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Early discrimination reversal learning impairment and preserved spatial learning in a longitudinal study of Tg2576 APPsw mice

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Abstract

To understand the relationship between amyloid-β and cognitive decline in Alzheimer's disease, we evaluated cortical and hippocampal function in a transgenic mouse model of amyloid over-expression in Alzheimer's disease, the Tg2576 mouse. Tg2576 mice and their non-transgenic littermates were assessed at both 6 and 14 months of age in a battery of cognitive tests: attentional set-shifting, water maze spatial reference memory and T-maze working memory. Spatial reference memory was not affected by Tg status at either age. Working memory was only affected by age, with 6-month-old mice performing better than 14-month-old ones. Older mice were also significantly impaired on reversal learning and on the intra- and extra-dimensional shift in attentional set-shifting. A significant transgene effect was apparent in reversal learning, with Tg2576 mice requiring more trials to reach criterion at 6 months old. These data indicate that the effects of normal aging in C57B6 × SJL F1 mice are most pronounced on putative frontal cortex-dependent tasks and that increasing Aβ load only affects discrimination reversal learning in our study.

Keywords: Frontal cortex; Hippocampus; Working memory; T-maze; Water maze; Set-shifting; Amyloid-β; Alzheimer's disease

1. Introduction

Tg2576 mice, which over-express the human APP Swedish mutation (K670N, M671L), are widely studied as an Alzheimer's disease (AD) transgenic mouse model [23]. This transgenic model develops a pathological hallmark of AD with increasing age, Aβ plaque deposition. Amyloid plaques are distributed in cortical and hippocampal regions after 10 months of age in a pattern similar to that observed in humans [24,50]. Levels of Aβ 1–40 and 1–42 also increase prior to plaque deposition, with measurable levels apparent by 6–8 months of age [24,53]. Age-dependent memory loss has been observed in Tg2576 mice [26,53], and some studies argue that the cognitive impairment is associated with soluble brain Aβ [27,32,53].

The predominant cognitive disorder in AD is in the memory domain, and therefore cognitive assessments in Tg2576 mice (and other AD mouse models) have focused on the effects of Aβ on hippocampal-dependent tasks like spatial reference memory [23,53]. However, AD patients also have deficits in cognitive domains that are related to the integrity of the frontal cortex. For example, AD patients suffer from prefrontal cortex (PFC)-dependent executive dysfunction and attentional impairment [3,4,7,16,31,41]. These PFC-dependent deficits attenuate the ability of AD patients to respond appropriately to the circumstances in everyday

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life, resulting in a decreased ability to independently perform activities of daily living [40]. One task used to detect PFC-dependent executive dysfunction and attentional shift-deficit in AD patients is the Wisconsin card sort test (WCST) [35,38,49]. The attentional set-shifting test is a variant of the WCST that has been modified for use in rodents [8]. The attentional-shift task has been shown to be dependent upon the integrity of the prefrontal cortex in rats, monkeys and humans [8,14,43].

If increasing levels of Aβ, whether soluble or insoluble, contribute to disruptions in neural circuitry with increasing age, deficits in cortical- and hippocampal-dependent functions should be observed in Tg2576 mice coinciding with increasing levels of Aβ. In order to observe the progression of both cortical and hippocampal functional changes in Tg2576 mice, we longitudinally tested the same mice at 6 and 14 months of age in a battery tests that have been demonstrated to be sensitive to hippocampal and/or cortical dysfunction in rodents. We tested Tg2576 mice and their non-transgenic littermates using an attentional set-shifting test, a water maze spatial learning test and a T-maze forced-alternation working memory test. This combination of tests uses different sensory modalities including olfaction, vision, and touch; and different types of motivation (food deprivation versus escape from the water). In addition, the longitudinal design helps to determine whether certain types of cognitive assessment are more amenable to longitudinal testing in normal aging and in transgenic mouse models of neurodegenerative disease.

