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Selective decline of neurotrophin and neurotrophin receptor genes within CA1 pyramidal neurons and hippocampus proper: correlation with cognitive performance and neuropathology in mild cognitive impairment and Alzheimer's disease

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Abstract

Hippocampal CA1 pyramidal neurons, a major component of the medial temporal lobe memory circuit, are selectively vulnerable during the progression of Alzheimer's disease (AD). The cellular mechanism(s) underlying degeneration of these neurons and the relationship to cognitive

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performance remains largely undefined. Here, we profiled neurotrophin and neurotrophin receptor gene expression within microdissected CA1 neurons along with regional hippocampal dissections from subjects who died with a clinical diagnosis of no cognitive impairment (NCI), mild cognitive impairment (MCI), or AD using laser capture microdissection (LCM), custom-designed microarray analysis, and qPCR of CA1 subregional dissections. Gene expression levels were correlated with cognitive test scores and AD neuropathology criteria. We found a significant downregulation of several neurotrophin genes (e.g., *Gdnf*, *Ngfb*, and *Ntf4*) in CA1 pyramidal neurons in MCI compared to NCI and AD subjects. In addition, the neurotrophin receptor transcripts *TrkB* and *TrkC* were decreased in MCI and AD compared to NCI. Regional hippocampal dissections also revealed select neurotrophic gene dysfunction providing evidence for vulnerability within the hippocampal proper during the progression of dementia. Downregulation of several neurotrophins of the NGF family and cognate neurotrophin receptor (*TrkA*, *TrkB*, and *TrkC*) genes correlated with antemortem cognitive measures including the Mini-Mental State Exam (MMSE), a composite global cognitive score (GCS), and Episodic, Semantic, and Working Memory, Perceptual Speed, and Visuospatial domains. Significant correlations were found between select neurotrophic expression downregulation and neuritic plaques (NPs) and neurofibrillary tangles (NFTs), but not diffuse plaques (DPs). The data suggest that dysfunction of neurotrophin signaling complexes have profound negative sequelae within vulnerable hippocampal cell types, which play a role in mnemonic and executive dysfunction during the progression of AD.

Keywords

brain-derived neurotrophic factor; microarray; neuritic plaques; neurofibrillary tangles; Trk receptors

Introduction

Dr. Leyla deToledo-Morrell dedicated a significant portion of her research career to utilizing quantitative imaging techniques to perform *in vivo* assessments of the hippocampus and entorhinal cortex during the progression of dementia. Her work focused on examining disease related-changes in medial temporal lobe structures and their relation to cognitive function, especially in prodromal stages of Alzheimer's disease (AD) (deToledo-Morrell et al., 2004, 2007; Dickerson et al., 2011; Stoub et al., 2010, 2012, 2014). We commemorate her efforts and recognize her generosity of spirit by contributing a study that dovetails her interests in early detection of AD-like pathology and correlation with cognitive and neuropathological criteria, albeit at the single population and hippocampal regional level and not by imaging techniques. We are confident that Leyla would have encouraged our efforts, and we dedicate this research article in her honor.

Factors initiating central nervous system neurodegeneration are varied, but a loss of trophic support has been strongly associated with cell death, decreases in dendritic growth and synaptogenesis, and age-dependent decline in hippocampal volume (Lu et al., 2013; Mufson et al., 2007b, 2016a). Neurotrophins are prominent trophic factors that act through their cognate receptors to play key roles in neuronal survival, differentiation, and growth (Kaplan

and Miller, 2000; Teng and Hempstead, 2004). Neurotrophins mediate a balance between neuronal survival or death depending upon the cellular milieu, type of neurotrophin receptor, and the downstream signaling pathways activated (Chao et al., 1998; Mitre et al., 2017). Nerve growth factor (NGF) was the first member of the neurotrophin family to be identified, which also includes brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NTF3), neurotrophin-4 (NTF4), and neurotrophin-5 (NTF5), among others (Chao et al., 1998; Scharfman and Chao, 2013; Teng and Hempstead, 2004). Neurotrophins exert biological effects by interacting with cell surface receptors, the cognate receptors of the tropomyosin-related tyrosine kinase (Trk) family of receptor tyrosine kinases and the pan-neurotrophin receptor ($p75^{NTR}$) (Deinhardt and Chao, 2014; Meeker and Williams, 2014; Mitre et al., 2017). Neurotrophins and their receptors, particularly BDNF and TrkB, are potent regulators of synaptic plasticity, learning and memory (Conner et al., 2009; Cowansage et al., 2010; Gomez-Palacio-Schjetnan and Escobar, 2013; Leal et al., 2015; Lu et al., 2013; Magby et al., 2006; Naito et al., 2017; Tapia-Arancibia et al., 2008; Yoshii and Constantine-Paton, 2010). Changes in the levels or activities of neurotrophins and neurotrophin receptors are common features of many neurodegenerative and neuropsychiatric disorders (Altar et al., 2009; Autry and Monteggia, 2012; Thompson Ray et al., 2011), including Alzheimer's disease (AD) (Allen et al., 2011; Erickson et al., 2010; Tanila, 2017). Several independent research laboratories, including our group, posit that deficiencies in neurotrophic activity leads to selective vulnerability of specific neuronal populations, whereas gain of neurotrophic function may facilitate recovery by targeting mechanisms of neuroplasticity (Mufson et al., 2007a, 2007b, 2015; Nagahara et al., 2009).

