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Toward evidence-based severity assessment in mouse models with repeated seizures: I. Electrical kindling

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ABSTRACT

Objective: Ethical decisions about an allowance for animal experiments need to be based on scientifically sound information about the burden and distress associated with the experimental procedure and models. Thereby, species differences need to be considered for recommendations regarding evidence-based severity assessment and refinement measures.
Methods: A comprehensive analysis of behavioral patterns and corticosterone or its metabolites in serum and feces was completed in kindled mice. The impact of kindling via two different stimulation sites in the amygdala and hippocampus was determined. Data were compared to those from naive and electrode-implanted groups.
Results: Amygdala and hippocampus kindled mice exhibited comparable behavioral patterns with increased activity in the open field, reduced anxiety-associated behavior in the elevated-plus maze, and increased anhedonia-associated behavior in the saccharin preference test. In addition, repeated stimulation of the hippocampus caused a reduction in burrowing behavior and an increase in active social interaction. Levels of corticosterone and its metabolites were not altered in serum or feces, respectively. A comparison of mouse data with findings from amygdala kindled rats confirmed pronounced species differences in behavioral patterns associated with the kindling process.
Significance: Taken together the findings suggest a severity classification for the mouse kindling paradigms as moderate regardless of the stimulation site. The outcome of the species comparison provides valuable guidance for species selection for studies exploring behavioral comorbidities. In this context, it is emphasized that the mouse kindling paradigms seem to be well suited for studies exploring the link between ictal events and network alterations on the one hand, and hyperactivity and anhedonia-associated behavior on the other hand. Moreover, the underlying pathophysiological mechanisms and the impact of therapeutic interventions on these behavioral alterations can be studied in these paradigms providing guidance for the clinical management of respective psychiatric comorbidities in patients.

1. Introduction

Since its first description by Graham Goddard [1] the kindling paradigm has been frequently applied in experimental epilepsy research. Based on its excellent predictive validity, it has contributed to the identification of various anti-seizure drugs, which have been licensed since the first description of the animal model [2,3]. While kindling has been predominantly applied in rats for many years and decades since its first description, the availability of genetically modified mice has raised interest in mouse kindling as a tool to study the consequences of a molecular alteration providing valuable information about pathophysiological mechanisms of ictogenesis and hyperexcitability with lowered seizure thresholds [3–5].

Moreover, it is of particular relevance to include different species in drug candidate screening programs considering that pharmacokinetics and -dynamics may differ tremendously between species.

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Related to the complexity of hyperexcitable networks, animal models remain the mainstay of preclinical anti-seizure drug development [3]. However, ethical approval by responsible authorities requires a harm–benefit analysis ideally based on scientifically sound information about the burden of a model and a low level of uncertainties [6]. We recently reported that kindled rats exhibit only minor behavioral alterations with an impact of nest complexity and soiling as well as selected parameters in tests assessing anxiety-associated behavioral patterns. Mice may respond differently to external stressors and may habituate slower than larger animals to handling and fixation procedures [7–10] such as those necessary to connect to the kindling stimulation cable.

Thus, we addressed the hypothesis that, in comparison with rat kindling, mouse kindling may cause a different level of distress, which may be reflected by more intense and divergent alterations of behavioral and biochemical parameters applied for severity assessment in laboratory rodents.

In addition, we tested, whether the choice of a specific implantation site can serve as a refinement measure in studies, in which the localization of the stimulation and recording electrode does not matter considering the research question. As both amygdala- and hippocampal-kindling are frequently applied in rodents [4,11,12], we compared the impact of implantation as well as stimulation in these two target brain regions. Our hypothesis that the distress associated with kindling via the amygdala versus hippocampus may differ is further underlined by the functions of these brain regions. While the amygdala plays a key role in the processing of anxiety and fear, the hippocampus contributes to episodic memory, and therefore regulates contextual processing of threat information and affects the generation of "learned" fear [13–15].

Here, we have analyzed a set of behavioral and biochemical parameters providing information about the level of distress associated with the kindling procedure. The comprehensive data sets that we obtained for the amygdala and hippocampal kindled mice also provide valuable information about the model's face validity regarding behavioral patterns reflecting psychiatric comorbidities in patients.

2. Materials and methods

2.1. Ethical statement

All investigations were conducted in line with the German Animal Welfare Act and the EU directive 2010/63/EU. The procedures and reporting were performed according to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines [16] and the Basel declaration including the 3R concept. All investigations were approved by the government of Upper Bavaria (license number ROB-55.2-2532.Vet_02-17-68). The calculation of the required sample size was carried out before the start of the study.

2.2. Animals

In total 80 female mice (HsdWin:NMRI, Envigo, Horst, Netherlands) at the age of ten weeks with 25–30 g of bodyweight at arrival were used. Mice were single-housed in open Macrolon type III cages (Zoonlab, Castrop-Rauxel, Germany) receiving food (Ssniff Spezialdiäten GmbH, Soest, Germany) and tap water *ad libitum*. Wood chip bedding material was provided (Premium Scientific Bedding J. Rettenmaier & Söhne GmbH + Co KG, Rosenberg, Germany). Additional cage enrichment comprised two Nestlets (Zoonlab, Castrop – Rauxel, Germany) and a mouse house (Zoonlab, Castrop – Rauxel, Germany) per cage. Mice were housed under environmentally controlled conditions (22 ± 2 °C, 55 ± 10% humidity) with a 12-h light–dark cycle (lights on at 5:00 a.m.). They were weighed weekly and controlled according to severity assessment schemes including the Grimace scale [17] and a modified Irwin score [18]. The electrical resistance of vaginal mucosa was measured to assess the estrous stage [19]. Cages including embedding and nesting material were changed once a week. After arrival the animals were distributed to the cages in a randomized order and also the order of cages in the animal facility was randomized (www.randomizer.org).

In total, ten animals were excluded from the experiment: three animals died during surgery, two animals had to be euthanized after surgery with respect to humane endpoints. Data from four animals had to be excluded due to incorrect electrode localization. One kindled animal did not exhibit consistent seizures as defined before the study (for more information regarding kindling procedure see supplementary material) and therefore its data were excluded.

2.3. Experimental design

Five groups of 11–15 animals were investigated. Four of these groups underwent stereotactic electrode implantation with two groups receiving an electrode in the right amygdala, and two in the right dorsal hippocampus CA1 region. Kindling stimulations were initiated in one of the amygdala-implanted groups and one of the hippocampus-implanted groups following a postsurgical recovery period of two weeks. In these groups, in the following termed amygdala-kindled and hippocampus-kindled, the initial afterdischarge duration (ADT) was determined on the first stimulation day. Animals were then kindled with daily stimulations from Monday to Friday between 1 and 3 p.m. as described in the supplementary material. Behavioral analyses were completed in the morning (for an overview of the experimental design see Fig. 1A). The other implanted groups, in the following termed AM sham and HIP sham, were exposed to the same handling procedures as the kindled animals except for the stimulations. The fifth group was a naïve group, which neither received electrode implantations nor stimulations.

