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RESEARCH PAPER

# Soluble βamyloid$_{1-42}$: a critical player in producing behavioural and biochemical changes evoking depressive-related state?

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**Background and purpose:** Depression is common in early phases of Alzheimer's disease (AD) and may represent prodromal symptoms of dementia. Recent reports suggest that early memory deficits and neuropsychiatric symptoms are caused by soluble rather than aggregated βamyloid (Aβ). Thus, we investigated the effects of soluble Aβ$_{1-42}$ on working memory and depressive/anxiety-related behaviour in rats and on 5-hydroxytryptaminergic neurotransmission and neurotrophin content in various brain regions.

**Experimental approach:** Behavioural reactivity to novel object recognition, open field, elevated plus maze and forced swimming test were assessed 7 days after i.c.v. injection of Aβ$_{1-42}$ or its vehicle. BDNF (brain-derived neurotrophic factor) and NGF (nerve growth factor) mRNA and protein levels and 5-hydroxytriptamine (5-HT) content were measured in the prefrontal cortex (PFC), striatum (STR) and nucleus accumbens (NAc).

**Key results:** Aβ$_{1-42}$ did not affect the ability to distinguish between familiar and novel objects, but Aβ-treated rats exhibited an increase in forced swimming immobility. No differences were revealed between experimental groups in the elevated plus maze test or in self-grooming (evaluated in the open field). In the PFC, but not STR or NAc, Aβ-injected rats exhibited a selective reduction in 5-HT content, BDNF and NGF expression.

**Conclusions and implications:** Our data suggest that soluble Aβ-treated rats have a depressive, but not anxiogenic-like, profile, accompanied by brain region-dependent alterations in the expression of neurotrophins and 5-hydroxytryptaminergic neurotransmission. Hence, these alterations induced by soluble Aβ might be sensitive indicators of early phases of AD and possible risk factors for the expression of neuropsychiatric symptoms in AD.
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**Keywords:** soluble βamyloid; depression; prefrontal cortex; 5-hydroxytryptamine; BDNF; NGF; rats

**Abbreviations:** 5-HT, 5-hydroxytryptamine; Aβ, βamyloid$_{1-42}$; AD, Alzheimer's disease; BDNF, brain-derived neurotrophic factor; NAc, nucleus accumbens; NGF, nerve growth factor; PFC, prefrontal cortex; STR, striatum

## Introduction

Mounting evidence suggests that depression is a risk factor for Alzheimer's disease (AD) (Ownby *et al.*, 2006; Sun *et al.*, 2008). Indeed, depression is common in pre-clinical AD, and may represent an early manifestation of this disease before any cognitive impairments appear (Geerlings *et al.*, 2000; Visser *et al.*, 2000). In particular, subjects with mild cognitive impairment and depression have more than twice the risk of developing Alzheimer-type dementia than patients without depression (Modrego and Ferrandez, 2004). Neuropsychiatric symptoms are not just an emotional reaction to the awareness of the dementing diseases, but may derive from neurobiological changes in specific brain areas and may be prodromal symptoms of dementia (Andersen *et al.*, 2005).

Recent studies suggest that early memory deficits and neuropsychiatric symptoms may be explained by the presence of soluble forms of βamyloid (Aβ) rather than the aggregated form which develops into insoluble plaques (Rowan *et al.*, 2005). Accordingly, in the early stages of AD, significant

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cognitive deficits have been directly attributed to soluble Aβ fragments (Mattson, 2004; Cleary *et al.*, 2005) and increased levels of soluble Aβ oligomers caused synaptic dysfunction (Hardy and Selkoe, 2002; Selkoe and Schenk, 2003), raising the possibility that increased brain soluble Aβ levels may contribute to the development of non-cognitive symptoms.

The preservation of key neurotransmitters and neurotrophic factors is considered crucial for the regulation of synaptic plasticity and neuronal survival, and a decline in their function is related to neurodegenerative disorders like AD (Mattson, 2004). Several studies suggest that a decrease in the levels of brain-derived neurotrophic factor (BDNF) could be associated with the pathogenesis of AD (Siegel and Chauhan, 2000; Fumagalli *et al.*, 2006; Tapia-Arancibia *et al.*, 2008), but the role for this factor is still to be established. BDNF has a pleiotropic influence on nerve cells, with effects ranging from synaptic transmission and neuronal plasticity, to regulation of differentiation and survival of specific neuronal populations (Thoenen, 2000; Burbach *et al.*, 2004). Moreover, BDNF has been frequently linked to mood disorders (Altar, 1999; Castren *et al.*, 2007; Kalueff *et al.*, 2007; Monteggia *et al.*, 2007) and, in animal models of depression, centrally administered BDNF produces antidepressant-like activity (Shirayama *et al.*, 2002). Therefore, BDNF has been proposed as a bridge between depression and AD (Tsai, 2003). Other neurotrophins have also been linked to depression and AD, such as nerve growth factor (NGF) (Schulte-Herbruggen *et al.*, 2007). A significant decrease in serum NGF has been observed in patients with mild cognitive impairment, suggesting that the availability of NGF might be reduced at the onset of any neurodegenerative processes (Schaub *et al.*, 2002). Neurotrophins and 5-hydroxytryptamine (5-HT) are close and reciprocally regulated signals and the central 5-hydroxytryptaminergic system has been shown to be altered in AD patients (Garcia-Alloza *et al.*, 2004); hence, changes in the levels of neurotrophin might contribute to degenerative disorders through modifications of the 5-hydroxytryptaminergic system (Tapia-Arancibia *et al.*, 2008).

In addition, Christensen *et al.* (2008) observed changes in BDNF levels and the function of the 5-hydroxytryptaminergic system 83 days after a single intra-hippocampal injection of $A\beta_{1\text{-}42}$ peptide in its aggregated form; in particular, the lower hippocampal 5-$HT_{2A}$ receptor levels and frontal cortical BDNF concentrations were affected.

The aim of the present study was to investigate whether an acute intracerebroventricular (i.c.v.) injection of soluble Aβ might induce alterations in non-cognitive domains as well as impair short-term memory in rats. Although the evaluation of mood-related disturbances in animal models is not straightforward, several paradigms for investigating symptoms related to depression and anxiety in rodents have been developed (Pellow *et al.*, 1985; Pellow and File, 1986; Choleris *et al.*, 2001; Cryan *et al.*, 2005). Therefore, the current study was designed to evaluate the effects of soluble Aβ on the working memory, motor activity, anxiety- and depression-related behaviours of young adult male rats. The levels of BDNF and NGF mRNA and protein were also measured in rat prefrontal cortex (PFC), striatum (STR) and nucleus accumbens (NAc). In addition, as the 5-hydroxytryptaminergic system may be primarily involved in the development of non-cognitive symptoms (Gage and Springer, 1981; Steckler and Sahgal, 1995), we investigated whether 5-hydroxytryptaminergic neurotransmission in the rat PFC, STR and NAc was affected by soluble Aβ treatment.

