Elevation of BACE in an Aβ rat model of Alzheimer’s disease: exacerbation by chronic stress and prevention by nicotine

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Abstract

In Alzheimer’s disease (AD), progressive accumulation of β-amyloid (Aβ) peptides impairs nicotinic acetylcholine receptor (nAChR) function by a mechanism that may involve α7 and αβ2–nAChR subtypes. Additionally, the beta-site amyloid precursor protein (APP)-cleaving enzyme (BACE), the rate-limiting enzyme in the pathogenic Aβ production pathway, is expressed at high levels in hippocampal and cortical regions of AD brains. We measured hippocampal area CA1 protein levels of BACE and α7 and αβ2–nAChR subunits using an Aβ rat model of AD (14-d osmotic pump i.c.v. infusion of 300 pmol/d Aβ peptides) in the presence and absence of chronic stress and/or chronic nicotine treatment. There was a significant increase in the levels of BACE in Aβ-infused rats, which were markedly intensified by chronic (4–6 wk) stress, but were normalized in Aβ rats chronically treated with nicotine (1 mg/kg b.i.d.). The levels of the three subunits α7, α6 and β2 were significantly decreased in Aβ rats, but these were also normalized in Aβ rats chronically treated with nicotine. Chronic stress did not further aggravate the reduction of nAChRs in Aβ-infused rats. The increased BACE levels and decreased nAChR levels, which are established hallmarks of AD, provide additional support for the validity of the Aβ i.c.v.-infused rat as a model of AD.

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Introduction

Dysfunctional cholinergic mechanisms are common in dementia disorders, including Alzheimer’s disease (AD) (Kasa et al. 1997; Perry et al. 2000). The brains of AD patients exhibit marked losses of nicotinic acetylcholine receptors (nAChRs), particularly α7 and αβ2–nAChR subtypes (Lahiri et al. 2002; Mattson, 2004; Utsuki et al. 2002) of pre-synaptic terminals in neocortical and hippocampal regions (Sze et al. 1997; Terry et al. 1991), which are correlated with progressive cognitive decline. Immunohistochemical, biochemical, and pharmacological data suggest that the high-affinity binding of Aβ1–42 to α7 and αβ2–nAChRs plays a critical role in AD pathogenesis (Wang et al. 2000a, b). It is suggested that chronic stimulation of α7-nAChRs by Aβ1–42 causes: (1) disruption of ERK2-MAPK signalling (Dineley et al. 2002); (2) internalization and intracellular accumulation of Aβ1–42 (Nagele et al. 2002); (3) modulation of GABAergic signalling (Alkondon et al. 2000), and/or (4) excessive glutamate receptor stimulation (Parpura-Gill et al. 1997). Experiments using exogenous Aβ administration, transgenic mice, and gene-targeting mouse models demonstrate correlations between excessive Aβ accumulation, impaired nAChR function (Mattson, 2004), and deficits in learning, memory, and long-term potentiation (LTP) (Chen et al. 2000; Freir et al. 2001). Collectively, these findings suggest that Aβ disrupts memory and LTP by impairing nAChR function.

Whereas nicotine up-regulates nAChRs and enhances memory (Levin & Rezvani, 2000; Mugnaini et al. 2002; Parker et al. 2004), stress and/or stress hormones, down-regulate nAChRs (Pauly & Collins, 1993; Takita et al. 1999) and impair memory (Aleisa...
et al. 2006b; Alzoubi et al. 2009; Gerges et al. 2004b; Park et al. 2001; Srivareerat et al. 2009a; Yun et al. 2010). Chronic nicotine treatment prevents stress-induced down-regulation of central nAChRs (Takita et al. 1999), which suggests a mechanism by which nicotine prevents stress-induced impairment of memory and LTP. Furthermore, that stress-induced atrophy of hippocampal neurons reversibly impairs cognitive function (McEwen et al. 1997), suggests chronic nicotine treatment may reduce the impact of excitatory amino acids and glucocorticoids, and subsequently prevent permanent damage and cognitive decline.