2.4. Experimental procedures and interventions

Upon arrival and before surgery, animals had ten days of acclimatization to the new animal facility and of habituation to handling by experimenters. For a detailed description of the surgical procedure as well as the behavioral evaluation and the statistical analysis see supplementary material. Overall several time points for investigations were of interest: time point 1, i.e. baseline values, time point 2, i.e. in the second postsurgical week, time point 3, i.e. in the early kindling phase of kindled animals and time point 4, i.e. in the late kindling phase.

2.5. Statistical analysis

Statistical analysis (GraphPad Prism, Version 5.04; GraphPad, USA) and all behavioral and sampling protocols were defined before the study has been started. Two group comparisons were performed with an unpaired *t*-test. For comparison of data between the two groups of kindled animals, electrode-implanted control groups (sham) and the naive control group, we applied a two-way analysis of variance (ANOVA) for parametric data. Post-hoc testing was based on a Bonferroni test. Repeated measures ANOVA was used for comparison of data with different time points like the burrowing test and fecal corticosterone analysis. Two-way ANOVA for repeated measurements with Bonferroni test for post-hoc testing was used for the statistical analysis of the kindling progress (dependent factor: time, independent factor: localization/experimental group). For nonparametric data, we

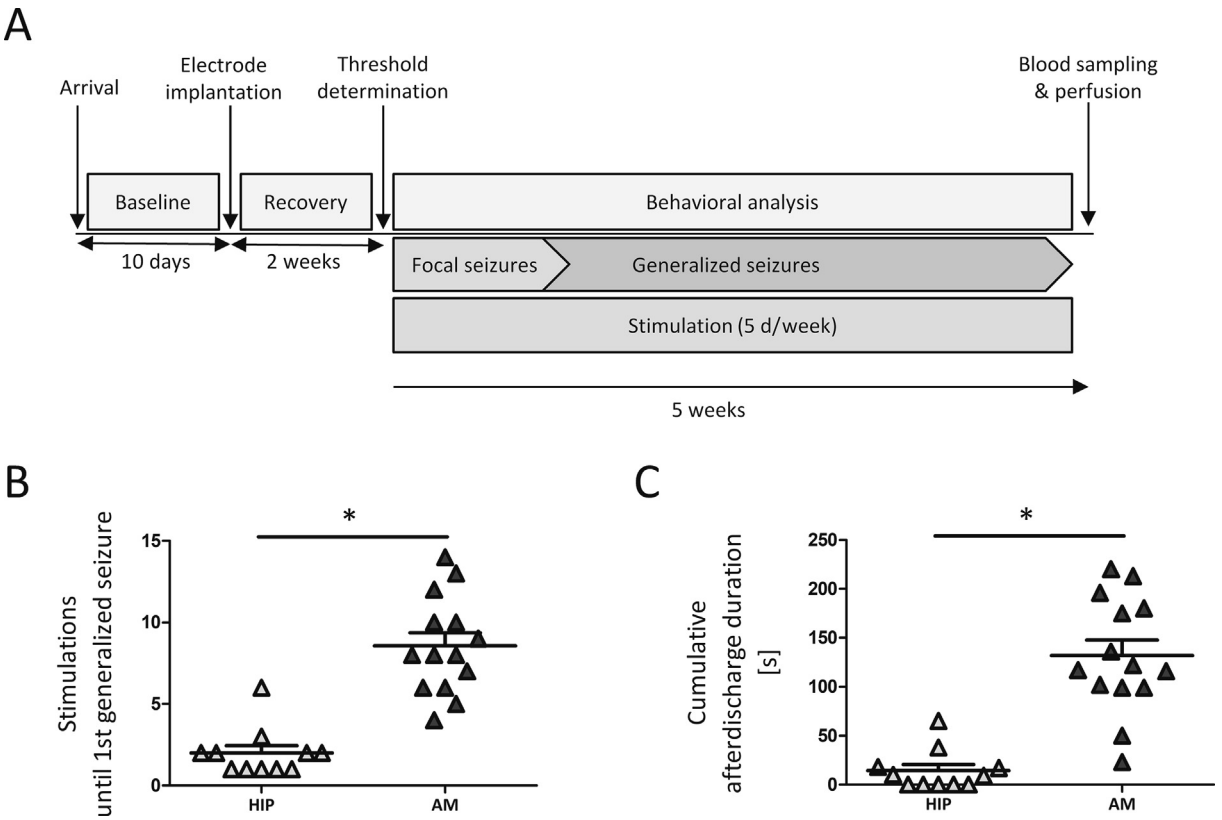


Fig. 1. Experimental design and kindling progression. (A) Timeline of kindling study. (B) Number of stimulations until induction of first generalized seizure. (C) Cumulative afterdischarge duration (hippocampus (HIP) $n = 11$ animals, amygdala (AM) $n = 14$ animals). Data represent the values of individual animals and SEM for B, mean and SEM for C. Analyzed by unpaired t-test for comparison between two groups (B, C). Significant differences are indicated by an asterisk (*). (B, C) $p < .0001$.

applied a Kruskal–Wallis test with Dunn's test for multiple comparisons. All tests were used two-sided and $p < 0.05$ was considered as a threshold for statistically significant differences. A Spearman correlation matrix has been created using R version 3.3.2.10. and visualized using the R package "corrplot"[20]. Principal component analysis (PCA) was calculated and visualized using the R-package "made 4"[21]. A forest plot has been created using R version 3.6.2 and visualized using the R-package "ggplot2"[22].

3. Results

3.1. Kindling

Mice with hippocampal stimulations (hippocampus-kindling group) exhibited early generalized seizures following a mean of two stimulations. In contrast, generalized seizures were only observed following a higher number of stimulations in mice with amygdala stimulation (amygdala-kindling group) (Fig. 1B, $p = <.0001$, mean ± SEM of hippocampus-kindling group = 2.000 ± 0.447, mean ± SEM of amygdala-kindling group = 8.571 ± 0.796). The duration of seizures increased along with repeated stimulations in both groups. However, the progression rate of seizure duration differed between both groups with amygdala-kindled mice showing longer seizures than hippocampus-kindled mice (Fig. 1C, after discharge duration: time effect: $p = .0002$, localization: $p < .05$). The respective cumulative afterdischarge duration in amygdala-kindled mice exceeded that in hippocampus-kindled mice (Fig. 1C, $p < .0001$, mean ± SEM of hippocampus-kindling group = 14.27 ± 6.195, mean ± SEM of amygdala-kindling group = 132.0 ± 15.70).

