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Supplemental information

A mathematical model of neuroimmune interactions in epileptogenesis for discovering treatment strategies

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S1 Stability analysis

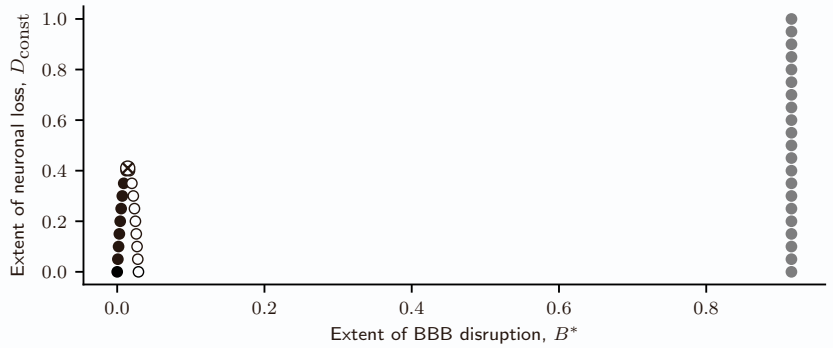

Figure S1: **Saddle node bifurcation illustrated with collision (crossed circle) of stable (black circles) and unstable (white circles) steady states at critical value of the extent of neuronal loss (** $D_{\text{const}} = D_{\text{critical}} \approx 0.41$**), related to Section 'Model design', STAR Methods and Figure 7.** The third fixed point (gray circles) shows low sensitivity to change in neuronal loss due to a low value of $\kappa_{D \to R}$. For values $D_{\text{const}} > D_{\text{critical}}$, only one stable steady state (gray circles) exist.

S2 Model parameter choice

Parameter	Description	Value	Units
τ_I	Timescale of neuroinflammatory reaction	1	day
τ_B	Timescale of BBB recovery	10	days
τ_D	Timescale of neuronal death process	10	days
τ_R	Timescale of circuit remodeling	10	days
$\kappa_{I\to B}$	Scaling parameter for effect of neuroinflammation on BBB permeability	0.1	-
$\kappa_{B\to I}$	Scaling parameter for proinflammatory effect of BBB leakage	1	-
$\kappa_{I\to D}$	Scaling parameter for neurotoxic effect of overactivated glia	8	-
$\kappa_{B\to R}$	Scaling parameter for effect of BBB leakage on circuit remodeling	1	-
$\kappa_{D\to R}$	Scaling parameter for effect of neuronal loss on circuit remodeling	0.0005	-
D_{max}	Maximum possible extent of neuronal loss	1	-
Θ	Neurotoxicity threshold of overactivated glia	0.25	-
$\kappa_{I\to S}$	Scaling parameter for strength of seizure-promoting effects of neuroinflammation	2	-
$\kappa_{R\to S}$	Scaling parameter for strength of seizure-promoting effects of circuit remodeling	2	-
$K_{S\to B}$	Scaling parameter for seizure burden on BBB integrity	0.875	-
T_{seiz}	Seizure duration	5	minutes
λ_{max}	Homeostatic upper bound of daily seizure number	15	$\frac{\text{seizures}}{\text{day}}$
$\kappa_{S\to B}$	Burden of single seizure on BBB integrity	16.8	-

Table S1: Model parameter descriptions and values, related to Section 'Model design', STAR Methods and Figures 1, 3-9.

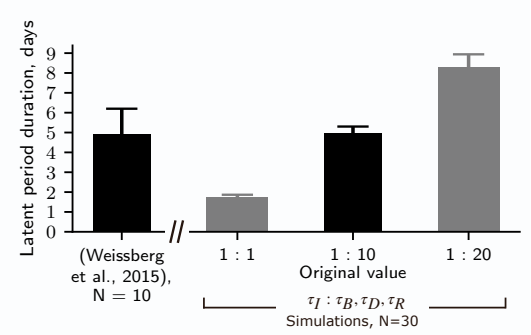

Figure S2: **Comparison of the model fit to the animal model data (left black bar) for the original ratio of timescales (right black bar) and two alternative values (gray bars), related to STAR Methods.** Data are represented as mean ± SEM.

