CHARLES UNIVERSITY
Second Faculty of Medicine

Summary of the Dissertation

Permeability of blood brain barrier during cerebral ischemia

Prostupnost hematoencefalické bariéry při mozkové ischémii

Jan Svoboda

Prague, 2025

The Dissertation was written during full-time doctoral study programme Kinesiology and Rehabilitation at the Department of Pathophysiology ,Second Faculty of Medicine, Charles University.

Supervisor: prof. MUDr. Jakub Otáhal, Ph.D., Department of Pathophysiology ,Second Faculty of Medicine, Charles University

Advisor:

Opponents: Doc.MUDr.Vladimír Riljak, Ph.D. (1.LF UK) Institute of Physiology, First Faculty of Medicine, Charles University

Doc.Ing.Monika Šorfová, Ph.D. (FTVS UK) Department of Biomedical Foundation in Kinanthropology, Faculty of Physical Education and Sport, Charles University

The defence will take place before the Board for the Defence of the Subject Area Board Kinesiology and Rehabilitation on .... in .... from .... hours

prof. MUDr. Alena KOBESOVÁ, Ph.D

Chairman of the Board for the Defence of Dissertations in doctoral study programme Kinesiology and Rehabilitation

prof. MUDr. Jan LACZÓ, Ph.D.

The Chairman of Subject Area Board and guarantor of the doctoral study programme Neurosciences

The Dean of the Faculty: prof. MUDr. Marek Babjuk, CSc.

This work has been supported by grant:
Czech Health Research Council NU21-02-00289.
NPO - no.LX22NPO5107 from the MEYS of the Czech Republic: Financed by EU – Next Generation EU

The dissertation is available for inspection at the Department for Ph.D. Study of the Dean´s Office, Second Faculty of Medicine, Charles University, V Úvalu 84, 150 06 Praha 5 (phone 224 435 836).

Permeability of blood brain barrier during cerebral ischemia

Abstract

Cerebral ischemia remains a leading cause of long-term disability and mortality worldwide, with blood-brain barrier (BBB) dysfunction playing a crucial role in its pathophysiology. This dissertation investigates the mechanisms of BBB permeability changes during ischemic stroke through both clinical and experimental approaches, providing new insights into stroke progression and potential therapeutic interventions. The clinical study involved MRI-based analysis of BBB disruption in ischemic stroke patients during the subacute phase (7–12 days post-stroke). By utilizing advanced imaging techniques, we assessed the localization and extent of BBB impairment in relation to ischemic lesion subregions. The findings indicate a strong correlation between lesion volume and BBB leakage, with significant gadolinium extravasation observed in FLAIR positive regions. The experimental study complemented these findings by employing a middle cerebral artery occlusion (MCAO) model in rodents, closely mimicking clinical stroke conditions and enabling a controlled investigation of BBB permeability changes. By comparing different rat strains, we identified significant variations in cerebrovascular responses, infarct size, and survival rates. Fluorescence spectrometry quantified BBB permeability by measuring Evans Blue (EB) extravasation, providing spaciotemporal evaluation of BBB disruption. Utilizing MRI, we assessed water diffusion abnormalities, offering insights into tissue integrity, edema formation, and the extent of ischemic damage. The combined clinical and experimental approach enhances our understanding of BBB disruption in ischemic stroke and its long-term consequences. The findings provide valuable markers for early detection of BBB dysfunction and contribute to the advancement of precision medicine in stroke care, emphasizing the importance of BBB integrity in optimizing patient outcomes and reducing post-stroke complications.

Keywords

blood-brain barrier, ischemic stroke, MRI, cerebral ischemia, vascular permeability, MCAO, neurovascular unit, post-stroke epilepsy, BBB disruption, experimental stroke model.

Prostupnost hematoencefalické bariéry při mozkové ischémii

Abstrakt

Mozková ischemie zůstává jednou z hlavních příčin dlouhodobé invalidity a mortality po celém světě, přičemž dysfunkce hematoencefalické bariéry (BBB) hraje klíčovou roli v její patofyziologii. Tato disertační práce zkoumá mechanismy změn permeability BBB během ischemické cévní mozkové příhody prostřednictvím klinických i experimentálních přístupů, čímž poskytuje nové poznatky o progresi cévní mozkové příhody a potenciálních terapeutických intervencích. Klinická studie zahrnovala analýzu narušení BBB u pacientů s ischemickou cévní mozkovou příhodou v subakutní fázi (7–12 dní po příhodě) pomocí magnetické rezonance (MRI). Využitím pokročilých zobrazovacích metod jsme hodnotili lokalizaci a rozsah poškození BBB v závislosti na subregionech ischemické léze. Výsledky ukázaly silnou korelaci mezi objemem léze a extravazací BBB, přičemž významná extravazace gadolinia byla pozorována ve FLAIR pozitivních oblastech. Experimentální studie tyto poznatky doplnila využitím modelu okluze střední mozkové arterie (MCAO) u hlodavců, který věrně napodobuje klinické podmínky cévní mozkové příhody a umožňuje kontrolované zkoumání změn permeability BBB. Porovnáním různých kmenů potkanů jsme identifikovali významné rozdíly v cerebrovaskulárních reakcích, velikosti infarktu a míře přežití. Fluorescenční spektrometrie kvantifikovala permeabilitu BBB měřením extravazace Evansovy modři (EB), což umožnilo prostorově-časové hodnocení narušení BBB. Pomocí MRI jsme analyzovali abnormality v difuzi vody, což poskytlo cenné informace o integritě tkáně, tvorbě edému a rozsahu ischemického poškození. Kombinovaný klinický a experimentální přístup rozšiřuje naše porozumění narušení BBB při ischemické cévní mozkové příhodě a jejím dlouhodobým důsledkům. Získané poznatky přispívají k rozvoji precizní medicíny v péči o pacienty po cévní mozkové příhodě, přičemž zdůrazňují důležitost integrity BBB pro optimalizaci výsledků léčby a snížení rizika komplikací po mozkové ischemii.

Klíčová slova

hematoencefalická bariéra, ischemická cévní mozková příhoda, MRI, mozková ischemie, cévní propustnost, MCAO, neurovaskulární jednotka, epilepsie po cévní mozkové příhodě, narušení BBB, experimentální model cévní mozkové příhody

Table of content

1 Introduction

Stroke remains one of the most severe and pressing health challenges worldwide, despite the advancements in medical technologies. It is currently the second-leading cause of death globally. Every year, approximately 12 million new cases of stroke occur, leading to 6 million deaths, underscoring the immense personal, familial, and societal impact of this condition. In 2021, there were approximately 102 million stroke cases worldwide, with ischemic stroke accounting for 70 million of those cases (He *et al.*, 2024). Furthermore, the incidence of ischemic stroke has been on a significant upward trajectory, rising from 4.07 million cases in 1990 to 7.86 million cases in 2021 (Li *et al.*, 2024). Projections indicate that this number will continue to increase, with estimates suggesting nearly 9.62 million new ischemic stroke cases by 2030 (Pu *et al.*, 2023). Despite advancements in acute stroke interventions, ischemic stroke remains a leading cause of long-term disability, with survivors often facing lifelong consequences. While immediate post-stroke treatments, such as thrombolysis and thrombectomy, have improved acute outcomes, the chronic consequences of stroke remain underexplored, and long-term recovery is often incomplete. This highlights the critical importance of high-quality, in-depth research that delves into the chronic effects of stroke, particularly in understanding the underlying pathophysiological processes that contribute to long-term disability. One such key area is the impairment of the blood-brain barrier (BBB), which plays a central role in ischemic stroke. BBB dysfunction is a hallmark of ischemic stroke, contributing to the progression of tissue damage, cerebral edema, and secondary neuroinflammation. Understanding the temporal and regional aspects of BBB impairment, especially during the subacute and chronic phases, is crucial for identifying therapeutic targets that could mitigate these long-lasting effects.

2 General background

2.1 Brain ischemia

Ischemic stroke occurs when there is a sudden and significant decrease in regional cerebral blood flow (rCBF), which can result from a variety of causes. This reduction in blood flow leads to a critical shortage of oxygen supply and a failure to remove metabolic waste products. During the acute phase of ischemia, a wide range of pathophysiological processes are set into motion, contributing to the ischemic damage observed in the affected cerebral tissue. One of the first effects is the rapid suppression of energetic metabolism, which quickly leads to a substantial drop in adenosine triphosphate levels. This energy deficit causes ionic imbalances, overproduction, and efflux of excitatory amino acids, as well as the development of lactic acidosis(Dirnagl, 2012). Calcium overload triggers vast cellular pathophysiological cascades including the massive release of reactive oxygen and nitrogen species, which modifies sensitive cellular targets, alternating the function of mitochondria and regulation of blood supply to the brain (neurovascular coupling) (Otáhal *et al.*, 2014). It is worth mentioning that these pathophysiological processes run on various levels affecting not only the ischemic core where the blood flow is under the ischemic threshold but also surrounding tissue. The above-described mechanisms lead to irreversible cell damage, affecting neuronal and glial cells, but also components of the vascular wall (Sarvari *et al.*, 2020). The most relevant manifestations of vascular impairment are a decline in vascular mechanical properties, neurovascular dysregulation, and blood-brain barrier (BBB) breakdown. Moreover, cerebral edema either due to vascular dysfunction (vasogenic edema) or cellular damage (cytotoxic and later ionic edema) is a common complication of cerebral infarction and the major cause of mortality (Yao *et al.*, 2021).

