

Supplementary Results

Initial insult and epileptogenesis

Angular bundle stimulation (AB). Immediately after termination of electrical stimulation, periodic epileptiform discharges (PEDs) were visible on the EEG and occurred at a frequency of 1-2 Hz, characteristic for SE. PEDs disappeared within a few minutes after diazepam injection in all rats, but reappeared in 3 of 16 animals during the night, about 7 h after diazepam injection. SE lasted 9.5-12 h in these 3 animals, while in all other animals it was limited to 2.5 hours. SE severity and duration was not different between rats that developed spontaneous recurrent seizures (SRSs) and rats that did not (5.5 ± 1.5 vs 2.5 ± 0 h; mean \pm SEM).

Continuous (24/7) EEG recordings revealed that none of the rats had SRSs during the first EEG recording period (0-9 days after SE). Rats that developed SRSs had 3 ± 1 seizures/week (mean \pm SEM) during the second EEG recording period (6-8 weeks after SE) and 4 ± 1 seizures/week mean \pm SEM during the third period (14-15 weeks after SE). The last SRS occurred at least 25 hours before sacrifice (on average 6 ± 2 days).

Amygdala stimulation (AMY). Amygdala stimulation evoked generalized motor seizures and self-sustaining SE in all animals, which was confirmed using vEEG recordings. One hour after stimulation, animals received a diazepam injection. If SE did not subside within one hour, animals received additional injections of diazepam. Termination of self-sustaining SE was confirmed by vEEG.

Animals were continuously (24/7) vEEG monitored for the whole 1st and 3rd months after stimulation and SRSs were observed in only a subset of them. Animals that developed SRSs experienced on average 47 ± 32 seizures during the whole monitoring time (mean \pm SEM), with a duration of 69 ± 8 seconds (mean \pm SEM). These animals had 8 ± 3 seizures (mean \pm SEM) in the

last 2 weeks of recording. Animals that were categorized as non-epileptic did not present any SRS in the 3 months after stimulation.

Lithium-pilocarpine (Li-pilo). SE-induced motor seizures disappeared after the first administration of the diazepam/phenobarbital/scopolamine cocktail, i.e., the duration of SE was identical in both groups of animals that subsequently did or did not develop epilepsy. The average latency, seizure-free period in rats that later developed epilepsy (that is, SRSs) was 24 ± 6 days (mean \pm SEM). In these animals, frequency of SRSs increased over time; during the first 2 weeks of monitoring, it was 2 ± 1 SRSs per week (mean \pm SEM); during the 2 weeks of monitoring at 2 months after SE it was 9 ± 2 (mean \pm SEM). The average SRS duration was 38 ± 9 s in the first 2 weeks of monitoring, and 48 ± 5 s in the 2 weeks of monitoring at 2 months.

Lateral fluid-percussion (LFP). The mean impact pressure in the TBI group was 3.27 ± 0.08 atm (n=16, median 3.3, range 3.2-3.4 atm). This induced a post-impact apnea lasting 35 ± 15 s (n=16, median 30.0, range 15- 65 s). Acute post-impact seizure-like behavior was observed in 6 of 16 rats with TBI, lasting 23 ± 10 s (median 28, range 5-30 s).

Seven TBI rats with late unprovoked spontaneous electrographic seizures and nine TBI rats without seizures were included in the study. In rats with PE, the average SRSs number during the 30-d monitoring period was 5.6 ± 6.2 (n=7, median 3, range 1 - 16). Of these 7 rats, 2 had 1 seizure, 3 had 2-5 seizures, and 2 had more than 5 seizures during the 4-week EEG monitoring. The average SRS duration was 93 ± 44 s (n=7, median 85 s, range 34 - 154 s). The average Racine score was 2.1 ± 1.0 s (n=7, median 2.3, range 1.0 – 3.5 s). None of the sham-operated animals had spontaneous seizures.

miRNA and isomiR plasma analysis 9 days after an epileptogenic insult

Principal component analysis. PCA showed that the plasma miRNA expression profile 9 days after the initial insult separated the samples from the four different models (AB vs AMY vs Lipilo vs LFP) as well as the control samples into different clusters ([Supplementary Figure 7](#)). In contrast, the PCA did not separate Epi and Non-Epi samples in any of the models. A similar pattern was observed for isomiRs ([Supplementary Figure 8](#)).