Gene-targeting experiments and transgenic animal studies have consistently demonstrated impairment of learning, memory, and hippocampal LTP following altered gene expression of selected protein molecules (Bach et al. 1995; Molinari et al. 1996; Son et al. 1996). In AD, progressive accumulation of Aβ peptides in the brain impairs spatial learning, memory and LTP (Alkadhi et al. in press; Pham et al. 2010; Srivareerat et al. 2009a,b).

BACE is a membrane-bound aspartyl protease that is ubiquitously expressed in adult peripheral tissues and various subregions in the brain. Aβ is derived from amyloid precursor protein (APP) via cleavage by two proteases, β- and γ-secretase (Vassar et al. 2009). The β-secretase has been identified as a novel aspartic protease named BACE1 (beta-site APP-cleaving enzyme 1 that initiates Aβ peptide formation (Vassar et al. 2009). BACE is expressed at high levels in hippocampal and cortical regions of AD brains (Fukumoto et al. 2002, Vassar et al. 1999). In APP transgenic mice, BACE overexpression increases Aβ formation (Bodendorf et al. 2002), whereas BACE knockout mice produce little or no Aβ peptide (Luo et al. 2001; Roberds et al. 2001), indicating that BACE expression plays a critical role in Aβ peptide biosynthesis. In this study, the effect of chronic stress and/or nicotine on the hippocampal levels of BACE and α2 and αβ2 nAChRs were evaluated in an Aβ rat model of AD. Results of this study provide additional support for the validity of this model of AD, and further characterize the interactive effects of stress and nicotine after Aβ infusion.

Methods

We used adult male Wistar rats, weighing 200–225 g at the beginning of the experiment (Charles River Laboratories). Rats were housed six per cage in a temperature-controlled room (25°C) with food and water available ad libitum, under a 12-h light/dark cycle (lights on 07:00 hours). Experiments were performed between 09:00 and 17:00 hours. All animal procedures were performed in accordance with the National Research Council’s Guide for The Care and Use of Laboratory Animals and with approval of The University of Houston IACUC.

Treatments

Seven experimental groups were used in the present study: control, stress, nicotine, Aβ, nicotine/Aβ, stress/Aβ and nicotine/stress/Aβ. All groups have been through a 7-d acclimation period. Immediately after the acclimation period, the stress, stress/Aβ and nicotine/stress/Aβ groups were subjected to 6 wk of chronic psychosocial stress. The nicotine, nicotine/Aβ, and nicotine/stress/Aβ groups were subjected to 6 wk of nicotine treatment. [−]-Nicotine (Sigma Aldrich, USA) was dissolved in saline, and subcutaneously injected twice daily at a dose of 1 mg/kg. Saline vehicle was administered to control, stress, Aβ, and stress/Aβ animals twice per day for 6 wk. Four weeks after the start of chronic stress and chronic nicotine treatment, all animal groups were implanted with 14-d osmotic pumps. The Aβ, stress/Aβ and nicotine/stress/Aβ animals were infused with Aβ peptides (osmotic pump, i.c.v.) for 2 wk during weeks 5 and 6 of the stress period. The control group was infused with an inactive reverse peptide (Alkadhi et al. 2010).

Chronic psychosocial stress

Chronic stress was induced using an intruder psychosocial stress model as described previously (Aleisa et al. 2006b; Alzoubi et al. 2009; Gerges et al. 2001). Briefly, rats of each stress group were allowed to remain with the same cage-mates for at least 1 wk to allow establishment of a social hierarchy within the group. Then, psychosocial stress was induced by daily random exchange of two rats from one cage into another for a period of 6 wk. The rat intruder model of psychosocial stress, used in this study, mimics psychosocial stress in humans, as it has been shown to increase plasma levels of corticosterone (Gerges et al. 2001) and to significantly elevate blood pressure (Alkadhi et al. 2005).

Control and Aβ groups were kept with the same cage-mates, and thus, remained unstressed.