## Methods

*Animals*

The experiments were conducted using 193 young-adult male Wistar rats (Harlan, S. Pietro al Natisone, Udine, Italy) weighing 250–300 g. The animals were randomly assigned to the experimental groups, one for each behavioural, neurochemical and biochemical analysis and they were allowed to acclimatize to the animal house for at least 7 days before the experiments. They were housed in pairs with food and water available *ad libitum* and handled during the weekly care procedures. They were housed at a constant room temperature (22 ± 1°C) and relative humidity (55 ± 5%) under a 12-h light/dark cycle (lights on from 7 h 00 min to 19 h 00 min). The experiments involving the animals and their care conformed to the institutional guidelines in compliance with national (D. L. N°. 116, G. U., Suppl. 40, February 18, 1992, Circ N°. 8, G. U., July 14, 1994) and International laws and policies (EEC Council Directive 86/609, OJ L 358, 1, 12 December 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996). Every effort was made to minimize the number of animals used and their suffering.

*$A\beta_{1\text{-}42}$ administration*

The $A\beta_{1\text{-}42}$ peptide was obtained from Tocris (Bristol, UK). The peptide was dissolved daily in sterile double-distilled water (vehicle) at a concentration of 4 μM as previously described (Trabace *et al.*, 2007). Briefly, rats were anaesthetized by administration of Equithesin (composition: 1.2 g sodium pentobarbital; 5.3 g chloral hydrate; 2.7 g $MgSO_4$; 49.5 mL propylene glycol; 12.5 mL ethanol and 58 mL distilled water), 3.6 mL·kg$^{-1}$ i.p., and secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Skin was shaved, disinfected and cut with a sterile scalpel and a hole was drilled to insert the injection needle (30-gauge stainless steel tubing; Cooper's Needles, Birmingham, UK). The i.c.v. injections were made using the following coordinates relative to bregma: AP = –0.5, ML = +1.2, DV = –3.2 with the incisor bar set at –3.3 mm (Paxinos and Watson, 1998).

$A\beta_{1\text{-}42}$ (5 μL) was delivered through a 25 μL Hamilton microsyringe at an infusion rate of 2 μL·min$^{-1}$ for 2.5 min. Control rats were injected with vehicle only, as reverse $A\beta_{42\text{-}1}$, used in preliminary experiments, was indistinguishable from vehicle alone (unpublished observations). The injection needle was left in place for an additional 5 min to prevent reflux. Placement of the needle track was verified at the time of dissection. All experimental procedures (behavioural and biochemical experiments) were performed 7 days after i.c.v. administration (sham-operated or Aβ-treated groups) or i.p. injection (Equithesin, intact group).

*Novel object recognition test*
The test was performed according to Giustino *et al.* (1996). Briefly, 30 rats were submitted to two habituation sessions (intersession interval: 24 h) where they were allowed 5 min to explore the apparatus. Twenty-four h after the last habituation, a session of two 3-min trials separated by a 1-min intertrial interval was carried out. In the first trial (T1), rats were exposed to two identical objects (white glasses or light bulbs). During the second trial (T2), rats were exposed to one familiar (F) object but the second familiar object was replaced by a new, differently shaped, object (N). In each trial each object was placed at an equidistant position between the centre and the wall of the arena. The position of the two objects was counterbalanced and randomly permutated during T2. At the beginning of each trial the rats were placed near the centre of the arena with their heads oriented in the opposite direction to the objects. Exploration of the objects was defined as sniffing or touching the object with the nose. Turning around or sitting on the object was not considered to be exploration. Object exploration was quantified as: exploratory activity, total time spent exploring both objects during each trial (T1 and T2); index of discrimination, expressed as ratio between the net time spent exploring the new (N–F) over the total exploration time (N–F/N+F). Objects and arena were carefully cleaned between each session to avoid confounding olfactory stimuli.

*Open field spontaneous locomotor activity*
The apparatus consisted of a circular arena, 75 cm diameter, made of dark plastic under dim lighting, as previously described by Monteggia *et al.* (2007). The experimental sessions were videotaped by a camera fixed above the arena. Thirty animals were acclimatized to the test room for 1 h before each test. Motor activity was measured by placing the rat into the centre of the arena before a 20 min session. The scoring was performed using a video-tracking motion analysis system (Ethovision, Noldus Information Tecnology, Wageningen, the Netherlands). To assess general locomotor activity, the following behavioural parameters (expressed as frequency on 5 min counts) were scored: number of square limit crossings with both forepaws, rearing (standing with the body inclined vertically, forequarters raised), and wall rearing (standing on the hind-limbs and touching the walls of the apparatus with the forelimbs). To investigate anxiety-related behaviour, we measured time spent performing general grooming activity consisting of: face grooming (strokes along the snout), head washing (semicircular movements over the top of the head and behind the ears) and body grooming (body fur licking) (Choleris *et al.*, 2001).

*Forced swimming test*
On the first of the two test days, thirty animals were placed individually in inescapable Perspex cylinders (diameter 23 cm; height 70 cm) filled with a constant maintained 25°C temperature water at a height of 30 cm (Cryan *et al.*, 2005). During the preconditioning period, the animals were observed for 15 min. Then, rats were removed and dried before returning them to their home cages. Twenty-four h later, each rat was returned to the water-filled cylinder for 5 min. This session was video-recorded and subsequently scored by an observer blind to the treatment groups. During the test sessions, the time that rats spent performing the following behaviours was measured: struggling (time spent in tentative of escaping), swimming (time spent moving around the cylinder) and immobility (time spent remaining afloat making only the necessary movements to keep its head above the water).

*Elevated plus-maze test*
The experimental procedures were performed according to Pellow *et al.* (1985). Briefly, the Plexiglas apparatus consisted of two opposite open arms (50 × 10 cm) without side walls and two closed arms (50 × 10 × 40 cm) extending horizontally at right angles from a central area (10 × 10 cm). Our maze was situated in a separate brightly lit room illuminated with four, 32-W fluorescent overhead lights each, which produce consistent illumination within the room. The apparatus had similar levels of illumination on both open and closed arms as reported by Walf and Frye (2007). The maze was elevated to a height of 50 cm in this lit room. At the beginning of the experiment, thirty animals were placed at the centre of the plus-maze, facing the open arm. During a 5-min observation period the following parameters were measured: number of open and closed arm entries and percentage of time spent on open arms. An arm entry was counted when both rat forepaws were placed into the given arm. The % open arm time, an inverse measure of anxiety-like behaviour, was calculated as (time in open arms/total time in arms) × 100.

*Tissue 5-hydroxytryptamine content*
Eighteen rats were killed by decapitation and brain immediately removed for dissection. Brains were placed dorsal side up in an ice-chilled rat brain matrix (World Precision Instruments, Inc., Aston, Stevenage, UK) with slits spaced at 1 mm. Using an ice-chilled razor blade, the target regions (PFC, STR and NAc) were dissected according to the atlas of Paxinos and Watson (1998). Tissues were frozen and stored at –80°C until analysis was performed. Samples were homogenized in 10 volumes ($w \cdot V^{-1}$) of perchloric acid 0.1 M. The homogenates were stored on ice for 30 min and then centrifuged at 10 000 *g* for 10 min at 4°C. The supernatants were filtered and properly diluted for analysis. 5-HT concentrations were determined by high performance liquid chromatography (HPLC) coupled with a coloumetric detector (ESA, Coulochem II, Bedford, MA, USA). Analysis was performed through a LC18 reversed phase cartridge column (15 cm × 4.6 mm, 3 micron; Supelco, Milan, Italy). The mobile phase was 130 mM $CH_3COONa$, 13 mM citric acid, 0.1 mM EDTA, acetonitrile 6%, in distilled water, buffered at pH 5.1 with $CH_3COOH$ and properly filtered. The flow rate, maintained by an isocratic pump (Shimadzu LC-10AD, Kyoto, Japan), was 1 $mL \cdot min^{-1}$. Cell potentials were set at $E_1$ = +50 mV and $E_2$ = +250 mV.

