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TAU DOWN-REGULATES BDNF EXPRESSION IN ANIMAL AND CELLULAR MODELS OF ALZHEIMER'S DISEASE

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Abstract

In Alzheimer's disease (AD), soluble tau accumulates and deposits as neurofibrillary tangles (NFTs). However, a precise toxic mechanism of tau is not well understood. We hypothesized that over-expression of wild-type tau down-regulates brain-derived neurotrophic factor (BDNF), a neurotrophic peptide essential for learning and memory.

Two transgenic mouse models of human tau expression and human tau (hTau40)-transfected human neuroblastoma (SH-SY5Y) cells were used to examine the effect of excess or pathologically modified wild-type human tau on BDNF expression. Both transgenic mouse models, with or without NFTs, as well as hTau40-SH-SY5Y cells significantly down-regulated BDNF mRNA compared to controls. Similarly, transgenic mice over-expressing amyloid- β significantly down-regulated BDNF expression. However, when crossed with tau knockout mice, the resulting animals exhibited BDNF levels that were not statistically different from wild-type mice.

These results demonstrate that excess or pathologically modified wild-type human tau down-regulates BDNF and that neither a mutation in tau nor the presence of NFTs is required for toxicity. Moreover, our findings suggest that tau at least partially mediates amyloid- β -induced BDNF down-regulation. Therefore, AD treatments targeting A β alone may not be effective without considering the impact of tau pathology on neurotrophic pathways.

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Keywords

Alzheimer's disease; Tau; Amyloid- β ; Brain-derived neurotrophic factor; Tauopathy; Transgenic mice

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that presents as global cognitive decline with associated memory loss and loss of executive function. The neuropathological hallmarks of AD include amyloid plaques composed of accumulated and deposited amyloid- β (A β) peptides and neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein. There is increasing evidence that although a primary insult in AD may be increased A β , it may ultimately lead to cognitive dysfunction and neurodegeneration by inducing alterations in tau (Iqbal and Grundke-Iqbal, 2008; Lewis, et al., 2001). Other neurodegenerative diseases are also characterized by pathological accumulations of aggregated protein, including α -synuclein in Parkinson's disease (PD) (Polymeropoulos et al., 1997; Spillantini et al., 1997), huntingtin in Huntington's disease (HD) (Davies et al., 1997), and TDP-43 in amyotrophic lateral sclerosis (ALS) (Neumann et al., 2006; Kwong et al., 2007). Recent evidence demonstrates that accumulation of tau is a downstream consequence of protein aggregation and a feature of all these disorders (Yang et al., 2003; Lei et al., 2010; Constantinescu et al., 2011; Fernandez-Nogales et al., 2014; Sengupta et al., 2015). Furthermore, tauopathies such as frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), Pick's disease (PiD), corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP) are also characterized by intracellular accumulations of tau protein (Crowther and Goedert, 2000; Goedert and Spillantini, 2011; Iwatsubo, et al., 1994; Lee, et al., 2001; Pollock, et al., 1986). While the phenotypic manifestation of specific cell types and brain areas affected vary among tauopathies, the presence of accumulated tau protein in the absence of other pathological hallmarks in tauopathies substantiates the toxicity of tau independent of A β .

The ratio of three-repeat (3R) and four-repeat (4R) tau isoforms and the phosphorylation status of tau are altered in several tauopathies, presumably resulting ultimately in accumulation of insoluble tau aggregates or NFTs (Janus, 2008; Lee, et al., 2001). In a healthy adult, the ratio of 3R:4R tau isoforms is approximately 1:1 in most regions of the brain (Goedert and Jakes, 1990; Hong, et al., 1998; Kosik, et al., 1989). Deviations from this ratio are characteristic of neurodegenerative tauopathies (Conrad, et al., 2007; Delacourte, et al., 1998; Ginsberg, et al., 2006; Hutton, et al., 1998; Ingelsson, et al., 2007; Sergeant, et al., 1997). Furthermore, tau hyperphosphorylation and aberrant phosphorylation at certain sites is characteristic of AD (Augustinack, et al., 2002; Bramblett, et al., 1993; Matsuo, et al., 1994). Hyperphosphorylated tau is prone to aggregation and is the main component of NFTs (Ballatore, et al., 2007; Lee, et al., 1991). Historically, NFTs were thought to be the neurotoxic form of tau (Eckermann, et al., 2007; Giannakopoulos, et al., 2003; Grober, et al., 1999; Mocanu, et al., 2008). However, recent evidence suggests that toxicity could be spread by soluble, low-molecular-weight oligomers (Kopeikina, et al., 2012; F $\acute{a}$ et al., 2016), which are recognized as NFT precursors (Lasagna-Reeves, et al., 2012).