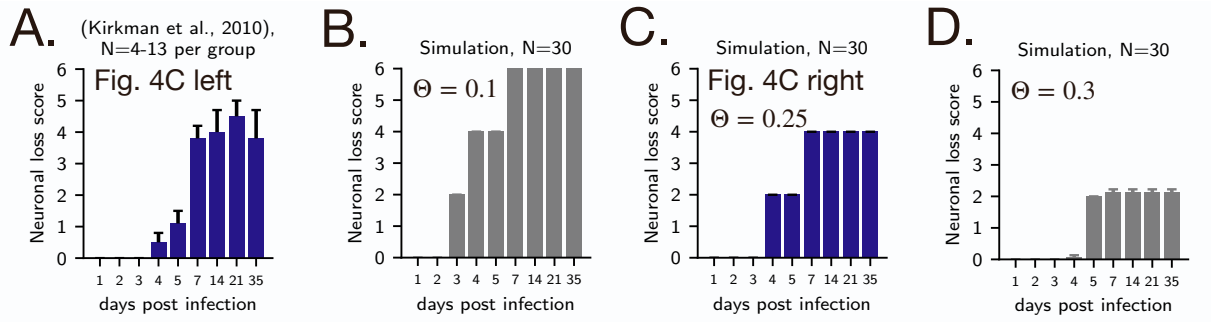

Figure S3: **Comparison of the model fit to the animal model data (A., imported from Fig. 4C left) for the originally chosen value of neurotoxicity threshold (C., imported from Fig. 4C right) and two alternative values (B. and D.), related to STAR Methods.** Data are represented as mean ± SEM.