Previous regional postmortem human brain studies have shown that *Bdnf*, a complex transcript with multiple isoforms, is decreased in cortex, hippocampus, and basal forebrain in end stage AD (Alvarez et al., 2014; Garzon et al., 2002; Holsinger et al., 2000; Michalski et al., 2015; Murray et al., 1994; Phillips et al., 1991). In contrast, *Ngf* levels have not shown marked differences in AD cortex (Fahnestock et al., 1996; Jette et al., 1994; Mufson et al., 2003). Data from our group has demonstrated that downregulation of genes encoding the neurotrophin receptors *TrkA*, *TrkB* and *TrkC*, but not $p75^{NTR}$ are significantly decreased during the progression of dementia in cholinergic basal forebrain (CBF) neurons and hippocampal CA1 pyramidal neurons during the progression of AD (Ginsberg et al., 2006, 2010). However, systematic evaluation of the NGF family of neurotrophins and their cognate receptor expression in the hippocampus is lacking, especially in relation to specific cognitive domains during the progression from mild cognitive impairment (MCI) to AD.

To gain a greater understanding of alterations in neurotrophin and neurotrophin receptor expression during the progression of dementia and their relation to cognitive impairment and AD neuropathology, we examined expression profiles of CA1 pyramidal neurons and the surrounding hippocampal formation using custom-designed microarray and qPCR analysis applied to postmortem human brain tissue obtained from the Rush Religious Orders Study (RROS), a longitudinal clinical pathological study of aging and dementia in retired Catholic clergy (Bennett and Launer, 2012; Bennett et al., 2002; Mufson et al., 1999, 2012a, 2016a). Each RROS participant received an annual detailed clinical evaluation, including a battery of tests for function in five cognitive domains (orientation, attention, memory, language, and perception) as well as detailed postmortem neuropathological evaluation, enabling clinical

pathological assessment of gene expression levels to cognitive performance and neuropathology.

Materials and Methods

Brain tissue accession and clinical pathological assessment

Clinical and neuropsychological evaluation criteria for the RROS cohort have been published previously (Bennett and Launer, 2012; Bennett et al., 2002; Mufson et al., 2000, 2002b, 2016a, 2016b). Upon entry into the RROS cohort, subjects were deemed not to have any comorbid conditions contributing to cognitive impairment. Antemortem cognitive assessments were performed each year before death, which included the Mini Mental State Exam (MMSE) as part of a battery of 17 neuropsychological tests, including assessments in Episodic, Semantic, and Working Memory, Perceptual Speed, and Visuospatial domains (Arvanitakis et al., 2008; Bennett et al., 2002; Malek-Ahmadi et al., 2016; Mufson et al., 2016a; Wilson et al., 2011). A global cognitive *z*-score (GCS) was compiled for each subject based on the neuropsychological battery composite scores of the 17 individual cognitive tests related to the five domains of cognition (Arvanitakis et al., 2008; Bennett et al., 2002; Malek-Ahmadi et al., 2016; Wilson et al., 2011). A board-certified neurologist made a clinical diagnosis for each RROS participant. Subjects were clinically categorized as NCI, MCI insufficient to meet criteria for dementia, and AD. Although there are no consensus criteria for the clinical classification of MCI (Winblad et al., 2004), the present MCI population was defined as subjects with impaired cognitive testing who were not found to have frank dementia by an examining neurologist (DeKosky et al., 2002; Mufson et al., 2000), similar to criteria used by other independent experts in the field (Petersen and Negash, 2008; Reisberg et al., 2008). The majority of AD subjects from the RROS cohort in this study were classified as mild to moderate AD based upon cognitive criteria. Within the RROS cohort, 23% of individuals progressed to MCI, and 36% of individuals progressed to probable AD at autopsy (Bennett and Launer, 2012). An additional cohort of end-stage AD subjects and aged-matched nondemented controls obtained from the Center for Neurodegenerative Disease Research (CNDR) at the University of Pennsylvania School of Medicine (Philadelphia, PA) was also used for the custom-designed microarray studies as a comparator to the mild to moderate RROS AD cases.

At autopsy, tissue blocks containing the hippocampal complex were immersion-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2 for 24–72 hours at 4 °C, paraffin embedded, and cut on a rotary microtome at 6 μm thickness (Ginsberg et al., 2010, 2012). Adjacent tissue slabs were also snap-frozen for qPCR. A series of tissue sections were prepared for neuropathological evaluation including visualization and quantitation of neuritic plaques (NPs), diffuse plaques (DPs), and NFTs using a modified Bielschowsky silver stain, Thioflavine-S, and antibodies directed against amyloid-β peptide (Aβ; 4G8, monoclonal, BioLegend, San Diego, CA) and tau (PHF1, monoclonal, a gift of Dr. Peter Davies, Hofstra Northwell School of Medicine) (Ginsberg et al., 2010; Malek-Ahmadi et al., 2016; Mufson et al., 2000, 2002a). Exclusion criteria included frontotemporal dementia, Lewy body disease, mixed dementias, Parkinson's disease, tauopathies, and stroke. A board-certified neuropathologist blinded to the clinical diagnosis performed the neuropathological

evaluation. Neuropathological designations were based on NIA Reagan, CERAD, and Braak staging criteria (Braak and Braak, 1991; Hyman and Trojanowski, 1997; Mirra et al., 1991). For amyloid burden and tangle load, summary counts for each subject were obtained for total number of NPs, DPs and NFTs as determined by the modified Bielschowsky silver stain in one square mm area (100× magnification) per entorhinal and hippocampal CA1 region (Malek-Ahmadi et al., 2016; Mufson et al., 2016b). Apolipoprotein E (ApoE) genotype was determined as described previously (Bennett et al., 2004; Counts et al., 2007; Mufson et al., 2000, 2016b).