3.2. Impact on activity, locomotion, and anxiety-associated behavior

The open-field test was carried out to obtain information about exploratory and locomotor activity in an unfamiliar environment. Comparison of all groups showed an overall difference between experimental groups, i.e. kindled and non-kindled animals, for distance moved (Fig. 2A, $F(1,51) = 14.33$, $p = .0004$) and velocity (Fig. S3A, $F(1,51) = 14.33$, $p = .0004$). In both kindling groups, a hyperlocomotion was evident in comparison with electrode-implanted mice (Figs. 2A and S3B, amygdala-kindled vs. AM sham $p < .05$; hippocampus-kindled vs. HIP sham $p < .05$). An overall difference between experimental groups and also between localizations of the electrode was found for time spent in immobility (Fig. S3B, experimental group: $F(1,51) = 14.85$, $p = .0003$; localization: $F(1,51) = 4.832$, $p = .0327$) and for rearing frequency (Fig. 2B, experimental group: $F(1,51) = 14.33$, $p = .0004$; localization: $F(1,51) = 7.885$, $p = .0070$). Whereas phases with immobility proved to be reduced in kindled animals in comparison with the respective electrode-implanted groups (Fig. S3B, amygdala-kindled vs. AM sham $p < .01$; hippocampus-kindled vs. HIP sham $p < .05$), rearing frequency reached higher levels in kindled mice exceeding those in electrode-implanted and naïve mice (Fig. 2B, amygdala-kindled vs. AM sham $p < .01$; hippocampus-kindled vs. HIP sham $p < .05$, amygdala-kindled vs. naïve $p < .05$).

In the elevated-plus maze test, kindled animals spent more time on the open arms and in the outer third of the open arms (Fig. 2C, $F(1,51) = 16.62$, $p = .0002$, Fig. S5A, $F(1,51) = 4.634$, $p = .0361$). However, direct comparison of individual groups only confirmed a difference for the amygdala-kindling group, which spent more time on open arms and their outer third than their electrode-implanted control group (Fig. 2C, amygdala-kindled vs.

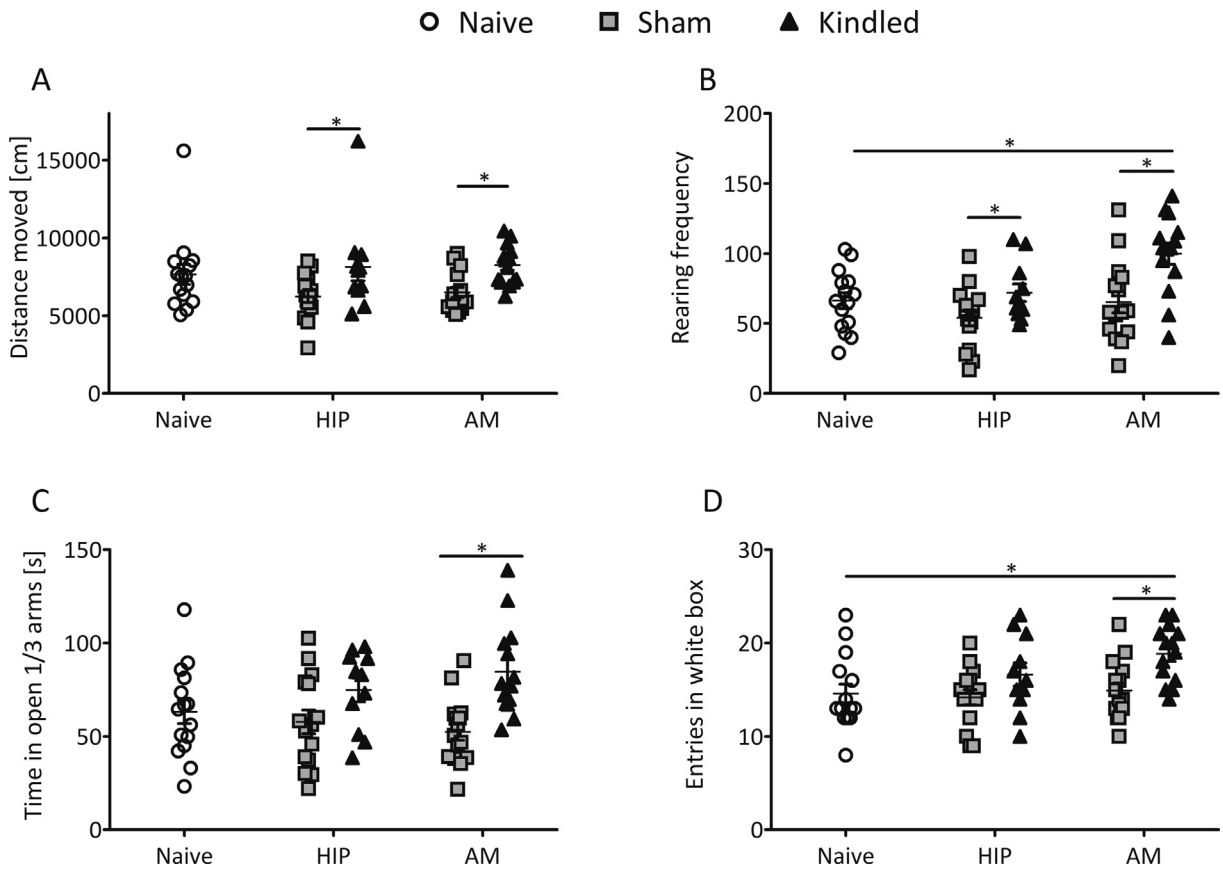


Fig. 2. Locomotion and anxiety-associated behavior. (A) Distance moved in centimeters in the open field. Kindled animals showed a longer distance moved than respective sham control groups ($p < .05$). (B) Rearing frequency. Kindled animals showed an increased rearing frequency as compared to their sham control groups and the naïve group. (Naive $n = 13$ animals, AM sham $n = 13$ animals, HIP sham $n = 14$ animals, amygdala-kindled $n = 13$ animals, hippocampus-kindled $n = 11$ animals, amygdala-kindled vs. AM sham $p < .01$; hippocampus-kindled vs. HIP sham $p < .05$, amygdala-kindled vs. naïve $p < .05$). (C) Time spent in the outer third of the open arms in seconds. Amygdala-kindled animals spent more time in the outer third of the open arms as compared to their sham control group ($p < .01$). (D) Entries in the white compartment. Amygdala-kindled animals exhibited an increased number of entries as compared to their sham control group and naïve control group (Amygdala-kindled vs. AM sham $p < .05$, Amygdala-kindled vs. naïve $p < .05$). Data represent mean, SEM, and values of individual animals. Two-way ANOVA with Bonferroni posthoc test was used for comparison between the groups. Significant differences are indicated by an asterisk (*).

AM sham $p < .001$, Fig. S5A, amygdala-kindled vs. AM sham $p < .01$). An overall difference between kindled and non-kindled animals was found for head dip behavior (Fig. S5B, $F(1,51) = 25.59$, $p < .0001$).

In the black-white box, the frequency of entries into the white box was increased in kindled animals (Fig. 2D, $F(1,51) = 12.29$, $p = .0010$). Direct comparison of the groups revealed a higher frequency of entries into the white box in amygdala-kindled mice in comparison with the respective control groups and the naïve group (Fig. 2D, amygdala-kindled vs. AM sham $p < .05$, amygdala-kindled vs. naïve $p < .05$). Further parameters did not differ between groups (Fig. S4A–C).