2.2 Structure and function of BBB

The blood-brain barrier (BBB) is a highly specialized structure that regulates the exchange of molecules between the circulatory system and the central nervous system (CNS), maintaining brain homeostasis and preventing neurotoxic elements from entering neural tissue (Barichello *et al.*, 2019; Kadry *et al.*, 2020; Tajes *et al.*, 2014). It is a component of the neurovascular unit (NVU), which includes endothelial cells, pericytes, astrocytes, microglia, neurons, and the extracellular matrix, working collectively to maintain BBB permeability through cellular and biochemical signaling (McConnell *et al.*, 2017; Muoio *et al.*, 2014). The BBB is structurally reinforced by tight junctions (TJ) composed of transmembrane proteins, such as claudins, occludins, and junctional adhesion molecules, anchored to scaffold proteins like zonula occludens (ZO-1, ZO-2, ZO-3) (Kadry *et al.*, 2020; Tajes *et al.*, 2014; Zlokovic, 2008). Among them, claudin-5 is crucial for maintaining BBB integrity, limiting the passage of molecules larger than 800 Da. Other claudins, such as claudin-3 and claudin-12, also contribute to barrier function, though their specific roles remain under investigation. Occludin, another essential TJ protein, regulates permeability but is not indispensable for BBB integrity (Kadry *et al.*, 2020; Zlokovic, 2008). Junctional adhesion molecules (JAM-A, JAM-B, JAM-C) and zonula occludens proteins further reinforce tight junctions, stabilizing endothelial barriers (Engelhardt and Sorokin, 2009). Adherent junctions (AJ), particularly vascular endothelial (VE)-cadherin, also play a significant role in BBB integrity. The BBB tightly regulates the transport of molecules through several mechanisms. Small, lipophilic molecules such as oxygen and carbon dioxide can diffuse freely across the endothelial membrane (Sweeney *et al.*, 2019; Zlokovic, 2008). Essential nutrients like glucose and amino acids are transported via solute carrier transporters, ensuring adequate metabolic supply to the brain (Kadry *et al.*, 2020).

2.3 Disruption of BBB in brain ischemia

Within minutes of ischemia, ATP depletion leads to Na^+/K^+-ATPase failure, intracellular sodium accumulation, and cytotoxic edema (Jiang *et al.*, 2014; Mohr *et al.*, 2010). Tight junction proteins, including occludin and claudin-5, undergo phosphorylation and degradation, causing increased paracellular permeability. However, in this early stage, the BBB primarily loses ion selectivity while remaining structurally intact against large plasma proteins (Mansour *et al.*, 2020; Obenaus and Badaut, 2022).

As ischemia persists beyond the first few hours, endothelial dysfunction further impairs BBB integrity, leading to the leakage of Na^+, Cl^-, and other small ions into the extracellular space. This shift is primarily mediated by the upregulation of the Na^+-K^+-$2Cl^-$ cotransporter (NKCC1) on endothelial cells (Jayakumar *et al.*, 2011). Furthermore, astrocytes begin to swell due to excessive sodium uptake via NKCC1 and dysregulated aquaporin-4 (AQP4) channel activity (Rutkowsky *et al.*, 2011; Thrane *et al.*, 2011) which lead to impaired water clearance from the extracellular space, further contributing to edema formation (Hawkins *et al.*, 2013). This marks the transition from purely intracellular swelling cytotoxic edema to a state where extracellular ionic disturbances drive further water influx into both cells and the perivascular space (ionic edema). Importantly, at this stage, while the BBB is still relatively intact against large proteins, the loss of ion selectivity creates osmotic gradients that facilitate uncontrolled water accumulation, setting the stage for increased brain tissue pressure and further neuronal injury.

Recanalization, while restoring blood flow, can exacerbate injury via so called “reperfusion injury”. At the BBB level, an early reperfusion sets off a rapid upregulation of matrix metalloproteinases especially MMP-9 facilitated by cytokines (TNF-α, IL-1β, IL-6) and chemokine signals (Jin *et al.*, 2018). MMP-9 and MMP-2 degrade type IV collagen, laminin, and fibronectin within the basement membrane, while also cleaving tight junction proteins (claudin-5, occludin, ZO-1) (Jin *et al.*, 2018; Liu *et al.*, 2017). As these proteins deteriorate, the normally restrictive endothelial barrier becomes more permeable, and vasogenic edema starts to develop. Moreover, endothelial cells themselves may become "blebbed" or detached from the basement membrane in patches due to cytoskeletal damage and disrupted integrin signaling.

As the stroke lesion matures, BBB disruption becomes more extensive due to inflammatory infiltration, microglial activation (Li *et al.*, 2022), and enzymatic degradation of vascular structures (Curry *et al.*, 2010). At this stage, the BBB transitions from selective permeability loss to profound structural breakdown, increasing the risk of hemorrhagic transformation.

Over time, the nature of the inflammatory infiltrate and the fate of the BBB evolve. By approximately one to two weeks after stroke onset, the predominantly pro-inflammatory M1-like microglial and macrophage profile may shift, at least in part, to a more anti-inflammatory or reparative M2-like phenotype. These cells produce factors such as IL-10 and TGF-β that can encourage vascular remodeling and aid in the partial reconstitution of the BBB (Chen *et al.*, 2019).

Persistent BBB disruption can remain in certain patients, particularly those with large infarcts, hemorrhagic transformations, or comorbidities (hypertension, diabetes, hypercholesterolemia). Chronically elevated levels of MMPs or sustained oxidative stress hamper efforts at restoring normal endothelial function. Some capillaries may undergo "pseudo-normalization" structurally yet retain functional deficits in tight junction arrangement and transendothelial electrical resistance (Di *et al.*, 2021).

While it is convenient to categorize the evolution of ischemic stroke and recanalization-associated injury into discrete phases, it is important to note that "in reality" these processes overlap extensively.

3 Aims of thesis

The **primary objective** of this thesis is to investigate the permeability of the blood-brain barrier during cerebral ischemia, with a particular focus on the acute and subacute phases of stroke. The specific goals of the study are as follows:

- To characterize changes in BBB permeability in human patients following ischemic stroke, as assessed by MRI. This includes a detailed analysis of the temporal and spatial dynamics of BBB impairment in the subacute period (7-12 days after symptom onset), which is crucial for understanding the progression of ischemic damage and identifying potential therapeutic windows.

- To establish a clinically relevant experimental model of ischemic stroke with reperfusion, specifically targeting the middle cerebral artery occlusion (MCAO) model. This model is intended to replicate human ischemic stroke conditions and to study the detailed mechanisms of BBB dysfunction in vivo. By refining this model, we aim to facilitate future research on stroke pathophysiology and therapeutic interventions.

- To create a standardized methodology for evaluating BBB disruption in experimental models of ischemic stroke. This includes the use of advanced MRI techniques to quantify and map BBB permeability, offering a reliable tool for assessing the efficacy of potential therapeutic strategies targeting BBB repair or protection.

4 Clinical study

4.1 MRI sequence overview

T1-weighted and T2-weighted imaging are primary MRI sequences, differing in cerebrospinal fluid (CSF) appearance: dark on T1-weighted images and bright on T2-weighted ones (Wintermark *et al.*, 2008). Fluid-Attenuated Inversion Recovery (FLAIR), similar to T2-weighted imaging but with longer echo time (TE) and repetition time (TR), enhances abnormality detection by suppressing CSF signals (Tedyanto *et al.*, 2022).

Gadolinium-enhanced T1-weighted imaging improves visualization of vascular structures and blood-brain barrier disruptions. This paramagnetic contrast agent shortens T1 relaxation time, appearing bright on scans (Lin and Liebeskind, 2016).

Diffusion-weighted imaging (DWI) detects water molecule movement, emphasizing restricted diffusion within cells. During ischemia, sodium-potassium pump failure leads to intracellular sodium accumulation, causing an osmotic shift that restricts water movement. DWI effectively highlights these changes, making it ideal for identifying acute ischemia (Lin and Liebeskind, 2016; Srinivasan *et al.*, 2006).

DWI, combined with apparent diffusion coefficient (ADC) mapping, identifies ischemic changes within minutes of vessel occlusion. DWI shows ischemic tissue as bright, while ADC maps confirm restricted diffusion by displaying dark areas, distinguishing acute infarcts from vasogenic edema and other T2-based effects. This ensures accurate stroke diagnosis (Lin and Liebeskind, 2016; Srinivasan *et al.*, 2006; Tedyanto *et al.*, 2022).

4.1 Methods

We collaborated on this part of the thesis in tight cooperation with Ing. David Kala PhD who created the data analysis which was included in his in his doctoral thesis (Kala, 2022) .

Eighty-six patients after cerebral ischemia underwent MRI scanning 7-12 days after the insult. The ischemic lesion was evaluated and delineated in FLAIR and DWI images. Lesions were segmented using semiautomatic algorithms in both FLAIR and DWI images. Regions with hyperintensity in FLAIR and DWI were classified as FLAIR+ and DWI+, respectively. Regions without hyperintensity were classified as FLAIR- and DWI-. Lesions were further divided into three subregions based on the overlap of FLAIR+ and DWI+ volumes (FLAIR+DWI+, FLAIR+DWI-, FLAIR-DWI+). BBB integrity was evaluated on T1w images taken before and after contrast application. SeLECT score was evaluated to estimate the risk of development of vascular epilepsy.

