

Regular Article

**Late Onset Loss of Hippocampal 5-HT and NE is Accompanied by Increases in  
BDNF Protein Expression in Mice Co-Expressing Mutant APP and PS1**

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## Abstract

Transgenic mice expressing both mutant amyloid precursor protein (APP<sup>swe</sup>) and presenilin-1 (PS1 $\Delta$ E9) develop amyloid deposits as early as 4 months of age and preliminary evidence suggests that this may be associated with degenerative changes in serotonin axons innervating the dentate gyrus of the hippocampus. In the present investigation, which focused on further delineating the effects of amyloid deposition on hippocampal neurochemistry, decreases in serotonin neurotransmitter levels (-25%) were discovered to be present at 18 months in APP<sup>+</sup>/PS1<sup>+</sup> mice, while norepinephrine was reduced in the hippocampus of 12 (-30%) and 18 month-old (-45%) APP<sup>+</sup>/PS1<sup>+</sup> double mutants. In addition, brain derived neurotrophic factor (BDNF) protein levels were investigated since changes in BDNF are reported to occur in AD, and BDNF has been shown to have trophic effects on serotonin and norepinephrine neurons. In doubly, but not singly mutant mice, hippocampal BDNF levels were increased at 12 (+70%) and 18 months (+170%). Furthermore, in a different model of serotonergic and noradrenergic degeneration, BDNF protein levels were similarly increased in response to depletions in hippocampal serotonin and norepinephrine caused by the chemical neurotoxin 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine (2'-NH<sub>2</sub>-MPTP). These findings show that early amyloid deposition in mice expressing mutant human APP and PS-1 is associated with a progressive loss of serotonin and norepinephrine neurotransmitter levels in the hippocampus later in life. Furthermore, BDNF protein levels are increased in APP<sup>+</sup>/PS1<sup>+</sup> and 2'-NH<sub>2</sub>-MPTP-treated mice, possibly as a

compensatory response to serotonergic and noradrenergic neurodegeneration in a brain region important for learning and memory.

**Keywords** (10 keywords):

Serotonin  
Norepinephrine  
Monoamine  
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Presenilin 1  
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A $\beta$  Peptide  
2'-NH<sub>2</sub>-MPTP

## 1. Introduction

Familial Alzheimer's disease (FAD) is associated with mutations in the genes encoding amyloid precursor protein (APP), presenilin 1 (PS1) or presenilin 2 (PS2) (Levy, 1990; Van Broeckhoven, 1990; Campion, 1995; Cruts, 1995; Levy-Lahad, 1995a; Levy-Lahad, 1995b; Price, 1998a). Specific mutations in these genes lead to altered proteolytic processing of APP with missense mutations in the presenilins affecting  $\gamma$ -secretase, an enzyme that cleaves APP at the C-terminus (De Strooper, 1998; Wolfe, 1999). Altered metabolism of APP results in increased production of total  $\beta$ -amyloid ( $A\beta$ ) (Gordon, 2002) and specifically, the highly amyloidogenic  $A\beta_{42}$  peptide (Duff, 1996; Citron, 1997; Kurt, 2001; Qi, 2003).  $A\beta$  aggregates to form extracellular plaques and together with neurofibrillary tangles, these constitute the neuropathological hallmarks of familial and sporadic AD (Hanger, 1992; Lantos, 1992).

Neurodegeneration associated with plaque formation in AD has been widely investigated (for reviews see: (Kanazawa, 2001; Hardy, 2002)). However, while much attention has focused on degeneration of basal forebrain cholinergic neurons (Whitehouse, 1982; Kopelman, 1986; Koliatsos, 1994), other neuronal subtypes have been found to sustain degenerative insult. A meta-analysis of post-mortem studies in AD patients demonstrated a significant loss of neurons not only in the cholinergic nucleus basalis, but also in the dorsal raphe and the locus coeruleus containing the serotonergic and noradrenergic cell bodies, respectively (Lyness, 2003). In addition, a small loss of dopamine neurons originating in the substantia nigra also was observed. The neurotoxic effects of  $\beta$ -amyloid on monoamine neurotransmitter systems is further

reflected by decreases in serotonin, norepinephrine and dopamine neurotransmitter levels detected in multiple brain regions in AD patients (Herregodts, 1989; Nazarali, 1992; Storga, 1996) with Herregodts *et al.* concluding that the serotonin system sustains the greatest degenerative loss of neurons in AD. Loss of serotonergic neurons also has been associated with the formation of senile plaques in rhesus monkeys (Kitt, 1989). Thus the progressive memory loss and cognitive impairments indicative of AD most likely result from neurodegeneration occurring in multiple forebrain neurotransmitter systems (Arai, 1984; Herregodts, 1989; Engelborghs, 1997; Lyness, 2003).

In addition to research in humans and nonhuman primates, many strains of genetically engineered mice have been produced to investigate the effects of accelerated cerebral amyloid deposition on neurodegeneration (for reviews see: (Duff, 1998; Price, 1998b; Wong, 2002)). Specifically, three lines of mice coexpressing mutant APP and PS1 have been produced (Borchelt, 1997; Lee, 1997; Holcomb, 1998; Jankowsky, 2004). Among these, transgenic (Tg) mice expressing both the APP<sub>swe</sub> (APP695: K595N and M596L mutations; APP<sup>+</sup>) and the PS1ΔE9 (PS1<sup>+</sup>) mutations show highly accelerated Aβ deposition and neuritic pathology in hippocampus at 6 months of age, which is not present in age-matched mice expressing either mutation alone (Jankowsky, 2004). Preliminary data suggests that this latter strain of APP<sup>+</sup>/PS1<sup>+</sup> Tg mice also exhibits degeneration of serotonin axons innervating the dentate gyrus of the hippocampus at 12 months of age, with evidence of continued axon loss occurring at 18 months (Yoo, 2002). Furthermore, 12 month-old doubly mutant APP<sub>swe</sub><sup>+</sup>/PS1<sup>+</sup> (A246E) Tg mice, but not singly mutant mice show impaired performance in the Morris water maze, suggesting

that a decrease in hippocampal-dependent spatial memory occurs in mice with early accumulation of A $\beta$  (Puolivali, 2002; Savonenko, 2003).

