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Toward Evidence-Based Severity Assessment in Mouse Models with Repeated Seizures: (II.) Impact of Surgery and Intrahippocampal Kainate

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Abstract
Introduction: Chronic epilepsy models require neurosurgical procedures including depth electrode implants. The intrahippocampal kainate model is a frequently used chronic paradigm, which is based on chemoconvulsant administration and status epilepticus induction during the surgical procedure. This experimental approach raises the question of the extent to which this approach affects postsurgical recovery. In addition to the short- and long-term impact of the surgical intervention, a potential impact of highly frequent electrographic seizure events needs to be considered in the context of severity assessment. ***Methods:*** Various behavioral, biochemical, and telemetric parameters were analyzed in four experimental groups of mice: 1st naive, 2nd with transmitter implants, 3rd with transmitter and electrode implants, and 4th with transmitter implants, electrode implants, and kainate-induced status epilepticus. ***Results:*** During the early postsurgical phase, transmitter implants caused a transient impact on Mouse Grimace scores and intragroup increase of fecal corticosterone metabolites. Additional craniotomy was associated with an influence on total heart rate variability and fecal corticosterone metabolites. Heart rate and Irwin score increases as well as a prolonged increase in Mouse Grimace scores pointed to an added burden related to the induction of a nonconvulsive status epilepticus. Data from the chronic phase argued against a relevant influence of frequent electrographic seizures on behavioral patterns, fecal corticosterone metabolites, heart rate, and its variability. However, Irwin scores indicated long-term changes in some animals with increased reactivity, body tone, and Straub tail. Interestingly, selected behavioral and telemetric data from the early poststatus epilepticus phase correlated with the frequency of electrographic seizure events in the chronic phase. ***Conclusion:*** In conclusion, our findings argue against the pronounced impact of highly frequent electrographic seizures on the well-being of mice. However, an increased level of nervousness in a subgroup of animals should be considered for handling procedures and refinement measures. In the early postsurgical phase, several parameters indicate an influence of the interventions with evidence that the nonconvulsive status epilepticus can negatively affect the recovery. Thus, the development and validation of refinement efforts should focus on this experimental phase. Finally, the datasets suggest that simple readout parameters may predict the long-term consequences of the epileptogenic insult. Respective biomarker candidates require further validation in the follow-up studies in models with subgroups of animals with or without epilepsy development.

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Introduction

Chronic epilepsy models are often based on the induction of a status epilepticus (SE) as a trigger for epileptogenesis with subsequent development of spontaneous recurrent seizures. These models require surgical procedures for implantation of electrodes allowing continuous electroencephalographic recordings as a basis for reliable seizure detection. In addition, some models are based on local administration of chemoconvulsants in susceptible brain regions via guide cannulas.

Since its first description, the use of the mouse intrahippocampal kainate model has increased steadily. The number of publications per year reached 116 in 2020 (search string: see online supplementary material S1; see www.karger.com/doi/10.1159/000522156 for all online suppl. material). Based on its characteristic features, the model has been classified as a model of mesial temporal lobe epilepsy [1, 2]. Epileptogenesis is triggered by unilateral injection of kainate in the dorsal hippocampus, which induces an SE followed by the development of recurrent paroxysmal epileptiform discharges. The presence and length of a latency period are influenced by the strain and sex of the animals [3]. In the chronic phase of the model, electrographic epileptiform episodes reach a high frequency with up to 60 paroxysmal events per hour [4]. In contrast, generalized motor seizures are a rather rare event in the intrahippocampal kainate model.

The interest in the intrahippocampal kainate model has been triggered by studies reporting poor responsiveness to several standard antiepileptic drugs including carbamazepine [5, 6]. This led to the suggestion that the model might be suitable to select drug candidates with improved efficacy in difficult-to-treat or drug-resistant epilepsy [7, 8]. As a consequence, the kainate model was also integrated into the reorganized Epilepsy Therapy Screening Program of the National Institute of Neurological Disorders and Stroke [9].

As previously discussed in the context of other rat and mouse epilepsy models, ethical decisions need to weigh the expected gain-in-knowledge against the burden for the animals. Thereby, decisions about the approval of an animal experiment would ideally be based on an evidence-based assessment of severity. Unfortunately, standard procedures for severity assessment have been limited to the application of clinical scoring systems without the assessment of parameters that aim to provide more detailed information about the affective state of an animal. However, as also emphasized by Jirkof et al. [10], a potential impact of an experimental intervention or model on the affective state must be considered highly relevant for conclusions about the well-being of a laboratory animal. In the context of a research consortium [11], we made relevant progress in the identification and validation of parameters that provide a basis for evidence-based severity assessment reaching a novel level of sensitivity [12–15]. Improved sensitivity of severity assessment approaches can be of particular relevance when it comes to animal welfare-based prioritization of models and the validation of refinement measures.

A focus on the intrahippocampal kainate model is of particular interest for two reasons. First, the model is based on the administration of the chemoconvulsant and induction of an SE during the surgical procedure with craniotomy for electrode and cannula implants. This raises the question of the extent to which exposure to kainate and prolonged seizure activity adds to the burden associated with the neurosurgical procedure. Moreover, it is of interest to assess the impact of the highly frequent electrographic paroxysmal events characterizing the chronic phase of the paradigm on the well-being of the animals. At the same time, a thorough characterization of an epilepsy model can provide valuable information for the selection of readout parameters for future study design and can point to parameters that might be linked pathomechanistically.

Thus, aiming to provide an improved basis for severity assessment and more comprehensive information about the characteristics of the intrahippocampal kainate model, we have evaluated various parameters providing information about an impact on behavioral patterns, activation of the hypothalamic-pituitary-gland axis as well as heart rate, and its total and short-term variability. By assessing the time-dependent course of selected parameters, we obtained information about possible predictive value of different readout parameters.

Materials and Methods

Ethical Statement

This study was approved by the government of Upper Bavaria (license number ROB-55.2-2532.Vet_02-17-86). The procedures and reporting were performed according to the Basel Declaration including the 3R concept and the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines [16]. All investigations were conducted in line with the German Animal Welfare Act and the EU directive 2010/63/EU.

Animals and Experimental Groups

In total, 36 female mice (HsdWin:NMRI; Envigo, Horst, The Netherlands) were used at the age of 4–5 weeks and with a body weight of 20–22 g upon arrival. Mice were single-housed under

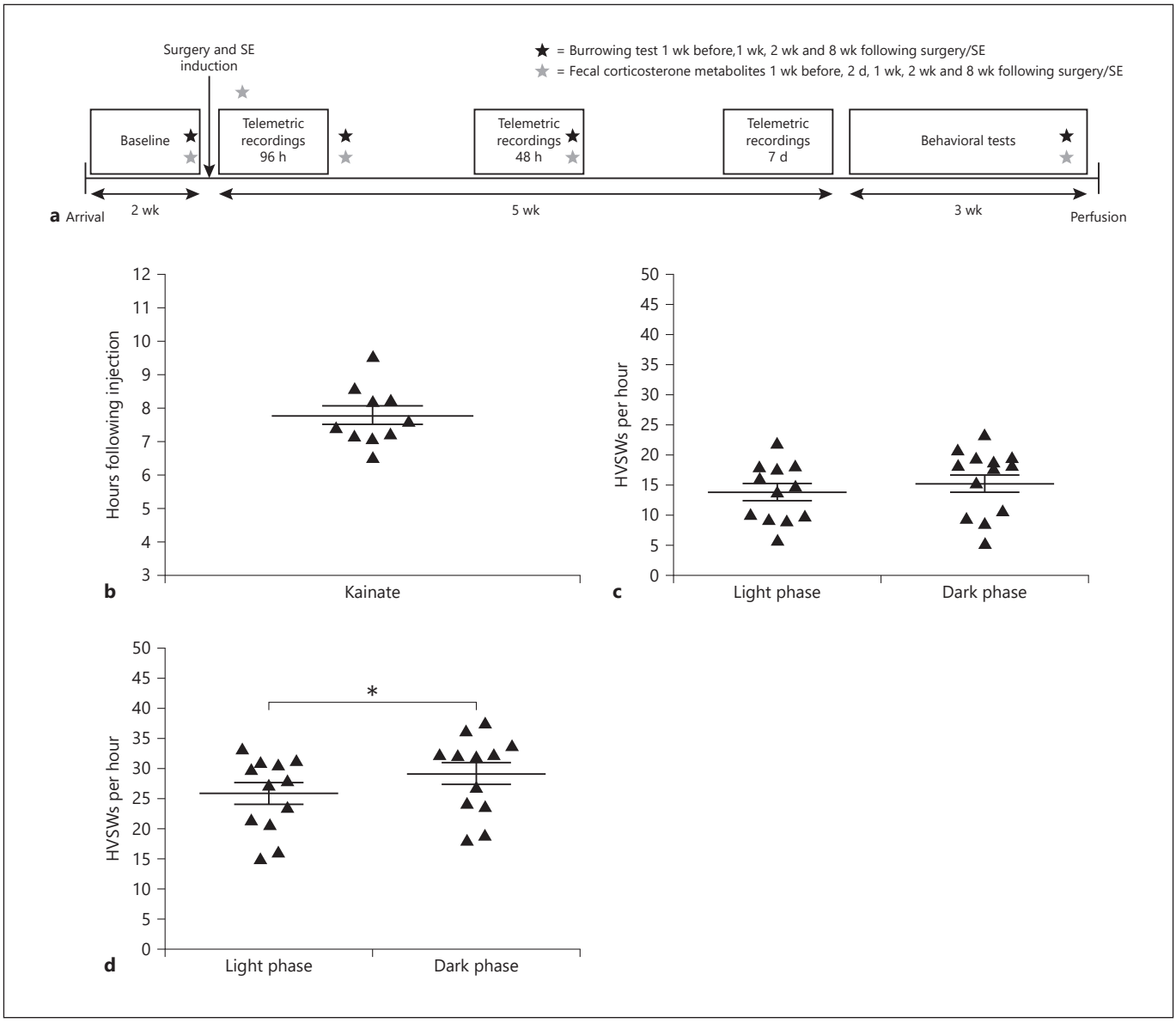

Fig. 1. Experimental design, SE, and epileptogenesis. **a** Timeline of study with telemetric recordings. **b** Onset of SE following intrahippocampal kainate injection (n = 10 animals). Number of HVSWs during light and dark phase (**c**) 2 weeks following SE, and (**d**) 4 weeks following SE (n = 12 animals). Data for **b–d** represent mean and SEM. Significant differences are indicated by an asterisk (*). SE, status epilepticus.