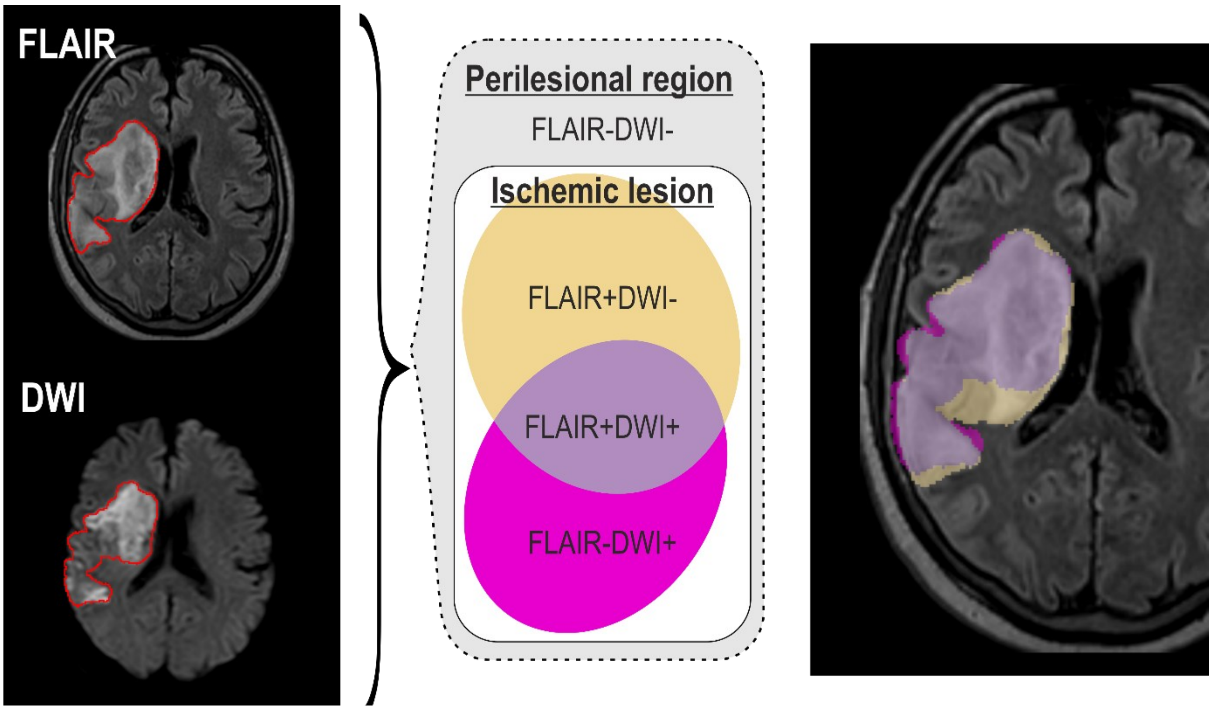

Figure 1 Lesion detection and definition of brain sub-regions (Kala, 2022, fig.10, edited).

4.2 Results

Ischemic lesions were detected in both groups by FLAIR or DWI hyperintensities and divided into three sub-regions: FLAIR+DWI+, FLAIR+DWI-, and FLAIR-DWI+. The median ischemic lesion volume in the leaking BBB group was 17.04 mL (IQR 40.04 mL), while in the intact BBB group, it was significantly lower at 4.59 mL (IQR 10.42 mL, p=0.001). The composition of lesions also varied between groups: the FLAIR+DWI- sub-region was significantly larger in the leaking BBB group (57% vs 31% in the intact BBB group). In the leaking BBB group, the volume of gadolinium extravasation (7.76 mL, IQR 16.13 mL) was compared to the ischemic lesion volume (21.6 mL, IQR 25.3 mL), showing a strong positive correlation. The spatial distribution of extravasated gadolinium was analyzed in the leaking BBB group. Sixty-three percent (IQR 59.4%) of the extravasated gadolinium was located in the FLAIR+DWI- sub-region, 22% (IQR 44.3%) in FLAIR+DWI+, and less than 1% in FLAIR-DWI+. A total of 96% (IQR 8.1%) of the gadolinium was inside the lesion, with only a small amount (0.6 mL, IQR 2.2 mL) detected in the perilesional tissue. Extravasated gadolinium was significantly higher in gray matter (2%, IQR 6%) compared to white matter (0.5%, IQR 2.4%).

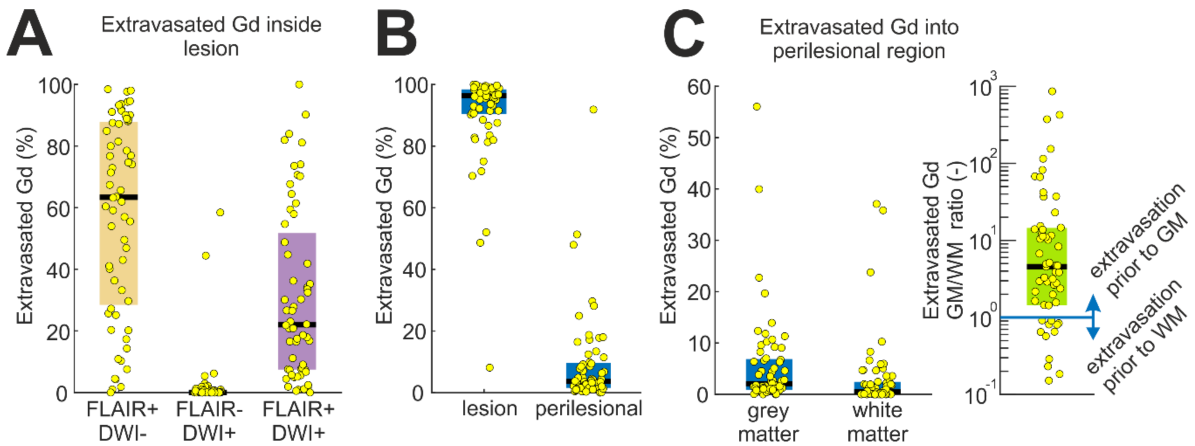

Figure 2 Localization of the BBB impairment in 60 patients with leaking BBB (Kala, 2022, fig.23, edited).

4.3 Discussion

In our clinical study, we thoroughly described the localization of the blood-brain barrier (BBB) impairment in relation to the ischemic lesion after a stroke at the time, when patients are typically discharged from the hospital.

In our data, the ischemic lesion was visible in FLAIR and DWI MRI with high contrast allowing segmentation to be performed. ADC maps are corrected for the T2 component and represent a direct evaluation of water diffusion, however, our datasets did not allow reliable segmentation of lesions. This likely corresponds with findings by Lansberg. (2001) where pseudonormalization of ADC together with maximal intensity in DWI during the second week after ischemia was reported (Lansberg *et al.*, 2001). Therefore, ADC wasn't used for lesion detection in this study.

We utilized the post-pre contrast method to assess and pseudo-quantify BBB leakage (Chassidim *et al.*, 2013; Kala *et al.*, 2017). While dynamic contrast-enhanced MRI (DCE-MRI) is commonly employed for quantitative permeability analysis (Thrippleton, 2019; Thrippleton *et al.*, 2019), it was not applied in this study.

BBB disruption was detected in 75% of patients (60/80). Patients with an intact BBB had smaller ischemic lesions, lower ASPECTS and SeLECT scores, and a lower risk of hemorrhagic transformation. Since the SeLECT score predicts post-stroke seizures, our data suggest that BBB disruption may increase the risk of vascular epilepsy. Additionally, a strong correlation was found between ischemic lesion volume and BBB disruption, consistent with experimental stroke models (Abo-Ramadan *et al.*, 2009).

The composition of ischemic lesions also varies between groups. In the group with leaking BBB, the FLAIR+DWI- sub-region extended over a significantly larger part of the ischemic lesion. A possible explanation is that while in patients with intact BBB FLAIR positivity is caused solely by gliosis as was reported for small vessel disease (Gouw *et al.*, 2011), in patients with leaking BBB

FLAIR+ region manifests both gliosis and vasogenic edema in the tissue where BBB was compromised (Lansberg *et al.*, 2015).

To further evaluate regional differences, we have segmented brain images into gray and white matter. Although higher susceptibility of white matter to BBB breakdown has been proposed (Bernier *et al.*, 2021), the analysis revealed higher extravasation of Gd in perilesional tissue in gray matter in comparison to white matter.

4.4 Conclusion

The study provides evidence that in the second week after ischemic insult the ischemic lesion still undergoes maturation, and the BBB is compromised in the majority of patients.

5 Experimental study

5.1 Methods

This study utilized 34 adult male rats (Wistar, Long Evans, and Sprague Dawley) to investigate middle cerebral artery occlusion (MCAO) and its impact on the blood-brain barrier (BBB). Approved by the Animal Care and Use Committee of the Institute of Physiology, Czech Republic, the protocol adhered to the European Community Council Directive 86/609/EEC and NIH humane standards. Efforts were made to minimize animal suffering and reduce numbers.

Anesthesia was induced with 3–4% Isoflurane and maintained at 1–2% during surgery, with body temperature controlled at 37.1±0.25°C. The surgical field was disinfected, and ophthalmic solution protected the eyes. Post-surgery, analgesics (Meloxicam and Nurofen) were administered for four days.

Cerebral blood flow (CBF) was monitored using a laser Doppler flowmeter (LDF) by carefully preparing the skull with a controlled drilling technique to optimize signal intensity. The LDF probe was secured over the middle cerebral artery (MCA) branch with a custom aluminum holder. The LDF signal was continuously recorded using a Periflow 5010 system, with data analyzed in Spike2 software (CED, Cambridge, UK).

MCA occlusion was performed under a surgical microscope. A nylon filament with a silicone tip (Doccol, USA) was advanced through the external carotid artery into the internal carotid artery until resistance was met, indicating MCA blockage. CBF drop was confirmed via LDF. After 60 minutes, the filament was withdrawn, and arteriotomy was ligated.

Noninvasive ischemic region assessment was conducted using microPET/CT (Albira, Bruker Biospin) in six Sprague-Dawley rats. Scans were performed 24 hours before and after MCAO following the adapted protocol of (Balsara *et al.*, 2014). Rats were fasted overnight and injected with ~18 MBq of 18F-FDG an hour before scanning. Data analysis using PMOD software and Schiffers MRI rat brain atlas (Schiffer *et al.*, 2006) allowed bilateral assessment of FDG activity. 3D ischemic lesion visualizations were generated using 3D Slicer software.

