Aus dem Institut für Neurophysiologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

# Altered network oscillations and synaptic plasticity in the blood-brain barrier-disrupted peri-infarct hippocampus are related to epileptiform activity and impaired GABAergic inhibition

zur Erlangung des akademischen Grades

Doctor medicinae (Dr. med.)

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von

Kristina Lippmann

aus Freiberg/Sachs.

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## Inhaltsverzeichnis

## Abstract

Erworbene Epilepsien in älteren Menschen resultieren häufig aus Hirnläsionen assoziiert mit Bluthirnschranken (BHS)-Störungen wie Schlaganfall, Trauma, Hirntumoren oder -entzündungen. Nach Schlaganfall entstandene Epilepsien können dabei mit kognitivem Abbau und schlechten neurologischen Ergebnissen verbunden sein. Krampfanfälle und Streudepolarisierungen (Spreading depolarizations) entstehen ferner oft als charakteristische Aktivität aus der ischämischen Läsion, können aber auch zur Läsionsausweitung und Reorganisation des anliegenden neuralen Netzwerkes beitragen und sind deshalb wegweisende elektrophysiologische Korrelate der Pathophysiologie. Als eine wichtige Struktur für die Gedächtniskonsolidierung aber auch als sensibles Areal der Epilepsieentstehung, ist der Hippokampus eine entscheidende Struktur um die pathophysiologischen Mechanismen zu untersuchen, die den BHS-Störung induzierten Veränderungen der synaptischen Plastizität und der neuralen Netzwerkaktivitäten unterliegen.

Zur mechanistischen Aufklärung der zugrundeliegenden Netzwerkpathophysiologie untersuchte ich verschiedene Aspekte der Veränderungen im Hippokampus der Ratte nach einer neokortikalen Photothrombose. Dabei verwendete ich vielseitige Methoden wie die Magnet Resonanz Tomographie, Hindruckmessungen, elektrophysiologische Ableitungen und Genexpressionsanalysen.

Die kortikale Photothrombose war mit einer früh auftretenden peri-ischämischen BHS-Störung im darunterliegenden, ipsilateralen Hippocampus und erhöhtem Hirndruck assoziiert, welche der Entwicklung eines vasogenen Ödems vorrausgingen. Intrahippokampale Feldpotentialableitungen präsentierten innerhalb der ersten Woche nach Schlaganfall in zweidrittel der Tiere elektrographische Krampfanfälle. Vor allem krampfende Tiere zeigten sowohl einen Anstieg in Theta- als auch einen Abfall in Gammafrequenzbändern, was auf eine gestörte inhibitorische Aktivität hinweist. Synaptische Interaktionen und Plastizität wurden nach 24 h und 7 Tagen nach Schlaganfall in parasagittalen hippokampalen Hirnschnitten untersucht. Feldpotentialableitungen in CA1 und CA3 deckten multiple ‚population spikes', epileptiforme Episoden und Streudepolarisierungen nach 24 h auf, welche nach 7 Tagen rückläufig waren. Allerdings zeigten Eingangs-Ausgangs-Analysen, dass die fEPSP-Spike Kopplung nach 7 Tagen signifikant erhöht war. Des Weiteren waren die feedback- und feedforward-Hemmungen in CA1 innerhalb der ersten Woche

vermindert. Am 7. Tag präsentierten Hirnschnitte mit epileptiformer Aktivität eine beeinträchtigte bidirektionale Langzeitplastizität nach hoch- und niederfrequenten Stimulationsprotokollen. Unterstützend für diese Erkenntnisse bestätigten Microarray- und PCR-Daten Expressionsveränderungen in Astrozyten-bezogenen Genen und deuteten eine verminderte Expression in $GABA_A$-Rezeptor Subtypen an.

Zusammenfassend lässt sich sagen, dass BHS-Störung im peri-Infarkt Hippokampus innerhalb der ersten Woche mit Übererregbarkeit, früher Disinhibition und abnormer synaptischer Plastizität assoziiert ist. Dadurch stellen die Daten eine enge Verbindung zwischen hippokampalen neuralen Netzwerken mit epileptiformer Aktivität und gestörter oszillatorischer Aktivität sowie Langzeitplastizität dar. Diese Erkenntnisse zeigen neue, etwaige diagnostische Überwachungsmöglichkeiten auf, um Patienten zu entdecken, die potentiell in Gefahr sind eine Epilepsie sowie kognitive Einschränkungen im Rahmen einer peri-läsionalen BHS-Störung zu entwickeln.

## Abstract

Aquired epilepsies in the elderly often result from brain lesions associated with blood-brain barrier (BBB)-disruption like stroke, trauma, tumors or brain infections. Post-stroke epilepsy can thereby be related to cognitive decline and poor neurological outcome. Seizures and spreading depolarization arise as characteristic activity from ischemic lesions but may also contribute to lesion progression and reorganization of the adjacent neural network and are therefore critical electrophysiological correlates of the pathophysiology. As an important structure for memory consolidation but also as a very sensitive area for epileptogenesis, the hippocampus is a crucial structure for investigating mechanisms underlying the pathophysiology of BBB-dysfunctional induced changes in synaptic plasiticity and neural network activity.

To elucidate mechanisms underlying the pathophysiology I investigated with a multi-methodological approach different aspects of changes in rat hippocampus following neocortical photothrombosis using magnetic resonance imaging, intracranial pressure and electrophysiological measurements as well as gene expression analysis.

Cortical photothrombosis was associated with early peri-ischemic BBB-dysfunction that included the underlying, ipsilateral hippocampus and increased intracranial pressure, both preceding the occurrence of vasogenic edema. Intrahippocampal field potential recordings revealed electrographic seizures within the first week in two thirds of animals. Predominantly seizing animals displayed an increase in theta and reduction in gamma frequency bands indicating disturbed inhibitory activity. Synaptic interactions and plasticity were studied in parasagittal hippocampal slices at 24 hrs and 7 days post-stroke. Field potential recordings in CA1 and CA3 uncovered multiple population spikes, epileptiform episodes and spreading depolarizations at 24 hrs declining at day 7. However, input-output analysis revealed that fEPSP-spike coupling was significantly enhanced at 7 days. In addition, CA1 feedback and feedforward inhibition were diminished over the first week. Slices generating epileptiform activity at 7 days revealed impaired bidirectional long-term plasticity following high and low frequency stimulation protocols. Supporting these findings, microarray and PCR data confirmed changes in expression of astrocyte-related genes and suggested downregulation in expression of $GABA_A$-receptor subunits.

In conclusion, BBB dysfunction in the peri-infarct hippocampus is within the first week related to hyperexcitability, early disinhibition and abnormal synaptic plasticity.

Thus, the data reveal a strong connection between hippocampal neural networks presenting epileptiform activity and disturbed oscillatory activity as well as long-term plasticity. These insights reveal possible new diagnostic monitoring opportunities in finding patients at risk for epileptogenesis and cognitive impairment associated with peri-lesional BBB-dysfunction.

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**Ausführliche Anteilserklärung an der erfolgten Publikation**

Publikation: **Lippmann K**, Kamintsky L, Kim SY, Lublinsky S, Prager O, Nichtweiß J, Salar S, Kaufer D, Heinemann U, Friedman A. Epileptiform activity and spreading depolarization in the blood-brain barrier-disrupted peri-infarct hippocampus are associated with impaired GABAergic inhibition and synaptic plasticity. J Cereb Blood Flow Metab, in press, 2016

Beitrag im Einzelnen: ~80 %, Kristina Lippmann (KL) entwarf die Studie, etablierte und führte alle in vivo Hindruckmessungen und intrahippokampalen Felpotential-ableitungen durch; KL führte den Großteil der MRT und der elektrophysiologischen Experimente im Hirnschnitt durch, operierte und bereitete die Tiere für die genetischen Analysen vor. KL analysierte alle Daten abgesehen von den Rohanalysen der MRT Scans, der intrahippocampalen in vivo Ableitungen und der Microarray/PCR Daten. KL interpretierte alle Daten, schrieb das Manuskript und korrigierte die Studie nach Maßgabe der Gutachteranmerkungen.

