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

DISSERTATION

Consequences of blood-brain barrier disruption

zur Erlangung des akademischen Grades
Medical Doctor – Doctor of Philosophy in Medical Neurosciences
(MD/PhD in Medical Neurosciences)

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Charité – Universitätsmedizin Berlin

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1. Abstract

The blood-brain barrier (BBB) enables the central nervous system to maintain a unique extracellular environment. Breakdown of the BBB is reported in numerous neurological diseases. However, consequences of BBB breakdown and its possible involvement in pathogenesis are not known. In my thesis I investigated the molecular and physiological consequences of BBB opening and the immediate and delayed effects on cortical function and structure.

As part of my thesis we established a rat model for focal, long lasting BBB disruption by in-vivo application of bile salts to the sensory-motor neocortex. We showed that penetration of serum albumin into the brain's extracellular space initiated a damage cascade resulting in chronic cortical hyperexcitability and delayed functional impairment. BBB disruption lead to rapid uptake of albumin by astrocytes, followed by alteration of protein expression and astrocytic functions. Downregulation of the astrocytic inward rectifier potassium channel Kir 4.1 was associated with compromised buffering of extracellular potassium. We showed that neuronal activity resulted in excess accumulation of potassium in the extracellular space and subsequent hyperexcitability. Several days after BBB breakdown or direct exposure of the cortex to albumin a focus of epileptiform activity developed. One month after treatment we found that the persisting epileptiform activity was associated with neuronal cell loss and deteriorated motor skills. Uptake of albumin into astrocytes could be prevented by blockade of transforming growth factor β receptors (TGF-βR), which also reduced the incidence of epileptogenesis following in-vivo exposure of the cortex to albumin. On the basis of our findings we propose that astrocytic uptake of albumin underlies BBB related epileptogenesis. We point out TGF-βRs as a potential therapeutic target for prevention of secondary epilepsies and neurodegeneration.

2. Introduction

Neuronal function is determined by the composition of the extracellular microenvironment [1]. The central nervous system (CNS) maintains a secluded, highly specialized environment in the presence of a rich blood supply and close juxtaposition of capillaries and parenchymal cells. This is achieved by the complex structural and functional unit of the BBB, which shields the brain effectively from blood ions, metabolites, proteins and cells and on the other hand provides sufficient metabolic supply [2]. The BBB consists of endothelial cells connected by tight junctions, surrounding pericytes and astrocytic processes which sheath the vessel from the parenchymal side and lengthen diffusion distance between endothelium and brain parenchyma [3,4].

A breakdown of BBB function is seen under various common neurological pathologies such as trauma, epilepsy, stroke, inflammation as well as neurodegenerative diseases [5,6]. However, the consequences of increased vascular permeability for CNS function are poorly understood. It has been recently speculated that BBB disruption may be a key trigger event leading to focal neocortical epilepsy [7,8]. In fact, patient studies indicated that regions of BBB leakage spatially corresponded with abnormal EEG activity [9,10]. However, due to the complexity of the clinical presentation in neurological patients it is difficult to isolate specific cause and effect.

Aims

The aim of this project was to investigate the consequences of primary BBB opening for cortical function and to understand the processes involved in these alterations. To study the changes induced by BBB dysfunction, our goals were (1) to establish an appropriate rat model of long lasting BBB opening with minimal damage to other brain components; (2) to study changes in cortical function and structure, using electrophysiological, morphological and molecular methods; (3) among the broad spectrum of processes following BBB opening to identify the most critical factors underlying the induced changes and to understand possible damage mechanisms.

3. Methods

We established a model for focal, long lasting BBB disruption in adult, male wistar rats (150-250g): A round craniotomy (4 mm diameter) is applied above the sensory-motor neocortex, the dura mater removed and the underlying cortex exposed for 30 minutes to artificial cerebrospinal fluid (aCSF) containing the bile salts dehydrocholate (DHC) or deoxycholate (DOC, 1–2 mM), which have the potency to open tight junctions in brain capillaries [11]. Sham operated rats were treated with aCSF without the bile salts.