3.3. Impact on social interaction and anhedonia-associated behavior

Results of the two-way ANOVA indicated an overall significant difference between experimental groups and between localizations of the electrode with regard to the time spent in active social interaction (Fig. 3A, experimental group: $F(1,21) = 11.92$, $p = .0024$; localization: $F(1,21) = 11.88$, $p = .0024$). Time spent in active social interaction in animals of the hippocampus-kindling group exceeded that in all other groups (Fig. 3A, hippocampus-kindled vs. all other groups $p < 0.05$).

The time animals spent in passive social interaction was at comparable low rates in all groups (Fig. S6).

Reduced uptake of saccharin solution observed in amygdala- and hippocampus-kindled animals demonstrated an increased anhedonia-associated behavior (Fig. 3B, $F(1,46) = 7.955$, $p = .0071$, amygdala-kindled vs. naïve $p < .05$; hippocampus-kindled vs. naïve $p < .05$).

3.4. Impact on burrowing paradigm and fecal corticosterone metabolites

Burrowing behavior and concentrations of fecal corticosterone metabolites were repeatedly assessed. Only at time point 4, i.e. in the late kindling phase of kindled animals, results of the two-way ANOVA indicated an interaction between electrode localizations and experimental groups (Fig. 3C and D; interaction: $F(1,47) = 6.49$, $p = .0142$, Fig. 3D, interaction: $F(1,47) = 10.44$, $p = .0013$). Furthermore, when comparing individual groups, the hippocampus-kindling group showed a reduced burrowing behavior during the 120-min exposure as compared to the respective sham group (Fig. 3C, hippocampus-kindled vs. HIP sham $p < .05$).

Evaluation of burrowing behavior at the different time points only revealed a difference in animals with amygdala stimulations at time point 4 (late kindling phase) as compared to all other time points (Supplementary Fig. S8H, $F(3,12) = 6.499$, $p < .05$). In the hippocampus-kindling group, a reduced burrowing behavior was evident at time points 3 and 4 (early and late kindling phase) as

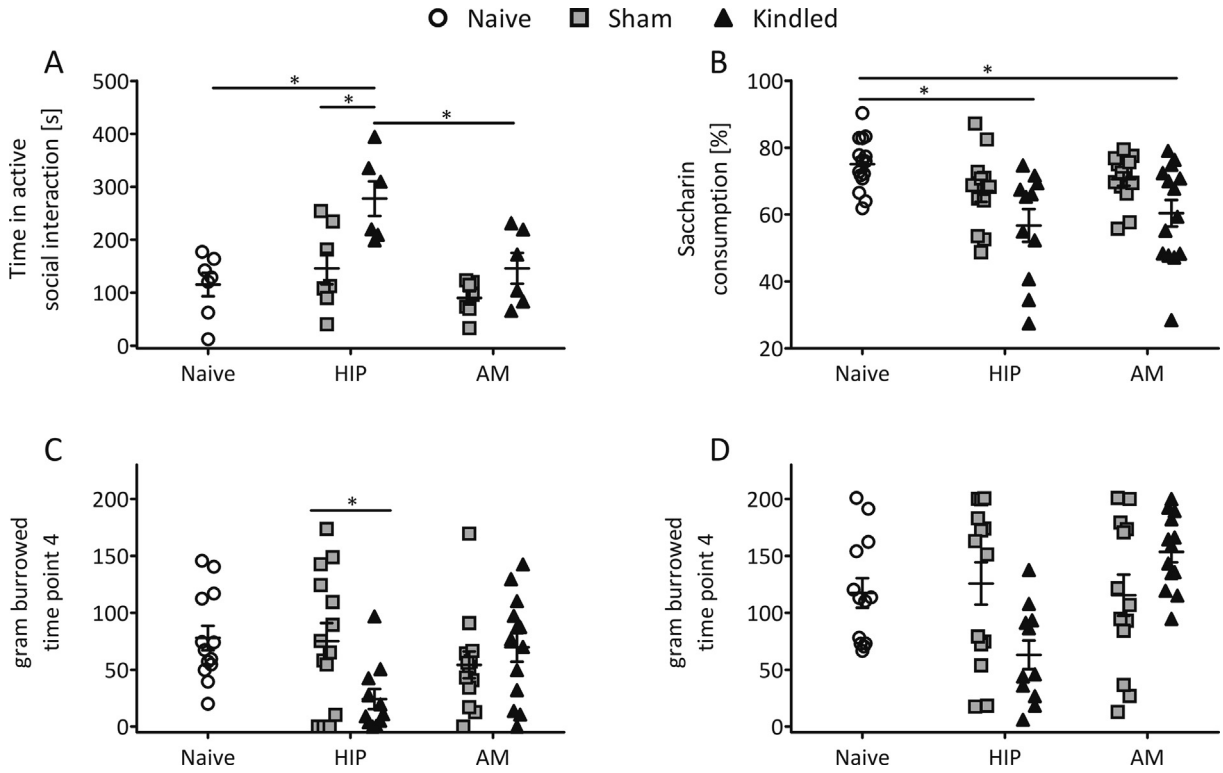


Fig. 3. Social interaction, saccharin consumption, and burrowing behavior. (A) Time spent in active social interaction. Hippocampus-kindled mice spent more time in active social interaction than the other experimental groups. (Naive $n = 7$ pairs, AM sham $n = 7$ pairs, HIP sham $n = 7$ pairs, amygdala-kindled $n = 6$ pairs, hippocampus-kindled $n = 6$ pairs, hippocampus-kindled vs. all other groups $p < .05$). (B) Saccharin consumption. Reduced uptake of saccharin solution in kindled animals. (Naive $n = 15$ animals, AM sham $n = 15$ animals, HIP sham $n = 15$ animals, amygdala-kindled $n = 14$ animals, hippocampus-kindled $n = 11$ animals, amygdala-kindled vs. naïve $p < .05$; hippocampus-kindled vs. naïve $p < .05$). (C, D) Burrowing behavior. (C) Burrowed amount in gram after 120 min during the late kindling phase (hippocampus-kindled vs. HIP sham $p < .05$). (D) Burrowed amount in gram overnight during the late kindling phase. (Naive $n = 15$ animals, AM sham $n = 15$ animals, HIP sham $n = 15$ animals, amygdala-kindled $n = 14$ animals, hippocampus-kindled $n = 11$ animals). Data represent the mean ± SEM, and values of individual or paired animals. Two-way ANOVA with Bonferroni posthoc test was used for comparison between the groups. Significant differences are indicated by an asterisk (*).

compared to their basal values (Fig. S8I, $F(3,10) = 8.403$, $p < .05$; Fig. S8J, $F(3,10) = 8.403$, $p < .05$).

Fecal corticosterone metabolites reached an overall difference at time point 2 between kindled animals and control groups (Fig. S9A, $F(1,50) = 6.095$, $p = .0170$). No difference was confirmed when comparing individual groups.

Serum corticosterone levels and estrous cycles were not affected in any group (Fig. S10A; data not shown).

