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Research report

# Differential susceptibility following β-amyloid peptide-(1–40) administration in C57BL/6 and Swiss albino mice: Evidence for a dissociation between cognitive deficits and the glutathione system response

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**Abstract**

Considerable evidence supports the role of oxidative stress in the pathogenesis of Alzheimer's disease (AD). Previous studies suggest that the central nervous system (CNS) administration of β-amyloid peptide, the major constituent of senile plaque in AD, induces oxidative stress in rodents which may contribute to the learning and memory deficits verified in the β-amyloid model of AD. In the present study, we compared the effects of a single intracerebroventricular (i.c.v.) injection of aggregated β-amyloid peptide-(1–40) ($A\beta_{1–40}$) (400 pmol/mouse) on spatial learning and memory performance, synaptic density and the glutathione (GSH)-dependent antioxidant status in adult male C57BL/6 and Swiss albino mice. Seven days after $A\beta_{1–40}$ administration, C57BL/6 and Swiss mice presented similar spatial learning and memory impairments, as evaluated in the water maze task, although these impairments were not found in $A\beta_{40–1}$-treated mice. Moreover, a similar decline of synaptophysin levels was observed in the hippocampus (HC) and prefrontal cortex (PFC) of both Swiss and C57BL/6 mice treated with $A\beta_{1–40}$, which suggests synaptic loss. C57BL/6 mice presented lower levels of glutathione-related antioxidant defences (total glutathione (GSH-t) levels, glutathione peroxidase (GPx) and glutathione reductase (GR) activity) in the HC and PFC in comparison to Swiss mice. Despite the reduced basal GSH-dependent antioxidant defences observed in C57BL/6 mice, $A\beta_{1–40}$ administration induced significant alterations in the brain antioxidant parameters only in Swiss mice, decreasing GSH-t levels and increasing GPx and GR activity in the HC and PFC 24 h after treatment. These results indicate strain differences in the susceptibility to $A\beta_{1–40}$-induced changes in the GSH-dependent antioxidant defences in mice, which should be taken into account in further studies using the Aβ model of AD in mice. In addition, the present findings suggest that the spatial learning and memory deficits induced by β-amyloid peptides in rodents may not be entirely related to glutathione-dependent antioxidant response.


*Keywords:* Alzheimer's disease; β-Amyloid peptide; Spatial learning; Glutathione (GSH); Gluthatione peroxidase (GPx); Glutathione reductase (GR); Synaptophysin; Mouse strain

## 1. Introduction

The pathological hallmarks of Alzheimer's disease (AD) include loss of synapses and the presence of senile plaques and neurofibrillary tangles. The senile plaques are primarily composed of β-amyloid (Aβ) peptide, which is a 39–43 amino acidic peptide formed upon proteolytic processing, by β- and γ-secretases [23], of the larger amyloid precursor protein (APP),

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a ubiquitously expressed transmembrane glycoprotein. The Aβ cascade hypothesis in AD pathogenesis postulates that increased accumulation of Aβ appears to be related to a gradual synaptic loss and neuronal death finally leading to cognitive impairments [25,52,57].

Recently, the actions of Aβ peptide on laboratory rodents have been extensively studied, specifically regarding its effects on the learning and memory processes. Cognitive deficits have been documented in both transgenic mice overexpressing mutant human APP [25,57] and in rodents with centrally administered synthetic peptides $A\beta_{1–40}$ [19,39,54,60] or $A\beta_{1–42}$ [26,54,61], analogous to peptides found in neuritic plaques in AD patients.

Despite the extensive study with Aβ peptide, the mechanism of Aβ-induced neurotoxicity and cognitive impairments remains unclear. There is considerable evidence suggesting that oxidative stress is involved in the mechanisms of Aβ-induced neurotoxicity [12,13] and AD pathogenesis [32,62]. For example, exposure to Aβ increases lipid peroxidation, protein oxidation and the formation of hydrogen peroxide in cultured cells [8]. Similarly, increases in lipid peroxidation, protein carbonyls and oxidation of mitochondrial DNA have been observed in the brains of AD patients [31]. Despite this evidence showing oxidative modifications in AD patients and in rodent models, a causal link still needs to be established. Nevertheless, studies on the potential therapeutic effects of antioxidants for the treatment of AD have produced promising results (for review see [41]).

Glutathione (GSH) is the major non-protein thiol antioxidant in mammalian cells [17], and it is considered to be the main intracellular redox buffer [48]. GSH protects cellular protein-thiols against irreversible loss, thus preserving protein function [22]. The notion that GSH takes part in the redox-sensitive signalling cascade has been receiving growing support [40]. One of the most important GSH-dependent detoxifying processes involves glutathione peroxidase (GPx), which plays a central role in the removal of hydrogen and organic peroxides and leads to the formation of oxidized glutathione (GSSG) [45]. GSSG is reduced back to its thiol form (GSH) by the ancillary enzyme glutathione reductase (GR), leading to the consumption of NADPH, which is mainly produced in the pentose phosphate pathway [50]. GSH also takes part in xenobiotic conjugation with the assistance of several glutathione *S*-transferase isoenzymes [24]. GSH conjugates or GSSG can be eliminated from the cell by the family of ATP-dependent transporter pumps [37].