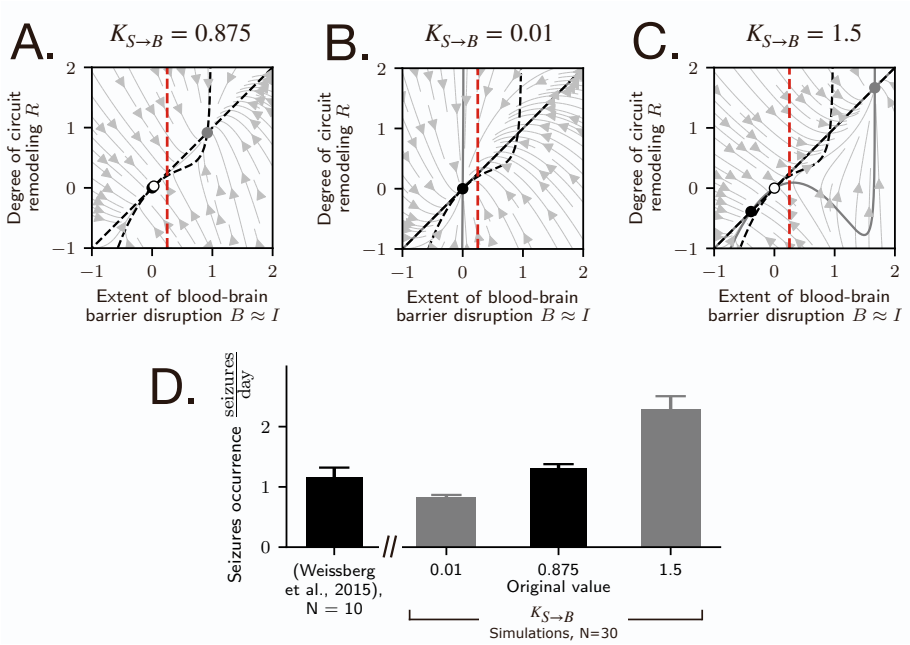

Figure S4: **Comparison of the model fit to the animal model data for originally chosen value of $K_{S\to B}$ and alternative values, related to STAR Methods: A.** State-space composition for the originally chosen value of $K_{S\to B} = 0.875$. Circles correspond to the steady states. Black dashed lines correspond to $\dot{B} = 0$ and $\dot{R} = 0$ nullclines. Red dashed line indicates the neurotoxicity threshold. **B.** State-space composition for the alternative value of $K_{S\to B} = 0.01$. Circles correspond to the steady states. Gray solid lines correspond to the nullclines. Black dashed lines correspond to the nullclines of the system with the original parameter values used as reference for comparison. Red dashed line indicates the neurotoxicity threshold. **C.** Identical to B. for the alternative value of $K_{S\to B} = 1.5$. **D.** Comparison of the model fit to the animal model data for the originally chosen value of $K_{S\to B} = 0.875$ (black bar) and two alternative values (gray bars). Data are represented as mean ± SEM.

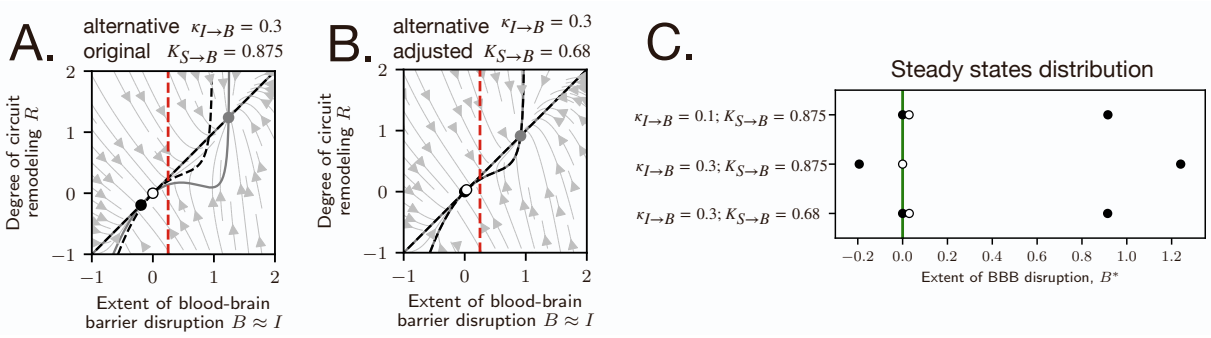

Figure S5: **Redundancy in the parameter space identified via choosing alternative value of $\kappa_{I\to B}\uparrow$ and adjusting $K_{S\to B}\downarrow$ accordingly, related to STAR Methods: A.** State-space composition for the alternative value of $\kappa_{I\to B}\uparrow = 0.3$ preserving original value of $K_{S\to B} = 0.875$. Circles correspond to the steady states. Gray solid lines correspond to the nullclines. Black dashed lines correspond to the nullclines of the system with the original parameter values used as reference for comparison. Red dashed line indicates the neurotoxicity threshold. **B.** State-space composition for the alternative value of $\kappa_{I\to B}\uparrow = 0.3$ and adjusted $K_{S\to B}\downarrow = 0.68$. Annotation identical to caption in A. **C.** Steady states distribution for the extent of BBB disruption B^* illustrated for the original parameter set (top); alternative parameter set with $\kappa_{I\to B}\uparrow$ (middle); and alternative parameter set with $\kappa_{I\to B}\uparrow$ and adjusted $K_{S\to B}\downarrow$ (bottom). Vertical green line illustrates the edge of the positive domain for the extent of the BBB disruption ($B^* = 0$).

