# INVESTIGATION OF THE EFFECT OF AGING ON BLOOD-BRAIN BARRIER MORPHOLOGY AND FUNCTION IN WISTAR RATS

Ágnes Bajza[1], Luca Bors[1], Barbara Hutka[1], Attila Csorba[2], Domokos Máthé[3], Gergely Orsi[4], Gábor Perlaki[4], Kinga Tóth[5] and Franciska Erdő[1]

*[1]Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest; [2]University of Szeged, Faculty of Pharmacy, Deparment of Pharmacognosy, Szeged; [3]Semmelweis University, Faculty of Medicine, Department of Biophysics and Radiation Biology, Budapest; [4]Diagnostic Center Pécs, NeuroCT Kft, Pécs; [5]Hungarian Academy of Sciences, Institute of Cognitive Neuroscience and Psychology, Budapest.*

*E-mail: erdo.franciska@itk.ppke.hu*

## Introduction

Several research articles reported increased permeability of blood-brain barrier (BBB) in advanced age. A close connection has also been described between the Alzheimer's pathology (especially the impaired beta-amyloid clearance) and the BBB dysfunction. The aim of our study was to compare the efflux transporter function, the microvascular morphology and the magnetic resonance image of the brain in young adult and aged Wistar rats.

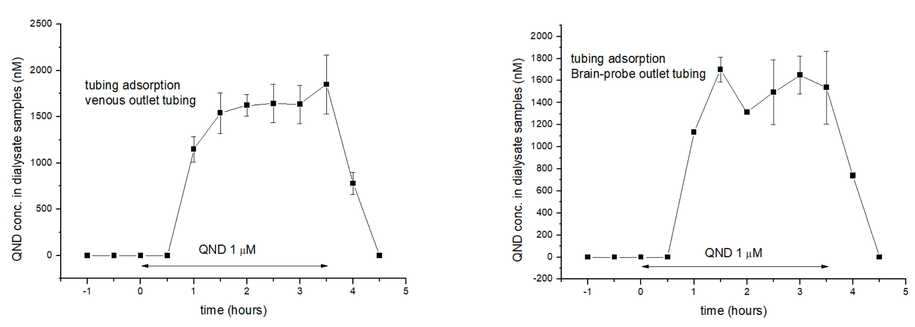


**Figure 1.** In vitro tubing absorption study for verification of non-specific binding of QND to the outlet tubing of the peripheral (left panel) and brain (right panel) microdialysis probes.

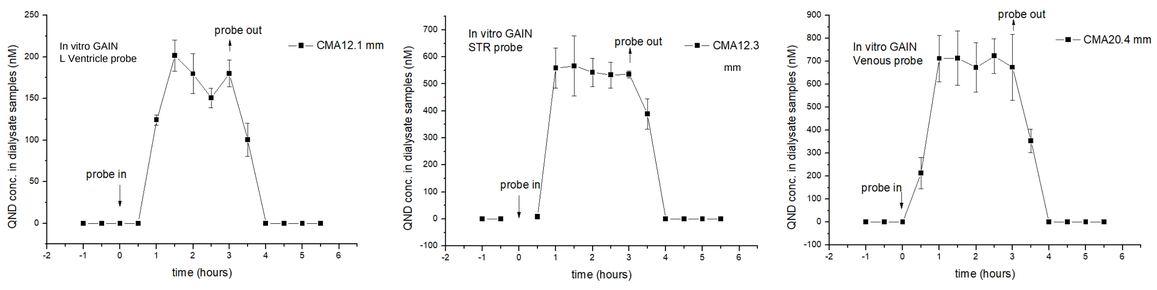


**Figure 2.** In vitro „gain" study for determination of relative recovery of microdialysis probes (CMA12.1; CMA12.3 and CMA20.4) for QND.

## Methods

The efflux transporter interactions were studied using a well-known P-glycoprotein (P-gp) substrate, quinidine (QND) and a specific P-gp inhibitor PSC-833 (valspodar) by in vivo microdialysis technique. The cerebral microvascular morphology was analyzed by electronmicroscopy (EM). The macroscopic differences between the brain structure of young and aged animals were studied by MR imaging.