It has been shown that GSH depletion renders cells more susceptible, while glutathione synthesis improves resistance against oxidative damage [17]. Previous studies have indicated that the GSH system may be activated as a response to oxidative stress in the brains of AD patients [3,30,43], as well as in Aβ-treated rodents [5,26]. Neuronal cell cultures treated with GSH precursors such as γ-glutamylcysteine ethyl ester (GCEE) diminish the pro-oxidative effects of Aβ by augmenting GSH levels [9,10]. Conversely, the inhibition of GSH synthesis leads to an increase in Aβ-induced cell death and intracellular Aβ accumulation [59]. Taken together, these data suggest that GSH-dependent antioxidant defences are mobilized by Aβ exposure and, possibly, are related to the changes observed in AD pathogenesis. In laboratory animal studies, the strain of choice represents an important concern that is frequently neglected. In this regard, the standard behavioural profile of learning and memory is widely diverse, depending on the mouse strain [63]. In addition, neurochemical and behavioural changes induced by xenobiotic neurotoxicants appear to be different in diverse mouse strains [21,47,51]. Of particular importance, Lehman et al. [28] have recently demonstrated that genetic background regulates APP processing, Aβ metabolism and Aβ deposition in the mouse brain. Therefore, the purpose of the present study was to compare the effects of i.c.v. administration of $A\beta_{1–40}$ on the spatial learning and memory, synaptic density and GSH-dependent antioxidant status in two strains of non-transgenic mice: Swiss (an outbred strain) and C57BL/6 mice (an inbred strain used widely as the background strain for transgenic and knockout mice).

## 2. Materials and methods

### 2.1. Subjects

Experiments were conducted using 3-month-old male Swiss albino and C57BL/6 mice weighing 25–45 g. They were kept in groups of 10 animals per cage and maintained in a room under controlled temperature (23 ± 1 °C). They were subjected to a 12 h light cycle (lights on 7:00 a.m.) with free access to food and water. All procedures used in the present study complied with the guidelines on animal care of the local Ethics Committee on the Use of Animals which follows the NIH publication "Principles of laboratory animal care".

### 2.2. β-Amyloid peptide administration

$A\beta_{1–40}$ (Tocris, MO, USA) and $A\beta_{40–1}$ (Bachem, CA, USA) were prepared as stock solution at a concentration 0.6 μg/μl in sterile 0.1 M phosphate-buffered saline (PBS) (pH 7.4), and aliquots were stored at −20 °C. $A\beta_{1–40}$ was aggregated by incubation in sterile distilled water at 37 °C for 4 days before use as described previously [35]. $A\beta_{1–40}$ (400 pmol/mouse), the reverse peptide $A\beta_{40–1}$ (400 pmol/mouse) or control solution (PBS) were administered by intracerebroventricular (i.c.v.) route using a microsyringe with a 28-gauge stainless-steel needle 3.0 mm long (Hamilton) according to the procedure previously described by Maurice et al. [35]. In brief, the needle was inserted unilaterally 1 mm to the right of the midline point equidistant from each eye, at an equal distance between the eyes and the ears and perpendicular to the plane of the skull. The injection volume (3 μl) of $A\beta_{1–40}$, $A\beta_{40–1}$ or PBS was delivered gradually. Mice exhibited normal behaviour within 1 min after injection. The injection placement or needle track was visible and was verified at the time of dissection. The present $A\beta_{1–40}$ and $A\beta_{40–1}$ dose is comparable to that of previous literature [27,61] on the use of $A\beta_{1–42}$.

### 2.3. Experimental design

The behavioural tasks (water maze and open field) and immunohistochemistry studies were performed 7 days after i.c.v. administration of $A\beta_{1–40}$ (400 pmol/mouse), $A\beta_{40–1}$ (400 pmol/mouse) or control solution (PBS). Independent groups of animals were sacrificed 24 h after i.c.v. injection of $A\beta_{1–40}$ (400 pmol/mouse), $A\beta_{40–1}$ (400 pmol/mouse) or PBS, and the prefrontal cortex (PFC) and hippocampus (HC) were removed for measurement of the GSH-t and the activity of the antioxidant enzymes GPx and GR.

### 2.4. Open field task

To verify the effects of i.c.v. treatment with $A\beta_{1–40}$ (400 pmol/mouse) on locomotor activity, the animals were placed for 5 min in the open field arena. The apparatus, made of wood covered with impermeable Formica, had a black floor of 30 cm × 30 cm (divided by white lines into nine squares of 10 cm × 10 cm) and transparent walls, 15 cm high. The experiments were conducted in a sound-attenuated room under low-intensity light (12 lx). Each mouse was placed in the

centre of the open field and the numbers of squares crossed and rearings were registered.

### 2.5. Water maze task

The apparatus was made of black painted fibreglass, 97 cm in diameter and 60 cm in height. For the tests, the tank was filled with water maintained at 23 ± 2 °C. The target platform (10 cm × 10 cm) was made of transparent Plexiglas and it was submerged 1–1.5 cm beneath the surface of the water. Starting points for animals were marked on the outside of the pool as north (N), south (S), east (E) and west (W). Four distant visual cues (55 cm × 55 cm) were placed on the walls of the water maze room. They were all positioned with the lower edge 30 cm above the upper edge of the water tank and in the standard setting, the position of each symbol marked the midpoint of the perimeter of a quadrant (circle = NE quadrant, square = SE quadrant, cross = SW quadrant and diamond = NW quadrant). The apparatus was located in a room with indirect incandescent illumination. A monitor and a video-recording system were installed in an adjacent room. The experiments were video-taped and the scores for latency of escape from the starting point to the platform and swimming speed were later measured through an image analyser (CEFET, Curitiba, PR, Brazil).

Mice were submitted to a spatial reference memory version of the water maze using a protocol that was adapted from one described previously [44]. The training session consisted of 10 consecutive trials during which the animals were left in the tank facing the wall and then allowed to swim freely to the submerged platform. The platform was located in a constant position (middle of the southwest quadrant), equidistant from the centre and the wall of the pool. If the animal did not find the platform during a period of 60 s, it was gently guided to it. The animal was allowed to remain on the platform for 10 s after escaping to it and was then removed from the tank for 20 s before being placed at the next starting point in the tank. This procedure was repeated 10 times, with the starting points (the axis of one imaginary quadrant) varying in a pseudo-randomized manner. The test session was carried out 24 h later and consisted of a single probe trial where the platform was removed from the pool and each mouse was allowed to swim for 60 s in the maze. The time spent in the correct quadrant (i.e. where the platform was located on the training session) was recorded and the percentage of the total time was analyzed.