Tissue preparation for microarray analysis

Acridine orange histofluorescence and bioanalysis (2100, Agilent Biotechnologies, Palo Alto, CA) (Ginsberg, 2008; Ginsberg et al., 1997, 1998) were performed on each brain to ensure that high-quality RNA was present in hippocampal tissue sections for downstream genetic analyses. 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). Deparaffinized tissue sections were blocked in a 0.1 M Tris (pH 7.6) solution containing 2% donor horse serum (DHS; Sigma, St. Louis, MO) and 0.01% Triton X-100 for 1 hour and then incubated with a primary antibody directed against nonphosphorylated neurofilament proteins (RMdO20) (Lee et al., 1987) in a 0.1 M Tris/2% DHS solution overnight at 4 °C in a humidified chamber. Sections were processed with the ABC kit (Vector Labs, Burlingame, CA) and developed with 0.05% diaminobenzidine (Sigma), 0.03% hydrogen peroxide, and 0.01 M imidazole in Tris buffer for 10 minutes (Ginsberg, 2005, 2008, 2014). Tissue sections were not coverslipped or counterstained and maintained in RNase-free 0.1 M Tris prior to laser capture microdissection (LCM).

LCM and RNA amplification

Individual CA1 pyramidal neurons were microaspirated via LCM (Arcturus PixCell IIe, ThermoFisher Scientific, South San Francisco, CA). Fifty cells were captured for population cell analysis as described previously (Ginsberg et al., 2010, 2012; Tiernan et al., 2016). CA1 pyramidal neurons were microaspirated from NCI (n=13), MCI (n=15), mild/moderate AD (n=9) RROS cases, and end stage AD (n=6) CNDR cases. The surrounding hippocampal formation, including the CA sectors, dentate gyrus, and subicular complex, was also microaspirated for comparison to the expression profile determined from CA1 pyramidal neurons. Regional hippocampal dissections were obtained from NCI (n=13), MCI (n=10), mild/moderate AD (n=10) RROS cases as well as end stage AD (n=6) and nondemented control (n=4) CNDR samples. RNAs extracted from the 50 cells per assay and the regional hippocampal dissections were amplified by the terminal continuation (TC) RNA amplification method developed by the Ginsberg laboratory (Alldred et al., 2008, 2009; Che and Ginsberg, 2004; Ginsberg, 2014). The TC RNA amplification protocol is available at http://cdr.rfmh.org/pages/ginsberglabpage.html. Microaspirated samples were homogenized in 100 μl of Trizol reagent (ThermoFisher Scientific, Waltham, MA), extracted with chloroform, and precipitated utilizing isopropanol (Alldred et al., 2009). RNAs were reverse transcribed in the presence of poly d(T) primer (100 ng/μl) and TC primer (100 ng/μl) in 1× first strand buffer (ThermoFisher Scientific), 2 μg of linear acrylamide (Applied Biosystems, Foster City, CA), 10 mM dNTPs, 100 μM dithiothreitol (DTT), 20 U of SuperRNase

Inhibitor (Applied Biosystems) and 200 U of reverse transcriptase (Superscript III, ThermoFisher Scientific). Single stranded cDNAs were digested and then placed in a thermal cycler using a solution consisting of 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 10 U RNase H (ThermoFisher Scientific) in a final volume of 100 μl. The thermal cycler program ran as follows: RNase H digestion step at 37 °C, 30 minutes; denaturation step 95 °C, 3 minutes; primer re-annealing step 60 °C, 5 minutes (Alldred et al., 2009; Che and Ginsberg, 2004). Samples were purified by column filtration (MilliporeSigma, Billerica, MA). Hybridization probes were synthesized by *in vitro* transcription using ^{33}P incorporation in 40 mM Tris (pH 7.5), 6 mM $MgCl_2$, 10 mM NaCl, 2 mM spermidine, 10 mM DTT, 2.5 mM ATP, GTP and CTP, 100 μM of cold UTP, 20 U of RNase inhibitor, 2 KU of T7 RNA polymerase (Epicentre Illumina, Madison, WI), and 120 μCi of ^{33}P-UTP (Perkin-Elmer, Boston, MA) (Alldred et al., 2009; Ginsberg, 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

Arrays were prehybridized (2 hours) and hybridized (12 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 the hybridization protocol, 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, Piscataway, NJ). All array phosphor images were adjusted to the same brightness and contrast levels for data acquisition and analysis (Alldred et al., 2012, 2015a).

