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Neurobiology of Aging 33 (2012) 1122.e23–1122.e39

NEUROBIOLOGY OF AGING

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# Impaired TrkB receptor signaling contributes to memory impairment in APP/PS1 mice

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Received 19 July 2011; received in revised form 25 October 2011; accepted 4 November 2011

**Abstract**

Brain-derived neurotrophic factor (BDNF) plays an important role in neuronal plasticity, learning, and memory. Levels of BDNF and its main receptor TrkB (TrkB.TK) have been reported to be decreased while the levels of the truncated TrkB (TrkB.T1) are increased in Alzheimer's disease. We show here that incubation with amyloid-$\beta$ increased TrkB.T1 receptor levels and decreased TrkB.TK levels in primary neurons. In vivo, APPswe/PS1dE9 transgenic mice (APdE9) showed an age-dependent relative increase in cortical but not hippocampal TrkB.T1 receptor levels compared with TrkB.TK. To investigate the role of TrkB isoforms in Alzheimer's disease, we crossed AP mice with mice overexpressing the truncated TrkB.T1 receptor (T1) or the full-length TrkB.TK isoform. Overexpression of TrkB.T1 in APdE9 mice exacerbated their spatial memory impairment while the overexpression of TrkB.TK alleviated it. These data suggest that amyloid-$\beta$ changes the ratio between TrkB isoforms in favor of the dominant-negative TrkB.T1 isoform both in vitro and in vivo and supports the role of BDNF signaling through TrkB in the pathophysiology and cognitive deficits of Alzheimer's disease.


*Keywords:* BDNF; Tyrosine kinase receptor; Amyloid; Memory; Hyperactivity

## 1. Introduction

Neuropathologically, Alzheimer's disease (AD) is characterized by amyloid plaques and neurofibrillary tangles, but the most likely proximal cause for the most characteristic symptom of the early stage of the disease, impaired memory for recent events, is loss of synapses (Selkoe, 2002). The molecular mechanisms whereby accumulation of amyloid-$\beta$ into the brain leads to synaptic loss are still incompletely known.

Neurotrophins, in particular brain-derived neurotrophic factor (BDNF) regulate neuronal survival, differentiation, and plasticity by activating the receptor tyrosine kinase TrkB (Huang and Reichardt, 2001). Reduced BDNF signaling

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doi:10.1016/j.neurobiolaging.2011.11.006

through TrkB leads to impaired spatial memory (Minichiello, 2009; Minichiello et al., 1999; Saarelainen et al., 2000b), while overexpression of TrkB enhances memory (Koponen et al., 2004). These observations have led to the suggestion that TrkB signaling might be involved in the pathophysiology of AD (Arancio and Chao, 2007; Castrén and Tanila, 2006; Schindowski et al., 2008; Zuccato and Cattaneo, 2009). BDNF messenger RNA (mRNA) and protein levels as well as protein levels for the full-length TrkB isoform have been found to be reduced in postmortem brain samples of AD patients (Connor and Dragunow, 1998; Connor et al., 1997; Ferrer et al., 1999; Phillips et al., 1991). In contrast, the truncated, dominant-negative isoform of TrkB (TrkB.T1) (Eide et al., 1996; Haapasalo et al., 2001) has been found to be increased (Connor et al., 1996; Ferrer et al., 1999). Importantly, BDNF levels are already reduced at the preclinical stages of the disease (Peng et al., 2005). Taken together, these findings implicate that BDNF signaling is impaired in AD patients. In contrast, studies in amyloid precursor protein (APP) transgenic mice modeling AD have yielded mixed results regarding brain BDNF levels (either mRNA or protein) with 2 lines displaying decreased levels (Peng et al., 2009), 1 line no change (Peng et al., 2009), and 2 lines showing increased levels (Burbach et al., 2004; Schulte-Herbrüggen et al., 2008; Szapacs et al., 2004). BDNF levels were reported to be increased in the APdE9 mouse line used in the present study. We are not aware of any study so far investigating TrkB receptor levels in transgenic AD mouse models.

