance: The present findings further confirm that burrowing behavior and saccharin preference might serve as valid parameters for severity assessment in chronic epilepsy models. Considering the course of alterations providing evidence for a more pronounced level of distress in the early phase following status epilepticus (SE), we suggest a classification of the electrical post-SE model as severe. This suggestion may serve as a guidance for laboratory-specific evaluations. Comparison between data from animals with tethered and telemetric recordings indicated an impact of the mode of recordings. However, further research is necessary to analyze the validity of telemetry as a putative refinement measure.

KEYWORDS

3R, basolateral amygdala, behavior, rodent, Stress, Funding information

Seiffert and van Dijk shared first authorship.

1 | INTRODUCTION

Post-status epilepticus (SE) models constitute important paradigms allowing study of the mechanisms of epilepsy development and identification and evaluation of biomarkers and target candidates for anticonvulsant, antiepileptogenic, and disease-modifying approaches.[1] Considering the complex cellular and network alterations characterizing epilepsy development and manifestation,[2] it is still impossible to completely replace in vivo experiments by in vitro studies in this field of research. It is therefore all the more important to consistently apply the other two concepts of the 3R principle, that is, reduction and refinement.[3–5] Refinement comprises the selection of the least burdensome model, which allows addressing the research hypothesis with a good scientific quality and informative data value.[6] Moreover, it is based on the application of measures that minimize the severity of the experimental procedures. Lidster and colleagues[2] have intensely discussed the necessity to assess specific refinement measures for rodent epilepsy models and have pointed out that a gain in knowledge in evidence-based severity assessment is urgently needed. Methods used to induce SE in epilepsy models are often invasive and severe, making it all the more important to pinpoint causes of distress that can be minimized to not only minimize animals suffering but also improve quality of scientific data derived from these animals.[2]

As described previously,[7] the assessment of severity in laboratory animals has become a specific goal in the revision of the European Union Directive on the protection of animals used for scientific purpose.[8] To this end, every animal experiment has to be classified prospectively and retrospectively to one of the following categories: "non-recovery," "mild," "moderate," and "severe" with regard to the respective pain, suffering, distress, or lasting harm to the animals (Article 38, 39, 54 and Annex VIII of Directive 2010/63/EU). The development and application of comprehensive severity assessment schemes will provide an improved basis for evidence-based severity classification, allowing the careful assessment of differences between models and the impact of putative refinement measures. Recently we provided the first scientific evidence for the classification of the kindling paradigm along with a recommendation for the application of selected parameters for a laboratory-specific evaluation.

In chronic models with spontaneous recurrent seizures, animals experience different experimental influences during various phases, from induction of the brain insult to the latency phase to the chronic phase with epilepsy manifestation.[1] Most of the research questions require thorough video–electroencephalography (EEG) monitoring to capture information about the frequency, severity, and duration of spontaneous seizures. Monitoring is thereby based on tethered recording with or without swivel systems or on telemetric recordings. Whereas the first method can result in mobility restrictions with tractive forces by the cable, telemetric recordings require additional subcutaneous or abdominal implantation of a transmitter.[2,9,10] Lidster et al.[2] have hypothesized that telemetric recordings may reduce the burden and stress for the animals during monitoring phases. However, respective scientific proof is still pending.

Key Points

- The data indicates that the assessment of burrowing behavior and saccharin preference can serve as indicators of severity
- The findings suggest classification of the electrical post-status epilepticus (SE) model as severe
- No clear indication was found for a decrease of severity due to telemetric recordings over tethered recordings in the model used

Electrical post-SE models had been developed as an alternate to the application of chemoconvulsants including pilocarpine or kainic acid, thereby avoiding potential influences of the proconvulsant compounds with test compounds.[1,11] Moreover, there has been the impression that electrical models may cause a milder phenotype associated with less-severe neuropathologic alterations, thereby resulting in a model that may be closer to the clinical scenario in human patients.[1,12]

In this study, we assessed the impact of an electrical post-SE model on a comprehensive set of behavioral, biochemical, and physiological parameters. These were chosen to give a comprehensive sense of the animals' distress and well-being, while at the same time being as objective and noninvasive as possible. In addition, suitable parameters should be generalizable to a wide range of animal models, allowing for easier classification of severity of different animal models (Keubler et al. 2019 submitted).

Besides severity assessment, the data generated in the different epilepsy animal models can be used to gain further insight into psychiatric comorbidities of epilepsy and the respective face validity of different animal models. Studies have demonstrated the existence of psychiatric comorbidities in patients, with matching behavioral alterations seen in rodent epilepsy models.[13–15] However, previous findings were not always consistent and robust,[16–18] so that a comprehensive analysis of behavioral alterations in different epilepsy models under identical conditions is of particular interest.

The electrical post-SE model has been developed with several modifications[12] based on an earlier approach by McIntyre et al.[19] It is based on 25-minute stimulation of the basolateral amygdala resulting in the induction of a self-sustained SE. It has been selected as a representative example of an electrical post-SE model for the current study. This study is a part of a series about the severity classification of three commonly used rat epilepsy models (I. Kindling model published by Möller et al.,[7,20] II. Chemical post-SE model, manuscript in revision by Koska et al., and the present study: III. Electrical post-SE model.)

2 | MATERIAL AND METHODS

2.1 | Animals

In total 63 female Sprague-Dawley rats (200-224 g, Envigo, The Netherlands) were used for the experiments. Additional information about the housing of the animals is provided in Appendix S1. Animals were randomly allocated to subgroups (www.randomizer.org). Forty-four animals (12 naive, 14 animals with electrode implantation [sham] and tethered connection and 18 electrode-implanted, stimulated animals [SE] with tethered recordings) were used in the "tethered subproject" and 19 animals with electrode and transmitter implantation (6 sham and 13 SE, both with telemetric recordings) were used for the "telemetry subproject." Examined time points in this project (see Figure 1 for a timeline) are the recovery phase post-surgery and three post-SE time points: the first week post-SE, the "post-insult phase," the second to seventh week post-SE "latency phase," and from the eighth week on to the end of study "chronic phase." Throughout the text, we refer to animals with a history of SE as "post-SE" animals in the early and latency phase. Following epilepsy manifestation, that is, in the chronic phase, animals are referred to as animals with epilepsy ("epilepsy"). In total, 18 animals (12 in the tethered and 6 in the telemetry subproject) of the initial 63 animals were excluded for the following reasons: Six animals died during or immediately following SE induction, four animals lost their electrodes, and four animals were killed due to other health concerns such as tumors or wound inflammation. Four animals (two in the tethered and two in the telemetry subproject) did not develop spontaneous recurrent seizures; these animals were excluded from the groupwise comparisons but included in the correlation matrix and principal component analysis.