Osmotic minipump implantation

Four weeks after the start of psychosocial stress, Aβ treatment-designated groups were implanted with 14-d Alzet osmotic minipumps as described previously (Alkadhi et al. 2010, in press; Nitta et al. 1994; Srivareerat et al. 2009a,b; Tran et al. 2010a,b). Human Aβ1–40 and Aβ1–42 peptides (AnaSpec Inc.,
USA) were dissolved in a solution containing 64.9% sterile distilled water; 35% acetonitrile and 0.1% trifluoroacetic acid (TFA) to prevent aggregation of Aβ peptides in the osmotic minipump. One day prior to implantation, osmotic pumps were primed as follows: pumps were filled with a mixture of 50% Aβ<sub>1-40</sub> and 50% Aβ<sub>1-42</sub> attached by a tubing to an L-shaped cannula, and left overnight in isotonic saline solution, at 37 °C. Rats were anaesthetized with an i.p. injection of a mixture of ketamine (100 mg/kg), xylazine (2.5 mg/kg), and acepromazine (2.5 mg/kg). Once anaesthetized, the skin over the implantation site was shaved, wiped with alcohol, and the rat was placed in a stereotaxic frame. Starting slightly behind the eyes, a 2.5 cm midline sagittal incision was made to expose the skull. A hole was drilled into the skull (A −0.3, L 1.1, V 3.6), above the left central ventricle, according to the atlas of Paxinos and Watson (Paxinos & Watson, 1986). The cannula was placed into the hole and held in place with dental cement. Then, the pump, which was attached to the catheter leading to the brain cannula, was inserted into a subcutaneous pocket in the back of the rat. The scalp wound was closed with wound clips, and tincture of iodine, followed by antibiotic ointment, was applied to the wound site to prevent bacterial infection. The osmotic pump continuously infused Aβ peptides for 2 wk. The stress/ Aβ, stress/Aβ/nicotine and Aβ groups received 300 pmol/d 1/1 mixture of human Aβ<sub>1-40</sub>/Aβ<sub>1-42</sub> while the control group received 300 pmol/d Aβ<sub>1-42</sub>-an inactive reverse peptide. The volume of infusion was 0.25 μl/h. Aβ<sub>1-40</sub> was used because of its high propensity for aggregation (Chiti & Dobson, 2006; Stravalaci et al, 2011), and the 1/1 mixture of Aβ<sub>1-40</sub>/Aβ<sub>1-42</sub> has recently been shown to produce the highest level of protofibrils, compared to several other mixture ratios (Wiberg et al, 2010), which undergo subsequent transition into insoluble extracellular plaques, characteristic of AD (Bitan et al. 2003; Goldsbury et al. 2005). Psychosocial stress continued throughout the duration of the experiment.

### Immunoblot analysis

Equal volumes of protein samples (10 μg total protein) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) on 12.5% Tris-glycine gels and electroblotted onto polyvinyl difluoride (PVDF) membranes (Immobilon, USA) as described previously (e.g. Gerges et al. 2004a, 2005; Norman et al. 2000). The intensity of the immunoreactive band was measured by densitometry using Fluorchem FC8800 software and expressed as a ratio to that of the glyceraldehyde phosphodehydrogenase (GAPDH, a loading control). Immunoblot intensities of molecules were expressed as the ratio to GAPDH intensities.

The following antibodies were used in this study: rabbit anti-α5 (1:500, Santa Cruz Biotechnology Inc., USA); rabbit anti-α4 (1:300, Santa Cruz Biotechnology); rabbit anti-β1 (1:300, Santa Cruz Biotechnology); rabbit anti-BACE (1:750, Abcam, USA) and mouse anti-GAPDH antibody (1:5000, RDI, Research Diagnostics Inc., USA).