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| ☐ | 2 | TRENDS COGN SCI | 1364-6613 | 20396 | 21.965 | 22.500 | 3.150 | 60 | 8.3 | 0.04585 | 9.498 |
| ☐ | 3 | BEHAV BRAIN SCI | 0140-525X | 7562 | 20.771 | 24.308 | 2.750 | 20 | >10.0 | 0.01103 | 10.686 |
| ☐ | 4 | ANNU REV NEUROSCI | 0147-006X | 13226 | 19.320 | 25.920 | 1.320 | 25 | >10.0 | 0.02423 | 13.624 |
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| ☐ | 6 | NEURON | 0896-6273 | 77446 | 15.054 | 16.839 | 3.221 | 399 | 8.2 | 0.22320 | 8.682 |
| ☐ | 7 | MOL PSYCHIATR | 1359-4184 | 14510 | 14.496 | 13.834 | 3.152 | 151 | 5.4 | 0.04317 | 5.007 |
| ☐ | 8 | TRENDS NEUROSCI | 0166-2236 | 19082 | 13.555 | 14.695 | 2.441 | 68 | >10.0 | 0.03363 | 6.266 |
| ☐ | 9 | ACTA NEUROPATHOL | 0001-6322 | 13098 | 10.762 | 9.762 | 2.500 | 116 | 6.7 | 0.03329 | 3.483 |
| ☐ | 10 | BIOL PSYCHIAT | 0006-3223 | 40812 | 10.255 | 10.359 | 2.667 | 210 | 7.7 | 0.07948 | 3.553 |
| ☐ | 11 | PROG NEUROBIOL | 0301-0082 | 11430 | 9.992 | 10.487 | 2.839 | 62 | 9.4 | 0.02014 | 3.553 |
| ☐ | 12 | ANN NEUROL | 0364-5134 | 32934 | 9.977 | 10.792 | 1.970 | 167 | 9.9 | 0.06054 | 4.176 |
| ☐ | 13 | J PINEAL RES | 0742-3098 | 6906 | 9.600 | 7.227 | 1.862 | 87 | 5.9 | 0.00816 | 1.120 |
| ☐ | 14 | BRAIN | 0006-8950 | 44379 | 9.196 | 10.418 | 2.422 | 244 | 8.5 | 0.08489 | 3.846 |
| ☐ | 15 | NEUROSCI BIOBEHAV R | 0149-7634 | 16868 | 8.802 | 10.528 | 1.657 | 198 | 6.4 | 0.04123 | 3.596 |
| ☐ | 16 | CEREB CORTEX | 1047-3211 | 26191 | 8.665 | 8.335 | 2.043 | 282 | 6.7 | 0.06927 | 3.290 |
| ☐ | 17 | SLEEP MED REV | 1087-0792 | 3919 | 8.513 | 9.258 | 2.062 | 48 | 6.6 | 0.00959 | 3.247 |
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| ☐ | 22 | CURR OPIN NEUROBIOL | 0959-4388 | 12732 | 6.628 | 7.284 | 1.604 | 164 | 8.2 | 0.03380 | 3.559 |
| ☐ | 23 | MOL NEURODEGENER | 1750-1326 | 2058 | 6.563 | 5.709 | 0.672 | 58 | 3.7 | 0.00873 | 1.797 |
| ☐ | 24 | NEUROIMAGE | 1053-8119 | 78028 | 6.357 | 7.289 | 1.500 | 1033 | 6.5 | 0.17239 | 2.257 |
| ☐ | 25 | J NEUROSCI | 0270-6474 | 173265 | 6.344 | 7.348 | 1.188 | 1486 | 8.0 | 0.38326 | 2.862 |
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| ☐ | 28 | BRAIN BEHAV IMMUN | 0889-1591 | 8218 | 5.889 | 5.895 | 1.278 | 205 | 4.7 | 0.02239 | 1.728 |
| ☐ | 29 | J PSYCHIATR NEUROSCI | 1180-4882 | 2491 | 5.861 | 6.789 | 0.902 | 41 | 6.0 | 0.00622 | 2.175 |
| ☐ | 30 | BRAIN STRUCT FUNCT | 1863-2653 | 2478 | 5.618 | 6.935 | 1.354 | 147 | 3.8 | 0.01071 | 2.435 |
| ☐ | 31 | MOL AUTISM | 2040-2392 | 577 | 5.413 | 5.821 | 0.442 | 52 | 2.4 | 0.00308 | 2.045 |
| ☐ | 32 | J NEUROINFLAMM | 1742-2094 | 5318 | 5.408 | 5.632 | 0.605 | 215 | 3.2 | 0.01820 | 1.524 |
| ☑ | 33 | J CEREBR BLOOD F MET | 0271-678X | 15903 | 5.407 | 5.455 | 0.974 | 232 | 8.0 | 0.02926 | 1.785 |
| ☐ | 34 | CURR OPIN NEUROL | 1350-7540 | 4789 | 5.307 | 5.004 | 0.828 | 87 | 6.0 | 0.01265 | 1.732 |
| ☐ | 35 | PAIN | 0304-3959 | 31705 | 5.213 | 6.241 | 1.239 | 284 | 9.6 | 0.05060 | 2.160 |
| ☐ | 36 | MOL NEUROBIOL | 0893-7648 | 4012 | 5.137 | 5.460 | 1.209 | 206 | 4.0 | 0.01026 | 1.509 |
| ☐ | 37 | CORTEX | 0010-9452 | 6768 | 5.128 | 5.014 | 0.966 | 178 | 5.8 | 0.01690 | 1.623 |
| ☐ | 38 | NEUROPHARMACOLOGY | 0028-3908 | 16524 | 5.106 | 4.823 | 1.182 | 417 | 6.0 | 0.03722 | 1.454 |
| ☐ | 39 | NEUROBIOL DIS | 0969-9961 | 12714 | 5.078 | 5.298 | 1.142 | 275 | 5.4 | 0.03304 | 1.662 |
| ☐ | 40 | NEUROTHERAPEUTICS | 1933-7213 | 2584 | 5.054 | 5.821 | 1.319 | 72 | 4.5 | 0.00909 | 1.829 |