Data collection and statistical analysis for custom-designed microarrays

Hybridization signal intensity was determined utilizing ImageQuant software (GE Healthcare). Briefly, each array was compared to negative control arrays utilizing the respective protocols without any starting RNA. Expression of TC amplified RNA bound to each linearized cDNA (18 neurotrophin and neurotrophin receptor genes on the array) minus background was then 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 the absolute quantity of probe (Eberwine et al., 2001; Ginsberg, 2008). These data do not allow the absolute quantitation of mRNA levels. However, an expression profile of relative changes in mRNA levels was generated.

qPCR

qPCR was performed on frozen micropunches from the hippocampal CA1 region NCI (n = 12), MCI (n = 13), and mild/moderate AD (n = 14) RROS cases. Taqman qPCR primers (Applied Biosystems) were utilized for the following genes: *Bdnf*, *Ngfb*, *Ntf3*, *TrkB*, *TrkC*, and the housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) as described previously (Alldred et al., 2015a, 2015b; Ginsberg et al., 2010, 2011). Standard curves and cycle threshold (Ct) were measured using standards obtained from total human

brain RNA. Samples were run in triplicate for the qPCR assessments. Negative controls consisted of the reaction mixture without input RNA.

Between-site transcript analysis

Comparison of neurotrophin and neurotrophin receptor expression profiles from the RROS and CNDR cohorts were performed for microaspirated CA1 pyramidal neurons (12 AD cases; n=6 RROS and n=6 CNDR) and regional hippocampal dissections (12 AD cases; n=6 RROS and n=6 CNDR) and 8 NCI cases (n=4 RROS and n=4 CNDR). Cases were matched for age at death and gender between cohorts.

Statistical analyses

Group differences for clinical diagnosis among neurotrophin and neurotrophin receptor mRNAs were analyzed using a Kruskall-Wallis test with a Dunn's post hoc test to correct for multiple group comparisons and to identify statistically significant groupwise comparisons (Dunn, 1964; Kruskal and Wallis, 1952). Transcripts demonstrating statistically significant group differences were analyzed using regression models using clinical diagnosis as the predictor and included age at death, gender, education level, and APOE ε4 carrier status to account for their effects. Linear and quadratic models were fit to each of the neurotrophin and neurotrophin receptor mRNAs in order to determine which model best represented the association. Model fit was determined by whether the linear or quadratic form had a higher R^2 value (Mertler and Vannatta, 2013). Several cases were used for both CA1 pyramidal neuron and regional hippocampal microarray analyses (NCI = 9; MCI = 8; AD = 6).

Spearman correlation analyses were used to assess linear associations between cognitive variables and each of the differentially regulated genes. Correlation analyses for select neurotrophin and neurotrophin receptor transcripts were also performed with NPs, DPs, and NFTs. A false discovery rate (FDR) (Benjamini and Hochberg, 1995) was employed to correct for multiple comparisons and maintain a significance level of $\alpha \leq 0.05$. Within the MCI group, amnestic (n=9) and non-amnestic (n=6) cases used for CA1 pyramidal neuron microaspiration and amnestic (n=8) and non-amnestic (n=2) cases used for regional hippocampal dissection analyses did not differ significantly for any of the neurotrophin or neurotrophin receptor transcripts evaluated (Supplemental Table ST1). Therefore, amnestic and non-amnestic MCI cases were combined into a single MCI group in order to increase statistical power.

Between-site analyses of the transcripts were carried out using a Mann-Whitney test. A FDR was also applied to these results in order to adjust for multiple comparisons. Boxplots and scatterplots were graphed using MedCalc (version 16.8.4; MedCalc Software bvba, Belgium).

Results

CA1 pyramidal neuron analyses

Demographic, cognitive, and neuropathological characteristics of the RROS cases used in the single population CA1 pyramidal neuron analyses are shown in Table 1. Age at death (p

= 0.68), education level (p = 0.19), and proportion of males and females (p = 0.33) was not significantly different between clinical groups. There was no significant difference in proportions of males and females between clinical groups (p = 0.33). The APOE ε4 allele was more prevalent in MCI and AD relative to NCI (p<0.001). Significant group differences were observed for all of the cognitive measures examined. The NCI group performed significantly better than both MCI and AD on the MMSE, Working Memory, Perceptual Speed, and Visuospatial domains (Table 1). MCI and AD groups were not significantly different. The NCI group performed significantly better than MCI and AD, and the MCI group performed significantly better than AD for Episodic, Semantic, and Working Memory domains (Table 1).

Postmortem interval (PMI) and brain weight at autopsy were not significantly different between groups (p = 0.45, p = 0.23, respectively). Median Braak stage for NCI was significantly lower compared to MCI and AD (Table 1). No significant difference was found between MCI and AD. For CERAD diagnosis, the Definite AD classification was more prevalent in MCI and AD groups relative to NCI, while the NIA-Reagan High Likelihood of AD classification was most prevalent in AD (Table 1). Table 2A shows differences for select mRNAs between clinical groups.

CA1 pyramidal neuron gene expression

A significant downregulation for the neurotrophins glial cell-derived neurotrophic factor (*Gdnf*), nerve growth factor beta isoform (*Ngfb*), and *Ntf4* was observed within CA1 pyramidal neurons in MCI compared to AD and NCI (Fig. 1A). Interestingly, AD and NCI levels were similar (Fig. 1A) suggesting upregulation in mild/moderate AD. No significant differences were found for *Gdnf*, *Ngfb*, and *Ntf4* expression between AD and NCI groups. In contrast, neurotrophin receptors *TrkB* and *TrkC* {both the extracellular domain (*ECD*) and tyrosine kinase (*TK* forms)} displayed significant downregulation in MCI and AD compared to NCI (Table 2A). The MCI and AD groups were not significantly different (Fig. 1B, C). Regression analyses revealed that group differences remained statistically significant after adjusting for age at death, education level, gender, and APOE ε4 status (Table 2B). Quadratic functions were used to fit *Gdnf*, *Ngfb*, and *Ntf4* and *TrkB ECD* expression levels while linear functions were used to fit *TrkB TK*, *TrkC ECD* and *TrkC TK* expression levels.