3.5. Correlation matrix

A cross-correlation analysis was completed to determine the informative value of different clinical and behavioral parameters. The respective information can help to conclude whether a specific parameter has an added value in composite scoring systems. Moreover, we analyzed to what extent clinical and behavioral parameters correlate with kindling parameters. With a focus on the respective research questions, the following correlations are of interest.

For amygdala-kindled animals (Fig. 4A), we observed correlations between kindling parameters and social interaction and the saccharin preference test as well as between kindling parameters and locomotion in the open field, anxiety-associated behavior in the elevated-plus maze and the Irwin score.

For hippocampus-kindled animals (Fig. 4B) similar to the amygdala-kindled animals, we observed correlations between kindling parameters and locomotion in the open field and anxiety-associated behavior in the elevated-plus maze. Moreover, correlations between kindling parameters and nest-building activity, as well as Irwin score and weight gain became evident. For a more detailed description of all correlations see the supporting information.

3.6. Principal component analysis

A principal component analysis was performed to structure, simplify, and illustrate the large data sets. For the PCA performed for amygdala kindled animals and the control animals (Fig. 5A), the first two principal components (PCs) explain 31.3% of the total variance in the data (PC1: 17.4%, PC2: 13.9%). Following the ANOVA, a Tukey post hoc test revealed a significant difference between the amygdala-kindled and naïve as well as sham animals ($p < 0.001$ and $p < 0.001$, respectively). For the PCA performed for hippocampus-kindled animals and the control animals (Fig. 5C), the first principal components (PCs) explain 30.6% of the total variance in the data (PC1: 16.3%, PC2: 14.3%). The ANOVA and a Tukey post hoc test revealed a significant difference between hippocampus-kindled and their sham animals along PC1 (PC1 ($F(2,38) = 4.7$, $p = 0.0120$) and between hippocampus-kindled animals and naive animals along PC2 (PC2 ($F(2,38) = 3.5$, $p = 0.0442$).

When focusing only on the two kindling groups (Fig. 5E), the first two principal components explain 34.1% of the total variance in the data (PC1: 20.0%, PC2: 14.1%). The ANOVA and a Tukey post hoc test revealed a significant difference between amygdala- and hippocampus-kindled animals along PC1 (PC1 ($F(1, 41) = 9.6$, $p = 0.005$) and PC2 (PC2 ($F(1, 23) = 6.8$, $p = 0.002$).

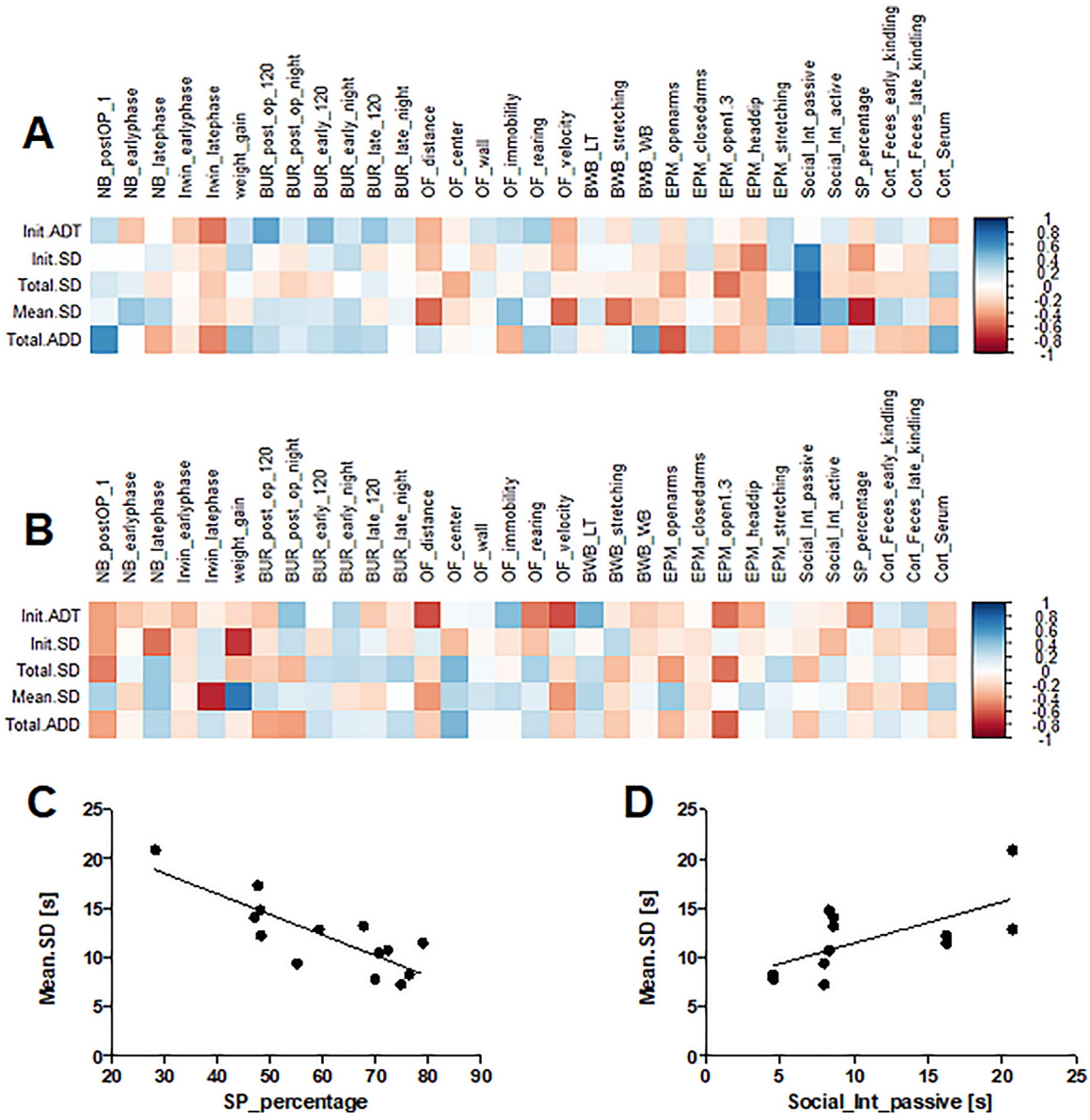
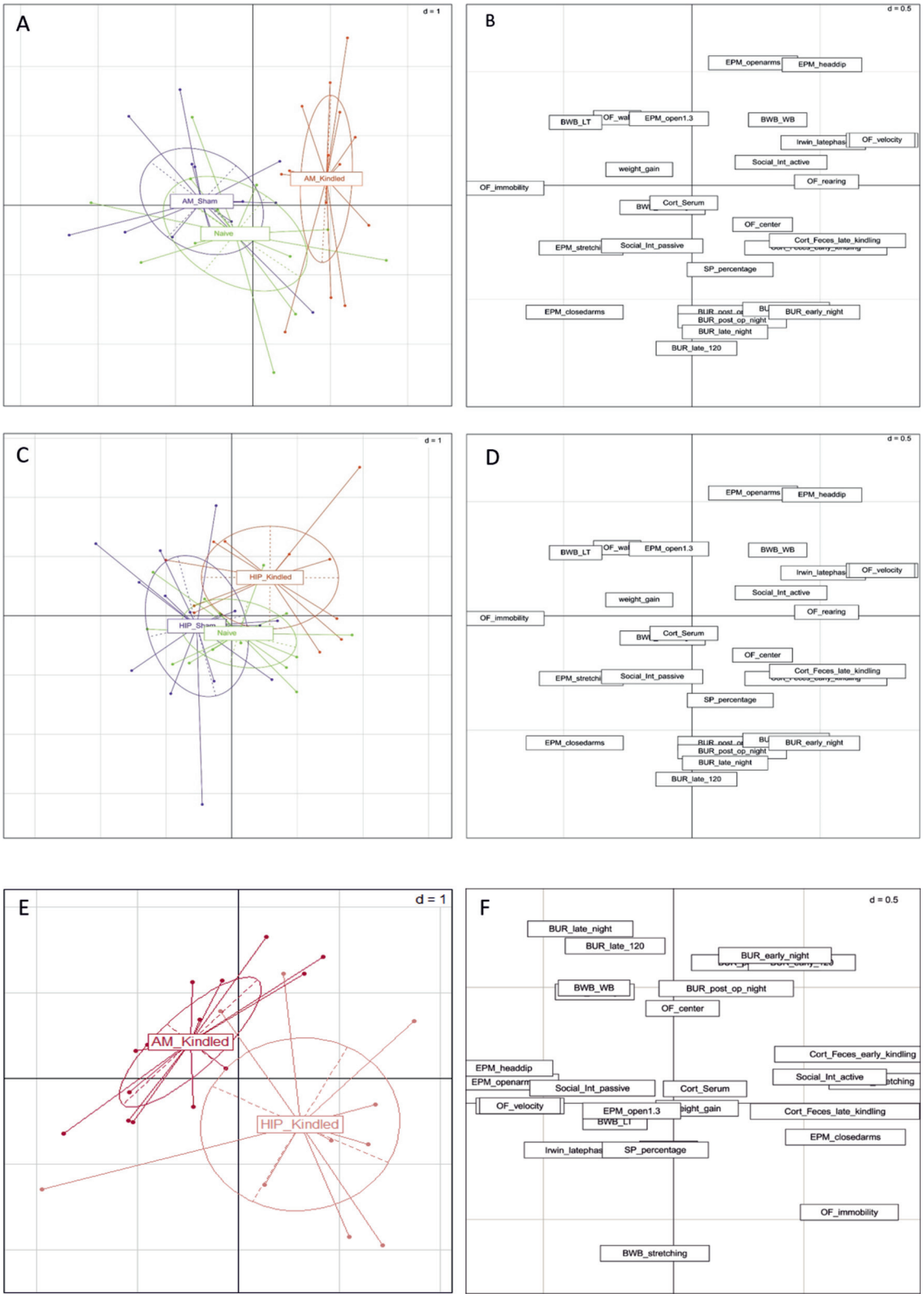