BBB permeability was assessed through Evans Blue extravasation and fluorescence spectrometry, adapted from (Wang and Lai, 2014). Thirty-two Wistar rats underwent MCAO, divided into groups based on post-ischemia timepoints (6h, 24h, 48h, 72h, 7d). Evans Blue dye (3 mg/kg) was intravenously administered and allowed to circulate for one hour before brain extraction. Tissue homogenization and spectrometric analysis were conducted using a Tecan Infinite 200 Pro plate reader (620 nm absorbance, 680 nm fluorescence). Data was analyzed with GraphPad Prism.

Experimental MRI was conducted on three Wistar rats 48 hours post-MCAO using a 7T Bruker Biospec 70/30 spectrometer at IKEM, Imaging included T2-weighted (T2w), T1-weighted (T1w), T1 mapping, and diffusion kurtosis imaging (DKI), with post-contrast T1 mapping and T1w at 10 and 20 minutes. T2-weighted imaging was used to identify edema and ischemic tissue damage, while post-contrast T1-weighted imaging and T1 mapping were employed to evaluate BBB impairment and vascular integrity. Diffusion Basis Spectrum Imaging (DBSI) was used to analyze water diffusion and tissue microstructure, differentiating axonal fibers, inflammation, and extracellular components (Chiang *et al.*, 2014; Wang *et al.*, 2019).

5.2 Resuts

The surgical procedure was consistent across all three rat strains, with no differences in vascular anatomy. Out of 34 animals, two Wistar rats (5.9%) died within four hours post-reperfusion, likely due to subarachnoid hemorrhage. A slight temperature increase (+0.3°C) was noted post-MCAO, and all strains experienced weight loss within 24 hours, with Wistar rats showing significant loss (20.7 ± 4.6 g, $P<0.05$). Intraluminal MCA occlusion resulted in ischemic injury in all animals. Sprague-Dawley and Wistar rats exhibited the most extensive infarcts (50.2±10.3% and 36.8±5.6%, respectively), while Long-Evans had significantly smaller infarcts (18.8±3.5%, $P<0.05$). The affected areas varied; Sprague-Dawley and Wistar rats had lesions in the caudoputamen, piriform, and entorhinal cortices, whereas Long-Evans showed moderate caudoputamen infarcts with slight sensorimotor cortex extension.

Regional cerebral blood flow (rCBF) followed expected changes during surgery. Initial MCA occlusion reduced rCBF to 48.0±6.2% in Wistar, 37.5±6.0% in Sprague-Dawley, and 63.2±5.9% in Long-Evans rats. Post-filament removal, rCBF returned to pre-occlusion levels or slightly higher (98.0±5.7% in Wistar, 112.2±19.9% in Sprague-Dawley, and 97.2±8.4% in Long-Evans). CCA clip release further increased rCBF (Wistar 131.1±13.0, Sprague-Dawley 143.2±26.0, Long-Evans 140.0±14.8%).

PET/CT revealed significant metabolic reduction in ischemic areas, visible as hypometabolic regions correlating with tetrazolium staining. FDG uptake ratios confirmed reduced activity in caudoputamen, amygdala, hypothalamus, diencephalon, and neocortical regions, with significant changes in the cingulate cortex.

Evans Blue spectrometry confirmed severe BBB disruption at all time points. Fluorescence signal intensity (arbitrary units) in the infarcted hemisphere was highest at 6 hours (885.5 ± 53.5 vs. 671.9 ± 26.9 in control, p<0.001) and remained elevated at 24 hours (512.8 ± 28.8 vs. 422.4 ± 24.6, p<0.001), 48 hours (674 ± 34 vs. 558.18. p<0.01), 72 hours (375.6 ± 44.3 vs. 298.6 ± 73.5, p<0.01), and 7 days (617.3 ± 73.5 vs. 486 ± 40, p<0.05). No significant differences between time points suggest Evans Blue reached an equilibrium, confirming BBB permeability but not its dynamic progression.

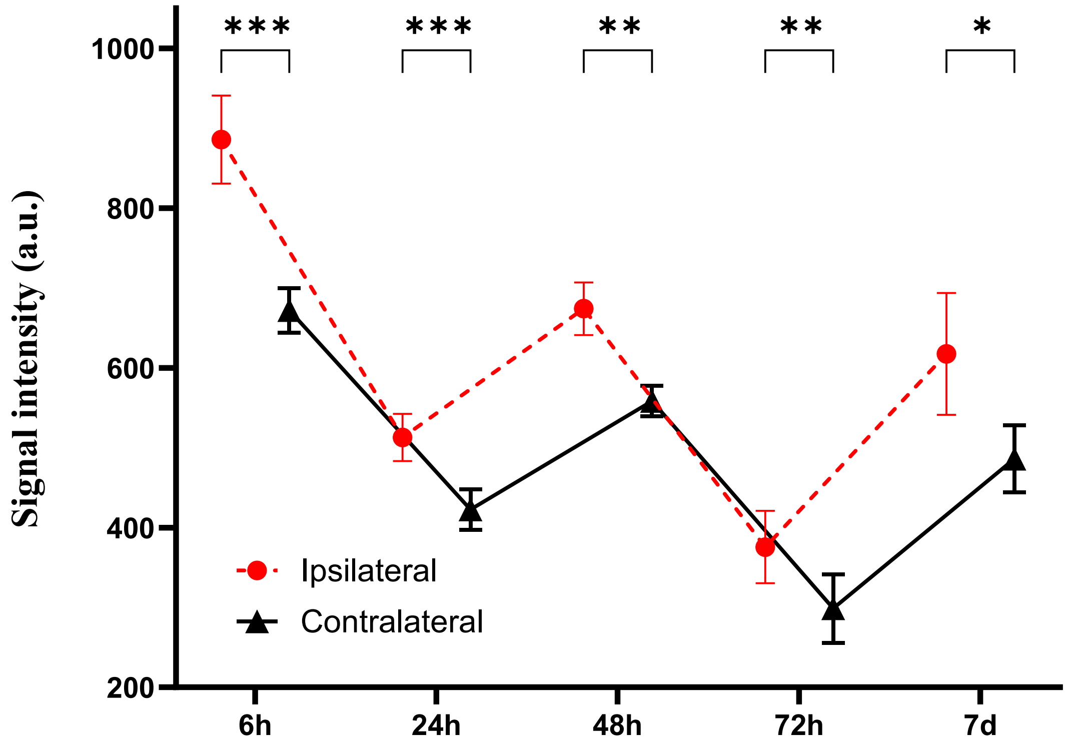

Figure 3 Evans Blue dye extravasation was present and statistically significant in all test groups at all time points (6h, 24h, 48h, 72h, 7d). Contralateral hemisphere was used as control. (*p<0.05, **p<0.01, ***p<0.001)

MRI at 48 hours post-MCAO showed ischemic lesions as hyperintense T2-weighted regions in the MCA territory, affecting the sensory cortex and caudoputamen, indicating vasogenic edema and fluid extravasation. Post-contrast T1-weighted imaging and T1 mapping showed enhanced signal in the ischemic lesion, confirming BBB impairment and Gd contrast leakage. Diffusion MRI revealed restricted diffusion with decreased ADC values, consistent with cytotoxic edema, while FA maps showed microstructural disorganization. DBSI analysis indicated reduced axonal integrity and increased extracellular water, highlighting tissue damage.