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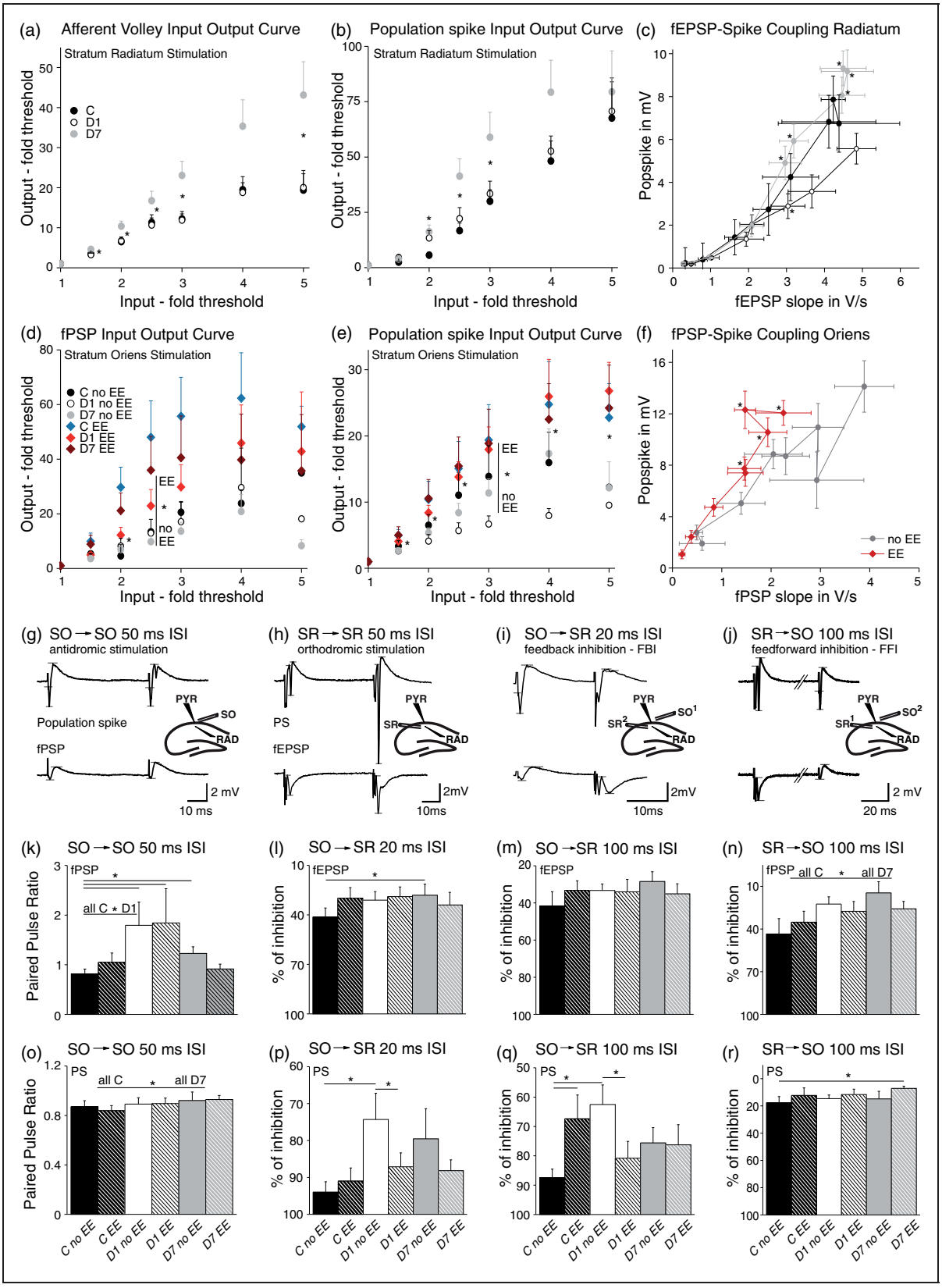


**Figure 4.** Increased input–output curves, fEPSP-spike (ES) coupling, and decreased feedback as well as feedforward inhibition in CA1 post-stroke. During antidromic stimulation, BBB-disrupted slices revealed increased paired pulse ratios and larger input–output curves, dependent on epileptiform activity. (a) Slices from D7 showed larger afferent volley amplitudes in SR recordings, indicating a

(continued)

slices (for 2.5–7: KW $p < 0.05$; MW post hoc: 2.5–7: D1 vs. D7; 2.5: C vs. D7 and 4: C vs. D1 $p < 0.0167$). By contrast, coupling between afferent volleys and fEPSP displayed no alteration in stroke animals, although afferent volleys had larger amplitudes at D7 compared to no significant changes in fEPSP amplitudes (data not shown).

We next analyzed SO-induced antidromic responses resulting from activation of pyramidal cell axons, which run within the alveus towards the subiculum and the fimbria fornix. SO stimulation induces via axonal collaterals GABAergic synaptic inhibition in CA1[32] and results in positive field potentials (fPSP) in the SR (Figures 3(a) and 4(g)). Interestingly, slope amplitudes were larger for intermediate stimulation intensities in slices with EEs, independent of treatment (Figure 4(d); for 2–2.5, EE vs. no EE: MW $p < 0.05$). Population spikes were also larger in slices with compared to those without EEs (Figure 4(e); for 1.5–5, MW $p < 0.05$). Because changes in ES-coupling for SR stimuli could either be due to changes in dendritic excitability or due to disinhibition, we tested whether there was an altered coupling between the amplitude of SO-evoked PSs and fPSPs. Indeed, slices with EEs showed increased fPSP-spike coupling compared to slices without EEs (Figure 4(f); for 3–7, MW $p < 0.05$), suggesting that reduced GABAergic inhibition within CA1 underlies hyperexcitability.

We then tested for paired pulse ratio in response to SR stimulation with 50 ms ISI. We always observed paired pulse facilitation with no difference between post-stroke slices and controls, suggesting a similar release probability of presynaptic vesicles (Figure 4(h)). In contrast, SO-induced fPSPs revealed a significant increase in paired pulse ratio at D1 compared to control (Figure 4(g) and (k); all C vs. D1: MW $p = 0.027$), while the ratio between PS amplitude was not affected. At D7 paired pulse ratio of PS and fPSP amplitude was increased in slices from treated animals compared to controls (Figure 4(o); C vs. D7: MW $p = 0.045$; Figure 4(k); C no EE vs. D7 no EE: MW $p = 0.032$). These experiments suggested a reduced GABAergic inhibition in the BBB dysfunctional hippocampus.

To further test for functional feedback inhibition, we paired SO and SR stimulation with an inter-stimulus interval of 20 ms (for $GABA_A$-mediated inhibition). This protocol stimulates CA1 pyramidal cell axonal collaterals that activate interneurons, which in turn inhibit CA1 pyramidal dendrites (circuitry in Figures 3(a) and 4). Vice versa, for induction of feedforward inhibition, SR preceded SO stimulation to activate – via Schaffer collaterals in SR – interneurons, which inhibit the incoming signal in dendrites of CA1 pyramidal cells (Figure 3(a)). The degree of inhibition was calculated as the ratio of the inhibited and the non-inhibited signal from the same stimulation site. In slices from controls, response to SR stimulation was significantly smaller compared to SR stimulation only when it followed SO stimulation, due to activation of GABAergic interneurons by SO stimulation (Figure 4(h) to (i)). At D1 PS and at D7, fEPSP amplitudes were significantly enlarged, suggesting a decline in feedback inhibition (Figure 4(p); PS MW: C no EE vs. D1 no EE: $p = 0.014$; Figure 4(l); fEPSP: C no EE vs. D7 no EE: $p = 0.041$). An increase in PS amplitude at D1 was also observed when SO-SR stimulus interval was 100 ms, suggesting that $GABA_B$-mediated late inhibition may also be affected (Figure 4(q): C no EE vs. D1 no EE: $p = 0.009$). We observed stronger