Two approaches were used to estimate BBB integrity in our model: (1) Injection of Evans blue (EB) solution into the peritoneum [12]. EB binds to serum albumin in the blood. Leakage into the brain parenchyma indicated BBB opening and was measured by spectroscopy or microscopy (Fig. 1). (2) In-vivo magnetic resonance imaging (MRI) [13]: T1 - weighed images were obtained with a 7 tesla small animal scanner before and after the injection of the BBB non-permeable agent gadolinium-DTPA (Gd-DTPA).

Figure 1 (modified from [12] Fig. 1 and [13], Fig. 1). BBB disruption following cortical bile salt application, indicated by Evans blue extravasation macroscopically (**a, b**) and microscopically (**c**, EB displays red fluorescence).

For electrophysiological brain activity measurements coronal brain slices (400 μm) were prepared in ice cold aCSF and kept in an interface chamber. Extra- and intracellular recordings were performed with glass electrodes in layer 2-5 of the neocortex; stimulation pulses were delivered to the border between white and gray matter by bipolar platinum electrodes [12]. Extracellular potassium concentration ($[K^+]_o$) was measured by ion sensitive microelectrodes (ISME), which were manufactured in our laboratory [14]. For local application of potassium we used double barreled iontophoresis electrodes [14].

To study cellular uptake properties, we prepared brain slices and applied fluorescently labeled albumin, dextran (70 kDa) or ovalbumin to the bathing solution. After variable times of incubation, slices were washed with oxygenated aCSF and fixated for further analysis.

For histological examination tissue was fixed and cut to 10-50 μm sections [12,15]. Nuclear DAPI and hematoxylin/eosin stainings were performed according to routine procedures. For immunohistochemistry we incubated slices with primary antibodies against GFAP, MAP2, potassium channel Kir 4.1 and GAD; signal detection was achieved using secondary biotinylated antibodies followed by a standard ABC-DAB development or Alexa Fluor 568 labeled secondary antibodies [12-14]. Digital images were obtained by epifluorescent or normal light microscopy. Image analysis and cell counting was performed manually by an unaware observer or automatized [13,15]. To verify double-labeling, we examined slices by confocal microscopy [14]. For dendritic branch counting experiments we performed iontophoretical biotin injection into acute brain slices in the interface chamber and allowed 8 h incubation for transport of biotin along the dendritic tree before fixation [13].

Gene expression was tested by quantitative reverse transcriptase-PCR (rt-PCR) by real-time kinetic analysis. Presented are percentages of gene expression levels of the treated hemisphere, as compared to the untreated hemisphere as control [14].

To evaluate motor skills we used a variety of neurological tests adapted to rats: Beam crossing, beam balancing, circle exit test, seeking behavior test and reflex evaluation [13,16]. For behavioural testing we tracked and analyzed animal movement in a 60x60x60 cm square arena [13], and tested decision making in a Y-maze, equipped with a guillotine door midways [13,17].

For pharmacological experiments drugs were added to the aCSF. To block potassium currents, bariumchloride in concentrations of 100 μM and 2 mM was dissolved in sulfate-free aCSF and applied to brain slices. To prevent neuronal potassium release secondary to Ba^{2+} induced neuronal depolarization, we blocked during these experiments synaptic transmission by 30 μM APV and 30 μM CNQX, $GABA_A$ receptors by 10 μM bicuculline and action potentials by 1 μM tetrodotoxin [18]. Bovine

serum albumin (Cohn fraction V, 96 % purity) was added to the aCSF at concentration between 0.004 – 0.4 mM, standard dose in most experiments was 0.1 mM, corresponding to 25% rat serum concentration [14]. Osmolarity of the albumin solutions was in a physiological range (308-312 mOsmol; Cryoscopic osmometer Osmomat 030, Gonotec, Berlin).

In statistical analyses groups were compared by the non-parametric U Mann–Whitney and paired Wilcoxon Signed Rank tests. Failure to perform the beam crossing test and percentage of slices displaying epileptiform activity was compared using the Pearson's χ^2 test. Statistical significance was assumed with p values <0.05. Values are presented as mean± standard error of the mean (SEM).