How tau might lead to cognitive decline and neurodegeneration is unclear. Loss-of-function resulting from tau detachment from microtubules and consequent microtubule instability (Alonso, et al., 1996; Dawson, et al., 2010) as well as gain-of-function resulting from aggregated tau physically interfering with intracellular transport (Wolfe, 2012) are two potential contributors to tau toxicity. However, among the most feasible hypotheses for tau-induced neurotoxicity is its ability to decrease trophic support for affected neurons (Fahnestock, 2011).

Brain-derived neurotrophic factor (BDNF) supports the survival and function of neurons and their connections that are vulnerable in diseases of the aging brain such as AD (Alderson, et al., 1990; Knusel, et al., 1991), PD (Hyman et al., 1991; Baquet et al., 2005) and HD (Altar et al., 1997; Baquet et al., 2004; Zuccato and Cattaneo, 2007; Baydyuk and Xu, 2014). Furthermore, in AD, PD and HD there is a significant down-regulation of BDNF mRNA (Phillips et al., 1991; Mogi et al., 1999; Holsinger et al., 2000; Howells et al., 2000; Zuccato et al., 2001; Garzon et al., 2002; Zuccato et al., 2008) and protein (Connor et al., 1997; Parain et al., 1999; Ferrer et al., 2000; Hock et al., 2000; Peng et al., 2005; Zuccato et al., 2008), which is strongly implicated in loss of memory and cognitive function (Fahnestock, 2011). Furthermore, BDNF polymorphisms have been associated with an increased risk of developing late-onset AD (Kunugi et al., 2001; Zhang et al., 2011; Ji et al., 2015). The exact mechanisms of BDNF down-regulation in AD have not been established, however, amyloid- β has been shown to decrease BDNF expression (DaRocha-Souto, et al., 2012; Garzon and Fahnestock, 2007; Rosa and Fahnestock, 2015; Tong, et al., 2004). In addition, recent findings showing that BDNF mRNA is down-regulated in the cortex of human non-AD tauopathy subjects (FTDP-17, PiD and CBD) (Belrose, et al., 2014) suggest that tau alone might decrease BDNF expression and hence, may mediate A β -induced BDNF down-regulation. However, there have been no *in vitro* investigations of tau-induced BDNF down-regulation, nor have transgenic models been utilized to confirm observations found in postmortem human tissue.

In this study, we used transgenic mouse models and tau transfected human neuroblastoma cells to determine if wild-type tau alone is capable of down-regulating BDNF. We also used A β over-expressing, tau knockout transgenic animals (APP23xTauKO) to determine whether tau mediates A β -induced BDNF down-regulation.

2. METHODS

2.1 Transgenic mice

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Nathan Kline Institute and NYU Langone Medical Centre and the Animal Ethics Committee of the University of New South Wales. All procedures complied with NIH guidelines and the statement on animal experimentation issued by the National Health and Medical Research Council of Australia. Cortical tissue from human tau-expressing mice (hTau and 8c-het mice), A β over-expressing APP23 mice and Tau knockout (KO) animals were compared to their respective, non-transgenic, wild-type littermates (either Swiss Webster/B6D2F1 compared to 8c-het and hTau mice, which are on a Swiss Webster/

B6D2F1 background, or non-transgenic C57BL6 mice compared to APP23 and APP23xTauKO mice which were on a C57BL6 background).

