Neuroimaging distribution of water molecules in brain

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ABSTRACT

In the present work we introduce the novel method, which allow evaluate the quantitative distribution of water molecules in brain tissue. The method is based on the labeling of water molecules by two-photon chromophore and fluorescence lifetime imaging technique (FLIM) [1-3]. FLIM allows direct visualization of the spatially dependent fluorescence decay and it is not dependent on the chromophore concentration, excitation intensity, and light-path length.

Three kinds of fluids are active in a brain: cerebrospinal, extracellular, and blood. The extracellular fluid compartment includes all water and electrolytes outside of cells (interstitial fluid, plasma, and lymph); cerebrospinal fluid (CSF) mostly contained water, and formed by ultrafiltration of blood in the choroid plexus (special cells that make up the walls of some collections of arteries) [4, 5].

Keywords: imaging; brain tissue; water; two-photon chromophore; fluorescence lifetime

1 EXPERIMENTAL PART

The chromophore, 4,4'-bis(9-carbazolyl)-distyrylbenzene (BND) was synthesized [6]. BND possesses large two-photon absorption cross section coefficient δ_{max} = 1050 x (10^{-50}) cm^4 s phot (GM) at 800 nm, broad emission band with the maximum intensity at 454 nm when excited at 378 nm (the quantum yield 0.68 in 10^{-5} M acetonitrile solution).

FLIM was performed using a Biorad Radiance 2100 MP system in combination with a Nikon TE 300 inverted microscope. Two photon excitation pulses were generated by a Ti:Sapphire laser (Coherent Mira) that was pumped by a 5 W Coherent Verdi laser. Pulse trains of 76 MHz (150 fs pulse duration, 800 nm center wavelength) were produced. The excitation light was directly coupled into the microscope and focused into the sample using a CFI Plan Apochromat 20 x (N.A. 0.75). Fluorescent light was detected using non-descanned single photon counting detection, which is the most sensitive solution for two-photon imaging. For the FLIM experiment the Hamamatsu R3809U MCP PMT was used, which has a typical time resolution around 50 ps. Bis-N-carbazolyl-distyrylbenzene (BND) emission was selected using a 450DF80 nm band-pass filter. Images with a frame size of 64*64 pixels were acquired using the SPC 830 module [7]. The average count rate was 2.10^4 photons/s, for the acquisition time of 90 seconds. From the intensity images obtained, complete fluorescence lifetime decays were calculated per pixel and fitted using a double exponential decay model.

Mice: female C57Bl mice (20gr) (Harlan) were kept in pathogen free conditions and given water and food *ad libitum*. Bis-*N*-carbazolyl-distyryl benzene (BND) was injected in the hippocampus area of two groups of mice. In each group, half the animals were subjected to conditions of water deprivation 12 hrs prior to use. The other group had free access to water.

Mice were anesthetized with sodium pentobarbital (3mg/100 gr body weight; intraperitoneally) and placed under a stereotaxic apparatus (Kopf Instruments). The site of injection was chosen according to a mouse brain atlas and coordinates of the hippocampus. Test materials (2 microliters) were injected into the hippocampus using a microsyringe inserted through a bore hole in the skull. Mice were sacrificed after 15 min and the brain was excised. Frozen sections (10 microns) were analyzed for fluorescence.

2 RESULTS AND DISCUSSION

2.1 The imaging of the water distribution in mouse brain.

For the investigation BND was injected in the hippocampus area. To investigate the fluorescence lifetime distribution of the incorporated BND, the contact area of hippocampus/lateral ventricle had been chosen: extracellular fluid is presented in hippocampus and cerebrospinal fluid filled the lateral ventricle. Blood vessels are located in both areas.

