MICROGLIA IN ACUTE BRAIN SLICES AND THEIR INFLUENCE ON NEURONAL NETWORKS

PhD thesis

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1. Introduction

The central nervous system (CNS) comprises a sophisticated ecosystem of neuronal and non-neuronal cells, with glia playing integral roles in homeostasis, metabolic support, and information processing. Microglia, the resident immune sentinels of the CNS parenchyma, are fundamental in orchestrating central immune responses and dynamically modulating neuronal activity through intricate cell-cell interactions. Importantly, their functional state is exquisitely sensitive to environmental cues, enabling rapid phenotypic transformation in response to trauma, infection, or disease. Acute brain slice preparations are an indispensable *ex vivo* model in neuroscience, preserving local tissue architecture and allowing for unparalleled experimental access for electrophysiological, imaging, and pharmacological studies. However, the mechanical act of slicing inevitably induces an acute injury, profoundly altering the microenvironment through cellular damage, blood-brain barrier disruption, and the release of damage-associated molecular patterns (DAMPs) like ATP. Consequently, microglia in acute slices are subjected to a continuous injury-related stimulus, yet the time-dependent evolution of their phenotype and its impact on network function have not been systematically characterized.

The investigations presented in this thesis underscore the critical nature of microglial phenotypes and interactions with neurons, governed by the profound sensitivity of microglia to diverse microenvironmental perturbations, including robust injury-related actions taking place in acute brain slices. Although this model does not fully replicate the physiological milieu observed *in vivo*, its inherent injury context renders it an invaluable tool for elucidating cellular responses to trauma. It also provides an instrumental platform for systematically examining the factors that govern microglial reactivity and their influence on other cells. Consequently, this work documents a robust injury-induced response while concurrently validating the acute brain slice preparation as an effective tool for dissecting underlying molecular, cellular and network mechanisms. The model thus offers significant potential for exploring novel therapeutic strategies aimed at modulating deleterious microglial phenotypes in pathological contexts.

2. Objectives

The overarching goal of this thesis was to systematically characterize the injury-induced behavior of microglia in acute slice preparations and to elucidate their functional impact on neuronal networks. The specific objectives were as follows:

1. **To establish a standardized acute slice time-series paradigm** for the controlled investigation of microglial dynamics over an experimentally relevant timeframe (0–5 hours), ensuring methodological consistency and enabling the assessment of generalizability by comparing samples from independent laboratories and preparation practices.

2. **To perform a time-dependent characterization of microglial phenotypes**, including their spatiotemporal distribution, morphological transformation, and electrophysiological properties. This involved quantitative analysis of cell migration, large-scale automated remodeling, patch-clamp electrophysiology and receptor expression in various brain regions and developmental stages.

3. **To investigate the molecular mechanisms underlying these phenotypic changes**, with a focus on purinergic (P2Y12R) and fractalkine (CX3CR1) signaling pathways. This was achieved through the use of genetic knockout models, pharmacological antagonists, and a novel, genetically encoded fluorescent ATP sensor to monitor extracellular purinergic dynamics in real-time.

4. **To assess the functional impact of microglial activity on neuronal network architecture and synchrony**. This objective sought to determine whether microglia influence injury-induced synaptic sprouting and contribute to the spontaneous emergence of synchronous network oscillations, specifically sharp wave-ripples (SWRs), in hippocampal slice preparations.

3. Methods

3.1. Animal models and slice preparation

Experiments utilized CX3CR1$^{+/GFP}$ reporter mice for microglial visualization, P2Y12$^{-/-}$ and CX3CR1$^{-/-}$ knockout mice, and a novel ATP1.0$^{\Delta Vglut1}$ transgenic line. A standardized protocol was established for preparing 300–450 µm thick horizontal hippocampal slices in ice-cold, carbogenated sucrose-based cutting solution. Slices were recovered in an interface-type chamber and immersion-fixed at designated time points (0 min, 20 min, 1 h, 2 h, 5 h) for time-series analysis. For microglia depletion studies, mice were treated with a diet containing the CSF1R inhibitor PLX3397 or PLX5622.

3.2. Immunohistochemistry and morphological analysis

Fixed slices were processed for pre- or post-embedding immunofluorescent labeling. Microglial distribution and morphology were analyzed from confocal z-stacks. A MATLAB-based automated tool, the Microglia Morphology Quantification Tool, was used for unbiased 3D reconstruction and extraction of 59 morphological parameters from individual microglia. Post-embedding immunolabeling provided high-resolution quantification of P2Y12R expression on microglial compartments and densities of glutamatergic (VGLUT1/Homer1) and GABAergic (VGAT/Gephyrin) synapses.

3.3. Real-time ATP imaging

Extracellular ATP dynamics were monitored using two-photon imaging of the fluorescent $GRAB_{ATP}$ sensor. This enabled the characterization of both large-scale ATP gradients and spontaneous, focal ATP release events. Simultaneous imaging of the ATP sensor and tdTomato-labeled microglia allowed for the analysis of microglial process recruitment to sites of ATP release.