*BDNF and NGF mRNA measurement*
Brain areas from 18 animals were collected as described above. mRNA was isolated using RNeasy mini kit (Qiagen, Hilden,

Germany) according to the manufacturer's instructions. Residual genomic DNA was removed using RNase-Free DNase set (Qiagen, Hilden, Germany). One μg total mRNA was reverse transcribed using the superscript II kit according to the manufacturer's instructions (Invitrogen, Milan, Italy). RT-PCR for BDNF and NGF was performed at 35 cycles. The primers used for BDNF were F 5′ GCG GCA GAT AAA AAG ACT GC 3′ and R 5′ GCC AGC CAA TTC TCT TTT TG 3′; for NGF were F 5′ GGA CGC AGC TTT CTA TCC TG 3′ and R 5′ AAA CAG TTT GGG GTC CAC AG 3′ (Invitrogen, Milan, Italy). RT-PCR gel (1%) was run at 100 V. Optical band density was analysed using ImageJ software (http://rsb.info.nih.gov/ij/).

*BDNF and NGF protein measurements*

PFC, STR and NAc were collected from 37 rats as described above. They were rapidly dissected, frozen on dry ice and stored at –80°C until assayed for neurotrophin content. BDNF and NGF protein was measured by ELISA using commercially available kits (BDNF and NGF Emax Immunoassay System, respectively, Promega, Milan, Italy) according to manufacturer's instructions. Briefly, tissues were homogenized and centrifuged at 12 000 *g* at 4°C for 30 min, and supernatants were diluted with a blocking buffer. ELISA was performed in 96-well plates (Iwaki, Funahashi, Japan). Colorimetric detection of peroxidase activity was achieved by adding TMB solution and peroxidase substrate, and incubating for 10 min at room temperature according to the manufacturer's instructions. The enzymatic reaction was stopped with HCl (1.0 M) and the optical density of each well was measured at 450 nm using a PowerWave XS plate reader (Bio-Tek, Winooski, VT, USA). Each analysis was performed in duplicate in the same assay to avoid inter-assay variations. Neurotrophin levels are expressed as percentage of intact controls.

*Statistical analysis*

All statistical analyses were performed using SigmaStat® 3.1 and Graph Pad® 5.0 for Windows. Data were tested for normality by the selection of parametric and non-parametric tests. Behavioural data were analysed by a two-way analysis of variance (ANOVA) for repeated measures or one-way ANOVA, followed by a Newman-Keuls Multiple Comparison's test, as required. For the *post-mortem* tissue analyses, data were analysed by one-way ANOVA followed by a Tukey's test. Differences were considered significant only when *P*-values were less than 0.05.

## Results

*Effect of Aβ on the novel object recognition test*

Two-way ANOVA revealed no differences between the treatment groups, whereas a significant difference was found only in time ($F_{t(1,27)} = 23.23$, $P < 0.001$, Figure 1A). A post-hoc test showed that all experimental groups spent less time in exploring objects in T2. When rats were re-exposed to the familiar and to the novel object, they did not show any significant difference between groups in total time spent for exploration of both objects during T2 (Figure 1B). In T2 two-way ANOVA revealed that all rats, independently of their respective group, spent more time in exploring the new than the familiar object ($F_{t(1,46)} = 33.64$, $P < 0.001$; Figure 1B). As shown in Figure 1C, no differences were found in discrimination index between the experimental groups (one way ANOVA, $F_{(2,23)} = 0.44$, n.s.; Figure 1C). However, statistical analysis revealed that Aβ-treated animals spent less total time exploring both objects (N and F) in both trials (T1 and T2) than controls ($F_{(2,23)} = 6.01$; $P < 0.01$; Figure 1D).

*Effect of Aβ on the open field test*

As shown in Figure 2, analysis of data revealed that there were no significant differences in the frequencies of crossing (two-way ANOVA for repeated measures: $F_{tr(2,69)} = 1.89$, n.s., $F_{t(3,69)} = 84.98$, $P < 0.001$, $F_{txtr(6,69)} = 0.41$, n.s.; Figure 2A), rearing ($F_{tr(2,81)} = 0.12$, n.s., $F_{t(3,81)} = 6.83$, $P < 0.01$, $F_{txtr(6,81)} = 0.84$, n.s.; Figure 2B) and wall rearing ($F_{tr(2,81)} = 2.22$, n.s., $F_{t(3,81)} = 70.08$, $P < 0.001$, $F_{txtr(6,81)} = 0.76$, n.s.; Figure 2C) between the experimental groups. Similarly, no differences were found across the groups with regard to the self-grooming behaviour (one way ANOVA, $F_{(2,25)} = 0.096$, n.s.; Figure 2D).

*Effect of Aβ on the forced swimming test*

Data showed that immobility frequency was significantly increased (one way ANOVA, $F_{(2,25)} = 5.59$, $P < 0.01$; Figure 3A) in Aβ-injected rats compared with either sham-operated or intact rats. Conversely, frequency in swimming activity was significantly decreased in Aβ-treated animals (one way ANOVA, $F_{(2,25)} = 3.63$, $P < 0.05$; Figure 3B). No difference in struggling activity was found between all the experimental groups considered (Figure 3C). Although a decrease in motor strength or endurance may affect the forced swimming test, no apparent motor deficit was noticed during habituation, consisting of 15 min of swimming in the cylinder 24 h before the test session (data not shown).

*Effect of Aβ on the elevated plus-maze test*

As shown in Figure 4, analysis of data revealed no significant differences in the percentage of time spent in the open arms, a measure of anxiety-like behaviour (one way ANOVA: $F_{(2,22)} = 0.05$, n.s., Figure 4A), or in the number of open arm entries ($F_{(2,20)} = 0.10$, n.s., Figure 4B) between the groups. Moreover, no significant differences were found in the number of entries into the closed arms, a measure of non-specific motor activity ($F_{(2,24)} = 0.06$, n.s.; Figure 4C) between the experimental groups.

*Effect of Aβ on tissue 5-HT levels*

One-way ANOVA revealed a significant difference in 5-HT content between intact or sham-operated and soluble Aβ-injected rats. Indeed, Tukey's post hoc test showed that soluble Aβ induced a significant reduction in 5-HT tissue concentrations in the PFC of rats ($F_{(2,17)} = 3.64$, $P < 0.05$; Figure 5A). Conversely, no significant differences in 5-HT content between the groups were found in the STR and NAc (Figure 5B,C).