**Figure 4.** Continued
greater number of activated presynaptic fibers (C n = 16 slices/7 animals, D1 n = 33/14, 7d n = 27/8; for fold threshold input of 1.5–3 and 5, KW: $p < 0.05$). (b) Population spike amplitudes were increased at D7, too (C n = 18/8, D1 n = 33/14, D7 n = 27/8; for 2–3-fold threshold: C vs. D7 and for 2.5: D1 vs. D7: KW $p < 0.05$). (C) ES-coupling was enhanced at D7 compared to a smaller coupling at D1 (for 2.5–7: KW $p < 0.05$; MW post hoc: 2.5–7: D1 vs. D7; 2.5: C vs. D7 and 4: C vs. D1 $p < 0.0167$). (d–f) For antidromic stimulation (SO), amplitudes of field postsynaptic potentials (fPSP) and population spikes as well as the fPSP-spike coupling were increased in slices displaying EE independent of their treatment (PS: for 1.5–5: all EE (n = 28/18) vs. all no EE (n = 15/11): MW $p < 0.05$; fPSP: for 2–2.5, MW $p < 0.05$; fPSP-spike coupling: for 3–7, MW $p < 0.05$). (g–j) Example traces of recordings during paired pulse stimulation with small schemes presenting the stimulation and recording sites. Horizontal lines in traces mark points between which amplitudes and slopes (fEPSP) were calculated. (g–h) Antidromic and orthodromic paired pulse stimulation (50 ms ISI) was done for investigating short-term plasticity. (i, j) Alternating stimulation, where an SO stimulus was followed by an SR stimulus and vice versa, was performed for testing for feedback and feedforward inhibition, respectively. (k, o) Paired pulse ratio of SO stimulation was increased in D1 and D7 post-stroke slices for fPSP and in D7 slices for PS (fPSP: all C n = 13/8, D1 n = 21/11; MW: p = 0.027; C no EE n = 7/6, D7 no EE n = 10/5; MW $p = 0.032$; PS: C n = 10/5, D7 n = 17/7, MW: $p = 0.045$). (l, p) $GABA_A$-mediated feedback inhibition (20 ms ISI) was declined at D1 for PS and at D7 for fEPSP in slices without EE. Slices from D1 with EE displayed stronger inhibition than without EE in PS (PS: C no EE n = 8/6, D1 no EE n = 6/5, D1 EE n = 17/11; MW: no EE: C vs. D1: $p = 0.014$; D1 no EE vs. D1 EE: $p = 0.042$; fEPSP: C no EE n = 8/6, D7 no EE n = 10/5, MW: no EE: C vs. D7: $p = 0.041$). (m, q) $GABA_B$-mediated feedback inhibition (100 ms ISI) of PS was smaller in D1 slices without EE and in controls with EE compared to controls without EE. D1 with EE revealed stronger inhibition than without EE (C no EE n = 7/6, C EE n = 5/4, D1 no EE n = 7/6, D1 EE n = 14/9; MW: no EE: C vs. D1: $p = 0.009$; C no EE vs. EE: $p = 0.028$; D1 no EE vs. EE: $p = 0.029$). fEPSP presented no differences. (n, r) $GABA_B$-mediated feedforward inhibition was lowered at D7 for fPSP and PS (fPSP: C n = 14/8, 7d n = 17/7, MW: $p = 0.035$; PS: C no EE n = 7/6, 7d EE n = 7/5, MW: $p = 0.049$).

disinhibition in treated slices without compared to slices with EE, which we cannot explicitly explain. Interestingly, effects of SO stimulation on fEPSPs were generally weaker compared to PSs (Figure 4(m)), indicating a stronger reduction in perisomatic feedback inhibition.

To test for feedforward inhibition, we first stimulated in the SR before the SO (20 ms ISI). No significant alterations were found in slices from PT-exposed rats, suggesting that $GABA_AR$-mediated disinhibition mostly involves feedback pathways (data not shown). At stimuli interval of 100 ms, fPSP and PS amplitudes were increased at D7, suggesting decreased $GABA_B$-mediated feedforward inhibition (Figure 4(j), N, R; MW: fPSP: all C vs. D7: $p = 0.035$; PS: C no EE vs. D7 EE: $p = 0.049$).

### *Impaired long-term plasticity*

Activation of astrocytes in the BBB-disrupted cortex[28] and early disinhibition as described here predict impaired synaptic plasticity. Indeed, previous studies described an altered induction of LTP in the peri-ischemic cortex.[33,34] We thus induced LTP in hippocampal slices from PT-treated rats and controls. In response to stimulation of the Schaffer collaterals, no significant differences were found in the percentage of slices in which stable LTP was induced in both the CA1 (92% of control, n = 36 slices/16 rats; 83% of D1, n = 24/13; 75% of D7 slices, n = 28/12) and CA3 (due to antidromic stimulation) regions (81% of control, n = 16/4; 92% of D1, n = 12/4; 54% of D7 slices, n = 11/3). Interestingly, while potentiation of both fEPSP and PS amplitude was unaltered at D1, PS potentiation was markedly reduced in both CA1 and CA3 at D7, predominantly in slices presenting EE (Figure 5(b): KW $p = 0.03$, post hoc: C no EE vs. D7 EE, $p = 0.0007$; Figure 5(c): planned comparison one-way ANOVA (pANOVA): within groups $p = 0.021$; GH post hoc: 24 h EE vs. 7d EE $p = 0.049$).

The reduction in potentiation at D7 raises the possibility that synaptic paths are already potentiated at this stage. We thus added a depotentiation protocol (1 Hz, 900 stimuli) after induction of a stable LTP.[26] Stable depotentiation (>5%, last 10′) of PS amplitude was measured in most slices from all groups (73% of control, n = 11/8; 62% of D1, n = 13/7; and 63% of D7 slices, n = 8/5). Population spikes in D7 slices with EE showed significantly less LTP and a strong tendency for impaired depotentiation compared to control slices (Figure 5(d), pANOVA $p = 0.049$, GH post hoc: C no EE vs. 7d EE $p = 0.009$), suggesting a bidirectional impaired long-term plasticity in D7 slices with hyperexcitability. Due to frequent occurrence of SDs in treated groups and differentiation into EE subgroups, the number of slices is rather small. Therefore, the observed tendency of the depotentiation data has to be confirmed in future studies.

### *Molecular analysis supports astrocytic activation and $GABA_AR$ downregulation in the BBB-disrupted hippocampus after PT*

The electrophysiological data suggest that in the BBB-disrupted peri-ischemic hippocampus, network excitability is increased, GABAergic inhibition is reduced, and synaptic plasticity is impaired. We therefore investigated gene expression for pathways potentially associated with these modifications. Consistent with previous reports on gene expression changes in the BBB-disrupted neocortex,[18] microarray data from ipsilateral hippocampus confirmed that genes associated with endothelial dysfunction (including the tight junction protein claudin) and transforming growth factor beta (TGFß) signaling molecules (Smad6, Fos, ACE) were upregulated at 12 h after PT with a subsequent decline at D1 (Figure 6(a), $> 1 \approx 2$-fold change). Genes associated with astrocytic transformation (GFAP, Vimentin and S100A) were up-regulated at D1 after PT. GFAP upregulation was confirmed using qPCR (Figure 6(d), Sham vs. D1, t-test: $p = 1.5 \times 10^{-5}$). In contrast to previous studies on the BBB-disrupted neocortex, we could not confirm downregulation of Kir4.1 mRNA, but did find a significant downregulation of the G protein-coupled inward rectifying potassium channel Kir3.1 at D7 (Figure 6(e), Sham vs. D7; $p = 0.025$). In agreement with the physiological data, microarray results confirmed a clear tendency for downregulation of $GABA_AR$-associated genes 12 h after PT (Figure 6(b)). qPCR confirmed a significant reduction of Gabrb2 and Gabrg1 receptor subunits at D1 (Figure 6(f) to (g), Sham vs. D1, MW: Gabrb2 $p = 0.0028$, Gabrg1 $p = 0.018$). No changes were found in the expression of Gabra4 and Gabrd (data not shown), important for tonic inhibition.[35] With respect to impaired LTP induction at D7, it is interesting to note that we did not find significant changes in the expression of AMPA or NMDA receptor-related genes (Figure 6(c)).

## Discussion

Cortical strokes present a risk factor for epileptogenesis and associated co-morbidities, including cognitive decline.[2] Using the photothrombotic stroke model we show here, that (1) cortical PT is associated with hippocampal BBB dysfunction and vasogenic edema (in contrast to the cytotoxic edema in the ischemic neocortex) and increase in ICP; (2) The BBB-disrupted hippocampus displayed spontaneous seizures in two-thirds of