Correlations of select mRNAs with cognitive measures, correcting for multiple comparisons are shown in Table 3 and Figure 2. Specifically, GCS correlated moderately with lower *TrkB ECD* (r = 0.55), *TrkB TK* (r = 0.51), and *TrkC TK* (r = 0.50) expression levels. Episodic Memory performance was moderately correlated with *TrkC TK* (r = 0.52) expression while *TrkB TK* and *TrkC TK* showed moderate correlations with the Visuospatial domain (r = 0.48, r = 0.51, respectively). No significant correlations were detected among transcripts from microdissected CA1 pyramidal neurons with NPs, DPs, or NFTs, including assessments within the NCI group (Supplemental Table ST2).

Regional hippocampal dissection analyses

Demographic, cognitive, and neuropathological characteristics of cases used in the hippocampal formation regional analyses are shown in Table 4. Age at death and education

level were not significantly different between clinical groups (p = 0.18, p = 0.27, respectively). No significant difference was found in the proportions of males and females between clinical groups (p = 0.74). The APOE ε4 allele was more prevalent in MCI and AD groups relative to NCI (p = 0.03). Significant group differences were observed for all cognitive variables (Table 4). The NCI group performed significantly better than both MCI and AD for MMSE, Semantic Memory, Working Memory, and Perceptual Speed. MCI and AD were not significantly different from each other. The NCI group performed significantly better than MCI and AD, while the MCI subjects performed significantly better than the AD cases for GCS, Episodic Memory, and Visuospatial domains (Table 4).

PMI and brain weight at autopsy were not significantly different between groups (p = 0.54, p = 0.14, respectively). Median Braak stage for NCI was significantly lower than both MCI and AD. No significant differences were found between MCI and AD. For CERAD diagnosis, Definite AD classification was more prevalent in MCI and AD relative to NCI while the NIAReagan High Likelihood classification was most prevalent in the AD group.

Regional hippocampal dissection gene expression

Differences between clinical groups for select neurotrophin and neurotrophin receptor mRNAs is shown in Table 5A. Significant downregulation of *Bdnf* was observed in MCI compared to AD and NCI. This downregulation was followed by a return to age-matched control levels. No significant differences were found for *Bdnf* between AD and NCI. In contrast, mRNAs for the neurotrophins ciliary neurotrophic factor (*Cntf*) and *Ngfb* and the neurotrophin receptors *TrkA ECD*, *TrkB ECD*, *TrkB TK*, *TrkC ECD*, and *TrkC TK* displayed significant downregulation in both MCI and AD compared to NCI (Fig. 3). MCI and AD were not significantly different from each other. Additionally, *TrkA TK* was significantly downregulated in AD compared to NCI. Regression analyses indicated group differences remained statistically significant after adjusting for age at death, education level, gender, and APOE ε4 status. Quadratic functions were used to fit *Bdnf*, *TrkB ECD*, *TrkB TK*, and *TrkC ECD* while linear functions were used to fit *Cntf*, *Ngfb*, *TrkA ECD*, *TrkA TK*, and *TrkC TK* (Table 5B).

Several statistically significant correlations were observed between neurotrophin and neurotrophin receptor transcripts and cognitive measures (Table 6). MMSE performance correlated moderately with *Cntf* (r = 0.60), *Ngfb* (r = 0.43), *TrkA TK* (r = 0.56), *TrkB ECD* (r = 0.59), and *TrkB TK* (r = 0.47) (Fig. 4). GCS performance correlated with *TrkB ECD* (r = 0.62) and *TrkB TK* (r = 0.70) expression (Fig. 5A, B) while moderate correlations were found for *Cntf* (r = 0.54), *Ngfb* (r = 0.52), *TrkA ECD* (r = 0.51), *TrkA TK* (r = 0.52), and *TrkC TK* (r = 0.42; Fig. 5C–5G) expression levels. Episodic Memory performance correlated moderately with *Ngfb* (r = 0.54), *TrkA ECD* (r = 0.55), *TrkA TK* (r = 0.61), *TrkB ECD* (r = 0.57), *TrkB TK* (r = 0.67), and *TrkC TK* (r = 0.43) (Supplemental Figure SF1). Semantic Memory correlated moderately with *Cntf* (r = 0.55), *TrkA ECD* (r = 0.44), *TrkA TK* (r = 0.53), *TrkB ECD* (r = 0.43), and *TrkB TK* (r = 0.48) (Supplemental Figure SF2). Working Memory correlated moderately with *TrkB TK* (r = 0.43) (Supplemental Figure SF3A) while Perceptual Speed showed moderate correlations with *TrkB ECD* (r = 0.40) and *TrkB TK* (r = 0.43) (Supplemental Figure SF3B, C). Moderate Visuospatial correlations

were observed with *Cntf* (r = 0.67), *Ngfb* (r = 0.44), and *TrkB TK* (r = 0.50) expression levels along with a strong correlation with *TrkB ECD* (r = 0.70) expression (Supplemental Figure SF4). No significant correlations were found between transcript levels and NPs, DPs, or NFTs within the NCI group.