Based on the potential contributions of serotonin, norepinephrine and dopamine neuronal degeneration to the cognitive impairments and other phenotypic abnormalities associated with AD, we investigated the effects of coexpression of APP<sub>swe</sub> and PS1 $\Delta$ E9 on monoamine neurochemistry. We analyzed serotonin, norepinephrine and dopamine levels and their major metabolites in various brain regions of 12 and 18 month-old mutant APP<sup>+</sup>, PS1<sup>+</sup> and APP<sup>+</sup>/PS1<sup>+</sup> transgenic mice. We also explored the effects of accelerated A $\beta$  formation on the neurotrophin, brain derived neurotrophic factor. BDNF is a 27 kD homodimeric protein that has been shown to have trophic effects on serotonergic (Mamounas, 2000), noradrenergic (Fawcett, 1998), dopaminergic (Lara, 2003) and cholinergic neurons (Koliatsos, 1994). Changes in BDNF expression appear to occur in Alzheimer's disease patients (for review see: (Murer, 2001)) and BDNF immunoreactivity is associated with senile plaques (Ferrer, 1999; Murer, 1999; Burbach, 2004). However, evidence to date has been conflicting and BDNF has been reported to be increased (Durany, 2000), as well as decreased (Connor, 1997; Hock, 2000; Michalski, 2003) in the hippocampus of human post-mortem Alzheimer's brain. Therefore, we investigated alterations in hippocampal BDNF protein levels associated with the neurodegenerative processes occurring in APP<sup>+</sup>/PS1<sup>+</sup> Tg mice.

To further investigate the selective effects of serotonergic and noradrenergic neurodegeneration on BDNF levels, the chemical toxin 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine (2'-NH<sub>2</sub>-MPTP) was employed. This amine-substituted

analog of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been shown to deplete forebrain serotonin and norepinephrine in mice (Andrews, 1993a; Andrews, 1993c; Andrews, 1993b; Andrews, 1996) and rats (Unger, 2002), while having no long-term effects on striatal dopamine. Furthermore, glial fibrillary acidic protein (GFAP), a marker of reactive gliosis, as well as argyrophilia are increased 2-3 days after 2'-NH<sub>2</sub>-MPTP administration (Luellen, 2003), both of which are markers of neurodegeneration. In the present study, the effect of 2'-NH<sub>2</sub>-MPTP on BDNF protein levels was investigated to determine whether chemically-mediated serotonergic and noradrenergic neurodegeneration alters hippocampal BDNF protein levels.

## 2. Materials and Methods:

### *Animals*

Transgenic mice expressing FAD-linked mutant amyloid precursor protein (APP) or FAD-linked mutant presenilin 1 (PS1) were developed at the Johns Hopkins University School of Medicine. These two strains of mice were crossbred to create doubly transgenic APP<sup>+</sup>/PS1<sup>+</sup> (Prp-APP/swe/Prp-PS1 $\Delta$ E9) mice (Borchelt, 1997; Lee, 1997). Four genotypes of mice (APP<sup>-</sup>/PS1<sup>-</sup>, APP<sup>+</sup>/PS1<sup>-</sup>, APP<sup>-</sup>/PS1<sup>+</sup>, APP<sup>+</sup>/PS1<sup>+</sup>) at 12 or 18 months of age were used for the present experiments (n=6-8/genoptype/age). Twelve week-old CD-1 male mice from Charles River Laboratories, Inc. (Wilmington, MA), weighing 30 to 40 g were used for the neurotoxin experiment. All mice were maintained at 20-22°C on a 12 hour light/dark cycle with food and water available *ad libitum*. Experimental protocols strictly adhered to the National Institutes of Health Animal Care Guidelines and were approved by the Johns Hopkins University School of Medicine and the Pennsylvania State University Institutional Animal Care and Use Committees.

### *Neurotoxin Treatment*

2'-NH<sub>2</sub>-MPTP was administered in four intraperitoneal injections of 20 mg/kg at 2-h intervals. Doses were calculated as the free base and given in a volume of 0.1 mL sterile water. Control animals received similarly timed injections of sterile water. These dosing regimens were selected because they have been shown to cause long-term depletions in cortical and hippocampal 5-HT and NE (Andrews, 1993a; Andrews, 1993c; Luellen,

2003). To determine the acute time course of the effects of 2'-NH<sub>2</sub>-MPTP on hippocampal neurochemistry and BDNF protein levels, two cohorts of mice (n=24) were treated with 2'-NH<sub>2</sub>-MPTP (n=14) or water (control, n=10) and a single cohort was sacrificed at 3 or 21 days post-treatment. The mice were killed by cervical dislocation and their brains were rapidly removed and dissected over ice to obtain hippocampus. The samples then were stored at -70 °C pending analysis. Half (n=7 for 2'-NH<sub>2</sub>-MPTP and n=5 for water) of the hippocampal samples were used for neurotransmitter analysis and the other half were reserved for BDNF protein analysis.