*Effect of Aβ on BDNF and NGF mRNA and protein levels*

As shown in Figure 6, one-way ANOVA followed by Tukey's comparison test revealed a significant reduction in BDNF

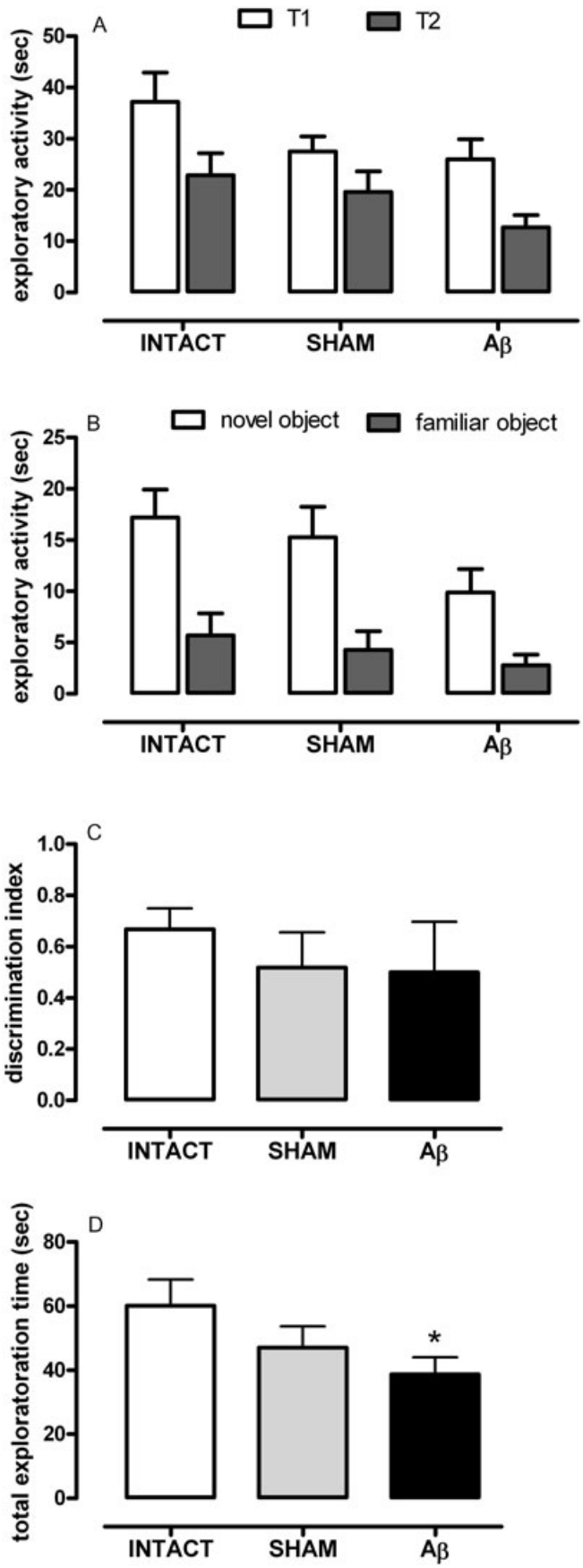


**Figure 1** (A) Exploratory activity during Trial 1 (T1) and Trial 2 (T2) (B) exploratory activity of novel and familiar objects during T2 (C) discrimination index and (D) total exploration time in the novel object recognition test of male Wistar rats 7 days after administration of Equithesin (3.6 mL·kg$^{-1}$ i.p.; INTACT), water (5 μL i.c.v.; SHAM) or $A\beta_{1\text{-}42}$ (4 μM, 5 μL i.c.v.; Aβ). Data are expressed as mean ± SEM of total frequency counts in 5 min intervals and total time in s ($n$ = 10 per group). (One-way ANOVA followed by Newman-Keuls multiple comparison's Test, *$P$ < 0.01 vs. intact and sham).

mRNA and protein levels in PFC of soluble Aβ-injected compared with intact or sham-operated rats ($F_{(2,15)}$ = 53.55, $P$ < 0.01 for mRNA; $F_{(2,20)}$ = 7.74, $P$ < 0.01 vs. intact and $P$ < 0.05 vs. sham for protein; Figure 6A,B). On the other hand, no difference was found in STR of Aβ-treated rats compared with both intact and sham-operated animals ($F_{(2,15)}$ = 0.45, n.s. for mRNA; $F_{(2,15)}$ = 0.09, n.s. for protein; Figure 6C,D).

NGF analysis revealed the same pattern showing a significant reduction in PFC mRNA and protein levels only in Aβ-injected rats ($F_{(2,15)}$ = 13.58, $P$ < 0.01 for mRNA; $F_{(2,15)}$ = 6.51, $P$ < 0.01 for protein; Figure 7A,B), while no differences were found in STR in all experimental groups ($F_{(2,15)}$ = 1.12, n.s. for mRNA; $F_{(2,15)}$ = 0.09, n.s. for protein; Figure 7C,D).

As previously reported in the literature (Conner *et al.*, 1997), the levels of BDNF and NGF mRNA in the NAc were barely detectable (data not shown). However, there were no significant differences in the protein levels of these neurotrophins in this area between the three experimental groups (BDNF: intact, 307 ± 44 pg·mg$^{-1}$ of tissue, sham, 384 ± 94, Aβ, 307 ± 22, $F_{(2,9)}$ = 0.53, n.s.; NGF: intact, 3269 ± 459, sham, 7727 ± 2207, Aβ, 8114 ± 1882, $F_{(2,9)}$ = 2.52, n.s.).

## Discussion and conclusions

The present study suggests that soluble Aβ-treated rats show a depressive-, but not anxiogenic-like, phenotype. These outcomes are accompanied by alterations in the expression of neurotrophins and 5-hydroxytryptaminergic neurotransmission in selective brain regions without an impairment in working memory.

Although the relationship among soluble Aβ, brain neurochemistry and depression remains complex, several studies have demonstrated an increased risk for the development of AD in individuals with late-life depression, suggesting a prodromal state of AD (Steffens *et al.*, 1997; Dal Forno *et al.*, 2005; Sun *et al.*, 2008). Aβ may have an effect on mood not limited to non AD patients, depression-like symptoms may indeed precede or accompany dementia (Starkstein *et al.*, 2008). On the other hand, $A\beta_{42}/A\beta_{40}$ ratio has been reported to correlate with severity of signs of AD pathogenesis in elderly individuals with late life major depression (Pomara *et al.*, 2006). Hence, the identification of early events in the development of AD-related depression would be an advance in our understanding of pathophysiological mechanisms underlying the prodromal phase of AD.