environmentally controlled conditions (22 ± 2°C, 55 ± 10% humidity) with a 12-h light-dark cycle (lights on at 6:00 a.m., CEST). Once a week, animals received a clean home cage (Makrolon type III cages; Zoonlab, Castrop-Rauxel, Germany) with wood chip bedding material (Grade 5; Altromin GmbH, Lage, Germany). Food (Ssniff Spezialdiäten GmbH, Soest, Germany) and tap water were available ad libitum. Additional cage enrichment comprised two nestlets (Zoonlab, Castrop-Rauxel, Germany) and one mouse house (Zoonlab, Castrop-Rauxel, Germany). After arrival, animals were allowed to acclimatize to the new animal facility for 10 days with daily handling by experimenters. Mice were weighed weekly and controlled daily according to severity assessment schemes including the Mouse Grimace Scale [17] and a modified Irwin Score [18]. For detailed information about the action units and Irwin parameters, see online supplementary Tables S1–S4.

Animals were randomly divided into four experimental groups, naive (n = 8 animals, without implants), telemetry (n = 8 animals, transmitter implanted), sham (n = 8 animals, electrode and transmitter implanted), and kainate (n = 12 animals, electrode and transmitter implanted and kainate-treated) group. When allocat-

ing mice to groups, the burrowed amount before the surgical intervention was used as a relevant parameter for stratified randomization (R software). For an overview of the experimental design, see Figure 1a.

Surgery and Intrahippocampal Injection of Kainate

Following acclimatization, animals underwent surgery with a telemetric transmitter (HD-X02; Data Sciences International, St. Paul, MN, USA) and electrode implantation. As analgesia mice received meloxicam (5 mg/kg s.c., Metacam®; Boehringer Ingelheim, Ingelheim am Rhein, Germany) 30 min before the start of anesthesia and again 24 h after the first injection, chloral hydrate (500 mg/kg i.p., solved in saline; Merck KGaA, Darmstadt, Germany) was used for anesthesia. Please note that this choice is related to the fact that previous studies reported an impact of inhalation anesthesia on SE induction in this model, resulting in the recommendation to use chloral hydrate [3]. Bupivacaine was administered for local anesthesia by subcutaneous infiltration of the incision area. The concentration used was 0.25% (Jenapharm®; Mibe GmbH, Brehna, Germany) for placement of the transmitter and leads and 0.25% + 0.0005% epinephrine (Jenapharm®; Mibe GmbH, Brehna, Germany) for electrode placement. The telemetric transmitter was placed subcutaneously after opening the skin in the dorsocaudal part of the scapula region. The negative ECG lead was fixed intramuscularly to the right pectoral muscle, the positive lead next to the xiphoid. Fixation of leads and closure of the skin was accomplished with absorbable sutures (Smi AG, St. Vith, Belgium). Animals were then fixed in a stereotactic frame (TSE Systems GmbH, Bad Homburg, Germany). Four holes were drilled in the skull bone. According to a protocol described by Gröticke et al. [19], kainate (50 nL of a 20 mM solution, i.e., 0.21 µg in 50 nL saline, i.e., 1 nmol, Sigma-Aldrich, Steinheim, Germany) was injected into the right CA1 area of the dorsal hippocampus (coordinates relative to bregma; ap: −1.8, lat: +1.6, dv:−1.7) using a 0.5-µL microsyringe. Animals of the sham group received saline instead of kainate. To avoid reflux, the needle of the syringe was maintained for additional 2 min at the injection site. Three screws were fixed into the skull and the negative EEG lead was connected to the screw positioned above the cerebellum. The positive EEG lead was connected to a Teflon-isolated stainless steel electrode before implanting it into the ipsilateral CA1 area, using the same coordinates as for kainate injection. Dental acrylic cement (Paladur®; Heraeus, Hanau, Germany) was used for fixation of the electrode and absorbable sutures for closing the skin. Additionally, a tissue adhesive (Surgibond®; Henry Schein Vet, Hamburg, Germany) was applied for closing the cut caused by transmitter placement. All surgical procedures were performed under aseptic conditions. To avoid hypothermia, animals were placed on a heating mat during and after surgery, and the temperature was controlled regularly.

Telemetric Recordings and Analysis

Immediately after surgery, mice were EEG, ECG, and video monitored over 96 h to record the nonconvulsive SE induced by kainate. Additional telemetric recordings were performed 2 weeks (for 48 h) and 4 weeks (for 7 days) post-SE. For telemetric recordings, home cages were placed on receiver plates, which were connected to a Ponemah® unit (Data Sciences International, St. Paul, MN, USA). Telemetric ECG and activity data of each animal were analyzed using Ponemah® software v. 6.41. To analyze telemetric EEG data, NeuroScore™ software v. 3.0 (Data Sciences International, St. Paul, MN, USA) was used. Generalized motor seizures were further confirmed using acquired videos by a video camera (Axis Communications, Lund, Sweden).

Behavioral Assessment

Assessment of nest-building behavior, burrowing behavior, and saccharin preference was conducted in the home cage in the animal facility. All additional behavioral tests including social interaction test, open field test, black-white box test, and elevated-plus maze test were performed in a behavior room with sound-isolated walls. A video-based (CCTV Camera; Panasonic, Suzhou, China) tracking software (EthoVision XT, version 8.5; Noldus, Wageningen, The Netherlands) was used for automated measurements. Mice were placed in the behavior room for habituation at least 30 min prior to testing. Between each trial, test arenas were cleaned with 0.1% acetic acid. The order of animals in all behavioral investigations was randomized (R software).

Nest Building

Nest-building behavior was assessed as described by Jirkof et al. [20]. Nest pictures were taken each day during the study in the light phase between 7:00 and 9:00 a.m. Based on these pictures, nest complexity was scored by a person blinded to group and treatment of animals. The scoring system was based on a standard operating protocol of the DFG research group FOR 2591: score 1 – the nestlet is almost untouched, >90% are intact; score 2 – the nestlet is partially torn up, 50–90% are still intact; score 3 – the nestlet is mostly destroyed, 50–90% of the nestlet is destroyed, <50% of the nestlet is intact, <90% of the nestlet is in one-quarter of the cage area; the cotton is not formed to a nest but distributed in the cage; score 4 – an identifiable, flat nest, >90% of the nestlet used for the nest, the material has the shape of a nest and is located in one-quarter of the cage area, the nest is flat, i.e., less than 50% of the circumference of the nest wall is higher than one-third of the height of the mouse house; score 5 – an almost perfect nest, >90% of the nestlet used for the nest, the nest resembles a crater with more than 50% of the circumference of the nest wall higher than one-third of the height of the mouse house; score 6 – perfect nest, >90% of the nestlet used for the nest, the nest resembles a crater with more than 90% of the circumference of the nest wall higher than one-third of the height of the mouse house.

Burrowing

The burrowing test was performed at four time points throughout the study. Baseline levels were assessed during the week before the surgical intervention and 48 h after a training session (first time point). The burrowing test was then repeated 1 week (second time point), 2 weeks (third time point), and 8 weeks after the surgical intervention (fourth time point). The experimental procedure was based on the protocol of Deacon et al. [21–23], adjusted by the research group FOR 2591. Empty round water bottles (Zoonlab, Castrop-Rauxel, Germany) with a length of 20 cm and an entrance of 3.5 cm diameter were filled with 200 ± 1 g food pellets (Ssniff Spezialdiäten GmbH, Soest, Germany). Two hours before the dark phase, a filled bottle was placed in the back left corner of each animal's home cage, the closed end facing the cage wall. After 2 hours, the remaining pellets in the bottle were weighed. Afterwards, the bottle with the remaining pellets was returned to the cage. At the end of the dark phase, the bottle was weighed again to evaluate the burrowed amount of pellets during the active phase.

Social Interaction

The test was performed according to a protocol by File and Hyde [24]. For two consecutive days, all animals underwent a habituation procedure during which animals were transferred to the behavioral room and placed individually into test cages (Makrolon Type III) for 10 min. The light intensity was set to 12 lux. On the test day, animals of the same experimental group were placed in the test arena with a weight-matched (±5 g) partner for 10 min. Due to the individual housing in the animal facility, none of the animals had direct contact with their interaction partner before the start of the test. A combined score was obtained for each pair of animals. The time spent in active social interaction (sniffing, grooming, playing, following, and walking on each other) and in passive social interaction (sitting or walking next to each other, but not interacting) was recorded in seconds.