#### *Monoamine Neurotransmitter Analysis*

In addition to the 2'-NH<sub>2</sub>-MPTP-treated mice described in the previous section, APP/PS1 mice were sacrificed by cervical dislocation and the brains were rapidly dissected on ice. Samples were stored at -70 °C prior to analysis. Brain region samples from one half of the brain (brainstem, hippocampus, frontal cortex and striatum) were analyzed for monoamine neurotransmitters and metabolites by high performance liquid chromatography with electrochemical detection (HPLC-ED) at +0.240 mV (Andrews, 1996). Serotonin (5-HT), its metabolite 5-hydroxyindoleacetic acid (5-HIAA), norepinephrine, dopamine, and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were separated and quantified in a single chromatogram. 5-Hydroxy-*N*-methyltryptamine oxalate was used as the internal standard. Relative peak areas of the sample peaks were compared to external standards

for quantitation. Total tissue protein levels were measured by the method of Lowry *et al.* (Lowry, 1951).

#### *Measurement of BDNF Protein Levels*

Hippocampus samples from the remaining side of the brain were analyzed using the BDNF Emax Immunoassay kit (Promega Co. Madison, WI) with an extraction procedure developed by us (Szapacs, 2004). Samples were weighed and homogenized in 2 mL of lysis buffer (100 mM PIPES pH 7, 500 mM NaCl, 0.2% Triton X-100, 0.1% NaN<sub>3</sub>, 2 mM EDTA, 200 μM PMSF, 10 μM leupeptin, 0.3 μM aprotinin, and 1 μM pepstatin). Samples were then divided and half of each sample was spiked (250 pg/mL BDNF) to quantify recovery. Using the manufacturer's extraction procedure, percent recoveries determined in previous experiments were only 2-5% (Szapacs, 2004). On the other hand, the modified procedure typically results in ~70% recovery of BDNF from hippocampal tissue. Samples then were centrifuged at 16,000 g for 30 minutes and the supernatants were analyzed for BDNF protein levels by ELISA according to the manufacturer's protocol (Promega, 2000).

#### *Drugs and Chemicals*

Components of the mobile phase and neurotransmitter standards were of HPLC grade or the highest quality obtainable from Sigma-Aldrich (St. Louis, MO). All chemicals used to make the BDNF extraction buffer and TBST wash buffer also were obtained from Sigma-Aldrich. 2'-NH<sub>2</sub>-MPTP was synthesized at The Pennsylvania State

University (University Park, PA) and is currently available from Sigma-Aldrich (A7969). The identity of 2'-NH<sub>2</sub>-MPTP was verified by <sup>1</sup>H-NMR and mass spectrometry. The purity of 2'-NH<sub>2</sub>-MPTP was ~99% by gas chromatography and was stored desiccated at 4 °C.

### 3. Results:

*Serotonin neurotransmitter levels are decreased in hippocampus at 18 months in APP<sup>+</sup>/PS1<sup>+</sup> transgenic mice*

At 12 month of age, no significant differences in 5-HT levels were detected in hippocampus [F(3,26)=1.7, p=0.19], frontal cortex [F(3,25)=2.9, p=0.054] or brain stem [F(3,26)=1.4, p=0.27] in APP<sup>+</sup>, PS1<sup>+</sup> or APP<sup>+</sup>/PS1<sup>+</sup> Tg mice compared to nonTg mice (Figure 1A). However at 18 months of age, PS1<sup>+</sup> Tg mice showed a statistically significant 20% decrease in 5-HT in hippocampus and APP<sup>+</sup>/PS1<sup>+</sup> Tg mice showed a 25% decrease in hippocampal 5-HT levels [F(3,23)=11, p<0.0001] (Figure 1B). No changes were observed in 5-HT in frontal cortex [F(3,23)=0.87, p=0.47] or brain stem [F(3,22)=0.79, p=0.51] at 18 months. Changes in 5-HIAA, the major metabolite of serotonin, were similar to those of 5-HT, except for the fact that decreases were only significant in the APP<sup>+</sup>/PS1<sup>+</sup> genotype at 18 months of age (-25%) [F(3,23)=4.7, p<0.01] (data not shown).

*Norepinephrine is significantly decreased at 12 and 18 months in APP<sup>+</sup>/PS1<sup>+</sup> mice*

In 12 month-old mice, NE levels in the hippocampus were significantly decreased by 25% in APP<sup>+</sup> Tg mice and by 30% in APP<sup>+</sup>/PS1<sup>+</sup> Tg mice compared to nonTg littermates ([F(3,26)=3.1, p<0.05], Figure 2A). By contrast, NE levels were increased by 25% in PS1<sup>+</sup> transgenic mice in frontal cortex [F(3,25)=6.0, p<0.01]. No changes in norepinephrine levels were detected in brain stem at 12 months of age [F(3,26)=1.8, p=0.18]. In 18 month-old mice, hippocampal NE levels were further decreased by 45% in APP<sup>+</sup>/PS1<sup>+</sup>

transgenic mice [ $F(3,23)=5.3$ ,  $p<0.01$ ] with no changes detected in frontal cortex [ $F(3,23)=2.8$ ,  $p=0.063$ ] or brain stem [ $F(3,22)=0.91$ ,  $p=0.45$ ] (Figure 2B).

*No alterations in striatal dopamine are present at either age*

The levels of dopamine (Figure 3) and its metabolites DOPAC and HVA (data not shown) were assessed in striatum in APP<sup>+</sup>, PS1<sup>+</sup> and APP<sup>+</sup>/PS1<sup>+</sup> Tg mice at 12 and 18 months of age. No statistically significant changes were detected in DA (12 month-old [ $F(3,26)=1.3$ ,  $p=0.31$ ], 18 month-old [ $F(3,22)=0.35$ ,  $p=0.79$ ]), DOPAC (12 month-old [ $F(3,26)=1.9$ ,  $p=0.15$ ], 18 month-old [ $F(3,22)=1.3$ ,  $p=0.30$ ]) or HVA (12 month-old [ $F(3,25)=0.71$ ,  $p=0.55$ ], 18 month-old [ $F(3,22)=0.08$ ,  $p=0.97$ ]) with respect to genotype.

