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Microarray analysis of CA1 pyramidal neurons in a mouse model of tauopathy reveals progressive synaptic dysfunction

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Abstract

The hTau mouse model of tauopathy was utilized to assess gene expression changes in vulnerable hippocampal CA1 neurons. CA1 pyramidal neurons were microaspirated via laser capture microdissection followed by RNA amplification in combination with custom-designed microarray analysis and qPCR validation in hTau mice and nontransgenic (ntg) littermates aged 11-14 months. Statistical analysis revealed ~8% of all the genes on the array platform were dysregulated, with notable downregulation of several synaptic-related markers including synaptophysin (*Syp*), synaptotagmin, and synaptobrevin, among others. Downregulation was also observed for select glutamate receptors (*GluRs*), *Psd-95*, *TrkB*, and several protein phosphatase subunits. In contrast, upregulation of tau isoforms and a calpain subunit were found. Microarray assessment of synaptic-related markers in a separate cohort of hTau mice at 7-8 months of age indicated only a few alterations compared to the 11-14 month cohort, suggesting progressive synaptic dysfunction occurs as tau accumulates in CA1 pyramidal neurons. An assessment of SYP and PSD-95 expression was performed in the hippocampal CA1 sector of hTau and ntg mice via confocal laser scanning microscopy along with hippocampal immunoblot analysis for protein-based validation of selected microarray observations. Results indicate significant decreases in SYP-immunoreactive and PSD-95-immunoreactive puncta as well as downregulation of SYP-immunoreactive and PSD-95-immunoreactive band intensity in hTau mice compared to age-matched ntg littermates. In summary, the high prevalence of downregulation of synaptic-related genes indicates that the moderately aged hTau mouse may be a model of tau-induced synaptodegeneration, and has profound effects on how we perceive progressive tau pathology affecting synaptic transmission in AD.

Keywords

Alzheimer's disease; hippocampus; laser capture microdissection; PSD-95; RNA amplification; synaptophysin; TrkB

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting >5.4 million people and is the 6th leading cause of death in the USA and AD-associated costs exceed \$183 billion dollars annually (Thies and Bleiler, 2011). AD is characterized by pathological hallmarks including extracellular accumulation of amyloid-beta (A β) peptides in senile plaques, and intracellular accumulation of the microtubule-associated protein tau in neurofibrillary tangles (NFTs). In addition, hippocampal disconnection is an invariant pathological hallmark of AD along with regionally selective synaptic loss (Hyman et al., 1984; Kordower et al., 2001). Tau protein is principally found within axons in normal brain, and is believed to promote microtubule assembly and stabilization (Ballatore et al., 2007; Duff, 2006). Tau localization to dendrites has also been identified, and is impacted by A β oligomers (Zempel et al., 2010). In AD, tau is redistributed into somatodendritic compartments, which include intracellular aggregates of NFTs formed by hyperphosphorylation and conformational alterations of tau (Binder et al., 2005; Cripps et al., 2006; Fischer et al., 2009; Jicha et al., 1997; Mondragon-Rodriguez et al., 2008). Tau hyperphosphorylation and intracellular accumulation may be directly related to synaptic loss, neuronal loss, and cognitive deficits in tauopathies (Binder et al., 2005; Duff, 2006; Hyman et al., 1984, 1990; Mufson et al., 2006).

A goal is to understand alterations in gene expression levels, and ultimately their protein products, in a mouse model of tauopathy, within a vulnerable cell type seen in AD. We are employing the hTau mouse model for single population expression profiling studies (Ginsberg, 2008; Ginsberg et al., 2006a). hTau mice are a mouse model of progressive tauopathy generated by overexpressing six human tau isoforms on a mouse null tau background (Andorfer et al., 2003; Duff et al., 2000). hTau mice develop pathological alterations including age-related phosphorylated intracellular tau accumulation within hippocampal and neocortical neurons (Andorfer et al., 2003, 2005). Delivery of minocycline, a derivative of tetracycline, reduces tau phosphorylation and aggregation (Noble et al., 2009), making this an attractive model to assess potential therapeutic interventions. hTau mice also develop moderate age-related dendritic alterations and cognitive deficits (Dickstein et al., 2010; Polydoro et al., 2009). Specifically, neuropathology progresses in the forebrain, and learning and memory deficits associated with cortical and hippocampal function manifest by 12 months of age (Polydoro et al., 2009).

The present study was undertaken to delineate cellular and molecular events that occur in hTau mice at two timepoints to help understand mechanisms that underlie neurodegeneration in human tauopathies, including AD. We employed custom-designed microarray analysis to evaluate multiple classes of transcripts relevant to neuroscience and neurodegeneration similar to our approach using postmortem human brains (Alldred et al., 2008, 2009; Ginsberg et al., 2006a, 2006b, 2010). Confirmation studies were performed using hTau and nontransgenic (ntg) littermates for select genes that demonstrated differential expression on the microarray platform via real-time quantitative PCR (qPCR). Furthermore, immunocytochemical evaluation of several synaptic-related marker protein products found to be dysregulated by microarray and qPCR assessments was performed via confocal laser scanning microscopy and immunoblot analysis.

Materials and Methods

Tissue preparation for microarray analysis

Animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Nathan Kline Institute/NYU Langone Medical Center and were in full accordance with NIH guidelines. A cohort of hTau (n = 15; 8M/7F) and ntg (n = 11; 6M/5F) mice 11-14 months of age and a cohort of hTau (n = 7; 4M/3F) and ntg (n = 7; 3M/4F) mice 7-8 months of age were given an overdose of ketamine and xylazine and perfused transcardially with ice-cold 4% paraformaldehyde buffered in 0.15 M phosphate buffer. Tissue blocks containing the dorsal hippocampus were paraffin embedded and 6 μ m-thick tissue sections were cut in the coronal plane on a rotary microtome for immunocytochemistry as described previously (Ginsberg, 2005a, 2010). RNase-free precautions were used throughout the experimental procedures, and solutions were made with 18.2 mega Ohm RNase-free water (Nanopure Diamond, Barnstead, Dubuque, IA).

Immunocytochemistry to identify CA1 neurons for subsequent microdissection was performed as described previously (Alldred et al., 2008; Ginsberg, 2005a; Ginsberg et al., 2010). Briefly, deparaffinized tissue sections were blocked in an RNase-free 0.1 M Tris (pH 7.6) solution containing 2% donor goat serum (DGS) and 0.01% Triton X-100 for 1 hour and then incubated with a primary monoclonal antibody directed against neurofilaments (RMdO20) which recognizes an epitope on the poorly phosphorylated NF-M/NF-H subunits (Lee et al., 1987) at a 1:200 dilution in a 0.1 M Tris/2% DGS solution overnight at 4 °C in a humidified chamber. An adjacent series was incubated with a primary monoclonal antibody directed against hyperphosphorylated tau (PHF1; a gift of Dr. Peter Davies; Jicha et al., 1997) at a 1:100 dilution. Sections were processed with the ABC kit (Vector Labs, Burlingame, CA) and developed with 0.05% diaminobenzidine, 0.03% hydrogen peroxide, and 0.01 M imidazole in Tris buffer for 10 minutes. Neurofilament-immunostained tissue sections were not coverslipped or counterstained, and were immersed in RNase-free 0.1 M Tris for microaspiration and subsequent TC RNA amplification.