4. Results

Focal cortical BBB disruption
Cortical application of bile salts induced a focal BBB disruption in all cortical layers 1 h after treatment (n = 8 animals), which was still present after 6 days, but not after 16 days (n = 11 animals), as indicated by EB leakage into the brain parenchyma (Fig. 1 in this manuscript and [12], Fig. 1) and Gd-DTPA enhancement in MRI ([13], Fig. 1). Histological examination 24 h after treatment showed that no structural cortical damage, bleeding or cell loss was induced by the treatment. However, a marked increase of GFAP expression indicated astrocytic activation ([12], Fig. 7; [14], Fig. 5 g-i). Intra- and extracellular electrophysiological recordings from brain slices during exposure to DOC and DHC showed no direct effect of the bile salts on neuronal function in the concentration range used for BBB disruption ([12], Fig. 2).

Epileptogenesis after BBB disruption
We tested the effects of BBB disruption on cortical function by electrophysiological recordings in acute cortical brain slice preparations obtained at different time points after bile salt application (2 h to 49 d). Regular cortical activity, indicated by brief population spikes in response to single electrical stimulation and the absence of spontaneous activity was recorded in the majority of slices from control (52 of 61 slices, 25 animals) and sham-

operated (37 of 41 slices, 13 animals) animals. From the fourth day and up to 49 days after BBB opening population spikes were followed by paroxysmal, all-or-none, long-duration (100–500 msec) field potentials (100 out of 139 slices, 51 animals), which propagated within the region of treatment (Fig. 2 below and [12], Fig. 3; [14], Fig. 1 c-f). In a subset of slices, these events occurred spontaneously without stimulation. Analysis of the underlying transmitter systems revealed contribution of excitatory glutamatergic signaling via both AMPA/KA and NMDA receptors in the presence of increased GABAergic inhibition ([12], Fig.4 and 5).

2 days　　　　　　　**4 days**　　　　　　　**49 days**

0.4 mV
0.2 s
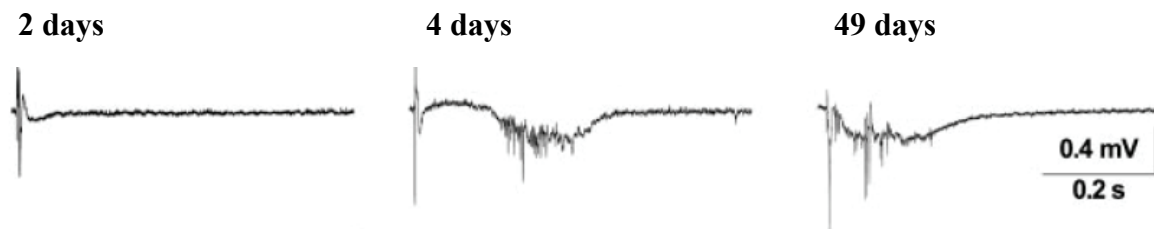
Figure 2 (modified from [12], Fig. 3): Representative traces showing evoked cortical activity at different times after BBB treatment (extracellular field potential recordings from brain slices).

We hypothesized that hyperexcitability after BBB disruption results from cortical exposure to serum components. Rat serum, denatured serum or aCSF containing albumin at 25% of serum concentration all induced paroxysmal hypersynchronous activity when applied directly to the cortex (67% of slices, n = 18; 77%, n = 13 and 72%, n = 50, respectively). Cortical exposure to a solution which contained electrolytes at serum concentrations had no effect on cortical function (n = 5 slices; [12], Fig. 6 a,b). This epileptogenic effect of albumin was not transmitted by BBB opening, since no Evans blue extravasation after *in-vivo* albumin application was detected ([12], Fig. 6 d). Additionally, hyperexcitability could be induced by albumin exposure of the brain slice preparation, in which no functional BBB exists ([14], Fig. 3).

Astrocytic albumin uptake via TGF-βR

To investigate the action of albumin we traced the protein in the cortex by two different methods: (1) In vivo by EB labeling of endogenous albumin after BBB disruption and (2) by exposure of brain slices to FITC-labeled albumin. In both cases albumin was observed in the extracellular space, but also inside cellular elements (see Fig. 3 below,