### 2.6. Immunohistochemistry

Seven days after i.c.v. injection of $A\beta_{1–40}$ (400 pmol/mouse), mice were perfused transcardially with PBS solution containing 4% paraformaldeyde (w/v). The brains were removed and kept overnight in the same solution. Immunohistochemical detection of synaptic changes was performed on the HC and PFC (5 μm slices) using monoclonal mouse anti-synaptophysin (1:400; Novocastra, Newcastle, UK). High temperature antigen retrieval was carried out by immersion of the slides in a water bath at 95–98 °C in 10 mM trisodium citrate buffer pH 6.0, for 45 min. The non-specific binding was blocked by incubating sections for 1 h with goat normal serum diluted in PBS. After overnight incubation at 4 °C with primary antibodies, the slides were washed with PBS and incubated with the secondary antibody Envision plus (Dako, CA, USA), ready-to-use, for 1 h at room temperature. The sections were washed in PBS, and the visualization was completed by use of DAB (3,3′-diaminobenzidine) (Dako, CA, USA) in chromogen solution and light counterstaining with Harris's hematoxylin solution. Images were captured with a microscope (Nikon Eclipse 50i) and Digital Sight Camera (DS-5M-L1, Nikon, NY, USA). Control and experimental tissues were placed on the same slide and processed under the same conditions. The settings for image acquisition were identical for control and experimental tissues. For each mouse, we obtained four images per section, one of the PFC and three images of the hippocampus. In the hippocampus we obtained one image from the stratum moleculare of the dentate gyrus (DG), stratum radiatum of CA1 area and stratum lucidum of CA3 area. Synaptophysin intensity of digitalized images were analyzed using NIH Image J 1.36b imaging software (national Institutes of Health, Maryland, USA). For each mouse, the values obtained for the PFC or the hippocampal subregions were averaged. The assessment of synaptic loss by this approach has been validated in previous studies using experimental models of neurodegeneration [14] and in human brains [34].

### 2.7. Enzyme assays

Twenty-four hours after i.c.v. treatment with $A\beta_{1–40}$ (400 pmol/mouse), $A\beta_{40–1}$ (400 pmol/mouse) or control solution (PBS), the animals were sacrificed by decapitation and the PFC and HC were rapidly dissected. Tissues were homogenized in HEPES 20 mM pH 7.0 and centrifuged at 20,000 × *g* for 30 min at 4 °C. The enzyme activity was determined in the supernatant in a Varian Cary 50 spectrophotometer. The GR activity was determined according to Carlberg and Mannervik [15]. Briefly, GR reduces GSSG to GSH at the expense of NADPH, the disappearance of which can be followed at 340 nm. GPx activity was measured indirectly by monitoring the consumption of NADPH at 340 nm according to Wendel [56]. The GPx uses GSH to reduce the *tert*-butylhydroperoxide, producing GSSG which is readily reduced to GSH by excess GR, thus consuming NADPH. The enzyme activity was expressed in mU/mg of total protein content, which was quantified according to Bradford [11], using bovine serum albumin as the standard.

### 2.8. Total glutathione determination

Twenty-four hours after i.c.v. treatment with $A\beta_{1–40}$ (400 pmol/mouse), $A\beta_{40–1}$ (400 pmol/mouse) or control solution (PBS), the animals were sacrificed by decapitation and PFC and HC were rapidly removed and homogenized in cooled 0.5 M perchloric acid. The homogenates were centrifuged at 15,000 × *g* for 2 min and the supernatant was separated and neutralized in potassium phosphate buffer (0.1 M, pH 7.4), after which it was submitted to the assay. Total glutathione (GSH-t), comprising the total of reduced (GSH) and oxidized (GSSG) forms, was determined by the GR-DTNB recycling assay according to the method originally described by Tietze [55].

### 2.9. Statistical analysis

The statistical evaluation of the results was carried out using two- or three-way analysis of variance (ANOVA) with strain, treatment and number of trials (repeated measure) as the independent variables. Following significant ANOVAs, multiple post hoc comparisons were performed using the Newman-Keuls test. The accepted level of significance for the tests was $P \leq 0.05$. All tests were performed using the Statistica® software package (StatSoft Inc., Tulsa, OK, USA).

## 3. Results

### 3.1. Effects of $A\beta_{1–40}$ administration on spatial learning and memory of Swiss and C57BL/6 mice

The effects of i.c.v. administration of $A\beta_{1–40}$ (400 pmol/mouse), $A\beta_{40–1}$ (400 pmol/mouse) or control solution (PBS) on the water maze performance of young adult (3-month-old) Swiss and C57BL/6 mice are summarized in Fig. 1. Three-way ANOVA (strain versus treatment versus repeated measures) revealed no significant inter-strain differences ($F_{1,40} = 1.14$; $P = 0.28$) in the escape latency to find the platform during the training session. However, it indicated a significant effect of treatment ($F_{2,40} = 192.52$; $P < 0.0001$) and repeated measures ($F_{9,360} = 184.70$; $P < 0.0001$) in the escape latency to find the platform during the training session. Subsequent Newman-Keuls tests indicated that the treatment with $A\beta_{1–40}$, but not with $A\beta_{40–1}$, induced similar spatial learning deficits in Swiss and C57BL/6 mice, as indicated by higher final escape latencies to find the platform in comparison to respective control-treated groups (Fig. 1A).