Single cell microaspiration and Terminal Continuation (TC) RNA amplification

LCM and TC RNA amplification procedures have been described in detail previously by our group (Alldred et al., 2008, 2009; Che and Ginsberg, 2004; Ginsberg, 2010; Ginsberg et al., 2010). Individual CA1 pyramidal neurons were microaspirated via LCM (Arcturus PixCell Ite, Applied Biosystems, Foster City, CA; Fig. 1A-D). Fifty cells were captured per reaction for population cell analysis (Alldred et al., 2008; Ginsberg, 2010; Ginsberg et al., 2010). A total of 2-6 microarrays (containing 50 LCM-captured CA1 neurons each) were performed per mouse brain. Reproducibility and linearity of the TC RNA amplification procedure has been published previously, including the use of CA1 neurons as input sources of RNA (Alldred et al., 2008; Che and Ginsberg, 2004; Ginsberg, 2008). The TC RNA amplification protocol is available at <http://cdr.rfmh.org/pages/ginsberglabpage.html>. This method entails synthesizing first strand cDNA complementary to the RNA template, re-annealing the primers to the cDNA, and finally *in vitro* transcription using the synthesized cDNA as a template (Fig. 1E). Microaspirated CA1 neurons were homogenized in 500 μ l of Trizol reagent (Invitrogen, Carlsbad, CA), extracted with chloroform, and precipitated utilizing isopropanol (Alldred et al., 2009). RNAs were reverse transcribed in a solution containing poly d(T) primer (100 ng/ μ l) and TC primer (100 ng/ μ l) in 1 $\times$ first strand buffer (Invitrogen), 2 μ g of linear acrylamide (Applied Biosystems), 0.5 mM dNTPs, 5 μ M DTT, 20 U of SuperRNase Inhibitor (Applied Biosystems), and 200 U of reverse transcriptase (Superscript III, Invitrogen). Single-stranded cDNAs were then subjected to RNase H digestion and re-annealing of the primers to generate cDNAs with double-stranded regions at the primer interfaces. Single stranded cDNAs were digested by adding the following and

then placed in a thermal cycler: 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, and 10 U RNase H (Invitrogen) in a final volume of 100 µl. RNase H digestion step at 37 °C, 30 minutes; denaturation step 95 °C, 3 minutes; primer re-annealing step 60 °C, 5 minutes (Che and Ginsberg, 2004). Samples were purified by column filtration (Montage PCR filters; Millipore, Billerica, MA). Column reservoirs were filled with 300 µl of 18.2 mega Ohm RNase-free water and the cDNA reaction was then added to the reservoir. The columns were then spun at 1000 × g for 15 minutes. To recover the cDNA, 20 µl of 18.2 mega Ohm RNase-free water was added to the columns, and the columns were inverted into clean microfuge tubes and spun at 1000 × g for 2 minutes (Aldred et al., 2008, 2009). Hybridization probes were synthesized by *in vitro* transcription using ³³P incorporation in 40 mM Tris (pH 7.5), 6 mM MgCl₂, 10 mM NaCl, 2 mM spermidine, 10 mM DTT, 2.5 mM ATP, GTP and CTP, 100 µM of cold UTP, 20 U of SuperRNase Inhibitor, 2 KU of T7 RNA polymerase (Epicentre, Madison, WI), and 120 µCi of ³³P-UTP (Perkin-Elmer, Boston, MA) (Ginsberg, 2005b, 2008). The reaction was performed at 37 °C for 4 hours. Radiolabeled TC RNA probes were hybridized to custom-designed cDNA arrays without further purification.

Custom-designed cDNA array platforms and array hybridization

Array platforms consist of 1 µg of linearized cDNA purified from plasmid preparations adhered to high-density nitrocellulose (Hybond XL, GE Healthcare, Piscataway, NJ) using an arrayer robot (VersArray, Bio-Rad, Hercules, CA) (Ginsberg, 2005b; Ginsberg, 2008). Each cDNA and/or expressed sequence-tagged cDNA (EST) was verified by sequence analysis and restriction digestion. Mouse and human clones were employed on the custom-designed array. Notably, all of the tau isoforms were derived from human sequences. Approximately 576 cDNAs/ESTs were utilized on the current array platform, organized into 19 gene ontology groups (Table I). The majority of genes are represented by one transcript on the array platform, although the neurotrophin receptors *TrkA*, *TrkB*, and *TrkC* are represented by ESTs that contain the extracellular domain (ECD) as well as the tyrosine kinase domain (TK) (Ginsberg et al., 2010; Ginsberg et al., 2006b).

Arrays were prehybridized (4 hours) and hybridized (16 hours) in a solution consisting of 6× saline–sodium phosphate–ethylenediaminetetraacetic acid (SSPE), 5× Denhardt's solution, 50% formamide, 0.1% sodium dodecyl sulfate (SDS), and denatured salmon sperm DNA (200 µg/ml) at 42 °C in a rotisserie oven (Che and Ginsberg, 2004; Ginsberg, 2008). Following hybridization, arrays were washed sequentially in 2× SSC/0.1% SDS, 1× SSC/0.1% SDS and 0.5× SSC/0.1% SDS for 15 min each at 37 °C. Arrays were placed in a phosphor screen for 24 hours and developed on a phosphor imager (GE Healthcare). All array phosphor images were adjusted to the same brightness and contrast levels for data acquisition and analysis.

Data collection and statistical analysis for custom-designed microarrays

Hybridization signal intensity was determined by utilizing ImageQuant TL (GE Healthcare). This array analysis program quantifies signal intensity, subtracts background by utilizing a spot edge average for each clone, and normalizes hybridization signal intensity. Statistical procedures for custom-designed microarray analysis have been described in detail previously (Ginsberg, 2007, 2009; Ginsberg and Mirnics, 2006). Briefly, expression of TC amplified RNA bound to each linearized cDNA (576 cDNAs/ESTs on the array platform) minus background was expressed as a ratio of the total hybridization signal intensity of the array (a global normalization approach). Global normalization effectively minimizes variation due to differences in the specific activity of the synthesized probe and absolute probe quantity (Eberwine et al., 2001; Ginsberg, 2008). These data do not allow the absolute quantitation of transcript levels. Rather, an expression profile of relative changes in mRNA

levels was generated. Relative changes in total hybridization signal intensity were analyzed by one-way analysis of variance (ANOVA) with post-hoc analysis (Neumann-Keuls test) and correction for multiple observations for individual comparisons. The level of statistical significance was set at the more conservative ($p < 0.01$, two-sided; p -values ($p < 0.02 - p < 0.05$ were considered as trends) to account for the large number of analyses performed (Ginsberg, 2010; Ginsberg et al., 2010). p -values were also adjusted using a false discovery rate controlling procedure (Reiner et al., 2003) to reduce sampling (Type I error) due to the large number of genes being analyzed simultaneously. Expression levels were graphed using a bioinformatics software package (GeneLinker Gold, Predictive Patterns, Kingston, ON).