Several correlations were found for select neurotrophin and neurotrophin receptor expression levels and region-specific hippocampal complex (entorhinal cortex and hippocampal CA1) counts of NPs and NFTs (Table 7). Moderate negative correlations were observed between higher entorhinal cortex NP load and reduced *TrkA ECD* (r = −0.46) and *TrkA TK* (r = −0.49) expression (Fig. 6A, B). Hippocampal CA1 NP load was moderately correlated with decreased *TrkA TK* (r = −0.56), *TrkB ECD* (r = −0.52) and *TrkB TK* (r = −0.50) expression (Fig. 6C–E). Entorhinal cortex NFT burden was moderately negatively correlated with *Ngfb* (r = −0.47), *TrkA TK* (r = −0.64), *TrkB ECD* (r = −0.48) and *TrkB TK* (r = −0.57) expression levels (Fig. 7). Hippocampal CA1 NFTs correlated moderately with *Cntf* (r = −0.49), *Ngfb* (r = −0.54), *TrkB ECD* (r = −0.45), and *TrkB TK* (r = −0.56) expression levels while a strong correlation was found for *TrkA TK* (r = −0.73) expression (Fig. 8). No significant correlations were found between neurotrophin mRNA levels and DPs.

Between-site transcript analysis

No significant differences were found for any of the neurotrophin or neurotrophin receptor transcripts between RROS or CNDR cohorts for CA1 pyramidal neurons or regional hippocampal dissections (Supplemental Table ST3).

qPCR

qPCR product analysis revealed downregulation of select transcripts that validated results obtained from the custom-designed microarray analyses. *Bdnf* was downregulated in both MCI and AD compared to NCI (p = 0.005; Fig. 9). MCI and AD were not significantly different. Despite low levels of *Ngfb*, this transcript was downregulated in AD compared to NCI (p = 0.001). MCI cases were unavailable for this qPCR analysis. Notably, the neurotrophin receptors *TrkB* and *TrkC* displayed downregulation during the progression of dementia by qPCR. Specifically, MCI and AD were significantly downregulated compared to NCI (*TrkB*, p<0.001; *TrkC*, p<0.001; Fig. 9). In addition, *TrkC* qPCR product levels were significantly less in AD than MCI, indicating progressive downregulation.

Discussion

Single population CA1 pyramidal neuron and hippocampal regional dissections combined with custom-designed microarray and qPCR of neurotrophin and neurotrophin receptor expression in postmortem brains indicate that select neurotrophic-related transcripts are significantly downregulated during the progression of AD. Essentially, the data revealed two separate populations of dysregulated genes. One group was comprised entirely of neurotrophin genes, including *Gdnf*, *Ngfb*, and *Ntf4* in CA1 pyramidal neurons and *Bdnf* in regional hippocampal dissections, which displayed downregulation only in MCI compared to AD and NCI. Upregulation in neurotrophin levels between MCI and AD suggest a form

of cellular plasticity during the progression of dementia. Interestingly, the hippocampal formation has been shown to be neuroplastic (DeKosky et al., 2002; Mufson et al., 2015) and cognitively resilient in the face of progressing AD pathology (Mufson et al., 2016b; Perez et al., 2015), at least at the early stage of the disease process. A similar rebound effect has been reported for hippocampal TrkA protein levels between MCI and AD (Mufson et al., 2012b). In addition, studies have shown a preservation of synapse number, synaptic protein expression, and dendritic spine size in the hippocampus of nondemented aged subjects with a wide range of Braak NFT stages (Mufson et al., 2015, 2016a). Whether the observed decrease in select neurotrophin expression levels in MCI, which recovers as the disease progresses is neuroplastic or aberrant merits further consideration, especially in light of our correlative analyses with multiple indices of cognitive performance.

A second group of neurotrophin and neurotrophin receptor genes, notably *TrkB* and *TrkC* in CA1 pyramidal neurons and *Cntf*, *Ngfb*, *TrkA*, *TrkB*, and *TrkC* in regional hippocampal dissections, are decreased in MCI and AD compared to NCI, indicating early and pervasive downregulation in this aspect of the medial temporal lobe memory circuit. These findings are consistent with our previous observations of alterations in Trk receptors, but not $p75^{NTR}$ in cholinergic basal forebrain and CA1 pyramidal neurons during the progression of dementia (Ginsberg et al., 2006, 2010). Decrements in select neurotrophins and in the cognate NGF, BDNF, and NT3 receptors may serve as a molecular marker for the transition from NCI to MCI, giving an indication of the progression of MCI to frank AD. Supporting this suggestion is a recent report showing that cerebrospinal fluid levels of proNGF, the precursor protein for NGF, marks the transition from NCI to MCI and AD (Counts et al., 2016). To date, AD-based studies have principally focused on the NGF and BDNF families of neurotrophins and neurotrophin receptors. The present results indicate that other growth factors, notably CNTF and GDNF, merit further consideration in their role(s) they play during the progression of dementia.