From a behavioural point of view, we found that soluble Aβ did not alter the ability of rats to distinguish between a familiar and a novel object. Surprisingly, Aβ-treated animals showed lower exploratory activity. Indeed, they spent less

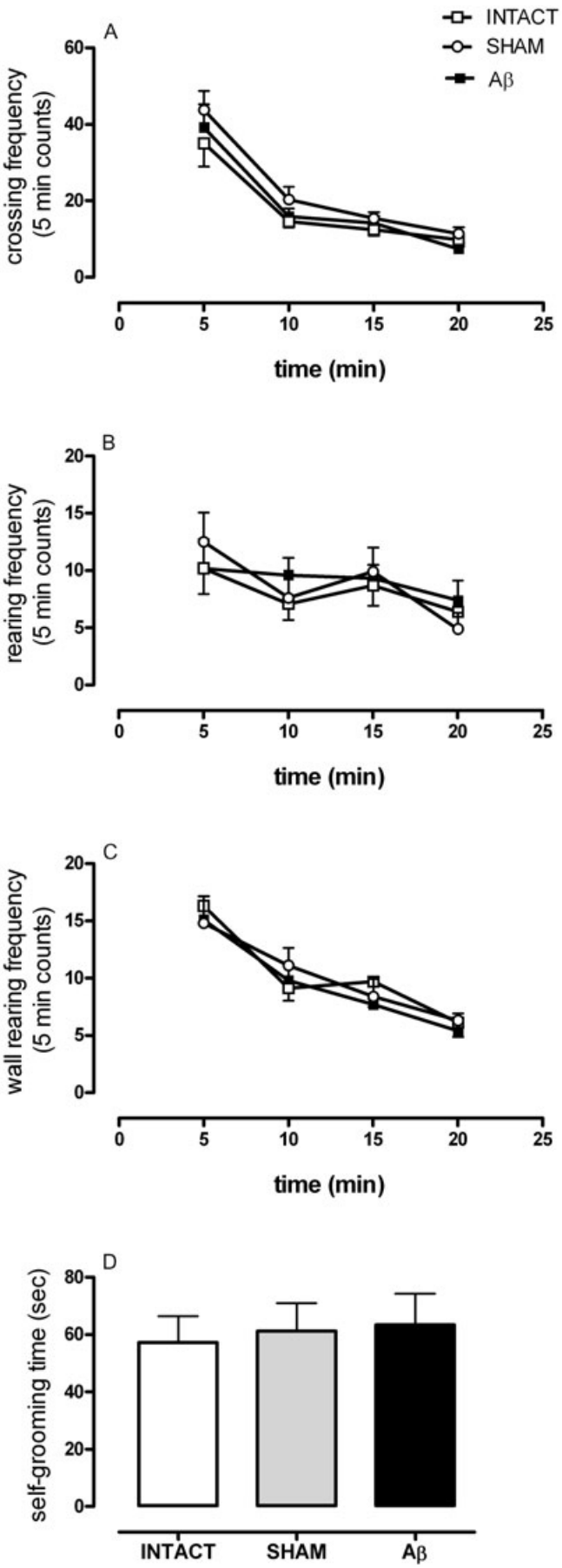


**Figure 2** (A) Crossing (B) rearing and (C) wall rearing frequency, and (D) self-grooming time, in the open field test in male Wistar rats 7 days after administration of Equithesin (3.6 mL·kg$^{-1}$ i.p.; INTACT), water (5 μL i.c.v.; SHAM) and A$\beta_{1\text{-}42}$ (4 μM, 5 μL i.c.v.; Aβ). Data are expressed as mean ± SEM of total frequency counts in 5 min and total time in s ($n$ = 10 per group).

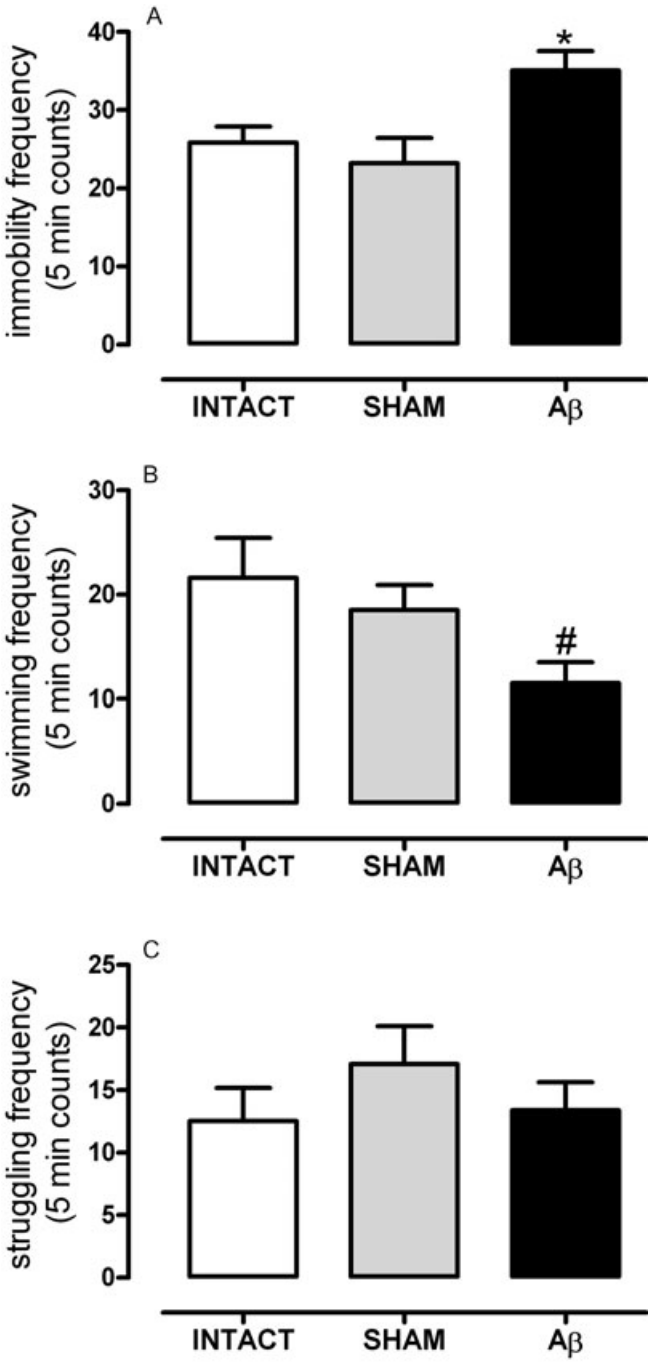


**Figure 3** (A) Immobility (B) swimming and (C) struggling frequency in the forced swimming test in male Wistar rats 7 days after administration of Equithesin (3.6 mL·kg$^{-1}$ i.p.; INTACT), water (5 μL i.c.v.; SHAM) and A$\beta_{1\text{-}42}$ (4 μM, 5 μL i.c.v.; Aβ). Data are expressed as mean ± SEM ($n$ = 10 per group). (One-way ANOVA followed by Newman-Keuls multiple comparison's test, *$P$ < 0.01, #$P$ < 0.05 vs. both intact and sham).

