Behavioural Brain Research 233 (2012) 237–243

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Research report

# Sensorimotor gating and memory deficits in an APP/PS1 double transgenic mouse model of Alzheimer's disease

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## HIGHLIGHTS

- APP/PS1 double transgenic mouse model of Alzheimer's disease (AD) was used.
- Sensorimotor gating was measured by prepulse inhibition test in AD mice.
- Prepulse inhibition was impaired in the 7- and 22-month-old AD mice.
- Memory impairment in the 7-month-old AD mice was detected in a water maze test.
- Sensorimotor gating in AD mice is impaired with the progressing of AD phenotype.

## ARTICLE INFO

*Article history:*
Received 16 March 2012
Received in revised form 2 May 2012
Accepted 6 May 2012
Available online 15 May 2012

*Keywords:*
Alzheimer's disease
APP/PS1 double transgenic mouse model
Sensorimotor gating
Prepulse inhibition
β-amyloid plaque
Memory

## ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder associated with cognitive deterioration and neuropsychiatric symptoms. Sensorimotor gating deficit has been identified in neuropsychiatric diseases. The aim of the present study was to evaluate the possible sensorimotor gating deficit and its correlation to memory impairment and cerebral β-amyloid (Aβ) plaque deposits in an amyloid precursor protein (APP)/presenilin-1 (PS1) double transgenic mouse model of AD. The sensorimotor gating in 3-, 7- and-22-month-old non-transgenic and transgenic mice was evaluated in a prepulse inhibition (PPI) task. Results revealed that the PPI was lower in the 7- and 22-month-old transgenic mice compared with the age-matched control, while the response to startle pulse-alone in the transgenic and non-transgenic mice was comparable. Congo red staining showed that Aβ neuropathology of transgenic mice aggravated with age, and the 3-month-old transgenic mice started to have minimum brain Aβ plaques, corresponding to the early stage of AD phenotype. Furthermore, memory impairment in the 7-month-old transgenic mice was detected in a water maze test. These results suggest that the sensorimotor gating is impaired with the progressing of AD phenotype, and its deficit may be correlated to cerebral Aβ neuropathology and memory impairment in the APP/PS1 transgenic mouse model of AD.



## 1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with cognitive deterioration and neuropsychiatric symptoms [1–3]. Sensorimotor gating deficit has been identified in neuropsychiatric disorders and a suppression of the P50 event-related potential of sensorimotor gating has been identified in AD patients [4–6]. Sensorimotor gating deficit in psychiatric patients such as schizophrenia has been evaluated by the prepulse inhibition (PPI) task [6,7]. A deficit in sensorimotor gating, reflected by reduced PPI, is well documented in schizophrenia, and this deficit has been proposed to contribute to sensory overload or flooding and, finally, cognitive fragmentation, thought disorder, and other psychotic symptoms [8,9]. Studies in animals have indicated that cortex that is affected in mild AD is involved in the regulation of PPI [10].

A pathologic hallmark of AD is the formation of senile plaque, in which β-amyloid peptide (Aβ) is a major component [11–14]. Aβ is derived through the processing of the large transmembrane β-amyloid precursor protein (APP) that is initially cleaved by the β-site APP cleaving enzyme 1 and subsequently by a presenilin (PS)-dependent γ-secretase complex in the transmembrane domain [15,16]. Genetic causes of AD include mutations in the

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http://dx.doi.org/10.1016/j.bbr.2012.05.007

APP, PS1 and PS2 genes [17]. Aβ triggered neuronal damage has been recognized as an important feature of AD pathology [18,19]. APP and/or PS1 transgenic mouse models of AD showing memory impairment and cerebral Aβ pathology, are useful to understand the mechanisms of clinical symptoms for AD [20–24]. Our previous studies have shown that the APP/PS1 transgenic mice start to have detectable brain Aβ plaques at 3 months of age, and their AD phenotype start to get into a progressive stage at 6 months of age [24,25].