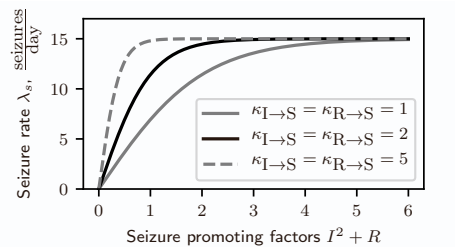

Figure S6: **Changing the parameters for the strength of seizure-promoting effects of neuroinflammation and circuit remodeling leads to scaling of the sigmoid function of seizure rate along the 'x' axis, related to STAR Methods.**

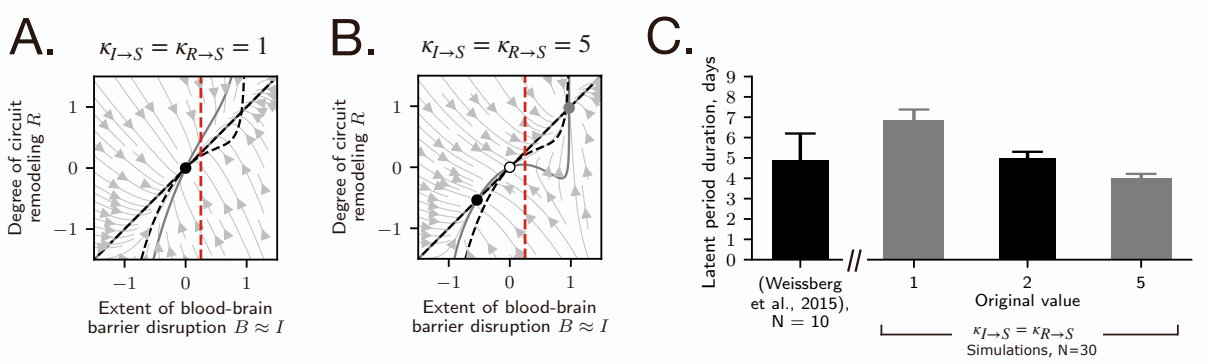

Figure S7: **The effects of changing the $\kappa_{I\to S}$ and $\kappa_{R\to S}$ parameter values on stability of the system and quality of the model fit, related to STAR Methods:** Effects of increasing (**A.**) and decreasing (**B.**) the values of $\kappa_{I\to S}$ and $\kappa_{R\to S}$ parameters on the stability of the system. Circles correspond to the steady states. Gray solid lines correspond to the nullclines. Black dashed lines correspond to the nullclines of the system with the original parameter values used as reference for comparison. Red dashed lines indicate the glial neurotoxicity threshold. **C.** Comparison of the model fit to the animal model data for the originally chosen values of $\kappa_{I\to S}$ and $\kappa_{R\to S}$ (black bar) and two alternative values (gray bars). Data are represented as mean $\pm$ SEM.

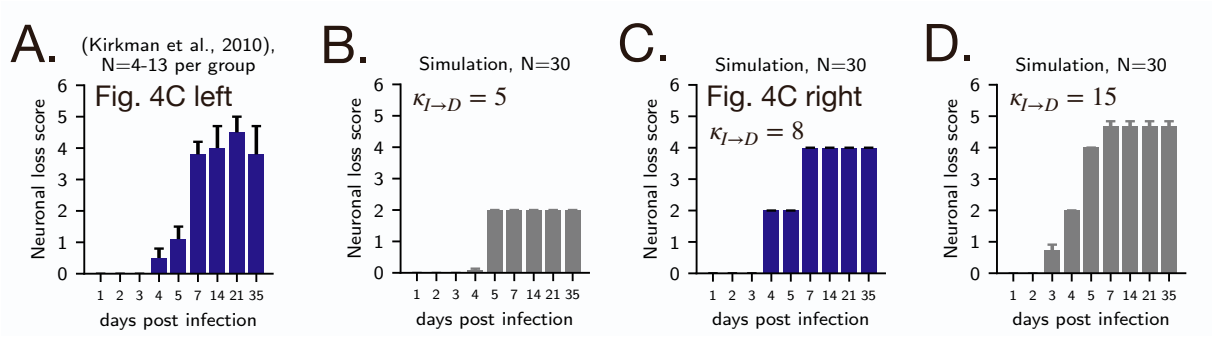

Figure S8: **Comparison of the model fit to the animal model data (A., imported from Fig. 4C left) for the originally chosen value of $\kappa_{I\to D}$ parameter (C., imported from Fig. 4C right) and two alternative values (B. and D.), related to STAR Methods.** Data are represented as mean ± SEM.