Fig. 4. Correlation matrix. (A) Correlation between kindling-related parameters and behavioral and biochemical parameters in amygdala-kindled animals. (B) Correlation between kindling-related parameters and behavioral and biochemical parameters in hippocampus-kindled animals. (C, D) Correlation between mean seizure duration and saccharin consumption (C, $p = .0003$) and time in passive social interaction (D, $p = .02$) in amygdala-kindled animals. Abbreviations used in Fig. 4 are listed in supplementary material.

3.7. Forest plot

For a direct comparison between species, data from an amygdala-kindling paradigm in rats[23] and the respective amygdala-kindling paradigm in mice were considered for the analysis (Fig. 6A and B). Here, we focus on those alterations that may reflect an increased level of distress in the animals. In rats (Fig. 6A), an increased anxiety-associated behavior became evident

Fig. 5. Principal component analysis (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. *d* shows the scale of the grid in the PCA plot. The ellipses show a summary of the point cloud. (A) PCA of data from the amygdala-implanted groups and naïve group. The distribution of the three groups is significantly different along PC1, individual comparisons indicate differences between kindled animals and the sham animals as well as between kindled animals and naïve animals. (B) Four of the top ten contributing variables contributing to PC1 were recorded in the open field, three in the elevated plus maze, two in the black-white box and one in the Irwin Score. (C) PCA of data from the hippocampus-implanted groups and naïve group. The distribution of the three groups is significantly different along PC1 and along PC2, individual comparisons reveal differences along PC1 between kindled animals and the sham animals and between kindled animals and naïve animals. Along PC2, individual comparisons reveal differences between kindled animals and naïve animals. (D) Four of the top ten contributing variables to PC1 were observed in the open field, two in the elevated-plus maze, two were observed in the fecal corticosterone metabolites analyzed at different time points (time point 2 and time point 3), one in the Irwin Score and one in the burrowing paradigm. For PC2, six of the contributing variables were recorded in the burrowing test, three in the elevated plus maze, and one in the saccharin preference test. (E) PCA of data from the two kindling groups. The distribution of the two groups is significantly different along PC1 and PC2. (F) Four of the top ten contributing variables to PC1 were recorded in the elevated-plus maze and three in the open field. Moreover, two variables were fecal corticosterone metabolites and one was social interaction. For PC2, six of the contributing variables were recorded in the burrowing test, two in the open field and two in the black-white box. Abbreviations used in Fig. 5 are listed in supplementary material.