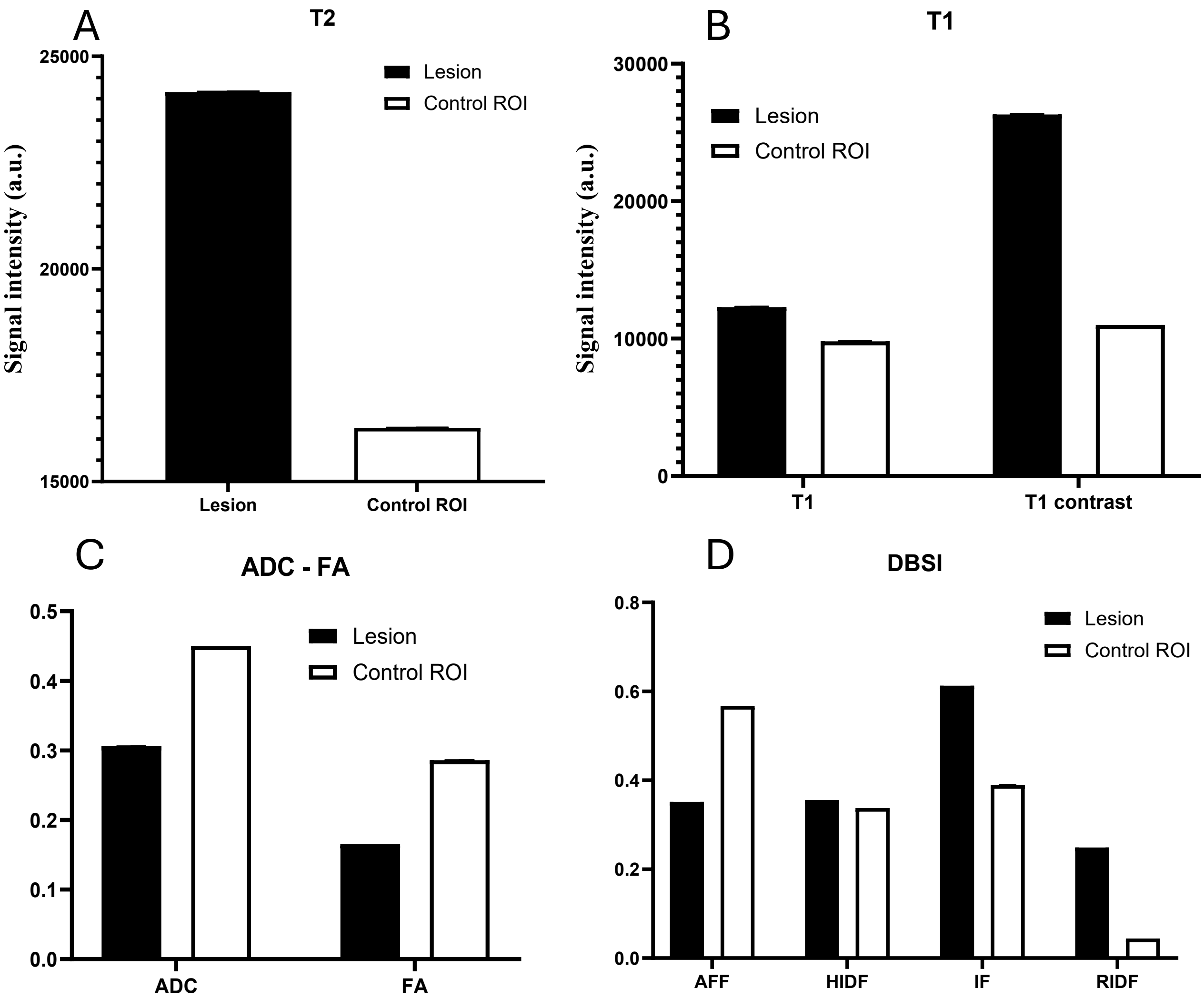

Figure 4 Graphs A-D illustrate ischemic damage, and tissue changes 48 hours post-MCAO in the rat model. **A)** Shows an increased T2 signal in the ischemic lesion compared to the contralateral side, indicating vasogenic edema. **B)** Highlights an increased T1 signal following Gd contrast administration, confirming BBB disruption. **C)** Demonstrates restricted diffusion with a decreased ADC signal and tissue disorganization with reduced FA values. **D)** Presents DBSI analysis, revealing an increased Axonal Fiber Fraction and decreased Restricted Isotropic Diffusion Fraction and Isotropic Fraction, indicating reduced axonal density and integrity, axonal injury in the ischemic core, altered cellular density, and increased extracellular water contribution. ADC - Apparent Diffusion Coefficient, FA - Fractional Anisotropy, AFF - Axonal Fiber Fraction, HIDF - Hindered Isotropic Diffusion Fraction, RIDF - Restricted Isotropic Diffusion Fraction

5.3 Discussion

This study demonstrated significant differences in ischemic region extent across rat strains. Despite similar brain vessel structures, regional cerebral blood flow (rCBF) varied, with Sprague-Dawley rats exhibiting the most pronounced drop (one-third of pre-occlusion levels), while Wistar and Long-Evans showed less reduction. Variability in rCBF likely stems from differences in collateral anastomoses (Oliff *et al.*, 1997). Sprague-Dawley rats provided the most reliable ischemic results.

Continuous rCBF monitoring with laser Doppler flowmetry (LDF) revealed a sharp decrease during occlusion and a rapid increase post-reperfusion. In two Wistar rats that died, a gradual rCBF rise suggested subarachnoid hemorrhage, confirmed by postmortem findings (Woitzik and Schilling, 2002). Reperfusion caused an rCBF increase beyond baseline, likely due to vasoactive substance release (Brožíčková and Otáhal, 2013; Iadecola and Nedergaard, 2007).

A 60-minute occlusion was selected as it aligns with clinical reperfusion therapy windows. Ischemic lesion extent corresponded with rCBF levels, with Sprague-Dawley rats exhibiting the most extensive infarcts (nearly half the hemisphere, affecting cortex and subcortical structures), while Long-Evans rats had smaller lesions primarily in the caudoputamen. The correlation between rCBF and ischemic region aligns with prior studies (Bardutzky *et al.*, 2005; Longa *et al.*, 1989; Taninishi *et al.*, 2015).

The increasing focus on rapid stroke reperfusion in clinical practice necessitates improved preclinical models. PET scanning at 24 hours post-stroke reliably delineated ischemic regions (Balsara *et al.*, 2014), supporting the use of Sprague-Dawley rats for further studies. PET imaging detected hypometabolism in affected regions, reflecting decreased glucose uptake, consistent with tetrazolium staining. Advanced PET analysis methods, such as Statistical Parametric Mapping, may enhance detection accuracy (Nie *et al.*, 2014).

Additionally, emerging radiotracers may help predict post-stroke complications like epilepsy (Bertoglio *et al.*, 2017).

Fluorescence spectrometry confirmed persistent BBB leakage up to seven days post-reperfusion. Despite significant differences between ischemic and control hemispheres, high background readings may stem from the absence of transcardial perfusion (Ryu *et al.*, 2018; Strbian *et al.*, 2008; Wang and Lai, 2014). The Evans Blue method, while effective in detecting BBB opening, lacks sensitivity for quantifying permeability over time (Saunders *et al.*, 2015).

MRI scans at 48 hours post-MCAO confirmed ischemic damage. T2-weighted imaging revealed hyperintensity in the MCA territory, indicating vasogenic edema (Alfadeel, 2020; Koch *et al.*, 2019). Gadolinium-enhanced T1 imaging confirmed BBB disruption (Matos Diaz *et al.*, 2017). Diffusion-weighted imaging (DWI) showed decreased ADC values, consistent with cytotoxic edema, while reduced FA values suggested loss of tissue integrity (Gaddamanugu *et al.*, 2022; Li *et al.*, 2019). DBSI further indicated axonal damage and extracellular water redistribution (Kim *et al.*, 2021; Matos Diaz *et al.*, 2017). DBSI provided more detailed microstructural insights than traditional DTI. Increased Axonal Fiber Fraction with reduced isotropic diffusion fractions suggested ongoing cellular damage (Kim *et al.*, 2021; Matos Diaz *et al.*, 2017). DBSI offers a valuable tool for assessing ischemic pathology, particularly in evaluating white matter integrity and edema.

A limitation of this MRI study is the small sample size, constrained by the recent availability of the Bruker 7T MRI at IKEM. Future studies with larger datasets will improve statistical significance and provide deeper insights into ischemic damage progression.

5.4 Conclusion

The study demonstrated strain-dependent differences in ischemic lesion extent and cerebral blood flow after MCAO, with Sprague-Dawley rats showing the most extensive infarcts and reliable ischemic outcomes. PET imaging effectively identified hypometabolic regions, while fluorescence spectrometry confirmed persistent BBB leakage, though Evans Blue had limitations in quantifying permeability.

MRI revealed vasogenic and cytotoxic edema, BBB disruption, and microstructural disorganization, with DBSI providing further insights into axonal damage and water redistribution. These findings highlight the importance of selecting suitable animal models and utilizing advanced imaging techniques for stroke research.

6 General conclusion

Clinical study

This study investigated blood-brain barrier (BBB) impairment in ischemic stroke patients during the subacute phase, particularly around hospital discharge. BBB disruption was observed in 75% of patients, predominantly within ischemic lesions, and was significantly associated with larger lesion volumes. Patients with intact BBB had smaller lesions, better ASPECTS scores, and a lower risk of hemorrhagic transformation, suggesting that BBB leakage correlates with more severe stroke outcomes and may increase the likelihood of vascular epilepsy. Ischemic lesions were identified using FLAIR and DWI MRI, with most BBB leakage occurring in FLAIR+ regions. Interestingly, Gd extravasation was also detected in perilesional gray matter, suggesting BBB dysfunction beyond the ischemic core.

Experimental study

In the experimental study, high variability between rat strains was observed in stroke induction using the intraluminal thread occlusion method. Sprague-Dawley rats proved to be the most reliable, whereas Wistar rats showed higher mortality, and Long-Evans rats developed significantly smaller infarcts. Positron emission tomography with 18F-fluorodeoxyglucose was validated as a suitable method for assessing ischemic lesion size. BBB disruption was evident at 6, 24, 48, 72 hours, and 7 days post-ischemia, with Evans Blue detection confirming BBB leakage. Finally, MRI revealed vasogenic and cytotoxic edema, BBB disruption, and microstructural disorganization, with DBSI providing further insights into axonal damage and water redistribution.

7 Summary

This thesis investigates blood-brain barrier permeability during cerebral ischemia, focusing on acute and subacute phases. Using clinical MRI data, it examines BBB dysfunction in stroke patients and develops an experimental model pipeline to replicate human stroke conditions.

Both clinical and experimental studies met their objectives. The clinical study showed widespread BBB impairment in ischemic stroke, correlating with larger infarcts, higher ASPECTS scores, and increased risk of hemorrhagic transformation. BBB leakage emerged as a potential biomarker for severe stroke outcomes and vascular epilepsy risk (SeLECT score). MRI sequences (FLAIR, DWI, post-contrast T1w) enabled precise lesion segmentation, revealing BBB disruption mainly in FLAIR-positive areas, extending into perilesional gray matter.

The experimental study validated the MCAO model as reliable for investigating BBB impairment. Fluorescence spectrometry and Evans Blue extravasation showed significant BBB disruption in the ischemic lesion, aligning with clinical MRI findings. These results support the role of BBB breakdown in extending ischemic damage and sustaining neuronal injury. The model confirmed BBB disruption at multiple time points, peaking in the acute and subacute phases.

The combined clinical and experimental results highlight the persistent nature of BBB impairment in ischemic stroke, even after reperfusion. BBB disruption is still present in the subacute phase when patients are typically discharged. This prolonged BBB impairment indicates that targeting BBB repair or stabilization could help prevent further neuronal damage and improve recovery.

