Final record

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Principal Investigator: Ákos Menyhárt PhD

Title: **Modulation of astrocyte spatial buffering: the role of cADPR-dependent inhibition of Cx43 phosphorylation**

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PhD students: **PhD dissertation of Rita Frank (Superv.: Eszter Farkas and Ákos Menyhárt)**
Scientific Student Conference: **Dr. Réka Tóth 2nd award on 35. OTDK 2021, Péter Szarvas 1st award on 35. OTDK 2021**

Research background:

Cerebral edema is a key prognosticator of unfavorable outcome in acute ischemic, hemorrhagic, or traumatic brain injury, in which spreading depolarizations (SDs) are involved. In addition, the robust correlate of lesion progression after acute brain injury is a characteristic pattern of SDs, which serves as an early biomarker of injury progression, and is considered as a pharmacological target[1]. SD is a slowly propagating wave (2-6 mm/min) of a near complete cellular depolarization followed by the transient depression of neural activity and cytotoxic edema[2]. In addition to the cytotoxic water translocation within the nervous tissue, ischemic SD initiates cerebrospinal fluid influx and drives acute brain swelling after middle cerebral artery occlusion in mice[3]. Although SD is primarily the profound ionic disturbance of neurons, intact astrocytic clearance mechanisms are essential for the recovery of the tissue from SD[2]. Under stress, the notable swelling of astroglia impairs K+ and glutamate clearance, which makes neurons susceptible for increased action potential firing, epileptiform activity, and SD, and compromises neuronal viability[4]. Moreover, astrocyte swelling in response to metabolic poisoning by fluorocitrate provoked spontaneous SD occurrence, and prolonged SD duration and neuronal injury in anesthetized rats[5].

Working hypotheses:

1. Cerebral cytotoxic edema is a key prognosticator of injury progression and unfavorable outcome in acute ischemic stroke
2. Astrocytes are pivotally involved in cytotoxic brain edema formation, because their membrane is especially permeable to water through AQP-4 channels
3. Pharmacological inhibition of astrocyte swelling, or hyperosmotic intervention reduces the focal expansion of injurious spreading depolarization and attenuates lesion progression is ischemic stroke

Main observations and results I.:

- We demonstrated in rodents that acute brain swelling upon cerebral ischemia impairs astroglial glutamate clearance and increases the tissue area invaded by spreading depolarization (SD)[6] (Figure 1.)
- We reported for the first time, that cytotoxic extracellular glutamate accumulation (>15 μM) predisposes an extensive bulk of tissue (4-5 mm2) for a yet undescribed simultaneous depolarization (SiD) (Figure 1.)
- We confirmed in rat brain slices exposed to osmotic stress that SiD is the pathological expansion of prior punctual SD foci (0.5-1 mm2), is associated with astrocyte swelling, and triggers oncotic neuron death.[6, 7] (Figure 1.)
- The blockade of astrocytic aquaporin-4 channels and Na+/K+/Cl- co-transporters, or volume-regulated anion channels mitigated slice edema, extracellular glutamate accumulation (<10 μM) and SiD occurrence[6]
- Furthermore, reversal of slice swelling by hyperosmotic mannitol counteracted glutamate accumulation and prevented SiD. In contrast, inhibition of glial metabolism or inhibition of astrocyte glutamate transporters reproduced the SiD phenotype[6]
- We demonstrated in the rodent water intoxication model of cytotoxic edema that astrocyte swelling and altered astrocyte calcium waves are central in the evolution of SiD. (Figure 1.)
- Finally, we discussed our results in the light of evidence for SiD in the human cortex (2 patients).