A
d = 1
AM_Kindled
AM_Sham
Naive
B
d = 0.5
EPM_openarms
EPM_headdip
BWB_LT
EPM_open1.3
BWB_WB
lrwin_latephas
OF_velocity
Social_Int_active
weight_gain
OF_rearing
OF_immobility
Cort_Serum
OF_center
EPM_stretchi
Social_Int_passive
Cort_Feces_late_kindling
SP_percentage
EPM_closedarms
BUR_early_night
BUR_post_op_night
BUR_late_night
BUR_late_120
C
d = 1
HIP_Kindled
HIP_Sham
Naive
D
d = 0.5
E
d = 1
AM_Kindled
HIP_Kindled
F
d = 0.5
BUR_late_night
BUR_late_120
BUR_early_night
BWB_WB
BUR_post_op_night
OF_center
Cort_Feces_early_kindling
EPM_headdip
Social_Int_active
Social_Int_passive
Cort_Serum
OF_velocity
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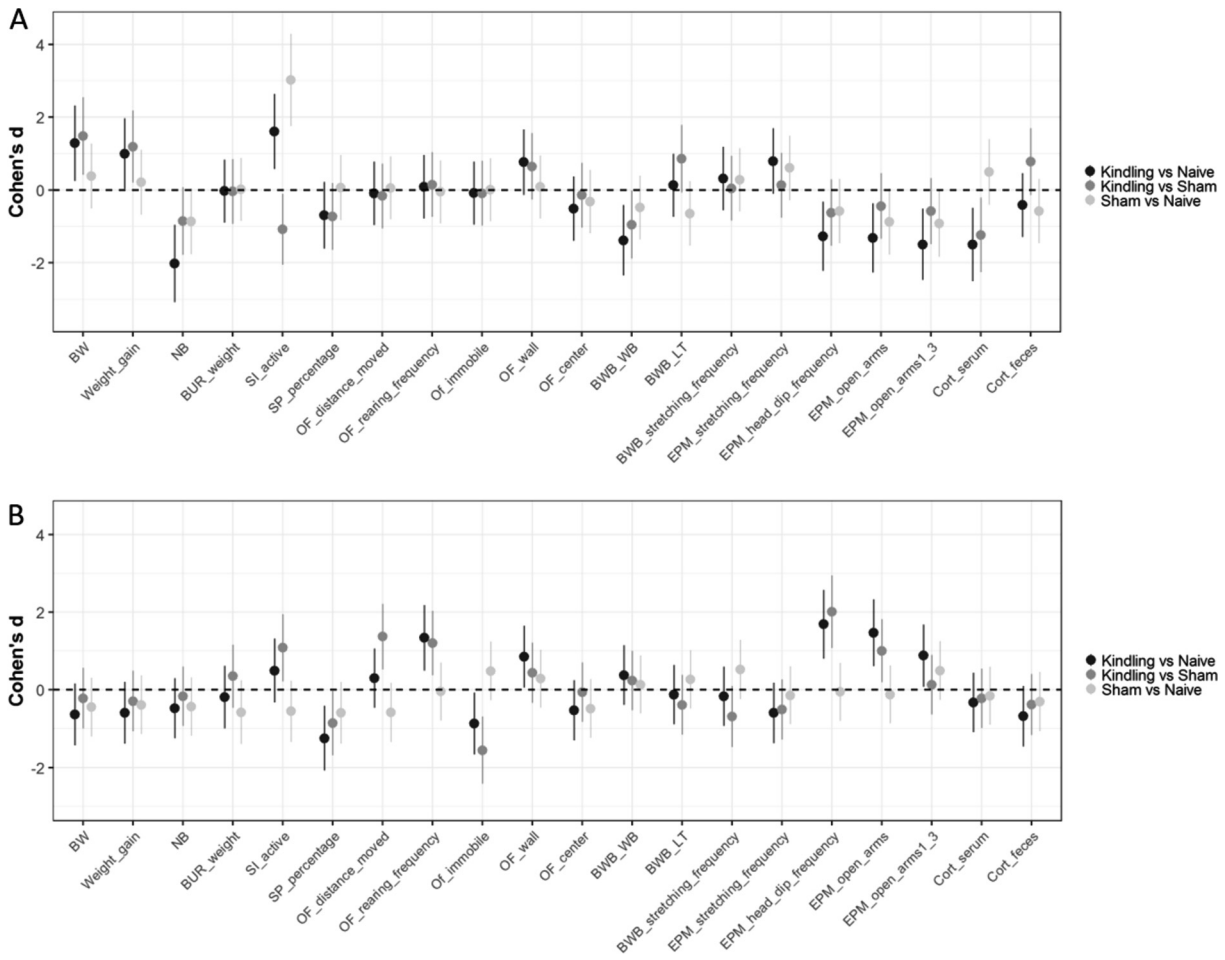

Fig. 6. Forest Plot illustrating the effect sizes of different parameters analyzed in the amygdala-kindling paradigm in rats (A) and mice (B). Parameters were assessed when all animals exhibited generalized seizures. Effect sizes were calculated as Cohen's d based on two groups (kindling vs sham, kindling vs naïve and sham vs naïve). Rat data had been published in Möller et al.[23]. Abbreviations used in Fig. 6 are listed in supplementary material.

in the black-white box and in the elevated-plus maze in comparison to naïve controls with less time spent in the white box ($F(2,27) = 3.84$, $p = 0.0337$, $d = -1.381$, ($CI_{95}[-2.346; -0.415]$)) and open arms ($F(2,31) = 4.82$, $p = 0.02$, $d = -1.316$ ($CI_{95}[-2.273; -0.359]$)). Additionally, rats showed a decreased nest-building activity ($F(2,31) = 12.96$, $p = .0015$, $d = -2.02$ ($CI_{95}[-3.087; -0.954]$)). In contrast, these tests did not reveal respective alterations in mice. However, as reported above other behavioral alterations became evident. Kindled mice showed a decreased uptake of sweetened solution (kindling vs sham $d = -0.861$ ($CI_{95}[-1.69; -0.032]$)), and a hyperlocomotion in the open-field test (kindling vs sham $d = 1.37$ ($CI_{95}[0.523; 2.217]$)). For detailed information about the readout parameters and the respective statistic information see above and in the supporting information.

In addition to behavioral parameters, we also compared alterations in corticosterone and its metabolites. In none of the species, the respective analysis of serum and feces indicated an increased level of distress in implanted or kindled animals. The only difference observed was a lower serum corticosterone level in kindled rats as compared to naïve control rats ($F(2, 29) = 5.03$, $p = 0.01$, $d = -1.495$ ($CI_{95}[-2.504; -0.486]$)).

4. Discussion

First, the selection of an appropriate animal model needs to consider the scientific aims of a study. However, whenever different alternate options meet the respective requirements, an animal-welfare based prioritization of models should be intensely considered from an ethical point of view for the choice of the model [24,25]. Therefore, it is of particular relevance to determine and compare the severity of different experimental models, and to assess possible refinement measures in an evidence-based manner. At the same time, comprehensive data sets provide valuable information about the validity of severity assessment parameters and about model characteristics, which can also guide the scientific selection process.

As pointed out by Lidster and colleagues [24] intracranial implantation procedures often add to the burden of epilepsy models. In line with previous findings in rats [23], the implantation of a depth electrode in mice remained without relevant long-term effects. However, symptoms in the early postsurgical phase indicated minor acute effects of the surgical intervention suggesting that the perisurgical analgetic management may require further optimization.

Electrical kindling paradigms are of particular interest in the assessment of seizure susceptibility in genetically modified mice as well as for the testing of drug candidates. While the originally described paradigm in rats was based on stimulation of the amygdala via implanted depth electrodes[1], subsequent studies explored different alternate electrode localizations including the hippocampus[11,12]. Considering the physiological functions of the respective brain regions, we hypothesized that the impact of the kindling process on the affective state and behavioral patterns should differ between the amygdala- and hippocampal-kindling

procedures in mice. Indeed differences in behavioral patterns became evident, but tended to be rather limited. In this context, one needs to consider the close anatomical relationship of the stimulated brain regions, and possible consequences of a relatively large field size of stimulation. Moreover, it is expected that at least some of the model-associated network changes characterize the brain of kindled animals regardless of the stimulation site within the temporal lobe, and that these network changes might shape behavioral patterns in a relevant manner.