The APPswe/PS1dE9 double transgenic (APdE9) mouse line is a widely used model of AD. These mice develop amyloid plaques in the cortex and hippocampus starting at 4 months of age (Garcia-Alloza et al., 2006), but memory impairment manifests only between 8 and 12 months of age (Minkeviciene et al., 2008; Savonenko et al., 2005). Thus these mice recapitulate the order of pathological events in AD patients (amyloid plaques develop gradually over years before memory impairment leads to the clinical diagnosis) as revealed by recent positron-emission tomography (PET) imaging studies with amyloid binding a ligand (Aizenstein et al., 2008; Kadir et al., 2012), in contrast to most other APP transgenic mice in which memory impairment usually precedes amyloid plaque formation (Van Dam et al., 2003; Westerman et al., 2002). This mouse line therefore offers an excellent model for studying molecular mechanisms downstream of amyloid plaque formation leading to memory impairment.

We have here investigated the role of TrkB signaling in amyloid-induced neuropathology leading to memory loss in the APdE9 mouse model of AD. We found that increased amyloid-beta (A$\beta$) peptide levels increase the expression of the truncated TrkB.T1 isoform in vitro in cultured neurons and in vivo in the brains of APdE9 mice. To further mimic impaired signaling through TrkB receptors as it occurs in AD, we cross-bred APdE9 mice with TrkB.T1 mice (Saarelainen et al., 2000a, 2000b), while crossing of APdE9 mice with those overexpressing the full-length TrkB.TK receptor (Koponen et al., 2004) was used to counteract the disturbed balance between truncated and full-length TrkB receptor. Importantly, all these mouse lines shared the same C57BL6/J background and had robust transgene expression in the cortex and hippocampus. Our results lend support to the idea that impaired TrkB signaling contributes to the memory impairment in AD.

## 2. Methods

### 2.1. Animals

The APPswe/PS1dE9 (APdE9) founder mice were obtained from Johns Hopkins University, Baltimore, MD, USA (D. Borchelt and J. Jankowsky, Dept. Pathology) and a colony was established at the University of Kuopio. These mice were generated by coinjection of chimeric mouse/human APPswe (mouse APP695 harboring a human A$\beta$ domain and mutations K595N and M596L linked to Swedish familial AD pedigrees) and human PS1-dE9 (deletion of exon 9) vectors controlled by independent mouse prion protein promoter elements (Jankowsky et al., 2004). This line was originally maintained in a hybrid C3HeJ × C57BL6/J F1 background, but the mice used in the present study were derived from backcrossing to C57BL6/J for 12 generations.

The development of mice overexpressing the truncated TrkB (TrkB.T1) or the full-length TrkB (TrkB.TK) receptors specifically in neurons (≥ 2-fold overexpression throughout cortex and hippocampus) have been described previously by Saarelainen et al. (2000a, 2000b) and Koponen et al. (2004), respectively. Expression of the transgenic receptor in both the TrkB.T1 and TrkB.TK mouse lines is highest in the cerebral cortex and hippocampus (Koponen et al., 2004; Saarelainen et al., 2000a), thus overlapping with the brain areas with the highest amyloid load in the APdE9 mouse (Jankowsky et al., 2004). In addition, both TrkB transgenic lines have moderate transgene expression in the thalamus, and the TrkB.TK line also moderate expression in the amygdala and cerebellum (Koponen et al., 2004; Saarelainen et al., 2000a). These mouse lines were originally maintained in a hybrid BALB/c × DBA/2 background, but the mice used in the present study were derived from backcrossing to C57BL6/J for 10 generations.

The housing conditions (National Animal Center, Kuopio, Finland) were controlled (temperature 22 °C, light from 7:00–19:00; humidity 50%–60%), and fresh food and water were freely available. The experiments were conducted according to the Council of Europe (Directive 86/609) and Finnish guidelines, and approved by the State Provincial Office of Eastern Finland.