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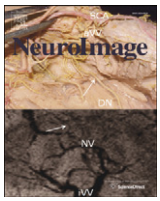

Imaging brain amyloid deposition using grating-based differential phase contrast tomography

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ABSTRACT

One of the core pathological features of Alzheimer's disease (AD) is the accumulation of amyloid plaques in the brain. Current efforts of medical imaging research aim at visualizing amyloid plaques in living patients in order to evaluate the progression of the pathology, but also to facilitate the diagnosis of AD at the prodromal stage. In this study, we evaluated the capabilities of a new experimental imaging setup to image amyloid plaques in the brain of a transgenic mouse model of Alzheimer's disease. This imaging setup relies on a grating interferometer at a synchrotron X-ray source to measure the differential phase contrast between brain tissue and amyloid plaques. It provides high-resolution images with a large field of view, making it possible to scan an entire mouse brain. Here, we showed that this setup yields sufficient contrast to detect amyloid plaques and to quantify automatically several important structural parameters, such as their size and their regional density in 3D, on the scale of a whole mouse brain. Whilst future developments are required to apply this technique *in vivo*, this grating-based setup already gives the possibility to perform powerful studies aiming at quantifying the amyloid pathology in mouse models of AD and might accelerate the evaluation of anti-amyloid compounds. In addition, this technique may also facilitate the development of other amyloid imaging methods such as positron emission tomography (PET) by providing convenient high-resolution 3D data of the plaque distribution for multimodal comparison.

Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly (Reitz et al., 2011). It is characterized by an insidious onset and a progressive decline of cognitive functions associated with predominant memory impairment. AD diagnosis is based mostly on the neuropsychological evaluation of the patient, which is always confirmed by the *post mortem* examination of the brain (McKhann et al., 1984). Indeed, two histopathological features characterize an AD brain: the amyloid plaques (AP) and the neurofibrillary tangles (NFTs). These lesions are formed of proteinaceous aggregates, which can be detected throughout the brain in the extracellular and the intracellular compartment, respectively. They appear early in the course of the disease several years before the occurrence of the first symptoms, and they spread to the temporo-parietal neocortex and subcortical nuclei following a typical pattern as the disease progresses (Braak and Braak, 1991; Delacourte et al., 1999; Thal et al., 2002). Therefore, detecting these two AD hallmarks through imaging techniques performed on the living patient is of major interest not only for diagnosis, but also for the evaluation of AD-modifying drugs.

Until recently, imaging techniques have been used mainly to exclude other causes of dementia (Petrella et al., 2003). However, significant technical progresses have been made, which now allow clinicians to use both structural and functional imaging to diagnose AD positively (Frisoni et al., 2010; Nordberg et al., 2010). New criteria for AD, mostly based on brain imaging, have even been proposed (Dubois et al., 2007, 2010; Reitz et al., 2011; Sperling et al., 2011). Among the recent progresses, the development of new tracers for Positron Emission Tomography (PET) dedicated to AD diagnosis like the flutemetamol, florbetapir, and florbetaben (Herholz and Ebmeier, 2011) offers the possibility to visualize the global averaged distribution of amyloid plaques in the brains of living patients. Unfortunately, this technique has a millimeter-range resolution and does not allow for the

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detection of amyloid deposits at a single-plaque level, which might result in a limited sensitivity, especially at the onset of the disease.

Imaging amyloid plaques at a single-plaque resolution remains a technical challenge because of their small size. They are spherical extracellular aggregates that range from <10 μm to >120 μm in diameter, depending on the brain region where they are observed with a major proportion of plaques around 10–40 μm in humans (Armstrong et al., 1995; Delaère et al., 1991). They are heterogeneous in shape and composition, and their morphology varies from diffuse (amorphous material) to focal deposits (dense aggregates), a typical plaque combining a dense core and a corona of fibrils (Dickson and Vickers, 2001; Wisniewski et al., 1989). Among current imaging techniques that could be used to detect amyloid plaques at a single-plaque resolution, Magnetic Resonance Imaging (MRI) has shown promising results. Several reports have indicated that microMRI devices are able to detect single amyloid plaques in the brain of transgenic mouse models of Alzheimer's disease, both *ex vivo* and *in vivo* (Braakman et al., 2006; Dhenain et al., 2009; Jack et al., 2005; Petiet et al., 2011; Vanhoutte et al., 2005; Wadghiri et al., 2003; Wengenack et al., 2011; Yang et al., 2011; Zhang et al., 2004) and in human brain tissue (Benveniste et al., 1999) (See Braakman et al., 2009 for an extensive review). However, this technique is currently limited by its relatively low spatial resolution when compared to the mean plaque size measured in human brains. Although this parameter varies depending on the device, the MRI sequence, and the time of acquisition, so far only large amyloid deposits, which represent a small fraction of the total amyloid burden, could be detected and quantified by this technique in small animals. Below this limit, the specificity of the signal remains to be established even if abnormal radiologic features could be detected (Diaz-de-Grenu et al., 2011; van Rooden et al., 2009).