arrows in a and c, and in [14], Fig. 2 a-d). Albumin was taken up by cells in a time course of minutes and subsequently concentrated in the nucleus (Fig. 3 b of this manuscript and [14], Fig. 2 b,c,g). This process was highly specific to albumin: Neither fluorescent dextran nor the protein ovalbumin, both of similar molecular weight, were taken up by cortical parenchymal cells (Fig. 3 c and [14], Fig. 2 c,d).
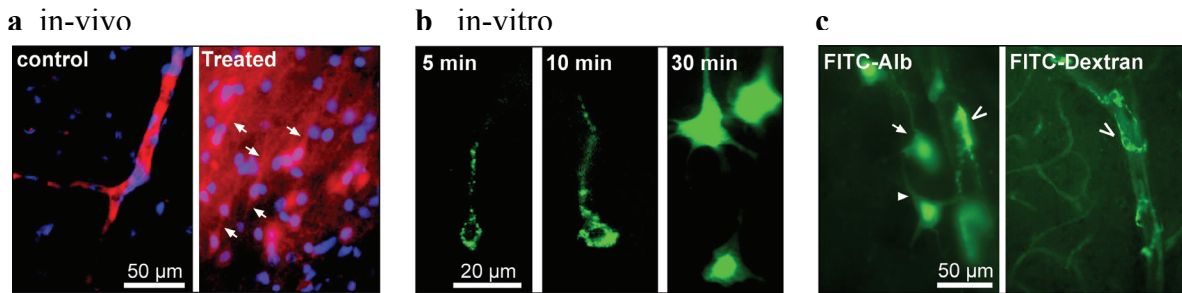
Figure 3 (modified from [14], Fig. 4): Cellular albumin uptake. (**a**) Microsection showing albumin-Evans blue complex inside cellular elements 6 h after BBB disruption (right panel, arrows), in comparison to an intact BBB (left panel). (**b**) Time course of uptake during exposure of brain slices to FITC-albumin. (**c**) Specific uptake of albumin by brain parenchymal cells (left panel, arrows), while perivascular cells (open arrows) display unspecific uptake of both dextran and albumin.

Immunohistochemistry revealed that astrocytes, but not neurons display albumin internalization ([14], Fig. 2 e,f). Competition experiments of FITC-albumin with non-labeled albumin for cellular uptake suggested saturable uptake kinetics ([14], Fig. 2 h). Specificity and kinetics suggested a receptor mediated uptake mechanism. Transforming growth factor β receptor (TGF-βR) was recently found to function as an albumin binding protein in lung endothelial cells [19]. We tested the hypothesis that TGF-βRs are involved in astrocytic albumin uptake. Incubation of slices with TGF-βR type 1 blocker or TGF-βR type 2 antibody prior to and during FITC-albumin exposure both reduced the number of FITC-albumin positive cells in a dose dependent manner ([14], Fig. 4 a-d). Moreover, application of TGF-βR blockers in-vivo reduced the frequency of albumin induced epileptogenesis one week following treatment ([14], Fig. 4 e,f).

Astrocytic dysfunction underlies neuronal hyperexcitability
In early stages (24 - 48 hours) after BBB disruption and before the onset of epileptiform activity, a marked astrocytic activation is present in our model (see above and [12], Fig. 7; [14], Fig. 5 g-i). Astrocytes serve an important role in maintenance of the neuronal

microenvironment, particularly by buffering rises of extracellular potassium concentration ($[K^+]_o$) during neuronal activity [20]. We tested the hypothesis that astrocytic K^+ buffering is impaired during the period of epileptogenesis and may therefore be involved in the generation of neuronal hyperexcitability. In recordings of $[K^+]_o$ with ion sensitive microelectrodes (ISME), we found a slowed clearance of potassium, released during tetanic electrical stimulation ([14], Fig. 5 a-c). This was due to a reduction of potassium inward rectifier conductance, as revealed by a reduced effect of low concentrations of barium on iontophoretically induced local increases of $[K^+]_o$ ([14], Fig. 5 d-f). Indeed, immunostainings and rt-PCR showed downregulation of the Kir 4.1 potassium inward rectifier channel subunit ([14], Fig. 5 g-i).