For immunoblotting of Aβ<sub>1-40</sub>, 150 μl of the homogenate was subjected to high-speed centrifugation at 100,000 g for 1 h at 4 °C. The supernatant was used to determine protein concentration by BCA assay (Pierce Chemical). Tissue homogenates were diluted with 4 × Laemmli buffer [50 mM Tris–HCl (pH 6.8), 10% glycerol, 2% SDS, 0.1 mg/ml Bromophenol Blue] and boiled for 3 min. Equal amounts of protein samples (10 μg) were separated by SDS–PAGE on 10–20% Tris-tricine gels and electroblotted onto PVDF membranes (Immobilon) and subsequently probed with rabbit polyclonal anti-Aβ<sub>1-40</sub> antibody (1:750 dilution, AnaSpec).

### Statistical analysis

All statistical analyses were performed using three-way ANOVA, followed by Tukey’s post-hoc test.
At the control animals. Thus, chronic nicotine treatment nor-

Fig. 1. Effect of stress on the basal levels of Aβ1–40 in hippocampal area CA1. Rats were stressed for 6 wk and during weeks 5 and 6 they were infused (osmotic minipump, i.c.v.) with Aβ peptides. Aβ-infused stressed rats were treated with nicotine for 6 wk (Nic/Str/Aβ group; 1 mg/kg s.c. twice daily). Nic, Aβ and Nic/Aβ groups (from Srivareerat et al. 2009a) are shown for comparison. Results are expressed as mean ± S.E.M. with 5–7 rats/group ( * p < 0.05 compared to control group; + p < 0.05 compared to all other groups). Insets are representative immunoblots.

Fig. 2. Basal levels of beta-amyloid precursor protein-cleaving enzyme (BACE) in area CA1. Chronic stress significantly increased basal levels of BACE in Aβ-infused rats. Nicotine prevented the increase in basal levels of BACE in both Aβ and Str/Aβ rats. Nic, Aβ and Nic/Aβ groups (from Srivareerat et al. 2009a) are shown for comparison. Values are mean ± S.E.M. from 6–8 rats; * p < 0.01 compared to all groups; & p < 0.05 compared to control group. Insets are representative immunoblots.

The BACE gene is associated with increased Aβ production (Vassar et al. 1999). We next investigated whether nicotine-induced reduction in the basal levels of Aβ1–40 in Aβ and stress/Aβ rats was correlated with decreased levels of BACE. Analysis of hippocampal CA1 homogenates by Western blot indicated that stress per se had no significant effect on the basal levels of BACE (Fig. 2). However, chronic stress significantly increased BACE levels in stress/Aβ animals, compared to Aβ animals and control animals (Fig. 2). These results strongly suggest that chronic stress potently modulates basal Aβ1–40 levels by a mechanism involving increased BACE protein concentration and consequently favouring production of pathogenic Aβ peptides.

In contrast to prolonged stress, 6 wk of concurrent nicotine treatment prevented the increase in the basal levels of BACE1 in nicotine/Aβ animals, and in nicotine/stress/Aβ animals such that they were not significantly different from those of control rats (Fig. 2).

**Levels of Aβ1–40 peptide**

In hippocampal area CA1 homogenates of stress/Aβ rats, chronic psychosocial stress significantly increased soluble Aβ1–40 levels compared to Aβ animals and control animals (Fig. 1) (control: 1.19 ± 0.04; Aβ: 1.53 ± 0.05; stress/Aβ: 1.67 ± 0.04; n = 9 rats/group). By contrast, chronic nicotine treatment significantly (p < 0.05) reduced the levels of Aβ1–40 in nicotine/Aβ and nicotine/stress/Aβ animals, compared to saline-treated Aβ and stress/Aβ animals (nicotine/Aβ: 1.15 ± 0.09; nicotine/stress/Aβ: 1.19 ± 0.09; n = 9–10 rats/group) (Fig. 1). The levels of Aβ1–40 detected in nicotine-treated animals were not significantly (p < 0.05) different from the corresponding levels in control animals. Thus, chronic nicotine treatment normalized basal levels of Aβ1–40 in Aβ and stress/Aβ rats, such that they are statistically equivalent to those of control animals.