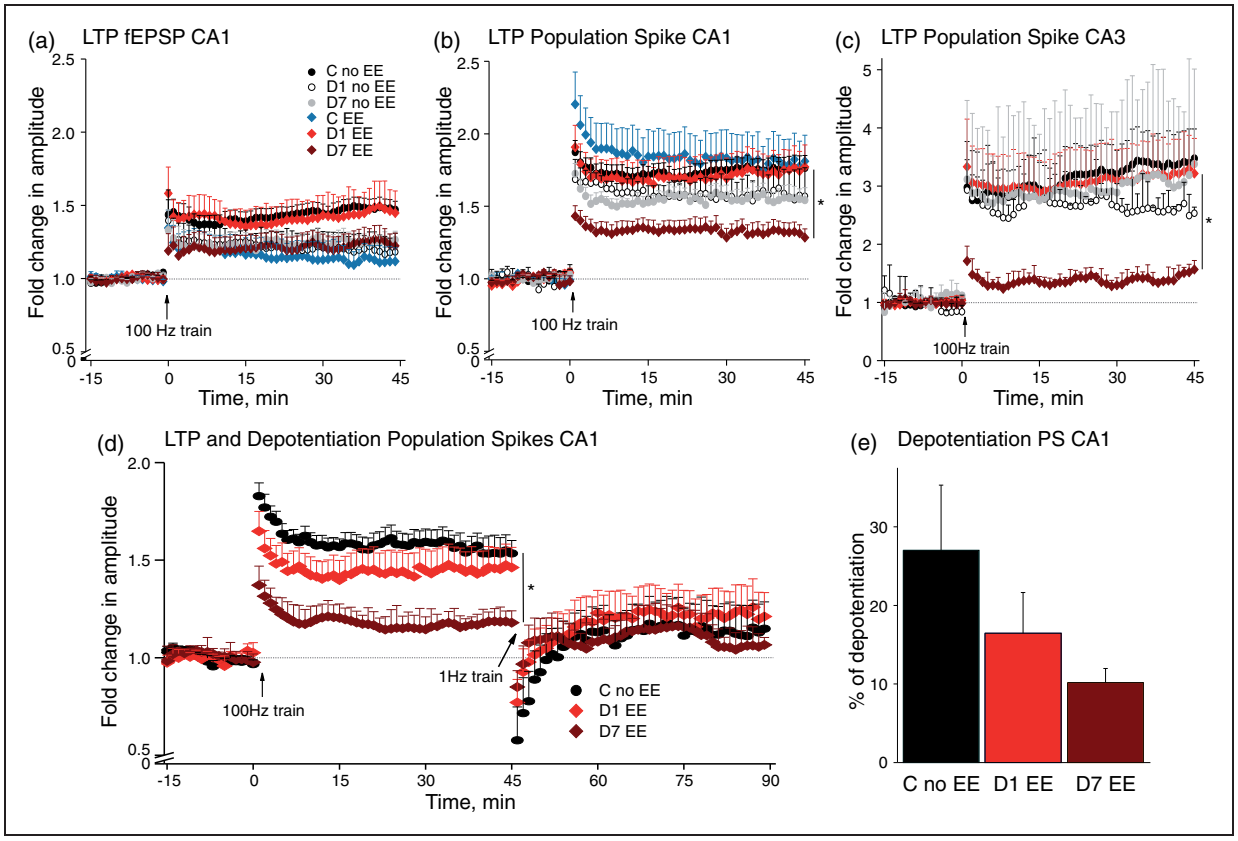


**Figure 5.** Bidirectional long-term plasticity was disturbed in slices from D7 post-stroke presenting with epileptiform activity. (a) Recordings from the dendritic area of CA1 revealed a modest, but insignificant reduction in LTP for all groups except D1 with EE compared to controls without EE. (b, c) LTP in PS recorded from the somatic area of CA1 and CA3 were strongly diminished in D7 slices with EE (b): KW $p = 0.03$, C no EE (n = 25/13) vs. D7 EE (n = 11/6), MW post hoc: $p = 0.0007$; (c): planned comparison one-way ANOVA (pANOVA): within groups $p = 0.021$; Games-Howell (GH) post hoc: 24 h EE (n = 8/3) vs. 7d EE (n = 4/3) $p = 0.049$). (d, e) Investigations of bidirectional long-term plasticity revealed reduced LTP and a tendency for less ability of depotentiation in D7 slices with EE ((d) LTP: pANOVA $p = 0.049$, GH post hoc: C no EE (n = 8/6) vs. 7d EE (n = 3/3) $p = 0.009$).

animals within the first week. In seizing animals, spectral analysis of hippocampal background activity showed a shift from gamma to theta frequency band; (3) Using ex vivo recordings, we confirmed hippocampal hyperexcitability after PT with SR-induced multiple PSs, epileptiform events and SDs already at D1, whereas facilitated afferent fiber activation and increased ES-coupling were recorded at D7; (4) Paired pulse experiments suggested decreased feedback and feedforward inhibition in CA1; (5) Impaired LTP and depotentiation were observed at D7 in slices with epileptiform events; (6) Molecular analysis confirmed upregulation in TGFß- and astrocyte-related genes and downregulation in $GABA_AR$ subunits.

Cortical PT leads to microvascular injury and BBB dysfunction within the first few hours after PT.[36] We measured ICP increase during the same time window, probably due to the associated influx of water molecules into the extracellular space. Concomitantly, Evans blue extravasation, changes in contrast enhanced-T1w, and T2w MRI scans were all consistent with early BBB dysfunction. While experiments in the open window technique (where there is likely no ICP increase) demonstrate BBB dysfunction in normally perfused brain adjacent to the ischemic lesion,[36] the associated edema and increase in ICP may contribute to facilitate further BBB breakdown in more remote areas, perhaps due to reduced cerebral blood flow. This supports craniotomy as a therapeutic option in ischemic stroke to prevent further BBB opening.[37] Other factors like propagating SDs and seizures, free radicals, and upregulation of MMPs may contribute further to BBB disruption, associated vasogenic edema, and astrocytic activation.[4,38–41] Changes in T2w scans and ADC maps followed those of contrast enhanced-T1w scans, suggesting the importance of contrast enhanced imaging for the identification of BBB damage as an early biomarker of tissue prognosis. The early drop in high ADC at 6 h may reflect "dendritic beading" induced by SDs,[4] which can lead to