Differential expression analysis. DEA showed less miRNAs upregulated or downregulated for each model at 9 days, as compared to 2 days after the initial insult, both when Epi samples were compared to control samples and when Non-Epi samples were compared to control samples ([Supplementary Figure 9](#)). A similar pattern was observed for isomiRs ([Supplementary Figure 10](#)).

Venn diagram analysis. Venn analysis displayed nearly no up- or downregulated model-specific miRNAs or isomiRs, and no miRNA or isomiR up- or downregulated in all models ([Supplementary Figures 11 and 12](#)).

Receiver-Operating Characteristic analysis. ROC analysis showed that specific miRNAs could be detected for each model that separated Epi, Non-Epi and Epi + Non-Epi samples from control samples with 100% sensitivity and 100% specificity (insult-specific miRNAs; [Figure 6A-H](#); AUC 1, $p < 0.05$). Interestingly, miR-222-3p could separate Epi samples (AUC 0.737, $p < 0.05$), Non-Epi samples (AUC 0.818, $p < 0.05$) and Epi + Non-Epi samples (AUC 0.780, $p < 0.05$) from controls for all models ([Figure 6I and J](#)). However, sensitivity and especially specificity were low (sensitivity 82 – 84%, specificity 65-77%).

ROC analysis also revealed specific miRNAs for each model that separated Epi from Non-Epi samples with high sensitivity and specificity (epileptogenesis-specific miRNAs; [Figure 6K-R](#)): e.g., miR-760-3p (AUC 0.938, $p < 0.05$) for the AB model, miR140-3p (AUC 0.893, $p < 0.05$)

for the AMY model, miR-145-3p (AUC 0.875, $p < 0.05$) for the Li-pilo model and miR-487b-3p (AUC 0.905, $p < 0.05$) for the LFP model. Let-7d-3p could separate Epi from Non-Epi samples (AUC 0.691, $p < 0.05$) for all models (**Figure 6S and T**), with low sensitivity (76%) and specificity (67%). A top-10 list of miRNAs is provided for each model individually and all models together in **Supplementary Table 3**.

The combination of the best ROCs of independent models, as well as a combination of the best 5 miRNAs based on AUCs across all models also did not display significant AUCs nor good sensitivity and specificity (respectively 73% and 49%, AUC 0.544 and 73% and 70%, AUC 0.678; **Supplementary Figure 13**).

For isomiRs, model-specific miRNAs could be detected that separated Epi as well as Non-Epi samples and Epi + Non-Epi samples from control samples with high sensitivity and specificity (insult-specific isomiRs; **Supplementary Figure 14**; AUC 0.960-1, $p < 0.05$): e.g., miR-434-3p_trim1 for the AB model, miR-144-3p_A_3prime for the AMY model, miR-144-3p_trim2 for the Li-pilo model and miR-106b-3p_AA_3prime for the LFP model. Furthermore, miR-18a-3p_G_3prime could separate with 78% sensitivity and 82% specificity Epi samples (AUC 0.789, $p < 0.05$) as well as Non-Epi samples (AUC 0.859 $p < 0.05$) and Epi + Non-Epi samples (AUC 0.826, $p < 0.05$) from control samples for all models (**Supplementary Figure 14**).

ROC analysis also revealed specific isomiRs for each model that separated Epi from Non-Epi samples with high sensitivity and specificity (epilepsy-specific isomiRs; **Supplementary Figure 15**): e.g., miR-130b-5p_trim2 (AUC 0.906, $p < 0.05$) for the AB model, miR-98-5p_trim4 (AUC 0.982, $p < 0.05$) for the AMY model, miR-3556a_TAC_5prime (AUC 0.891, $p < 0.05$) for the Li-pilo model and miR-160b-5p_trim2 (AUC 0.889, $p < 0.05$) for the LFP model. MiR-1249_miRNA could separate Epi from Non-Epi samples (AUC 0.721, $p < 0.05$) for all models (**Supplementary Figure 15**), with low sensitivity (77%) and specificity (61%) a. A

top-10 list of isomiRs is provided for each model individually and all models together in [Supplementary Table 4](#).

The combination of the best ROCs of independent models, as well as a combination of the best 5 miRNAs based on AUCs across all models also did not display significant AUCs nor good sensitivity and specificity (respectively 40% and 79%, AUC 0.560 and 87% and 58%, AUC 0.743; [Supplementary Figure 16](#)).