**Levels of BACE**

At the β-secretase site, cleavage of APP by BACE releases Aβ. It has been reported that overexpression of the BACE gene is associated with increased Aβ production (Vassar et al. 1999). We next investigated whether nicotine-induced reduction in the basal levels of Aβ1–40 in Aβ and stress/Aβ rats was correlated with decreased levels of BACE. Analysis of hippocampal CA1 homogenates by Western blot indicated that stress per se had no significant effect on the basal levels of BACE (Fig. 2). However, chronic stress significantly increased BACE levels in stress/Aβ animals, compared to Aβ animals and control animals (Fig. 2). These results strongly suggest that chronic stress potently modulates basal Aβ1–40 levels by a mechanism involving increased BACE protein concentration and consequently favouring production of pathogenic Aβ peptides.

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**nAChR subtypes**

Inasmuch as decreased nAChR activation is correlated with AD cognitive decline, we investigated protein levels of the predominant neuronal nicotinic receptor subtypes (α7 and α7β2ε nAChR) to determine if chronic nicotine pretreatment could up-regulate the basal levels of nAChR in Aβ and stress/Aβ rats. Consistent
In parallel, significant (p < 0.05) reductions in the immunoreactivity of the subunit levels in Aβ- and stress/Aβ groups compared to control animals (Fig. 4a). In parallel, significant (p < 0.05) reductions in the immunoreactivity of the subunit were observed in Aβ and stress/Aβ groups compared to control animals (Fig. 4b).

Chronic nicotine treatment, by itself, significantly up-regulated basal levels of α7-nAChR subunits in Aβ- and stress/Aβ rat groups. Chronic nicotine treatment up-regulated basal levels of α7 subunit and normalized the subunit levels in Aβ-treated groups. Nic, Aβ, and Nic/Aβ groups (from Srivareerat et al. 2009a) are shown for comparison. Data are means ± S.E.M. of 5–7 rats/group; * p < 0.001 indicates significant difference from all other groups; # p < 0.01 indicates significant difference from control group. Insets are representative immunoblots.

Discussion

We have previously reported that stress exacerbates Aβ-induced impairment of LTP and learning and memory, and that chronic nicotine treatment prevents these impairments in both Aβ and stress/Aβ animals with the observations of decreased numbers of [3H]nicotine, [3H]acetylcholine, and [125I]α-bungarotoxin binding sites in AD brains (Hellstrom-Lindahl et al. 1999), protein levels of α7-nAChR were significantly (p < 0.05) reduced in Aβ and stress/Aβ rats, compared to controls (Fig. 3). Additionally, immunoblot analysis revealed significant reductions of α7-nAChR protein in Aβ and stress/Aβ animals (Fig. 4a). In parallel, significant (p < 0.05) reductions in the immunoreactivity of the β7 subunit were observed in Aβ and stress/Aβ groups compared to control animals (Fig. 4b).

Chronic nicotine treatment, by itself, significantly up-regulated basal levels of α7, α6, and β2-nAChR subtypes compared to saline-treated controls. Furthermore, basal levels of α7 and α6 were normal in nicotine/Aβ and nicotine/stress/Aβ rats such that they were not significantly different from the levels in control rats (Fig. 3, 4a). Moreover, protein levels of the β2-subunits were significantly higher than those of the control rats in nicotine/Aβ and nicotine/stress/Aβ rats (Fig. 4b).

Fig. 3. Basal protein levels of nicotinic acetylcholine receptor (n-AChR) α7 subunits in the cell membrane of the hippocampal CA1 neurons of rats. Chronic stress up-regulated basal levels of α7 subunits and normalized the subunit levels in Aβ-treated groups. Nic, Aβ, and Nic/Aβ groups (from Srivareerat et al. 2009a) are shown for comparison. Data are means ± S.E.M. of 5–7 rats/group; * p < 0.001 indicates significant difference from all other groups; # p < 0.01 indicates significant difference from control group. Insets are representative immunoblots.