Hyperactivity and increased exploratory behavior, a reduction in anxiety-associated behavior in the elevated-plus maze, and saccharin preference was evident in, both, amygdala and hippocampal-kindled mice. Thereby, the increased time spent in aversive areas of the elevated-plus maze might be related to an overall increased activity and reduced risk assessment. An increase in activity patterns has previously also been reported in different post-status epilepticus models [26–28]. Hyperactivity in these animals models with repeated seizure induction reflects the increased prevalence for attention-deficit/hyperactivity disorder in pediatric and adult patients with epilepsy [29,30]. Reduced saccharin preference can be interpreted as evidence for anhedonia-associated behavior. A reduction or loss of the preference for sweetened solutions has previously been described in different chronic epilepsy models in laboratory rodents including chemical and electrical post-status epilepticus models [31–34] as well as a rat kindling paradigm [35]. These findings in different animals models are in line with the increased risk for depressive disorders in patients with temporal lobe epilepsy [36].

Additional behavioral alterations were only observed in the hippocampal-kindling model suggesting that molecular and network alterations triggered by stimulation of the hippocampal CA1 region exert more pronounced effects on behavioral patterns than stimulation of the amygdala. In contrast to saccharin preference and behavior in the open field and elevated-plus maze, active social interaction was only affected by hippocampal-kindling, but not in response to amygdala-kindling. Social isolation and disorganized social interaction complexity have been reported previously as a consequence of distress[37–39]. Thus, the increase in active social interaction in the absence of aggressive behavior is rather not interpreted as an indicator of an increased level of distress. However, the fact that hippocampal-kindled mice exhibited lower levels of burrowing activity might indicate that these animals have a reduced motivation to engage in behavioral patterns that are not essential for survival. Earlier studies revealed that burrowing activity in rodents can be a sensitive indicator of pain and distress in laboratory rodents[40–42].

In this context, the lack of a direct significant difference between amygdala- and hippocampal-kindled mice, softens any conclusions about a difference in the severity of both paradigms. Thus, while there is a trend for a difference, our data rather argue against robust differences in kindling-associated distress. Thus, the findings provide no basis to recommend for or against amygdala- or hippocampal-kindling from an animal welfare point of view. While no clear recommendation regarding prioritization of the two models can be given, both models seem to mimic different comorbidities of temporal lobe epilepsy. Therefore, they can be considered useful models to investigate behavioral alterations associated with ictal events and changes in neuronal networks. As discussed above, this in particular applies to hyperactivity and anhedonia-associated behavior. In contrast, only the hippocampal-kindling paradigm was associated with an impact on social interaction. However, as interaction proved to be increased the model does not reflect autism-like behavior, which has previously been reported based on a reduction of social interaction in chronic epilepsy models and as a consequence of early-life seizurs in mice (Lugo et al., 2014; Seo et al., 2013).

Prolonged kindling stimulation paradigms can be associated with a progressive increase in the occurrence of interictal spiking and with the onset of spontaneous ictal activity [43–45]. In future studies, it would be of interest to assess the correlation between behavioral alterations and interictal spiking and spontaneous ictal activity, which can be observed following prolonged kindling stimulation paradigms. Previous studies in different chemical models already provided first evidence for a link between interictal spiking and hyperactivity as well as cognitive deficits [46,47].

Considering the rather minor alterations on the one hand, and the duration of the kindling paradigm, on the other hand, we suggest a classification of the mouse kindling models as moderate according to the EU regulations and the report of the expert working group on severity classification. This conclusion is supported by the lack of any changes in fecal corticosterone metabolite levels arguing against moderate or severe chronic distress in kindled mice. In previous studies, fecal corticosterone metabolites were confirmed as an indicator of distress in laboratory rodents [48–51].

Species choice is frequently predetermined from a scientific point of view by factors that cannot be influenced such as differences in pharmacokinetics. However, in the absence of external factors and predictive validity aspects predetermining a specific species, species choice can constitute a relevant refinement measure in the sense of the 3R concept based on the selection of the least affected species [10,52]. A comparison of data from mice with previous findings in kindled rats revealed substantial differences in selected behavioral parameters with increases in anxiety-associated behavior and compromised nest-building only evident in rats and increases in anhedonia-associated behavior and open-field activity only evident in mice. Anxiogenic-like effects have been reported for different animal models of complex partial seizures including the kindling paradigm [53,54]. Respective effects have been at least partly attributed to the stimulation of brain regions involved in the modulation of anxiety and fear, such as the amygdala. However, alterations in the affective state can also be a consequence of repeated seizures with abnormal neuronal activity patterns affecting a network of different brain regions [55], and distress associated with experimental procedures [10,50,56]. The difference between rats and mice with anxiety-associated alterations developing in opposing directions might thus, be related to different influencing factors including differences in kindled networks or a different susceptibility to distress triggered by comparable experimental procedures. In this context, it also needs to be taken into account that electrodes were placed in the basolateral amygdala in rats, whereas placement in a specific amygdalar nucleus is impossible in mice considering the dimensions of the brain regions and the electrode size.

Supporting previous findings in rats [57], we recently demonstrated a lack of anhedonia-associated patterns in a kindling paradigm in rats [23]. In contrast, kindling in mice triggered anhedonia-associated behavioral patterns in the present study.

Another difference became evident when analyzing nest-building behavior in kindled rats and mice with compromised nest-building only observed in rats [58]. The higher sensitivity of nest building in rats might be related to the lower level of motivation to construct complex nest structures [59]. Related to their smaller body size, thermoregulation in mice depends more intensely on environmental factors, so that it might be that nest building is only affected at higher levels of distress in mice.

Taken together, the species comparison demonstrates that anxiogenic effects in rats and the induction of anhedonia-associated behavior in mice need to be considered as a burden related to kindling procedures in these species. These findings are also of particular relevance for species selection for studies focusing on mechanisms and therapeutic management of epilepsy-associated anxiety disorders or depression. In this context, it is important to

keep in mind that behavioral consequences can be affected by multiple factors including handling, age, strain, sex, and exact electrode localization. Mazarati and colleagues [35] have for instance described that kindling in immature rats can result in persistent depression-like behavioral patterns including a loss of preference for a sweet solution.

Finally, the data sets obtained in the present study provide information about the value of severity assessment parameters in mice. Our findings point to activity and anhedonia-associated behavior as the most interesting parameters in mice.

In summary, alterations in activity and anhedonia-associated behavior characterized both kindling models. The impact of kindling on behavioral patterns tended to be more pronounced in mice with hippocampal-kindling with an increase in social interaction and evidence for an influence on burrowing. Taken together the findings suggest a severity classification as moderate regardless of the stimulation site.

A species comparison considering previously published data from rats confirmed tremendous differences in amygdala kindling-associated behavioral alterations in rats versus mice. These findings provide valuable guidance for species selection for studies exploring behavioral comorbidities. In this context, it is emphasized that the mouse kindling paradigms seem to be well suited for studies exploring the link between ictal events and network alterations on the one hand, and hyperactivity and anhedonia-associated behavior on the other hand. Moreover, the underlying pathophysiological mechanisms and the impact of therapeutic interventions on these behavioral alterations can be studied in these paradigms providing guidance for the clinical management of respective psychiatric comorbidities in patients.

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Conflict of interest

None of the authors has any conflict of interest to disclose. 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.

Ethical publication statement

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yebeh.2020.107689.

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