Open Field

The open field test was used to examine the exploratory behavior and locomotor activity in an unfamiliar surrounding. The light intensity was set to approximately 12 lux. Two black cylinders (60 cm diameter, 40 cm height) were used simultaneously, in order to perform two runs in parallel. Mice were placed individually into the open field arena, facing the wall at a distance of 10 cm. Animals were video recorded (CCTV Camera; Panasonic) for 10 min using EthoVision XT software v. 8.5 Software (Noldus, Wageningen, The Netherlands). The software tracked the total distance moved, time of immobility, and time spent in different zones (wall, middle, center). Rearing behavior was scored manually by a person unaware of animals group allocation.

Black-White Box

In the black-white box test, each animal was assessed individually with a light intensity of 50 lux in the white compartment. The white compartment (40 × 40 cm) is connected to the black compartment (40 × 20 cm) by a 10 × 10 cm passage area. The black compartment is covered with a top lid. Trials lasting 5 min were monitored by video recording (CCTV Camera, Panasonic). In the beginning, each animal was placed in the white box with its head facing the black compartment. Latency to enter the black box, time spent in the black and white box, and stretching postures were scored manually by a person being unaware of the group allocation.

Elevated-Plus Maze

The elevated-plus maze consists of two open arms (40 lux), two closed arms with sidewalls of 12.5 cm height (20 lux), and a central platform arranged in the form of a plus sign. All arms are 40 cm long. At the start of the test, each animal was placed on the central platform with the head facing the same open arm. For each 5-min session, total distance moved, velocity, and time spent in closed and open arms were recorded by tracking software (EthoVision, version 8.5; Noldus, Wageningen, The Netherlands). The number of head dips (looking down from the open arms) was scored manually by a person being unaware of the group allocation.

Saccharin Preference

The test was performed according to a protocol by Klein et al. [25]. Two hundred grams of liquid was provided in each bottle (total volume of 250 mL) and a watering nipple with a diameter of 1 mm. To avoid side preference effects, two water bottles were attached to the cage during the entire study. The test was carried out on 4 consecutive days. On the first day, the water intake was evaluated. For that purpose, both bottles were filled with tap water and measured after 24 h. The following day, one of the two bottles was filled with 0.1% saccharin solution (Aldrich Saccharin ≥98%; Sigma-Aldrich Chemie GmbH, Germany) to assess the preference for the sweet solution. On day 3 of the experiment, both bottles were filled again with tap water. On the fourth day, the bottle on the other side was filled with 0.1% saccharin solution. The consumed amounts of liquid were assessed after 24 h for each testing day.

Analysis of Fecal Corticosterone Metabolites

To determine a baseline value, fecal samples were collected the week before the surgical intervention. Further samples were collected 2 days as well as 1, 2, and 8 weeks following SE. According to a protocol developed by the research group FOR 2591, the animals were placed in a fresh cage in the morning (7:00–9:00 a.m.) and all feces were collected. Feces were then stored frozen at –20°C, dried, and homogenized. Afterward, a 0.05 g portion of each sample was extracted with 1 mL 80% methanol. The analysis of the fecal corticosterone metabolites was carried out with a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay, as previously described by Touma et al. [26, 27].

Staging the Estrous Cycle

At the end of the study, the estrous cycle was determined using a commercial Ohmmeter (Multimeter Amprobe HEX60-D; Buerklin, Oberhaching, Germany) according to a method described by Ramos et al. [28].

Statistical Analysis

GraphPad Prism (Version 5.04; GraphPad, La Jolla, CA, USA) was used for statistical analysis. Statistical differences between two time points of one group were computed using a paired *t* test. Group differences between four groups were calculated by a one-way ANOVA followed by a Bonferroni multiple comparisons post hoc test. Repeated-measures ANOVA was used for comparison of data with different time points such as fecal corticosterone analysis and analysis of the burrowing performance. Normal distribution was confirmed by a Shapiro-Wilk test. For nonparametric data, the Kruskal-Wallis test was used, followed by Dunn's post hoc test for multiple comparisons. p values of <0.05 were considered as a threshold for statistically significant differences. The Spearman correlation matrix was created using R version 4.0.3 [29] and visualized using the R-package "corrplot" [30]. The Principal component analysis (PCA) was calculated and visualized using the R-package "made4" [31]. For the visualization of the telemetric recordings and the forest plot, the R-package "ggplot" was used [32]. Loess regression with a span of 0.15 was used to smooth the line of the graph in Figure 2 and online supplementary Figures S2–S5.

Results

SE and Epileptogenesis

The onset of SE was delayed by several hours (7.78 ± 0.85 h; Fig. 1b). Two and 4 weeks following SE, the mice exhibited frequent paroxysmal electrographic episodes, which met the definition of high voltage sharp waves

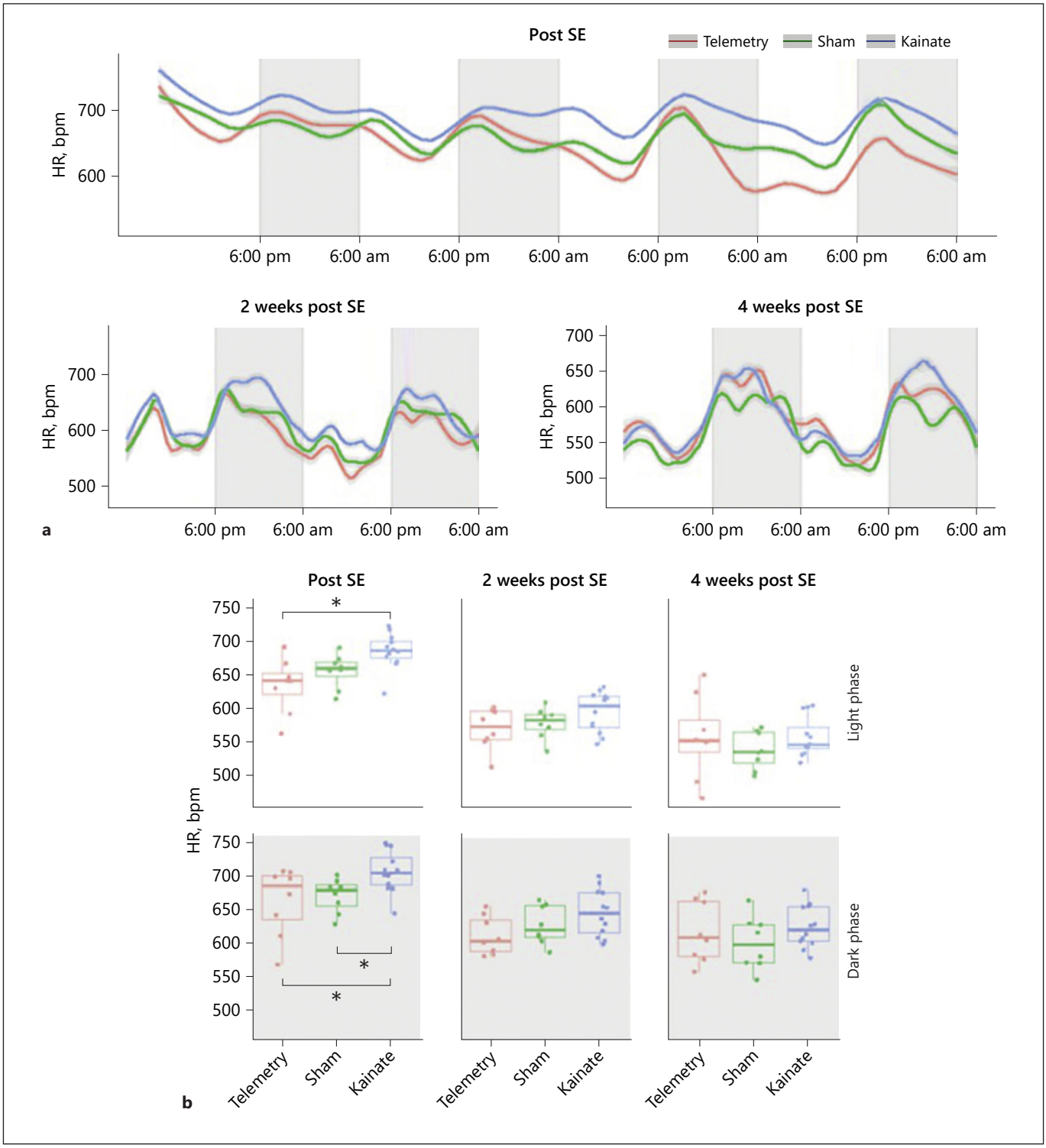

Fig. 2. HR and HR variability. **a**, **c** Telemetric recordings were performed at three different time points. The graph illustrates the time-based course of these parameters. **b**, **d** Mean values of light and dark phases were calculated and illustrated as boxplots for each time point. **a**, **b** Animals of the kainate group exhibited increased HRs in both light and dark phase in the early phase following surgery and SE (light phase, $p = 0.0029$; dark phase, $p = 0.0118$ and $p = 0.0413$). **c**, **d** SDNN was significantly decreased in animals of the sham group during the dark phase ($p = 0.0360$) and in animals of the kainate group during the light and dark phase (light phase, $p = 0.0222$; dark phase, $p = 0.0253$). Significant differences are indicated by an asterisk (*). Total n was: telemetry, $n = 8$; sham, $n = 8$; kainate, $n = 12$. HR, heart rate.