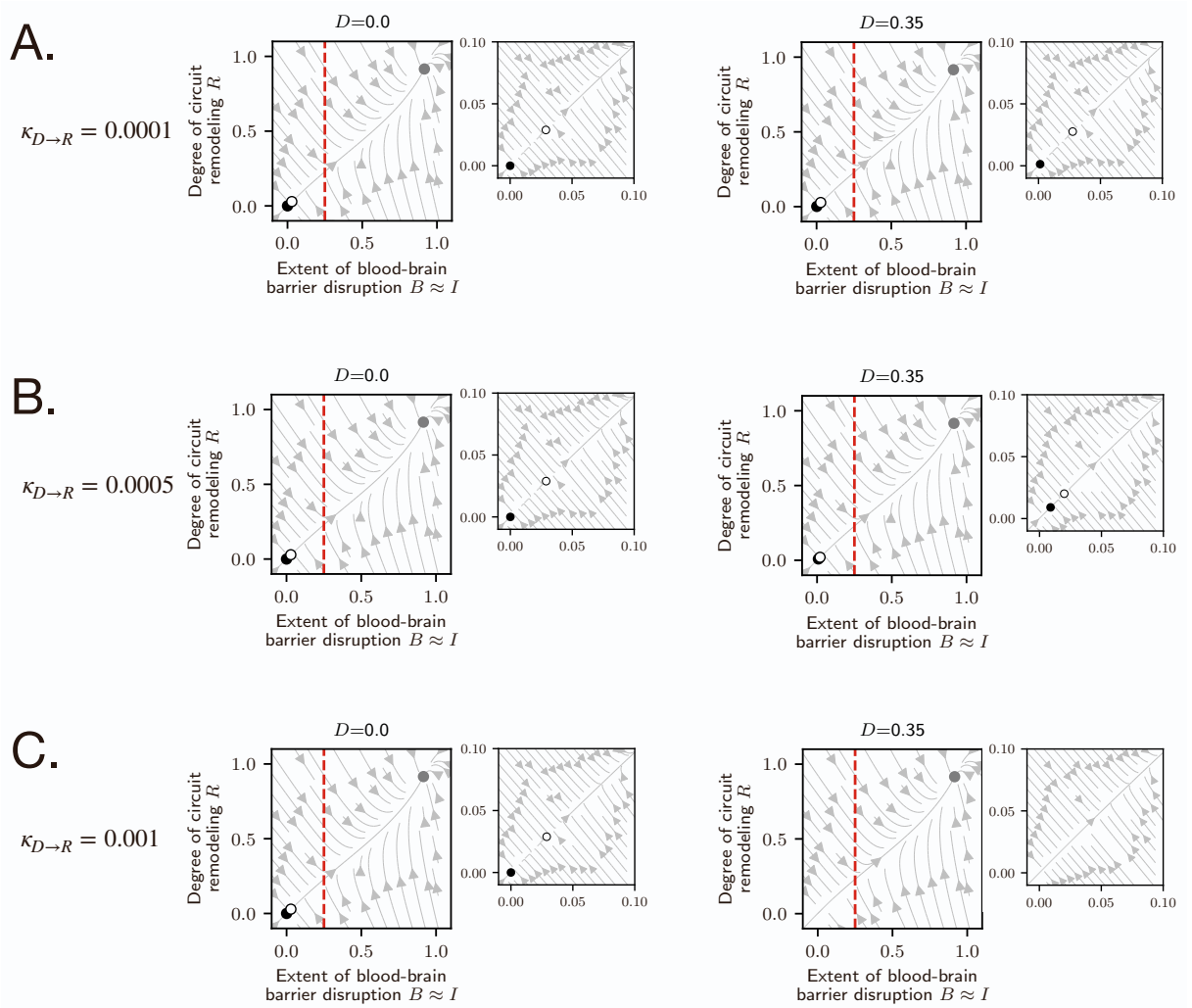

Figure S9: **The effect of $\kappa_{D\to R}$ parameter on the stability of the system illustrated by stability changes in conditions of non-zero neuronal loss, related to STAR Methods: A.** State-space plots for the alternative lower parameter value $\kappa_{D\to R} \downarrow = 0.0001$. Smaller plots correspond to figure insets. Left and right panels correspond to conditions of absent ($D = 0.0$) and present ($D = 0.35$) neuronal loss respectively. Red dashed lines correspond to glial neurotoxicity threshold. **B.** State-space plots for the original parameter value $\kappa_{D\to R} = 0.0005$. Annotation identical to caption in A. **C.