Three to 16 month-old hTau transgenic mice, which express human tau on a mouse tau knockout background (Andorfer, et al., 2005; Andorfer, et al., 2003; Levine, et al., 2009) and 8c-het transgenic mice, which express human tau on a heterozygous mouse tau background (Duff, et al., 2000), were used in this study. The distribution of ages of these transgenic mice is provided in Table 1. hTau mice develop NFTs in a similar pattern and distribution as seen in human tauopathies (Andorfer, et al., 2003) and experience age-dependent cognitive and physiological impairments similar to that observed in AD patients (Polydoro, et al., 2009). hTau mice exhibit insoluble pre-tangles at approximately 9 months of age (Andorfer, et al., 2003), neuronal loss in the piriform cortex between 8 and 17 months of age and NFT pathology beginning at 15 months of age (Andorfer, et al., 2005; Andorfer, et al., 2003; Levine, et al., 2009). In contrast, 8c-het transgenic mice exhibit increased tau phosphorylation and altered tau isoform expression prior to 8 months of age compared to wild-type mice but do not develop insoluble tau aggregates (Duff, et al., 2000). The genotypes of these animals were confirmed prior to analysis by Transnetyx Inc. (Cordova, TN), and Western blots were carried out demonstrating increased soluble tau in the 8c-het mice compared to both non-transgenic and hTau transgenic mice.

APP23 mice exhibit a 7-fold over-expression of mutated APP harboring the Swedish mutation, resulting in significantly increased soluble A β and plaques depositing at about 6 months of age (Sturchler-Pierrat, et al., 1997) with cognitive impairments beginning as early as 3 months of age (Van Dam, 2003). TauKO (*Mapt*^{-/-}) mice (Tucker, et al., 2001) lack tau but do not have any detectable phenotype. Mice used in this study were 24 months of age.

2.2 Cell culture

Human neuroblastoma SH-SY5Y cells (ATCC, Manassas, VA, USA) were grown in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (Gibco BRL, Carlsbad, CA, USA), 1% L-glutamine (Gibco) and 1% penicillin/streptomycin (Gibco), as described previously (Rosa and Fahnstock, 2015).

2.3 Cell Transfection

The human 4-repeat tau isoform, hTau40, was subcloned into pcDNA3.2/V5/DEST (Invitrogen) by Gateway[®] cloning and transfected into SH-SY5Y cells using Lipofectamine 3000[®] (Invitrogen). The hTau40 plasmid contains the longest isoform of tau, with exons 2, 3, and 10 present and a total size of 441 amino acids. Tau SH-SY5Y transfectants were selected with 250 μ g/ml G418 (BioShop, Burlington, ON, Canada) for 30 days (Pennanen and Gotz, 2005). To induce neuronal differentiation, cells were treated with 10 μ M retinoic acid (Sigma-Aldrich, Oakville, ON) for 10 days, as described previously (Garzon and Fahnstock, 2007; Rosa and Fahnstock, 2015). V5 expression was quantified via Western blotting to ensure hTau expression following differentiation, and total BDNF mRNA and BDNF transcript IV mRNA levels were quantified via qRT-PCR and compared to non-transfected control cells.

2.4 Quantitative Real-time Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Frozen cortical tissue from all transgenic mice and SH-SY5Y cells were sonicated (Sonic Dismembrator Model 100, Fisher Scientific) in a 1:20 w/v ratio in Trizol® (Invitrogen, Burlington, Ontario). Sonicates were centrifuged for 3 minutes at 9000 x g at 4°C, and RNA was extracted as previously described (Rosa, et al., 2015). Resulting RNA concentration and purity were determined by Multiskan GO and SkanIT software (Thermo Scientific, Nepean, ON, Canada) at 260/280 nm. 1 µg of RNA from each sample was used for reverse transcription with Superscript™ III, following the manufacturer's protocol (Invitrogen). Real-time PCR was carried out as described in Rosa & Fahnstock 2015, using 300 nM each forward and reverse BDNF primers [GenBank:NM_001709] (forward 5'-GCGGCAGATAAAAAGACTGC-3', reverse 5'-CTTATGAATCGCCAGCCAAT-3', Mobix, Hamilton, ON, Canada), BDNF Transcript IV primers [GenBank:NM_170733.3] (forward 5'-GAGTATTACCTCCGCCATGC-3', reverse 5'-ATTCACGCTCTCCAGAGTCC-3') or β-actin primers [GenBank:NM_001101] (forward 5'-AGCCATGTACGTAGCCATCC-3', reverse 5'-CTCTCAGCTGTGGTGGTGAA-3'), 10 µl SYBR Green qPCR Supermix UDG™ (Invitrogen), 30 nM of reference dye ROX (Stratagene, La Jolla, CA, USA or Invitrogen), and 1 µL of cDNA in a total volume of 20 µL. Real-time PCR standards were generated from purified PCR products using the primers listed. Amplifications of samples, standards and controls (no-RT and no-template controls) were run in triplicate as described previously (Rosa, et al., 2015; Rosa and Fahnstock, 2015).