We hypothesize that sensorimotor gating deficit occurs with the progression of disease in AD. In order to evaluate the possible sensorimotor gating deficit and its correlation to memory impairment and cerebral Aβ plaque deposits in AD, the sensorimotor gating in 3-, 7- and 22-month-old APP/PS1 transgenic mice and non-transgenic was evaluated in a prepulse inhibition (PPI) task. The spatial memory was measured by a water maze test, and cerebral Aβ plaque deposits were evaluated by Congo red staining in AD mice.

## 2. Materials and methods

### 2.1. Animals

All procedures with animals were performed in accordance with the guidelines established by the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Manitoba. APP/PS1 double transgenic and non-transgenic mice were generated from mating between single transgenic mice expressing human mutant $APP_{K670N/M671N}$ [21] and mutant $PS1_{M146L}$ [26], and chosen by the genotyping results of polymerase chain reaction (PCR) using APP or PS1 primers. The single APP and PS1 transgenic mice were originally obtained from AstraZeneca R&D. APP transgenic mice derive from B6SJLF1 background, while PS1 transgenic mice derive from B6D2F1 × Swiss Webster background, producing progeny with a mixture of these backgrounds. Total 69 female non-transgenic (Con) and APP/PS1 double transgenic (Tg) mice at 3 ($n$ = 17 in each group), 7 ($n$ = 12 in each group) and 22 ($n$ = 6 in Con group, $n$ = 5 in Tg group) months of age were used in the present study. They were housed three or four per cage, with free access to food and water under controlled laboratory conditions.

### 2.2. Prepulse inhibition (PPI) of the startle response test

The sensorimotor gating in 3-, 7- and 22-month-old AD transgenic and non-transgenic mice was evaluated in a PPI test [27]. PPI of the startle response provides an operational measure of sensorimotor gating, a putative neural mechanism that inhibits the processing of extraneous sensory, cognitive and motor information [28]. PPI refers to the normal reduction in startle magnitude that occurs when an abrupt startling stimulus is preceded 30–500 ms by a weak pre-stimulus [28].

Each mouse was placed into a small Plexiglas cylinder connecting motion sensors within a large sound-attenuating chamber (San Diego Instruments, San Diego, CA). The motion sensors recorded voltage changes in arbitrary units (mV) that were digitized and stored by the computer controlling the delivery of acoustic stimuli through loudspeakers mounted 21 cm above the plastic cylinders. Motion sensors were calibrated daily with the SR-LAB Standardization Unit (San Diego Instruments, San Diego, CA). The background sound level (70 dB) and calibration of the acoustic stimuli was confirmed daily with a digital sound level meter (RadioShack, Fort Worth, TX). Startle pulse was set to 120 dB and prepulse intensities were set to 3 dB (PP3), 6 dB (PP6) and 12 dB (PP12) above background noise level. The test session consisted of 64 trials and included five trial types (startle pulse-alone, PP3, PP6, PP12, and no-stimulus) in four blocks. Blocks 1 and 4 included six startle pulse-alone trials in each block, and block 2 and 3 include six startle pulse-alone trials, five PP3, five PP6, five PP12, and five no-stimulus in each block. Duration of acoustic stimuli was set to 20 ms for prepulses and 40 ms for startle pulses, and interstimulus interval between prepulse and startle pulse was set to 100 ms (from onset to onset).

After a 5 min habituation period, PPI-test sessions were conducted. The intertrial intervals with an average of 15 s were varied from 5 to 23 s in pseudo-random order in the session, and a total PPI test time for each mouse was approximately 23 min. The maximum response in 65 ms recording window was used as the startle amplitude for each trial. Levels of PPI at each prepulse sound level were calculated in blocks 2 and 3 as (1-averaged response amplitude in trials with a prepulse stimulus and startle stimulus/averaged response amplitude in trials with the startle stimulus alone) × 100%. The averaged response amplitude in trials with the startle stimulus alone in each block, and the averaged response amplitude in trials with no-stimulus in blocks 2 and 3 were also calculated.

### 2.3. Water maze test

One day after PPI test, spatial memory acquisition and retention in the 3- and 7-month-old mice were assessed by water maze test [24,29]. The water maze was performed in a plastic pool (120 cm in diameter; 50 cm high) filled with water (22 ± 1 °C). The maze was surrounded by various extra-maze cues, such as different laboratory benches and posters. An overhead CCD camera and a computerized tracking system with ANY-Maze™ Video Tracking Software (Stoelting Co., IL, USA) were used to track, record and analyze the movement of the mice in the test.