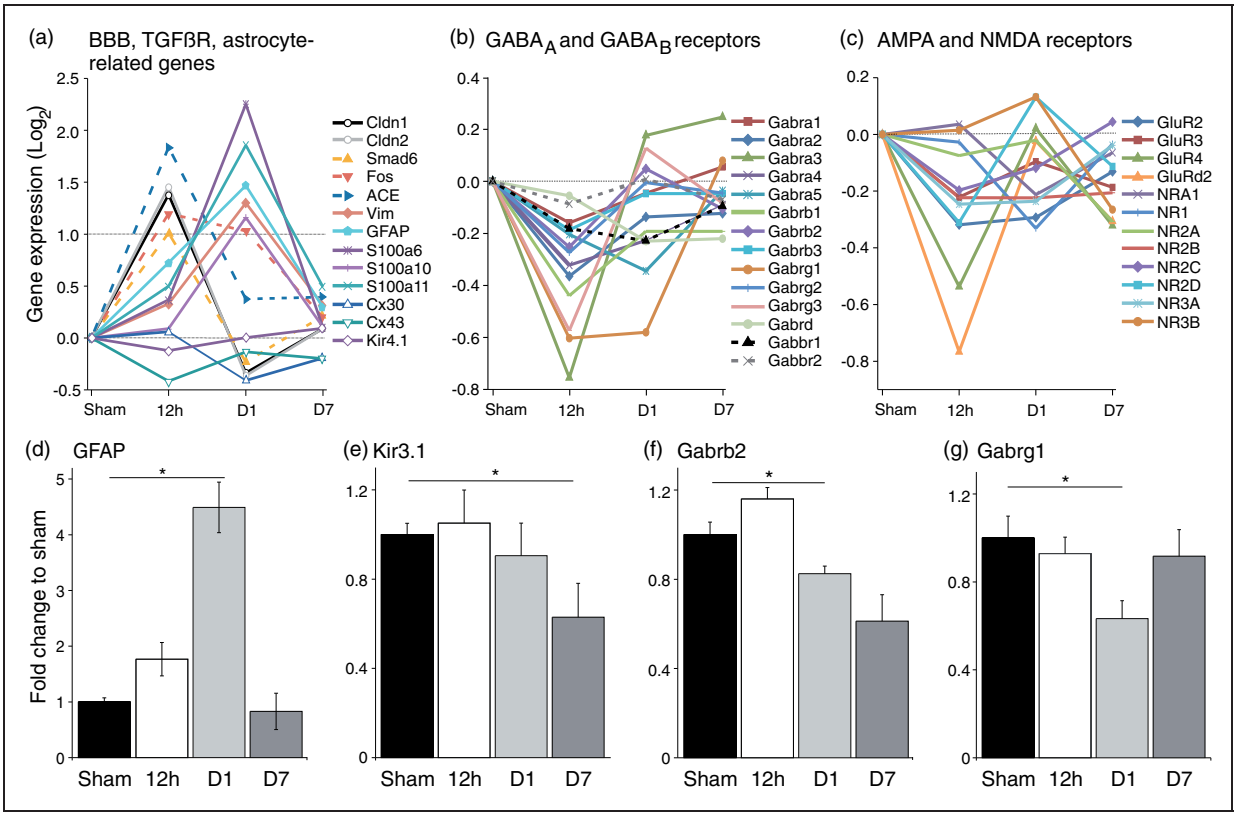


**Figure 6.** Microarrays and qPCR propose possible underlying causes for network hyperexcitability. (a) Genes associated with tight junctions and TGFß receptor signaling showed increased expression at 12 h post-stroke. Astrocyte-related genes were subsequent upregulated with their peak at D1 (n = 1 at 12 h, D1 and D7 for sham and stroke, respectively). (b) Subunits of $GABA_A$Rs presented as a group a tendential downregulation at 12 h. Subunits of $GABA_B$Rs were not altered. (c) Gene expression of postsynaptic NMDA and AMPA receptors was not affected by stroke. (d) qPCR results revealed at D1 a significant increase in expression of GFAP, indicating astrocytic transformation (Sham (n = 6) vs. D1 (n = 5); t-test: $p = 1.5 \times 10^{-5}$). (e) Gene expression of the G protein-coupled inwardly rectifying potassium channel Kir3.1 was lowered at D7, which is associated with $GABA_B$Rs (Sham (n = 6) vs. D7 (n = 4); t-test: $p = 0.025$). (f, g) Beta2 and gamma1 subunits of $GABA_A$Rs were downregulated at D1 post-stroke (each: Sham (n = 6) vs. D1 (n = 5), MW: Gabrb2 $p = 0.0028$, Gabrg1 $p = 0.018$).

dendritic injury and thereby also contribute to aberrant synaptic plasticity.[42] The on-going increase and slow oscillations in ICP are pointing to a yet unknown dynamic process, where effects of uptake and degradation of serum proteins, variations in perfusion pressure, and dynamic changes at the blood–brain interface may be involved. While we cannot exclude a direct damaging effect due to ICP probe insertion (diameter of 1.33 mm), this is unlikely as ICP baseline values are in line with previous reports,[30,31] and the extent of BBB dysfunction was not different than that observed in experiments with no ICP probe.

Importantly, we found BBB damage preceding the onset of spontaneous seizures in intrahippocampal recordings. These results are consistent with recent recordings from a patient with subarachnoid hemorrhage, in which spontaneous focal seizures were recorded using subdural opto-electrodes, were followed and co-localized with neocortical BBB breakdown shown by MR imaging.[43] It is thus suggested that the extent of microvasculopathy, specifically BBB dysfunction, predicts the occurrence of focal cortical seizures. This is consistent with our studies showing focal neocortical epileptiform activity days after experimental BBB opening or brain exposure to serum albumin.[17,44] Unfortunately, due to technical limitations we could not scan animals with implanted electrodes and could not test whether the large differences found between animals in the number of seizures reflects different susceptibility for BBB dysfunction. Hippocampal seizures were not related to the occurrence of periodic lateralized epileptiform discharges (PLEDs) as the duration of single events was much longer and the recurrence rate lower than those observed in stroke patients or rat focal ischemia.[45,46] Seizing animals experienced a significant shift in on-going background activity from gamma to theta, suggesting ictogenic network modifications. Continuous recordings of background activity and dynamic analysis

of power changes after an insult might therefore serve as predictors of seizures. The observed decrease in gamma band may reflect the susceptibility of fast-spiking inhibitory interneurons to metabolic stress and free radicals[38] and predicts impairment of feedforward inhibition required for the generation of high frequencyoscillations.[47,48] Thus, the in vivo monitoring of BBB integrity, ICP, and electrographic activity can all serve as biomarkers indicative of significant, perhaps irreversible, modifications in local brain networks associated with microvascular injury.

Our in vitro recordings confirmed pathological increase in excitability within the peri-ischemic hippocampal network, expressed as SDs and epileptiform discharges. That excitability increase was recorded in vitro under physiological ACSF, denoting it to be due to long-lasting intrinsic modifications and not solely due to transient environmental effects (e.g. increased ICP, reduced blood flow, BBB dysfunction). Interestingly, spontaneous EEs infrequently occurred also in slices from non-treated controls, presumably due to transient inhibitory failure in Wistar rats[49] or due to trauma involved in slice preparation. The occurrence rate and amplitude of EEs were most prominent at D1 after PT, declining at D7. Interestingly, in slices only from D1, EE amplitudes decreased significantly after LTP and even more after the depotentiation protocol, suggesting that high-frequency stimulation potentially reduces uncontrolled network firing. The occurrence of SDs was maximal at D1 and back to control level at D7. Likewise, patients with subarachnoid hemorrhage present their early maximum of SDs in the first two days, preceding the peak of seizure occurrence around day 8,[43,50] consistent with the onset of seizures in our in vivo data. Notably, most SDs were triggered following repetitive stimulation, when oxygen consumption increases and extracellular potassium rises. This could facilitate SD emergence also in remote areas from the core ischemic lesion,[51] when maintenance systems and neural networks are affected. Importantly, the threshold for SD induction and the occurrence of spontaneous SDs are only transiently lowered around D1 and not at D7, consistent with recordings in brain slices from epilepsy patients resected hippocampi and animal models showing increased threshold for SD induction in chronic epilepsy.[4,52,53]

We suggest an early functional GABAergic disinhibition in the BBB-impaired hippocampus around D1 after PT based on the following observations: (1) High-frequency gamma activity, dependent on fast-spiking inhibitory interneurons, was reduced in seizing animals; (2) SR stimulation frequently induced multiple PSs and SO stimulation revealed increased input–output curves and ES-coupling in slices presenting epileptiform activity. Impaired perisomatic inhibition mediated by, e.g. axo-axonic cells, which usually control back-propagating action potentials and are highly vulnerable to metabolic stress,[47,54] may thereby be involved; (3) SO stimulation displayed enhanced-paired pulse ratio. Thus, in addition to impaired perisomatic inhibition, dendrite-targeting inhibition controlling positive fPSPs may be affected as well; (4) Diminished $GABA_{A+B}$-mediated feedback inhibition shown by combined SO-SR stimulation and (5) reduced mRNA expression of $GABA_AR$ subunits support this hypothesis.

We describe changes in the function of the peri-ischemic hippocampal network, lasting for seven days (after BBB permeability was restored), that include: (1) increased afferent volley amplitude to stimulation, suggesting changes in fiber excitability; (2) lower threshold for SR-induced action potential generation and increased ES-coupling. This may be due to changes in intrinsic properties, dendritic morphology, reduced inhibition and excitatory synaptogenesis[55]; (3) diminished functional $GABA_BR$-mediated feedforward inhibition, and (4) limited bidirectional long-term plasticity in slices with epileptiform activity.