Although we found similar findings between microdissected CA1 neurons and hippocampus proper expression levels, a greater number of significant neurotrophic gene transcripts were downregulated in the regional hippocampal dissections. Since the latter consist of a heterogeneous mix of cell types, it is likely that multiple vulnerable hippocampal neuronal subtypes (e.g., CA1 neurons, stellate cells, and GABAergic interneurons, among others) and possibly non-neuronal cell types (e.g., astrocytes and microglia) are involved in the observed downregulation (Ginsberg et al., 2000, 2010, 2012; Ginsberg and Che, 2005; Kamme et al., 2003; Miller et al., 2013; Rice et al., 2015). Also, admixed population analysis cannot take into consideration cell loss (or cell proliferation). Therefore, the comparison of CA1 pyramidal neuron single population analysis is particularly informative relative to changes in the entire hippocampal region.

A major strength of the present study design is the ability to correlate antemortem cognitive measures (e.g., MMSE, GCS, and individual tests of Episodic, Semantic, and Working Memory, Perceptual Speed, and Visuospatial domains) with neurotrophin and neurotrophin receptor gene level expression from CA1 pyramidal neurons and regional hippocampal dissections. The power of these correlative studies is evident, as significant correlations were seen with select neurotrophin genes and Trk receptors (but not $p75^{NTR}$) with MMSE and

GCS performance as well as Episodic Memory and other cognitive domains. Moreover, correlations of gene expression levels with neuropathology revealed an informative pattern. Similar to cognitive parameters (Malek-Ahmadi et al., 2016; Mufson et al., 2016b), neurotrophic gene expression correlates better with hippocampal NFTs than NPs, and no significant correlations were observed with DPs. These results suggest that decreases in select neurotrophins/neurotrophin receptors are associated with an increase in NPs and NFTs likely indicating that this decrement contributes to hippocampal cellular dysfunction, particularly within vulnerable CA1 pyramidal neurons. A lack of correlation between neurotrophin and neurotrophin receptor transcripts and AD pathology within the NCI group is likely a function of small sample size, but is still informative given the increasing interest in preclinical AD (Epelbaum et al., 2017; Lim et al., 2016; Mufson et al., 2016a; Rentz et al., 2017). Noting the between-group differences found in the current study, it is likely that downregulation of select neurotrophins and neurotrophin receptors may be detected earlier within larger pre-clinical AD populations that have varying levels of AD pathology in future studies.

These data support the hypothesis that cell type specific decrements in select neurotrophin genes is an early event in MCI, which coincides with downregulation of their cognate Trk receptors during the earliest stages of the progression of dementia. Further, neuronal neurotrophin and Trk receptor dysfunction is associated with prodromal AD and correlates with multiple individual and global measures of cognitive decline and AD neuropathology. A lack of differences between the mild/moderate AD cohort (RROS) and end-stage AD cohort (CNDR) suggests that these neurotrophic transcript reductions plateau during the transition from MCI to AD. Other factors associated with the risk of developing MCI and AD, such as vascular dysfunction and metabolic syndrome (Allegri and Guekht, 2012; Marosi and Mattson, 2014; Passaro et al., 2015), among others, may also impinge on neurotrophic responses within vulnerable neurons, and are worthy of additional study. Taken together, these results suggest that increased neurotrophic activity, driven by elevated cognate Trk receptor expression, could support a neuroplastic response that restores deficits in hippocampal neurotrophic signaling, a concept that should be exploited by drug discovery and therapeutic intervention approaches (Longo et al., 2007; Mufson et al., 2012b; Tuszynski, 2007; Tuszynski et al., 2005) for the treatment of early AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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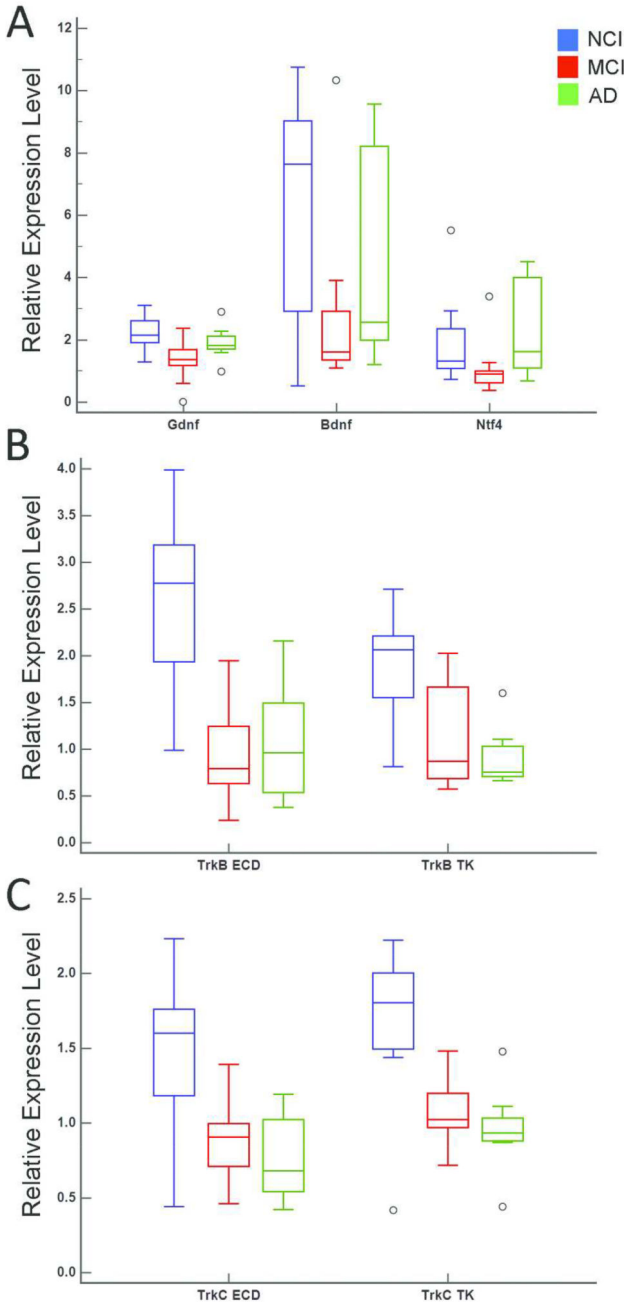

Figure 1.