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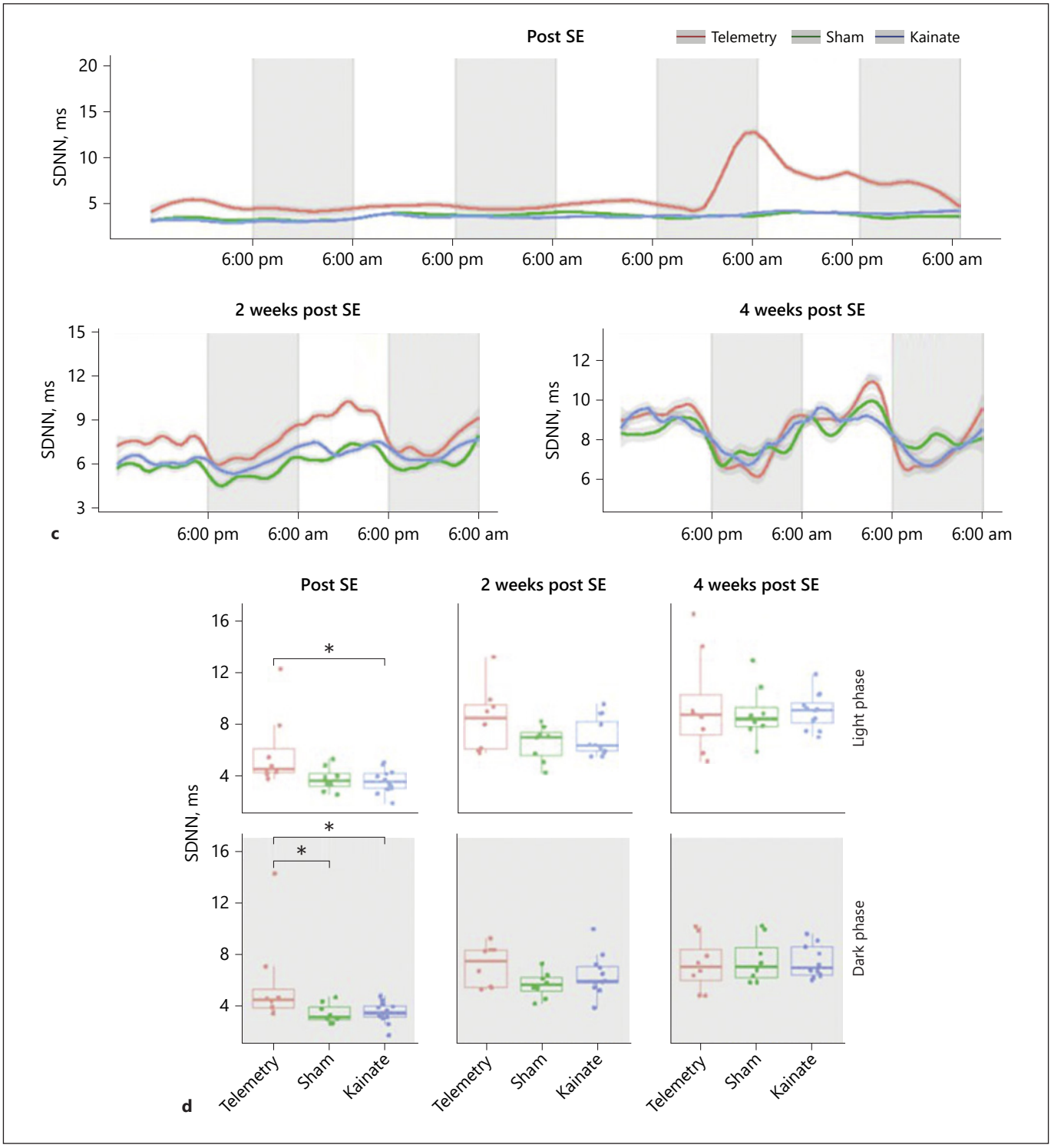

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(HVSWs) by Twele et al. [4]. The mean number of HVSWs during the recording periods of 48 h amounted to 15.40 per hour, 2 weeks and 29.26 per hour, 4 weeks following SE (Figure 1c, d; representative EEG recordings: see online suppl. Fig. S5). Four weeks following SE, a significant difference between the mean number of HVSWs during the dark phase versus light phase became evident ($p = 0.0119$).

The number of hippocampal paroxysmal discharges (HPDs), which met the criteria described by Twele et al. [4] was very low. HPDs were observed in 8 mice. In these animals, the total number of HPDs during the 48-h recording phase ranged between zero and two per hour. Generalized convulsive seizures were almost only observed in association with the handling of the animals. The number of animals with handling-associated seizures amounted to 7. The total number of handling-associated generalized convulsive seizures in these animals ranged between 1 and 4. During the recording phase, 2 weeks following SE, only one generalized convulsive seizure was detected in 2 animals.

Early Phase following Surgery and SE: Home Cage Activity, Heart Rate, and Heart Rate Variability

In the early phase following SE, telemetric recordings were compared between all groups that received transmitter implants. Activity patterns during the light and dark phase were neither affected by additional electrode nor by the kainate-induced SE (online suppl. Fig. S1). In apparent contrast, kainate exposure with induction of an SE exerted an impact on the heart rate, which reached increased levels in this experimental group during the light and dark phase (Fig. 2a, b; light phase, $p = 0.0029$; dark phase, $p = 0.0118$ and $p = 0.0413$). In contrast, animals with transmitter implants and with additional craniotomy exhibited comparable heart rate patterns.

To obtain information about the total variability of the heart rate, and hereby the adaptability of the autonomic nervous system, we analyzed the standard deviation of NN intervals (SDNN). Moreover, we analyzed short-term variability based on the root mean square of successive differences (RMSSD), percentage of subsequent NN intervals that deviate more than 6 ms (NN6), and the proportion derived by dividing NN6 by the total number of NN intervals (pNN6). None of the parameters of the short term were affected by the experimental interventions (online suppl. Fig. S2–S4). In comparison with animals with telemetry transmitter implants only, additional craniotomy with electrode and cannula implants exerted an impact on SDNN with a reduction during the dark phase. In animals with kainate exposure, a reduction of SDNN was evident during the light and dark phase (Fig. 2c, d).

Early Phase following Surgery and Status Epilepticus: Mouse Grimace Scale, Irwin Score, Nest-Building Performance, and Fecal Corticosterone Metabolites

In the early phase following surgery and SE induction, the Mouse Grimace Scale was applied to obtain information about the level of postsurgical pain (Fig. 3a). Increased Grimace scale levels were observed in animals with transmitter and electrode on days 1 and 2 following surgery (days 1 and 2: sham vs. naive $p < 0.05$). In animals with additional kainate exposure, an impact on the Grimace scale was observed until postsurgical day 4 (day 1–4: kainate vs. naive $p < 0.05$). From day 2 to 4, the respective group difference was not only evident in comparison with naive animals but also with animals with sole implantation of transmitters (day 2–4: kainate vs. telemetry $p < 0.05$). On day 1 following surgery and SE, all animals with surgical interventions exhibited scores from two or more. Only 3 animals from the kainate group still showed scores greater than two on day 2 following surgery.

Mice with intrahippocampal kainate injections exhibited elevated Irwin scores following surgery (Fig. 3b; kainate vs. all other groups $p < 0.05$). The parameters that were affected included the reaction to touching, nervousness and startle response, Straub tail, and increased body tone. Respective alterations were extending into the chronic phase.

As further explained below, nest building can be compromised as a consequence of postsurgical distress and pain [33]. A reduction in nest complexity was observed at postsurgical day 1 in mice with transmitter implants and in mice with kainate administration (Fig. 3c; kainate vs. naive $p < 0.05$; telemetry vs. naive $p < 0.05$). An increase in fecal corticosterone metabolite levels was demonstrated in all groups with surgical interventions. The group comparison revealed differences between animals with craniotomy or kainate exposure and naive animals (Fig. 3d; $F(3, 32) = 7.587$, $p = 0.0006$; sham vs. naive $p = 0.0023$; kainate vs. naive $p = 0.0006$).

Chronic Phase: Home Cage Activity, Heart Rate, and Heart Rate Variability

In the chronic phase, telemetric parameters were assessed at two time points 2 and 4 weeks following SE. At both time points, activity and heart rate showed the characteristic circadian rhythmicity with a peak during the dark phase. Whereas previous recordings of activity and heart rate in female Sprague Dawley rats revealed a characteristic pattern with two peaks during the dark phase, this pattern was not evident in the present recordings from female NMRI mice.

Home cage activity and heart rate during light and dark phase remained unaffected by any of the experimental conditions including chronic transmitter implants, electrode implants, and history of a kainate-induced SE with subsequent epileptogenesis (online suppl. Fig. S1;