This study deepens understanding of BBB dysfunction in stroke, emphasizing its role in progression and therapeutic potential. Advanced imaging in both clinical and experimental settings has provided key insights into BBB impairment, highlighting the need for future research on interventions to mitigate chronic stroke effects, including vascular epilepsy and neuronal degeneration.

8 Resumé

Tato práce se zabývá propustností hematoencefalické bariéry během mozkové ischemie se zaměřením na akutní a subakutní fázi. Na základě klinických dat z magnetické rezonance zkoumá dysfunkci BBB u pacientů s cévní mozkovou příhodou a vyvíjí experimentální model, který napodobuje podmínky lidské cévní mozkové příhody.

Klinické i experimentální studie splnily své cíle. Klinická studie prokázala rozsáhlé poškození BBB u ischemické cévní mozkové příhody, které koreluje s většími infarkty, vyšším skóre ASPECTS a zvýšeným rizikem hemoragické transformace. Únik BBB se ukázal jako potenciální biomarker pro závažné následky cévní mozkové příhody a riziko vaskulární epilepsie (skóre SeLECT). Sekvence MRI (FLAIR, DWI, postkontrastní T1w) umožnily přesnou segmentaci lézí a odhalily narušení BBB hlavně v oblastech s pozitivním FLAIR, které zasahovalo do perilesionální šedé hmoty.

Experimentální studie potvrdila, že model MCAO je spolehlivý pro zkoumání poškození BBB. Fluorescenční spektrometrie a extravazace Evansovy modři prokázaly významné narušení BBB v ischemické lézi, což se shoduje s klinickými nálezy MRI. Tyto výsledky podporují úlohu narušení BBB při prodlužování ischemického poškození a udržování neuronálního poškození. Model potvrdil narušení BBB ve více časových bodech, které dosáhlo vrcholu v akutní a subakutní fázi.

Kombinované klinické a experimentální výsledky poukazují na přetrvávající povahu poškození BBB u ischemické cévní mozkové příhody, a to i po reperfuzi. Narušení BBB je stále přítomno v subakutní fázi, kdy jsou pacienti obvykle propuštěni. Toto dlouhodobé poškození BBB naznačuje, že cílená oprava nebo stabilizace BBB by mohla pomoci zabránit dalšímu poškození neuronů a zlepšit zotavení.

Tato studie prohlubuje znalosti o dysfunkci BBB u cévní mozkové příhody a zdůrazňuje její roli v progresi a terapeutický potenciál. Pokročilé zobrazování v

klinických i experimentálních podmínkách poskytlo klíčové poznatky o poškození BBB a zdůraznilo potřebu budoucího výzkumu intervencí, které by zmírnily chronické následky mrtvice, včetně vaskulární epilepsie a degenerace neuronů

References

Abo-Ramadan, U., Durukan, A., Pitkonen, M., Marinkovic, I., Tatlisumak, E., Pedrono, E., Soinne, L., *et al.* (2009), "Post-ischemic leakiness of the blood-brain barrier: A quantitative and systematic assessment by Patlak plots", *Experimental Neurology*, Vol. 219 No. 1, pp. 328–333, doi: 10.1016/j.expneurol.2009.06.002.

Alfadeel, H.A. (2020), "Role of Diffusion Weighted Imaging in Enhancing MR Imaging in Recent Is-chemic Stroke Patients", *Al-Mukhtar Journal of Sciences*, Omar Al-Mukhtar University, Vol. 35 No. 2, pp. 104–115, doi: 10.54172/mjsc.v35i2.316.

Balsara, R.D., Chapman, S.E., Sander, I.M., Donahue, D.L., Liepert, L., Castellino, F.J. and Matthew Leevy, W. (2014), "Non-invasive imaging and analysis of cerebral ischemia in living rats using positron emission tomography with 18F-FDG", *Journal of Visualized Experiments*, Journal of Visualized Experiments, No. 94, doi: 10.3791/51495.

Bardutzky, J., Shen, Q., Henninger, N., Bouley, J., Duong, T.Q. and Fisher, M. (2005), "Differences in ischemic lesion evolution in different rat strains using diffusion and perfusion imaging", *Stroke*, Vol. 36 No. 9, pp. 2000–2005, doi: 10.1161/01.STR.0000177486.85508.4d.

Barichello, T., Collodel, A., Hasbun, R. and Morales, R. (2019), "An Overview of the Blood-Brain Barrier", *Neuromethods*, Vol. 142, Humana Press Inc., pp. 1–8, doi: 10.1007/978-1-4939-8946-1_1.

Bernier, L.P., Brunner, C., Cottarelli, A. and Balbi, M. (2021), "Location Matters: Navigating Regional Heterogeneity of the Neurovascular Unit", *Frontiers in Cellular Neuroscience*, Frontiers Media S.A., 30 June, doi: 10.3389/fncel.2021.696540.

Bertoglio, D., Verhaeghe, J., Santermans, E., Amhaoul, H., Jonckers, E., Wyffels, L., Van Der Linden, A., *et al.* (2017), "Non-invasive PET imaging of brain inflammation at disease onset predicts spontaneous recurrent seizures and reflects comorbidities”, *Brain, Behavior, and Immunity*, Academic Press Inc., Vol. 61, pp. 69–79, doi: 10.1016/j.bbi.2016.12.015.

Brožíčková, C. and Otáhal, J. (2013), “Effect of an inhibitor of neuronal nitric oxide synthase 7-nitroindazole on cerebral hemodynamic response and brain excitability in urethane-anesthetized rats”, *Physiological Research*, Vol. 62 No. SUPPL 1, doi: 10.33549/physiolres.932564.

Chassidim, Y., Veksler, R., Lublinsky, S., Pell, G.S., Friedman, A. and Shelef, I. (2013), *Quantitative Imaging Assessment of Blood-Brain Barrier Permeability in Humans FLUIDS AND BARRIERS OF THE CNS*.

Chen, Z., Chopp, M., Zacharek, A., Li, W., Venkat, P., Wang, F., Landschoot-Ward, J., *et al.* (2019), “Brain-Derived Microparticles (BDMPs) Contribute to Neuroinflammation and Lactadherin Reduces BDMP Induced Neuroinflammation and Improves Outcome After Stroke”, *Frontiers in Immunology*, Frontiers Media S.A., Vol. 10, doi: 10.3389/fimmu.2019.02747.

Chiang, C.W., Wang, Y., Sun, P., Lin, T.H., Trinkaus, K., Cross, A.H. and Song, S.K. (2014), “Quantifying white matter tract diffusion parameters in the presence of increased extra-fiber cellularity and vasogenic edema”, *NeuroImage*, Academic Press Inc., Vol. 101, pp. 310–319, doi: 10.1016/j.neuroimage.2014.06.064.

Curry, J.M., Hanke, M.L., Piper, M.G., Bailey, M.T., Bringardner, B.D., Sheridan, J.F. and Marsh, C.B. (2010), “Social disruption induces lung inflammation”, *Brain, Behavior, and Immunity*, Vol. 24 No. 3, pp. 394–402, doi: 10.1016/j.bbi.2009.10.019.

Di, Z., Wu, X., Xie, W. and Lin, X. (2021), “Effect of Pericytes on Cerebral Microvasculature at Different Time Points of Stroke”, *BioMed Research International*, Hindawi Limited, doi: 10.1155/2021/5281182.

Dirnagl, U. (2012), “Pathobiology of injury after stroke: The neurovascular unit and beyond”, *Annals of the New York Academy of Sciences*, Blackwell

Publishing Inc., Vol. 1268 No. 1, pp. 21–25, doi: 10.1111/j.1749-6632.2012.06691.x.

Engelhardt, B. and Sorokin, L. (2009), "The blood-brain and the blood-cerebrospinal fluid barriers: Function and dysfunction", *Seminars in Immunopathology*, November, doi: 10.1007/s00281-009-0177-0.

Gaddamanugu, S., Shafaat, O., Sotoudeh, H., Sarrami, A.H., Rezaei, A., Saadatpour, Z. and Singhal, A. (2022), "Clinical applications of diffusion-weighted sequence in brain imaging: beyond stroke", *Neuroradiology*, Springer Science and Business Media Deutschland GmbH, 1 January, doi: 10.1007/s00234-021-02819-3.

Gouw, A.A., Seewann, A., Van Der Flier, W.M., Barkhof, F., Rozemuller, A.M., Scheltens, P. and Geurts, J.J.G. (2011), "Heterogeneity of small vessel disease: A systematic review of MRI and histopathology correlations", *Journal of Neurology, Neurosurgery and Psychiatry*, BMJ Publishing Group, doi: 10.1136/jnnp.2009.204685.

Hawkins, B.T., Gu, Y.H., Izawa, Y. and Del Zoppo, G.J. (2013), "Disruption of dystroglycan-laminin interactions modulates water uptake by astrocytes", *Brain Research*, Vol. 1503, pp. 89–96, doi: 10.1016/j.brainres.2013.01.049.

He, Q., Wang, W., Zhang, Y., Xiong, Y., Tao, C., Ma, L., Ma, J., *et al.* (2024), "Global, Regional, and National Burden of Stroke, 1990–2021: A Systematic Analysis for Global Burden of Disease 2021", *Stroke*, Wolters Kluwer Health, doi: 10.1161/STROKEAHA.124.048033.

Iadecola, C. and Nedergaard, M. (2007), "Glial regulation of the cerebral microvasculature", *Nature Neuroscience*, November, doi: 10.1038/nn2003.

Jayakumar, A.R., Panickar, K.S., Curtis, K.M., Tong, X.Y., Moriyama, M. and Norenberg, M.D. (2011), "Na-K-Cl cotransporter-1 in the mechanism of cell swelling in cultured astrocytes after fluid percussion injury", *Journal of Neurochemistry*, Vol. 117 No. 3, pp. 437–448, doi: 10.1111/j.1471-4159.2011.07211.x.

