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Review - Part of the Special Issue: Alzheimer's Disease - Amyloid, Tau and Beyond

Behavioral assays with mouse models of Alzheimer's disease: Practical considerations and guidelines

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ABSTRACT

In Alzheimer's disease (AD) basic research and drug discovery, mouse models are essential resources for uncovering biological mechanisms, validating molecular targets and screening potential compounds. Both transgenic and non-genetically modified mouse models enable access to different types of AD-like pathology in vivo. Although there is a wealth of genetic and biochemical studies on proposed AD pathogenic pathways, as a disease that centrally features cognitive failure, the ultimate readout for any interventions should be measures of learning and memory. This is particularly important given the lack of knowledge on disease etiology – assessment by cognitive assays offers the advantage of targeting relevant memory systems without requiring assumptions about pathogenesis. A multitude of behavioral assays are available for assessing cognitive functioning in mouse models, including ones specific for hippocampal-dependent learning and memory. Here we review the basics of available transgenic and non-transgenic AD mouse models and detail three well-established behavioral tasks commonly used for testing hippocampal-dependent cognition in mice – contextual fear conditioning, radial arm water maze and Morris water maze. In particular, we discuss the practical considerations, requirements and caveats of these behavioral testing paradigms.

Contents

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized clinically by progressive cognitive decline (reviewed in [1]). Currently, AD is the most common type of dementia worldwide; and since age is the biggest risk factor, the prevalence is expected to greatly increase over the next few decades with aging population structures. Unfortunately, despite decades of research, the etiology of AD is unknown, and many fundamental questions remain unanswered. Continuing research into the basic underlying biology of AD as well as renewed efforts in developing disease-modifying drugs are necessary to address this problem. In both the basic research and translational arenas, animal models of the disease are critical. In particular, genetic and non-genetic mouse models of AD pathology have become key research tools for discovering disease pathways and targets as well as testing new therapeutic approaches (reviewed in [2,3]).

Ultimately, as a disease of synaptic and cognitive failure (reviewed in [4,5]), both preclinical hypotheses and translational developments in AD research need to address the crucial therapeutic endpoint – amelioration and/or prevention of cognitive dysfunction. Indeed, the most striking characteristic of Alzheimer's is the progressive decline of cognitive functioning that is caused by massive loss of neurons and synapses. Most importantly, focusing on the behavioral phenotype offers the advantage of avoiding assumptions on the etiopathogenesis of the disease, ones which may be disproved by future studies. The currently-approved medications for AD, which include acetylcholinesterase inhibitors and a N-methyl-D-aspartate receptor (NMDAR) antagonist, offer only minimal temporary improvements in this regard. In testing new therapeutic targets and compounds, mouse models are key resources for providing access to AD-type pathology in vivo concurrently with behavioral testing options. The mouse models offer the ability to validate molecular targets and screen potential compounds on the translational pathway leading to clinical testing.

Several cognitive assays are available for assessing mice, particularly in their hippocampal-dependent learning and memory abilities. Given that AD pathology initiates and is most severe in the hippocampus and entorhinal cortex of the medial temporal lobe (reviewed in [6]), these murine cognitive assays that are hippocampal-dependent are ideally-suited for AD research. The use of these cognitive assays versus other readouts maximizes the likelihood of selecting a target or compound that is relevant for memory systems in vivo.

Among the cognitive assays that test murine learning and memory, we will highlight and discuss three behavioral tasks that are commonly used to examine associative memory and spatial memory: fear conditioning (FC), radial arm water maze (RAWM), and Morris water maze (MWM). Among their advantages, these tasks are straightforward in implementation and allow for the relatively fast assessment of several batches of mice in a short period of time. However, key parameters need to be well-controlled in order to minimize variability in the results and maximize reproducibility between experiments. In this review, we focus on the practical considerations of these assays – the protocols, guidelines and caveats based on our experience with various AD mouse models.