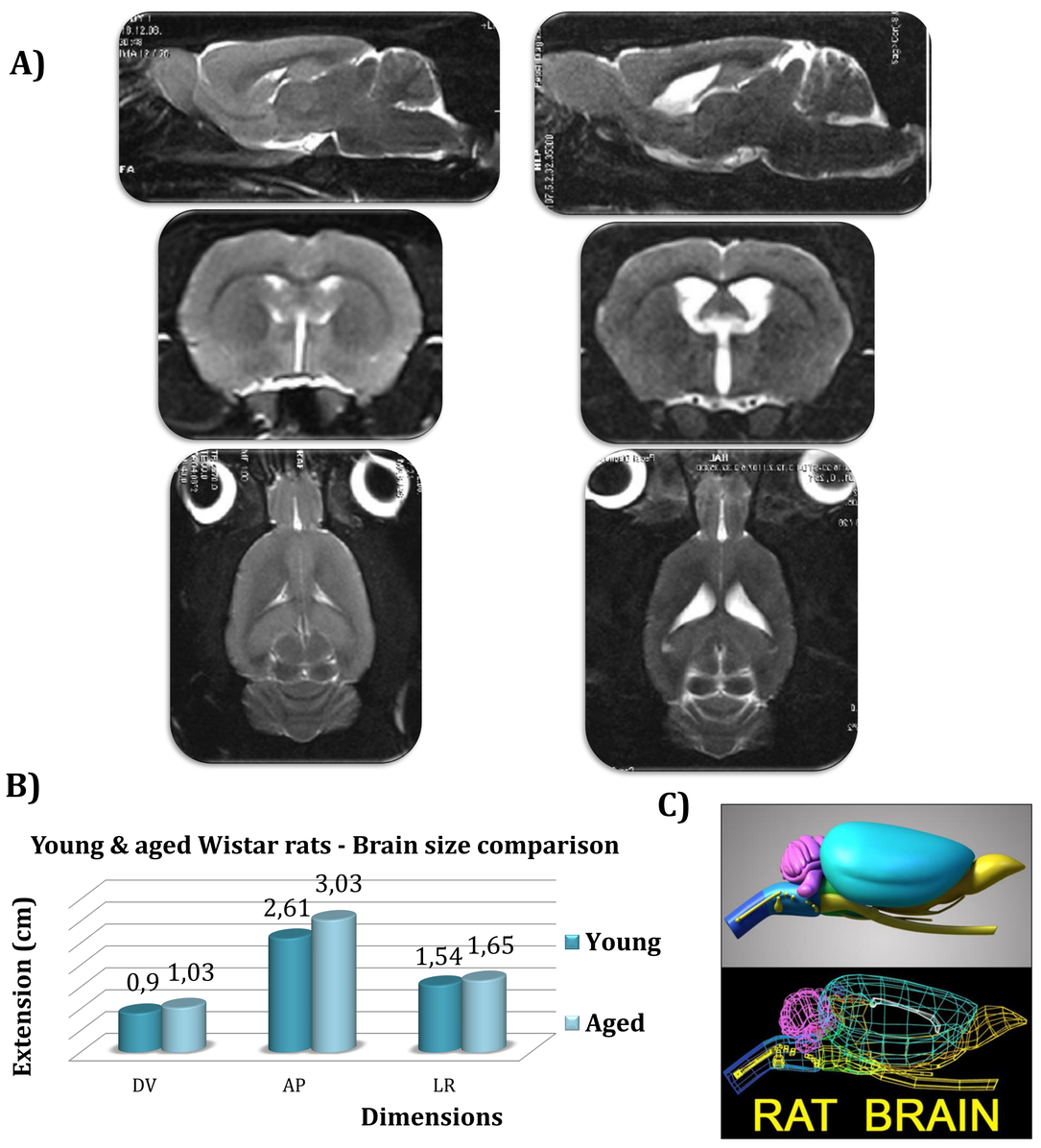


**Figure 3. A)** MR Images of young and aged rat-brains to determine the stereotaxic coordinates. These images show that the ventricles expand in older animals. Significant changes in the brain size can only be observed in the anterior-posterior direction. (Top images: sagittal view, Middle images: frontal view and Bottom images: horizontal view.) Figure 3 **B)** Brain size comparison in young and aged rats: dorsoventral (DV), anterior-posterior (AP), and Lateral (LR) extension of the brain. Aged rat (>14 months old) compared to young adults (3-4 months old). **C)** Rat brain solid and mesh model.