Fig. 4. Basal protein levels of α7- and β2-nAChR subunits in the cell membrane of the hippocampal CA1 neurons of rats. (a) α7-nAChR subunit is markedly decreased in Aβ and stress/Aβ rat groups. Chronic nicotine treatment up-regulated basal levels of α7 subunit and normalized the subunit levels in Aβ-treated groups. Data are means ± S.E.M. of 5–7 rats/group. * p < 0.001 indicates significant difference from all groups. (b) Basal levels of β2-nAChR subunit in area CA1 were decreased in Aβ and stress/Aβ groups, but were markedly increased in all nicotine-treated animals, even in the presence of Aβ and stress. Nic, Aβ, and Nic/Aβ groups (from Srivareerat et al. 2009b) are shown for comparison. * p < 0.05–0.01 indicates significant difference compared to control animals. Data are presented as means ± S.E.M. of 5–7 rats/group. Insets are representative immunoblots.

(Alkadhi et al. 2010, in press; Srivareerat et al. 2009a, b; Tran et al. 2010a, b). The present study shows that stress increases Aβ5-40 and aggravates the elevation of BACE levels and the reduction of α7- and α7β2-nAChR levels in Aβ-treated rats. Nicotine treatment, on the other hand, prevents the Aβ- and stress/Aβ-induced changes in the levels of these molecules. The elevated levels of BACE and the reduced levels of nAChRs, which are hallmarks of AD, provide further support for the validity of our i.c.v. Aβ infusion as a model for AD.
The accumulation of microglial cells in the amyloid plaques is a hallmark of the innate response to Aβ deposits (Hartlage-Rubsamen et al. 2003; McGeer & McGeer, 2003). That activated microglia are immunopositive for inflammatory cytokines, e.g. interleukin-1 (IL-1), IL-6, APOE, and complement proteins, suggests that Aβ triggers neuroinflammatory responses, which induce neuronal death and AD progression (Del Bo et al. 1995; Mark et al. 1995). In fact, the BACE promoter contains binding sites for several important transcription factors, including nuclear factor-κB (NF-κB) (Sambamurti et al. 2004). Furthermore, since NF-κB-like consensus sequences are also located in the promoter region of the APOE gene (Bales et al. 1999), several investigators have postulated that altered NF-κB-directed gene expression may contribute to the neurodegeneration and inflammatory responses that occur in AD. For example, in rat cortical neurons, constitutive NF-κB activity decreased in a concentration- and time-dependent fashion, following Aβ exposure; conversely, exposure of rat astrocytes to Aβ results in activation of NF-κB, which is also concentration- and time-dependent (Bales et al. 1998). Furthermore, it has been reported that cytoplasmic IκB protein levels are significantly decreased, and BACE levels are concurrently increased, in guinea-pig astrocytic cells, following 48 h exposure to Aβ peptides (Bourne et al. 2007). Together, the data suggest that NF-κB mediates Aβ-induced neurotoxicity and/or astrocyte activation by increasing basal protein and/or activity levels of BACE. In accordance with that, results of the current study show elevation of BACE levels in the hippocampus of Aβ and stress/Aβ rat groups. Additionally, since stress significantly aggravates Aβ-induced elevation of BACE levels, it is likely that stress exacerbation of Aβ-induced deficient cognition and synaptic plasticity (Alkadhi et al. 2010, in press; Srivareerat et al. 2009a; Tran et al. 2010a,b) is mediated through a BACE-dependent mechanism, which could predispose neurons to Aβ-induced neurotoxicity and neuroinflammation. Nicotine on the other hand, has been shown to normalize the hippocampal levels of BACE, and to prevent Aβ- and stress/Aβ-induced cognitive and synaptic plasticity impairments (Alkadhi et al. 2010, in press; Srivareerat et al. 2009b).