*Hippocampal BDNF protein levels are elevated at 12 and 18 months of age in APP<sup>+</sup>/PS1<sup>+</sup> mice*

BDNF has been shown to cause regenerative sprouting of damaged serotonergic axons in the adult brain (Mamounas, 1995; Mamounas, 2000). Furthermore, genetically induced decreases in the expression of BDNF result in accelerated age-dependent degeneration of serotonergic innervation to the forebrain (Lyons, 1999). Thus, we explored whether BDNF protein levels may be altered in response to serotonergic and possibly, noradrenergic terminal degeneration in hippocampus in APP<sup>+</sup>/PS1<sup>+</sup> Tg mice. Determination of BDNF protein levels by quantitative ELISA demonstrated a significant increase in hippocampal BDNF levels (+70%) in 12 month-old APP<sup>+</sup>/PS1<sup>+</sup> Tg mice compared to APP<sup>+</sup> and PS1<sup>+</sup> transgenic mice and nonTg littermates [ $F(3,26)=6.7$ ,  $p<0.01$ ] (Figure 4). In 18 month-old APP<sup>+</sup>/PS1<sup>+</sup> Tg mice, BDNF protein levels were elevated to

an even greater extent (+170%) compared to nonTg levels in hippocampus [F(3,21)=20.72, p<0.001] (Figure 4).

*5-HT and NE levels are reduced and BDNF protein levels are increased in the hippocampus following 2'-NH<sub>2</sub>-MPTP treatment*

In this experiment, we utilized the potent and selective serotonin and norepinephrine neurotoxin 2'-NH<sub>2</sub>-MPTP to model hippocampal serotonergic and noradrenergic neurodegeneration occurring in APP/PS1 transgenic mice (Andrews, 1993a; Andrews, 1993c; Andrews, 1993b; Andrews, 1996; Unger, 2002; Luellen, 2003). Serotonin levels were significantly decreased by 80% 3 days post-treatment [F(3,22)=45.5, p<0.001] and 50% 21 days post-treatment [F(3,22)=45.5, p<0.001] with 2'-NH<sub>2</sub>-MPTP in hippocampus. Norepinephrine also was significantly depleted by 80% 3 days post-treatment [F(3,21)=31.8, p<0.001] and 70% 21 days post-treatment [F(3,21)=31.8, p<0.001].

To determine if hippocampal BDNF was altered in response to 2'-NH<sub>2</sub>-MPTP, BDNF protein levels were measured using ELISA. These data revealed a significant increase in BDNF (+75 %) between 2'-NH<sub>2</sub>-MPTP-treated and control mice at 3 days ([t(10)=-3.8, p<0.01]; Fig. 5). On the other hand, hippocampal BDNF levels were not significantly different between the two groups 21 days after 2'-NH<sub>2</sub>-MPTP treatment [t(10)=-1.4, p=0.19].

#### 4. Discussion

The major findings of this study are three-fold. First, loss of monoamine neurotransmitters in mice coexpressing FAD-linked APP and PS1 occurred specifically in the hippocampus where 5-HT, 5-HIAA and NE levels were decreased in an age-dependent manner. Second, losses of 5-HT and NE was concomitant with an age-dependent increase in BDNF protein levels in hippocampus in APP<sup>+</sup>/PS1<sup>+</sup> mice. Third, mice with reductions in hippocampal serotonin and norepinephrine levels caused by the neurotoxin 2'-NH<sub>2</sub>-MPTP also showed a time-dependent increase in BDNF.

Preliminary immunocytochemical studies by others in APP<sup>+</sup>/PS1<sup>+</sup> Tg mice suggest that a decrease in serotonin axon density occurs in the dentate gyrus of the hippocampus beginning at 12 months of age and this becomes more pronounced by 18 months (Yoo, 2002). In the present investigation, decreases in serotonin neurotransmitter levels became evident by 18 months of age. Tissue serotonin levels have been shown to underestimate neuronal damage detectable by immunocytochemistry, particularly when degeneration is limited to specific subregions (Lyons, 1999). Moreover, the ability of the brain to compensate for the loss of serotonergic innervation by increasing the synthesis of 5-HT in remaining neurons may mask changes reflective of the early stages of axonal degeneration in 12 month-old APP<sup>+</sup>/PS1<sup>+</sup> Tg mice and result in only a modest (25%) decrease in 5-HT in 18 month-old APP<sup>+</sup>/PS1<sup>+</sup> Tg mice.

Previous studies have reported decreases in 5-HT cell body numbers in brain stem in postmortem human Alzheimer patients (Lyness, 2003), however, we detected

no change in brain stem 5-HT levels in doubly mutant APP<sup>+</sup>/PS1<sup>+</sup> Tg mice. Once again, increased serotonin synthesis may account for these findings. Alternately, they may be indicative of the time course over which plaques form in transgenic mice (~12 months) compared to the duration of AD in the human patients studied (3-11 years with exposure to amyloid plaques presumably predating the diagnosis of AD). Shorter  $\beta$ -amyloid exposure in mice may not allow sufficient time for axonal damage to cause retrograde degeneration of 5-HT cell bodies. Furthermore, serotonergic neuronal perikarya may be relatively resistant to degeneration in mice (Andrews, 1993a; Mamounas, 2000). Overall however, the current data lend support to the hypothesis that early onset A $\beta$  deposition in mice is associated with degeneration of 5-HT axons projecting to the hippocampus.

Notably, the present study also revealed a decrease in hippocampal NE at both 12 and 18 months in APP<sup>+</sup>/PS1<sup>+</sup> Tg mice, signifying that degeneration of noradrenergic axons potentially occurs. Significant differences in striatal levels of DA or its major metabolites were not detected, suggesting that the nigrostriatal dopamine system may be relatively unaffected by amyloid plaque formation in APP<sup>+</sup>/PS1<sup>+</sup> Tg mice. Immunocytochemical studies using antibodies directed against tyrosine hydroxylase and serotonin are planned to further assess neurodegenerative changes in noradrenergic, dopaminergic and serotonergic forebrain innervation in APP<sup>+</sup>/PS1<sup>+</sup> transgenic mice.