We tested whether reduced potassium buffering 24 hrs after BBB disruption has consequences on neuronal function. Since in the resting brain slice preparation the ionic milieu is determined by the perfusion solution, we created a steady state balance between potassium release and uptake during stimulation trains at low frequencies (0.4 – 0.67 Hz). In fact, in the BBB treated slices potassium accumulated to higher levels (see Fig. 4 a below). This was accompanied by generation of NMDA-R dependent depolarizing after-potentials in intracellular recordings, as well as in field potential recordings (Fig. 4 b), indicating that increased excitability of the network correlates with activity dependent dysbalance of the neuronal microenvironment during epileptogenesis (also in [14], Fig. 6).
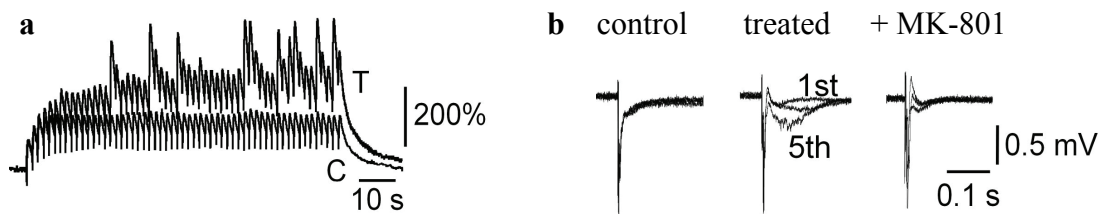
Figure 4 (modified from [14], Fig. 6). (**a**) Recordings of $[K^+]_o$ show excess K^+ accumulation during repetitive stimulation 24 h following albumin exposure. (**b**) Superimposed traces of evoked population activity at different times during the same train demonstrate that elevated $[K^+]_o$ in the albumin treated cortex is associated with neuronal after-potentials, sensitive to the NMDA-R blocker MK-801.

Long-term changes following cortical albumin exposure

To investigate long-term consequences of BBB disruption, we compared functional and structural changes in acute and chronic stages (one day up to one month post treatment). During the first 2 weeks after treatment, MRI measurements showed an increase of cortical volume in the presence of an opened BBB, indicating vasogenic edema. In chronic stages (one month post treatment), the BBB had recovered and cortical volume appeared to be reduced ([13], Fig. 1 and 4 a). This was associated with loss of both excitatory and inhibitory neurons in chronic stages, reduced dendritic branching and an increased number of astrocytes ([13], Fig 3 b-d). Propagation velocity of epileptiform activity in the treated cortex was significantly faster one month after BBB disruption ([13], Fig. 2), and the threshold for induction of cortical spreading depression (CSD) was increased ([13], Fig. 3). To assess whether these changes were functionally significant, we performed neurological and behavioral tests. In fact, chronic animals displayed deterioration of motor skills, while changes in behavior and cognition were not noticed ([13], Fig. 5 and 6).

5. Discussion

The main goal of my thesis research was to investigate the consequences of primary cortical BBB disruption and to understand the underlying mechanisms. We found that (1) spatially confined BBB disruption leads to the development of focal epileptiform activity in the neocortex. (2) Uptake of serum albumin by cortical astrocytes is a crucial factor in the cascade of epileptogenesis and is regulated by TGF-βR. (3) Astrocytic activation and dysfunction is an early change during the period of epileptogenesis, resulting in an altered neuronal microenvironment and enhanced excitability. (4) Long-term changes following BBB breakdown include neuronal cell loss and deterioration of cortical function.

Our study shows for the first time that primary BBB opening leads to delayed development of epileptiform activity (see Fig. 2 in this manuscript). Direct action of the bile salts as an underlying mechanism is unlikely, since the concentrations we used (<3 mM) had no acute effect on neuronal function ([12], Fig. 2), and cortical exposure to

serum albumin induced similar changes ([14], Fig. 1 c). Our model shares characteristics with chronic animal models of seizure (e.g. the chronically injured cortex [21] or pilocarpine treatment [22]) with a several day window of regular activity before the onset of hypersynchronous activity. This activity clearly differs from normal activity and is characterized by all-or-none occurrence of depolarizing, propagating events in response to threshold stimulation (shown in [14], Fig. 1 d and [13], Fig. 2 c,d). While EEG recordings are still required to characterize the in vivo correlates of the paroxysmal responses observed in vitro, spontaneous seizures observed in few of the BBB-treated rats support the relevance of our model for the investigation of epileptogenesis.