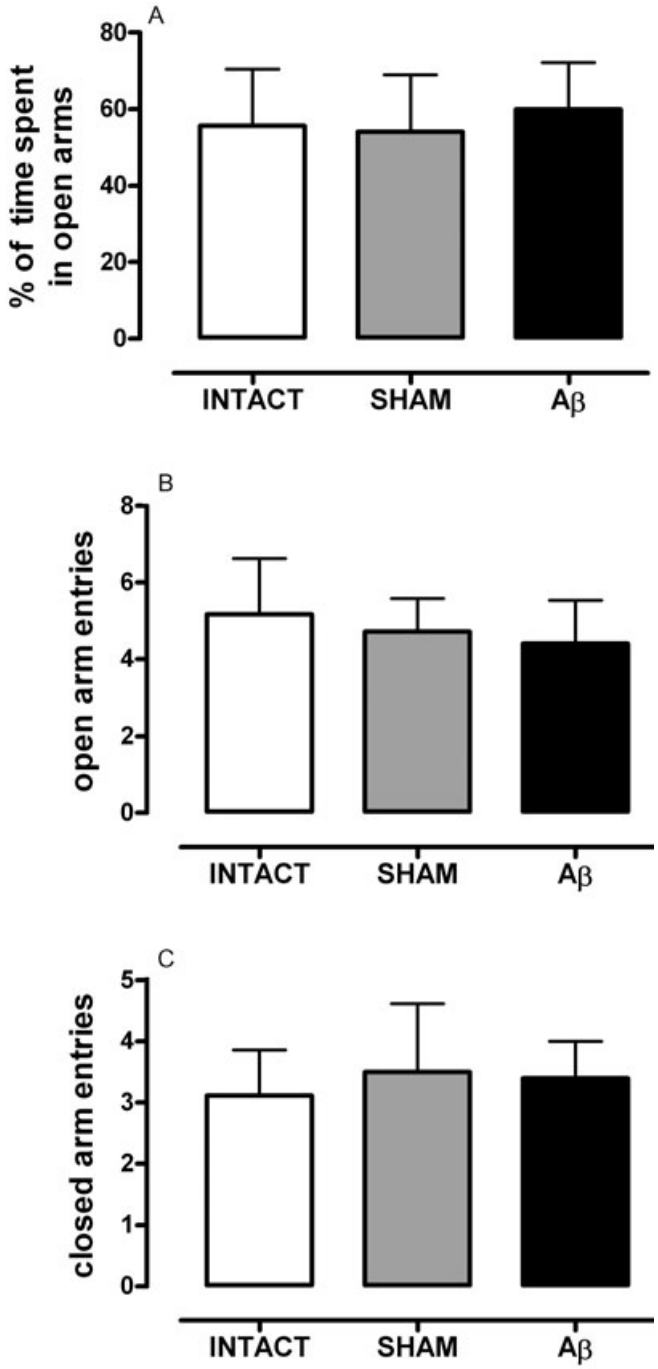


**Figure 4** (A) Amount of time (% of total time) spent in open arm (B) number of open arm entries and (C) number of closed arm entries in the elevated plus-maze test in male Wistar rats 7 days after administration of Equithesin (3.6 mL·kg$^{-1}$ i.p.; INTACT), water (5 μL i.c.v.; SHAM) and $A\beta_{1\text{-}42}$ (4 μM, 5 μL i.c.v.; Aβ). Data are expressed as mean ± SEM ($n$ = 10 per group).

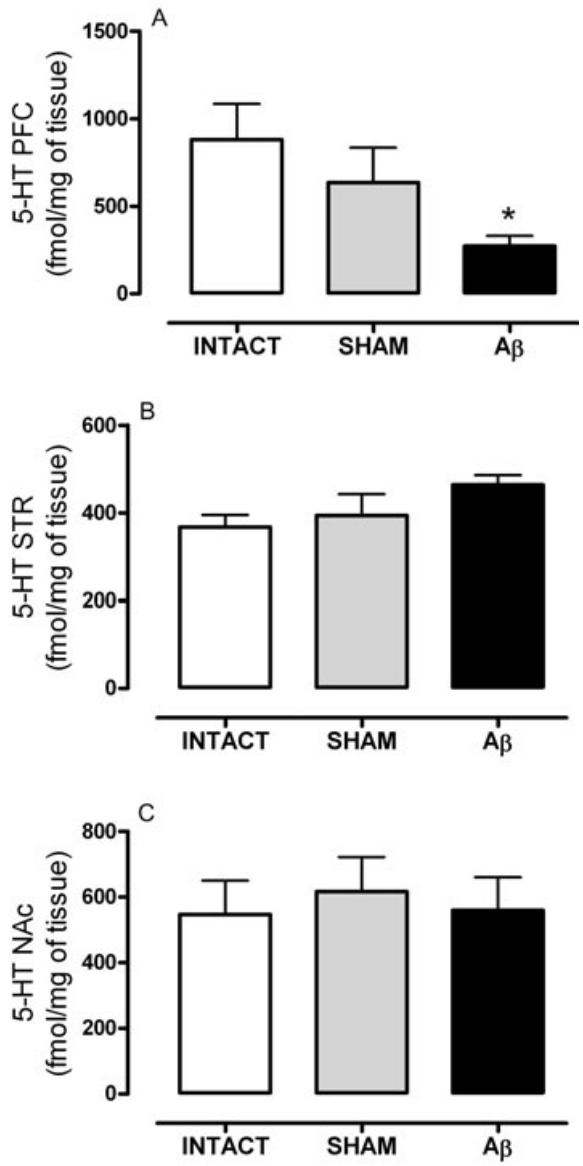


**Figure 5** 5-hydroxytryptamine (5-HT) levels in (A) PFC (B) STR and (C) NAc of male Wistar rats 7 days after administration of Equithesin (3.6 mL·kg$^{-1}$ i.p.; INTACT), water (5 μL i.c.v.; SHAM) and $A\beta_{1\text{-}42}$ (4 μM, 5 μL i.c.v.; Aβ). Data are expressed as mean ± SEM ($n$ = 6 per group). (One-way ANOVA followed by Tukey's test, *$P$ < 0.05 vs. intact or sham).

time exploring both objects in all experimental sessions than controls, suggesting these animals are less inclined to explore but can still recognize a novel object when encountered, in the context of normal levels of general motor activity. A possible interpretation of the reduced explorative behaviour during working memory evaluation is that soluble Aβ, before the appearance of cognitive impairments, might induce motivational deficits. Accordingly, others have reported that non-cognitive symptoms often accompany behavioural changes in AD (Assal and Cummings, 2002; Egashira *et al.*, 2005). Interestingly, we found that soluble Aβ significantly affected rat behaviour when they were placed in a cylinder of water, during the FST, a method for inducing a behavioural state resembling depression in rats by exposing them to a mildly aversive situation from which there is no possibility of escape (Porsolt *et al.*, 1977). Aβ-treated rats exhibited a marked increase in forced swimming test-induced immobility time compared with controls, reflecting a state of behavioural despair or hopelessness. Although obtained in a different animal model, our results are in line with those from studies reporting that mice over-expressing APPswe/PS1, at an age