The study was approved by the Government of Upper Bavaria (reference number 55.2-1-54-2532-105-2016, 55.2-1-54-2532-011-2015) and was conducted in accordance with the German Animal Welfare act and the EU directive 2010/63/EU. All procedures followed the Basel declaration including the 3R concept and were reported according to the Animal Research: Reporting of In Vivo Experiments guidelines.

2.2 | Electrode and telemetry implantation, electrical stimulation

Both the electrode implantation in the right basolateral amygdala and the induction of the SE were performed as described previously by Walker et al.[21] (BLA; anteroposterior [AP] −2.2, lateral [L] +4.7, ventral [V] +8.5 mm). The implantation of the telemetry transmitter and cables was completed as reported in Möller et al.[20] (For a detailed description of the implantation of the transmitter and the electrical stimulation, see Appendix S1.)

2.3 | Tethered and telemetric recordings

To detect spontaneous recurrent seizures, all animals with SE induction were subjected to combined 14-day video-EEG monitoring 8 weeks following SE. The tethered group was monitored as described by Walker et al.[21] In animals with telemetry transmitters, EEG, electrocardiography (ECG), and activity of the sham and SE animals were not only recorded during the 14-day phase 8 weeks following SE, but also for 48 hours in the phase before SE induction, and at two additional time points following SE (post-insult and latency phase). (A description of the telemetric monitoring as well as the analyses of time domain and frequency domain data is provided in Appendix S1.)

2.4 | Analysis of behavioral and biochemical parameters

Analysis of all behavioral and biochemical parameters was performed as previously described by Möller et al.[7] A timeline can be found in Figure 1. Detailed information about the statistical approach is provided in Appendix S1.

3 | RESULTS

3.1 | Induction of status epilepticus and development of spontaneous recurrent seizures

Twenty-five (tethered, 14; telemetry, 11) of 29 stimulated rats developed a type 3 SE, whereas four animals from the tethered group developed a type 2 SE according to Brandt et al.[12] Twenty-one rats with SE induction (tethered, 12; telemetry, 9) reached the chronic phase. Fifteen rats exhibited seizures during the EEG monitoring (Figure S1; tethered, n = 8; telemetry, n = 6) with a mean seizure duration of 623 seconds (standard deviation [SD] 718, median 291, mean/day 44 seconds, SD 51, median 21) in the tethered and 581 seconds (SD 722, median 283, mean/day 41 seconds,

