SPREADING DEPOLARIZATION IN THE ISCHEMIC RAT BRAIN:

AGE-DEPENDENCE, AND MECHANISMS BEHIND THE ASSOCIATED HEMODYNAMIC RESPONSES

The proposal originally formulated the following goal:

"Our overall aim is to improve our understanding of the evolution of the hemodynamic and metabolic correlates of spreading depolarization (SD) during ischemia, and to identify vasoregulatory mechanisms of the SD-associated hemodynamic response. A key component of the study will be to determine the impact of age on SD occurrence. The proposed research will rely on conventional electrophysiology and novel imaging techniques applied to relevant young and aged rat models of cerebral ischemia."

This goal has been fully achieved, as detailed below in the order of the research questions formulated in the proposal:

Original Hypothesis (**H**), to be tested along a defined Objective (**O**):
H1: The hemodynamic response to SD is achieved by a fine balance between constrictive and dilator vasoregulatory mechanisms. Ischemic insults shift this balance toward vasoconstriction, which leads to hampered hyperemia, or even hypoperfusion (spreading ischemia) with SD.
O1: We will determine the kinetics of SD and associated hemodynamic responses in established cerebral ischemia models in order to identify features inflicted by ischemia, which may also be responsible for the pathogenic effect of SD.

Cerebral ischemia was induced by bilateral common carotid artery occlusion in isoflurane anesthetized rats. Sham-operated animals of both age groups served as control. Electrocorticogram, direct current potential, and cerebral blood flow (CBF) variations were acquired via a small craniotomy or a large closed cranial window above the parietal cortex. SDs were elicited by KCl through a second craniotomy distal to the recording site. Local CBF variations were monitored either with laser-Doppler flowmetry, or laser speckle contrast analysis (LASCA). Pharmacological manipulations were implemented to unravel the molecular mechanisms of CBF adjustment is reposne to SD.
The relative amplitude of hyperemia markedly decreased, while its duration more than doubled under ischemia with respect to baseline. The hyperemic element of the cerebral blood flow response to spreading depolarization was found to be effectively modulated by tissue pH in the intact rat cerebral cortex, and this coupling proved dysfunctional under ischemia. Pharmacological manipulations in combination with the use of potassium-sensitive microelectrodes suggested that large-conductance Ca^{2+}-activated potassium (BK) channels and L-type voltage-gated calcium channels played significant roles in the SD-coupled, marked initial vasoconstriction under elevated baseline potassium. Potassium efflux through BK channels has emerged as a central component in the devastating neurovascular effects of SDs in tissue at risk.
In conclusion, we found evidence for both metabovascular (e.g. tissue pH) and neurovascular coupling (e.g. potassium) with SD, which are impaired by ischemia. This leads to the insufficiency of the CBF response to SD in the ischemic cerebral cortex, which is thought to facilitate the progressions of ischemic neurodegeneration. Our data and those of others prompted us to propose the concept, that the CBF response associated with spreading depolarization is essentially determined by metabolic mediators, which probably outweigh neurovascular coupling (Fig. 1).

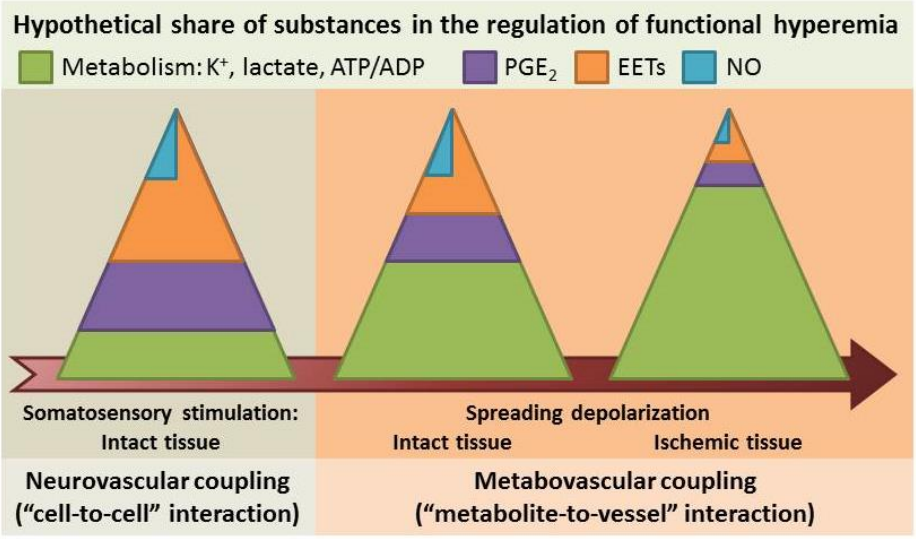

Figure 1. The CBF response associated with spreading depolarization is essentially determined by metabolic mediators, which outweigh neurovascular coupling. Abbreviations: EETs, epoxyeicosatrienoic acids; NO, nitric oxide; PGE_2, prostaglandin E2.