We demonstrated that albumin is specifically taken up into astrocytes (Fig. 3 above, also in [14], Fig. 2). Blockade of TGF-βRs prevented albumin uptake by astrocytes (see [14], Fig. 4). Modification of albumin uptake by TGF-βRs was also reported in kidney tubule [23] and lung endothelial cells [19], and these receptors are expressed on astrocytes [24]. However, we cannot clarify yet, whether the uptake is mediated directly by the receptors, e.g. by albumin binding to the receptor (as suggested by [19]) and internalization, or if TGF-β signaling modulates a distinct, but yet specific uptake mechanism. In addition, future experiments are awaited to investigate whether the effect on astrocytes is mediated by TGF-βR signaling (with albumin as a possible agonist), or by direct interactions of proteins within the cell. However, since TGF-βR blockade reduced the frequency of albumin mediated epileptogenesis in our model, we conclude that these receptors play an integral role in the pathogenesis after BBB disruption and suggest this pathway as a promising target for therapeutic intervention.

In our model, astrocytic activation was associated with compromised buffering of extracellular potassium on the basis of reduced barium sensitive potassium inward rectifier current (I_{KIR}, see in [14], Fig. 5). I_{KIR} plays a crucial role in spatial buffering of $[K^+]_o$ by astrocytes [25]. Our findings are in line with previous studies showing a loss of I_{KIR} in reactive astrocytes after traumatic brain injury [26], freeze lesion [27] and in hippocampi from epileptic patients [28]. Downregulation of the potassium inward rectifier channels subunit Kir 4.1 (see [14], Fig. 5 g-i), which is expressed mostly on astrocytes at processes surrounding synapses and blood vessels [29,30] confirms our results and may be an anatomical correlate of reduced I_{KIR}.

Deficient regulation of $[K^+]_o$ was observed in the chronic epileptic brain and suggested as a mechanism of epileptogenesis [31-33]. In our model reduction of potassium buffering was strongest 24 h after BBB disruption, at a time point where we recorded apparently normal neuronal activity in response to single stimulation. During repetitive stimulation neuronally released K^+ accumulated in the extracellular space and resulted in generation of NMDA-R dependent afterdepolarizations (Fig. 4 above, see also [14], Fig. 5 a and 6). Under in-vivo conditions with ongoing neuronal activity in a closed system, this mechanism may result in further elevation of $[K^+]_o$ and prolonged NMDA-R activation, which could induce the generation of a chronic hyperexcitable epileptic network by non-specific synaptic plasticity.

One month after BBB disruption, we found reduced cortical volume and loss of excitatory and inhibitory neurons ([13], Fig. 4 a-c). Cell death is observed in chronic epileptic tissue from human [34] and may be a consequence of excitotoxicity during seizure activity [35]. Alternatively, after BBB disruption serum derived toxic derivatives may initiate delayed inflammatory responses eventually leading to irreversible cellular damage. Our neurological tests demonstrate functional deterioration of the affected sensory-motor cortex which may be a consequence of cell death. This finding parallels functional impairment in patients with temporal lobe epilepsy, who are found to suffer from a decline in cognitive and memory functions [36]. The delayed progressive nature of the deterioration, even after the BBB resumed its normal function, suggests disruption as a triggering event for a continuing, self-perpetuating damage.

Our research demonstrates a novel mechanism of epileptogenesis and delayed impairment of cortical function on the basis of serum albumin exposure and astrocytic dysfunction, and points out a central role of the neurovascular unit in CNS pathogenesis. Interference with astrocytic albumin uptake may constitute a new therapeutic strategy to prevent secondary neurological damage following BBB disruption.

6. References

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7. Declaration of own contribution to the submitted publications

The contributions of the doctoral student Sebastian Ivens to the submitted publications present as follows:

- **Publication 1:**

  Seiffert E, Dreier JP, Ivens S, Bechmann I, Tomkins O, Heinemann U, Friedman A.
  Lasting blood-brain barrier disruption induces epileptic focus in the rat somatosensory cortex.
  J Neurosci 24:7829-7836, 2004.

  Contribution: approx. 20 percent

  Detailed Contribution:
  Participation in planning and conducting the experiments (Operational model of blood-brain barrier disruption, preparation of brain slices, electrophysiological recordings, preparation of tissue for histological analysis), data analysis, preparation and correction of the manuscript including figures, processing the peer review.

- **Publication 2:**

  Ivens S, Kaufer D, Flores LP, Bechmann I, Zumsteg D, Tomkins O, Seiffert E, Heinemann U, Friedman A.
  TGF-β receptor-mediated albumin uptake into astrocytes is involved in neocortical epileptogenesis.
  Brain 130:535-547, 2007.