Decreases in serotonin and norepinephrine levels may contribute to learning and memory deficits previously reported to occur in APP<sup>+</sup>/PS1<sup>+</sup> Tg mice (Puolivali, 2002).

Reductions in both serotonergic and/or noradrenergic neurotransmission have been found to affect learning and memory in humans and rats (Richter-Levin, 1989; Nilsson, 1990; Chen, 1992; Clayton, 2000; Parvizi, 2001; Madhyastha, 2002; Myhrer, 2003; Porter, 2003; Collier, 2004). In a clinical trial, acute depletions of tryptophan, the precursor to 5-HT caused impairment in working memory in Alzheimer-type senile dementia patients and control patients (Porter, 2003). Other studies have indicated that the combined loss of serotonergic and cholinergic neurons leads to a spatial learning deficit in rats (Richter-Levin, 1989; Nilsson, 1990) that can be reversed by grafting both serotonergic and cholinergic neurons into the hippocampus (Nilsson, 1990). Finally, one study implicated serotonin and norepinephrine in spatial learning and memory tasks with as little as a 10-15% decrease in tissue levels of 5-HT and NE leading to impairment (Madhyastha, 2002). Together these data suggest that degeneration in the serotonin and/or noradrenergic systems, possibly in combination with the loss of cholinergic innervation, has profound effects on cognitive brain function.

Phillips et al. first reported a decrease in BDNF mRNA in the hippocampus in patients with AD (Phillips, 1991). Subsequent studies demonstrated reductions in BDNF protein, its preprotein and specific BDNF mRNA transcripts in the parietal cortex of Alzheimer's disease patients at the time of death (Fahnestock, 2002; Garzon, 2002; Michalski, 2003). However, postmortem studies conducted in humans offer only a snapshot of the changes occurring in BDNF in the late stages of the disease. On the other hand, transgenic mouse models such as APP<sup>+</sup>/PS1<sup>+</sup> mice present the opportunity to investigate alterations in BDNF during earlier stages of  $\beta$ -amyloid deposition. The

current findings show an ongoing increase in BDNF protein levels in 12 versus 18 month-old mice coexpressing mutant APP and PS1 transgenes linked to FAD. Increases in BDNF protein levels may reflect an early onset compensatory mechanism in response to neuronal degeneration associated with A $\beta$  deposition. Infusion of A $\beta$ <sub>42</sub> directly into the brain has been shown to increase BDNF mRNA expression in the hippocampus (Tang, 2000). Furthermore, studies on the effects of *p*-chloroamphetamine, a serotonergic neurotoxin, demonstrated a toxin-induced decrease in the number of 5-HT immunoreactive fibers in hippocampus concomitant with an increase in BDNF mRNA in the dentate gyrus, CA1 and CA3 pyramidal cell regions of the hippocampus (Zetterstrom, 1999). These findings, together with the present data from mice treated with 2'-NH<sub>2</sub>-MPTP support the idea that an increase in BDNF occurs in response to damage to serotonergic, and possibly noradrenergic axons projecting to the hippocampus.

An alternate explanation for our findings in doubly mutant APP<sup>+</sup>/PS1<sup>+</sup> mice is that  $\beta$ -amyloid plaque formation may impair BDNF retrograde transport in degenerating serotonergic and noradrenergic neurons projecting from the brain stem to the hippocampus. Cooper *et al.* have demonstrated that failed retrograde transport of another neurotrophin, nerve growth factor (NGF) occurs in basal forebrain cholinergic neurons in a Down's Syndrome model of cholinergic degeneration (Cooper, 2001). Many studies have addressed the subject of retrograde BDNF transport (DiStefano, 1992; Mufson, 1994; Sobreviela, 1996) and Mufson and colleagues have summarized these data in a recent review (Mufson, 1999). BDNF is transported from dorsal

hippocampus to hypothalamic, diagonal band and basal forebrain cholinergic nuclei, entorhinal cortex, contralateral hippocampus and adjacent subfields of the ipsilateral hippocampus (Sobreviela, 1996; Mufson, 1999). However, these authors did not find evidence of BDNF retrograde transport directly from hippocampus to the medial or dorsal raphe regions of the brain stem where the serotonergic cell bodies are located, or to the locus coeruleus noradrenergic cell bodies. By contrast, others have found evidence for anterograde BDNF transport in the CNS (Altar, 1997; Conner, 1997; Fawcett, 1998; Spalding, 2002). Double labeling studies for dopamine  $\beta$ -hydroxylase (a marker of noradrenergic neurons) and BDNF showed that these two proteins are highly co-localized in brain stem (Fawcett, 1998) and the authors postulated that these data, in combination with previous *in situ* hybridization studies (Castren, 1995) indicate that BDNF is synthesized in norepinephrine cell bodies and anterogradely transported into noradrenergic axons and terminals. Thus trafficking of BDNF is complex and does not appear to involve direct retrograde transport from hippocampus to brain stem. In light of this, uncovering possible alterations in BDNF trafficking in APP<sup>+</sup>/PS1<sup>+</sup> mice will require detailed comprehensive studies.