3.4. Electrophysiology

Whole-cell and perforated patch-clamp recordings from microglia were performed to assess time-dependent changes in resting membrane potential and input resistance. The

contribution of the NKCC1 ion transporter to microglial membrane properties was investigated using NKCC1$^{-/-}$ mice and osmotic challenges. Local field potential (LFP) recordings were performed in the hippocampal CA3 pyramidal layer to monitor the emergence and characteristics of spontaneous sharp wave-ripple (SWR) activity by comparing control and microglia depleted or loss of microglial function acute slices.

3.5. Modeling and statistical Analysis

A 3-dimensional bipartite model was developed to investigate how changes in microglial morphology constrain their ability to form somatic junctions with neurons. All quantitative data were analyzed by observers blinded to the experimental conditions. Statistical significance was assessed using appropriate parametric or non-parametric tests (e.g., ANOVA with post-hoc tests, Mann-Whitney U-test), with $p < 0.05$ considered significant.

4. Results

4.1. Microglia undergo rapid, injury-induced migration and morphological transformation

We first established that acute slice preparation induces a robust and directed migratory response in microglia. Over a 5-hour period, microglial cell bodies progressively translocated towards the top surface of the slice, with their density in the most superficial layer increasing by 75%. This migratory behavior was critically dependent on fractalkine signaling, as it was significantly impaired in CX3CR1$^{-/-}$ mice. This process was paralleled by a similar polarization of microglial processes toward the surface of slice preparations, which was robustly modulated by both P2Y12R and CX3CR1 pathways. Concurrently, microglia throughout the tissue underwent a dramatic morphological transformation from a ramified to an amoeboid phenotype. Quantitative analysis revealed that microglial sphericity doubled, and the number of process endpoints decreased by ~80% within 1–2 hours post-slicing. This transformation was highly reproducible across animal age, slice preparation parameters, and different laboratories, establishing it as a universal feature of the acute slice model. This process was modulated by both purinergic and fractalkine pathways, being attenuated in P2Y12R$^{-/-}$ mice and accelerated in CX3CR1$^{-/-}$ mice.

4.2. Electrophysiological correlates of microglial activation

The phenotypic transformation was accompanied by a functional shift in microglial membrane properties, as whole-cell recordings demonstrated a gradual but significant depolarization of the microglial resting membrane potential over the 5-hour incubation period, consistent with the observed phenotypic transition in morphology. Using perforated-patch recordings and NKCC1$^{-/-}$ mice, we identified the NKCC1 ion transporter as a key regulator of microglial chloride homeostasis and membrane properties, suggesting its role in shaping the microglial response to the osmotic and ionic challenges inherent to the slice environment.

4.3. Spatiotemporal ATP dynamics orchestrate microglial responses

Utilizing a genetically encoded ATP sensor, we identified two distinct modes of purinergic signaling post-injury. First, a sustained extracellular ATP gradient was established, with highest concentrations near the cut surfaces, which likely serves as the primary chemoattractant signal for the observed large-scale microglial migration. Second, we discovered spontaneous, focal ATP release events occurring deep within the tissue, which we classified as "flashes" (smaller, faster) and "surges" (larger, slower). Microglia rapidly recruited their processes to these endogenous ATP events in a P2Y12R- and CX3CR1-dependent manner, demonstrating that these receptors mediate microglial surveillance and response to local, ongoing injury signals.

4.4. Microglia actively remodel neuronal circuits post-injury

Microglial transformation directly impacted microglia-neuron interactions. P2Y12R expression on microglial processes was rapidly downregulated within the first hour, yet microglia remained responsive to ATP signals. While the overall prevalence of somatic junctions with neurons decreased over time — a finding our computational model attributed to the reduced physical reach of amoeboid microglia — the somatic area covered by microglial processes transiently doubled immediately after slicing, indicating an acute protective response. At the synaptic level, microglia dynamically altered their contacts, increasing interactions with glutamatergic synapses while retracting from GABAergic ones. Most strikingly, we found that microglia were indispensable for the synaptic sprouting observed in acute slices. While control slices exhibited a time-dependent increase in both glutamatergic and GABAergic synapse density, this process was completely abolished for glutamatergic synapses and dysregulated for GABAergic synapses in microglia-depleted slices. This demonstrates a pivotal role for microglia in actively driving injury-induced structural plasticity.

4.5. Microglia are essential for the emergence of network synchrony

Finally, we established a direct link between these microglial functions and the recovery of integrated network activity. The spontaneous emergence of hippocampal sharp wave-ripples (SWRs), a hallmark of synchronous network function, occurred only after 2 hours of incubation, coinciding with the peak of microglia-mediated synaptic sprouting.