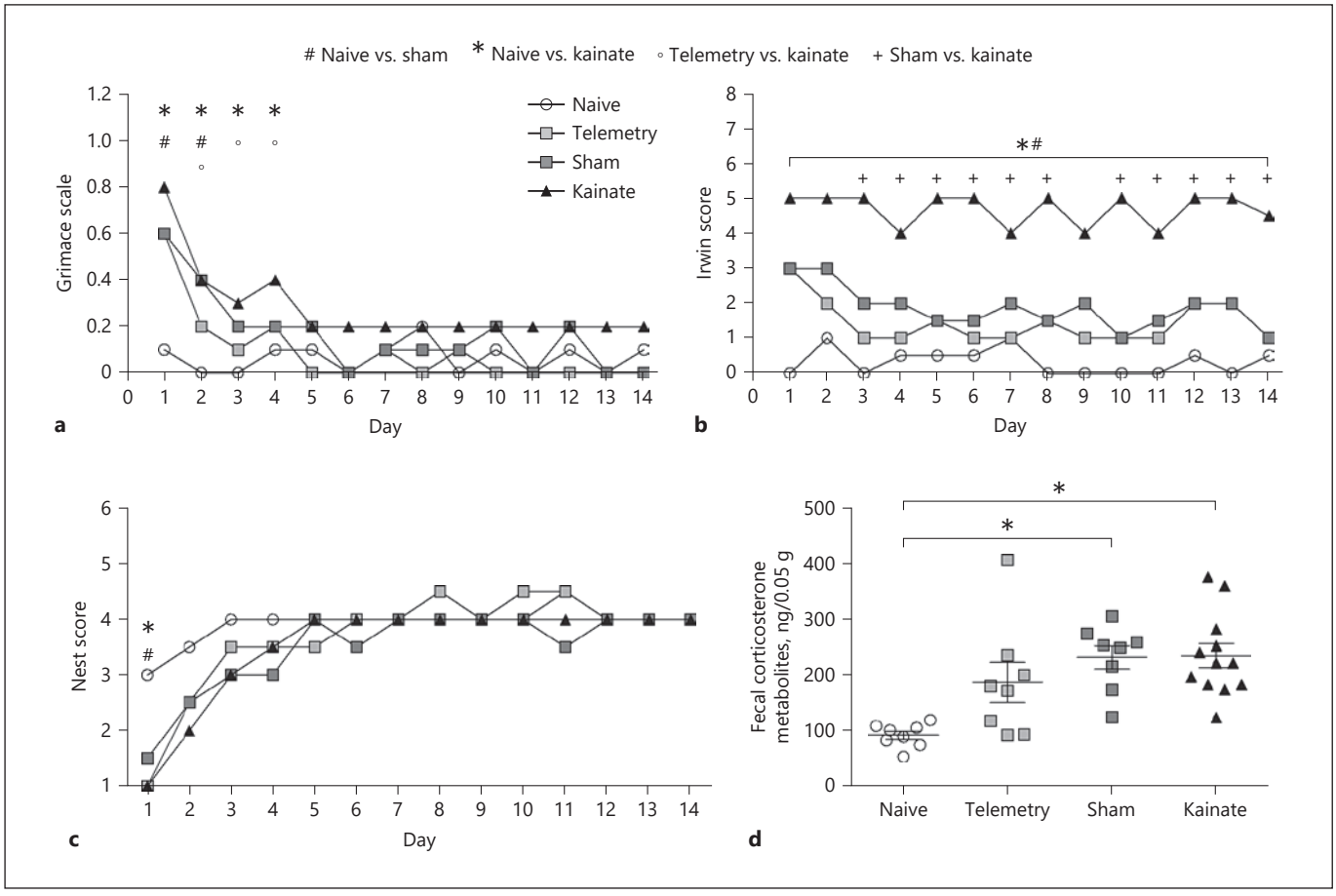


Fig. 3. Mouse Grimace Scale, Irwin score, nest-building performance, and fecal corticosterone metabolites in the early phase following surgery and SE. **a** Mouse Grimace Scale 14 days following surgery and SE. Data represent the median (naive, $n = 8$; telemetry, $n = 8$; sham, $n = 8$; kainate, $n = 12$). **b** Irwin score in the early phase following surgery and SE. Animals with intrahippocampal kainate injections exhibited elevated Irwin scores. Data represent the median (naive, $n = 8$; telemetry, $n = 8$; sham, $n = 8$; kainate, $n = 12$). **c** Nest complexity score in the early postsurgical phase. A significant reduction of nest complexity was observed in animals from the kainate and telemetry groups. Data represent the median (naive, $n = 8$; telemetry, $n = 8$; sham, $n = 8$; kainate, $n = 12$). **d** Fecal corticosterone metabolites 2 days following surgery and SE. Significant differences were detected between animals with craniotomy or kainate exposure and naive animals. Data represent the mean ± SEM, and values of individual animals (naive, $n = 8$; telemetry, $n = 8$; sham, $n = 8$; kainate, $n = 12$). All significant differences are indicated by different signs ($p < 0.05$). All animals from the kainate, sham, and telemetry groups received telemetry transmitters.

Fig. 2a, b). As described above, we analyzed SDNN as a parameter of total heart rate variability and RMSSD, NN6, and pNN6 as parameters of short-term heart rate variability. None of these parameters was affected by the experimental interventions and procedures (Fig. 2c, d; online suppl. Fig. S2–S4).

Chronic Phase: Open Field Activity and Anxiety-Associated Behavior

Analysis of behavioral patterns in the open field provided information about exploratory behavior, activity, and locomotion in an unfamiliar environment. The group comparison argued against any long-term alterations in horizontal and vertical activity as well as in the time spent in different zones of the open field as a consequence of the transmitter implant, the electrode implant, or i.h.c. kainate-induced SE (online suppl. Fig. S6). The fact that we did not detect differences in the time in the center or thigmotaxis provided first evidence that anxiety-associated behavior is comparable among the different experimental groups. However, in this context, it is important to consider that the open-field paradigm with very dim light

conditions was set up to focus on the analysis of exploration and activity with a limited influence on anxiety.

Thus, we have additionally analyzed the behavioral patterns in the black-white box and the elevated-plus maze to obtain more information about anxiety-associated behavior (online suppl. Fig. S7, S8). Animals of all experimental groups exhibited a normal exploratory behavior with no difference in the time spent in the aversive areas of the paradigms. Moreover, a comparable number of stretching postures indicated a lack of differences in risk assessment.

Chronic Phase: Nest Building, Burrowing, Social Interaction, and Anhedonia-Associated Behavior

Mice show a high natural motivation for nest-building and burrowing behavior even though these behavioral traits are non-essential for survival in standard experimental animal facilities. A reduction in respective behavioral patterns can indicate compromised well-being for instance related to distress or pain [33]. Repeated analysis of nest-building performance in the chronic phase argued against any relevant impact of the experimental procedures on nest complexity scores (online suppl. Fig. S9). High nest complexity scores were rapidly reached following the weekly introduction of new nest material.

Testing of the burrowing performance did not reveal group differences in the chronic phase of the paradigm with repeated assessment 1 week, 2 weeks, and 8 weeks following SE. Analysis of the development of burrowing during the experiment demonstrated an intragroup increase in the group with a kainate-induced SE. This increase became evident during the chronic phase with more pellets removed from the burrowing tube at the last time point in comparison with the earlier time points following SE (online suppl. Fig. S10; $F(3, 33) = 5.013$, $p = 0.0052$).

Sickness behavior and distress can affect patterns of social interaction [34]. The analysis of interaction patterns in mouse pairs from the different experimental groups points to the lack of a relevant influence of the implants or the SE and epileptogenesis. Both, the time spent in active and passive interaction proved to be in a comparable range in all groups (online suppl. Fig. S11).

Alterations in the preference for sweet solutions can provide valuable information about anhedonia-associated behavior. Based on the analysis of saccharin and water consumption on different days with a switch in the bottle position, we were able to exclude a side preference as a potential bias for all groups. The mean consumption of saccharin solution in the different groups ranged between 40.6 and 88.1% of the total fluid consumption without relevant group difference (online suppl. Fig. S12).

Chronic Phase: Fecal Corticosterone Metabolites

Fecal corticosterone metabolites were repeatedly analyzed during the chronic phase with recurrent electrographic and rare motor seizures. None of the experimental groups exhibited increased levels of fecal corticosterone metabolites indicating that neither chronic transmitter and electrode implants nor the history of a kainate-induced SE with subsequent epileptogenesis cause chronic activation of the hypothalamic-pituitary-adrenal axis (online suppl. Fig. S13).

Correlation Matrix

A cross-correlation analysis was completed to obtain information about an association between different parameters. Thereby, it was of particular interest to check for evidence that complex parameters and parameters, which require invasive approaches, can be replaced by simple parameters, which are easy to assess. Moreover, a correlation across different time points is of interest to explore a potential predictive value of selected parameters.

At the early time point, a strong correlation with a correlation coefficient r exceeding ± 0.7 was observed between the Mouse Grimace Scale and the Irwin score. Interestingly, the Irwin scores at earlier time points, i.e., 2 days and 2 weeks following SE correlated with Irwin scores reached at later time points during the experiment (Fig. 4; for a detailed description of all correlations, see online suppl. Table S5 in the supporting information).

Concerning telemetric parameters, the analysis pointed to a correlation between activity, heart rate, or the heart rate variability parameters SDNN, NN6, and pNN6 recorded in the early phase following SE and different behavioral parameters analyzed in the chronic phase of the model. Moreover, a correlation was observed between heart rate or SDNN and the Mouse Grimace Scale, and Irwin score evaluated during the initial phase following SE. However, the respective level of correlation proved to be only moderate with a correlation coefficient ranging between ± 0.5 and 0.7 (online suppl. Fig. S14).