## Results

The brain penetration of QND increased significantly after pretreatment with PSC-833 in young rats. With similar chemical knocking out, the brain exposure to QND was less increased compared to the baseline in the aged rats determined by dual –probe microdialysis. The post-mortem studies by EM revealed decreased tight junction protein expression between the endothelial cells in the brain capillaries of old animals compared to the young ones. The size of astrocyte endfeet surrounding endothelial layer enlarged with aging and also the thickness of the basal membrane increased in old rats. The MRI investigations showed a remarkable increase in the volume of all cerebral ventricles with advanced age. The total size of the brain changed minimally, but in the bodyweight of young and aged animals tested, a more than 3 fold difference was determined.

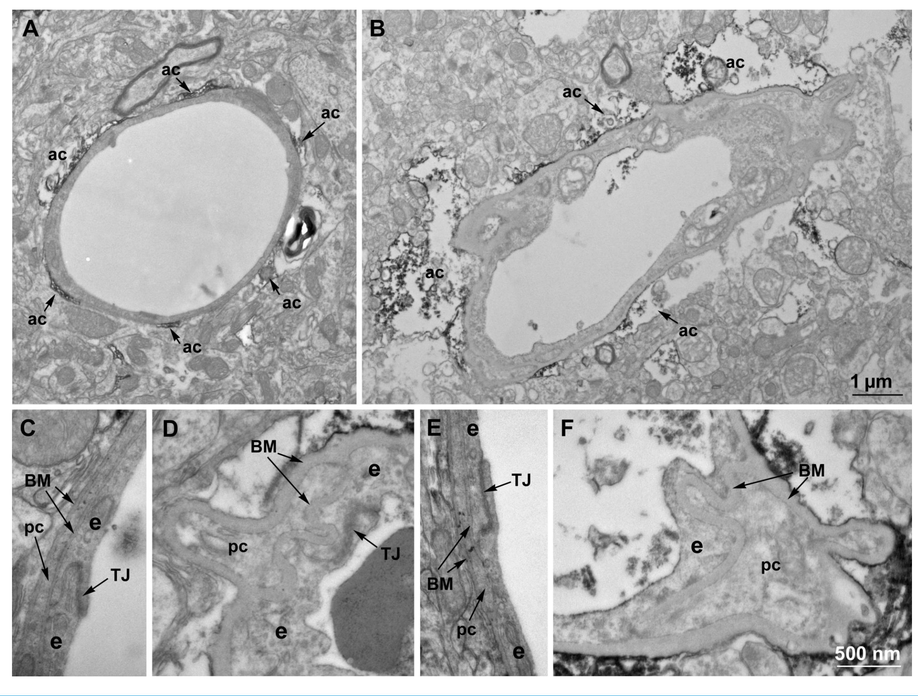


**Figure 4. A**: Electromicroscopic image of brain capillaries in young (A) and aged (B) rats. **C, E**: High magnification (30.000x) images of capillary-wall in young rat. The tight junctions (TJ) can be observed between the endothelial cells (e), the opposing side of the lumen, a pericyte (pc) can be seen embedded into the basal membrane (BM) **D, F**: The brain capillary in an aged animal (30.000x magnification) There are fewer tight junctions and the BM is significantly thicker than in the young animal. Enlarged pericytes can also be seen surrarounding the endothelial cells, but in less number (Scales: A-B: 1 μm; C-F: 500 nm) **.**