Epidemiological studies reported a beneficial effect of nicotine in certain inflammatory diseases. It has been suggested that nicotine promotes anti-inflammatory effects through activation of α7 (Nizri et al. 2009) and α1β2- nAChRs, by reducing NF-κB activation (Hosur et al. 2009). Furthermore, it has been reported that nicotine-induced production of the anti-apoptotic protein, Bcl-2, prevents the Aβ-induced apoptosis of PC12 cells (Marrero & Bencherif, 2009). Thus, in addition to the variety of effects including anti-oxidant properties in the hippocampus, which can contribute to its neuroprotective effect (Liu & Zhao, 2004), and stimulation of liver-metabolizing enzymes (Yildiz, 2004), nicotine also possesses a prominent anti-inflammatory effect. It is recognized that nicotine triggers the ‘cholinergic anti-inflammatory’ pathway through activation of α7-nAChRs to inhibit inflammatory cytokine production in murine and human macrophages and in several models of inflammatory disease in vivo (Mabley et al. 2009; Rosas-Ballina et al. 2009).

Under our experimental conditions, continuous Aβ-infusion alone, and in combination with chronic stress, decreased protein levels of α7- and α1β2-nAChRs. The deficit of nAChRs may be related to alterations of turnover and synthesis of nAChRs on different levels, e.g. transcription, translation, and post-translational modifications. Infusion of low concentrations of Aβ activates presynaptic α7-nAChRs causing increased Ca2+ influx through both the nAChR and voltage-gated Ca2+ channels (Dougherty et al. 2003), therefore, excessive Ca2+ entry into neurons may be a mechanism of Aβ-induced neurotoxicity (Shimohama & Kihara, 2001). Prolonged activation of nAChRs by Aβ may cause desensitization (without subsequent up-regulation), leading to an irreversible decline of the nAChR population (Chen et al. 2006).

Contrary to our findings, are reports that show activation and up-regulation in nAChRs by Aβ. For example, in Tg2576 animals, α7-nAChRs were temporally up-regulated in the hippocampus at age 4 months, and continued to increase in the dentate gyrus (DG) and area CA1, up to 20 months in the absence of measurable changes in α1β2-nAChRs (Dineley et al. 2001). Furthermore, in Tg2576 mice, increased expression of α7-nAChRs was correlated with deficits in Morris water maze performance. The up-regulation of nAChRs may reflect over-activation of the nicotinic receptor by Aβ at a different stage of AD than that measured in the present study. Thus, nAChR up-regulation may be an initial compensatory response of the cholinergic system to AD neuropathology. Furthermore, it is possible that while the number of nAChRs and cholinergic neurons is preserved or even increased in early AD (as a compensatory mechanism), their function may be altered. Exhaustion of this compensatory mechanism and/or neuronal death may eventually lead to a decrease in nAChRs and other cholinergic markers at the late stages of the disease.
Nicotine influences neuronal activity, synaptic communication, and behaviour through activation of nAChRs. Acute exposure to nicotine activates presynaptic α4β2 and α4-nAChRs of the Schaffer collaterals to increase glutamate release and, thus, increases excitability of the pyramidal cells of area CA1. However, chronic exposure to nicotine in habitual users of tobacco products, induces rapid and persistent loss of nAChR functional activity in the brain (desensitization) (Dani & Heinemann, 1996; Lukas, 1991; Wonnacott, 1990), which may lead to increases in α4β2- and α7-nAChR subtypes (up-regulation) in most brain regions, including the hippocampus (Mugnaini et al. 2002; Parker et al. 2004).

Activation of α7- and α4β2-nAChRs may be responsible for the nicotine-mediated neuroprotection. The α7- and α4β2-nAChRs are down-regulated in response to chronic agonist exposure and up-regulated in response to chronic exposure to antagonist. It is not clear whether the up-regulation of nicotine-induced nAChRs observed under our experimental conditions resulted in an increase or decrease in nicotinic cholinergic function. However, the finding that nAChRs were up-regulated concurrently with significantly improved cognitive ability and LTP may indicate that chronic, low-dose nicotine treatment in Aβ and stress/Aβ rats induced a functional up-regulation of neuronal nAChRs. The nicotine-induced up-regulation of nAChRs may be due to increased receptor synthesis or assembly, increased membrane incorporation from a reserve pool of receptors, or decreased degradation (Peng et al. 1994). In the hippocampus and cortex of APPswe mice, α7-nAChRs exhibit a progressive temporal up-regulation, reaching a 3- to 4-fold increase at age 9 months, followed by a decrease, at 12 months, which may be correlated with an increased Aβ peptide burden competing for α7-nAChR sites (Jones et al. 2006).