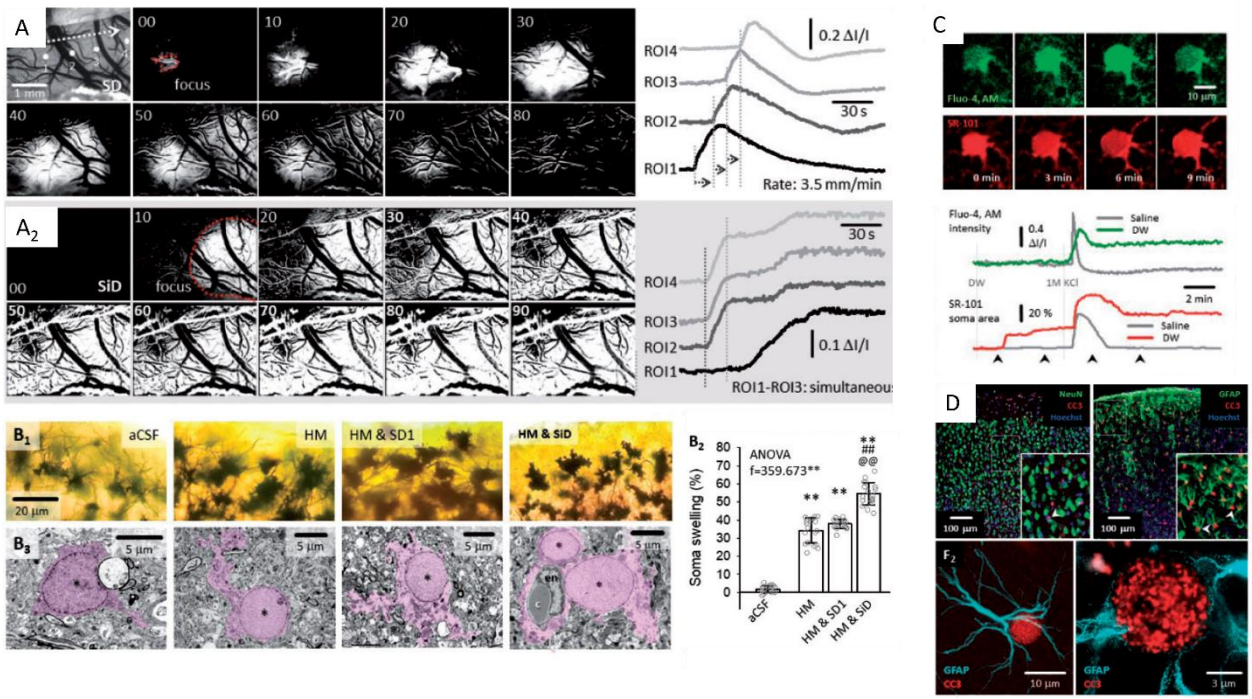

Figure 1. The evolution of simultaneous depolarization (SiD) upon anoxia induction is linked to acute tissue swelling. Anoxia-triggered spreading depolarization (recurrent, SD, rSD; 6 of 11 rats). Background subtracted intrinsic optical signal (IOS) image sequences show SD propagation from a confined focus at the site of elicitation with 1M KCl (A1). Traces derived from four regions of interest (ROIs) confirm the propagation of the SD. In contrast, anoxia triggered SiD that appeared at ROI2, ROI3 and ROI4 in synchrony, and involved a considerable part of field of view simultaneously (A2). Astrocyte swelling: Golgi-Cox-stained sections (B1) and electron photomicrographs (B2) of

astrocytes in aCSF, HM after SD1 in HM and after SiD in HM (asterisk: astrocyte nucleus; purple shading astrocyte plasma and nucleus, c: capillary lumen filled with an erythrocyte). The soma swelling of astrocytes was measured in Golgi-Cox-stained preparations and have been expressed relative to soma size in aCSF (B3). Two-photon imaging of intracellular calcium waves (Fluo-4, AM) in astrocytes, and astrocyte soma volume (SR-101) in the cerebral cortex of anesthetized mice. (C) Representative traces derived from the experiment presented in Panel C show the intracellular accumulation of Ca^{2+} and astrocyte soma swelling with SD after water intoxication (DW) with respect to control (saline). Arrowheads beneath the traces show the time instant of the images in Panel C. Immunocytochemical co-localization of cleaved caspase-3 (CC3) with cell nuclei (Hoechst) of neurons (NeuN) and astrocytes (GFAP) shows predominant glial apoptosis after SiD (D). Super-resolution (STED) microscopy unravels the nuclear localization of CC3.

The clinical management of cerebral edema in acute brain injury currently aims at the reduction of intracranial pressure and the maintenance of cerebral perfusion pressure by sedation, hyperventilation, osmotherapy, hypothermia, and in the most severe cases decompressive craniectomy.[8] Our results that preventive hyperosmotic intervention reduced the excitability of the nervous tissue and most importantly, averted SiD, provide pathophysiological insight into this empirical clinical strategy for the first time, and emphasize the need to invent new ways of preventive osmotherapy in the treatment of acute brain injury.

Main observations and results II.:

- Our observations reported the non-synchronized astrocyte intracellular calcium oscillations after SD for the first time *in vivo*[9] (Figure 2.)
- Non synchronized astrocyte calcium oscillations occurred coincidentally with arteriolar dilation upon SD