2. Methods

2.1. Subjects

The subjects were female Tg2576 mice and their non-transgenic (NTg) littermates on a C57B6 × SJL F1 background [23]. Male Tg2576 mice are too aggressive to be group housed for extended periods of time, thus not included in this longitudinal study. Tg2576 APPsw mice over-expressed a 695 amino acid splice form (Swedish mutation K670N M671L) of the human amyloid precursor protein (APP_{695}) that resulted in an five-fold increase in Aβ 1–40 and a 14-fold increase in Aβ 1–42 with increasing age [23]. Subjects were maintained on a 12-h light/dark cycle and had *ad lib* access to food and water unless indicated otherwise. One week prior to testing, all subjects were taken out of group housing cages to be individually housed and then returned to group housing with their original cage mates between testing at 6 and 14 months of age. During behavioral evaluation, the experimenters were blinded to the Tg status of the subjects. At the beginning of the study, there were 15 NTg and 13 Tg2576 mice, all negative for the retinal degeneration mutation. Two NTg and 3 Tg2576 mice died in the 8 months between the first and second behavioral evaluation, leaving a final N of 13 NTg and 10 Tg2576 mice. However, 1 NTg and 1 Tg2576 mice failed water maze cue training at 6 months of age and another Tg2576 mouse failed at 14 months of age. These three mice were also eliminated from statistical analysis. Therefore, the total number of mice analyzed for longitudinal analysis was 12 NTg and 8 Tg2576 mice. The APP genotype of the mice was re-confirmed using DNA from tail biopsies taken prior to behavioral analysis. DNA was isolated using DNeasy Tissue Kit (Qiagen) and amplified with the PCR reaction performed using the HotMaster Mix (Eppendorf). Primers were obtained from Integrated DNA Technologies.

The mice were evaluated in the following sequence: attentional set-shifting, water maze (visible and hidden platform) and T-maze working memory test. Due to the concerns of long term food restriction effect on mice, two food restricted tests (attentional set-shifting and T-maze test) were separated by the non-food restricted water maze test. The complete test battery took 9 weeks. The tests were administered in the same order at 6 and 14 months of age.

2.2. Behavioral tests

2.2.1. Attentional set-shifting

Mice were maintained on a restricted diet of 85% *ad lib* body weight and water remained freely available in the home cage. Mice were weighed before the testing and fed immediately after testing in order to maintain body weight at 85%. Mice were trained to dig in glass pots (4.5 cm diameter and 2.2 cm depth) to obtain a food reward (one 25 mg chocolate pellet, Bio-Serv, NJ, USA) that was buried in the pot 2 cm beneath the surface of the digging medium. Absorbent sticks scented with essential oil were taped outside each pot. The tests were performed inside the mouse's home cage using a Plexiglas rack to transport the pots in and out of the cage. The rack and mouse were separated by an opaque Plexiglas sliding door. A trial began after the sliding door was lifted.

When mice were 6 months old, they went through a 7-day set-shifting protocol. On day 1, mice were food-deprived and habituated to the test apparatus. Habituation consisted of placing the empty rack and sliding door into each home cage for 10 min per day. Habituation to the digging pots occurred on days 1–3 by placing the pots filled with a mixture of the media and several chocolate pellets into the home cages overnight. On day 4, pots filled with mixed medium containing several chocolate pellets were put into the home cage using the rack. The pots were re-baited until the mice were digging reliably for chocolate pellets. On day 5 and 6, the mice were trained on two simple discrimination (SD) tests each day. Mice received two SDs using odor as the positive (rewarded) dimension on one of these days, and two SDs using medium as the positive dimension on the other day (counterbalanced across mice). Each discrimination problem was trained to a criterion of six consecutive correct trials. The sequence of medium or odor pairs and the positive stimulus were counterbalanced between the NTg and Tg2576 groups. These stimuli were never used again. All phases of the attentional set-shifting test were run on day 7 including: simple