Statistical analysis of the probe test scores revealed no significant inter-strain differences ($F_{1,40} = 1.91$; $P = 0.17$). However,

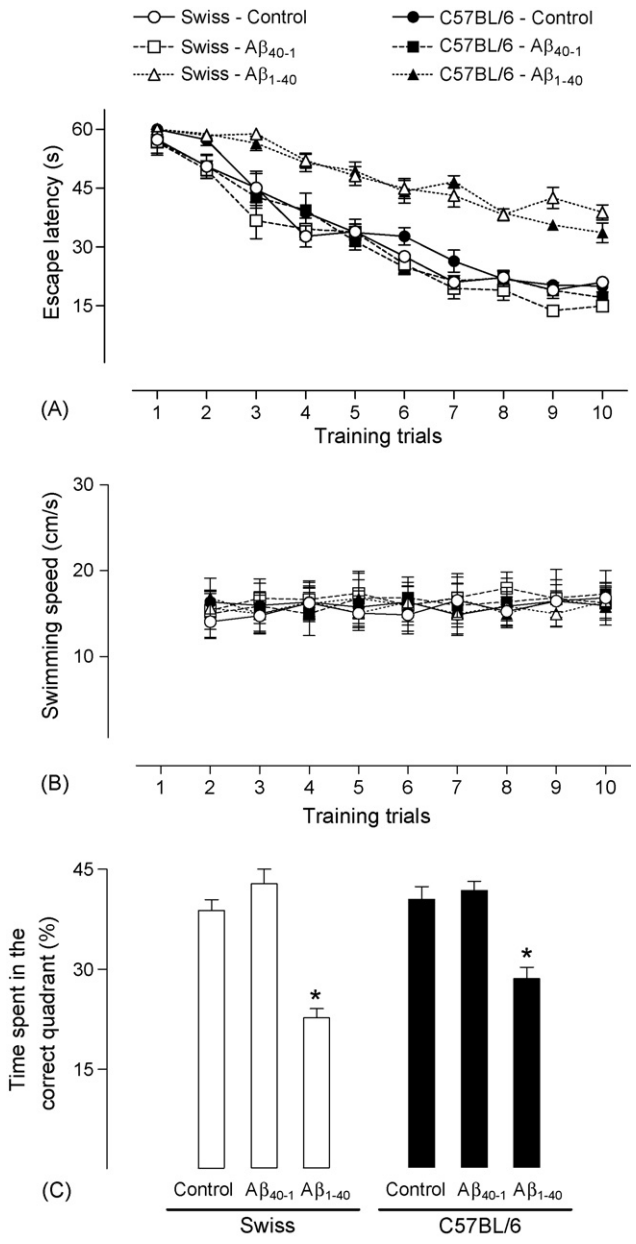


Fig. 1. The effects of i.c.v. administration of $A\beta_{1–40}$ (400 pmol/mouse), $A\beta_{40–1}$ (400 pmol/mouse) or control solution (PBS) on the water maze performance of Swiss and C57BL/6 mice. Training trials were carried out on day 7 after treatments. Data are presented as means ± S.E.M. latency, in seconds, for escape to a submerged platform (A) and swimming speed (cm/s) (B) ($n = 7–8$ animals in each group). (C) The probe test session was performed 24 h after the training trials. Data are presented as means ± S.E.M. of the time spent in the correct quadrant. $^{*}P \leq 0.05$ compared to the control-treated group of the same strain (Newman-Keuls test).

a clear spatial memory impairment was observed in the $A\beta_{1–40}$-treated mice ($F_{2,40} = 107.81$; $P < 0.0001$), regardless of the strain (Swiss or C57BL/6), with a significant reduction in the percentage of time spent in the correct quadrant (i.e. where the platform was located during the training session) (Fig. 1C).

Moreover, the effects of i.c.v. administration of PBS, $A\beta_{40–1}$ or $A\beta_{1–40}$ (400 pmol/mouse) on water maze performance of Swiss and C57BL/6 mice were not directly related to motor impairments, since no alterations were observed in the swimming speeds ($F_{2,40} = 0.88$; $P = 0.36$) in the water maze (Fig. 1B) or in the total squares crossed ($F_{2,45} = 0.10$; $P = 0.90$) and rearing ($F_{2,45} = 0.05$; $P = 0.95$) in the open field arena (Table 1).

Table 1
The effects of i.c.v. administration of $A\beta_{1–40}$ (400 pmol/mouse), $A\beta_{40–1}$ (400 pmol/mouse) or control solution (PBS) on locomotor activity of Swiss and C57BL/6 mice tested in the open field

| Strain/treatment | Squares crossed | Rearing | *N* |
|---|---|---|---|
| Swiss mice | | | |
| Control | 76.0 ± 4.6 | 27.8 ± 2.5 | 10 |
| $A\beta_{40–1}$ | 80.4 ± 3.7 | 36.2 ± 5.3 | 8 |
| $A\beta_{1–40}$ | 77.2 ± 3.6 | 30.1 ± 2.9 | 9 |
| C57BL/6 mice | | | |
| Control | 71.6 ± 3.6 | 30.1 ± 3.3 | 9 |
| $A\beta_{40–1}$ | 75.9 ± 2.9 | 28.7 ± 1.6 | 7 |
| $A\beta_{1–40}$ | 72.2 ± 4.5 | 29.0 ± 2.2 | 8 |

Experiments were carried out during 5 min on day 7 after treatments. Data are expressed as the mean + S.E.M. of the total squares crossed and rearing. N = number of animals.