Previous experiments assessing cholinergic degeneration in a separate line of doubly mutant APP<sup>+</sup>/PS1<sup>+</sup> mice (APP<sup>swe</sup>/PS1:M146L) have been mixed. In one report using p75<sup>NTR</sup>-immunoreactivity as a marker for cholinergic basal forebrain innervation, no changes in cholinergic axons projecting to the hippocampus of doubly mutant mice compared to nontg mice were detected (Jaffar, 2001). However, in a second study utilizing an antibody directed against the vesicular acetylcholine transporter, a modest

decrease in the size of hippocampal cholinergic synapses was detected at 8 months of age in doubly mutant mice that was not accompanied by a change in cell body number. Additional in depth histological analyses of neurodegenerative changes in the cholinergic neurotransmitter system, as well as the monoamine systems over a wide range of ages in APP<sup>swe</sup>/PS1 $\Delta$ E9 mice appears warranted, as well as studies aimed at investigating the molecular mechanisms underlying the increased expression of BDNF occurring in response to coexpression of these mutant forms of APP and PS1 proteins.

In conclusion, the two time-points studied herein reveal increasingly pronounced changes in hippocampal neurochemistry in mice with a greater density of A $\beta$  deposits (18 months of age) compared to mice with fewer A $\beta$  deposits (12 months of age) (Borchelt, 1997). We observed decreases in NE levels and increases in BDNF as early as 12 months of age in the hippocampus of APP<sup>+</sup>/PS1<sup>+</sup> Tg mice, suggesting that even at lower densities, plaque formation adversely affects hippocampal neurochemistry. In 18 month-old APP<sup>+</sup>/PS1<sup>+</sup> Tg mice with continued advancement of plaque deposition, 5-HT neurotransmitter levels were decreased, NE levels were further reduced and BDNF protein levels were elevated to a greater extent implying that a *progressive neurodegenerative pathology* is present in APP<sup>+</sup>/PS1<sup>+</sup> Tg mice. In further support of these data, BDNF protein levels also were increased as early as 3 days after neurotoxic damage to the forebrain serotonergic and noradrenergic systems by 2'-NH<sub>2</sub>-MPTP, however, they began to return to normal by 21 days after this type of acute insult.

In light of the present findings on BDNF in APP<sup>+</sup>/PS1<sup>+</sup> Tg mice, it appears that increases in the expression of this neurotrophic factor occur in response to hippocampal

neurodegeneration. Drugs that inhibit the reuptake of serotonin (i.e. Prozac, Paxil), which are commonly used in the treatment of mood and anxiety disorders also have been reported to increase BDNF mRNA (Nibuya, 1995) and more recently, to stimulate hippocampal neurogenesis (Santarelli, 2002). Therefore,  $\beta$ -amyloid associated increases in BDNF may act to stimulate the generation of new neurons in APP<sup>+</sup>/PS1<sup>+</sup> Tg mice as part of a compensatory response to continuing degeneration. APP<sup>+</sup>/PS1<sup>+</sup> Tg mice will serve as a good model to further explore these hypotheses, especially with regard to the development and testing of novel neuroprotective or neuro-regenerative therapeutic strategies (Altar, 1999).