2.5 Protein Extraction and Western Blotting

3.65×10⁵ hTau-transfected SH-SY5Y cells were lysed in 150 µL of lysis buffer [50mM Tris pH 7.4, 150mM NaCl, 5mM EDTA, 1% Triton, 1 complete EDTA-free tablet (Roche, Mississauga, ON, Canada) and 1 complete PhosSTOP Phosphatase Inhibitor Cocktail Tablet (Roche) per 10ml cell lysis buffer] as described previously (Rosa and Fahnstock, 2015) prior to Western blotting.

Twelve percent sodium dodecyl sulfate (SDS) polyacrylamide gels were used to separate 25 µg of total protein under reducing conditions before transferring to polyvinylidene fluoride membranes (Bio-Rad, Hercules, CA, USA). As described previously (Rosa and Fahnstock, 2015), membranes were blocked with a 1:1 solution of phosphate-buffered saline (PBS) pH 7.4 and Odyssey Blocking Buffer (Cedarlane, Burlington, Ontario, Canada) for 1 hour. After blocking, the blots were probed overnight at 4°C with Anti-V5 (Invitrogen; diluted 1:5000 in 1:1 solution of PBS-T and Blocking Buffer). After washing with PBS containing 0.5% Tween-20 (PBS-T), blots were incubated with the secondary antibody IRDye 800CW-conjugated goat anti-mouse (LI-COR Biosciences, Lincoln, NE, USA; diluted 1:8000 in 1:1 solution of PBS-T and Blocking Buffer) for 1 hour at room temperature, washed with PBS-T, and scanned using an Odyssey Infrared Imaging System (LI-COR Biosciences). Band intensities were quantified by densitometry with local background subtraction using LI-COR Odyssey Software, version 2.0.

2.6 Statistical Analysis

Statistical analyses were carried out using IBM Statistics 22 software (SPSS, Chicago, IL, USA). Two-sample *t*-tests, assuming equal variances, ANOVAs with *post-hoc* Tukey's test

for pairwise comparisons or Pearson correlations were used according to the experimental design. PCR product synthesis for the 8c-het and hTau mouse data was modeled as a function of mouse genotype using a general linear model (GLM; (McCulloch, 2011)). Significance was judged at the level ($\alpha = 0.05$), two-sided.

3. RESULTS

3.1 Expression of wild-type human tau in transgenic mice down-regulates BDNF

hTau and 8c-het transgenic mice both express human, wild-type tau, but 8c-het mice are on a heterozygous mouse tau background whereas hTau mice are on a homozygous mouse tau knockout (KO) background (Andorfer, et al., 2003; Duff, et al., 2000). In both of these transgenic tau-expressing mouse models, BDNF mRNA was significantly down-regulated compared to non-transgenic control mice of the same background [hTau mice vs. wild-type mice (GLM and *post hoc* Tukey's test, $p=0.006$) and 8c-het mice vs. wild-type mice (GLM and *post hoc* Tukey's test, $p=0.017$); Figure 1]. BDNF mRNA levels, however, did not differ between TauKO mice and non-transgenic controls (GLM and *post hoc* Tukey's test, $p=0.537$, Figure 1).

