Methods 53 (2011) 201–207

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Monitoring protein aggregation and toxicity in Alzheimer's disease mouse models using in vivo imaging

Tara L. Spires-Jones *, Alix de Calignon [1], Melanie Meyer-Luehmann [2], Brian J. Bacskai, Bradley T. Hyman

Massachusetts General Hospital/Harvard Medical School, 114 16th Street, Charlestown, MA 02129, USA

ARTICLE INFO

Article history:
Available online 14 December 2010

Keywords:
Alzheimer
Tau
Plaque
Multiphoton
In vivo imaging

ABSTRACT

Aggregation of amyloid beta peptide into senile plaques and hyperphosphorylated tau protein into neurofibrillary tangles in the brain are the pathological hallmarks of Alzheimer's disease. Despite over a century of research into these lesions, the exact relationship between pathology and neurotoxicity has yet to be fully elucidated. In order to study the formation of plaques and tangles and their effects on the brain, we have applied multiphoton in vivo imaging of transgenic mouse models of Alzheimer's disease. This technique allows longitudinal imaging of pathological aggregation of proteins and the subsequent changes in surrounding neuropil neurodegeneration and recovery after therapeutic interventions.

1. Introduction

Alzheimer's disease (AD), first described in 1907, is a devastating neurodegenerative disorder characterized by dementia and pathologically defined by the presence of two types of abnormal protein aggregates: intracellular neurofibrillary tangles (NFT) and extracellular senile plaques [1]. Plaques are composed largely of aggregated amyloid beta peptide (Aβ) derived from proteolytic cleavage of amyloid precursor protein (APP), while NFT are formed of hyperphosphorylated and misfolded tau protein [2]. Pathological protein aggregation is a common feature across the spectrum of neurodegenerative diseases, thus it is important to elucidate the relationship between protein aggregation and degeneration of the brain in order to develop therapeutic strategies.

In AD, the presence of plaques is an early occurrence in the disease process and mutations in amyloid precursor protein and presenilins 1 and 2, proteins involved in cleaving APP to generate Aβ, can cause rare familial forms of AD strongly implicating amyloid as the initiating factor in the disease [3]. NFT accumulate and spread through the brain later in the disease process, however their presence correlates better with cognitive decline, synapse loss, and neuronal loss than amyloid burden, arguing the importance of NFT in the degenerative process [4]. These post-mortem data implicating amyloid in disease initiation and NFT in neuronal death are correlative and not definitive since by their nature these experiments cannot determine the precise timing and order of events. Using transgenic mouse models that accumulate plaque or tangle pathology, we have developed a method combining in vivo multiphoton imaging of pathology with viral infections to fill neurons with fluorophores, proteins postulated to be involved in neurodegeneration, or functional indicators to study the timing of plaque and tangle formation and the degeneration associated with them (see outline of the method in Fig. 1).

2. Materials and methods

2.1. Animal models

In order to study aggregation of Alzheimer-related proteins, we take advantage of transgenic mouse models expressing human amyloid precursor protein, presenilin, or tau with mutations associated with familial forms of AD or frontotemporal dementia. We have used several plaque-bearing mouse models including Tg2576 mice which express the 695 amino acid isoform of APP containing the 'Swedish' double mutation Lys670-Asn, Met671-Leu [5], PDAPP mice expressing an APP minigene with the V717F mutation [6], and APP/PS1 mice expressing a mutant human presenilin 1 (DeltaE9) and a chimeric mouse/human APP with the Swedish double mutation [7]. These mice all develop senile plaques but at different ages and on different strain backgrounds, so investigating aggregation across several models allows confirmation of the

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid beta; NFT, neurofibrillary tangle; APP, amyloid precursor protein.

* Corresponding author. Fax: +1 617 724 1480.
E-mail address: tspires@partners.org (T.L. Spires-Jones).
[1] Present address: University of Oxford, Department of Physiology, Anatomy, and Genetics, Oxford, UK.
[2] Present addresses: German Center for Neurodegenerative Diseases (DZNE), 80336 Munich, Germany; and Adolf-Butenandt-Institute, Biochemistry, Ludwig Maximilians-University, 80336 Munich, Germany.