For fighting vasogenic edema, albumin uptake into astrocytes is probably essential[40] and seems to involve TGFß signaling leading to alterations in astrocytic properties.[18,56] Our microarray data confirm the involvement of TGFß signaling molecules, like SMAD6, and a subsequent astrocytic transformation in the BBB-disrupted peri-ischemic hippocampus, as previously shown in the cortex in models for BBB dysfunction.[18,28,56] These results are also consistent with studies showing alterations in hippocampal potassium homeostasis after albumin treatment.[20] The cortical astrocytic transformation was also shown to contribute to reduced buffering of glutamate and potassium, leading to their extracellular accumulation in an activity-dependent manner and leading, at least temporarily, to reduction in the spatial specificity of synaptic transmission.[28] Glutamate and GABA could thereby leak from the synaptic cleft and interact with extrasynaptic receptors and presynaptic terminals. The reduced expression of Kir3.1 mRNA indicates reduced sensitivity of $GABA_BR$-mediated signaling enhancing glutamate and GABA release.[57] The augmented potassium accumulation will additionally prolong presynaptic depolarization and increase transmitter release and may cause reduced chloride extrusion from neurons. Interneuronal dysfunction and reduced expression of postsynaptic GABARs can also contribute towards a shift between excitation and inhibition in favor of excitation. Together, these astrocytic-mediated effects on neuronal functions are expected to lower the threshold

for hypersynchronous epileptic discharges and SDs, as well as alter network plasticity.

Our data from LTP experiments present an example for excitation-induced changes in plasticity. Potentiation and depotentiation of PSs were particularly impaired in D7 slices with epileptiform activity. The concomitant reduction in gamma activity recorded in vivo and impaired long-term plasticity in animals presenting epileptiform activity is an interesting characteristic of the affected hippocampal network and may underlie observed cognitive dysfunctions.

In conclusion, the remote and transient opening of the BBB in the hippocampus adjacent to the injured neocortex is associated with reduced inhibition and hyperexcitability of the neuronal network. This is associated with an early and temporary reduction in the threshold for spreading depolarization and epileptiform discharges, and delayed, perhaps lasting changes in plasticity and oscillatory activity, which may contribute to cognitive co-morbidities in patients with focal cortical injury.

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## Authors' contribution

KL: planning and execution of surgery, in vivo measurements, ex vivo electrophysiology and analyses, writing the ms.; LK: development of seizure detection algorithm and analysis of intrahippocampal recordings; SYK: analysis of expression changes; SL: analysis of MRI scans; OP: contributed to MRI and ICP experiments; JFN: contributed to LTP measurements; SS: contributed to electrophysiological control data; DK: planning and supervising the expression analysis, editing the ms.; UH and AF: planning and supervising the experiments and data analysis, writing the ms.

## References

1. Herman S. Epilepsy after brain insult: targeting epileptogenesis. *Neurology* 2002; 59(9 Suppl 5): S21–S26.
2. Bladin CF, Alexandrov AV, Bellavance A, et al. Seizures after stroke: a prospective multicenter study. *Arch Neurol* 2000; 57: 1617–1622.
3. Schoknecht K, David Y and Heinemann U. The blood-brain barrier-gatekeeper to neuronal homeostasis: clinical implications in the setting of stroke. *Semin Cell Dev Biol* 2015; 38: 35–42.
4. Dreier JP and Reiffurth C. The stroke-migraine depolarization continuum. *Neuron* 2015; 86: 902–922.
5. Friedman A and Heinemann U. Role of blood-brain barrier dysfunction in epileptogenesis. In: Noebels JL, Avoli M, Rogawski MA, et al. (eds) *Jasper's basic mechanisms of the epilepsies*. Bethesda, MD: National Center for Biotechnology Information (US) Michael A Rogawski, Antonio V Delgado-Escueta, Jeffrey L Noebels, Massimo Avoli and Richard W Olsen, 2012.
6. Tanaka E, Yamamoto S, Kudo Y, et al. Mechanisms underlying the rapid depolarization produced by deprivation of oxygen and glucose in rat hippocampal CA1 neurons in vitro. *J Neurophysiol* 1997; 78: 891–902.
7. Muller M and Somjen GG. Na(+) and K(+) concentrations, extra- and intracellular voltages, and the effect of TTX in hypoxic rat hippocampal slices. *J Neurophysiol* 2000; 83: 735–745.
8. Erdemli G, Xu YZ and Krnjevic K. Potassium conductance causing hyperpolarization of CA1 hippocampal neurons during hypoxia. *J Neurophysiol* 1998; 80: 2378–2390.
9. Fleidervish IA, Gebhardt C, Astman N, et al. Enhanced spontaneous transmitter release is the earliest consequence of neocortical hypoxia that can explain the disruption of normal circuit function. *J Neurosci* 2001; 21: 4600–4608.
10. Vyskocil F, Kritz N and Bures J. Potassium-selective microelectrodes used for measuring the extracellular brain potassium during spreading depression and anoxic depolarization in rats. *Brain Res* 1972; 39: 255–259.
11. Heinemann U and Lux HD. Ceiling of stimulus induced rises in extracellular potassium concentration in the cerebral cortex of cat. *Brain Res* 1977; 120: 231–249.
12. Hinzman JM, DiNapoli VA, Mahoney EJ, et al. Spreading depolarizations mediate excitotoxicity in the development of acute cortical lesions. *Exp Neurol* 2015; 267: 243–253.
13. Strong AJ, Venables GS and Gibson G. The cortical ischaemic penumbra associated with occlusion of the middle cerebral artery in the cat: 1. Topography of changes in blood flow, potassium ion activity, and EEG. *J Cereb Blood Flow Metab* 1983; 3: 86–96.
14. Hossmann KA. Viability thresholds and the penumbra of focal ischemia. *Ann Neurol* 1994; 36: 557–565.
15. Shin HK, Dunn AK, Jones PB, et al. Vasoconstrictive neurovascular coupling during focal ischemic depolarizations. *J Cereb Blood Flow Metab* 2006; 26: 1018–1030.