3.2 Soluble tau-induced BDNF down-regulation is independent of age

Tau-induced neurotoxicity is thought to precede the formation of NFTs (Gomez-Isla, et al., 1996; Yoshiyama, et al., 2007). Loss of BDNF has been reported to be an early event in the progression of AD, occurring in mild cognitive impairment (MCI) and in mouse models of amyloid- β and tau over-expression prior to the formation of plaques and NFTs (Francis, et al., 2012; Peng, et al., 2005). In this study, mice sampled were between 3 and 16 months of age. This age range was intentionally large to determine if BDNF expression preceded the development of NFTs in the hTau animals. Our findings support the early loss of BDNF expression in both 8c-het and hTau mouse models. There was no correlation between age and BDNF expression in non-transgenic Swiss Webster/B6D2F1 control animals (Pearson correlation $r=0.22$, $p=0.438$; Figure 2A), 8c-het mice (Pearson correlation $r=-0.24$, $p=0.345$; Figure 2B) or hTau mice (Pearson correlation $r=-0.12$, $p=0.690$; Figure 2C). While too few animals were available to obtain statistically significant differences in BDNF expression at each age examined, the lack of correlation between BDNF expression and age within each mouse genotype, and the overall reduction in BDNF expression when ages were averaged within each genotype (Figure 1), suggest that tau-induced BDNF loss is independent of age and occurs early in development. Furthermore, our results support the toxicity of soluble tau, prior to the formation of NFTs in hTau mice (Figure 2).

3.3 Over-expression of wild-type tau in transfected human cells down-regulates BDNF Transcript IV

Full-length human tau-transfected, differentiated SH-SY5Y cells expressed significant amounts of V5 protein compared to non-transfected, V5-negative controls (Student's t-test, $p<0.00001$; Figure 3A), which was indicative of the presence of hTau40-V5-pcDNA3.2 plasmid in the transfected cells following differentiation.

BDNF mRNA was significantly down-regulated in wild-type human tau (hTau40)-transfected SH-SY5Y cells compared to non-transfected controls (Student's *t*-test, $p < 0.0001$; Figure 3B). Additionally, BDNF Transcript IV mRNA was significantly decreased in these wild-type tau over-expressing human neuroblastoma cells compared to controls (Student's *t*-test, $p < 0.0001$; Figure 3C).

3.4 Tau partially mediates A β -induced BDNF down-regulation

BDNF mRNA was significantly reduced in APP23 mice compared to wild-type animals (one-way ANOVA and *post hoc* Tukey's test, $p = 0.017$; Figure 4). In agreement with previous reports of A β -induced BDNF down-regulation *in vitro* and *in vivo* (Garzon and Fahnestock, 2007; Tong, et al., 2004), the over-expression of A β in APP23 mice was sufficient to significantly decrease BDNF expression. Crossing APP23 mice with TauKO mice partially rescued BDNF levels, as BDNF expression in APP23xTauKO mice was intermediate between non-transgenic control mice and APP23 mice and was not statistically different from either non-transgenic control mice of the same background (one-way ANOVA and *post hoc* Tukey's test, $p = 0.162$; Figure 4) or TauKO mice (one-way ANOVA and *post hoc* Tukey's test, $p = 0.990$; Figure 4).

4. DISCUSSION

In neurodegenerative disease, although the primary insult may be protein accumulation and aggregation, ultimately cognitive dysfunction and neurodegeneration may result from alterations in tau (Gotz, 2001; Iqbal and Grundke-Iqbal, 2008; Lewis, et al., 2001; Masliah, et al., 2001; Pennanen and Gotz, 2005; Roberson, et al., 2007). However, mechanisms that lead from alterations in tau to cognitive decline associated with AD are not clear. We previously showed that there is decreased BDNF mRNA and protein in the parietal cortex of human tauopathy (PiD and CBD) patients (Belrose, et al., 2014). In tauopathies, we reported down-regulation of BDNF transcript IV, which is largely regulated by cyclic AMP response element binding protein (CREB)-mediated transcription (Pruunsild, et al., 2011; Shieh, et al., 1998; Tao, et al., 1998). In the present study, we demonstrate that soluble, wild-type tau significantly down-regulates BDNF transcription *in vitro* and *in vivo*. Furthermore, we determine that tau down-regulates BDNF transcript IV *in vitro*. Finally, we show that tau knockout at least partially rescues A β -induced BDNF down-regulation. These data demonstrate that tau alone, and as a mediator of A β toxicity, is capable of down-regulating BDNF, which likely contributes to learning and memory deficits characteristic of AD.