The results are detalied in relevant publications:

1. Menyhárt, Á., Makra, P., Szepes, B.É., M. Tóth, O., Hertelendy, P., Bari, F., **Farkas, E.** (2015) High incidence of adverse cerebral blood flow responses to spreading depolarization in the aged ischemic rat brain. Neurobiol. Aging, 36(12), 3269-77.
2. Menyhárt, Á., Zölei-Szénási, D., Puskás, T., Makra, P., Bari, F., **Farkas, E.** (2017) Age or ischemia uncouples the blood flow response, tissue acidosis, and the direct current potential signature of spreading depolarization in the rat brain. Am. J. Physiol. Heart Circ. Physiol. 313(2), H328-H337.
3. Menyhárt, Á., Zölei-Szénási, D., Puskás, T., Makra, P., M.Tóth, O., Szepes, B.É., Tóth, R., Ivánkovits-Kiss, O., Obrenovitch, T.P., Bari, F., **Farkas, E.** (2017) Spreading depolarization remarkably exacerbates ischemia-induced tissue acidosis in the young and aged rat brain. Sci Rep. 7(1), 1154.
4. Hertelendy P., Menyhárt Á., Makra P., Süle Z., Kiss T., Tóth G., Ivánkovits-Kiss, O., Bari, F., **Farkas., E.** (2017) Advancing age and ischemia elevate the electric threshold to elicit spreading depolarization in the cerebral cortex of young adult rats. J. Cereb. Blood Flow Metab. 37(5), 1763-1775.
5. Toth, P., Szarka, N., **Farkas, E.**, Ezer, E., Czeiter, E., Amrein, K., Ungvari, Z., Hartings, J.A., Buki, A., Koller, Á. (2016) Autoregulatory dysfunction and spreading depression-related neurovascular un-coupling following traumatic brain injury: pathomechanisms, therapeutic implications, and perspectives. Am. J. Physiol. Heart. Circ. Physiol. 311(5), H1118-H1131.

H2: Vasoactive arachidonic acid metabolites are potential mediators of the SD-associated CBF response. Cerebral ischemia modulates the synthesis of these metabolites, thereby altering the kinetics of the hemodynamic response.

O2: We will dissect potential vasoregulatory mechanisms mediating the hyperemic CBF response to SD, with the help of pharmacological manipulations. We will resolve whether the identified mechanisms are compromised by cerebral ischemia.

The significance of prostanoid signaling in neurovascular coupling during somatosensory stimulation is increasingly more appreciated, yet its involvement in mediating the CBF response to SD has remained inconclusive. In our experiments, selective cyclooxygenase (COX) enzyme inhibitors (NS-398, SC-560), an

antagonist of the EP4 receptor of prostaglandin E2 (L161,982) or an antagonist of the FP receptor of prostaglandin F2α (AL-8810) were applied topically to a cranial window over the parietal cortex of isoflurane-anesthetized rats. In the same model system presented at H1/O1, we collected experimental evidence for the role of prostanoid signalling in the regulation of the CBF response to SD.
We found that EP4 receptor antagonism significantly decreased peak hyperemia and augmented post-SD oligemia in the intact but not in the ischemic cortex. AL-8810 augmented peak hyperemia amplitude under ischemia. When the brain tissue is exposed to ischemia, the early transient hypoperfusion element of the CBF response to SD becomes dominant, and only hypoemia can be observed. AL-8810 significantly increased the minimum point of the hypoemic response.
In cocnlusion, potential agonsism of EP4 receptors or the antagonism of FP receptors emerges as a promising approach to inhibit the evolution of injurious SDs in cerebral ischemia.

The results are detalied in relevant publications:

1. Varga, D.P., Szabó, Í., Varga, V.É., Menhyárt, Á., Bálint, A.R., Kozma, M., Krizbai, I.A., Bari, F., **Farkas, E.** (2020) The antagonism of prostaglandin FP receptors inhibits the evolution of spreading depolarizations in an experimental model of global forebrain ischemia. Neurobiol. Dis., in preparation.
2. Szabó, Í., M.Tóth, O., Török, Z., Varga, D.P., Menyhárt, Á., Frank, R., Hantosi, D., Hunya, Á., Bari, F., Horváth, I., Vigh, L., **Farkas, E.** (2019) The impact of dihydropyridine derivatives on the cerebral blood flow response to somatosensory stimulation and spreading depolarization. Br. J. Pharmacol. 176(9), 1222-1234.
3. Menyhárt, Á., Farkas, A.E., Varga, D.P., Frank, R., Tóth, R., Bálint, A.R., Makra, P., Dreier, J.P., Bari, F., Krizbai, I.A., **Farkas, E.** (2018) Large-conductance Ca2+-activated potassium channels are potently involved in the inverse neurovascular response to spreading depolarization. Neurobiol. Dis. 119, 41 52.
4. Varga, D.P., Puskás, T., Menyhárt, Á., Hertelendy, P., Zölei-Szénási, D., Tóth, R., Ivánkovits-Kiss, O., Bari, F., **Farkas, E.** (2016) Contribution of prostanoid signaling to the evolution of spreading depolarization and the associated cerebral blood flow response. Sci Rep. 6:31402. doi: 10.1038/srep31402.

H3: Tissue acidosis that typically evolves during ischemic insults, is capable of modifying the hemodynamic response to SD. Various levels of tissue acidosis produce heterogeneous kinetics of the hemodynamic response during ischemia.
O3: We will identify the role of ischemia-related pH changes in altering the kinetics of SD and related hemodynamic responses. We will adapt our imaging system to incorporate pH imaging among our tools. We will introduce pH-sensitive microelectrodes to validate NR imaging for tissue pH changes.

We introduced tissue pH imaging with using pH sensitive microelectrodes as reference. Even though pH-sensitive microelectrodes provide excellent extracellular pH (pHe) signal, additional, spatial resolution is also required to follow SDs that occur spontaneously at unpredictable sites in the ischemic cortex, and propagate across regions of various metabolic status. Neutral Red (NR) is a vital dye that indicates changes of intracellular pH (pHi) in the brain, and has been applied successfully to follow spreading acidification and depression in the cerebellum. We adapted NR imaging to monitor pHi changes with SD in the rat cerebral cortex, and combined it with laser speckle contrast analysis (LASCA)-based cerebral blood flow (CBF) imaging to directly relate tissue pH changes to CBF variations. We have found a good correlation between intra- and extracellular acidosis during SD.
We have shown in the cerebral cortex of isoflurane anesthetized laboratory rats, that SDs propagating under ischemic penumbra-like conditions decrease intra and- extracellular tissue pH transiently to levels, which have been recognized to cause tissue damage. Further, tissue pH after the passage of each

spontaneous SD event remained acidic for an extended period of time. Correlation analysis between tissue pH and the SD-coupeled hyperemia suggested that the hyperemic element of the cerebral blood flow response to spreading depolarization is effectively modulated by tissue pH.

The results are detalied in relevant publications:

1. M. Tóth, O., Menyhárt, Á., Varga, V.É., Hantosi, D., Iványkovits-Kiss, O., Varga, D.P., Szabó, Í., Janovák, L., Dékány, I., **Farkas, E.**, Bari, F. (2019) Chitosan nanoparticles release nimodipine in response to tissue acidosis to attenuate spreading depolarization evoked during forebrain ischemia. Neuropharmacology, under revision.
2. Menyhárt, Á., Zölei-Szénási, D., Puskás, T., Makra, P., Bari, F., **Farkas, E.** (2017) Age or ischemia uncouples the blood flow response, tissue acidosis, and the direct current potential signature of spreading depolarization in the rat brain. Am. J. Physiol. Heart Circ. Physiol. 313(2), H328-H337.
3. Menyhárt, Á., Zölei-Szénási, D., Puskás, T., Makra, P., M.Tóth, O., Szepes, B.É., Tóth, R., Iványkovits-Kiss, O., Obrenovitch, T.P., Bari, F., **Farkas, E.** (2017) Spreading depolarization remarkably exacerbates ischemia-induced tissue acidosis in the young and aged rat brain. Sci Rep. 7(1), 1154.

H4: The hyperemic response to SD becomes less pronounced in the aged brain. Aging rearranges the balance between vasoconstrictive and vasodilator mechanisms, and resets the balance with a dominating vasoconstrictive component.
O4: We will consider the impact of aging on the evolution of SD and related hemodynamic responses, with a major focus on the old ischemic brain. We will determine whether vasoregulatory mechanisms implicated in the CBF response to SD are altered by aging. We will examine how age influences SD and ischemia-related tissue pH variations, which may reflect injury progression in ischemia.