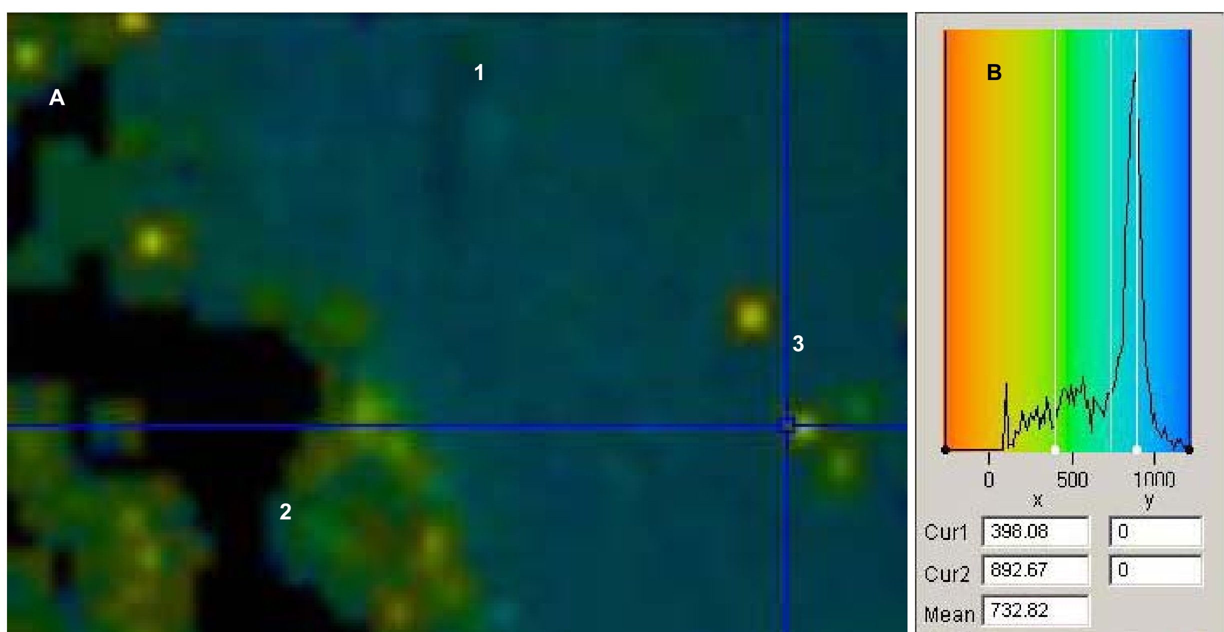

Figure 1. A - The typical FLIM picture: BND fluorescence lifetime distribution in the hippocampus (1), lateral ventricle (2) and blood vessels (3) areas; (B) - the statistical distribution and color scale of the fluorescence lifetime.

Figure 1,a presents the typical image of the fluorescence lifetime distribution in the investigated area. As can be seen, mainly three different colored regions are shown. The region of the hippocampus is blue colored, lateral ventricle (choroid plexus) is green colored and the regions belonged blood vessels are yellow colored. The Figure 1,b displays the correlation of the colors scale with the duration of the fluorescence decay. Therefore, fluorescence lifetime within the time scale - 900-1000ps shown as blue colored; correspondingly, 400-700 ps - green colored; 400 ps and less – is yellow colored. Thus, different colored fluorescence lifetime imaging in the lateral ventricle/hippocampus means, that BND fluorescence lifetime in hippocampus (extracellular fluid) - 900 ± 50ps, lateral ventricle (choroid plexus) (cerebrospinal fluid) – 520 ± 50 ps; and blood vessels – 400 ps – 150ps within the areas were observed. The BND fluorescence lifetime in acetonitrile solution 1170 ps was found.

As it was shown earlier, the BND blue fluorescence excited in *n*-π* energy transition is due to de-excitation S_1 to S_0. The weak binding property of BND has recently been demonstrated [8]. Based on this finding we assume that interaction of BND with biological species - erythrocytes**,** leukocytes, platelet, proteins, and also small molecules such as glucose, has low probability and it does not affect the fluorescence decay. Addition of water significantly quenched the BND fluorescence [9] displaying the strong BND - water molecules interaction and as a consequence –decrease of the fluorescence lifetime.

Comparison of the FLIM of the hippocampus area filled with the extracellular fluid and the lateral ventricle area with choroid plexus filled with the cerebrospinal fluid, demonstrate that the changes in the fluorescence lifetime of the chromophore indeed reflect the amount of water present in each area.