### *3.2. Effects of $A\beta_{1–40}$ administration on the synaptic density in the hippocampus and prefrontal cortex of Swiss and C57BL/6 mice*

To evaluate the neuronal integrity after i.c.v. injection of aggregated $A\beta_{1–40}$ (400 pmol/mouse), we performed immunohistochemistry analysis for the pre-synaptic protein synaptophysin in the PFC and the hippocampal stratum moleculare of the dentate gyrus (DG), stratum lucidum of area CA3 and stratum radiatum of area CA1. Two-way ANOVA (strain versus treatment) revealed no significant inter-strain differences in synaptophysin levels in CA1 ($F_{1,8} = 0.76$; $P = 0.41$), CA3 ($F_{1,8} = 0.87$; $P = 0.38$), DG ($F_{1,8} = 2.17$; $P = 0.18$) or PFC ($F_{1,8} = 0.36$; $P = 0.56$) (Fig. 2).

Moreover, as shown in Fig. 2, the i.c.v. injection of $A\beta_{1–40}$ (400 pmol/mouse) induced a significant reduction of synaptophysin levels in the CA1 ($F_{1,8} = 35.04$; $P < 0.001$), CA3 ($F_{1,8} = 44.74$; $P < 0.001$), DG ($F_{1,8} = 48.27$; $P < 0.001$) and PFC ($F_{1,8} = 13.58$; $P < 0.01$) of both Swiss and C57BL/6 mice, suggesting a disruption of synaptic integrity in these areas.

### *3.3. Effects of $A\beta_{1–40}$ administration on the glutathione-dependent antioxidant system in Swiss and C57BL/6 mice*

The results for the effects of i.c.v. treatment with $A\beta_{1–40}$ (400 pmol/mouse), $A\beta_{40–1}$ (400 pmol/mouse) or control solution (PBS) on the GSH-dependent antioxidant parameters in Swiss and C57BL/6 mice are illustrated in Fig. 3. Two-way ANOVA (strain versus treatment) revealed significant inter-strain differences in the GSH-dependent antioxidant parameters for the HC (GR: $F_{1,27} = 54.40$; $P < 0.0001$; GPx: $F_{1,27} = 34.36$; $P < 0.0001$; GSH-t: $F_{1,27} = 36.53$; $P < 0.0001$) and PFC (GR: $F_{1,27} = 29.64$; $P < 0.0001$; GPx: $F_{1,27} = 22.12$; $P < 0.0001$; GSH-t: $F_{1,27} = 6.34$; $P < 0.05$). Subsequent Newman-Keuls tests comparing control-treated animals indicated that C57BL/6 mice

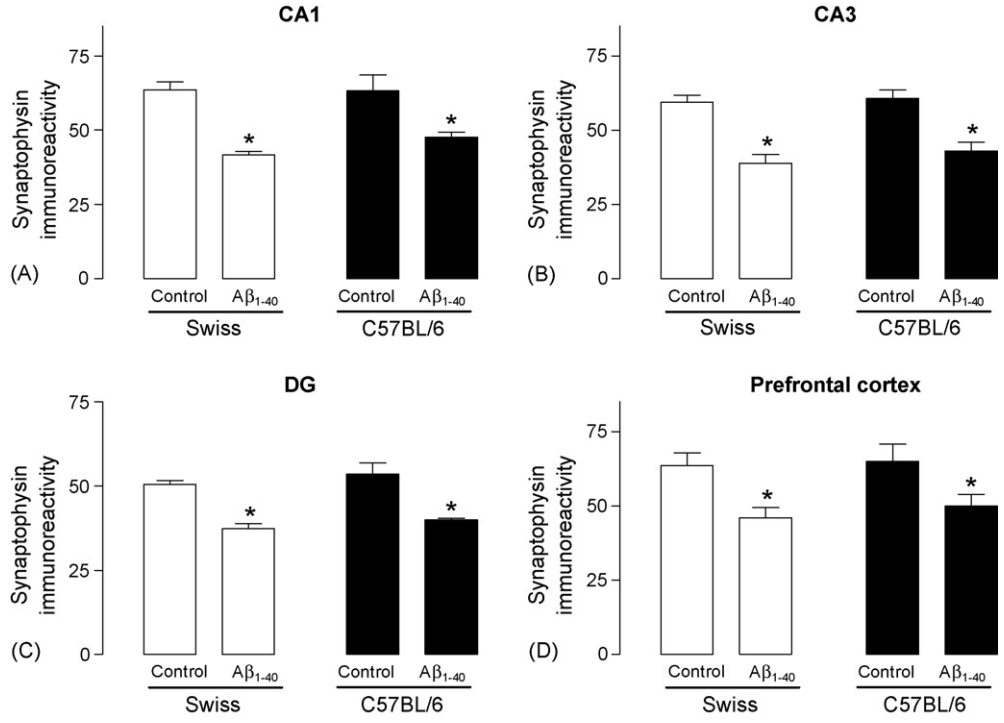


Fig. 2. The effects of i.c.v. administration of $A\beta_{1–40}$ (400 pmol/mouse) or control solution (PBS) on the synaptic density in the hippocampus and prefrontal cortex of Swiss and C57Bl/6 mice. Immunohistochemistry analysis for the pre-synaptic protein synaptophysin in the hippocampal stratum radiatum of area CA1 (A), stratum lucidum of area CA3 (B), stratum moleculare of the dentate gyrus (DG) (C) and prefrontal cortex (D) were carried out on day 7 after $A\beta_{1–40}$ administration ($n = 3$ animals in each group). $^{*}P \leq 0.05$ compared to the control-treated group of the same strain (Newman-Keuls test).

presented lower basal levels of GSH-t and GPx and GR activity in the HC and PFC when compared to Swiss mice.

