Evidence for Altered Copper Availability at the Brain Fluid Barrier Systems in Alzheimer's disease

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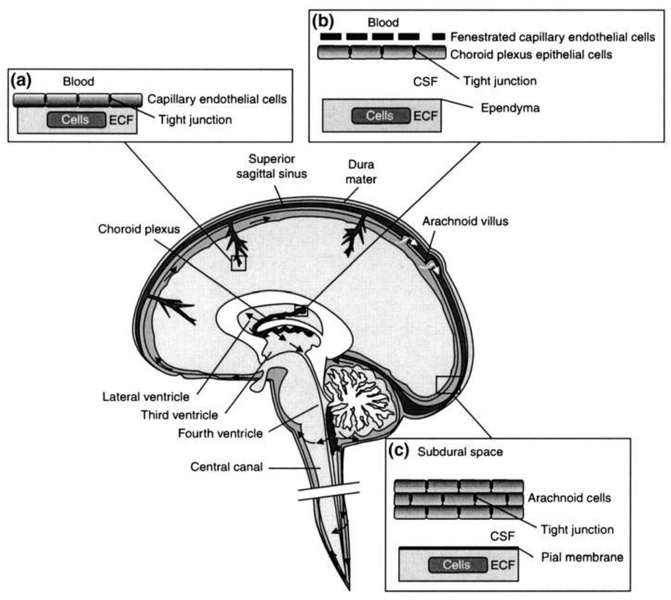
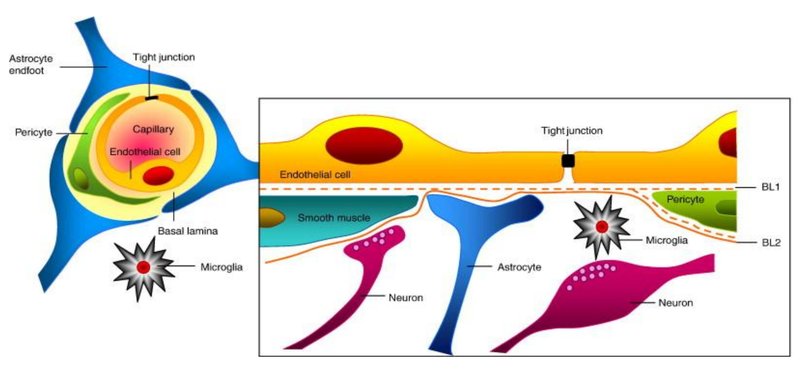
Abstract

Alzheimer's disease (AD) is a progressive neurological disorder associated with extracellular amyloid-ß (Aß) deposits and oligomerization, intracellular neurofibrillary tangles (NFTs), synaptic toxicity, oxidative stress, and metal dyshomeostasis. Analysis of post-mortem human brains showed that Cu and Zn are associated with Aß plaques and Fe levels elevated within NFTs. Despite an extensive understanding of how metals promote these aspects in vitro, an adequate explanation for the origin of interactions, and evolution as they pertain to AD is still lacking. We were specifically interested in determining whether altered copper bioavailability in AD originates at the blood-brain-barrier (BBB) and/or the blood-CSF-barrier (BCSFB). Here, we present results from experiments where we determined bulk and localized changes in copper concentrations/distributions in AD and control brains. We utilized X-ray fluorescence microscopy which has attogram sensitivity, submicron resolution and is fully quantitative to image brain sections of control and Tg2576 mice, an animal model for AD. We determined the copper distribution and concentration around the lateral ventricle as well as control areas. To our surprise, we found large, tightly localized concentrations of Cu around the lateral ventricle in both Tg2576 and healthy controls. High resolution scans clearly showed that these Cu 'hot spots' were not in choroidal epithelial cells but rather in fiber tracts and caudoputamen in close proximity to the lateral ventricle, perhaps even in the ependymal cells surrounding the lateral ventricle. We determined the Cu concentration in these areas in both symptomatic Tg2576 and age-matched controls and found that the Cu hot spots contain >100 fold more Cu than the surrounding tissue. The Cu concentration in controls was on average 1.6x (40%) higher than in Tg2576 mice (9400(!) µg/g and 5800(!) µg/g wet tissue, respectively). We consequently performed bulk analysis of these and other regions for post-mortem human tissue using ICPMS. The results provide insights into AD by deciphering the complex distribution, concentration, age as well as disease related changes of copper in AD brains and controls.