Jiang, Z., Li, C., Arrick, D.M., Yang, S., Baluna, A.E. and Sun, H. (2014), "Role of nitric oxide synthases in early blood-brain barrier disruption following transient focal cerebral ischemia", *PLoS ONE*, Public Library of Science, Vol. 9 No. 3, doi: 10.1371/journal.pone.0093134.

Jin, Z., Liang, J., Li, J. and Kolattukudy, P.E. (2018), "Novel Role of MCP-Induced Protein 1 in Ischemic Brain Damage in Animals of Transient Focal Ischemia", 11 December, doi: 10.20944/preprints201812.0128.v1.

Kadry, H., Noorani, B. and Cucullo, L. (2020), "A blood–brain barrier overview on structure, function, impairment, and biomarkers of integrity", *Fluids and Barriers of the CNS*, BioMed Central Ltd, 1 December, doi: 10.1186/s12987-020-00230-3.

Kala, D. (2022), *Image Processing Pipelines for Analysis of Clinical Human MRI Doctoral Thesis*, Czech Technical University in Prague, October.

Kala, D., Svoboda, J., Litvinec, A., Pošusta, A., Lisý, J., Šulc, V., Tomek, A., *et al.* (2017), "/GD-Tracker/ A software for blood-brain barrier permeability assessment", *Lekar a Technika*, Vol. 47 No. 2.

Kim, J.H., Kim, S.Y., Kim, B., Lee, S.R., Cha, S.H., Lee, D.S. and Lee, H.J. (2021), "Prospects of therapeutic target and directions for ischemic stroke", *Pharmaceuticals*, MDPI AG, Vol. 14 No. 4, doi: 10.3390/ph14040321.

Koch, S., Mueller, S., Foddis, M., Bienert, T., von Elverfeldt, D., Knab, F., Farr, T.D., *et al.* (2019), "Atlas registration for edema-corrected MRI lesion volume in mouse stroke models", *Journal of Cerebral Blood Flow and Metabolism*, SAGE Publications Ltd, Vol. 39 No. 2, pp. 313–323, doi: 10.1177/0271678X17726635.

Lansberg, M.G., Thijs, V.N., O'brien, M.W., Ali, J.O., De Crespigny, A.J., Tong, D.C., Moseley, M.E., *et al.* (2001), *Evolution of Apparent Diffusion Coefficient, Diffusion-Weighted, and T2-Weighted Signal Intensity of Acute Stroke*, *AJNR Am J Neuroradiol*, Vol. 22.

Lansberg, M.G., Wintermark, M., Kidwell, C.S., Warach, S. and Albers, G.W. (2015), “Magnetic Resonance Imaging of Cerebrovascular Diseases”, *Stroke: Pathophysiology, Diagnosis, and Management*, Elsevier Inc., pp. 768–789, doi: 10.1016/B978-0-323-29544-4.00048-7.

Li, C., Lan, C., Zhang, X., Yin, L., Hao, X., Tian, J., Lin, L., *et al.* (2019), “Evaluation of Diffusional Kurtosis Imaging in Sub-acute Ischemic Stroke: Comparison with Rehabilitation Treatment Effect”, *Cell Transplantation*, SAGE Publications Ltd, Vol. 28 No. 8, pp. 1053–1061, doi: 10.1177/0963689719837919.

Li, S., Wernersbach, I., Harms, G.S. and Schäfer, M.K.E. (2022), “Microglia subtypes show substrate- and time-dependent phagocytosis preferences and phenotype plasticity”, *Frontiers in Immunology*, Frontiers Media S.A., Vol. 13, doi: 10.3389/fimmu.2022.945485.

Li, X. yu, Kong, X. meng, Yang, C. hao, Cheng, Z. feng, Lv, J. jie, Guo, H. and Liu, X. hong. (2024), “Global, regional, and national burden of ischemic stroke, 1990–2021: an analysis of data from the global burden of disease study 2021”, *EClinicalMedicine*, Elsevier Ltd, Vol. 75, doi: 10.1016/j.eclinm.2024.102758.

Lin, M.P. and Liebeskind, D.S. (2016), *Imaging of Ischemic Stroke*, *Continuum (Minneap Minn)*, Vol. 22.

Liu, X., Su, P., Meng, S., Aschner, M., Cao, Y., Luo, W., Zheng, G., *et al.* (2017), “Role of matrix metalloproteinase-2/9 (MMP2/9) in lead-induced changes in an in vitro blood-brain barrier model”, *International Journal of Biological Sciences*, Ivyspring International Publisher, Vol. 13 No. 11, pp. 1351–1360, doi: 10.7150/ijbs.20670.

Longa, E.Z., Weinstein, P.R., Carlson, S. and Cummins, R. (1989), “Reversible Middle Cerebral Artery Occlusion Without Craniectomy in Rats”, *Stroke*, No. 20, pp. 84–97.

Mansour, T., Sileem, S., El-Barody, M. and Okasha, A. (2020), “Role Of Magnetic Resonance Imaging With Diffusion-Weighted Images In Assessment Of Patients with Pre-eclampsia In Pre And Postpartum Periods”, *Al-Azhar International Medical Journal*, Al-Azhar University, Vol. 0 No. 0, pp. 0–0, doi: 10.21608/aimj.2020.43406.1324.

Matos Diaz, I., Liang, J.W., Ostojic, L.V. and Tansy, A.P. (2017), “Acute Stroke Multimodal Imaging: Present and Potential Applications toward Advancing Care”, *Seminars in Neurology*, Thieme Medical Publishers, Inc., Vol. 37 No. 5, pp. 558–565, doi: 10.1055/s-0037-1607355.

McConnell, H.L., Kersch, C.N., Woltjer, R.L. and Neuwelt, E.A. (2017), “The translational significance of the neurovascular unit”, *Journal of Biological Chemistry*, American Society for Biochemistry and Molecular Biology Inc., 20 January, doi: 10.1074/jbc.R116.760215.

Mohr, C., Brady, J.D. and Rossi, D.J. (2010), “Young age and low temperature, but not female gender delay ATP loss and glutamate release, and protect Purkinje cells during simulated ischemia in cerebellar slices”, *Neuropharmacology*, Vol. 58 No. 2, pp. 392–403, doi: 10.1016/j.neuropharm.2009.09.013.

Muoio, V., Persson, P.B. and Sendeski, M.M. (2014), “The neurovascular unit - concept review”, *Acta Physiologica*, Blackwell Publishing Ltd, doi: 10.1111/apha.12250.

Nie, B., Liu, H., Chen, K., Jiang, X. and Shan, B. (2014), “A statistical parametric mapping toolbox used for voxel-wise analysis of FDG-PET images of rat brain”, *PLoS ONE*, Public Library of Science, Vol. 9 No. 9, doi: 10.1371/journal.pone.0108295.

Obenaus, A. and Badaut, J. (2022), “Role of the non-invasive imaging techniques in monitoring and understanding the evolution of brain edema”, *Journal of Neuroscience Research*, John Wiley and Sons Inc, 1 May, doi: 10.1002/jnr.24837.

Oliff, H.S., Coyle, P. and Weber, E. (1997), *Journal of Cerebral Blood Flow and Metabolism Rat Strain and Vendor Differences In Collateral Anastomoses*.

Otáhal, J., Folbergrová, J., Kovacs, R., Kunz, W.S. and Maggio, N. (2014), "Epileptic focus and alteration of metabolism", *International Review of Neurobiology*, Vol. 114, Academic Press Inc., pp. 209–243, doi: 10.1016/B978-0-12-418693-4.00009-1.

Pu, L., Wang, L., Zhang, R., Zhao, T., Jiang, Y. and Han, L. (2023), "Projected Global Trends in Ischemic Stroke Incidence, Deaths and Disability-Adjusted Life Years From 2020 to 2030", *Stroke*, Wolters Kluwer Health, Vol. 54 No. 5, pp. 1330–1339, doi: 10.1161/STROKEAHA.122.040073.

Rutkowsky, J.M., Wallace, B.K., Wise, P.M., O'donnell, M.E. and O'donnell, M.E. (2011), "Effects of estradiol on ischemic factor-induced astrocyte swelling and AQP4 protein abundance", *Am J Physiol Cell Physiol*, Vol. 301, pp. 204–212, doi: 10.1152/ajpcell.00399.2010.-In.

Ryu, H.W., Lim, W., Jo, D., Kim, S., Park, J.T., Min, J.J., Hyun, H., *et al.* (2018), "Low-dose evans blue dye for near-infrared fluorescence imaging in photothrombotic stroke model", *International Journal of Medical Sciences*, Ivyspring International Publisher, Vol. 15 No. 7, pp. 696–702, doi: 10.7150/ijms.24257.

Sarvari, S., Moakedi, F., Hone, E., Simpkins, J.W. and Ren, X. (2020), "Mechanisms in blood-brain barrier opening and metabolism-challenged cerebrovascular ischemia with emphasis on ischemic stroke", *Metabolic Brain Disease*, Metabolic Brain Disease, doi: 10.1007/s11011-020-00573-8.

Saunders, N.R., Dziegielewska, K.M., Møllgård, K. and Habgood, M.D. (2015), "Markers for blood-brain barrier integrity: How appropriate is Evans blue in the twenty-first century and what are the alternatives?", *Frontiers in Neuroscience*, Frontiers Media S.A., doi: 10.3389/fnins.2015.00385.

Schiffer, W.K., Mirrione, M.M., Biegon, A., Alexoff, D.L., Patel, V. and Dewey, S.L. (2006), "Serial microPET measures of the metabolic reaction to a microdialysis probe implant", *Journal of Neuroscience Methods*, Vol. 155 No. 2, pp. 272–284, doi: 10.1016/j.jneumeth.2006.01.027.