BDNF is critical for synaptic plasticity, cognition, and memory consolidation, and for the survival of neurons that degenerate in a number of neurodegenerative disorders (Alderson et al., 1990; Hyman et al., 1991; Knusel et al., 1991; Altar et al., 1997; Lu, 2003; Baquet et al., 2005; Allen et al., 2011; Fahnestock, 2011). In AD in particular, BDNF expression is decreased early in the progression of the disease, and the amount of BDNF decrease is directly correlated with the degree of cognitive decline (Francis, et al., 2012; Peng, et al., 2005). In this study, we show that tau-induced BDNF down-regulation is independent of the formation of NFTs. It has been shown that significant synaptic loss can occur prior to NFT formation (Yoshiyama, et al., 2007). Furthermore, in AD before the formation of NFTs,

neuronal loss can be detected along with the presence of tau oligomers (Gomez-Isla, et al., 1996), suggesting that soluble, aggregated tau species are neurotoxic. In addition, both 8c-het mice (Andorfer, et al., 2005) and a *Drosophila* tauopathy model (Wittmann, et al., 2001) exhibit neurodegeneration despite their lack of NFT-like tau pathology. Lastly, hTau mice begin to experience neuronal cell loss prior to the onset of NFT pathology, supporting the notion that NFTs are not the primary cause of neurodegeneration (Andorfer, et al., 2005). However, the mechanism of soluble tau-induced cell death is not clear. In this study, we have shown that both 8c-het and hTau mice down-regulate BDNF, and moreover, that their reduction of BDNF is roughly equal. These findings provide further evidence that NFTs are not required for toxicity. Rather, soluble tau exerts its toxicity, at least in part, via reduction in BDNF expression. In addition, while the transgenic animals examined in this study differ by the presence or absence of NFTs, both 8c-het and hTau mice are known to express hyperphosphorylated tau protein (Duff et al., 2000; Andorfer et al., 2003). Tau hyperphosphorylation is thought to be the most relevant and impactful post-translational modification of tau, decreasing its ability to promote microtubule polymerization (Lindwall and Cole, 1984) and promoting the somatodendritic mislocalization of tau into the soma (Delacourte and Buee, 2000; Goedert 2004). This suggests that while NFTs are not required for BDNF down-regulation, tau hyperphosphorylation, which is present in both transgenic animal models examined, may play an important role in tau-induced BDNF down-regulation. Our current findings also indicate that tau-induced BDNF down-regulation is an early event, which takes place prior to the formation of NFTs in hTau mice, consistent with previous reports of neurodegeneration in this model (Allred, et al., 2012; Andorfer, et al., 2005). The present report is significant in that we demonstrate early and persistent neurotrophin dysregulation by soluble tau, prior to, and independent of, the formation of NFTs.

We also demonstrate that expression of human wild-type tau devoid of mutations is sufficient to cause neurotrophin dysregulation. Historically, the majority of research into understanding tau toxicity has focused on both transgenic animal and cell culture models harboring disease-causing tau mutations, such as transgenic mice expressing the P301L tau mutation (Lewis, et al., 2000) or mutated Tau441 cell lines (Loffler, et al., 2012). In contrast, the current study focuses on wild-type tau toxicity, which highlights that without any mutation, the expression of wild-type human tau, whether induced by A β or some other toxic insult, is capable of down-regulating BDNF. This result is highly relevant to understanding the pathophysiology of sporadic AD, by far the most common form of this neurodegenerative disorder.