** State-space plots for the alternative higher parameter value $\kappa_{D\to R} \uparrow = 0.001$. Annotation identical to caption in A. For the lowered parameter value of $\kappa_{D\to R}$, system loses sensitivity to changes in neuronal loss (see absence of the steady state location changes between panels of A.). For the elevated parameter value of $\kappa_{D\to R}$, system loses the stability (see disappearance of the steady states between panels of C.).

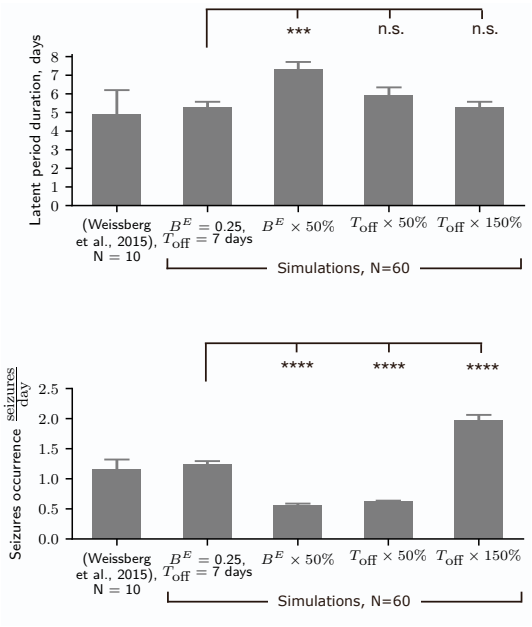

Figure S10: **Two-fold increase of the number of simulations in cohort does not lead to major differences in simulation results, related to STAR Methods:** Re-run of simulation from Fig. 3A,B with twice higher number of simulations in cohort ($N = 60$). Data are represented as mean $\pm$ SEM. The unpaired two-group Mann-Whitney U test was performed: ***$p < 0.001$; ****$p < 0.0001$.