Srinivasan, A., Goyal, M., Al Azri, F. and Lum, C. (2006), "State-of-the-art imaging of acute stroke", *Radiographics*, October, doi: 10.1148/rg.26si065501.

Strbian, D., Durukan, A., Pitkonen, M., Marinkovic, I., Tatlisumak, E., Pedrono, E., Abo-Ramadan, U., *et al.* (2008), "The blood-brain barrier is continuously open for several weeks following transient focal cerebral ischemia", *Neuroscience*, Vol. 153 No. 1, pp. 175–181, doi: 10.1016/j.neuroscience.2008.02.012.

Sweeney, M.D., Zhao, Z., Montagne, A., Nelson, A.R. and Zlokovic, B. V. (2019), "Blood-brain barrier: From physiology to disease and back", *Physiological Reviews*, Vol. 99 No. 1, pp. 21–78, doi: 10.1152/physrev.00050.2017.

Tajes, M., Ramos-Fernández, E., Weng-Jiang, X., Bosch-Morató, M., Guivernau, B., Eraso-Pichot, A., Salvador, B., *et al.* (2014), "The blood-brain barrier: Structure, function and therapeutic approaches to cross it", *Molecular Membrane Biology*, Informa Healthcare, doi: 10.3109/09687688.2014.937468.

Taninishi, H., Jung, J.Y., Izutsu, M., Wang, Z., Sheng, H. and Warner, D.S. (2015), "A blinded randomized assessment of laser Doppler flowmetry efficacy in standardizing outcome from intraluminal filament MCAO in the rat", *Journal of Neuroscience Methods*, Elsevier, Vol. 241, pp. 111–120, doi: 10.1016/j.jneumeth.2014.12.006.

Tedyanto, E.H., Tini, K. and Pramana, N.A.K. (2022), "Magnetic Resonance Imaging in Acute Ischemic Stroke", *Cureus*, Springer Science and Business Media LLC, doi: 10.7759/cureus.27224.

Thrane, A.S., Rappold, P.M., Fujita, T., Torres, A., Bekar, L.K., Takano, T., Peng, W., *et al.* (2011), "Critical role of aquaporin-4 (AQP4) in astrocytic Ca2+ signaling events elicited by cerebral edema", *Proceedings of the National*

Academy of Sciences of the United States of America, Vol. 108 No. 2, pp. 846–851, doi: 10.1073/pnas.1015217108.

Thrippleton, M.J. (2019), "MRI measurement of blood–brain barrier leakage: minding the gaps", *Journal of Physiology*, Blackwell Publishing Ltd, 1 February, doi: 10.1113/JP277425.

Thrippleton, M.J., Backes, W.H., Sourbron, S., Ingrisch, M., van Osch, M.J.P., Dichgans, M., Fazekas, F., *et al.* (2019), "Quantifying blood-brain barrier leakage in small vessel disease: Review and consensus recommendations", *Alzheimer's and Dementia*, Elsevier Inc., 1 June, doi: 10.1016/j.jalz.2019.01.013.

Wang, H.L. and Lai, T.W. (2014), "Optimization of Evans blue quantitation in limited rat tissue samples", *Scientific Reports*, Nature Publishing Group, Vol. 4, doi: 10.1038/srep06588.

Wang, Q., Wang, Y., Liu, J., Sutphen, C.L., Cruchaga, C., Blazey, T., Gordon, B.A., *et al.* (2019), "Quantification of white matter cellularity and damage in preclinical and early symptomatic Alzheimer's disease", *NeuroImage: Clinical*, Elsevier Inc., Vol. 22, doi: 10.1016/j.nicl.2019.101767.

Wintermark, M., Albers, G.W., Alexandrov, A. V., Alger, J.R., Bammer, R., Baron, J.C., Davis, S., *et al.* (2008), "Acute stroke imaging research roadmap", *American Journal of Neuroradiology*, Vol. 29, doi: 10.1161/STROKEAHA.107.512319.

Woitzik, J. and Schilling, L. (2002), "Control of completeness and immediate detection of bleeding by a single laser-Doppler flow probe during intravascular middle cerebral artery occlusion in rats", *Journal of Neuroscience Methods*, Vol. 122 No. 1, pp. 75–78.

Yao, Y., Zhang, Y., Liao, X., Yang, R., Lei, Y. and Luo, J. (2021), "Potential Therapies for Cerebral Edema After Ischemic Stroke: A Mini Review", *Frontiers in Aging Neuroscience*, Vol. 12, doi: 10.3389/fnagi.2020.618819.

Zlokovic, B. V. (2008), "The Blood-Brain Barrier in Health and Chronic Neurodegenerative Disorders", *Neuron*, 24 January, doi: 10.1016/j.neuron.2008.01.003.

Overview of Author’s publications

Publications related to thesis topic

Svoboda, J., Litvinec, A., Kala, D., Pošusta, A., Vávrová, L., Jiruška, P. and Otáhal, J. (2019), “Strain differences in intraluminal thread model of middle cerebral artery occlusion in rats”, *Physiological Research*, Czech Academy of Sciences, Vol. 68 No. 1, pp. 37–48, doi: 10.33549/physiolres.933958.

IF:1.9

Kala, D., Šulc, V., Olšerová, A., Svoboda, J., Prysiazhniuk, Y., Pošusta, A., Kynčl, M., *et al.* (2022), “Evaluation of Blood-Brain Barrier Integrity by the Analysis of Dynamic Contrast-Enhanced MRI – a Comparison of Quantitative and Semi-Quantitative Methods”, *Physiological Research*, Czech Academy of Sciences, Vol. 71, pp. S259–S275, doi: 10.33549/physiolres.934998.

IF:1.9

Kala, D., Svoboda, J., Litvinec, A., Pošusta, A., Lisý, J., Šulc, V., Tomek, A., *et al.* (2017), “/GD-Tracker/ A software for blood-brain barrier permeability assessment”, *Lekar a Technika*, Vol. 47 No. 2.

Svoboda, J., Kala, D., Litvinec, A., Jiruška, P., Otáhal, J. (2017) Mozek – svět za bariérou. *Vesmír*, Vol. 96, 564 - 565

Publications under peer-review

Michal, L., Kala, D., Olšerová, A., Šulc V., Prysiazniuk Y., Svoboda, J., Šanda, J., Kynčl, M., Otáhal, J., Tomek, A. Spatio-Temporal Dynamics of MRI Diffusion Metrics in Ischemic Brain Regions in Stroke Patients with Hemorrhagic Transformation Following Endovascular Therapy. *European Stroke Journal*

Kala, D., Prysiazhniuk, Y., Jánský, P., Olšerová, A., Šrámková, T., Benešová, K., Magerová, H., Šulc, V., Schwabová, P., Holubová, Z., Svoboda, J., Michal, L., Kynčl, M., Šanda, J., Marusič, P., Otáhal, J., Tomek, A. Postischemic Cavity Development: A Potential Predictor of Clinical Outcomes? *PlosOne*

Publications not directly related to thesis topic

Daněk, J., Danačíková, Š., Kala, D., Svoboda, J., Kapoor, S., Pošusta, A., Folbergrová, J., *et al.* (2022), "Sulforaphane Ameliorates Metabolic Changes Associated With Status Epilepticus in Immature Rats", *Frontiers in Cellular Neuroscience*, Frontiers Media S.A., Vol. 16, doi: 10.3389/fncel.2022.855161.

IF:4.2

Faridová, A.T., Heřman, H., Danačíková, Š., Svoboda, J. and Otáhal, J. (2023), "Serum Biomarkers Of Hypoxic-Ischemic Brain Injury", *Physiological Research*, Czech Academy of Sciences, Vol. 72, pp. S461–S474, doi: 10.33549/physiolres.935214.

IF:1.9

Kapoor, S., Kala, D., Svoboda, J., Daněk, J., Faridová, A., Brnoliaková, Z., Mikulecká, A., *et al.* (2022), "The Effect of Sulforaphane on Perinatal Hypoxic-Ischemic Brain Injury in Rats", *Physiological Research*, Czech Academy of Sciences, Vol. 71 No. 3, pp. 401–411, doi: 10.33549/physiolres.934878.

IF:1.9

Curriculum Vitae

Personal data

Name: Jan Svoboda, Mgr. Date of birth: 18. srpen 1989

Phone: (+420) 607100141

E-mail: jan.svoboda@lfmotol.cuni.cz

Education

Graduated:

- Specialization in Health Care, Physiotherapy, master's degree, Faculty of Physical Education and Sport, Charles University in Prague (2013-2015)
- Specialization in Health Care, Physiotherapy, Faculty of Physical Education and Sport, Charles University in Prague (2009-2013)
- Gymnasium Přípotoční (2005- 2009, matriculation exam - Čj, B, Che, Aj)

Current study:

- Doctoral study program Biomechanics, Faculty of Physical Education and Sport, Charles University in Prague and Institute of Physiology, Academy of Sciences of the Czech Republic (from 2015 to present)

Courses taken outside the scope of study

- Massage course - sports, relaxation and reconditioning massage UK FTVS
- Dynamic neuromuscular stabilization, part A, B
- Training course for obtaining qualification and professional competence in the field of experimental animals according to §15d (3) of Act No. 246/1992 Coll. - design of experiments and experimental projects

Work experience

- masseur at Aeditus (from 2012 to 2013)
- physiotherapist at CKP Roseta s.r.o. (from 2013 to 2015)
- physiotherapist VO2MAX (from 2015 to 2016)
- PhD student, Department of Developmental Epileptology, Academy of Sciences of the Czech Republic (2015 to present)
- Academic staff member of the Department of Anatomy and Biomechanics, Faculty of Physical Education, Charles University (2015-2021)
- Researcher, Department of Pathological Physiology, 2nd Faculty of Medicine, Charles University (2022 to present)