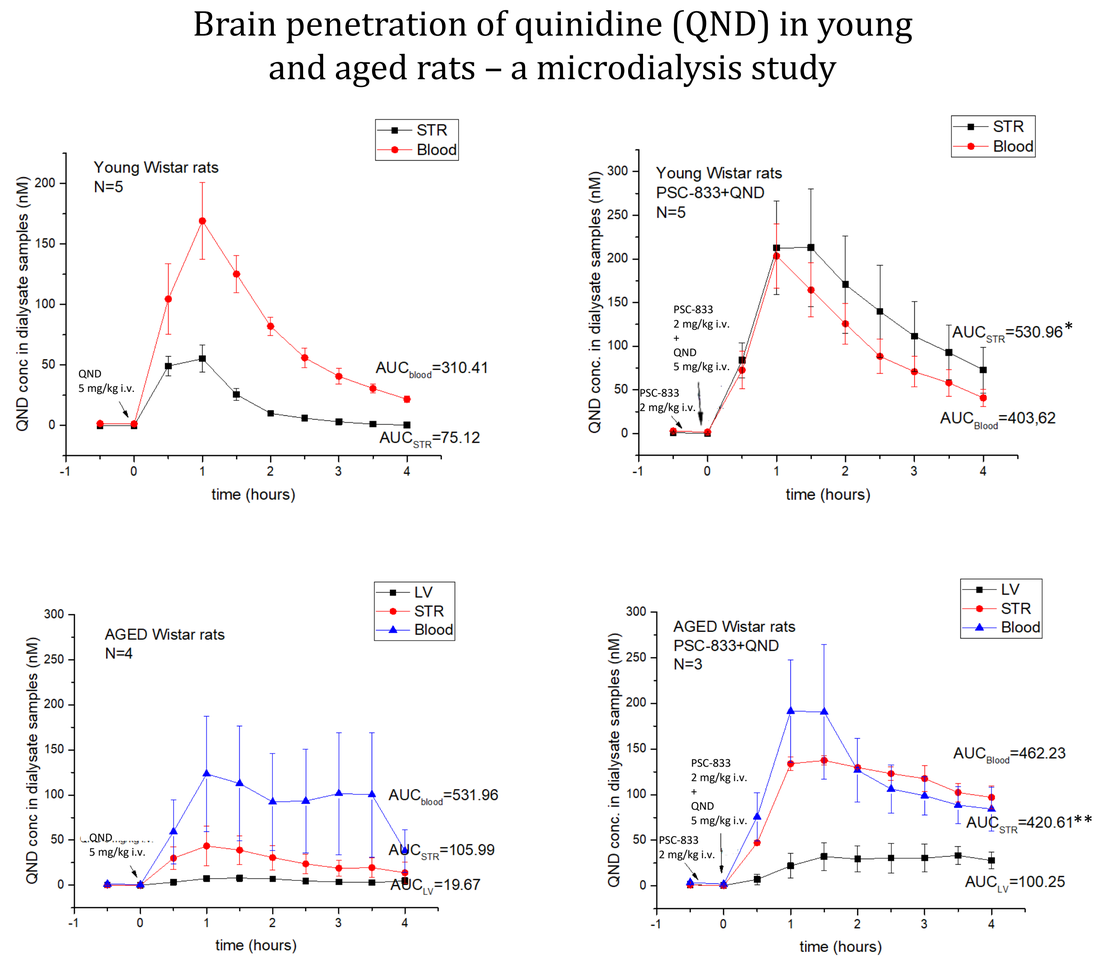


Figure 5. Concentration-time profiles of QND in young (upper panels) and aged (lower panels) Wistar rats, measured by dual- or triple-probe microdialysis. Both the striatal and the ventricular levels of QND increased in a statistically significant manner if the QND treatment (left panels) was combined with PSC-833 treatment (right panels). The basic brain levels of QND seem to be higher in aged animals ($AUC_{STR}$ = 105.99 in aged vs. 75.12 in young), while after the administration of the inhibitor, the striatal concentration reached a higher level in young, than in aged rats ($AUC_{STR}$ = 530.96 in young vs. 420.61 in aged)

## Conclusion

Our results indicate that the BBB permeability changes during aging. This can be the consequence of the increased paracellular transport between the endothelial cells (decreased number of tight junctions). But other factors, like enlarged thickness of the basal lamina and the better protective function of P-gp against QND entrance into the brain suggest that the protective function of some elements of the BBB is preserved or even improved with aging.

## References

Janelidze S et al, Neurobiol Aging. 2017 Mar;51:104-112

van de Haar HJ et al, Neurobiol Aging. 2016 Sep;45:190-6

Wu H et al, Brain Res. 2016 Apr 15;1637:154-67.

## Acknowledgements:

The research has been partially supported by the European Union, co-financed by the European Social Fund **(EFOP-3.6.3-VEKOP-16-2017-00002)**.

Pázmány Péter Catholic University
**Faculty of information Technology & Bionics**

**Pécs Diagnostic Center**

**University of Szeged**

**Hungarian Academy of Sciences, Institute of Cognitive Neuroscience and Psychology**