qPCR

qPCR was performed on microdissected paraffin-embedded tissue sections {adjacent to the tissue sections used for microarray analysis (Alldred et al., 2008; Ginsberg, 2008)} containing the hippocampal CA1 region from 11-14 month hTau ($n = 9$; 5M/4F) and ntg ($n = 7$; 3M/4F) mice. Taqman qPCR primers (Applied Biosystems, Foster City, CA) were utilized for the following genes: *Arc* (mM00479619_g1), synaptophysin (*Syr*; mM00436854_m1), *TrkB* (mM01341760_m1), *Gria1* (mM01342711_m1), *Gria2* (mM01220174_m1), *Gria3* (mM01322408_m1), and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*; mM99999915_g1). Samples were assayed on a real-time qPCR cyclor (7900HT, Applied Biosystems) in 96-well optical plates as described previously (Alldred et al., 2008; Ginsberg et al., 2006b, 2010; Jiang et al., 2010). Standard curves and cycle threshold (Ct) were generated using standards obtained from total mouse brain RNA. The ddCT method was employed to determine relative gene level differences between hTau and ntg mice with *Gapdh* qPCR products used as a control (ABI, 2004; Alldred et al., 2008; Ginsberg et al., 2010; Jiang et al., 2010). A total of 3-5 independent samples per subject were run in triplicate for the qPCR assessments. Negative controls consisted of the reaction mixture without input RNA. Alterations in PCR product synthesis were analyzed by one-way ANOVA with post-hoc analysis (Neumann-Keuls test; level of statistical significance was set at $p < 0.05$).

Confocal laser scanning microscopy

hTau ($n = 8$) and ntg ($n = 6$) mice 12-14 months of age were perfused transcardially with ice-cold 4% paraformaldehyde and post fixed for 2 hours in 4% paraformaldehyde followed by immersion in sucrose solutions (12%, 18%, and 30% in PBS for 24 hours each) prior to frozen sectioning in a cryostat. Tissue sections were cut at 20 μ m for immunohistochemical labeling and mounted onto Superfrost plus glass slides (Thermo Fisher Scientific, Waltham, MA). Double label immunofluorescence for subsequent confocal microscopy was performed as described previously (Alldred et al., 2005; Jiang et al., 2010). Briefly, tissue sections were permeabilized with 0.3% Triton X-100, 10% normal horse serum (NHS) in PBS for 1 hour and then incubated with primary antibodies directed against PSD-95 (MA-0146; monoclonal; Thermo Fisher Scientific, Waltham, MA; 1:400 dilution) and synaptophysin (101-002; rabbit polyclonal; Synaptic Systems, Goettingen, Germany; 1:200 dilution) overnight in 0.05% Triton X-100, 10% NHS in PBS at 4 °C in a humidified chamber. Sections were washed three times in PBS containing 0.05% Triton X-100 and incubated with secondary antibodies coupled to Alexa 488 and Alexa 594 dyes, respectively (Molecular Probes Invitrogen, Eugene, OR). Sections were washed in PBS and coverslipped with buffered glycerol. Control experiments included omission of primary antisera and swapping Alexa dyes for the respective primary antibodies (Alldred et al., 2005; Ginsberg et al., 1995; Jiang et al., 2010).

Fluorescent images were captured with a confocal laser scanning microscope (LSM 510 Meta, Carl Zeiss, Thornwood, NY) with a Plan-Apochromat 100 $\times$ (N.A. 1.4) objective.

PSD-95-immunoreactive and synaptophysin-immunoreactive puncta were assessed using an unbiased estimation approach within stacks of optical z-sections acquired by confocal microscopy. Image acquisition parameters were adjusted for each section (1024×1024), with one slice analyzed within each of two z-stacks (10 optical slices per $10 \mu\text{m}$) taken per section. Three sections were analyzed per subject (a total of 6 slices per subject were employed). AxioVision Automeasure software (Carl Zeiss) was utilized for stereologic assessments, measuring area (μm^2), total puncta number, and mean intensity of the puncta. Area, number and intensity of puncta were quantified if they measured within the target area between $0.04 \mu\text{m}^2$ and $3.0 \mu\text{m}^2$. The mean signal intensity was compared to background measurements taken for each image (Alldred et al., 2005; Jiang et al., 2010). Alterations in immunoreactive puncta intensity, size, and number were analyzed by one-way ANOVA with post-hoc analysis (Neumann-Keuls test; level of statistical significance was set at $p < 0.05$).

Immunoblot analysis

Frozen microdissected CA1 sector hippocampal tissue samples obtained from hTau and ntg mice aged 3-5 months (hTau; $n = 10$; 5M/5F) (ntg; $n = 5$; 3M/2F), 7-10 months (hTau; $n = 8$; 5M/3F) (ntg; $n = 6$; 3M/3F), and 12-16 months (hTau; $n = 11$; 7M/4F) (ntg; $n = 9$; 5M/4F). Hippocampal tissues were homogenized in a 20 mM Tris-HCl (pH 7.4) buffer containing 10% (w/v) sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), 5 mM ethylene glycol-bis (β -aminoethylether)-N,N,N',N'-tetra-acetic acid (EGTA), 2 mg/ml of the following: (aprotinin, leupeptin, and chymostatin), 1 mg/ml of the following: {pepstatin A, antipain, benzamidine, and phenylmethylsulfonyl fluoride (PMSF)}, 100 $\mu\text{g/ml}$ of the following: {soybean trypsin inhibitor, Na-p-tosyl-L-lysine chloromethyl ketone (TLCK), and N-tosyl-L-phenylalanine chloromethyl ketone (TPCK)}, 1 mM of the following: (sodium fluoride and sodium orthovanadate) and centrifuged as described previously (Counts et al., 2004; Ginsberg, 2005a, 2010). All protease inhibitors were purchased from Sigma (St. Louis MO). Identical amounts of homogenates (10 μg) were loaded into a gel electrophoresis apparatus, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 4-15% gradient acrylamide gels; Bio-Rad), and transferred to nitrocellulose by electroblotting (Mini Transblot, Bio-Rad). Nitrocellulose membranes were blocked in blocking buffer (LiCor, Lincoln, NE) for 1 hour at 4°C prior to being incubated with antibodies directed against GRIA1 (101-002; rabbit polyclonal; Synaptic Systems; 1:1,000 dilution), GRIA2/3 (09-307; rabbit polyclonal; Millipore; 1:1,000 dilution), GRIN1 (NMDA R1; sc-1467; goat polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA; 1:1,000 dilution), PSD-95 (MA-0146; monoclonal; Thermo Fisher Scientific; 1:1,000 dilution), SYP (S-5768; monoclonal; Sigma; 1:1,000 dilution), tau (CP27; monoclonal; gift of Dr. Peter Davies; 1:2,500 dilution; (Cripps et al., 2006; Jicha et al., 1997), or β -tubulin (TUBB; monoclonal antibody; T-5293; Sigma, 1:1,000 dilution) in blocking buffer overnight at 4°C . Membranes were developed with affinity-purified secondary antibodies conjugated to IRDye 800 (Rockland, Gilbertsville, PA) and visualized using an infrared detection system (Odyssey, LiCor). Immunoblots were quantified by densitometric software supplied with the instrument. Signal intensity of immunoreactive bands was normalized to TUBB immunoreactivity for each assay. Alterations in immunoreactive band intensity were analyzed by one-way ANOVA with post-hoc analysis (Neumann-Keuls test; level of statistical significance was set at $p < 0.05$).