2. Alzheimer's disease pathology

In Alzheimer's disease, there are two primary histopathological features evident upon post-mortem examination of brain tissue – amyloid plaques and neurofibrillary tangles (NFTs) (reviewed in [1,7]). The plaques consist of insoluble extracellular deposits of the amyloid-β (Aβ) peptide and can be observed throughout the cortex. Neurofibrillary tangles consist of aggregates of hyperphosphorylated tau, a microtubule-binding protein. Evident with both the plaques and NFTs, the misfolding and aberrant aggregation of the constituent protein exemplifies a key pathogenic feature of the disease.

Although amyloid plaques were observed histopathologically in AD brains since Alois Alzheimer's early descriptions [8], the composition of the plaques remained unknown for decades. In 1985, researchers were finally successful in purifying Aβ and identifying it as the predominant constituent of the plaques [9]. A vast amount of subsequent research implicated Aβ as the main molecular culprit in AD pathogenesis, in what is classically known as the "amyloid cascade hypothesis". Isolating the peptide then led to the identification and sequencing of the amyloid precursor protein (APP), from which Aβ is produced [10].

APP is a type I transmembrane glycoprotein that is abundantly expressed in the brain, particularly by neurons. APP can undergo a series of cleavages by secretase enzymes, one pathway of which results in the production of Aβ peptides. APP contains α-, β- and γ-secretase cleavage sites. Processing at the α-secretase site releases a large portion of the ectodomain and precludes the formation of Aβ since the cleavage occurs within the Aβ sequence. Alternatively, APP can be cleaved at the β-secretase site, which together with an intramembrane γ-secretase cleavage, produces Aβ peptides. In neurons, the sole β-secretase is β-site APP cleaving enzyme 1 (BACE1), a transmembrane aspartyl protease that generates the N terminus of Aβ. The γ-secretase complex is comprised of four subunits: presenilin 1 or 2 (PS1/PS2), nicastrin, presenilin enhancer 2 (PEN-2) and anterior pharynx-defective 1 (APH-1). There are multiple γ-cleavage site possibilities, which result in the production of Aβ peptides with varying lengths (usually 37–43 amino acids). Approximately 90% of secreted Aβ is 40 amino acids long ($A\beta_{40}$). However, there is also a smaller proportion of 42 residue-long Aβ peptides ($A\beta_{42}$) that make up <10% of the total Aβ pool. In a series of studies, $A\beta_{42}$ was found to have a much higher propensity for aggregation compared to the shorter peptides, leading to a focus on $A\beta_{42}$ as the main amyloidogenic species in AD [11–14].

A strong body of evidence indicates that soluble oligomers of Aβ, consisting of 2–12+ peptides, are a primary neurotoxic culprit in AD pathogenesis (reviewed in [5]). In contrast to amyloid plaques, which do not correlate well with cognitive decline, soluble Aβ species are significantly correlated with disease symptoms and severity [15,16]. These aggregates can be formed from synthetic or natural Aβ peptides, including those secreted by cells or directly isolated from the brain tissue of AD patients and transgenic mouse models. Many studies have established that Aβ oligomers can exert detrimental effects on neuronal physiology and synaptic transmission. For example, Aβ oligomers appear to preferentially bind to or cluster at synapses, with one study observing that >90% of Aβ oligomer binding in neurons occurs at dendritic spines at sites positive for PSD-95, a marker for post-synaptic compartments [17]. On a structural level, Aβ oligomers have been shown to cause changes in spine morphology and decreases in spine density [18,19]. This loss of synapses is highly relevant for underlying the cognitive impairments of AD, and indeed, it has been found to be a major structural correlate of dementia [20].

Although the receptor(s)/binding partner(s) and downstream signaling mechanisms induced by Aβ oligomers are not fully elucidated, the functional effects on cognition are well-established. High concentrations of Aβ (>nanomolar) – whether chronically or acutely present, synthetic or naturally-derived – can markedly impair neuronal physiology and synaptic plasticity [e.g. long-term potentiation (LTP)] [21–29], an electrophysiological correlate of memory. More importantly, pathological Aβ exposure can strongly impact behavior, including performance in learning and memory