Moreover, ANOVA revealed a significant effect for the treatment factor on the GSH-dependent antioxidant parameters in the HC (GR: $F_{2,27} = 11.22$; $P < 0.001$; GPx: $F_{2,27} = 7.35$; $P < 0.01$; GSH-t: $F_{2,27} = 17.57$; $P < 0.0001$) and PFC (GR: $F_{2,27} = 9.09$; $P < 0.001$; GPx: $F_{2,27} = 21.52$; $P < 0.0001$; GSH-t: $F_{2,27} = 3.60$; $P < 0.05$) of mice. Post hoc comparisons indicated a significant increase of GR and GPx activity in the HC and PFC of Swiss $A\beta_{1–40}$-treated mice 24 h after treatment, accompanied by a decrease in the levels of GSH-t in both structures. However, in contrast to the results obtained with Swiss mice, the i.c.v. administration of $A\beta_{1–40}$ (400 pmol/mouse) did not significantly affect the GR and GPx activity or the GSH-t content in the HC and PFC of C57BL/6 mice (Fig. 3).

## 4. Discussion

The present findings demonstrate spatial learning and memory deficits induced by a single i.c.v. administration of the β-amyloid ($A\beta_{1–40}$) peptide in mice, extending previous results obtained with the peptides $A\beta_{1–42}$ [26,61] and $A\beta_{25–35}$ [35]. These cognitive impairments appear to be associated with synaptic loss, since a significant reduction of synaptophysin levels was observed in the hippocampus (HC) and prefrontal cortex (PFC) of $A\beta_{1–40}$-treated mice. More importantly, our results demonstrate, for the first time, an inter-strain antioxidant response elicited by $A\beta_{1–40}$ treatment in C57BL/6 and Swiss mice. Moreover, background differences in the glutathione-dependent antioxidant defences in the HC and PFC of C57BL/6 and Swiss mice were also observed.

It is generally accepted that there are multiple memory systems. Two of the most studied examples are the hippocampal and the basal ganglia memory systems, which process and store information independently and in different styles. According to this view, the hippocampal system processes spatial-temporal memories involving relations among environmental cues (e.g. episodic memory in humans), while the basal ganglia system is involved in habit learning in which a single stimulus is repeatedly associated with a response [36,42,58]. Two different versions of the Morris water maze task have proved to be particularly suitable for the testing of spatial memory or habit learning in rodents. In the spatial version, rodents learn to escape to a submerged platform that is maintained in the same location in the water maze from the beginning to the end of the experiment. In this case the animals need to make associations among the spatial environmental cues in order to form a cognitive map that helps them to find where the platform is [38]. In the habit version, the animals learn to associate the position of a white ball attached to the platform, protruding above the water. The platform position is changed randomly among trials. In this case, a single stimulus (the ball) is repeatedly associated with a response of approaching the platform. Spatial memory depends critically on the integrity of the HC but not of the dorsal striatum, whereas habit learning depends critically on the integrity of the dorsal striatum but not of the HC [42,58].

In the present study, the i.c.v. injection of $A\beta_{1–40}$ (400 pmol/mouse), but not $A\beta_{40–1}$ (400 pmol/mouse), 7 days