Results

Microarray analysis identifies selectively altered genes including synaptic-related markers and glutamate receptors within CA1 neurons in aged hTau mice

CA1 pyramidal neurons in hTau mice displayed profuse intracellular accumulation of phosphorylated tau as compared to ntg littermates (Fig. 1F, G). Expression profiling was performed on a total of 90 custom-designed microarrays for hTau and ntg mice 11-14 months of age (50 CA1 neurons were analyzed per microarray) following the TC RNA amplification protocol. Quantitative analysis revealed differential regulation of 48 genes out of a total 576 (8.3%) on the custom-designed array (Table II). Notably, expression profiling results show upregulation of all 6 human isoforms of tau in the hTau mouse model compared to ntg littermates (Fig 2A). This observation effectively serves as a positive control, and is consistent with previous results from the hTau mouse that demonstrated an increase in tau protein expression levels (Andorfer et al., 2003, 2005). No significant effect of gender was detected in the cohorts of hTau and ntg mice.

Classes of transcripts that were selectively downregulated included synaptic-related markers and glutamate receptors (GluRs), indicating deficits in excitatory synaptic transmission. Alterations included genes encoding proteins located within presynaptic and postsynaptic membranes. Specifically, several synaptic-related genes displayed downregulation in aged hTau CA1 neurons including synapsin 3 (*Syn3*; 1.8 fold; $p < 0.004$), synaptobrevin (*Syb*; 2.2 fold; $p < 0.003$), synaptotagmin (*Synj*; 2.2 fold; $p < 0.001$), synaptosomal-associated protein 29 kD (*Snapt29*; 1.9 fold; $p < 0.004$), syntaxin 4A (*Stx4a*; 2.8 fold; $p < 0.001$), syntaxin 7 (*Stx7*; 1.8 fold; $p < 0.002$), and synaptophysin (*Syp*; 1.7 fold; $p < 0.01$; Fig. 2B). In addition, GluRs that were downregulated included α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunits *Gria1* (1.6 fold; $p < 0.006$), *Gria2* (2 fold; $p < 0.001$), and the kainate (KA) receptor *Grik4* (1.6 fold; $p < 0.006$; Fig. 2C). There was no differential regulation of AMPA receptors *Gria3*, *Gria4*, N-methyl-D-aspartate (NMDA) receptor subunits, and metabotropic GluRs (mGluRs). The GluR-interacting protein/postsynaptic density protein *Psd95* was also downregulated (1.6 fold; $p < 0.006$). No differential regulation of the GluR-interacting proteins *Grip1* or *Grip2* was observed (Fig. 2C).

Dysregulation of the brain derived neurotrophic factor (BDNF) receptor *TrkB* was also found within aged hTau CA1 neurons, including significant downregulation of the extracellular domain (*TrkBectd*; 2.3 fold; $p < 0.002$) and the tyrosine kinase domain (*TrkBtk*; 2.2 fold; $p < 0.003$). No significant alterations were found for gene expression of the pan NGF receptor *p75^{NTR}*, nerve growth factor receptor *TrkA* or the neurotrophin-3 receptor *TrkC* (Fig. 2D). Downregulation of fragile $\times$ mental retardation 1 gene (*Fmr1*; 1.9 fold; $p < 0.008$), G-protein q polypeptide (*Gnaq*; 2.7 fold; $p < 0.001$), and galanin receptor 1 (*Galr1*; 2.5 fold; $p < 0.002$) was found along with downregulation of the insulin-like growth factors *Igf1* (1.8 fold; $p < 0.01$), and *Igf2* (2 fold; $p < 0.01$). Additionally, genes within the GABAergic neurotransmission class displayed selective downregulation including *Gad67* (2.2 fold; $p < 0.001$) and GABA A receptor-associated protein like 1 (*Gabarap-1l*; 2 fold; $p < 0.004$). Interestingly, downregulation of genes that encode protein phosphatase (PP) PP1 and PP2 subunits that regulate tau dephosphorylation including PP1 catalytic subunit γ (*Ppp1cc*; 1.5 fold; $p < 0.01$), PP2 regulatory subunit α (*Ppp2r1a*; 2.4 fold; $p < 0.001$), and PP2 catalytic subunit α (*Ppp2ca*; 1.7 fold; $p < 0.01$; Fig. 2E) were observed along with downregulation of the kinases protein kinase C β 1 (*Prkcb1*; 2.5 fold; $p < 0.002$) and a trend for inositol-(1,4,5) trisphosphate 3-kinase A subunit (*Ipk3a*; 1.6 fold; $p < 0.03$).

Transcripts that displayed upregulation within CA1 neurons in hTau mice included the large subunit of the protease calpain 1 (*Capn1*; 3.1 fold; $p < 0.001$) and a trend for the

intramembrane protease nicastrin (*Ncstn*; 1.6 fold; $p < 0.03$) as well as the cell cycle regulatory gene cyclin E1 (*Ccne1*; 2.2 fold; $p < 0.004$), aquaporin 1 (*Aqp1*; 2 fold; $p < 0.004$), steroid-related gene 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*Hmgcr*; 2.1 fold; $p < 0.005$), and the cannabinoid receptor *Cnr1* (1.8 fold; $p < 0.01$). Upregulation of the transcription factors embryonic lethal abnormal vision Drosophila-like (ELAV) *Elav1* (2.23 fold; $p < 0.001$), *Elav4* (1.9 fold; $p < 0.002$), and nuclear respiratory factor 1 (*Nrf1*; 1.8 fold; $p < 0.01$) were also observed. Moreover, differential regulation of the immediate-early genes *Arc* (downregulated; 2.1 fold; $p < 0.001$) and *Fosb* (upregulated; 1.9 fold; $p < 0.004$; Fig. 2E) was identified in CA1 neurons. In summary, the classes of transcripts that displayed the most significant alterations in hTau mice included synaptic-related markers, select GluR subunits, select PP1 and PP2 subunits, and several transcription factors/immediate-early genes among other individual genes that did not represent a single gene ontology grouping.