The Fluid Barrier System in the brain



Barriers of the brain. There are three principal barrier sites between blood and brain. (a) The BBB proper, which is created at the level of the cerebral capillary endothelial cells by tight junction formation. (b) The blood–CSF barrier (BCSFB) lies at the choroid plexuses in the lateral, third and fourth ventricles of the brain where tight junctions are formed between the epithelial cells at the CSF-facing surface (apical surface) of the epithelium. Some drugs and solutes enter the brain principally across the choroid plexuses into CSF, while others enter via both the BBB and BCSFB. (c) The arachnoid barrier. The arachnoid is a multi-layered epithelium with tight junctions between cells of the inner layer that form an effective seal. Transport across the arachnoid membrane is not an important route for the entry of solutes into the brain. Reproduced with permission from : N. Joan Abbott , Adjanie A.K. Patabendige , Diana E.M. Dolman , Siti R. Yusof , David J. Begley; **Structure and function of the blood/brain barrier;** Neurobiology of Disease Volume 37, Issue 1 2010 13 – 25, http://dx.doi.org/10.1016/j.nbd.2009.07.030



The cell associations at the BBB. The cerebral endothelial cells form tight junctions at their margins which seal the aqueous paracellular diffusional pathway between the cells. Pericytes are distributed discontinuously along the length of the cerebral capillaries and partially surround the endothelium. Microglia are the resident immunocompetent cells of the brain. Reproduced with permission from : N. Joan Abbott , Adjanie A.K. Patabendige , Diana E.M. Dolman , Siti R. Yusof , David J. Begley; **Structure and function of the blood/brain barrier;** Neurobiology of Disease Volume 37, Issue 1 2010 13 – 25, http://dx.doi.org/10.1016/j.nbd.2009.07.030

Typical Copper Levels in Human

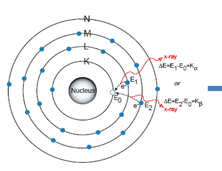
Normal:
- Brain 3.32±1.50 (n=43) (Lech et al. Biol Trace Elem Res (2007) 118:10–15)
- Liver 3.47±1.51 (n=79) (Lech et al. Biol Trace Elem Res (2007) 118:10–15)
- CSF: 8.67 – 22.50 ng/ml (Av 14.16 ng/ml (SD 6.0))
- Blood: 0.54 - 2.6 mg/ml
- Urine: 11-120 mg / 24hrs

In AD Subjects (n=38):
- CSF: 3.73 – 23.66 (Av 11.57 +/- 0.29 ng/ml (SD 3.98)

In WD disease:
- CSF: 76.25 (+/- 5.95 ng/ml)

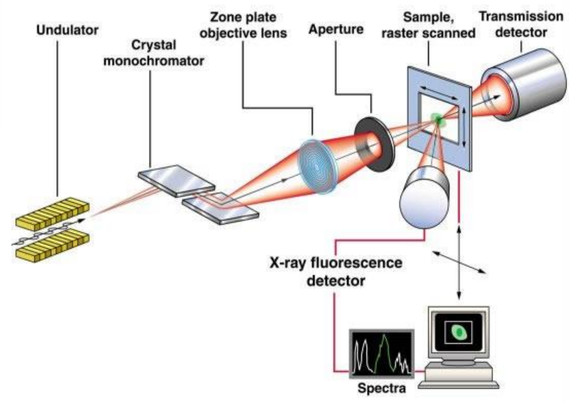
X-ray Fluorescence Microscopy

Technique
Inner shell electrons of an atom are ionized into the continuum. Higher shell electrons relax back into the 'hole' emitting a fluorescent photon.