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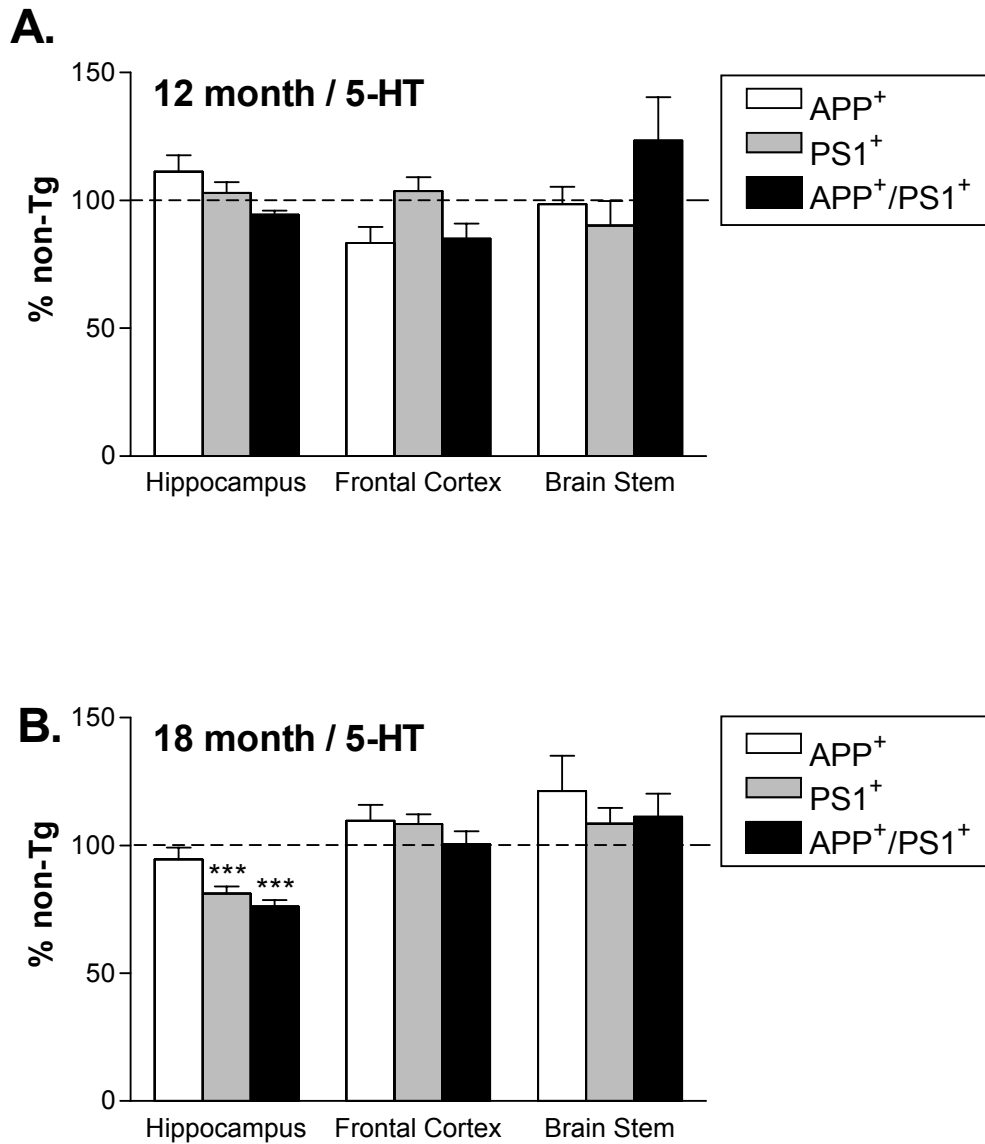
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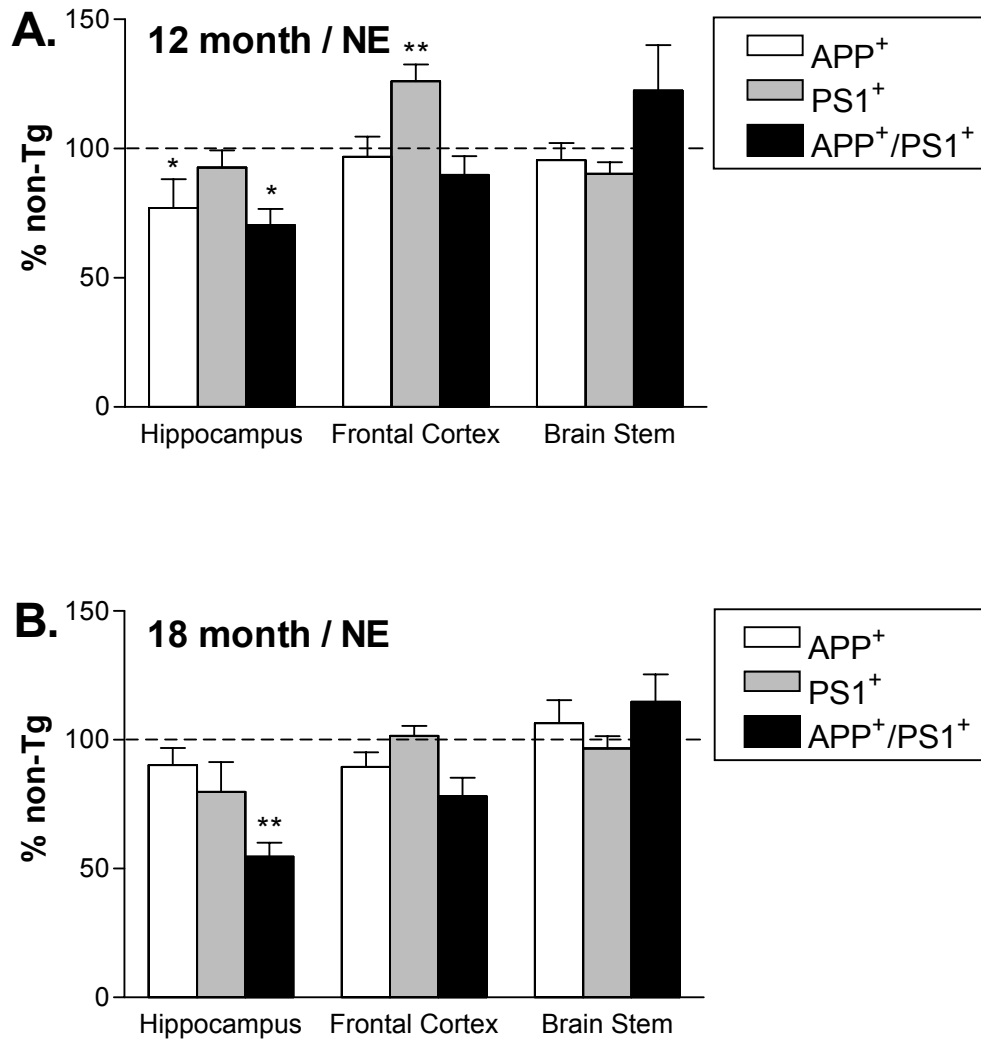
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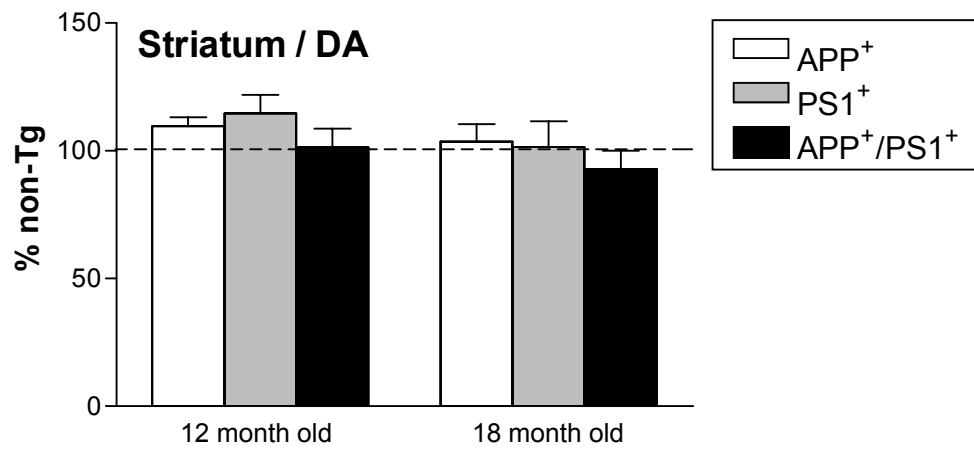
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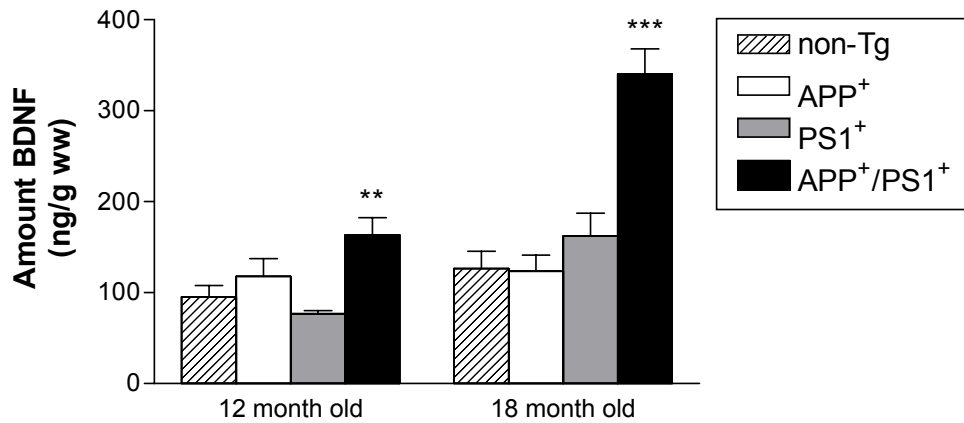
**Figure 1.** Serotonin levels measured by HPLC-ED in hippocampus, frontal cortex and brain stem of (A) 12 month-old and (B) 18 month-old APP<sup>+</sup> (n=7,7 respectively), PS1<sup>+</sup> (n=8,7) or APP<sup>+</sup>/PS1<sup>+</sup> (n=7,7) Tg mice graphed as percent of nonTg littermate levels (n=8,6). Control group means  $\pm$  SEM in hippocampus, frontal cortex and brain stem respectively, were as follows: 12 mo. old,  $5.7 \pm 0.4$ ,  $5.1 \pm 0.3$  and  $11 \pm 1$  and 18 mo. old,  $6.5 \pm 0.2$ ,  $5.5 \pm 0.4$  and  $8.2 \pm 0.6$  ng/mg protein. The probabilities indicated in the figure are: \*\*\*p<0.001 for differences from nonTg values.