Importantly, BDNF transcript IV (formerly transcript III) is down-regulated in human AD cortical tissue (Garzon, et al., 2002), in mouse models of AD (Peng, et al., 2009) and in SH-SY5Y cells treated with A β (Garzon and Fahnstock, 2007). BDNF transcript IV, one of 17 different BDNF transcripts, accounts for approximately half of the total BDNF mRNA found in the cortex (Garzon and Fahnstock, 2007; Pruunsild, et al., 2007). BDNF transcript IV is transcriptionally regulated by CREB (Pruunsild, et al., 2011; Shieh, et al., 1998; Tao, et al., 1998). CREB transcription is reduced in AD post-mortem hippocampal tissue, in A β -treated rat hippocampal neurons (Pugazhenth, et al., 2011) and in A β -treated SH-SY5Y cells (Rosa and Fahnstock, 2015). The current investigation demonstrates that over-expression of tau

alone is capable of down-regulating BDNF and that it does so via transcript IV, which is in parallel to our previous finding that amyloid- β also significantly down-regulates BDNF transcript IV (Garzon and Fahnstock, 2007).

This study furthermore demonstrates that A β -induced BDNF down-regulation is at least partially rescued by depletion of tau in APP23xTauKO mice. Prior to the current investigation, it was known that increased A β down-regulates BDNF in both cell culture (DaRocha-Souto, et al., 2012; Yoshiyama, et al., 2007; Garzon and Fahnstock 2007; Rosa and Fahnstock 2015) and in transgenic mice (Peng, et al., 2009). The latter is further supported by our current findings that APP23 transgenic mice, which over-express A β , also experience a significant reduction in BDNF mRNA compared to non-transgenic control animals. However, tau is thought to be down-stream of A β and therefore a more direct mediator of neurodegeneration (Ittner, et al., 2010; Lewis, et al., 2001; Masliah, et al., 2001; Pennanen and Gotz, 2005). Specifically, it has been shown that NFT-like changes can be induced by A β *in vivo* (Gotz, et al., 2001) and *in vitro* (Busciglio, et al., 1995). Furthermore, A β -induced neurodegeneration is prevented in primary neuronal cultures from TauKO mice (Rapoport, et al., 2002), and knocking out tau in a transgenic AD mouse model can block A β -induced cognitive impairments (Roberson, et al., 2007). Whether the rescue of cognitive impairment is mediated by BDNF was not tested. In this study we demonstrate that tau mediates A β -induced BDNF down-regulation, as knocking out tau in APP23 mice restores BDNF levels nearly to control values. BDNF levels in APP23xTauKO animals are not statistically significantly different from controls or from TauKO mice; however, they are intermediate between APP23 and control animals. Thus, while our findings show that tau mediates A β -induced BDNF down-regulation, we cannot rule out the possibility that A β also affects BDNF levels independently of tau. We have previously shown that A β significantly down-regulates BDNF via down-regulation of CREB transcription (Rosa and Fahnstock 2015). However, the mechanism of tau-induced BDNF down-regulation appears to be independent of CREB transcription or activation and is not mediated by either decreased CREB-binding protein (CBP) or inactive CREB-regulated transcription co-activator 1 (CRTC1) (*data not shown*). While these findings do not provide a singular mechanism of tau-induced BDNF down-regulation alone, they do suggest that A β and tau may work both together and independently to down-regulate BDNF.

Loss of BDNF in neurodegenerative disorders is a key mediator of synaptic dysfunction, neurodegeneration and subsequent cognitive decline. Our current findings reveal that expression of soluble, wild-type tau, devoid of pathological mutations and in the absence of NFTs, down-regulates BDNF. Furthermore, tau at least partially mediates A β -induced BDNF down-regulation. These results, as well as our previously published findings (Garzon and Fahnstock, 2007; Peng, et al., 2009; Rosa and Fahnstock, 2015), suggest that both A β and tau are responsible for BDNF down-regulation. Moreover, our finding that BDNF loss may mediate tau neurotoxicity down-stream of A β has profound implications for therapeutic intervention in AD. Clinical trials targeting A β have, so far, been ineffective (Soejitno et al., 2015; Gody et al., 2016). This suggests that current monotherapies used to alleviate AD symptoms by targeting A β pathology alone may not be sufficient, and that combined treatments targeting multiple molecules, including tau, may be desirable (Mufson et al., 2016). Lastly, the recent discovery that the pathological accumulation of tau protein is a

consequence of protein aggregation in a variety of neurodegenerative diseases (Yang et al., 2003; Lei et al., 2010; Constantinescu et al., 2011; Fernandez-Nogales et al., 2014; Sengupta et al., 2015) suggests that tau-induced BDNF down-regulation is a common mechanism of cognitive dysfunction in these disorders, and consequently, our findings have implications beyond the scope of AD.