  Contribution: approx. 65 percent

  Detailed Contribution:
  Construction of ion sensitive electrodes, planning and conducting the majority of experiments (Operational model of blood-brain barrier disruption, cortical application of albumin and TGF- βR antagonists in-vivo and in-vitro, preparation of brain slices, electrophysiological recordings, preparation of tissue for histological analysis, fluorescence microscopy), data analysis, preparation and correction of the manuscript including figures, processing the peer review.

- **Publication 3:**

  Tomkins O, Friedman O, <u>Ivens S</u>, Reiffurth C, Major S, Dreier JP, Heinemann U, Friedman A.
  Blood-brain barrier disruption results in delayed functional and structural alterations in the rat neocortex.
  Neurobiol Dis 25:367-377, 2007.

  Contribution: approx. 20 percent

  Detailed Contribution:
  Participation in planning and conducting the majority of experiments (Operational model of blood-brain barrier disruption, preparation of brain slices, electrophysiological recordings, iontophoretical dendrite staining, preparation of tissue for histological analysis), data analysis, preparation and correction of the manuscript including figures, processing the peer review.

  Prof. Dr. Uwe Heinemann   Sebastian Ivens

8. Publications

In the following the publications are inserted according to their order of appearance in section 7 ("Declaration of own contribution to the submitted publications").

9. Curriculum Vitae

My curriculum vitae is not published in the electronic version of my thesis due to data privacy regulations.

10. List of own publications which are included in the thesis with impact factors

1. ***Seiffert E, Dreier JP, <u>Ivens S</u>, Bechmann I, Tomkins O, Heinemann U, Friedman A.***
Lasting blood-brain barrier disruption induces epileptic focus in the rat somatosensory cortex.
J Neurosci 24:7829-7836, 2004.

Impact Factor 2004: **7.907**

2. ***<u>Ivens S</u>, Kaufer D, Flores LP, Bechmann I, Zumsteg D, Tomkins O, Seiffert E, Heinemann U, Friedman A.***
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3. ***Tomkins O, Friedman O, <u>Ivens S</u>, Reiffurth C, Major S, Dreier JP, Heinemann U, Friedman A.***
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List of further publications

Journals

4. ***Zimmerman G, Njunting M, <u>Ivens S</u>, Tolner EA, Behrens CJ, Gross M, Soreq H, Heinemann U, Friedman A:***
Acetylcholine-Induced Seizure-like Activity and Cholinergic Modified Gene Expression in Chronically Epileptic Rats.
Eur J Neurosci 27(4):965-75, 2008.

Impact Factor 2006: **3.709**

<u>Oral presentations</u>

1. ***Ivens S, David Y, Heinemann U, Friedman A.***
   Mechanisms underlying epileptogenesis in the blood-brain barrier disruptetd cortex.
   Berlin Brain Days, November 26 – 30 2008, Berlin, Germany.

2. ***Ivens S, Bechmann I, Kaufer D, Heinemann U, Friedman A.***
   Astrocytic-Neuronal Interactions during Epileptogenesis in the Rat Neocortex.
   7th European Congress on Epileptology, International League against Epilepsy, ILAE, Helsinki, Finnland, July 2-6, 2006.

3. ***Ivens S, Seiffert E, Kaufer D, Bechmann I, Heinemann U, Friedman A.***
   Epileptogenesis after BBB-disruption: Astrocytic dysfunction as a trigger.
   Berlin Neuroscience Symposium, Berlin, Germany, February 2006.

4. ***Ivens S, Seiffert E, Kaufer D, Heinemann U, Friedman A.***
   Abnormal K+ buffering in the blood brain-barrier disrupted cortex.
   Joint Symposium of the DFG Neuroscience Graduate Schools, Göttingen, Germany, February 2005.

5. ***Ivens S, Dreier J, Heinemann U Friedman A.***
   Abnormal K+ buffering after blood-brain barrier disruption.
   Autumn Neuroscience Symposium, Berlin, Germany, November 2004.

6. ***Ivens S, Seiffert E, Heinemann U, Friedman A.***
   Epileptiform activity and cortical spreading depression in the blood-brain-barrier disrupted cortex.
   European Students Conference, Berlin, Gemany, November 2003.