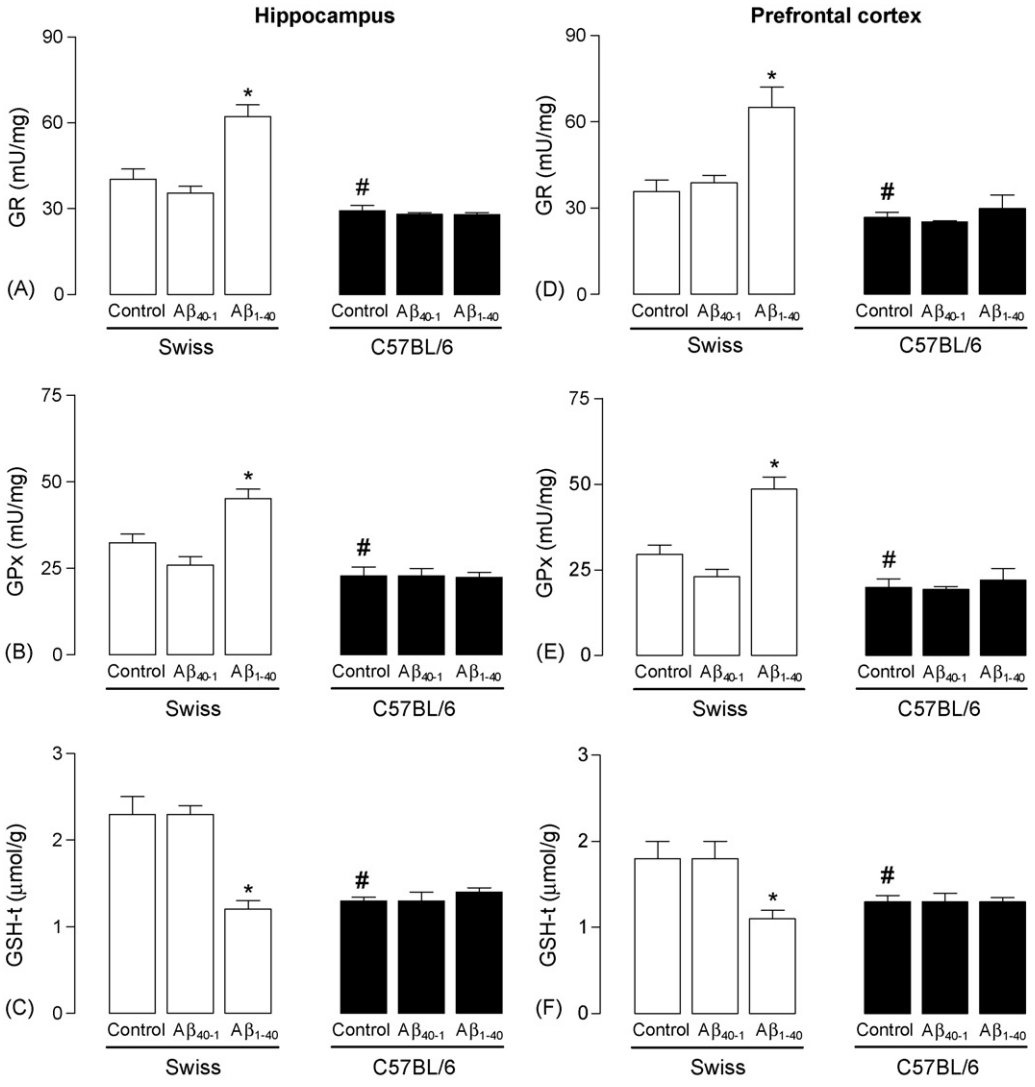


Fig. 3. The effects of i.c.v. administration of $A\beta_{1–40}$ (400 pmol/mouse), $A\beta_{40–1}$ (400 pmol/mouse) or control solution (PBS) on GSH-dependent antioxidant parameters in Swiss and C57BL/6 mice. Activities of antioxidant enzymes GR (A and D) and GPx (B and E) and GSH-t levels (C and F) were measured in the hippocampus and prefrontal cortex of mice 24 h after treatments. The values represent the mean ± S.E.M. of GR and GPX activity (mU/mg protein) ($n = 5–7$ animals in each group) and GSH-t levels (µmol/g wet tissue) (5–7 animals in each group). $^{*}P \leq 0.05$ compared to the control-treated group of the same strain. $^{\#}P \leq 0.05$ compared to the control-treated group of the Swiss strain (Newman-Keuls test).

prior to training, resulted in similar spatial learning and memory decline in Swiss and C57BL/6 mice, as indicated by higher final escape latencies to find the platform during the training session and reduced percentage of time spent in the correct quadrant in the probe test session, respectively. The water maze task is a complex paradigm where the performance of animals is influenced by factors related to attention and motivation as well as to the sensorimotor function, and these factors collectively determine the success or failure to find the platform. Therefore, it is important to emphasize that $A\beta_{1–40}$-treated mice, regardless of the strain (Swiss or C57BL/6), did not differ from control-treated mice in ambulation and rearing in the open field task. These results suggest that a single i.c.v. injection of $A\beta_{1–40}$ (400 pmol/mouse) has no effect on motor function and exploratory activity in mice. Furthermore, in the water maze, neither the escape latency to find the platform during the first training trial nor the swimming speed during the training trials were affected by i.c.v. $A\beta_{1–40}$ treatment. This response suggests that there are no major changes in swimming ability. Taken together, it is likely that the impairment of performance in $A\beta_{1–40}$-treated C57BL/6 and Swiss mice is due to learning and/or memory deficits.

The exact mechanism responsible for this cognitive deficit induced by $A\beta_{1–40}$ administration in mice is still unknown. Converging evidence suggests that increased accumulation of Aβ appears to be related to a gradual synaptic loss and neuronal death, finally leading to cognitive impairments [25,52,57]. Highly complex behaviours, such as those evaluated in the water maze test, depend on the coordinated function of several brain structures. For practical reasons, we addressed in the present study the neuronal integrity in the HC and PFC of Swiss and C57BL/6 mice after i.c.v. injection of $A\beta_{1–40}$ (400 pmol/mouse) through immunohistochemistry analysis for the pre-synaptic protein synaptophysin. A similar decline in synaptophysin levels was observed in Swiss and C57BL/6 mice at seven days after

$A\beta_{1-40}$ administration, suggesting a reduction in synaptic density. On the other hand, no alteration was observed for caspase-3 protein immunostaining, indicating that neuronal apoptosis was probably not activated, at least not up to 7 days following $A\beta_{1-40}$ treatment (data not shown). These results reinforce the notion that synaptic dysfunction precedes the neuronal death seen in AD and suggests that the synaptic loss may be responsible, at least in part, for the cognitive impairments induced by $A\beta_{1-40}$ in mice.

Glutathione is an abundant intracellular antioxidant and scavenger of reactive oxygen species (ROS), providing the neuronal cell with important protection against oxidative damage [17]. Previous studies have shown that the GSH system may be activated as a response to oxidative stress in the brains of AD patients [3,30,43], as well as in the brains of Aβ-treated aging mice [26]. Moreover, recent findings suggest that Aβ might initiate a cascade of events resulting in a severe depletion of GSH [1]. The inter-strain difference in the GSH-dependent antioxidant defences in C57BL/6 and Swiss mice under basal conditions was remarkable. When compared to Swiss mice, C57BL/6 mice presented lower levels of GSH-t and reduced GPx and GR activity in the HC and PFC. The relevance for such differences cannot be anticipated, but it can be predicted that lower antioxidant status may result in elevated susceptibility to oxidative challenge. On the contrary, considering that antioxidant defences counterbalance to basal ROS production, it is possible to infer that C57BL/6 mice have lower basal ROS production and consequently needs lower antioxidant defences. Taking into account the fact that mitochondrion is one of the primary sources of ROS in the normal functioning cell [7], it would be interesting to compare mitochondrial ROS production between C57BL/6 and Swiss mice.