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ABBREVIATIONS

AD	Alzheimer's disease
Aβ	Amyloid-beta
ALS	Amyotrophic lateral sclerosis
BDNF	Brain-derived neurotrophic factor
CBD	Corticobasal degeneration
CBP	CREB-binding protein
CRTC1	CREB-regulated transcription coactivator 1
CREB	Cyclic AMP response element binding protein
DMEM	Dulbecco's modified Eagle's medium
FTDP-17	Frontotemporal dementia and parkinsonism linked to chromosome 17
GLM	General linear model
hTau	Human tau
HD	Huntington's Disease
NFT	Neurofibrillary tangle
PD	Parkinson's Disease
PiD	Pick's disease
PSP	Progressive supranuclear palsy
proNGF	Pro-nerve growth factor
qRT-PCR	Quantitative Real-time Reverse Transcription-Polymerase Chain Reaction
SDS	Sodium dodecyl sulfate

TauKO Tau knockout**REFERENCES**

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Highlights

- Excess or pathological tau down-regulates BDNF and BDNF Transcript4 expression *in vivo* and *in vitro*
- Non-mutated tau down-regulates BDNF prior to the formation of NFTs
- Tau knockout rescues A β -induced BDNF down-regulation
- Tau-induced BDNF down-regulation could contribute to memory loss in many disorders

Figure 1. Expression of wild-type tau in transgenic mice down-regulates BDNF expression
 Both 8c-het (* $p=0.017$) and hTau (** $p=0.006$) transgenic mice show significantly down-regulated BDNF mRNA compared to non-transgenic controls. There was no significant difference in BDNF expression between TauKO mice and non-transgenic controls ($p=0.537$). Generalized Linear Model (GLM) and *post hoc* Tukey's test. Error bars represent S.E.M. Animals ranged in age from 3–16 months, as indicated in Table 1. $n=11-15$ /group.

Figure 2. Tau-induced BDNF loss is an early event, prior to formation of NFTs in hTau mice
 Both 8c-het and hTau transgenic mice show significantly down-regulated BDNF expression, compared to control mice, which appears to begin at the earliest developmental age tested. There is no correlation between BDNF expression (normalized to β -actin) in these tau-expressing transgenic models with age: **A**) non-transgenic control mice (Pearson correlation $r=0.22$, $p=0.438$, **B**) 8c-het mice (Pearson correlation $r=-0.24$, $p=0.345$) or **C**) hTau animals (Pearson correlation $r=-0.12$, $p=0.690$)

Figure 3. Human neuroblastoma cells over-expressing wild-type human tau down-regulate BDNF and BDNF Transcript IV expression

A) SH-SY5Y cells transfected with full-length hTau40-V5-pcDNA3.2 plasmid (H) express V5-tagged protein that was absent from non-transfected control cells (C) (Student's *t*-test, ***p*<0.00001). Tau over-expressing SH-SY5Y cells express significantly decreased **B)** BDNF mRNA (normalized to β-actin) compared to controls (Student's *t*-test, ***p*<0.0001) and **C)** BDNF Transcript IV mRNA (normalized to β-actin) compared to controls (Student's *t*-test, ***p*<0.0001). Error bars represent S.E.M. n=9/group

Figure 4. Tau partially mediates A β -induced down-regulation of BDNF expression

Over-expression of A β in APP23 transgenic mice resulted in a significant reduction of BDNF mRNA compared to non-transgenic animals (one-way ANOVA and *post hoc* Tukey's test * $p=0.017$). However, APP23xTauKO mice expressed levels of BDNF mRNA that were intermediate between wild-type and APP23 mice and were no different from non-transgenic animals (one-way ANOVA and *post hoc* Tukey's test $p=0.162$). Error bars represent S.E.M. $n=5-9$ /group

Table 1

Distribution of tau transgenic samples assessed by genotype and age.

	3–5 months	6–10 months	11–16 months
Non-transgenic	n=6	n=5	n=4
8c-het	n=4	n=5	n=2
hTau	n=4	n=4	n=5