In contrast to the marked downregulation of select synaptic-related markers and GluRs in CA1 pyramidal neurons microaspirated from 11-14 month old hTau mice (Fig. 2B, C), only a few select genes were downregulated in hTau mice aged 7-8 months compared to ntg littermates via assessment of 37 custom-designed microarrays. Specifically, downregulation was observed for *Syp* (1.6 fold; $p < 0.01$) and *Psd95* (1.7 fold; $p < 0.01$) with a trend for downregulation for *Synj* (1.5 fold; $p < 0.03$) and *Arc* (1.5 fold; $p < 0.03$). No differential regulation was seen for the other identified synaptic-related markers downregulated at 11-14 months, including *Syn3*, *Syb*, *Snap29*, *Stx4a*, and *Stx7* the or GluRs *Gria1*, *Gria2*, and *Grik4*, suggesting the alterations seen in these genes at 11-14 months are due to progressive tau accumulation during aging.

qPCR validation of select gene expression alterations in the CA1 sector of the hippocampus in hTau mice

Based upon results gleaned from the custom-designed microarray analysis of CA1 pyramidal neurons in 11-14 month old mice, select gene expression levels were assessed via qPCR using microdissected hippocampal CA1 tissue harvested from hTau and ntg mice. The ddCT method was employed to determine relative gene level differences using *Gapdh* qPCR products as a control (Alldred et al., 2008, 2009; Ginsberg, 2008; Ginsberg et al., 2010). No differences in *Gapdh* expression levels were found between hTau and ntg mice, consistent with previously published observations using the CA1 sector of the hippocampus from mouse models of neurodegeneration (Alldred et al., 2008, 2009; Ginsberg, 2005a, 2010). Data was presented as percent of ntg $\pm$ standard deviation (SD). qPCR analysis independently validated the microarray observations using a separate cohort of mice, including downregulation of the synaptic-related marker *Syp* ($p < 0.001$), immediate early gene *Arc* ($p < 0.04$), and the neurotrophin receptor *TrkB* ($p < 0.04$) within the hippocampal CA1 region in hTau mice (Fig. 3A). qPCR analysis also demonstrated a statistically significant decrease in expression levels for *Gria1* ($p < 0.03$) and *Gria2* ($p < 0.02$), but not *Gria3*, consistent with microarray findings in aged mice (Fig. 3B). Two additional genes that did not differ between hTau and ntg mice on the microarray platform, the synaptic-related marker synaptopodin (*Synpo*) and the neurotrophin receptor *TrkA* were also examined. However, both transcripts were at the limit of resolution for qPCR assessment, which precluded microarray confirmation.

SYP and PSD-95 levels are reduced within puncta in the CA1 hippocampus of aged hTau mice

Following qPCR validation, we analyzed whether downregulation of *Syp* and/or *Psd95* mRNA levels would result in changes in protein levels denoted by loss of punctate synaptic clusters and/or protein localization within postsynaptic sites. Dual fluorescence confocal microscopy was performed within the pyramidal cell layer of the CA1 region in hTau and

ntg mice 12-14 months of age to assess SYP-immunoreactive and PSD-95-immunoreactive puncta size, number, and staining intensity. Confocal microscopy demonstrated a colocalized punctate pattern for SYP and PSD-95 (Fig. 4A). Quantitative morphometric analyses identified a significant decrease in the staining intensity for both SYP ($p < 0.02$; Fig. 4B) and PSD-95 ($p < 0.03$; Fig. 4C) in hTau mice, indicating that the reduction in gene expression for these two transcripts seen in the microarray and qPCR studies also reflects a loss at the respective protein expression level. Although a significantly lower expression level of SYP and PSD-95 per puncta was observed in hTau mice, no differences were observed in the total number of immunoreactive puncta or the punctal area in hTau mice compared to ntg littermates (Fig. 4B, C).

Downregulation of select synaptic and GluR protein levels within the hippocampal CA1 sector in aged hTau mice

Protein-based assessment was performed via immunoblotting using well-established antibodies combined with frozen hippocampal CA1 sector punches to assess whether transcriptional alterations, particularly in aged hTau mice, resulted in protein level changes. Expression level data was normalized to TUBB expression $\pm$ SD. No significant changes were observed within hTau mice at 3-5 months of age (data not shown). Consistent with microarray analysis at 7-8 months of age, hTau mice displayed significant downregulation of SYP ($p < 0.01$) and PSD-95 ($p < 0.008$) expression in a separate cohort of 7-10 month old hTau mice compared to age-matched ntg littermates, with no significant changes in GluR expression levels (Fig. 5A). Moreover, quantitative analysis of select GluRs and GluR-interacting proteins demonstrated a significant downregulation of GRIA1 ($p < 0.01$), GRIA2/3 ($p < 0.01$), and PSD-95 ($p < 0.005$), but not GRIN1 (NMDA R1) in 12-16 month old hTau mice (Fig. 5B, C), consistent with microarray and qPCR findings in the CA1 region. Significant down regulation of SYP was also observed ($p < 0.005$; Fig. 5B, C), validating (along with the PSD-95 immunoblot analysis) the tissue-based confocal observations in aged hTau mice (Fig. 4).

Discussion

Postmortem brain and biomarker studies of tau in human cerebrospinal fluid indicate the importance of assessing tau biology during AD pathogenesis. The hTau mouse line was created to model this pathology by expression of the normal human tau gene in the absence of mouse tau. hTau mice display pathological changes including accumulation of phosphorylated tau in the somatodendritic compartment of neurons, age-related increases in tau phosphorylation, and conformational alterations of tau (Andorfer et al., 2003, 2005). NFTs are not likely to occur solely due to tau overexpression, as another mouse model of tauopathy, 8c mice, has human tau and mouse tau overexpression, but does not develop overt tau cytopathology (Andorfer et al., 2003, 2005; Yuan et al., 2008). We are interested in identifying mechanisms of how tau dysfunction affects vulnerable neuronal populations. To this end, we performed microarray analysis, qPCR, and protein-based validation studies on CA1 neurons in hTau mice prior to and following an established timepoint where a behavioral phenotype has been identified (Andorfer et al., 2003, 2005; Polydoro et al., 2009).

The single population expression profiling paradigm employed in this study enabled an extensive, concurrent representation of hundreds of genes relevant to neurodegeneration and neuroscience. Notably, upregulation of the 6 isoforms of tau was seen within CA1 neurons in hTau mice, commensurate with increased tau protein levels in the hippocampus (Andorfer et al., 2003, 2005). Approximately 8% of the genes interrogated on the custom-designed array platform were dysregulated in 11-14 month old hTau mice compared to ntg littermates, including a disproportionately large number of synaptic-related markers and

discrete AMPA and KA GluR subunits displaying downregulation which progressed from 7-8 months of age where only a few genes (including *Syp* and *Psd95*) were initially observed to be dysregulated. Interestingly, dysfunction of excitatory neurotransmission has been observed in aged hTau mice in behavioral and physiological paradigms (Andorfer et al., 2003; Polydoro et al., 2009), and these deficits coincide with dendritic alterations observed in CA1 neurons of older tauopathic mice (Dickstein et al., 2010; Gotz et al., 2010; Luebke et al., 2010). Notably, alterations in expression profiles and cytoarchitecture may not be as dependent on the biophysics of NFT formation as much as on the accumulation of tau within the somatodendritic domain (Berger et al., 2007; Luebke et al., 2010; Rocher et al., 2010). This has basic, translational, and drug development implications, as our data support the emerging concept that developing methods to regulate progressively accumulating intracellular tau levels may be a key aspect to therapeutic interventions. Further support of this hypothesis comes from *in vitro* studies whereby transfection of tau in hippocampal neurons causes somatodendritic tau redistribution with concomitant synaptodegeneration, including spine loss and decrements in presynaptic and postsynaptic markers similar to our *in vivo* observations (Thies and Mandelkow, 2007). It is noteworthy that several key proteins are translated locally at synaptic sites, including mRNAs present directly within dendritic processes as well as somatic mRNAs that are transported to synaptic zones for local translation (Steward et al., 1998; Swanger and Bassell, 2011). Although our single cell microarray analysis is more sensitive than global or regional microarray analyses (Ginsberg et al., 2011), we cannot formally exclude the possibility of under representing expression level changes at local synaptic sites. Notwithstanding this caveat, the present findings at the genomic and protein levels provide a cellular backdrop for the aforementioned behavioral and morphological measures, implicating a loss of integral synaptic and GluR subunits, among others, in the progressive development of tauopathy.