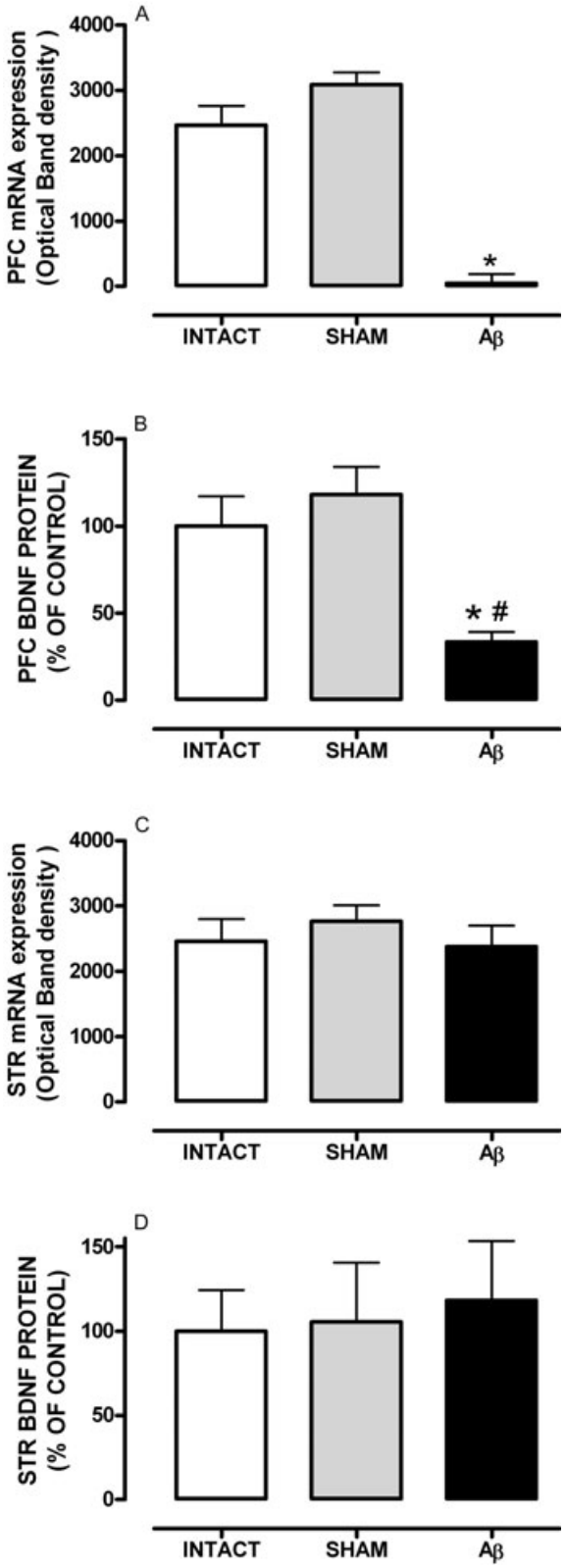


**Figure 6** Brain-derived neurotrophic factor (BDNF) expression of mRNA in (A) PFC and (C) STR, and protein in (B) PFC and (D) STR of male Wistar rats 7 days after administration of Equithesin (3.6 mL·kg$^{-1}$ i.p.; INTACT), water (5 μL i.c.v.; SHAM) and A$\beta_{1\text{-}42}$ (4 μM, 5 μL i.c.v.; Aβ). Data are expressed as mean ± SEM ($n = 6$ per group). (One-way ANOVA followed by Tukey's test, *$P < 0.01$ for mRNA vs. intact and sham; *$P < 0.01$ vs. intact and #$P < 0.05$ vs. sham for protein).

characterized by high levels of soluble Aβ (Marutle *et al.*, 2002), showed an increased duration of immobility in FST (Filali *et al.*, 2009). Nevertheless, in our experimental conditions, a possible anxiogenic effect of soluble Aβ is doubtful, as it had no effect in the elevated plus-maze test, a behavioural model of anxiety-like behaviour (Pellow *et al.*, 1985; Pellow and File, 1986). In addition, under the appropriate conditions, behavioural reactivity in the open field test can be used as an index of increased anxiety (Britton and Britton, 1981; Choleris *et al.*, 2001), because rats engage in repetitive grooming in response to anxiogenic situation (Spruijt *et al.*, 1988). Again, in this test, no differences were revealed between Aβ-treated and control animals confirming a lack of anxiety-like behaviour. Therefore, the enhanced immobility time of Aβ-treated animals is unlikely to have been caused by a deficit in motor function or a change in their anxiety level.

The mechanism by which soluble Aβ peptide induces depressive-like behaviour is not yet clear, but the present results raise the possibility that the modulation of 5-hydroxytryptaminergic neurotransmission is involved. Soluble Aβ-treated rats showed a selective reduction in swimming, and not struggling, behaviour in the FST. Accordingly, selective 5-HT reuptake inhibitors share the common feature of selectively increase swimming, but not struggling, behaviour, whereas selective noradrenaline reuptake inhibitors and tricyclic antidepressants preferentially increase struggling, but not swimming behaviour (Detke *et al.*, 1995; Cryan *et al.*, 2005).

To assess directly whether soluble Aβ inhibits 5-hydroxytryptaminergic neurotransmission as the above data suggest, we measured 5-HT levels in several brain areas. As expected, we found that 5-HT content was selectively reduced in the PFC of Aβ-treated animals, but not in the STR or NAc. These results are supported by demonstrations that impairments of the 5-hydroxytryptaminergic system are present in the very early stages of AD (Versijpt *et al.*, 2003; Egashira *et al.*, 2005; Kepe *et al.*, 2006). In this regard, it is well known that impaired 5-hydroxytryptaminergic neurotransmission in the prefrontal area is central to depressive disorders (Krishnan and Nestler, 2008), but could also have an important role in the pathogenesis of several neurodegenerative diseases (Mattson *et al.*, 2004; Egashira *et al.*, 2005). In particular, there is substantial *post-mortem* and clinical evidence of disruptions of the 5-hydroxytryptaminergic system in AD (Morgan *et al.*, 1987; Lanctot *et al.*, 2001). Furthermore, the risk of developing AD is higher in individuals with a history of depression (Kessing and Andersen, 2004). This area needs to be evaluated further in molecular studies of 5-hydroxytryptaminergic function and by investigating the responsiveness of soluble Aβ-treated animals to antidepressant-like activity of selective 5-HT reuptake inhibitors.

The Aβ-induced changes may result in dysfunction in multiple neurotransmitter systems and their associated interactions. Indeed, our and other previous studies have shown