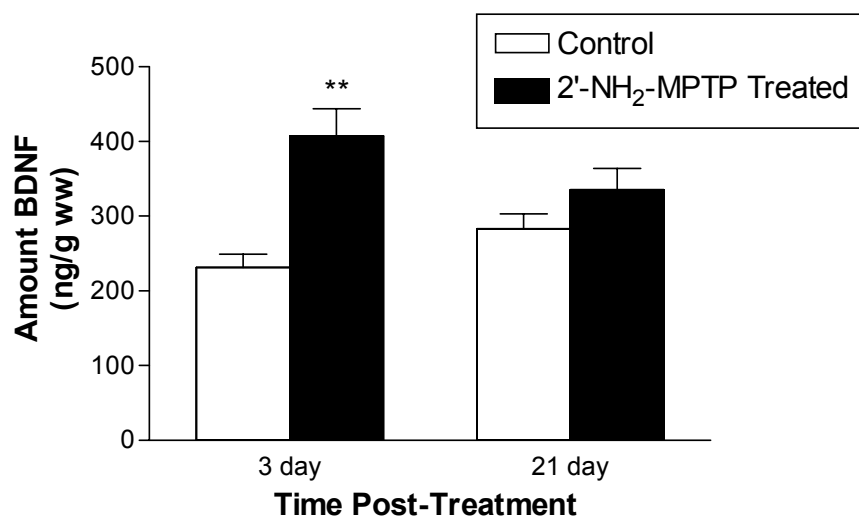
**Figure 2.** Norepinephrine levels measured by HPLC-ED in the hippocampus, frontal cortex and brain stem of (A) 12 month-old and (B) 18 month-old APP<sup>+</sup> (n=7,7 respectively) PS1<sup>+</sup> (n=8,7) or APP<sup>+</sup>/PS1<sup>+</sup> (n=7,7) Tg mice graphed as percent of nonTg littermate levels (n=8,6). Control group means  $\pm$  SEM in the hippocampus, frontal cortex and brain stem respectively, were as follows: 12 mo. old,  $3.6 \pm 0.2$ ,  $3.1 \pm 0.2$  and  $9.1 \pm 0.9$  and 18 mo. old,  $3.9 \pm 0.3$ ,  $3.0 \pm 0.3$  and  $8.0 \pm 0.7$  ng/mg protein. The probabilities indicated in the figure are: \*p<0.05 and \*\*p<0.01 for differences from nonTg values.



**Figure 3.** Dopamine levels measured by HPLC-ECD in striatum of 12 and 18 month-old APP<sup>+</sup> (n=7,7 respectively), PS1<sup>+</sup> (n=8,7) or APP<sup>+</sup>/PS1<sup>+</sup> (n=7,7) Tg mice graphed as percent of nonTg littermate levels (n=8,6). Control group means  $\pm$  SEM in the striatum were as follows: 12 mo. old,  $87 \pm 5$  and 18 mo. old  $110 \pm 6$  ng/mg of protein.



**Figure 4.** BDNF levels measured by ELISA in the hippocampus of 12 and 18 month-old nonTg (n=8,5 respectively), APP<sup>+</sup> (n=7,7), PS1<sup>+</sup> (n=8,6) or APP<sup>+</sup>/PS1<sup>+</sup> (n=7,7) Tg mice in ng BDNF/ g wet tissue weight. The probabilities indicated in the figure are: \*\*p<0.01 and \*\*\*p<0.001 for differences from nonTg values.



**Figure 5.** BDNF levels measured by ELISA in the hippocampus of CD-1 mice treated with 2'-NH<sub>2</sub>-MPTP or control at 3 day (n=7,5 respectively) and 21 day (n=7,5 respectively) post-treatment. BDNF levels are reported as ng BDNF/ g wet tissue weight. The probabilities indicated in the figure are: \*\*p<0.01 for differences from control animals.