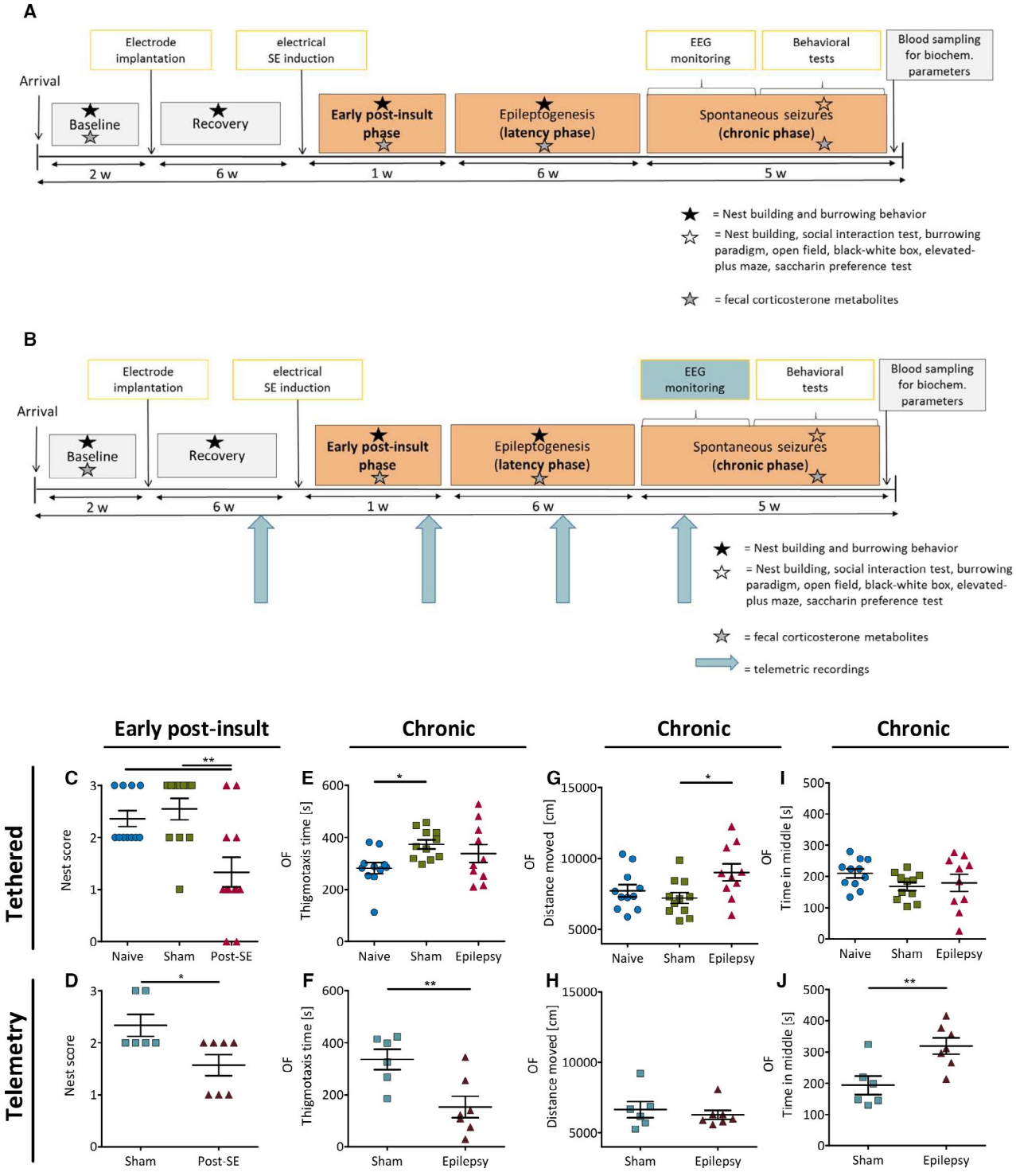

FIGURE 1 Timeline of study, nest building activity, and open field locomotor activity. A, timeline of the subproject with tethered recordings. B, timeline of the subproject with telemetric recordings. C, D, nest building scores are significantly reduced for stimulated animals 1 week post-status epilepticus (SE) induction in, both, the tethered (C, $H(2) = 10.68$, $P = 0.005$, epilepsy against both control groups $P < 0.05$) and the telemetry (D, $P = 0.0399$) group. E-J, measures of locomotor activity in the open field. In the tethered subproject, sham animals with electrode implantation spent significantly more time at the wall compared to naive animals (E, $F(2,29) = 3.524$, $P = 0.0427$, sham against naive $P < 0.05$), whereas in the telemetry subproject, sham animals spent significantly more time at the wall compared to animals with epilepsy (F, $P = 0.0086$). Animals with epilepsy and previous tethered recordings moved greater distances compared to the sham animals (G, $F(2,29) = 3.791$, $P = 0.2073$, epilepsy against sham $P < 0.05$). No group differences were evident in the telemetry subproject (H). Finally, animals with epilepsy of the telemetry group spent more time in the middle of the arena compared to the sham group (J, $P = 0.0086$), whereas no respective differences were observed in the tethered subproject (I). Error bars indicate standard error of the mean. $^*P < 0.05$ and $^{**}P < 0.01$. Total n for the tethered subproject: naive n = 11, sham n = 11, post-SE n = 12, epilepsy n = 10. Total n for the telemetry subproject: sham n = 6, epilepsy n = 7

SD 52, median 20) in the telemetry group. During the 14-day monitoring, the tethered and the telemetry group exhibited a mean seizure frequency of 13, respectively, 9.6 seizures (tethered: SD 18.7, median 6, mean/day 0.9 seconds, SD 1.3, median 0.4; telemetry: SD 12.5, median 5, mean/day 0.7 seconds, SD 0.9, median 0.4) in 14 days.

3.2 | Impact on nest building and soiling

In animals with SE, a reduction of nest complexity became evident only 1 week following SE (Figure 1C,D). No alterations were observed at later time points during epileptogenesis (Figure S2). Following SE, the level of soiling

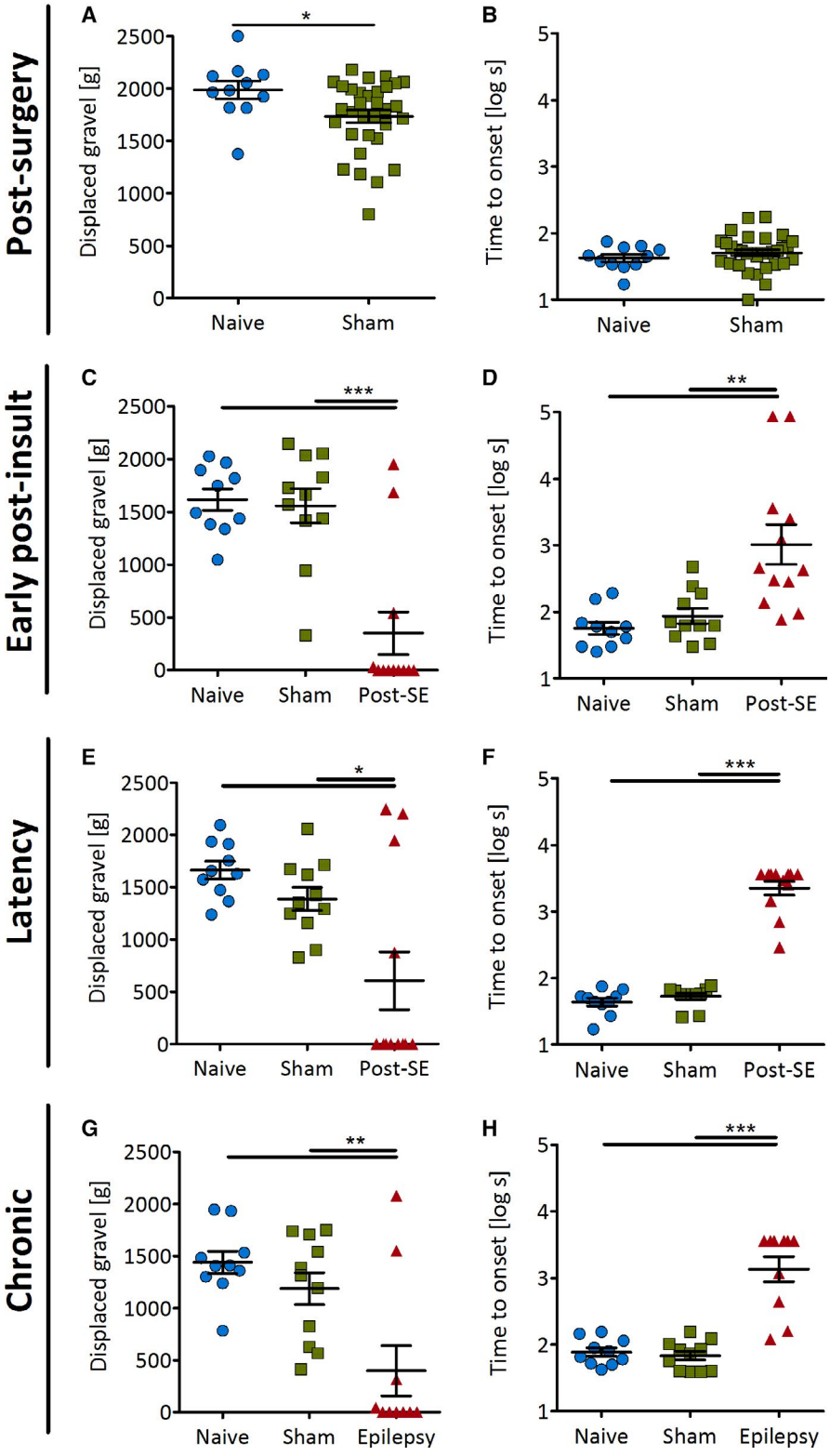

FIGURE 2 Burrowing behavior of the tethered subproject. A, C, E, G, amount of burrowed gravel. B, D, F, H, latency to start burrowing. Implanted animals burrowed significantly less gravel 1 week post-surgery (A, $P = 0.0313$) but exhibited no differences in latency to start burrowing (B). Following status epilepticus (SE) induction, stimulated animals burrowed significantly less gravel compared to both naive and sham animals during the early post-SE phase (C, $F(2,30) = 18.93$, $P < 0.0001$, epilepsy against both control groups $P < 0.001$), latency phase (E, $F(2,30) = 8.438$, $P = 0.0002$, epilepsy against both control groups $P < 0.05$), and chronic phase (G, $F(2,28) = 9.477$, $P = 0.0007$, epilepsy against both control groups $P < 0.01$). In addition, stimulated animals exhibited a longer latency to start burrowing during the three post-SE phases (D, $F(2,30) = 11.19$, $P = 0.0002$, epilepsy against both control groups $P < 0.01$; F, $F(2,30) = 162.1$, $P < 0.0001$, epilepsy against both control groups $P < 0.001$ & H, $F(2,28) = 37.44$, $P < 0.0001$, epilepsy against both control groups $P < 0.001$). Error bars indicate standard error of the mean. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. Total n for the tethered subproject: naive n = 10, sham n = 11, post-SE n = 12, epilepsy n = 10

(Figure S3) reached higher levels during the latency phase in animals prepared for tethered recordings (Figure S3D). A decreased distribution of feces was observed in the early phase following SE in animals with telemetry transmitters (Figure S3C).