S3 Detailed specifications of performed simulations

Animal model / Condition simulated	**Modification**	**Model type**	**Input type and intensity**	**Time of injury onset** T_{on}	**Time of injury offset** T_{off}	**Num. of simulations,** N	**Fig.**
Blood-brain barrier leakage rodent model	-	stochastic	$B^E = 0.25$	0 days	7 days	30	3A-D
	descreased injury intensity (50% concentration)	stochastic	$B^E = 0.125$	0 days	7 days	30	3A,B
	descreased injury intensity (50% time window duration)	stochastic	$B^E = 0.25$	0 days	3.5 days	30	3A,B
	increased injury intensity (150% time window duration)	stochastic	$B^E = 0.25$	0 days	10.5 days	30	3A,B
	-	rate	$B^E = 0.25$	0 days	7 days	1	3E,F
	increased injury intensity (150% time window duration)	rate	$B^E = 0.25$	0 days	10.5 days	1	3E,F
	descreased injury intensity (50% time window duration)	rate	$B^E = 0.25$	0 days	3.5 days	1	3E,F
	descreased injury intensity (25% time window duration)	rate	$B^E = 0.25$	0 days	1.75 days	1	3E,F
Theiler's murine encephalomyelitis virus (TMEV) mouse model	-	stochastic	$I^E = 0.4$	0.9 day	6 days	30	4, 5
	testing intervention with suppression of seizure effect on BBB integrity $K_{S\to B} \downarrow = \frac{K_{S\to B}}{100}$ applied in different time intervals	rate	$I^E = 0.4$	0.9 day	6 days	5 (4 + 1 reference)	9A-E
	testing intervention with suppression of activation of glia by factors infiltrating the parenchyma $\kappa_{B\to I} \downarrow = \frac{\kappa_{B\to I}}{100}$ applied in different time intervals	rate	$I^E = 0.4$	0.9 day	6 days	5 (4 + 1 reference)	9F-H
Chemically-induced (pilocarpine) status epilepticus rodent model	-	stochastic	D^E=1; B^E=1.65	0 days	2 days	30	6
	-	rate	D^E=1; B^E=1.65	0 days	2 days	1	6D-F
	testing intervention with suppression of seizure effect on BBB integrity $K_{S\to B} \downarrow = \frac{K_{S\to B}}{100}$ applied in different time intervals	rate	D^E=1; B^E=1.65	0 days	2 days	7 (6 + 1 reference)	8
Neuronal loss	Supracritical extent of neuronal loss	stochastic	Initial conditions $D_0 = 1$	-	-	5	7A
		rate	Initial conditions $D_0 = [0.45;\ 0.5;\ 0.6\ 0.7;\ 0.8;\ 0.9;\ 1]$	-	-	7	7C
	Subcritical extent of neuronal loss	stochastic	Initial conditions $D_0 = 0.3$	-	-	5	7A

Table S2: Detailed specifications of performed simulations, related to Section 'Model design', STAR Methods and Figures 3-9.

S4 Neuronal loss score computation with masking procedure

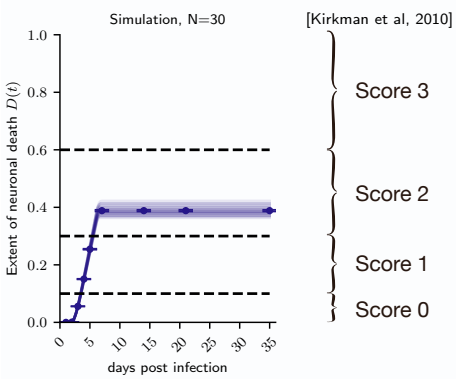

Figure S11: **Neuronal loss score computation (masking procedure) from Kirkman et al. (2010), related to Figure 4:** Raw neuronal death data from TMEV model simulation (left) and neuronal loss score computation scheme (right). Horizontal dashed lines on the left correspond to 10%, 30% and 60% extent of neuronal loss, which are the border values separating score values in the scheme from Kirkman et al. (2010). In Kirkman et al. (2010), neuronal loss score data are presented as a sum of scores for 2 hippocampi (maximum score: $3 \times 2 = 6$). Thus, the neuronal loss score computed for simulated TMEV animals was multiplied by factor of 2 for comparability with experimental data. Absence of variability (0 SEM) in Fig. 4C is explained by 'masking out' of variability in neuronal loss score computation (left).

S5 Seizure rate dependence

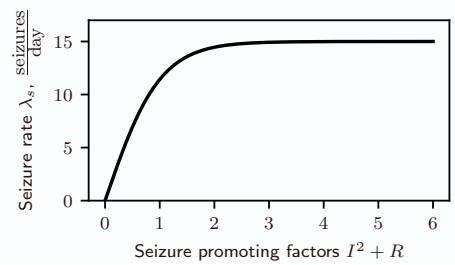

Figure S12: **Seizure rate dependence on seizure promoting factors: neuroinflammation and circuit remodeling, related to STAR methods section, Equation 3.**