Importantly, our result showed that the generation of SWRs was profoundly dependent on microglia. The prevalence of SWR-generating slices dropped from 50% in controls to just 11% in microglia-depleted preparations, and the amplitude of the remaining SWRs was severely attenuated. The loss of P2Y12R or CX3CR1 function also significantly impaired SWR emergence and properties, underscoring the importance of these pathways in the microglial-dependent restoration of network function.

5. Discussion

The findings of this thesis collectively demonstrate that microglia in acute brain slices are not in a quiescent state but are engaged in a dynamic, injury-induced program that is fundamentally neuroprotective and essential for the functional recovery of the neuronal network *ex vivo*. We provide a comprehensive, time-resolved map of this process, from the initial injury signals to the resulting changes in network synchrony.

Our characterization of extracellular ATP dynamics reveals a multi-layered purinergic signaling environment that orchestrates the microglial response. The transient ATP gradient drives global, large-scale cell migration, while actively released, focal "flash" and "surge" events mediate local surveillance and process motility. Ultimately, this chemotactic response to injury signals is mediated by P2Y12R and CX3CR1.

Crucially, our work repositions microglia from passive bystanders to essential active players in the *ex vivo* environment. Their indispensable role in synaptic sprouting reveals that microglia actively facilitate the structural reorganization of neuronal circuits in response to injury. This structural remodeling, coupled with their known neuroprotective functions, culminates in the restoration of complex, synchronous network activity. The near-complete absence of SWRs in microglia-depleted slices is a stark demonstration of this dependency, indicating that microglia create a permissive environment for neuronal networks to regain functional integrity after injury.

These results have important implications for the interpretation of studies using acute brain slice preparations. It is now evident that such experiments are conducted within a dynamic context of evolving microglial phenotypes and changes in microglia-neuron interactions, which must be considered in studies investigating microglia function, synaptic plasticity, and network physiology. Our detailed baseline characterization could provide a useful framework for designing and interpreting these questions in future *ex vivo* research. Moreover, by elucidating the roles of key signaling pathways like P2Y12R and CX3CR1 in this recovery process, this work offers insights that may be translatable to *in vivo* scenarios of acute brain injury, such as stroke and trauma.

6. Conclusions

This thesis provides a systematic investigation into the time-dependent behavior of microglia in acute brain slices and their influence on neuronal networks. The main conclusions are:

1. Acute slice preparation induces a universal and rapid phenotypic transformation of microglia, characterized by directed migration, morphological shift to an amoeboid state, and membrane depolarization. This response is driven by injury-related signals and is consistently observed across different experimental conditions.
2. A complex purinergic signaling landscape, comprising both sustained ATP gradients and spontaneous focal ATP release events, orchestrates the microglial response. Microglial motility and process recruitment are mediated by P2Y12R and CX3CR1 signaling pathways.
3. Microglia actively remodel neuronal circuits following the slicing injury. They are essential for the subsequent synaptic sprouting of both excitatory and inhibitory synapses, demonstrating a critical role in injury-induced structural plasticity.
4. The neuroprotective and remodeling functions of microglia are indispensable for the functional recovery of neuronal network activity *ex vivo*. The spontaneous emergence of synchronous network activity (SWRs) is severely impaired in the absence of microglia or microglia-specific functions, establishing their role as key facilitators of network resilience.

7. Publications

Presented in this thesis:

1. ***Péter Berki***, *Csaba Cserép*, Zsuzsanna Környei*, Balázs Pósfai, Eszter Szabadits, Andor Domonkos, Anna Kellermayer, Miklós Nyerges, Xiaofei Wei, Istvan Mody, Araki Kunihiko, Heinz Beck, He Kaikai, Wang Ya, Nikolett Lénárt, Zhaofa Wu, Miao Jing, Yulong Li, Attila I Gulyás, Ádám Dénes (2024):*
Microglia contribute to neuronal synchrony despite endogenous ATP-related phenotypic transformation in acute mouse brain slices.
Nature Communications, *15*(1), 5402. *:co-first authors

2. *Krisztina Tóth, Nikolett Lénárt, **Péter Berki**, Rebeka Fekete, Eszter Szabadits, Balázs Pósfai, Csaba Cserép, Ahmad Alatshan, Szilvia Benkő, Dániel Kiss, Christian A Hübner, Attila Gulyás, Kai Kaila, Zsuzsanna Környei, Ádám Dénes (2022):*
The NKCC1 ion transporter modulates microglial phenotype and inflammatory response to brain injury in a cell-autonomous manner.
PLoS biology, *20*(1), e3001526.

Other publications:

3. *Virág Takács, Zsuzsanna Bardóczi, Áron Orosz, Abel Major, Luca Tar, **Péter Berki**, Péter Papp, Márton I Mayer, Hunor Sebők, Luca Zsolt, Katalin E Sos, Szabolcs Káli, Tamás F Freund, Gábor Nyiri (2024):*
Synaptic and dendritic architecture of different types of hippocampal somatostatin interneurons.
PLoS Biology, *22*(3), e3002539.