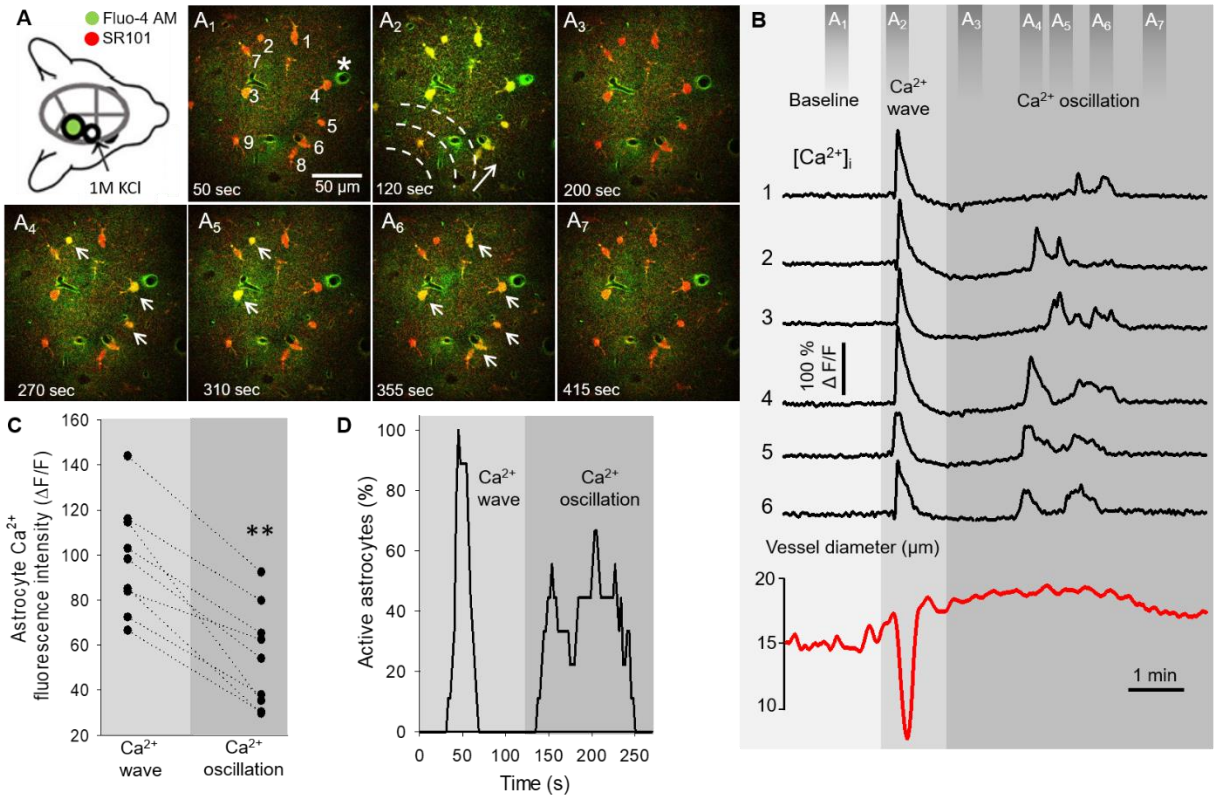

Figure 2. Astrocyte Ca2+ dynamics during spreading depolarization (SD) in the mouse somatosensory cortex. (A) Schematic illustration of the closed cranial window preparation indicates the position of the imaging site (green). SD events were triggered by topical application of KCl in a smaller rostral open craniotomy (open circle). Images (A1–7) demonstrate astrocyte Ca2+ changes (Fluo-4 AM, green) associated with SD. Astrocytes (numbered 1–6 on A1) were selectively labeled by SR101 (red). Dashed lines and arrow (A2) denote the direction of SD propagation; white arrows are pointing at astrocyte somata displaying Ca2+ oscillations (A4–6). (B) Astrocyte Ca2+ changes (i.e., wave and oscillations) extracted from regions of interests (numbered 1–6 in A1) coincide with arteriole diameter changes (labeled with * in A1) during SD. Dark grey bars (top) indicate time points of the corresponding images (A1–7). (C) Ladder plot shows the peak fluorescence maximum (ΔF/F) of Ca2+ waves and subsequent oscillations derived from nine cells. (D) Percentage of astrocytes (n = 9 in total) displaying Ca2+ changes at each time point during acquisition. Ca2+ events were defined as ΔF/F ≥ 13% with respect to baseline fluorescence. Images were taken at a cortical depth of 55–75 µm. Data are given as mean ± st.dev. Two-tailed paired t-test was used for statistical analysis with the level of significance set at p ** < 0.01.

Our results provide the first in vivo characterization of delayed astrocyte Ca2+ oscillations following SD and are complementary to the in vitro findings of Wu et al. [10]. Although SD is primarily a neuronal depolarization wave, the role of astrocytes in the electrophysiological restoration of the nervous tissue after SD, and in ischemia, is increasingly more recognized [11]. Therefore, better understanding of astroglial biology is expected to improve our understanding of SD and its consequences and may offer more specific therapeutic targets to treat acute cerebrovascular disorders in which SD is relevant.

Taken together, I want to express my gratitude for the honorable support of NKFIH.

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Direct research output:

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