Although the number of dysregulated genes in the hTau mouse at 11-14 months of age is moderate (and even less so at 7-8 months of age) compared to our previous microarray studies in NFT-bearing CA1 neurons obtained postmortem from AD brains, where we observed alterations in approximately 24%-36% of interrogated genes depending on the array platform (Ginsberg et al., 2000, 2006a, 2010, 2011), there is considerable overlap in the dysregulation of genes encoding integral synaptic proteins and GluR subunits (Table III), indicating the utility of this model to mimic synaptodegeneration found in AD. Notably, a consistent finding in both regional and single cell hippocampal analyses within the AD brain is the downregulation of genes encoding several synaptic-related markers (including *Syp*, synapsins, and syntaxins, among others) (Callahan et al., 1999; Colangelo et al., 2002; Ginsberg et al., 2000, 2006a, 2011; Gutala and Reddy, 2004; Mufson et al., 2002). Gene expression alterations are also reflected in respective protein level deficits, and importantly, correlation with cognitive decline (Counts et al., 2006; Heffernan et al., 1998; Heinonen et al., 1995; Masliah et al., 1991; Sze et al., 1997). Thus, hippocampal synaptodegeneration (particularly in CA1 neurons) is becoming recognized as one of the cardinal observations of AD pathogenesis, although the relationship(s) of this degeneration to tauopathy and amyloid deposition remain speculative and in need of further characterization in relevant animal and cellular models. Furthermore, down regulation of discrete GluRs and GluR-interacting proteins has been recognized as a feature of AD pathology (Armstrong et al., 1994; Carter et al., 2004; Ginsberg et al., 2004, 2006a; Ikonovic et al., 1995; Proctor et al., 2010; Yasuda et al., 1995) that is reflected in gene expression and protein level changes in discrete AMPA and KA subunits as well as in PSD-95 within the hTau mouse. Combined with independent physiological and morphological assessments (Andorfer et al., 2003; Dickstein et al., 2010; Noble et al., 2009; Polydoro et al., 2009), the present results underscore the observation that the hTau mouse is emerging as a model of progressive, age-related synaptic dysregulation within vulnerable hippocampal neurons, which can be exploited for the assessment of therapeutic approaches aimed at restoring synaptic integrity, and warrants future high-

density microarray analysis in the hTau mouse and parallel studies in AD postmortem brains. Moreover, the hTau mouse is devoid of amyloid-related pathology, which is perceived as a decided advantage, as effects of tau overexpression can be delineated in the absence of potential confounds of amyloidosis, as these two pathological hallmarks may have both distinct, as well as overlapping, effects on hippocampal circuitry and vulnerability (Small and Duff, 2008). Models of synaptodegeneration independent of APP and/or A β overproduction are increasingly becoming an area of great interest, since anti-amyloid therapies have not been effective to date in AD patients, as evidenced by the apparent lack of success of high-profile clinical trials of γ -secretase inhibitors and A β vaccination therapies (Imbimbo and Giardina, 2011; Sambamurti et al., 2011; Schor, 2011).

CA1 pyramidal neuron expression profiling at 11-14 months of age also demonstrated significant downregulation of select PP subunits, consistent with our previous human CA1 neuron molecular fingerprinting studies in AD (Ginsberg et al., 2000, 2006a; Mufson et al., 2002), and supports the concept that decrements in phosphatase activity are associated with the progression of AD (Goedert et al., 1995; Liu et al., 2005; Pei et al., 1994; Sontag et al., 2004; Vogelsberg-Ragaglia et al., 2001). Moreover, upregulation of *Capn1* in aged hTau mice is consistent with observations of dysregulated protease activity in AD and in AD models, particularly within the calpain activation system (Di Rosa et al., 2002; Liang et al., 2010; Nixon, 2003; Rao et al., 2008). Although a description of the relevance of each gene change in the hTau mouse is beyond the scope of this report, it is worth noting that many of the expression level changes are relevant to AD pathology and disrupted synaptic plasticity, including the dysregulation of the neurotrophin receptor *TrkB* and the immediate-early gene *Arc*. Interestingly, the single population expression profiling paradigm in CA1 neurons within postmortem AD and mild cognitive impairment brains demonstrated a highly significant downregulation of *TrkB* (both the *TrkBcd* and *TrkBik* forms; Table III) that correlated with levels of cognitive decline (Ginsberg et al., 2010). *TrkB* expression is widespread throughout the BDNF-responsive cortical mantle and hippocampal formation (Mufson et al., 2006), and may be a novel site for pharmacotherapeutic interventions via small molecule activators and/or *TrkB* transactivation (Longo et al., 2007; Rajagopal et al., 2004; Skaper, 2008). Based on the present study, employing the aged hTau mouse, with particular emphasis on hippocampal CA1 pyramidal neurons, is an exciting site of research for translational development of *TrkB*-based therapeutics for amelioration of neurodegeneration and associated cognitive decline during the progression of AD.

In summary, the coordinated microarray, qPCR, confocal microscopy, and immunoblotting studies presented herein indicate that the moderately aged hTau mouse has relevance towards understanding the source(s) of expression level changes found in human AD and related tauopathies. Specifically, the present results identified progressive gene and protein level changes in a vulnerable hippocampal cell type critical for learning and memory that indicates the hTau mouse is a model of progressive synaptodegeneration associated with tauopathy. Moreover, these findings in the hTau mouse at 11-14 months (which show progressive increases from observations starting at 7-8 months of age) potentially mimic intracellular tau level changes consistent with mild cognitive impairment and/or mild AD levels, and emphasize the importance of studying synaptic-related markers and GluRs in animal models of neurodegenerative disorders, particularly at the single population level.

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Abbreviations

AD	Alzheimer's disease
Aβ	amyloid- β peptide
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
APP	amyloid- β precursor protein
ANOVA	analysis of variance
BDNF	brain derived neurotrophic factor
Ct	cycle threshold
DGS	donor goat serum
ESTs	expressed sequence-tagged cDNAs
ECD	extracellular domain
ELAV	embryonic lethal abnormal vision Drosophila-like
GluRs	glutamate receptors

GAPDH	glyceraldehyde-3 phosphate dehydrogenase
IACUC	Institutional Animal Care and Use Committee
KA	kainate
LCM	laser capture microdissection
NFTs	neurofibrillary tangles
ntg	nontransgenic
NMDA	N-methyl-D-aspartate
NHS	normal horse serum
qPCR	real-time quantitative polymerase-chain reaction
PP	protein phosphatase
SSPE	6× saline–sodium phosphate–ethylenediaminetetraacetic acid
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TC	terminal continuation
TK	tyrosine kinase domain
TLCK	Na-p-tosyl-L-lysine chloromethyl ketone
TPCK	N-tosyl-L-phenylalanine chloromethyl ketone
TUBB	β-tubulin

Research Highlights

Microarrays, qPCR, and confocal microscopy assessed CA1 neurons in tauopathic mice. Analysis of hTau CA1 pyramidal neurons revealed progressive synaptic dysfunction. Downregulation was observed for select glutamatergic markers at 11-14 months of age. qPCR and confocal analysis validated PSD-95 and TrkB microarray results in hTau mice.