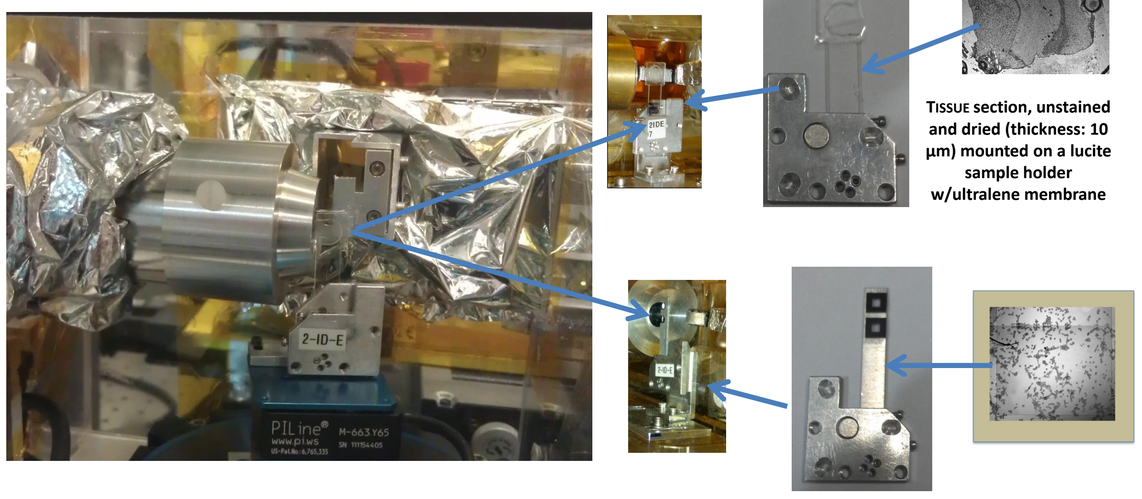


Advantages
- element specific and quantitative,
- sensitive with low background,
- provides subcellular resolution,
- measures multiple elements simultaneously, (think "2D ICPMS")
- samples are measured in their 'native' state

Disadvantages
Requires highly brilliant, high energy, 3rd generation synchrotron X-ray sources which are currently available only at very few user facilities worldwide. Beamtime is scarce, measuring sufficient biological replicates is challenging.



BL 2-ID-e , APS, Argonne National Lab

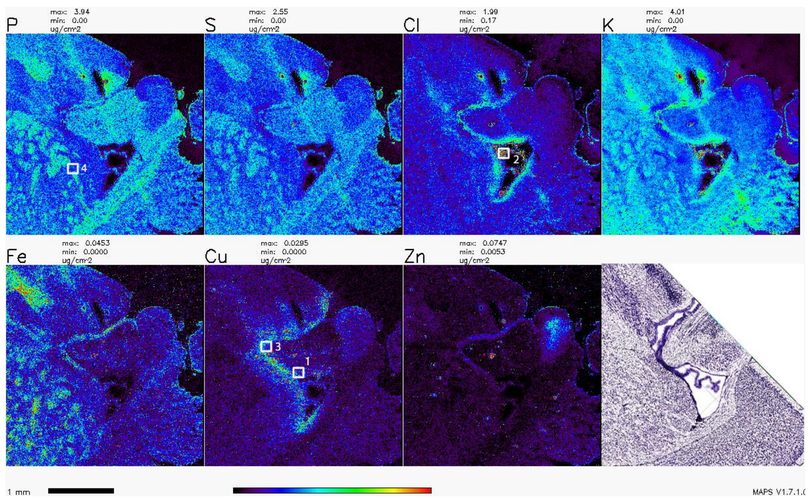


TISSUE section, unstained and dried (thickness: 10 µm) mounted on a lucite sample holder w/ultralene membrane

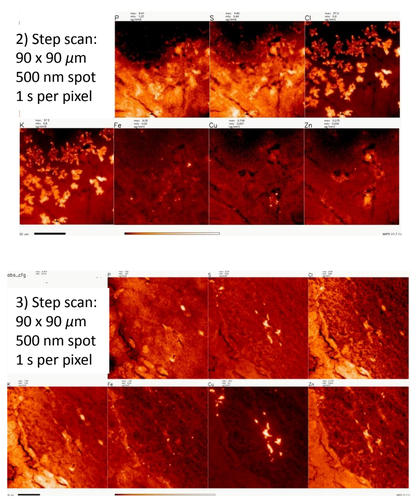
CELLS grown and fixed on a SiN membrane (2 x 2 mm)