We also addressed the question of whether the data suggested an association between the frequency of electrographic HVSW episodes and behavioral patterns. The respective analysis indicated an association with a reduced thigmotaxis, an increased number of head dips, and an increased time in aversive areas of different behavioral paradigms (including the center of the open field,

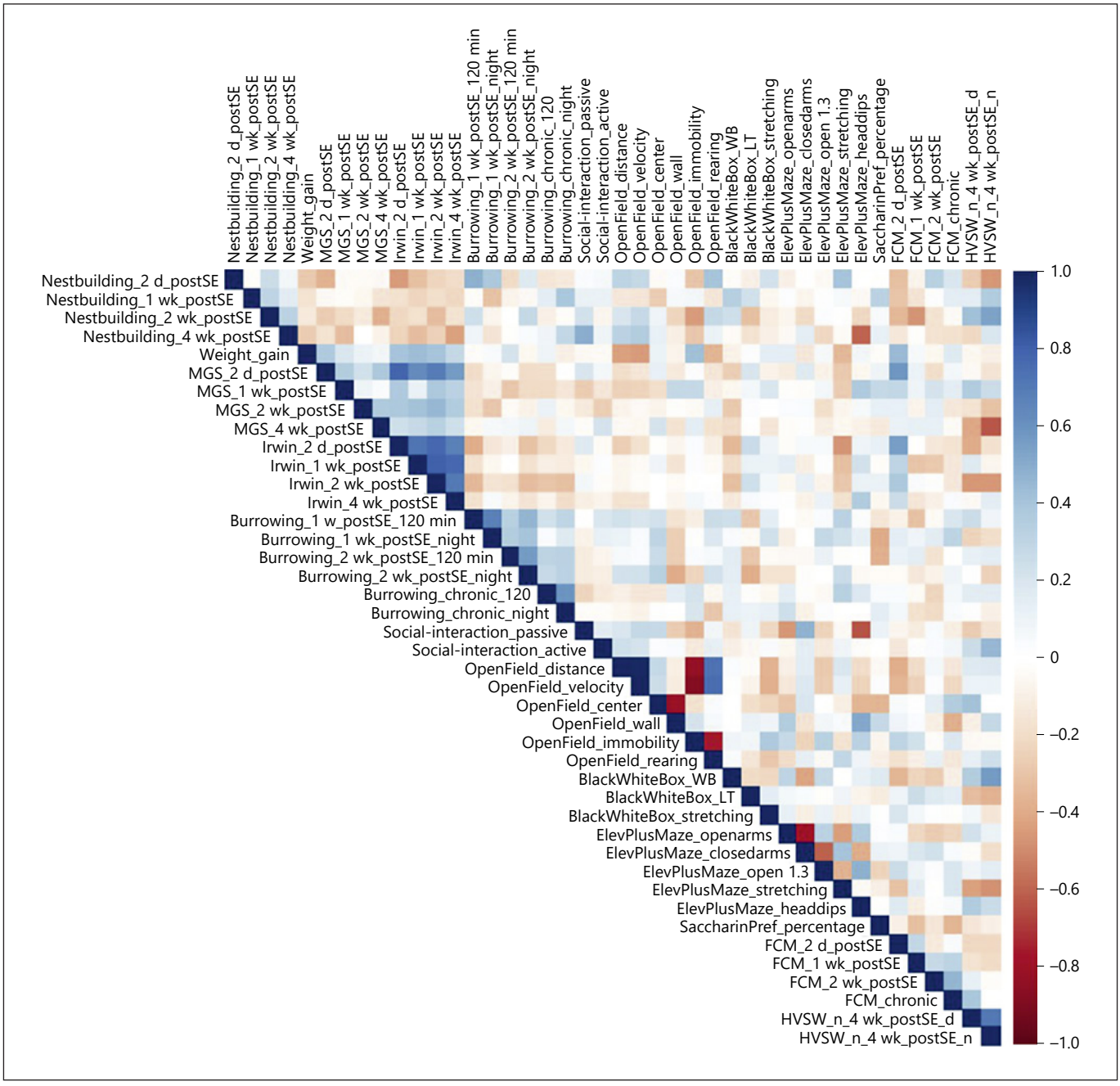


Fig. 4. Correlation matrix. Spearman correlations between HVSWs, behavioral, and biochemical parameters are illustrated with a heat map. Abbreviations used in Figure 4 are listed in the online supplementary material.

white area of the black-white box, and the outer third of the open arms of the elevated-plus maze).

Principal Component Analysis

PCA was performed to structure and illustrate the large data set. For the PCA performed for all four experimental groups (Fig. 5), the first two principal components (PCs) explained 34.48% of the total variance in the data (PC1: 22.09%, PC2: 12.38%). For none of the PCs, a significant difference between the groups was detected. For detailed information about the variables contributing to the PCs, see online supplementary Tables S6 and S7 in the supporting

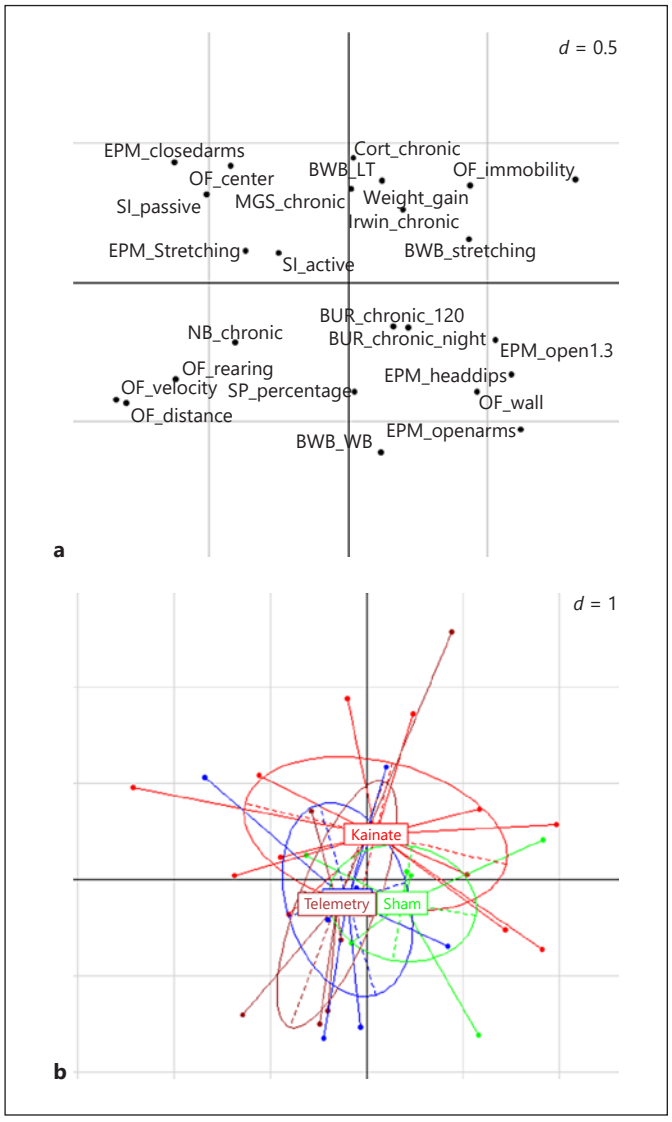


Fig. 5. PCA of all measured variables. PCA with PC1 on the *x*-axis and PC2 on the *y*-axis. Individual animals for each group are shown. **a** Five of the top ten contributing variables to PC1 were recorded in the open field, four in the elevated-plus maze, and one in the social interaction test. For PC2, five of the contributing variables were observed in the open field, two in the elevated-plus maze, and one in the black-white box. Moreover, one variable was fecal corticosterone metabolites and one was from the saccharin preference test. **b** PCA of all five experimental groups. Distribution of the four groups was not significantly different neither along PC1 nor along PC2. Abbreviations used in Figure 5 are listed in the online supplementary material.

information. Additional PCAs were performed for groups with surgical interventions and the naive group (sham vs. telemetry; sham vs. naive; telemetry vs. naive; data not shown). Again, no significant differences were detected.

Forest Plot

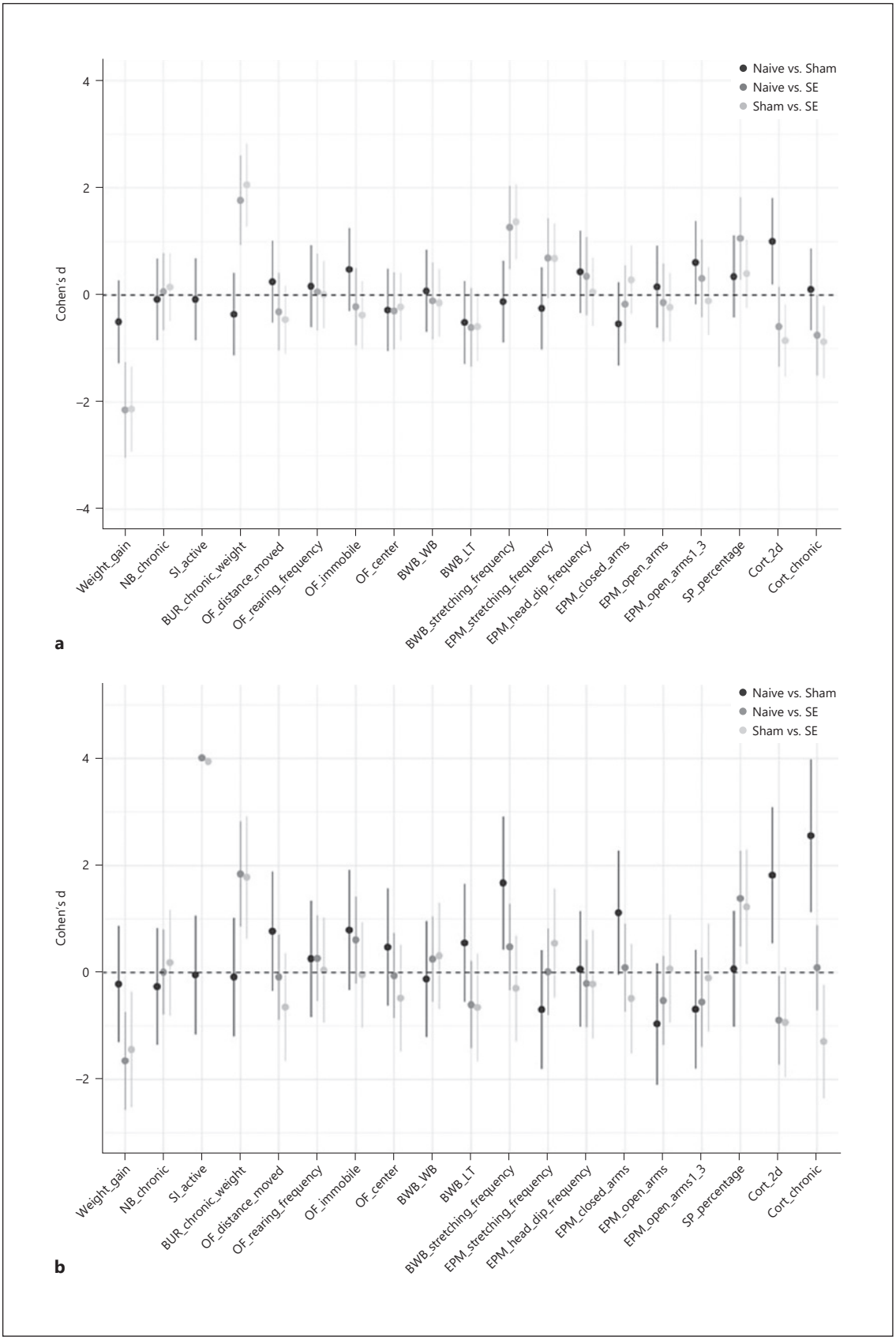
Data from two different post-SE models in rats [13, 14] and the intrahippocampal kainate model in mice were considered for a comparison between species and models (Fig. 6a–c). In this context, differences between seizures types and frequencies characteristic for the models need to be considered.