Stress may exacerbate AD by a variety of possible mechanisms including: (1) Activation of glucocorticoid (type II) receptors by high levels of corticosteroids during stressful conditions enhances Ca2+ influx and inhibits CA1 pyramidal cell excitability, thus worsening the Aβ-induced disruption of Ca2+ homeostasis (e.g. Conrad et al. 1999). (2) In area CA1 of the hippocampus, chronic stress is known to significantly decrease brain-derived neurotrophic factor (BDNF), which plays a major role in neuronal survival (Zuccato & Cattaneo, 2009). (3) Chronic stress is known to cause a severe reduction in the levels of nerve cell adhesion molecule (NCAM) (Jin et al. 2004), which has been shown to be increased in the brains of AD patients, indicating neurogenesis as a possible defence mechanism (see Sandi, 2004 for review). (4) Stress may alter the processing and production of various AD-related proteins. Exposure to stress or glucocorticoids increases the levels of APP, C99, and BACE, suggesting that stress drives the processing of APP towards the amyloidogenic pathway, which may account for the increased levels of Aβ and the increased amount of plaque formation (Lee et al. 2009) that are also observed with stress (Catania et al. 2009; Green et al. 2006; Srivareerat et al. 2009b).

The solubility profiles of C-terminal Aβ1-40 and Aβ1-42 peptides differ considerably, with Aβ1-40 being more kinetically soluble than Aβ1-42 (Barrow & Zagorski, 1991; Burdick et al. 1992). This suggests that co-incipubation of kinetically soluble, Aβ1-40, with insoluble, Aβ1-42 would seed amyloid plaque formation, in vivo. Thus, 24 h prior to implantation, osmotic pumps were primed with a mixture containing equal concentrations of Aβ1-40 and Aβ1-42, and left overnight at 37 °C, in order to ‘seed’ formation of amyloid deposits, in vivo. Prior to implantation, there was no evidence of flocculation of the peptide during priming, indicating a soluble initial state of the infused peptides. However, since the physiological concentration of CSF and plasma Aβ and peptide structure (monomeric, oligomeric, fibrillar) were not determined, pre- or post-infusion, it is extremely difficult to determine which species and/or structure of Aβ was responsible for the impairment of learning and memory, and synaptic function observed in our study. Additionally, it is of note that learning and memory performance in Aβ animals cannot always be correlated with brain Aβ levels. For example, Kotilinek et al. (2002) observed that administration of BAM-10 antibody (recognizing the N-terminus of Aβ) significantly improved memory in Tg2576 mice, in absence of significant changes in brain Aβ levels. Thus, it is possible that in early stages of AD, memory function may be affected by mechanisms other than Aβ accumulation.

In this study, we only evaluated the levels Aβ1-40, which is the most abundant Aβ species in the brain (e.g. Lewczuk & Willfang, 2008; Spies et al. 2010). The increased BACE levels/activity may explain the Aβ1-40 elevation shown in the current study. Another possibility is that the 2-wk infusion of peptides may have
overwhelmed the normal Aβ catabolic processes thus causing accumulation of endogenous Aβ42.

Collectively, the results of the current study show that stress intensifies Aβ-induced elevation in BACE1, and reduction in α2- and α4β2-nAChR levels, whereas chronic nicotine treatment prevents these changes. The findings of the current study confirm previous results and could help to further characterize the Aβ i.c.v.-infused rat as a model of AD.

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Statement of Interest
None.

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