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

The Grimace scale reached scores between 0.8 and 0.9 on the first postsurgical day. Scores gradually decreased, reaching baseline between the second and third postsurgical day (Figure S4A,B). Surgery did not result in significant weight loss (Figure S4C,D). In contrast, significant weight loss occurred following SE. This was regained over the following 2 weeks (Figure S4E,F). From the third week until the end of the experiments, the stimulated animals exhibited an increased body weight as compared to both control groups.

In the open field paradigm applied in the chronic phase, electrode-implanted rats without SE spent more time in the outer ring (Figure 1E) and less time in the center of the open field (Figure S5A). Animals with epilepsy and previous tethered recordings exhibited an increased distance moved (Figure 1G) and a reduced frequency of immobility phases

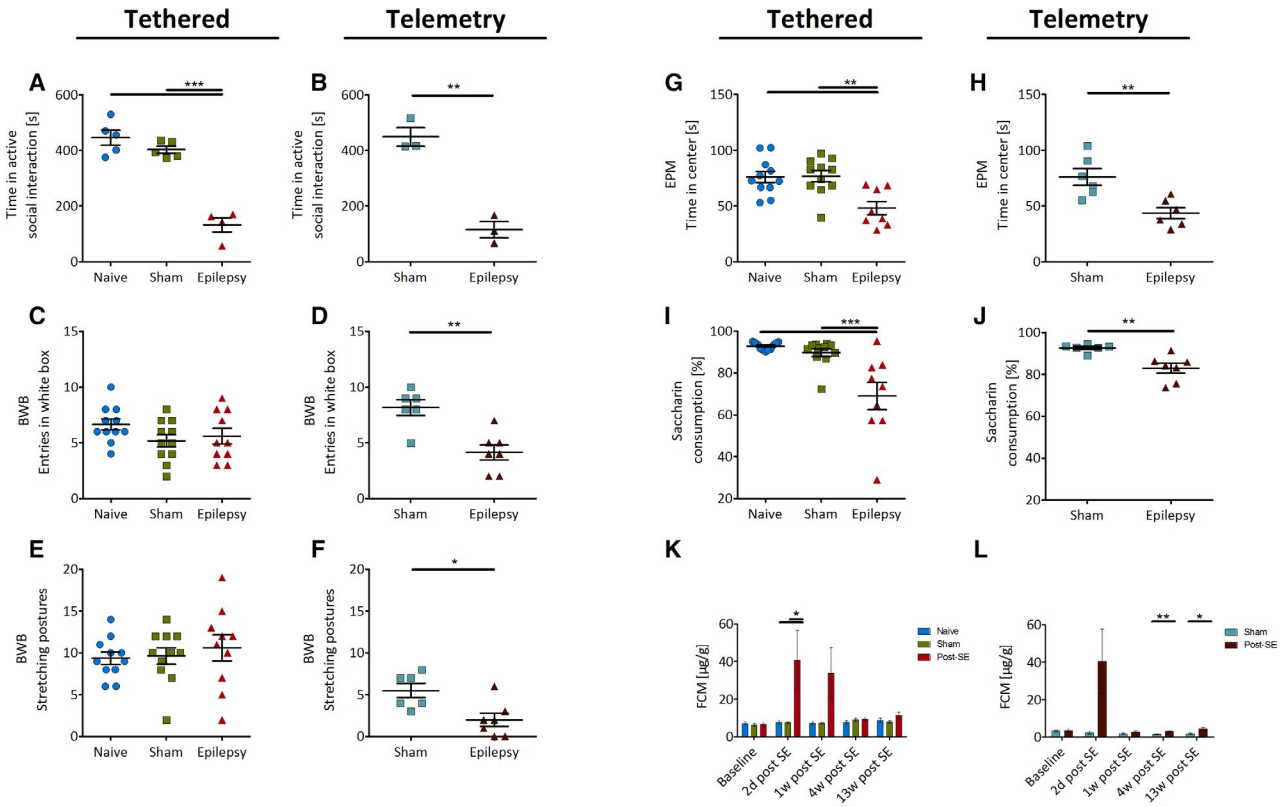

FIGURE 3 Social interaction, anxiety-associated, anhedonia-associated behavior, and fecal corticosterone metabolite concentration (FCM). A, B, time spent in social interaction, animals with epilepsy spent significantly less time in active social interaction compared to the corresponding control groups in both the tethered (A, $H(3) = 8.966$, $P = 0.0113$, epilepsy against naive $P < 0.01$) as well as the telemetry subproject (B, $P = 0.0049$). C-F, performance in the black-white box (BWB). Animals from the tethered subproject exhibited no differences between groups for both the number of entries into the white box (C) as well as the number of stretching postures (E). In the telemetry subproject, animals with epilepsy showed significantly fewer entries into the white box (D, $P = 0.0017$) and fewer stretching postures (F, $P = 0.0115$). G, H, elevated plus maze (EPM) performance, in both the tethered (G, $F(2,27) = 8.706$, $P = 0.0012$, epilepsy against both control groups $P < 0.01$) and the telemetry (H, $P = 0.0049$) subproject animals with epilepsy spent significantly less time in the center area of the EPM compared to both control groups. I, J, saccharin preference test, in both the tethered (I, $F(2,28) = 12.86$, $P = 0.0001$, epilepsy against both control groups $P < 0.001$) and telemetry (J, $P = 0.0036$) subproject. Animals with epilepsy showed lower preference for saccharin compared to the control groups. K, L, fecal corticosterone metabolite levels. Feces were collected at various stages during the study. In the tethered subproject (K) elevated fecal corticosterone metabolite levels were found in the stimulated animals 2 days post-SE induction ($F(2,30) = 4.975$, $P = 0.0136$, epilepsy against both control groups $P < 0.05$). In the telemetry subproject (L), elevated levels were found in the stimulated animals 4 weeks ($P = 0.0073$) and 13 weeks post-SE ($P = 0.0188$) induction. Error bars indicate standard error of the mean. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Total n for the tethered subproject: naive n = 11, sham n = 11, post-SE n = 12, epilepsy n = 10. Total n for the telemetry subproject: sham n = 6, epilepsy n = 7. Total number of pairs in the social interaction test for the tethered subproject: naive n = 5 pairs, sham n = 5 pairs, epilepsy n = 4 pairs and the telemetry subproject: sham n = 3 pairs, epilepsy n = 3 pairs

(Figure S5E). In contrast, animals with epilepsy and telemetric recordings showed reduced thigmotaxis and increased time in the middle ring (Figure 1F,J).