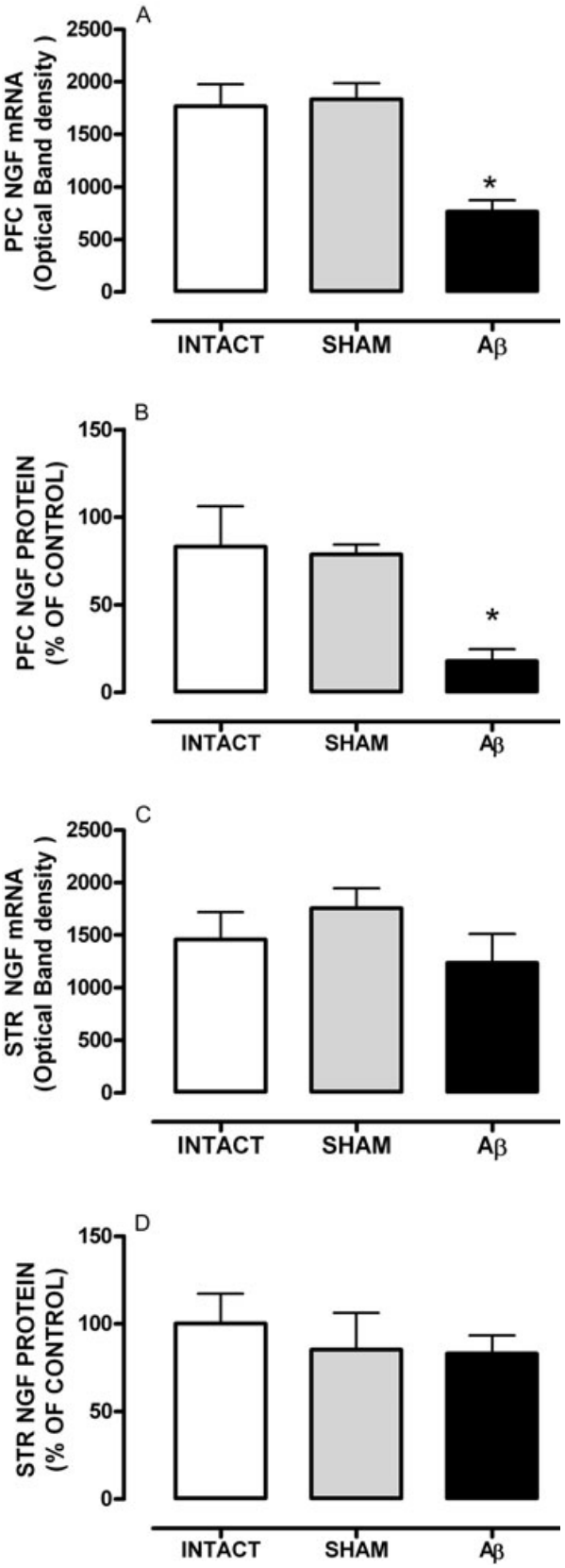


**Figure 7** Nerve growth factor (NGF) expression of mRNA in (A) PFC and (C) STR, and protein in (B) PFC and (D) STR of male Wistar rats 7 days after administration of Equithesin (3.6 mL·kg$^{-1}$ i.p.; INTACT), water (5 μL i.c.v.; SHAM) and $A\beta_{1\text{-}42}$ (4 μM, 5 μL i.c.v.; Aβ). Data are expressed as mean ± SEM ($n$ = 6 per group). (One-way ANOVA followed by Tukey's test, *$P$ < 0.01 vs. intact and sham for mRNA; *$P$ < 0.05 vs. intact and sham for protein).

deficits in dopaminergic system in soluble Aβ-treated rats both in PFC (Trabace *et al.*, 2007) and in NAc (Preda *et al.*, 2008). Of note, functional interactions between dopaminergic and 5-hydroxytryptaminergic neuronal systems in the rat PFC have been observed. In particular, dopamine release was facilitated by fluoxetine, a drug which increases 5-HT concentrations, administration (Matsumoto *et al.*, 1999). Basic science research has shown that 5-HT receptors modulate dopaminergic function (Alex and Pehek, 2007). Therefore, it is conceivable that the neuromodulatory action of soluble Aβ on both the 5-hydroxytryptaminergic and dopaminergic system in PFC might profoundly disrupt the functioning of this area, potentially leading to impairment of mood control.

Another important factor that undergoes significant changes in AD is represented by BDNF (Lanctot *et al.*, 2001). Many reports have documented evidence of decreased expression of BDNF in neurological diseases (Murer *et al.*, 2001; Lang *et al.*, 2004). Moreover, relative levels of BDNF mRNA and proteins are decreased in frontal (Ferrer *et al.*, 1999), temporal (Connor *et al.*, 1997), parietal (Garzon *et al.*, 2002; Michalski and Fahnestock, 2003) and entorhinal cortex (Narisawa-saito *et al.*, 1996) in severe AD.

To determine whether the observed BDNF decrease is an early event during the progression of cognitive decline in AD, we measured the BDNF expression of either its mRNA or protein in the PFC, STR and NAc after an acute injection of soluble Aβ, and observed a selective reduction of both in the PFC of Aβ-injected rats, but not in the STR.

These data on the Aβ-induced decrease in BDNF and 5-HT content in PFC are supported by previous observations, in which exogenous delivery of BDNF promoted the function and sprouting of 5-hydroxytryptaminergic neurons in adult rat brains (Mamounas *et al.*, 1995), and BDNF-deficient mice were also deficient in 5-hydroxytryptaminergic innervation (Lyons *et al.*, 1999). On the other hand, neurotrophins are known to regulate synaptic plasticity, neurogenesis and neuronal survival in the adult brain. These two signals co-regulate each other such that 5-HT stimulates the expression of BDNF, and BDNF enhances the growth and survival of 5-HT neurons (Mamounas *et al.*, 1995).

In addition, the content of NGF in the brain has been shown to change during the time course of neurodegeneration in AD. *Post mortem* studies have indicated a lack of NGF action in early stages of AD, whereas NGF concentrations were found to be enhanced in brains of patients with severe AD, thought to be partly due to the pathologically altered axonal transport of NGF in neurons (Schulte-Herbruggen *et al.*, 2008). In the present study, we found a reduction in the expression of NGF in PFC of soluble Aβ-injected animals. No alterations were observed in the STR. In accord with previous

published data (Conner *et al*., 1997), mRNA neurotrophin levels in the NAc were below the limit of detection in the present study, but no differences were observed in either the BDNF or NGF protein content.

Our findings support the concept that Aβ has detrimental effects in its soluble form even before plaque formation and before the occurrence of neurodegeneration (Moechars *et al*., 1999). Previously, using Hoechst immunocytochemistry, we showed that no gross signs of neurodegeneration occur within the area of Aβ diffusion in the periventricular parenchyma (site of injection) and in the PFC (Trabace *et al*., 2007). However, the possibility that Aβ treatment induces more subtle signs of toxicity, such as synaptic degeneration and neurite retraction, cannot be exclude from our present results and future studies are warranted in this regard. The actions of soluble Aβ on the central nervous system are complex, and much more detailed assessments of these phenomena, accounting for length of exposure, route of administration and experimental periods, are clearly needed.

The findings from the present study differ in some important aspects from previous data on the role of soluble Aβ in mediating behavioural, neurochemical and biochemical alterations. Indeed, the novelty of this study is apparent from the observed acute effect of Aβ in its soluble form and, by showing that soluble $A\beta_{1\text{-}42}$ might have pro-depressive properties, our data provide some novel outcomes that contribute to research on the non-cognitive symptoms of AD.

To the best of our knowledge, this is the first report showing that soluble Aβ can selectively inhibit the expression of BDNF and NGF, and can selectively reduce 5-HT content in the PFC, while inducing a state of despair in healthy adult rats. In addition, motor function, working memory and anxiety-related emotional response were normal. We interpret these results to signify that the soluble Aβ-induced impairments might be considered as sensitive markers of an early dysfunction observed in adult animals without neuropathological plaques, thus suggesting that these changes might be risk factors for the expression of neuropsychiatric symptoms in AD.

In conclusion, the findings described here suggest that soluble Aβ represents a critical player in producing functional and biochemical deficits in rats showing a depressive-like, but not an anxiogenic-like, phenotype. Such an approach should help to provide a fruitful basis for questioning not only the mechanisms underlying the effects of soluble Aβ, but also possible targets for therapeutic intervention very early in the disease process.

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## Conflict of interest

The authors declare no conflict of interest.

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