16. Woitzik J, Pinczolits A, Hecht N, et al. Excitotoxicity and metabolic changes in association with infarct progression. *Stroke* 2014; 45: 1183–1185.
17. Friedman A, Kaufer D and Heinemann U. Blood-brain barrier breakdown-inducing astrocytic transformation: novel targets for the prevention of epilepsy. *Epilepsy Res* 2009; 85: 142–149.
18. Cacheaux LP, Ivens S, David Y, et al. Transcriptome profiling reveals TGF-beta signaling involvement in epileptogenesis. *J Neurosci* 2009; 29: 8927–8935.
19. Stoll G, Kleinschnitz C, Meuth SG, et al. Transient widespread blood-brain barrier alterations after cerebral photothrombosis as revealed by gadofluorine M-enhanced magnetic resonance imaging. *J Cereb Blood Flow Metab* 2009; 29: 331–41.
20. Lapilover EG, Lippmann K, Salar S, et al. Peri-infarct blood-brain barrier dysfunction facilitates induction of spreading depolarization associated with epileptiform discharges. *Neurobiol Dis* 2012; 48: 495–506.
21. Dorr A, Sled JG and Kabani N. Three-dimensional cerebral vasculature of the CBA mouse brain: a magnetic resonance imaging and micro computed tomography study. *Neuroimage* 2007; 35: 1409–1423.
22. Watson BD, Dietrich WD, Busto R, et al. Induction of reproducible brain infarction by photochemically initiated thrombosis. *Ann Neurol* 1985; 17: 497–504.
23. Bar-Klein G, Cacheaux LP, Kamintsky L, et al. Losartan prevents acquired epilepsy via TGF-beta signaling suppression. *Ann Neurol* 2014; 75: 864–75.
24. Karhunen H, Bezvenyuk Z, Nissinen J, et al. Epileptogenesis after cortical photothrombotic brain lesion in rats. *Neuroscience* 2007; 148: 314–324.
25. Bragin A, Jando G, Nadasdy Z, et al. Gamma (40-100 Hz) oscillation in the hippocampus of the behaving rat. *J Neurosci* 1995; 15(1 Pt 1): 47–60.
26. Behrens CJ, van den Boom LP, de Hoz L, et al. Induction of sharp wave-ripple complexes in vitro and reorganization of hippocampal networks. *Nat Neurosci* 2005; 8: 1560–1567.
27. Taube JS and Schwartzkroin PA. Mechanisms of long-term potentiation: EPSP/spike dissociation, intradendritic recordings, and glutamate sensitivity. *J Neurosci* 1988; 8: 1632–1644.
28. David Y, Cacheaux LP, Ivens S, et al. Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis? *J Neurosci* 2009; 29: 10588–10599.
29. Zhao S and Fernald RD. Comprehensive algorithm for quantitative real-time polymerase chain reaction. *J Comput Biol* 2005; 12: 1047–1064.
30. Guild SJ, McBryde FD and Malpas SC. Recording of intracranial pressure in conscious rats via telemetry. *J Appl Physiol* 2015; 119: 576–581.
31. Murtha LA, McLeod DD, Pepperall D, et al. Intracranial pressure elevation after ischemic stroke in rats: cerebral edema is not the only cause, and short-duration mild hypothermia is a highly effective preventive therapy. *J Cereb Blood Flow Metab* 2015; 35(4): 592–600.
32. Engel D, Schmitz D, Gloveli T, et al. Laminar difference in GABA uptake and GAT-1 expression in rat CA1. *J Physiol* 1998; 512(Pt 3): 643–649.
33. Hagemann G, Redecker C, Neumann-Haefelin T, et al. Increased long-term potentiation in the surround of experimentally induced focal cortical infarction. *Ann Neurol* 1998; 44: 255–258.
34. Mittmann T and Eysel UT. Increased synaptic plasticity in the surround of visual cortex lesions in rats. *Neuroreport* 2001; 12: 3341–3347.
35. Ferando I and Mody I. In vitro gamma oscillations following partial and complete ablation of delta subunit-containing GABAA receptors from parvalbumin interneurons. *Neuropharmacology* 2015; 88: 91–98.
36. Schoknecht K, Prager O, Vazana U, et al. Monitoring stroke progression: in vivo imaging of cortical perfusion, blood-brain barrier permeability and cellular damage in the rat photothrombosis model. *J Cereb Blood Flow Metab* 2014; 34: 1791–1801.
37. Zweckberger K, Juettler E, Bosel J, et al. Surgical aspects of decompression craniectomy in malignant stroke: review. *Cerebrovasc Dis* 2014; 38: 313–323.
38. Dirnagl U, Iadecola C and Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci* 1999; 22: 391–397.
39. Gursoy-Ozdemir Y, Qiu J, Matsuoka N, et al. Cortical spreading depression activates and upregulates MMP-9. *J Clin Invest* 2004; 113: 1447–1455.
40. Kuroiwa T, Cahn R, Juhler M, et al. Role of extracellular proteins in the dynamics of vasogenic brain edema. *Acta Neuropathol* 1985; 66: 3–11.
41. Rosenberg GA and Yang Y. Vasogenic edema due to tight junction disruption by matrix metalloproteinases in cerebral ischemia. *Neurosurg Focus* 2007; 22: E4.
42. Risher WC, Ard D, Yuan J, et al. Recurrent spontaneous spreading depolarizations facilitate acute dendritic injury in the ischemic penumbra. *J Neurosci* 2010; 30: 9859–9868.
43. Winkler MK, Chassidim Y, Lublinsky S, et al. Impaired neurovascular coupling to ictal epileptic activity and spreading depolarization in a patient with subarachnoid hemorrhage: possible link to blood-brain barrier dysfunction. *Epilepsia* 2012; 53(Suppl 6): 22–30.
44. Seiffert E, Dreier JP, Ivens S, et al. Lasting blood-brain barrier disruption induces epileptic focus in the rat somatosensory cortex. *J Neurosci* 2004; 24: 7829–7836.
45. Hartings JA, Williams AJ and Tortella FC. Occurrence of nonconvulsive seizures, periodic epileptiform discharges, and intermittent rhythmic delta activity in rat focal ischemia. *Exp Neurol* 2003; 179: 139–149.
46. Neufeld MY, Vishnevskaya S, Treves TA, et al. Periodic lateralized epileptiform discharges (PLEDs) following stroke are associated with metabolic abnormalities. *Electroencephalogr Clin Neurophysiol* 1997; 102: 295–298.
47. Kann O, Papageorgiou IE and Draguhn A. Highly energized inhibitory interneurons are a central element for information processing in cortical networks. *J Cereb Blood Flow Metab* 2014; 34: 1270–1282.
48. Zemankovics R, Veres JM, Oren I, et al. Feedforward inhibition underlies the propagation of cholinergically

induced gamma oscillations from hippocampal CA3 to CA1. *J Neurosci* 2013; 33: 12337–12351.
49. Penttonen M, Nurminen N, Miettinen R, et al. Ultra-slow oscillation (0.025 Hz) triggers hippocampal after-discharges in Wistar rats. *Neuroscience* 1999; 94: 735–743.
50. Dreier JP, Major S, Pannek HW, et al. Spreading convulsions, spreading depolarization and epileptogenesis in human cerebral cortex. *Brain* 2012; 135(Pt 1): 259–275.
51. Pomper JK, Haack S, Petzold GC, et al. Repetitive spreading depression-like events result in cell damage in juvenile hippocampal slice cultures maintained in normoxia. *J Neurophysiol* 2006; 95: 355–368.
52. Maslarova A, Alam M, Reiffurth C, et al. Chronically epileptic human and rat neocortex display a similar resistance against spreading depolarization in vitro. *Stroke* 2011; 42: 2917–2922.
53. Tomkins O, Friedman O, Ivens S, et al. Blood-brain barrier disruption results in delayed functional and structural alterations in the rat neocortex. *Neurobiol Dis* 2007; 25: 367–377.
54. Dugladze T, Schmitz D, Whittington MA, et al. Segregation of axonal and somatic activity during fast network oscillations. *Science* 2012; 336: 1458–1461.
55. Weissberg I, Wood L, Kamintsky L, et al. Albumin induces excitatory synaptogenesis through astrocytic TGF-beta/ALK5 signaling in a model of acquired epilepsy following blood-brain barrier dysfunction. *Neurobiol Dis* 2015; 78: 115–125.
56. Ivens S, Kaufer D, Flores LP, et al. TGF-beta receptor-mediated albumin uptake into astrocytes is involved in neocortical epileptogenesis. *Brain* 2007; 130(Pt 2): 535–547.
57. Lujan R and Aguado C. Localization and targeting of GIRK channels in mammalian central neurons. *Int Rev Neurobiol* 2015; 123: 161–200.

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

## Publikationen

Epileptiform activity and spreading depolarization in the blood-brain barrier-disrupted peri-infarct hippocampus are associated with impaired GABAergic inhibition and synaptic plasticity. **Lippmann K**, Kamintsky L, Kim SY, Lublinsky S, Prager O, Nichtweiß J, Salar S, Kaufer D, Heinemann U, Friedman A.; J Cereb Blood Flow Metab. 2016, in press, IF: 5.41

Synaptic plasticity in area CA1 of rat hippocampal slices following intraventricular application of albumin. Salar S, Lapilover EG, Müller J, Hollnagel JO, **Lippmann K**, Friedman A, Heinemann U. Neurobiol Dis. 2016; 91:155-165; IF: 5.08

Differential participation of pyramidal cells in generation of spontaneous sharp wave-ripples in the mouse subiculum in vitro. Maslarova A, **Lippmann K**, Salar S, Rösler A, Heinemann U. Neurobiol Learn Mem. 2015; 125:113-119; IF: 3.65

Blood-brain barrier dysfunction can contribute to pharmacoresistance of seizures. Salar S, Maslarova A, **Lippmann K**, Nichtweiss J, Weissberg I, Sheintuch L, Kunz WS, Shorer Z, Friedman A, Heinemann U. Epilepsia 2014; 55:1255-63; IF: 4.57

Peri-infarct blood-brain barrier dysfunction facilitates induction of spreading depolarization associated with epileptiform discharges. Lapilover EG, **Lippmann K**, Salar S, Maslarova A, Dreier JP, Heinemann U, Friedman A. Neurobiol Dis. 2012; 48:495-506; IF: 5.62

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