One week after surgery, electrode-implanted animals showed decreased burrowing behavior compared to naive animals (Figure 2A). Following SE, animals exhibited a prolonged latency to initiate burrowing behavior and a reduced amount of gravel burrowed (Figure 2C,D). The impact proved to be evident throughout all phases of epileptogenesis regardless of the EEG monitoring approach, that is, telemetric (Figure S6) or tethered (Figure 2E-H).

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

In animals with epilepsy manifestation, the time spent with active social interaction proved to be significantly reduced (Figure 3A,B).

Animals with tethered recordings did not exhibit behavioral alterations in the black-white box (BWB) paradigm (Figures 3C,E and S7E,G). In contrast, we observed a lower number of stretching postures and entries in the white box in animals with telemetric recordings (Figure 3D,F).

In the elevated plus maze (EPM), an increased level of activity was evident in animals with epilepsy and telemetric recordings (Figure S7B,D). In contrast, activity proved to be in the control range in animals with epilepsy and tethered recordings. Both groups spent less time in the center of the maze as compared to their respective control groups (Figure 3G,H).

Anhedonia-associated behavior was assessed based on saccharin consumption. Regardless of the preceding EEG-monitoring approach, animals with epileptic seizures showed a comparable volume consumption, but a reduced preference of saccharin in comparison with the control groups (Figures 3I,J and S8).

3.5 | Impact on biochemical parameters

Neither adrenal gland weight nor any of the serum and hair biochemical parameters were affected in the chronic phase (Figures S9 and S10).

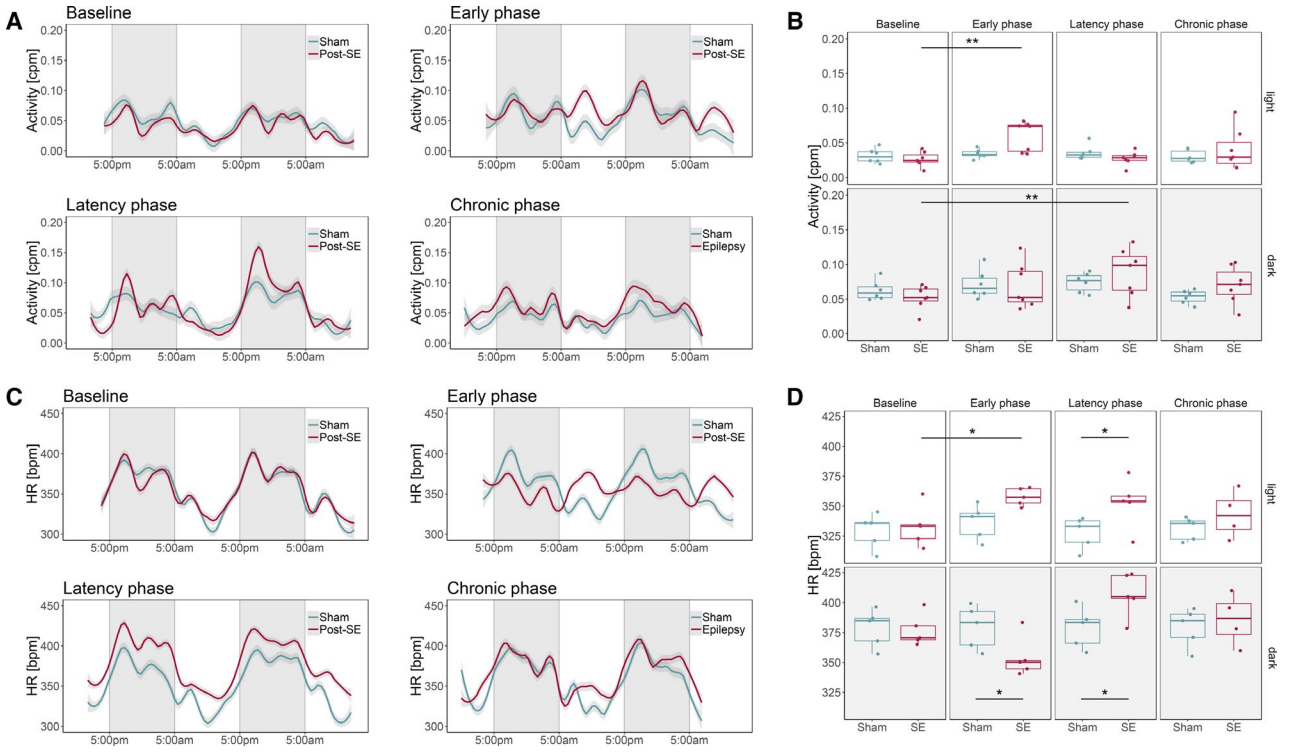

FIGURE 4 Home cage activity and heart rate. A, C, timeline depicting 2 days of recordings at four different time points. B, D, mean values calculated for both the combined light and dark periods for each of the four time points. A, B, during the early post-insult phase stimulated animals showed increased activity compared to baseline measurements in the light phase ($P = 0.0093$) (= resting phase). During the latency phase stimulated animals showed increased activity compared to baseline measurements in the dark phase ($P = 0$ 0.0057). C, D, increased heart rate was observed in post-SE animals in the early phase compared to baseline measurements during the light phase ($P = 0.0341$). Furthermore, heart rate was significantly increased in the latency phase during both the light ($P = 0.0330$) and dark phase ($P = 0.0200$) compared to the sham animals, whereas post-SE animals showed lower heart rate during the dark phase in the early post-insult phase compared to sham animals ($P = 0.0309$). Total n for the telemetry subproject: sham n = 6, epilepsy n = 5. *$P < 0.05$ and **$P < 0.01$

Because we wanted to avoid invasive sampling during the experiment, analysis during earlier experimental phases has been restricted to feces samples (Figure 3K,L). Two days following SE, we demonstrated increased concentrations of fecal corticosterone metabolites in animals prepared for tethered recordings and a trend for a respective increase in animals prepared for telemetric recordings. Moreover, elevated fecal corticosterone metabolite levels were evident before and following telemetric monitoring in the respective group of rats.

Considering the early increase of fecal corticosterone metabolites following SE, we were interested in additionally analyzing serum corticosterone during other experimental phases. Serum samples were available from rats of a different study, in which animals with electrode implantation were sacrificed 2 and 10 days following SE (Figure S11). Analysis of their serum samples revealed increased corticosterone levels 2 days following SE.