Here, we focused on alterations that may reflect an increased level of distress in the animals. In rats (Fig. 6a, b), an increased anhedonia-associated behavior became evident with a reduced preference for sweet solutions in the saccharin preference test {chemical post-SE model: $F(2, 34) = 14.18$, $p = 0.0003$, SE vs. naive $d = 1.056$ (Cl_{95} [0.287; 1.824]), SE vs. sham $d = 0.399$ (Cl_{95} [−0.243; 1.042]); electrical post-SE model: $F(2, 30) = 4.975$, $p = 0.0136$, SE vs. naive $d = 1.381$ (Cl_{95} [0.487; 2.276]), SE vs. sham $d = 1.316$ (Cl_{95} [−0.555; 2.077])}. Additionally, rats spent less time in active social interaction {chemical post-SE model: $F(2, 16) = 44.87$, $p = 0.2580$, SE vs. naive $d = 4.511$ (Cl_{95} [3.213; 5.808]), SE versus sham $d = 4.601$ (Cl_{95} [3.407; 5.796]); electrical post-SE model: $F(2, 11) = 52.32$, $p < 0.0001$, SE vs. naive $d = 4.013$ (Cl_{95} [2.621; 5.405]), SE vs. sham $d = 4.128$ (Cl_{95} [2.911; 5.345])}. In contrast, these tests did not reveal respective alterations in mice.

In addition to behavioral parameters, we also compared alterations in corticosterone and its metabolites. In rats, as well as in mice, the respective analysis of feces indicated an increased level of distress 2 days following SE {chemical post-SE model: $F(2, 35) = 10.72$, $p = 0.0002$, SE versus naive $d = -0.590$ (Cl_{95} [−1.335; 0.155]), SE versus sham $d = -0.852$ (Cl_{95} [−1.523; −0.180]); electrical post-SE model: $F(2, 30) = 4.975$, $p = 0.0136$, SE vs. naive $d = 0.006$ (Cl_{95} [−0.789; 0.802]), SE versus sham $d = -1.066$ (Cl_{95} [−1.790; −0.341]); intrahippocampal kainate model: kainate versus naive $d = -2.387$ (Cl_{95} [−3.631; −1.142])}. For detailed information about the readout parameters and the respective statistic information, see above and online supplementary Table S8 in the supporting information.

Discussion

Different chronic rat and mouse models have been categorized as models of temporal lobe epilepsy. The list of respective paradigms includes kindling models with repeated electrical or chemical induction of seizures and post-SE models with induction of an epileptogenic SE triggering epileptogenesis with the development of spontaneous recurrent seizures. While these models share dif-



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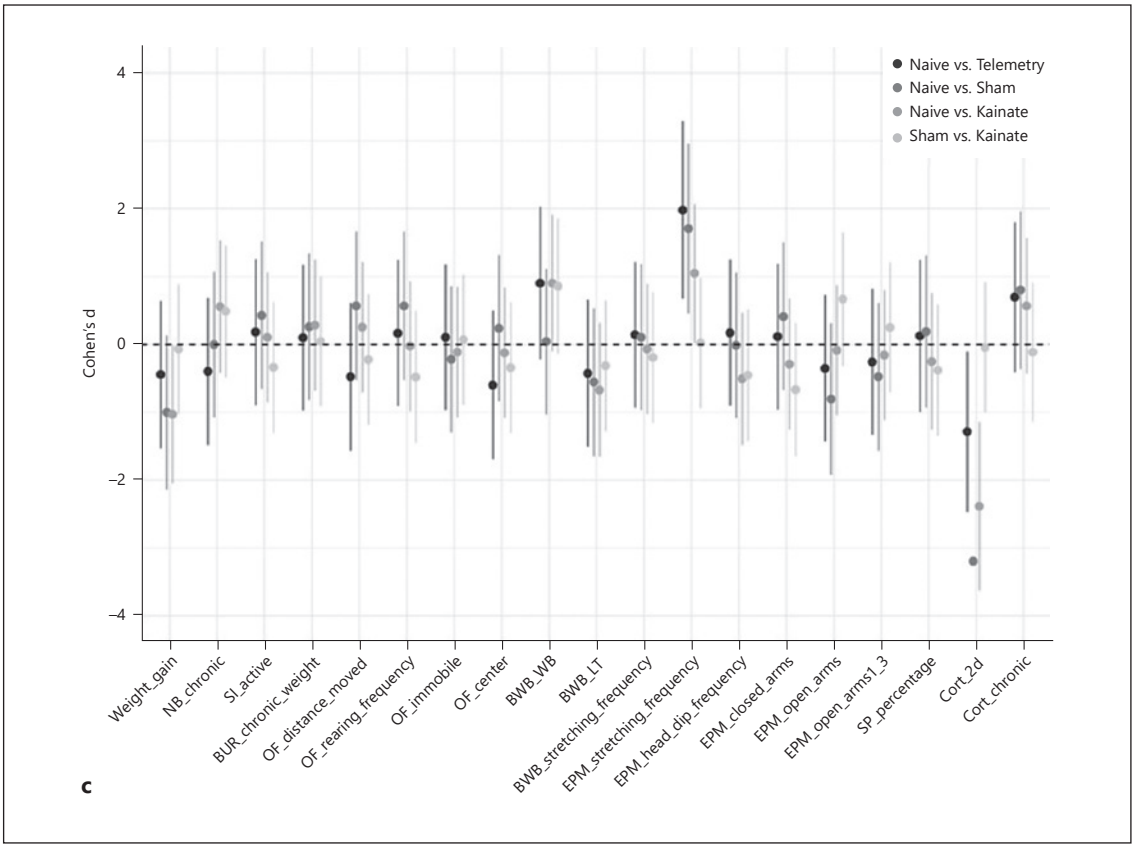

Fig. 6. Forest plot illustrating the effect size of different parameters analyzed in different post-SE models in rats (**a**, **b**) and mice (**c**). Parameters were assessed in the chronic phase. Effect sizes were calculated as Cohen's d based on two groups (**a**, **b**: naive vs. sham, naive vs. SE and sham vs. SE; **c**: naive vs. telemetry, naive vs. sham, naive vs. kainate and sham vs. kainate). Rat data had been published in Koska et al. [13] (**a**) and Seiffert et al. [14] (**b**). Abbreviations used in Figure 6 are listed in the online supplementary material.

ferent features including the need for neurosurgical procedures, they also largely differ regarding the model-specific spectrum of characteristics that determines their face validity.

As stated above, the intrahippocampal kainate model is based on the administration of the chemoconvulsant and induction of SE during the craniotomy procedure with depth electrode implants [19]. This approach raises the question of the extent to which kainate exposure and prolonged seizure activity might compromise the early recovery phase of the animals and increase the distress of the animals. During the early phase following surgery, an influence of the interventions in the different experimental groups became evident for several readout parameters. The group with sole implants of transmitters exhibited transiently increased Mouse Grimace scores and an intragroup increase of fecal corticosterone metabolites. Moreover, nest-building data indicate a slight delay in nest-building activity. However, data from the following days argued against a longer lasting effect. While the lack of group differences in corticosterone metabolites, and the lack of significant alterations in the Irwin score rather argue against a high level of discomfort and pain, the findings nevertheless indicate a relevant level of residual discomfort and pain. Thus, it is recommended to further assess refinement measures aiming to optimize the minimal-invasive surgical procedures, postsurgical care, and pain management. As recently discussed by Kumstel et al. [35] and also previously concluded based on own findings from rats [13, 14], the invasive nature of telemetric approaches argues against its value for severity assessment. This conclusion is now further supported by the fact that we confirmed a moderate correlation between telemetric readout parameters and parameters that can be

easily assessed without a surgical intervention including the Mouse Grimace Scale and the Irwin score. Thus, it does not seem to be worthwhile to conduct telemetric recordings as there is no evidence that it can further increase the sensitivity of severity assessment or add relevant informative value provided that more comprehensive and detailed composite scores combining different behavioral parameters are applied. An approach to select parameters and design a composite measure score has been previously published along with a ready-to-use online tool [15].