1046-2023/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymeth.2010.12.009

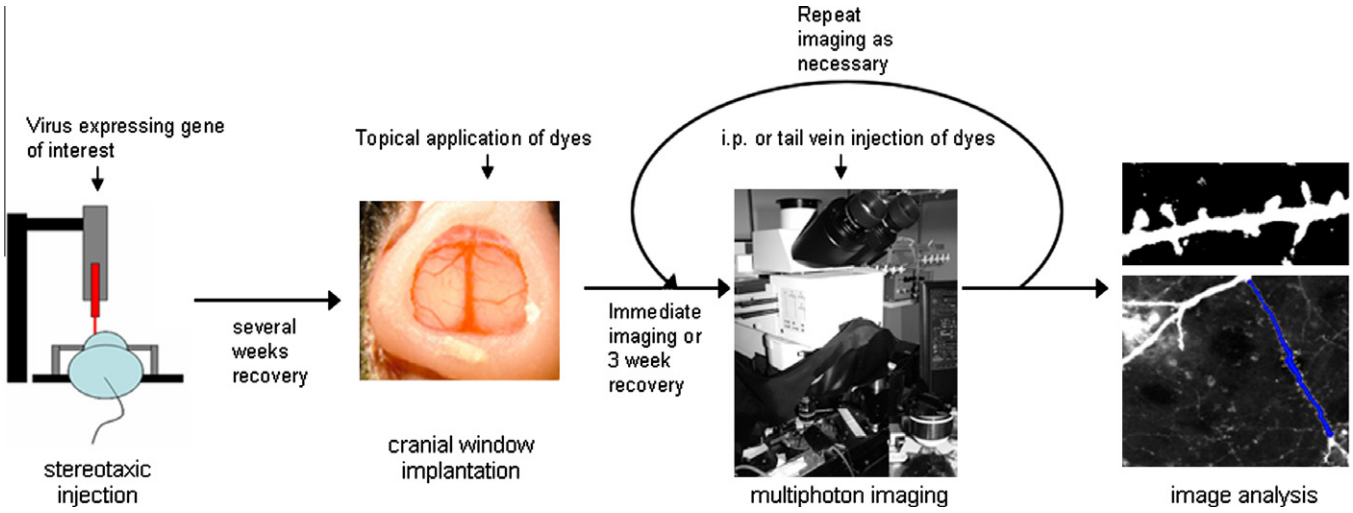

Fig. 1. Schematic of in vivo imaging.

relevance of findings to the disease pathogenesis. We study NFT formation and toxicity in the rTg4510 mouse model expressing human tau with the P301L mutation associated with frontotemporal dementia [8]. This model has the advantage of being regulatable – the transgene can be suppressed with doxycycline administration in the food – allowing investigation of the reversibility of effects of NFT on the brain.

Transgenic mouse models not directly related to Alzheimer's pathology are also very useful for imaging the effects of AD pathology on the brain when crossed with AD model mice. For example, animals transgenic for fluorescent proteins can be used to study the effects of pathology on neuronal structure [9], mice expressing immediate early genes could be used to asses the response of neurons to stimulation [10], mice with fluorescent mitochondria could be used to study the effects of pathology on mitochondrial localization [11], mice with fluorescent microglia have been used to observe glial changes around plaques [9,12], etc.

All animal work described here conforms to NIH and institutional IACUC regulations.

2.2. Instrumentation

2.2.1. Surgical equipment

For surgery and imaging, the mouse must be anesthetized and the head stabilized in a stereotaxic device. Since these are long-term experiments, we are careful not to place the ear bars into the ears of the animal to avoid rupturing the tympanic membranes which is painful for the animal. Instead ear bars are placed in the notch on the skull immediately anterior to the ears. For injection of virus into the brain, a standard stereotaxic frame with a syringe holder and pump are ideal (stereotaxic apparatus – David Kopf instruments. Tujunga, CA; injector system – Stoelting Co, Wood Dale, IL). Similarly, for craniotomy and cranial window implantation, standard stereotaxic devices can be used. For imaging on the microscope, specialized stereotaxic frames mounted on a base that fits into the microscope stage can be used, or a small steel bar with a screw hole can be implanted adjacent to the cranial window and a small screw used to secure the animal onto a holder mounted on the microscope stage [13].

For cranial window implantation, we use a dissecting scope (for example Zeiss, Stemi SV6) to visualize the surgical area and use illuminators with light guides (Fiber Light, Dolan-Jenner Industries, Boxborough, MA). Standard microsurgical tools are used (from Fine Science Tools and Harlan Tekland).

2.2.2. Multiphoton microscope system

Imaging with two-photon laser excitation allows penetration of the laser to subcortical areas up to several hundreds of microns deep (to layer V) without phototoxicity that would be induced by visible light lasers. For in vivo multiphoton imaging, we have used 2 systems, (1) a BioRad 1024 system mounted on an upright Olympus BX50WI microscope with a custom built three channel photomultiplier array and (2) an Olympus Fluoview 1000MPE mounted on an Olympus BX61WI upright microscope with four photomultiplier detectors. Both systems use a mode-locked tunable femtosecond pulsed titanium/sapphire laser (Mai Tai, Spectraphysics) for excitation. A 20× high numerical aperture (0.95) dipping objective is used due to its long working distance and large light gathering capacity.

2.3. Surgical procedures

The specialized surgical techniques for in vivo imaging are difficult to replicate based on written procedures. Several groups around the world specialize in this type of imaging and regularly train scientists in the surgical techniques. Watt Webb from Cornell and Winfried Denk at the Max Planck Institute first implemented multiphoton microscopy for imaging live cells. Other investigators routinely using cranial window implantation and multiphoton imaging of the brain include Karel Svoboda at Howard Hughes Medical Institute [13], and Mark Hubener and Tobias Bonhoeffer at the Max Planck Institute [14]. There are also video protocols that are very useful in learning the technique that have been published in the Journal of Visualized Experiments [15–17].

Before surgical procedures, sterilize the work area and stereotax with 70% ethanol. Surgical instruments should be sterilized in a bead sterilizer. Prepare sterile PBS on ice and soak gel foam cut into small squares (2–3 mm) in sterile PBS for keeping the skull moist.

2.3.1. Anesthesia

Either injection or inhalant anesthetics may be used for surgery and repeated imaging of animals, however, several factors should be considered before choosing an anesthetic regimen. It is important to maintain body temperature near 37 °C particularly in tau expressing animals since hypothermia has been shown to enhance tau phosphorylation [18]. Isoflurane, a commonly used inhalant anesthetic is more dangerous to workers than injectibles (particularly pregnant women) since they are exposed to some amounts of the anesthetic which is known to have detrimental effects on the