3.6 | Impact on home cage activity and heart rate

Home cage activity (Figure 4A,B) assessed by telemetric recordings during the light phase (= resting phase) proved to be increased in the early post-insult phase when compared to baseline measurement. Following epilepsy manifestation,

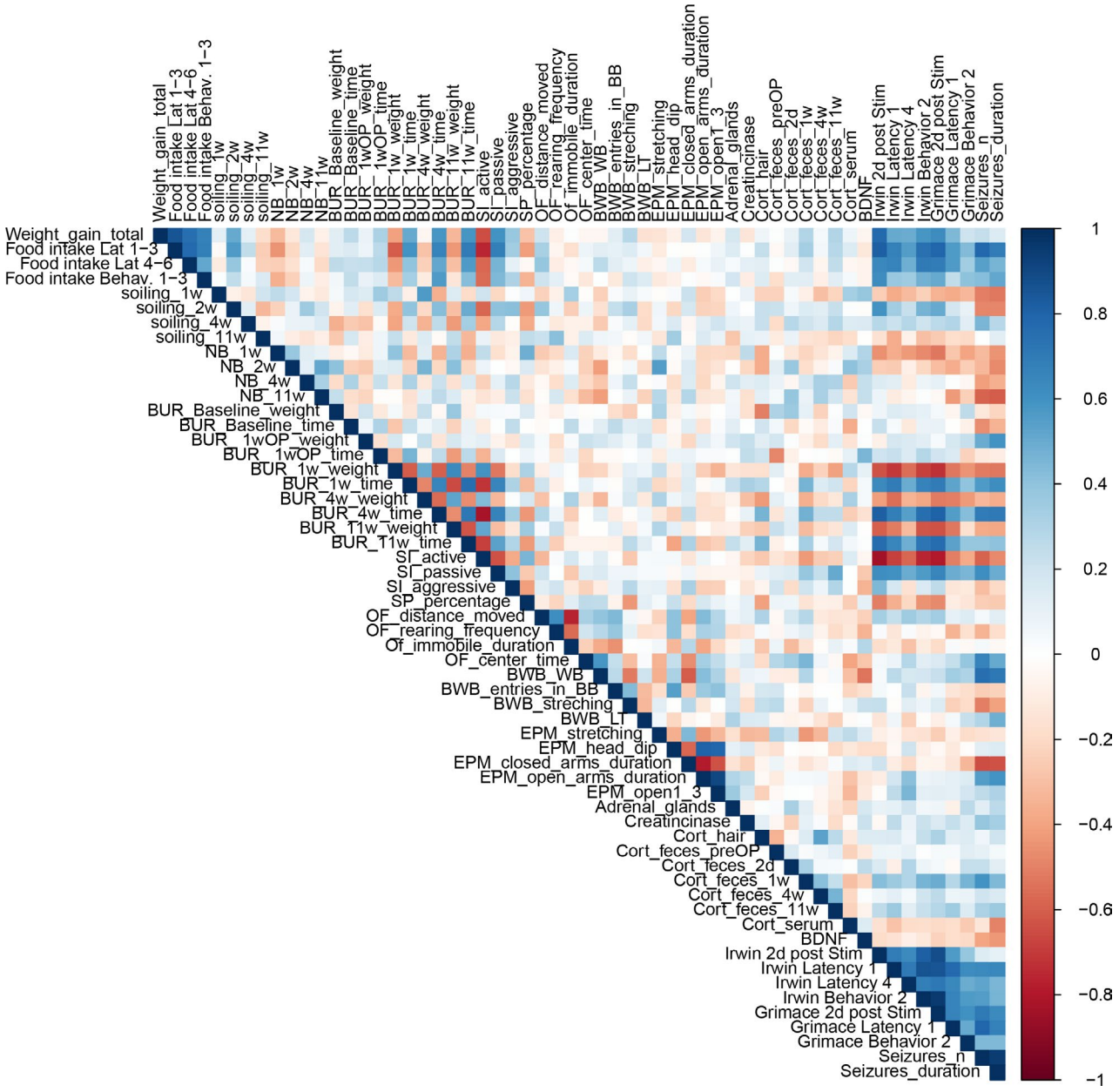

FIGURE 5 Correlation matrix. A heat map illustrating the correlation between different parameters (Spearman correlation coefficient). Abbreviations are described in Appendix S1

analysis of recordings during the dark phase (= activity phase) demonstrated activity levels exceeding those during baseline recordings in the latency phase.

In the early phase following SE, comparison with respective baseline data revealed an increase in heart rate associated with a decrease in the NN interval (Normal to Normal R-peak interval) during the resting phase of the animals (Figures 4C,D and S12A,B). Moreover, in the active phase, the NN interval proved to be decreased 4 weeks following SE. Group differences between implanted rats and rats with SE were observed only during the early post-insult (dark phase) and the latency phase (light and dark phases).

Aiming to obtain information about the total variability of the heart rate, we analyzed the SD of NN intervals (= SDNN). In both groups, SDNN remained in the range of baseline measurements throughout the experiment (Figure S13). Differences between groups were evident during selected experiment phases. However, it needs to be considered that a group difference was already evident during the baseline dark phase recordings.

Information about short-term variability and spontaneous adjustment of heart rate was obtained by analyses of further parameters (root mean square of successive differences [RMSSD]; percent of subsequent NN intervals, which deviate more than 9 msec [NN9]; proportion derived by dividing NN9 by the total number of NN intervals [pNN9]). None of these parameters proved to be affected by the experimental procedures (data not shown).

An additional analysis of the frequency domain did not reveal relevant experiment-associated alterations in comparison with baseline measurements (Figure S14). A group difference was evident only in the latency phase, with animals following SE exhibiting a lower low-frequency band to high-frequency band ratio (LF/HF with LF = 0.1–1.0 Hz and HF = 1.0–3.5 Hz).

3.7 | Correlation matrix of all measured variables

Two correlation matrices were created, one comparing all variables recorded in animals with a tethered recording (Figure 5) and one with all variables recorded in animals with a telemetric recording (Figure S15). Because the number of significant correlations is too high to be listed completely, we only point out selected noteworthy findings.

Several pairs or groups of variables stand out for sharing a high number of correlations with other variables. Total weight gain and food intake measured at various time points highly correlate with each other and, in turn, both correlate with a number of other measures such as the latency to start burrowing, social interaction, and both the Irwin and Grimace scores. Likewise, both social interaction and burrowing behavior correlate with each other and both show strong correlations with the different scoring schemes.

Seizure frequency and duration correlate with some but not all of the mentioned parameters. Seizure duration correlates significantly with early burrowing behavior, food intake, and the Grimace score but not with social interaction or saccharin preference. Seizure frequency correlates with social interaction, the Grimace score, but only with burrowing onset and not with saccharin preference. Adjusting the corresponding *P* values for false discovery rate (Benjamini and Hochberg[22]) retains the same overall trends between the described parameters.