Mouse Brain Tissue

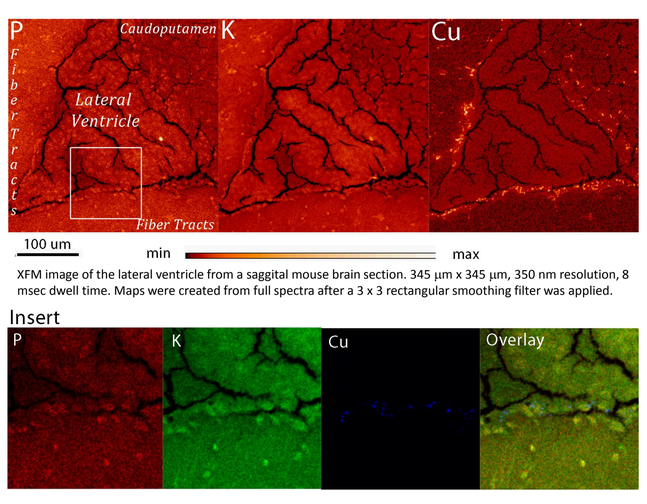
Fly Scan Mode



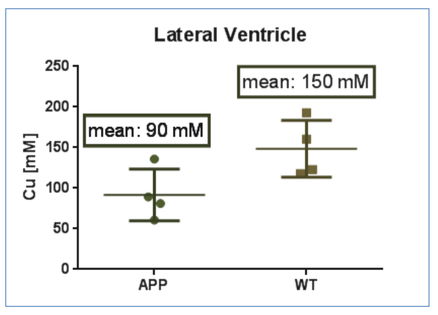
Step Scan Mode



Fly Scan Mode w/ cellular resolution



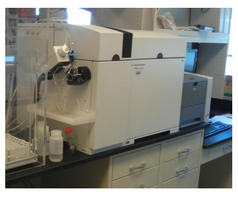
XFM image of the lateral ventricle from a saggital mouse brain section. 345 µm x 345 µm, 350 nm resolution, 8 msec dwell time. Maps were created from full spectra after a 3 x 3 rectangular smoothing filter was applied.

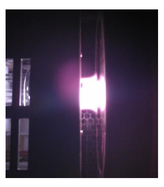


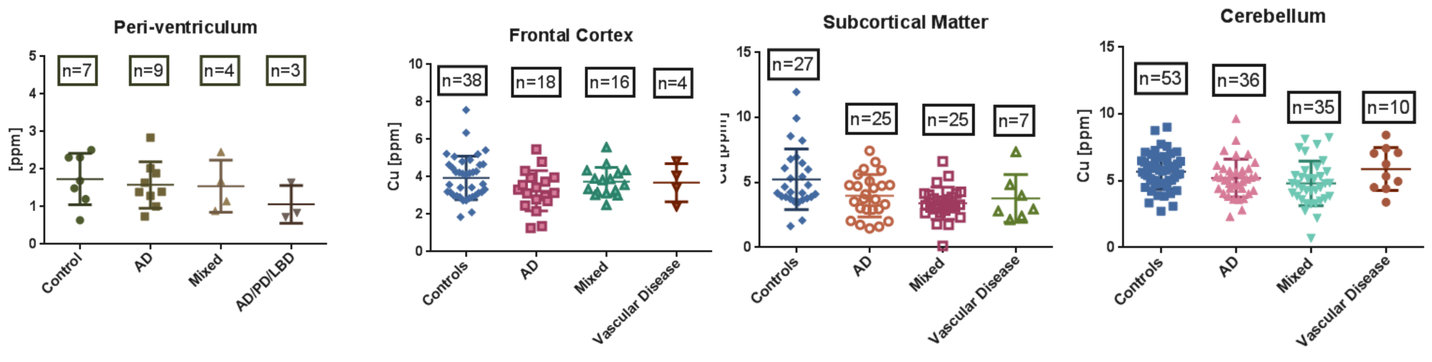
The lateral ventricle is extremely rich in Cu
These Cu measurements complement earlier reports by Betty Eipper et al. (*Neuroscience. (2006), Neurobiol Dis. (2007)*) that Atp7a is overexpressed around the ependymal cells at the lateral ventricle.

XFM confirmed that the average copper concentration in mouse brains is ~ 50 – 100 µM but it also revealed local differences: the copper concentration around the lateral ventricles is ~ 100 mM(!).

Copper concentration in human brains by ICPMS:







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