Figure 1.

LCM of CA1 neurons and TC RNA amplification.

A. LCM was performed on 6 μ m thick tissue sections of hTau and ntg mice. The CA1 region of hippocampus was immunostained with antibodies directed against neurofilaments for hippocampal pyramidal neuron detection and subsequent microaspiration. Scale bar: 25 μ m.

B. CA1 neurons were isolated by applying laser pulses (black circles) over desired neurons. Note that tissue sections were dehydrated and not coverslipped to enable proper execution of the LCM process. Scale bar: 20 μ m.

C. CA1 region of hippocampus seen after microaspiration, with cells captured by LCM removed and adjacent immunostained CA1 neurons remaining in the tissue section. Scale bar: 40 μ m.

D. Captured CA1 neurons were visualized on a clean slide for contrast. Scale bar: 25 μ m.

E. Schematic overview of the TC RNA amplification procedure.

F. Representative photomicrograph illustrating intensely labeled PHF1-immunoreactive CA1 neurons within the hippocampus of an aged hTau mouse. The inset depicts profuse intracellular phospho-tau immunoreactivity within the cell body of CA1 neurons.

G. PHF1 immunoreactivity was localized principally to neuropil surrounding the CA1 pyramidal layer in age-matched ntg littermates compared to the intracellular labeling in hTau mice. Scale bar F, G: 100 μm . Inset scale bar F, G: 50 μm .

Figure 2.

Color-coded heatmaps depicting differential regulation of select transcripts in 11-14 month old hTau mice compared to age-matched ntg littermates.

A. Upregulation of the 6 tau isoforms were found in hTau mice. Asterisk denotes ($p < 0.05$), double asterisk denotes ($p < 0.005$), and triple asterisk denotes ($p < 0.001$).

B. Downregulation of a high percentage of individual synaptic-related markers was observed (e.g., *Syn3*, *Syb*, *Syp*, *Synj*, *Stx4a*, *Stx7*, and *Snape29*), making this class of transcripts the most affected overall within the aged hTau mouse. Asterisk denotes ($p < 0.01$), double asterisk denotes ($p < 0.004$), triple asterisk denotes ($p < 0.003$), quadruple asterisk denotes ($p < 0.002$), and quintuple asterisk denotes ($p < 0.001$).

C. Downregulation of select AMPA receptor subunits (*Gria1* and *Gria2*), a KA receptor (*Grik4*), and *Psd95* is demonstrated. No differential regulation of NMDA receptors was found. Asterisk denotes ($p < 0.006$) and double asterisk denotes ($p < 0.001$).

D. Downregulation of the BDNF receptor *TrkB*, but not *p75*, *TrkA*, or *TrkC* was observed along with the galanin receptor *Galr1*. In contrast, significant upregulation of the large subunit of the protease calpain 1 (*Capn1*) was found in hTau mice. Asterisk denotes ($p < 0.002$) and double asterisk denotes ($p < 0.001$).

E. Selective downregulation of PP1 (*Ppp1cc*) and PP2 (*Ppp2r1a* and *Ppp2ca*) subunits that regulate tau dephosphorylation were observed in hTau mice. Additionally, significant downregulation of the immediate-early gene *Arc* contrasted the up regulation immediate-early gene *Fosb* in hTau mice. Asterisk denotes ($p < 0.01$), double asterisk denotes ($p < 0.004$), and triple asterisk denotes ($p < 0.001$).

Figure 3. qPCR validation of microarray observations in microdissected CA1 pyramidal neurons in 11-14 month old hTau and age-matched ntg littermates. The ddCT method was used for quantitative analysis and data is depicted as percentage of ntg expression $\pm$ SD. A. *Syp*, *Arc*, and *TrkB* PCR products displayed significant downregulation in hTau mice compared to ntg littermates. Asterisk denotes ($p < 0.04$) and double asterisk denotes ($p < 0.01$). B. Downregulation of *Gria1* and *Gria2*, but not *Gria3* PCR products was found, confirming the microarray results. Asterisk denotes ($p < 0.03$) and double asterisk denotes ($p < 0.02$).

Figure 4.

Confocal microscopy combined with unbiased quantitative morphometric analysis was performed within the CA1 region of hippocampus for two synaptic proteins (SYP and PSD-95), which were downregulated via microarray and qPCR analyses.

A. Representative confocal images depicting downregulation of SYP (red) puncta and PSD-95 (green) puncta in hTau mice compared to ntg littermates. A merged image indicates the overlapping distribution of SYP and PSD-95 (yellow) puncta within the CA1 pyramidal layer.

B. Morphometric analyses of the confocal images confirmed the expression profiling results, as significantly lower signal intensity levels for SYP were found in hTau mice. No significant differences were found in the number of puncta in hTau and ntg mice. Moreover, no significant differences exist between hTau and ntg mice in terms of punctal area measurements. Asterisk denotes ($p < 0.02$).

C. Similar to the SYP-immunoreactive results, down regulation of PSD-95-immunoreactivity was found in hTau mice. No significant differences in the number of puncta or punctal area measurements were observed. Asterisk denotes ($p < 0.03$).

Figure 5.

Immunoblotting using hippocampal CA1 sector punches to assess whether selected transcriptional alterations resulted in commensurate protein level changes in hTau mice compared to ntg littermates at 7-10 months of age (A) and 12-16 months of age (B).

Expression level data was normalized to TUBB expression $\pm$ SD.

A. Downregulation of SYP and PSD-95 expression was observed in hTau mice compared to age matched littermates. Asterisk denotes ($p < 0.01$) and double asterisk denotes ($p < 0.008$).

B. Downregulation of GRIA1, GRIA2/3, PSD-95, and SYP was observed within the CA1 sector in 12-16 month old hTau mice, consistent with microarray, qPCR, and confocal analyses. Asterisk indicates ($p < 0.01$) and double asterisk indicates ($p < 0.005$).

C. Representative immunoblots obtained from 13-14 month old hTau and ntg mice demonstrating downregulation of GRIA1, GRIA2/3, PSD-95, and SYP in hTau mice, consistent with microarray and qPCR findings in the CA1 region. Upregulation of tau is demonstrated in the hTau mice as a positive control.