It has more recently been discussed whether standard pain management for human patients undergoing craniotomy is sufficient to control postsurgical pain [36]. Among other aspects, the discussion has focused on the need for opioid analgesics, which are often avoided in craniotomy procedures due to their potential to increase cerebral pressure. The fact that we observed an impact of additional craniotomy on total heart rate variability and a group difference in fecal corticosterone metabolites between mice with craniotomy and naive mice suggests that the electrode and cannula implants add to the surgical consequences and cause a pain state that seems to be more difficult to control than pain following a sole transmitter implant. Whereas the efficacy of separate administration of an NSAID or the opioid buprenorphine has been compared for craniotomy in mice [37], there is still an apparent lack of data and evidence-based recommendations for multimodal regimes developed to control pain in mice with interventions such as craniotomy [38]. In a future study, we will therefore compare the efficacy of different multimodal analgesic regimes to provide an improved basis for perioperative and preemptive pain management in mice with craniotomy.

The impact of the nonconvulsive SE induced by intrahippocampal kainate is by no means comparable to that of a convulsive SE resulting in the need for intense care in the early post-SE phase [13, 14, 39]. Nevertheless, our data indicate that a nonconvulsive SE can also add to the burden during the early postsurgical phase. This additional burden was reflected by heart rate increases, a prolonged increase in Mouse Grimace scores, and the impact on the Irwin score. While it remains impossible to conclude whether these data reflect a negative impact on the postsurgical recovery or are a more direct consequence of the SE, the findings point to the fact that efforts to develop refinement measures should focus on the early postsurgical phase. Depending on the aim of the study, one theoretical option to limit the burden after SE would be a delayed transmitter implantation. However, in the model using the intrahippocampal injection of kainic acid requires a craniotomy with a transient insertion of a cannula, and the electrode is placed during the same procedure as this does not add to the invasiveness of the procedure and does not prolong the surgical intervention in a relevant manner.

Moreover, we can conclude that the need for refinement does not only exist in models with a convulsive SE but also in models with a nonconvulsive SE. The particular relevance of the early phase following SE has already been pointed out by Lidster and colleagues [40]. Their suggestions for potential refinement measures during this experimental phase comprised a limitation of the duration of the SE to the length required to trigger epileptogenesis as well as efforts to nurse animals with maintenance of temperature and hydration following SE [40]. While the SE induced by intrahippocampal kainate will probably not be associated with a relevant impact on hydration and temperature due to the lack of convulsive seizures, it might be worthwhile to invest more time to determine the minimum duration of SE, resulting in epilepsy manifestation in the majority of animals, and thereby providing the opportunity to limit the prolonged seizure activity as far as possible.

The chronic phase of the intrahippocampal kainate model is characterized by rather rare generalized convulsive seizures and by highly frequent electrographic epileptiform episodes that develop rapidly following SE [3, 6, 41]. Our findings confirm a high rate of paroxysmal electrographic epileptiform episodes developing in female NMRI mice as a consequence of kainate exposure. As previously described by Twele et al. [4], HVSWs were more frequent than HPDs. In this context, it is important to note that EEG analysis in animals without kainate exposure demonstrated the lack of comparable epileptiform episodes. This finding is in line with a previous report, in which respective electrographic seizure-like events were not detected in male sham control NMRI mice [42]. Moreover, it supports the conclusion that the paroxysmal electrographic episodes are specific for the induced epilepsy in the intrahippocampal kainate model.

The extremely high number of electrographic seizure-like events raises concerns about the impact on the well-being of the animals. Previously, we have identified several severity assessment parameters that can significantly increase the sensitivity of severity assessment in mice and rats. In particular, these parameters can provide valuable information about a potential impact of an intervention or model on the affective state of the animals [15]. The application of these preselected behavioral and biochem-

ical parameters to the intrahippocampal kainate model did not point to a relevant influence in the chronic phase. These data are in apparent contrast to our previous findings from rat post-SE models, in which we for instance demonstrated hyperactivity and a reduction in the engagement in pleasant activities including burrowing, social interaction, and saccharin preference [13, 14, 43, 44]. Comparison with the present dataset suggests that the burden of the chronic phase of the intrahippocampal kainate models seems to be very low considering a normal clinical score and no relevant group differences in a comprehensive set of behavioral parameters. Among others, these were assessing activity patterns, exploratory, anxiety-associated, anhedonia-associated behavior, and social interaction. Moreover, fecal corticosterone metabolites in the normal range, argue against chronic distress in the animals. These metabolites constitute a valuable non-invasive indirect measure of adrenocortical activity [45]. In this context, it is of interest that previous findings revealed an impact of various interventions and models on levels of fecal corticosterone metabolites confirming the validity of the readout parameter in the context of distress in laboratory animals.

The fact that an intragroup increase in burrowing was observed in mice with a history of a kainate-induced SE might be related to a trend towards a slight drop in burrowing during early phases following SE. However, the lack of a significant impact during the early phase and the lack of group differences argues against a relevant impact on this parameter. Burrowing behavior has previously been suggested as a highly sensitive severity assessment parameter in mice [46, 47].

The only relevant effect that was consistently observed as a short-term and long-term consequence of a kainate-induced SE was an increase in Irwin scores. The Irwin score has been developed in the 1960s as a tool for safety pharmacology exploring the impact of a drug candidate on the autonomous, peripheral, and central nervous system [18, 48, 49]. In the context of severity assessment, the Irwin scoring system can be considered as an extension of a standard clinical score, providing more detailed information about behavioral patterns based on simple tests that are not very time-consuming. We have therefore integrated Irwin scores in our studies focused on the development of evidence-based severity assessment schemes [12–14, 50]. Findings from these studies already pointed to a favorable relationship between the time requirement and the gain in information. The parameters that contributed to increased scores in mice with kainate exposure comprised reactivity, increased body tone, and Straub tail. Considering that these parameters seem to indicate a persistent increase in the level of nervousness, it is concluded that gentle handling procedures are of utmost relevance when handling mice, particularly in this epilepsy model. In this context, it should, for instance, be considered that tunnel handling can reduce the level of distress associated with experimental procedures as well as standard husbandry procedures such as cage change [51, 52].

In human patients, temporal lobe epilepsy is associated with an increased risk for psychiatric comorbidities such as depression and anxiety disorders [53]. While this is reflected by behavioral alterations in several chronic models of temporal lobe epilepsy, this does not seem to be the case for the mouse intrahippocampal kainate model. Our data rather argue against a good face validity in the context of psychiatric comorbidities. Thus, it cannot be recommended to use the model in experimental studies aiming to explore the mechanisms of the psychiatric disorders or aiming to assess the impact of a preventive or therapeutic approach on the full range of possible symptoms of temporal lobe epilepsy including psychiatric consequences. These findings are in line with previous data that also reported only limited behavioral alterations in the kainate paradigm [19]. The only relevant effects were an impact on behavior in the forced swim test, on learning performance in the Morris water maze [19], and on the sucrose preference test [25]. Both paradigms were avoided in the present study as distress-associated with exposure to water would have introduced a potential bias for the other readout parameters. Taken together, the current data suggest a more pronounced impact of the model in the early postsurgical and post-SE phase and very limited alterations in the chronic phase. Based on these data, we are suggesting a severity classification of moderate for this model.

Despite the lack of pronounced behavioral alterations in the chronic phase, it was interesting to note that telemetric parameters from the early phase including activity, heart rate, and heart rate variability showed a correlation with behavioral patterns in the chronic phase. These findings suggest that the immediate impact of the SE on activity, heart function, and autonomous nervous system function, may predict the long-term consequences of an SE. Even more interestingly, early data from the telemetric and behavioral analyses showed a high level of correlation with the long-term manifestation of electrographic epileptiform episodes. This finding is of particular interest, as this might allow preselection of animals for pharmacological studies aiming to assess preventive, disease-

modifying, or antiseizure effects of novel therapeutic approaches. Previous reviews discussing models and avenues for future preclinical validation and selection of drug candidates for epilepsy management have already pointed to the need for respective markers to limit the expenses in terms of time associated with the use of chronic epilepsy models with spontaneous seizure development [54].

Moreover, the data may provide evidence that simple readout parameters can predict the risk for epileptogenesis. However, the experimental and translational value requires the follow-up studies with a comparison between animals with and without the development of epileptiform episodes and seizures.

In conclusion, our findings argue against a pronounced impact of highly frequent electrographic seizures on the well-being of mice. However, an increased level of nervousness in subgroups of animals should be considered for handling procedures and refinement measures. In the early postsurgical phase, several parameters indicate an influence of the interventions with evidence that kainate exposure and the nonconvulsive SE can negatively affect the recovery. Thus, the development and validation of refinement efforts should focus on this experimental phase. In this context, it will also be of particular relevance to analyze the impact of sex, thereby considering the more severe convulsive SE and more frequent HPDs in male mice.

Finally, the fact that early telemetric and behavioral data correlated with long-term seizure development suggests that simple readout parameters may predict the long-term consequences of the epileptogenic insult. Respective biomarker candidates require further validation in the follow-up studies in models with subgroups of animals with or without epilepsy development.

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Statement of Ethics

The study was approved by the government of Upper Bavaria (license number: AZ 55.2-2532-17-86) and was planned and conducted in line with the German Animal Welfare Act and the EU directive 2010/63/EU. All procedures and reporting were performed according to the ARRIVE guidelines and the Basel Declaration including the 3R concept.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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Author Contributions

H.P., V.B., I.K., and C.P. contributed to the concept and design of the study. V.B., I.K., and C.P. performed the experiments and completed the statistical analysis. S.R.T. helped with the data analysis. R.P. performed the analysis of fecal corticosterone metabolites. H.P. and V.B. drafted the manuscript. All authors read and approved the final version.

Data Availability Statement

All data generated or analyzed during this study are included in this article and/or its online supplementary material files and/or will be available in the severity assessment repository of the DFG research unit 2591. Further inquiries can be directed to the corresponding author.

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