Cerebral ischemia was induced by bilateral common carotid artery occlusion in young (8-9 weeks old), and old (2 year old) isoflurane anesthetized rats. Sham-operated animals of both age groups served as control. Electrocorticogram, direct current potential, and cerebral blood flow (CBF) variations were acquired via a small craniotomy or a large closed cranial window above the parietal cortex. SDs were elicited by KCl through a second craniotomy distal to the recording site.
The impact of age was most of all reflected by the prominent decrease of the magnitude of hyperemia under all three phases of the experiments (i.e. baseline, ischemia and reperfusion). In another set of experiments we observed that CBF decreased progressively during ischemia in the old but not in the young animals during ischemia, and inverse neurovascular coupling with SD evolved frequently in the old but not in the young group of animals.
The analysis of the coupling between tissue pH and the hyperemic response to SD revealed that old age uncoupled the amplitude of hyperemia from that of the DC potential shift and acidosis. Aging dissociated the duration of acidosis from the duration of the DC potential shift and hyperemia under Ischemia. The age-related uncoupling of acidosis from the other variables was clearly due to the marked elongation of the duration of acidosis relative to the other variables in the old group.
In summary, aging impaired the CBF response to SD, probably because the coupling between tissue pH and CBF becomes inefficient in the aged.

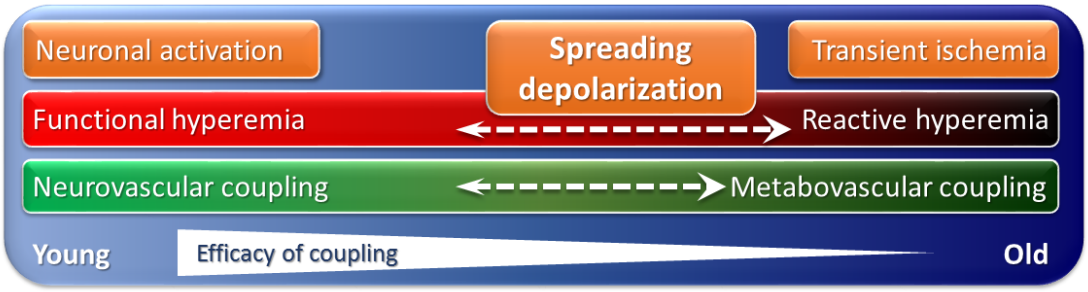

Figure 2. Conceptual overview of the type of coupling between spreading depolarization (SD) and the associated hyperemia in the context of aging. Our result corroborate the notion that metabolic signaling plays a major role in the mediation of hyperemia in response to SD. we propose, that on a hyperemia spectrum with functional and reactive hyperemia as its two end points, the nature of the SD-coupled hyperemic response falls closer to reactive than to functional hyperemia. This would be especially relevant for SD events, which produce a sudden, transient drop of perfusion prior to the evolution of hyperemia. Finally, we have shown that aging considerably weakens metabovascular coupling with SD.

The results are detalied in relevant publications:

1. Hertelendy, P., Varga, D.P., Menyhárt, Á., Bari, F., Farkas, E. (2019) Susceptibility of the cerebral cortex to spreading depolarization in neurological disease states: The impact of aging. Neurochem. Int. 127, 125-136.
2. Makra, P., Menyhárt, Á., Bari, F., Farkas, E. (2018) Spectral and multifractal signature of cortical spreading depolarisation in aged rats. Front. Physiol. 9, 1512.
3. Hertelendy P., Menyhárt Á., Makra P., Süle Z., Kiss T., Tóth G., Ivánkovits-Kiss, O., Bari, F., Farkas., E. (2017) Advancing age and ischemia elevate the electric threshold to elicit spreading depolarization in the cerebral cortex of young adult rats. J. Cereb. Blood Flow Metab. 37(5), 1763-1775.
4. Menyhárt, Á., Zölei-Szénási, D., Puskás, T., Makra, P., Bari, F., Farkas, E. (2017) Age or ischemia uncouples the blood flow response, tissue acidosis, and the direct current potential signature of spreading depolarization in the rat brain. Am. J. Physiol. Heart Circ. Physiol. 313(2), H328-H337.
5. Menyhárt, Á., Zölei-Szénási, D., Puskás, T., Makra, P., M.Tóth, O., Szepes, B.É., Tóth, R., Ivánkovits-Kiss, O., Obrenovitch, T.P., Bari, F., Farkas, E. (2017) Spreading depolarization remarkably exacerbates ischemia-induced tissue acidosis in the young and aged rat brain. Sci Rep. 7(1), 1154.
6. Menyhárt, Á., Makra, P., Szepes, B.É., M. Tóth, O., Hertelendy, P., Bari, F., Farkas, E. (2015) High incidence of adverse cerebral blood flow responses to spreading depolarization in the aged ischemic rat brain. Neurobiol. Aging, 36(12), 3269-77.