In the acquisition (hidden-platform) test, the hidden clear Plexiglas® platform (10 cm in diameter) was kept constant in the middle of one quadrant throughout training. The training consisted of 4 blocks of trials (4 trials/block, with a 30 min trial interval). Each trial lasted 60 s or until the mouse climbed onto the hidden platform when the escape latency was recorded. The retention (probe) test was carried out without the platform 24 h after the last training trial. Each mouse was put into the pool for 60 s, and the time spent in the target quadrant (the platform located quadrant during the training trials) was recorded. The floating mice in the water maze test were excluded from statistical analysis.

### 2.4. Histology and quantification of amyloid deposition

On the day following behavioral test, mice were perfused through the ascending aorta with cold 0.1 M phosphate-buffered saline (PBS, pH 7.4) under a deep surgical level of anesthesia with isoflurane. The hemispheres (excluding cerebellum, pons and medulla oblongata) were separated by cutting at the midline. The left hemisphere was post-fixed in 4% paraformaldehyde in PBS, and then cryoprotected in 30% sucrose in PBS. Finally, the left hemisphere was cut into 30 μm thick coronal sections on a freezing, sliding microtome. To confirm the stage of AD in the 3-, 7- and 22-month-old transgenic mice, brain sections were stained with Congo red solution to identify Aβ plaques. A commercially available Congo Red kit (Sigma–Aldrich, St. Louis, MI) was used after counterstained with Mayer's hematoxylin solution according to the manufacturer's protocol. There was no Congo red positive staining in the brains of non-transgenic control mice.

The total number of amyloid plaques in the cortex and the hippocampus of the Congo red stained sections (four sections at levels of −1.58, −1.76, −1.94 and −2.12 mm to Bregma [30] in each mouse) were counted manually under using a Zeiss Imager-A1 microscope at 100× magnification [24,25]. The plaques were counted in the whole cortex and hippocampus areas of the selected sections. The size of the total counted area (the cortex or hippocampus) was collected using Olympus BH2-RFCA microscope fitted with a Spot-RT digital camera (Diagnostic Instruments, Sterling Heights, MI), and analyzed using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). Plaques for which the intensity was not sufficient above the background for proper threshold were eliminated. Plaque counting was performed in a blind fashion. The plaque number was calculated as the total number of plaques counted per 10 $mm^2$ area of the cortex or hippocampus.

### 2.5. Statistical analysis

All results were expressed as means ± S.E.M. The significance of differences was determined by two- and one-way ANOVA, followed by a Protected Fisher's LSD post hoc test for multiple comparisons. A two-tailed $t$-test for independent samples was used for two-group comparisons. A $P$ value of less than 0.05 was regarded as statistically significant.

## 3. Results

### 3.1. Impaired PPI in 7- and 22-month-old AD transgenic mice

To investigate the sensorimotor gating in mice, a prepulse inhibition (PPI) test was performed. For the 3-month-old mice (Fig. 1A), two-way ANOVA showed that genotype (non-transgenic or transgenic) had no effect on the PPI (%), although prepulse intensity [$F(2,96) = 8.09$, $P = 0.0006$] produced a significant change on the PPI (%).

For the 7-month-old mice (Fig. 1B), two-way ANOVA showed that genotype [$F(1,66) = 8.95$, $P = 0.0039$] and prepulse intensity [$F(2,66) = 23.05$, $P < 0.0001$] produced a significant change on the PPI (%). A post hoc analysis indicated that PPI (%) at PPI6 was significantly lower in the transgenic mice compared with non-transgenic control (Fig. 1B).

For the 22-month-old mice (Fig. 1C), two-way ANOVA showed that genotype [$F(1,27) = 17.14$, $P = 0.0003$] and prepulse intensity [$F(2,27) = 3.46$, $P = 0.046$] produced a significant change on the PPI (%). A post hoc analysis indicated that PPI (%) at PPI3 and PPI6 was significantly lower in the transgenic mice compared with non-transgenic control (Fig. 1C).