The observed resistance of young adult C57BL/6 mice to $A\beta_{1-40}$-induced alterations in GSH-dependent antioxidant parameters is in contrast to the response of 15-month-old C57BL/6 mice that have demonstrated significant increase in GR and GPx activity in the HC and PFC following a single i.c.v. administration of $A\beta_{1-42}$ [26]. This discrepancy with early data may be explained by differences in the age (3-month-old versus 15-month-old) of the subjects and/or the Aβ peptides ($A\beta_{1-40}$ versus $A\beta_{1-42}$) utilized to evaluate alterations in antioxidant parameters. Moreover, the current response obtained in Swiss mice is consistent with the induction of GPx and GR mRNA [3,4] and the increased activity of these enzymes [30] in vulnerable regions of the AD brain. AD patients have lower erythrocyte GSH levels [29], added to the fact that GSH is depleted in experimental models using Aβ [1,59], indicating that GSH is actively involved in these processes. Interestingly, GSH has been shown to be induced by $A\beta_{25-35}$ in PC12 cells [6], indicating that Aβ is able to promote glutathione synthesis. Altogether, these evidences indicate that in the progress of AD or shortly after Aβ treatment in experimental models, cells respond by a general up-regulation of antioxidant defences. We can further speculate that this response is counterbalanced by an oxidative burden [8,30] that possibly increases GSH demand, leading to its consumption.

GPx is an antioxidant defence enzyme that can directly detoxify $H_2O_2$ and lipid hydroperoxides at the expense of GSH. The increased GPx activity induced by $A\beta_{1-40}$ treatment could possibly reflect an adaptive attempt to counteract increased lipid peroxide production. Corroborating with this view, Ran et al. [46] have recently demonstrated that overexpression of GPx 4 protects neurons against oxidative injury and $A\beta_{25-35}$-induced cytotoxicity. Increased GPx activity may lead to GSSG load, since augmented ROS production is a common finding in AD models [46]. To counteract this possible GSSG load, GR reduces GSSG back to two GSH, contributing directly to the protection and repair of protein thiols in cells under oxidative stress [17]. Therefore, the increased GR activity observed in the HC and PFC of $A\beta_{1-40}$-treated Swiss mice may be an indicator of cellular response to GSH depletion, and possibly to an increased modification of thiol-containing proteins. In agreement with these data, previous studies have reported increased GR activity in different encephalic structures after pro-oxidative insults [18,20], including $A\beta_{1-42}$-induced oxidative damage [26]. In vitro studies with GSH precursors such as γ-glutamylcysteine ethyl ester (GCEE) have diminished the pro-oxidative effects of Aβ by up-regulating GSH levels in neuronal cultures [9,10]. Conversely, the inhibition of GSH synthesis leads to an increase of Aβ-induced cell death and intracellular Aβ accumulation [59]. Taken together, these findings suggest that prevention of GSH depletion associated with the increase of GSH-dependent antioxidant enzymes (GPx and GR) activity may represent important cellular responses to counteract Aβ-induced neurotoxicity.

The underlying mechanism responsible for the observed strain differences in the susceptibility of $A\beta_{1-40}$-induced changes in the GSH-dependent antioxidant defences in C57BL/6 and Swiss mice still needs to be elucidated. However, some considerations can be given. Lehman et al. [28] have recently demonstrated that genetic background regulates APP processing, Aβ metabolism and Aβ deposition in the mouse brain. Moreover, an earlier study designated to optimize Aβ immunization in mice has indicated that C57BL/6 mice are less responsive to Aβ immunization in comparison to other mouse strains [53]. In rats, the response to Aβ following i.c.v. infusion [27], or even after a single i.p. injection [33], was a reduction in antioxidant enzymes, in a clear association with an increase in oxidative markers. Thus, the antioxidant response can be triggered by an inflammatory reaction [16,33], which might differ in distinct species and among different strains, especially in the comparison of inbred and outbred species, and may represent a possible explanation for the differences found in the two strains. Interestingly, Agardh et al. [2] reported no changes in the retinal tissue levels of GSH in C57BL/6 mice with long-standing hyperglycemia and hyperlipidemia, conditions that favour oxidative stress and lipid peroxidation. The authors emphasize the importance of further studies to explore whether this response can be explained by an increased expression of alternative systems of protection in C57BL/6 mice. In a genetic model of AD, using C57BL/6 as the background strain, markers of oxidative stress such as lipid peroxidation products (e.g. 4-hydroxynonenal) and impaired superoxide dismutase activity were increased [49]. The absence of response (no changes in GR and GPx activity), despite clear signs of oxidative stress [9,26,30], points to a possible lack of response. In agreement with our data,

the activities of GR and GPx were not altered in this APP transgenic mouse model [49]. Therefore, our findings reinforce the strain of choice as an important concern in animal research.

Another important discussion concerning the cognitive impairments in Aβ-treated rodents is whether these symptoms are directly associated with oxidative stress or whether these two phenomena present distinct mechanisms. Our results indicate that the reduced susceptibility of C57BL/6 mice to $A\beta_{1–40}$-induced changes in the GSH-dependent antioxidant defences was not correlated with altered sensitivity to $A\beta_{1–40}$-induced spatial learning or memory impairment in the water maze test. Synaptophysin immunocontent was also decreased equally in Swiss and C57BL/6 mice. Thus, our study suggests that regardless of an adaptive increase in glutathione-dependent enzymes following Aβ administration, learning and memory impairments are produced, and possibly the antioxidant response specificity may be related to an alternative factor, such as immunological response. However, it must be conceded that regarding $A\beta_{1–40}$-induced cognitive impairments, additional learning and memory tasks are necessary to completely discard the existence of strain differences between Swiss and C57BL/6 mice.

In conclusion, the present findings demonstrate that a single i.c.v. administration of $A\beta_{1–40}$ peptide causes similar spatial learning and memory impairments in adult C57BL/6 and Swiss mice and that these alterations are associated with synaptic loss in the HC and PFC. More importantly, our results reinforce the notion that GSH-dependent antioxidant defences represent important cellular markers of Aβ-induced neurotoxicity, demonstrating inter-strain differences in antioxidant parameters of C57BL/6 and Swiss mice. Thus, strain differences in mice should be taken into account in further studies using Aβ as a model of AD.

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