Table I
Classes of transcripts

Gene Category	# of genes	Gene Category	# of genes
AD-related genes	42	GluRs, transporters, & interacting	42
calcium, potassium, & sodium channels	20	immediate early genes	12
catecholamine synthesis, receptors, & transporters	28	neurotrophins & neurotrophin receptors	20
cell death-related genes	28	protease-related markers	60
cytoskeletal elements	24	protein phosphatases & kinases	40
development-related markers	24	steroid synthesis & receptors	34
G-protein subunits	42	synaptic-related markers	24
GABA synthesis, receptors, & transporters	20	transcription factors & regulators	42
glial-associated markers	10	others	49
glucose utilization	15	TOTAL	576

Table II
Genes altered in moderately aged hTau mice (11-14 months) compared to ntg littermates

Unigene Annotation	Gene	Direction of Change
ADRA1B	(adrenergic alpha 1B receptor)	–
AQP1	(aquaporin 1)	+
ARC	(activity regulated cytoskeletal-associated protein)	–
CAPN1	(calpain 1, mu/I large subunit)	+
CCND2	(cyclin D2)	+*
CCNE1	(cyclin E1)	+
CNR1	(CB1 cannabinoid receptor)	+*
ELAVL1	(embryonic lethal abnormal vision like 1; Hu antigen R)	+
ELAVL4	(embryonic lethal abnormal vision like 4; Hu antigen D)	+
FMR1	(fragile × mental retardation 1)	–
FOSB	(FBJ osteosarcoma oncogene B)	+
GABARAP-L1	(GABA-A receptor-associated protein like 1)	–
GAD67	(glutamate decarboxylase isoform 67)	–
GALR1	(galanin receptor 1)	–
GNAQ	(G protein, q polypeptide)	–
GNB2	(G protein, beta polypeptide 2)	–*
GRIA1	(GluR1 AMPA1)	–
GRIA2	(GluR2 AMPA2)	–
GRIK4	(KA receptor 1)	–
HMGCR	(3-hydroxy-3-methylglutaryl-coenzyme A reductase)	+
IGF1	(insulin-like growth factor 1)	–*
IGF2	(insulin-like growth factor 2)	–*
IP3KA	(inositol-(1,4,5) trisphosphate 3-kinase A subunit)	–
MAOB	(monoamine oxidase B)	–*
MAPT1	(microtubule-associated protein tau 3Rtau 0 insert)	+
MAPT2	(microtubule-associated protein tau 4Rtau 0 insert)	+
MAPT3	(microtubule-associated protein tau 3Rtau 1 insert)	+
MAPT4	(microtubule-associated protein tau 4Rtau 1 insert)	+
MAPT5	(microtubule-associated protein tau 3Rtau 2 inserts)	+*
MAPT6	(microtubule-associated protein tau 4Rtau 2 inserts)	+
MENTHO	(STARD3; N-terminal like MLN64 homolog)	+
NCSTN	(nicastrin)	+*
NR3C1	(glucocorticoid receptor Grl)	–*
NRF1	(nuclear respiratory factor 1)	+
NTRK2ECD	(BDNF receptor TrkB, extracellular domain)	–
NTRK2TK	(BDNF receptor TrkB, tyrosine kinase domain)	–
PPP1CC	(protein phosphatase 1 catalytic subunit, γ isoform)	–
PPP2CA	(protein phosphatase 2 catalytic subunit, α isoform)	–*
PPP2R1A	(protein phosphatase 2 regulatory subunit A, α isoform)	–

Unigene Annotation	Gene	Direction of Change
PRKCB1	(protein kinase C, β 1 subunit)	–
PSD-95	(postsynaptic density protein 95; disc homolog 4)	–
RGS2	(regulator of G-protein signalling 2)	+*
RGS13	(regulator of G-protein signalling 13)	–*
SNAP-29	(synaptosomal-associated protein, 29 Kd)	–
STX4A	(syntaxin 4A)	–
STX7	(syntaxin 7)	–
SYB	(synaptobrevin 1; vesicle-associated membrane protein 1)	–
SYN3	(synapsin 3)	–
SYNJ	(synaptojanin)	–
SYP	(synaptophysin)	–

Data represents significant ($p < 0.01$ or greater) gene changes for hTau mice compared to ntg littermates.

+, up regulation; –, down regulation. Asterisk indicates a trend where ($p < 0.02 - p < 0.05$).

Table III
Genes altered within CA1 pyramidal neurons in the hTau mouse and in postmortem AD brain tissues

Unigene Annotation/Gene	hTau mice	AD
ARC (activity regulated cytoskeletal-associated protein)	–	–
FOSB (FBJ osteosarcoma oncogene B)	+	+*
GAD67 (glutamate decarboxylase isoform 67)	–	NS
GAT1 (GABA A transporter 1)	NS	–
GRIA1 (GluR1 AMPA1)	–	–
GRIA2 (GluR2 AMPA2)	–	–
GRIA3 (GluR3 AMPA3)	NS	NS
GRIA4 (GluR4 AMPA4)	NS	NS
GRIK4 (KA receptor 1)	–	NS
GRIK5 (KA receptor 2)	NS	NS
GRIN1 (NMDA receptor NR1)	NS	NS
GRIN2A (NMDA receptor NR2A)	NS	NS
GRIN2B (NMDA receptor NR2B)	–	–
GRIP1 (glutamate receptor interacting protein 1)	NS	NS
GRIP2 (glutamate receptor interacting protein 2)	–	–
NTRK2ECD (BDNF receptor TrkB, extracellular domain)	–	–
NTRK2TK (BDNF receptor TrkB, tyrosine kinase domain)	–	–
NTRK3ECD (NT-3 receptor TrkC, extracellular domain)	NS	–
NTRK3TK (NT-3 receptor TrkC, tyrosine kinase domain)	NS	–
P75NTR (pan-neurotrophin receptor)	NS	NS
PPP1CC (protein phosphatase 1 catalytic subunit, γ isoform)	–	–
PPP2CA (protein phosphatase 2 catalytic subunit, α isoform)	–*	–
PPP2R1A (protein phosphatase 2 regulatory subunit A, α isoform)	–	–
PRKCB1 (protein kinase C, β 1 subunit)	–	NS
PSD-95 (postsynaptic density protein 95; disc homolog 4)	–	–
SNAP-29 (synaptosomal-associated protein, 29 Kd)	–	–
SNCA (α -synuclein)	NS	–
STX1 (syntaxin 1)	NS	–*
STX4A (syntaxin 4A)	–	–
STX7 (syntaxin 7)	–	–
SYB (synaptobrevin 1; VAMP1)	–	–
SYN1 (synapsin 1)	NS	–
SYN3 (synapsin 3)	–	–
SYNGR1 (synaptogyrin 1)	NS	–*
SYNJ (synaptojanin)	–	–*
SYNPO (synaptopodin)	NS	–
SYP (synaptophysin)	–	–
SYT1 (synaptotagmin 1)	NS	–

Data represents significant ($p < 0.01$ or greater) gene changes for hTau mice compared to ntg littermates (left column) and AD compared to cognitively normal, age-matched controls (right column) as described in (Ginsberg et al., 2011; Ginsberg et al., 2000).

+, up regulation; –, down regulation. Asterisk indicates a trend where ($p < 0.02 - p < 0.05$). Note the high similarity in downregulation of several synaptic-related markers, GluRs, and PP subunits in hTau and AD CA1 pyramidal neurons.