The correlation matrix of the group of animals that underwent telemetric recordings shares many similarities with the correlation matrix of the animals that underwent tethered recordings. When focusing on the correlations with the heart rate and heart variability parameters, several notable observations can be made. First of all, the heart rate and heart rate variability parameters recorded 4 weeks following SE show significant correlations with the majority of behavioral and biochemical measures. Second, many of the behavioral variables measured at 11-12 weeks post-insult exhibit stronger correlations with heart rate variables measured during 4 weeks post-insult, as opposed to the time point closer to the insult: 1 week following SE or closer to the behavioral recordings, 9 weeks following SE.

Similar to the correlation matrix of the animals that underwent tethered recordings, seizure frequency and duration correlate with burrowing but not with social interaction or saccharin preference. In addition, several correlations can be found with heart rate and heart rate variability parameters. However, adjusting the corresponding *P* values for false discovery rate here loses most of these significances, with only the correlations between food intake and ECG recordings measured during the latency remaining.

3.8 | Principal component analysis

Using the combined data of the stimulated animals from the tethered as well as the telemetric recorded group, a principal component analysis (PCA) was performed using only those variables that were measured following the monitoring period. This allowed the investigation of any possible effect on behavior due to a difference in the impact of tethered and telemetric recordings (Figure 6). The first two principal components (PC1,2) represent a total of 41.44% of the variance (PC1, 24.80%; PC2, 16.63%). The two groups are diagonally separated along both PC1 ($F(3,17) = 3.91$, $P = 0.027$) and PC2 ($F(3,17) = 3.366$, $P = 0.043$). The behavioral paradigm that had a prominent impact on both PC1 and PC2 separating the two groups is the EPM. This paradigm represents the top four parameters along PC2 (number of head dips, time spent in the open arms [in total and in the outer one-third] and the

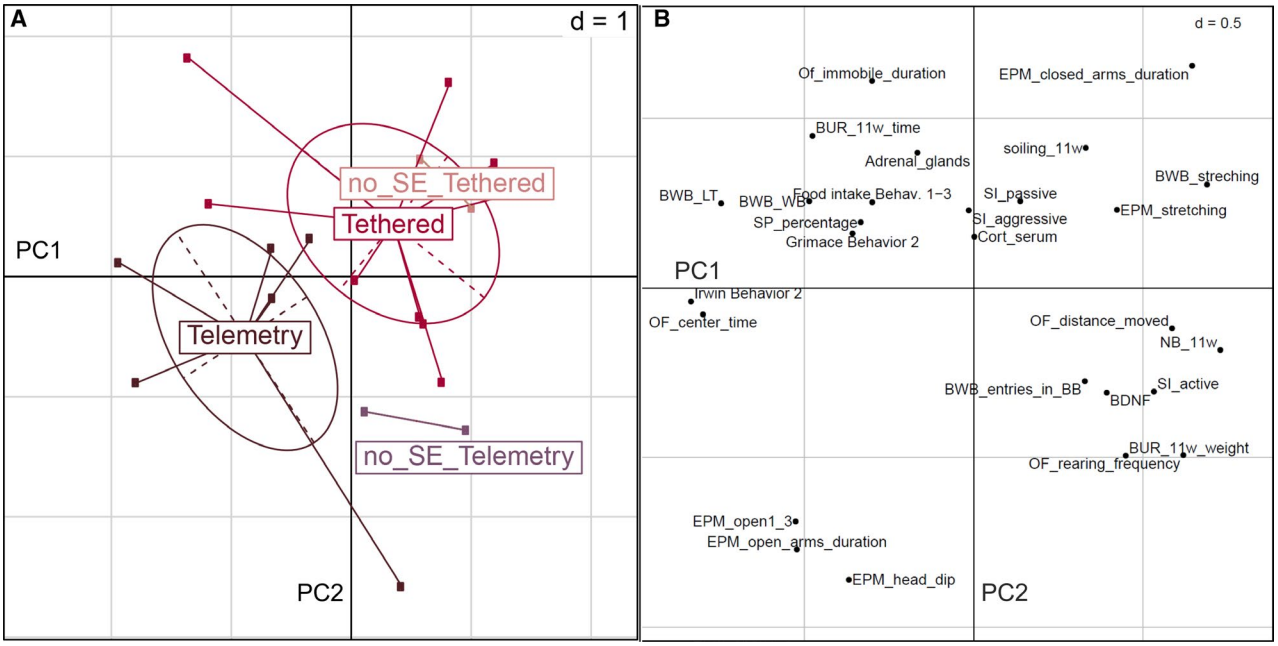

FIGURE 6 Principal component analyses (PCA) with data from all stimulated animals using variables measured following the monitoring period to determine the influence of the tethered vs telemetric recording. A, the *x*-axis shows principal component 1 (PC1) contributing 24.8% of variance, while the *y*-axis shows principal component 2 (PC2) contributing 16.6% of the variance. Each dot represents an individual animal placed along the first two principal components. Data from the tethered and telemetry group are significantly separated in a diagonal manner along both PC1 and PC2. B, the relative weight of individual variables on PC1 and PC2

time spent in the closed arms), while also contributing to PC1 (time spent in the closed arms and in the outer one-third of the open arms). The second most frequent paradigm is the open field test, which also features on both PC1 and PC2, with the total time spent in the center, the number of rearing postures, total distance moved, and the time spent immobile.

4 | DISCUSSION

Whereas electrical post-SE models in rodents constitute an important tool in experimental epileptology research,[1] the impact on experimental animals needs to be carefully considered for ethical evaluation of animal experiment proposals and for efforts to minimize the burden for the development of refinement measures.[2] Therefore, we have assessed behavioral, biochemical, and physiological parameters throughout different experimental phases based on a comprehensive set of parameters.

In most studies, the first intervention comprises stereotactic surgery with implantation of a depth electrode, which in electrical models might serve as the stimulation and recording electrode at the same time.[12,19,23–25] Following implantation of an electrode in the basolateral amygdala, we observed only minor acute and long-term behavioral alterations with a reduced amount of gravel burrowed 1 week following surgery and a decreased time in the center of the open field 18 weeks following surgery. Along with Grimace scale analysis during the first week following surgery, our data suggest a transient phase of compromised well-being in the early postsurgical phase. However, later analyses of behavior and biochemical data during the chronic phase did not indicate a chronically increased level of distress as a consequence of the implant. One caveat concerning the interpretation of the Grimace scale score is the putative bias caused by the electrode implants and the impossibility to blind the scorers because of it.

All parameters that could be repeatedly assessed without invasive procedures and with stable data despite repeated assessment were analyzed during the early post-insult phase and the latency phase of the post-SE model.