Another imaging technique that has been widely used for AD diagnosis is X-ray Computed Tomography (CT). Although MRI is now preferred to CT to assess macroscopic changes in AD patients – mainly because it is less invasive – a new set of experimental imaging techniques, also based on X-rays, is currently under development. In contrast to conventional absorption-based CT, these methods rely on sensing the phase shift induced by the interaction of X-rays with matter. The result is a considerable improvement of contrast in soft tissue (Zhou and Brahme, 2008). These methods include propagation-based methods (Cloetens et al., 1999; Groso et al., 2006), analyzer-based methods like diffraction enhanced imaging (DEI) (Chapman et al., 1997) and interferometric methods using a crystal (Bonse and Hart, 1965; Momose, 1995) or a grating interferometer (McDonald et al., 2009; Weitkamp et al., 2005). Among them, two setups have been shown to image amyloid plaques *ex vivo* in mouse models of AD (Connor et al., 2009; Noda-Saita et al., 2006). The setup presented here – based on a grating interferometer – provides higher quality images, giving the possibility to visualize both anatomical features and individual amyloid plaques at a micrometer resolution in the entire brain of a transgenic mouse model of AD, without using any contrast agents and in a nondestructive manner. With the high-quality images obtained from these scans, automated quantification of plaque features like size and density over the entire brain was possible. The measurement technique together with automated segmentation render large cross-sectional studies feasible and will facilitate multimodal comparison with other imaging techniques. The ability of this setup to further increase the resolution of images in selected areas by performing local tomography (McDonald et al., 2009) opens also new possibilities, such as visualizing the morphology of amyloid plaques in large brain areas.

Materials and methods

Animals and brain processing

The 5xFAD mouse line was generated in the laboratory of Dr Robert Vassar at Northwestern University, Chicago (Oakley et al., 2006) and was transferred to the École Polytechnique Fédérale de Lausanne (EPFL) in 2009 from Jackson Laboratories, US (Stock number: 006554). This transgenic line over-expresses both the amyloid precursor protein gene (*APP*) and the presenilin-1 gene (*PSEN1*), carrying familial AD mutations: APP K670N/M671L (Swedish), I716V (Florida) and V717I (London); PSEN1 M146L and L286V.

Animals were euthanized by intraperitoneal injection of pentobarbital (150 mg/kg) and transcardially perfused by cold phosphate buffered saline (PBS). Subsequently, the brains were harvested, fixed with 4% paraformaldehyde in PBS for a week at 4 °C and stored in sterile PBS until further processing. The brain samples were brought to the Swiss Light Source in order to perform the tomographic scanning, and then transferred back to EPFL for histology. All procedures were approved by the Committee on Animal Experimentation for the canton of Vaud, Switzerland, in accordance with Swiss Federal Laws on Animal Welfare and the European Community Council directive (86/609/EEC) for the care and use of laboratory animals.

Histology

In order to avoid tissue distortion during the histological process, thick brain sections (400 μm) were prepared using a vibratome (Leica VT1200S). Brain sections were next stained by Thioflavin S (Sigma-Aldrich) according to the following procedure. Free-floating sections were incubated in a solution of Thioflavin S 0.01% (w:v) in ethanol 50% for 8 min at room temperature under low agitation and were next washed in two baths of ethanol 50% (2×5 min) and two baths of PBS (2×10 min). Sections were next mounted in glycerol 80% using secure-sealed spacers (Invitrogen).

Fluorescence microscopy

Multi-photon fluorescence imaging

Thick brain sections stained by Thioflavin S were acquired on a LEICA SP5 Multi-photon microscope (Leica Microsystems, Germany) at the EPFL bioimaging and optics facility (http://biop.epfl.ch). A motorized platform was used to scan the whole sections at 8000 Hz with a Plan-Apochromat 10×/0.40 glycerine immersion objective (Leica #11506293). Excitation was performed at 800 nm and external Non Descanned Detectors (NDD) were used to capture the emitted fluorescence between 500 nm to 650 nm. 130 stacks covering the whole section were acquired at a frame resolution of 512×512 pixels every 4 μm (final voxel size in xyz: 2 μm×2 μm×4 μm), and were stitched together using the Leica Application Suite Software.

Processing of fluorescence images

The fluorescence images did not require any further normalization step. For noise reduction, a Lagrangian-of-Gaussian (LoG) filter, also known as Mexican-hat filter, was applied. This type of convolution filter is well known to serve as a detector for spherical particles in background noise (Sage et al., 2005). In our case, an isotropic Gaussian σ filter size of 2^3 voxels gave the best results. The LoG filter was applied to the image stack in 3D to emphasize the spherical plaques embedded in background noise.

Differential phase contrast (DPC) tomography

DPC image acquisition

In X-ray optics, the interaction of X-rays with typical materials is encoded in the material's index of refraction $n = 1\text{-}\delta + i\beta$, where δ is the decrement of the real part of the refractive index (responsible for the phase shift) while the imaginary part β describes the absorption properties of the tissue. Since biological tissue (e.g. brain matter) consists mainly of light elements like water and hydrocarbons, there is negligible absorption contrast, and thus conventional (absorption-based) X-ray tomography is not suited to distinguish anatomical or