

Diffusive spread of substance through brain extracellular space in *in vitro* model of sleep and awake brain states

S. Hrabetova*, A. Sherpa, F. Xiao

State University of New York Downstate Medical Center, Brooklyn, NY 11203, USA

*Email: sabina.hrabetova@downstate.edu

Brain function is based on communication between individual cells. This communication utilizes small neurotransmitter and neuromodulator molecules, which are transported through extracellular space (ECS). Substances released into the ECS spread predominantly by diffusion because the ECS lacks any active transport mechanism. Because its structure exerts a direct influence on the intercellular signaling and also on transport of nutrients, metabolites and therapeutic agents [1, 2], the ECS has fundamental importance for brain function.

Diffusion can be exploited experimentally to quantify two major structural parameters of the ECS, volume fraction and diffusion permeability [1, 3]. Volume fraction (α) is the proportion of the tissue occupied by the ECS. Diffusion permeability (θ) is defined as a ratio of the effective diffusion coefficient in the brain tissue and the free diffusion coefficient, and it quantifies hindrance imposed to diffusing molecules. ECS parameters have been quantified in many brain regions of different species [2] but all those studies were performed either in sleeping animals or in *in vitro* preparations of brain slices, which likely represent the sleep state. Recently, the ECS parameters were measured for the first time in brain of awake mice [4]. This study reported that α was 0.23 in the neocortical region of the sleeping mice but decreased to only 0.14 when the mice woke up; θ remained unchanged. The study also reported that α in awake animal increased from 0.14 to 0.23 when noradrenergic system in the neocortex was inhibited by a mixture of noradrenergic antagonists, implying that noradrenergic signaling is involved in regulation of α during sleep and awake brain states.

Activation of noradrenergic system is known to cause wakefulness, increase vigilance and facilitate the transition of cortical activity from the sleep state to the awake state [5]. We measured ECS parameters in a visual region of the rat neocortical slices under control conditions and after activation of noradrenergic system by the noradrenergic agonist isoproterenol. Using the Real-Time Iontophoretic (RTI) method [1], we found that α decreased from 0.22 to 0.18 when isoproterenol was applied, suggesting that activation of noradrenergic receptors mimics the awake state in a brain slice. Next, we utilized this experimental paradigm as an *in vitro* model of sleep and awake brain states to study diffusive spread of molecules with small and high molecular weights in these states. To this end, we quantified diffusion of a small cation tetramethylammonium (MW 74) with the RTI method and fluorescently-labeled macromolecule dextran (MW 3000) with Integrative Optical Imaging method [6] in the visual neocortex both under the control conditions and after application of isoproterenol. Our preliminary results show that θ_{TMA} remained constant (0.376 vs. 0.381) while θ_{dextran} decreased (0.346 vs. 0.289) after the application of isoproterenol.

In conclusion, our pilot study suggests that the diffusive spread of macromolecules in brain ECS is more restricted during the awake-like state than during the sleep-like state. This result has important implications for transport of growth factors, proteins and macromolecular therapeutics in brain ECS.

References

- [1] C. Nicholson, J. Phillips: *Ion diffusion modified by tortuosity and volume fraction in the extracellular microenvironment of the rat cerebellum*. J. Physiol. **321**, 225-257 (1981). [2] E. Sykova, C. Nicholson: *Diffusion in brain extracellular space*. Physiol. Rev. **88**, 1277-1340 (2008). [3] J. Hrabec, S. Hrabetova, K. Segeth: *A model of effective diffusion and tortuosity in the extracellular space of rat brain*. Biophys. J. **87**, 1606-1617 (2004). [4] L. Xie, H. Kang, Q. Xu, M.J. Chen, Y. Liao, M. Thiyagarajan, J. O'Donnell, D.J. Christensen, C. Nicholson, J.J. Illif, T. Takano, R. Deane, M. Nedergaard: *Sleep drives metabolite clearance from the adult brain*. Science **342**, 373-377 (2013). [5] C.M. Constantinople, R.M. Bruno: *Effects and mechanisms of wakefulness on local cortical networks* **69**, 1061-1068 (2011). [6] C. Nicholson, L. Tao: *Hindered diffusion of high molecular weight compounds in extracellular microenvironment measured with integrative optical imaging*. Biophys. J. **65**, 2277-2290 (1993).

Acknowledgement: Supported by Grant R01 NS047557 from NIH NINDS.