<u>Posters</u>

1. ***Ivens S, David Y, Kaufer D, Flores LP, Virgintino D, Heinemann U, Friedman A.***
   Altered astrocytic control of excitability during epileptogenesis following neocortical BBB disruption.
   Society for Neuroscience, annual meeting, San Diego, USA, Oct 3–7, 2007.

2. ***Ivens S, Voitcu R, David Y, Flores LP, Kaufer D, Virgintino D, Heinemann U, Friedman A.***
   Altered astrocytic control of neuronal excitability during BBB-related epileptogenesis.
   Signal transduction in the blood-brain barrier, International Symposium, Potsdam, Germany, September 13-16, 2007.

3. ***Ivens S, Bechmann I, Kaufer D, Heinemann U, Friedman A.***
Albumin mediated damage cascade during neocortical epileptogenesis.
Neurizons, International Max Planck Research School, Göttingen, Germany, May 31 – June 2, 2007.

4. ***Ivens S, Merkin V, Cagnano EB, Shelef I.***
Necrotic meningioma: Characteristic imaging findings in diffusion MRI and MR spectroscopy.
Brain Tumor 2006, Max Delbrück Communications Center, Berlin, Germany, December 7-8, 2006.

5. ***Ivens S, Kaufer D, Bechmann I, Tomkins O, Seiffert E, Heinemann U and Friedman A.***
Astrocytic albumin uptake induces epileptogenesis following blood-brain barrier disruption.
Signal Transduction in the Blood-Brain Barriers, International Symposium, Salzburg, Austria, September 07-10, 2006.

6. *Friedman A, **Ivens S, Bechmann I, Heinemann U.*** Glial-neuronal interactions during neocortical epileptogenesis.
International Neuroscience Symposium of the DFG Transregional Research Consortium SFB/TR 3, "Cellular Dynamics in the Normal and Epileptic Brain", Bonn, Germany, February 16-18, 2006.

7. ***Ivens S, Seiffert E, Dreier JP, Heinemann U, Friedman A.***
Astrocytic-Neuronal Interactions Underlie Epileptogenesis following Blood-Brain Barrier-Disruption.
Annual Meeting of the Israel Society for Neuroscience, Eilat, Israel, December 2005.

8. ***Ivens S, Seiffert E, Heinemann U, Friedman A.***
Abnormal K+ buffering in the blood-brain barrier disrupted cortex.
Annual Meeting of the Israel Society for Neuroscience, Eilat, Israel, November 2004.

9. ***Ivens S, Seiffert E, Heinemann U, Friedman A.***
Abnormal K+ buffering in the epileptic blood-brain barrier disrupted cortex.
Final Symposium DFG GRK 238 "Damage cascades in neurological disorders - studies with imaging techniques", Berlin, Germany, November 2004.

10. ***Ivens S, Seiffert E, Dreier JP, Heinemann U, Friedman A.***
Abnormal K+ buffering in the epileptic blood-brain barrier disrupted cortex.
Society for Neuroscience, annual meeting, San Diego, USA, October 2004.

11. ***Ivens S, Heinemann U, Friedman A.***
    Abnormal K^+ buffering in the epileptic BBB disrupted cortex.
    Signal transduction in the blood-brain barrier, International Symposium, Potsdam, Germany, September 2004.

12. ***Ivens S, Heinemann U, Friedman A.***
    Glial-neuronal interactions in the blood-brain barrier.
    4th forum of European Neuroscience, Lisbon, Portugal, July 2004.

13. ***Ivens S, Heinemann U, Friedman A.***
    Cholinergic dysfunction in pilocarpine model of mesial temporal lobe epilepsy.
    International Medical Chemical Defense Conference, Munich, Germany, April 2004.

14. ***Ivens S, Seiffert E, Heinemann U, Friedman A.***
    Glial-neuronal interactions in the blood-brain barrier disrupted cortex.
    Berlin Neuroscience Forum, Liebenwalde, Germany, April 2004.

15. ***Ivens S, Seiffert E, Heinemann U, Friedman A.***
    Epileptiform Activity and Cortical Spreading Depression in a Chronic model of Epilepsy.
    Annual Meeting of the Israel Society for Neuroscience, Eilat, Israel, December 2003.

11. Selbstständigkeitserklärung

„Ich, Sebastian Ivens, erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema:

„Consequences of blood-brain barrier disruption"

selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe."

Berlin, 11.1.2008   Sebastian Ivens