The preliminary explanation was found in the existence of BND exists as *E*,*E; E*,*Z;* and Z,Z isomers [9]. *E, E* isomer is most stable, however, corresponding *E*,*Z* and *Z*,*Z* isomers are considerably less stable than the *E*,*E* isomer (by 4.4 and 8.4 kcal/m, respectively, for the PM3 optimized structures). The stabilization of the less stable isomers, most probably, occurs involving formation of hydrogen bonds in the presence of water [9].

2.2 Effect of the dehydration on the BND fluorescence lifetime dispersed in blood

Here we also present the evaluation of the distribution of the fluorescence lifetime in the blood vessels in two groups of mice: one of them had free access to water and the second one was kept in dehydrated conditions during 12 hours prior experimental providing.

Figure 2 demonstrates the comparison of the fluorescence lifetime distribution in blood vessels, located in hippocampus/lateral ventricle area in normal and dehydrated cases. The major distribution of BND fluorescence lifetime was evaluated as 350 ps in the normal

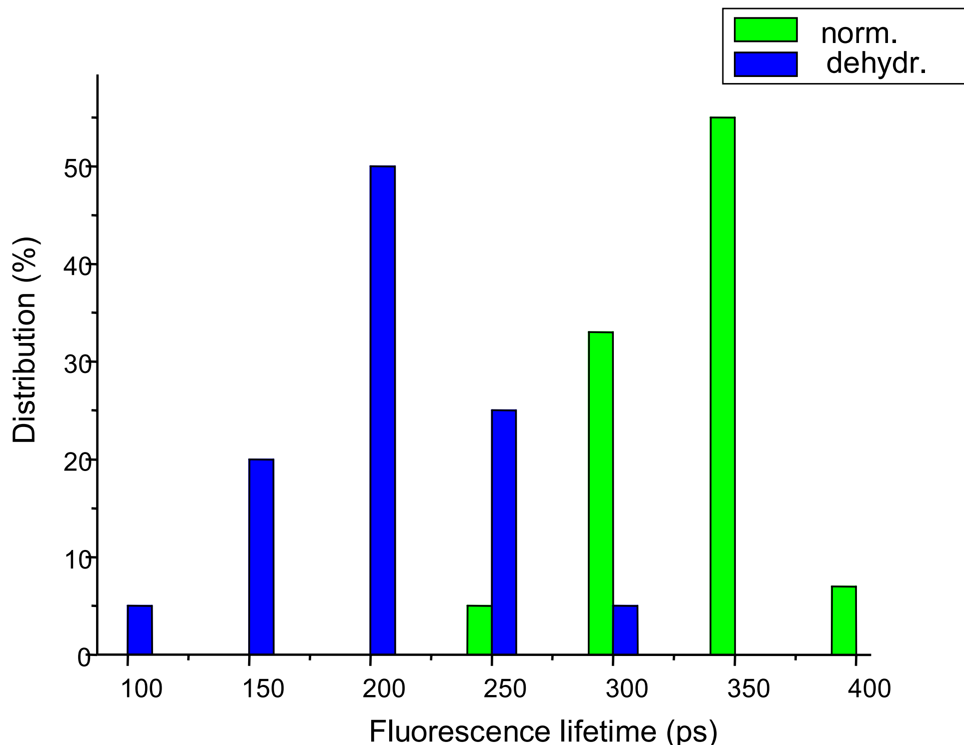

Figure 2. The statistical distribution of the fluorescence lifetime in a normal (green) and dehydrated (blue) cases.

case and much shorter around 200 ps in the dehydrated case.

The results presented (Figure 2) - decrease of the fluorescence lifetime in a case of dehydration pointed out the effect of the dehydration on the water content in a blood. Most probably, increase of the fluorescence lifetime in a case of dehydration caused by the increase of the concentration of the water molecules in a blood Noteworthy, the fluorescence lifetime in the blood vessels decreased down to 300 – 150 ps for the water deprived conditions, meaning increase in the amount of water in blood in most locations of the blood vessels in the investigated area

CONCLUSION

Interaction of BND with water upon irradiation leads to quenching of fluorescence and diminishing the fluorescence lifetime. The mechanism of this interaction is currently under investigation. The high sensitivity of the photochemical behavior of BND to the amount of water present allows effective mapping of biological tissues demonstrated in the present study by imaging of the mouse brain using the FLIM technique.

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