As expected, a transiently compromised well-being became evident based on reduced nest complexity as well as body weight 1 week following SE. Stimulated animals recuperated reaching a higher body weight starting from the third week following SE to the end of the project. Whereas nest complexity levels normalized in subsequent phases, burrowing behavior proved to be reduced throughout all post-SE phases including the latency phase. These data should be interpreted in the context of previous studies demonstrating that both nest building and burrowing behavior can be analyzed as indicators of distress and pain associated with experimental procedures in laboratory rodents.[26–29]

As a short- and long-term consequence of prolonged seizure activity, fecal corticosterone metabolite levels proved to be increased in different phases of the study. However, the alterations of this biochemical stress marker were not very

robust, as effects were often observed only in one of the animal groups prepared for tethered or telemetric recordings and were not always reflected by alterations in serum or hair corticosterone. The difference between animal groups might also be due to a high level of variance and the smaller n in the telemetry-implanted group.

As we have demonstrated in a separate group of animals, increased serum corticosterone can be observed in the early post-insult phase. For the interpretation of serum corticosterone levels and fecal corticosterone metabolite data, it needs to be considered that these might not only reflect stress but also a direct influence of seizure activity on the hypothalamic-pituitary-adrenal axis.[30]

Taken together, the data confirm a more pronounced detrimental impact on well-being in the early post-SE phase, and a milder influence during the latency phase. In the chronic phase, the more detailed analysis of behavioral parameters indicated an elevated level of anhedonia-associated behavior and reduced behavioral patterns, for which rodents normally exhibit a high level of motivation. When considering the entire experiment, the level of severity proved to be significantly higher as in the amygdala-kindling paradigm with repeated electrical induction of convulsive seizures,[7] and in a comparable range as in the pilocarpine post-SE model (Koska et al., manuscript in revision), although relevant differences were also observed between the chemical and electrical model. Considering the early post-SE phase with a transient more pronounced impact on the well-being of the animals, we suggest a classification as severe based on the European expert working group report.[31] As already emphasized in our previous publication,[7] the laboratory-specific conditions need to be taken into account, so that a suggestion can provide only a basal guidance for ethical committee members and scientists.

One main aim of our series of studies is to identify and validate parameters that are suitable for severity classification of epilepsy models, and that might be applied for assessment of new models as well as for evidence-based development of refinement measures according to the 3R principle. The present data further confirm burrowing as a more sensitive indicator of compromised well-being as compared to nest building. Moreover, we would like to highlight the saccharin preference test as an easy-to-apply test, which is performed in the home cage without any intervention necessary. Respective data are in line with our findings from the pilocarpine post-SE model (Koska et al., manuscript in revision).

Among the biochemical parameters, fecal corticosterone metabolite analysis seems to be more sensitive as compared to corticosterone analysis in serum or hair.[32] However, as discussed earlier the interpretation requires more complex considerations and caution in epilepsy models.

Only relatively limited effects were observed by additional telemetric analysis including an increased resting activity, heart rate, and NN interval in selected light phases and experimental phases. Alterations proved to be most pronounced in the early post-insult phase suggesting a disturbed resting phase with elevated activity levels and heart rate. Heart rate elevations have been discussed previously as a marker of distress in laboratory rodents exposed to surgical intervention, tumor models, or a sepsis model.[9,33–36]

In this context, it is of interest that heart rate variability has been reported to be differentially affected by chronic stress exposure with a decrease in time-domain indices and an increase in frequency domain indices.[37] The lack of any relevant and robust alterations in the electrical post-SE model may thus argue against a high level of stress.

The results of the burrowing test and saccharin preference tests proved to correlate with a large number of other measures such as food intake, nest building, social interaction, Irwin and Grimace scoring, as well as different measures of heart rate and heart rate variability parameters. Taking into account that these tests are easy to apply, these findings question the additional informative value of telemetric activity and electrocardiographic analysis considering the tremendous experimental effort and high costs of these analyses.

As the long-term aim of the study was the determination of robust severity assessment parameters, female rats were used due to their higher variance. Future studies are planned to test any potential sex difference in the identified parameters in different models. However, for the electrical post-SE model it also needs to be considered that male rats show a high mortality, so that female rats are normally preferred for this particular model.

Besides an assessment of severity, the current data set along with respective data sets from the two studies in the kindling model and the pilocarpine post-SE model also inform about the face validity of these epilepsy models regarding behavioral alterations reflecting psychiatric comorbidities in patients. In view of the fact that previous findings have not always been consistent and robust,[16–18] it is of particular interest that the current study series resulted in comprehensive, valuable data sets from three epilepsy models, which have been generated under identical conditions. These data sets may also help to resolve some of the discrepancies in the field. In addition, behavioral test batteries in the electrical post-SE and other epilepsy models can be used to assess correlations between potential biomarker candidates and behavioral parameters under standardized conditions.[38,39]

Finally, we were eager to obtain further information about whether replacement of tethered seizure monitoring by telemetric seizure monitoring reduces the burden for the animals as suggested by Lidster et al.[2] Our findings from the pilocarpine model revealed that minor differences can be observed between animals with tethered and telemetric recordings with evidence for a higher level of distress in the group with tethered monitoring (Koska et al., manuscript in revision).

In the present study, the more comprehensive surgical procedure with subcutaneous implantation of a telemetry

transmitter did not result in more pronounced behavioral alterations in the early postsurgical phase. These data indicate that additional implantation of the transmitter might not increase the severity of the surgical intervention in a significant manner. In the chronic phase, behavioral and biochemical alterations were rather comparable, when comparing rats with tethered vs telemetric monitoring.

In a PCA, the parameters predominantly contributing to the separation between the tethered and telemetric groups were recorded in the EPM and open field paradigm, also suggesting a difference in locomotion between the two groups. Of interest, it was the group with telemetric recordings that showed higher levels of velocity and distance moved as compared to their electrode-implanted control group. Whether this effect is due to the tethered group being somewhat restrained in their movement during the 2 weeks prior to behavioral testing, a difference in experienced stress, or a batch difference between the two experimental groups requires more detailed investigation.

In support of our findings from the pilocarpine post-SE model (Koska et al., manuscript in revision), the present data further confirm that burrowing behavior and saccharin preference might serve as valid parameters for severity assessment in chronic epilepsy models.

Considering the course of alterations in behavioral, biochemical, and physiological parameters with a transient more pronounced impact on well-being during the early post-SE phase, we suggest a classification as severe providing a guidance for laboratory-specific evaluations.

Comparison between data from animals with tethered and telemetric recording did not indicate pronounced differences in the level of distress among these groups. Thus, in view of previous findings from the pilocarpine post-SE model (Koska et al., manuscript in revision), the validity of telemetric recordings as a putative refinement measure might differ depending on the epilepsy model.

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DISCLOSURE

The authors declare that they have no conflict of interest. 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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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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