Boxplots illustrating statistically significant clinical group differences within CA1 pyramidal neurons for neurotrophins *Gdnf*, *Bdnf*, and *Ntf4* (**A**) neurotrophin receptors *TrkB ECD* and *TrkB TK* (**B**), and *TrkC ECD* and *TrkC TK* (**C**).

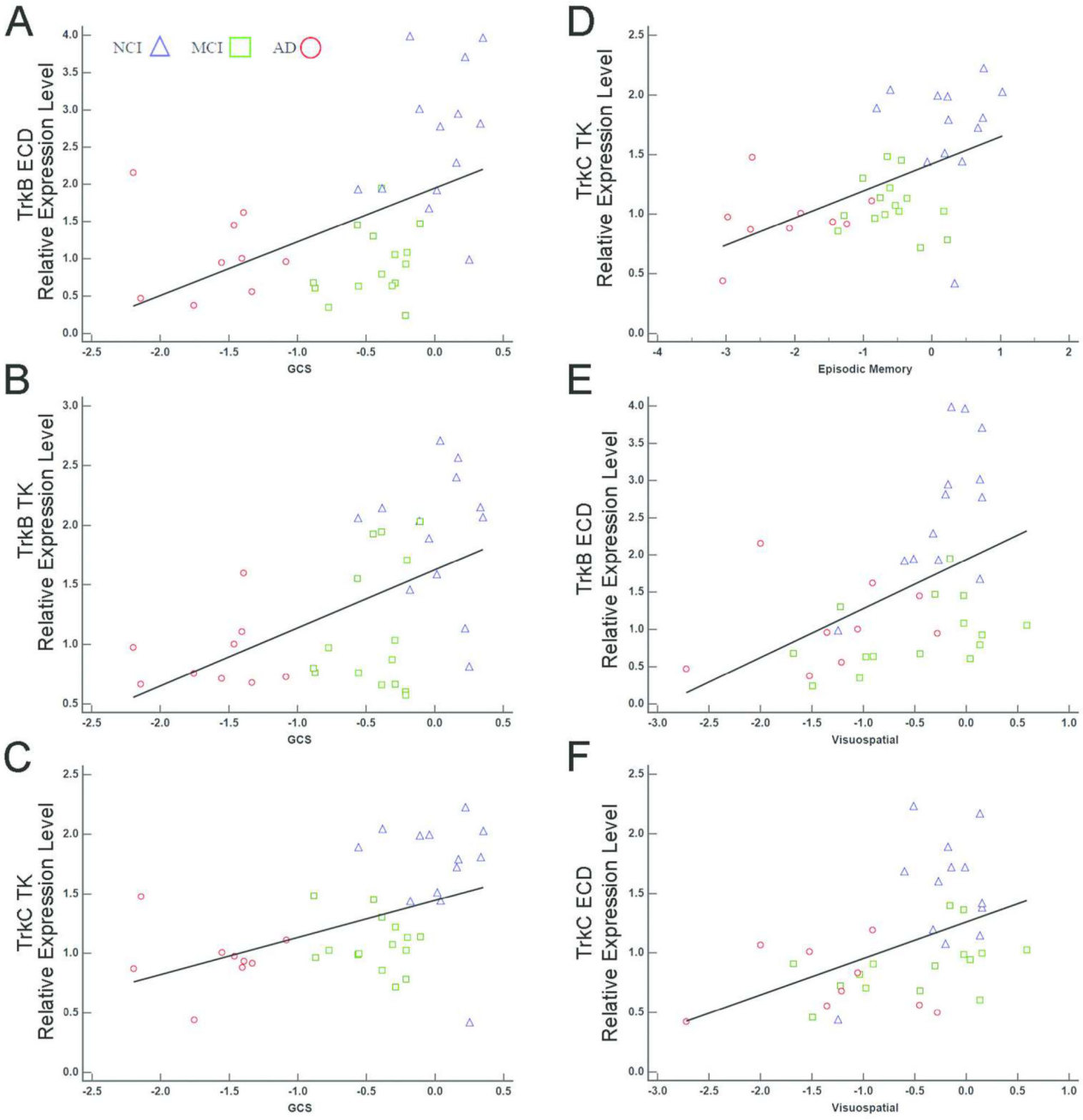

Figure 2.

Scatterplots highlighting significant correlations between *TrkB* and *TrkC* with cognitive decline. GCS performance correlated moderately with *TrkB ECD* (**A**), *TrkB TK* (**B**), and *TrkC TK* (**C**) expression levels. Episodic Memory performance was moderately correlated with *TrkC TK* (**D**) expression. In addition, *TrkB TK* (**E**) and *TrkC TK* (**F**) showed moderate correlations with the Visuospatial domain.

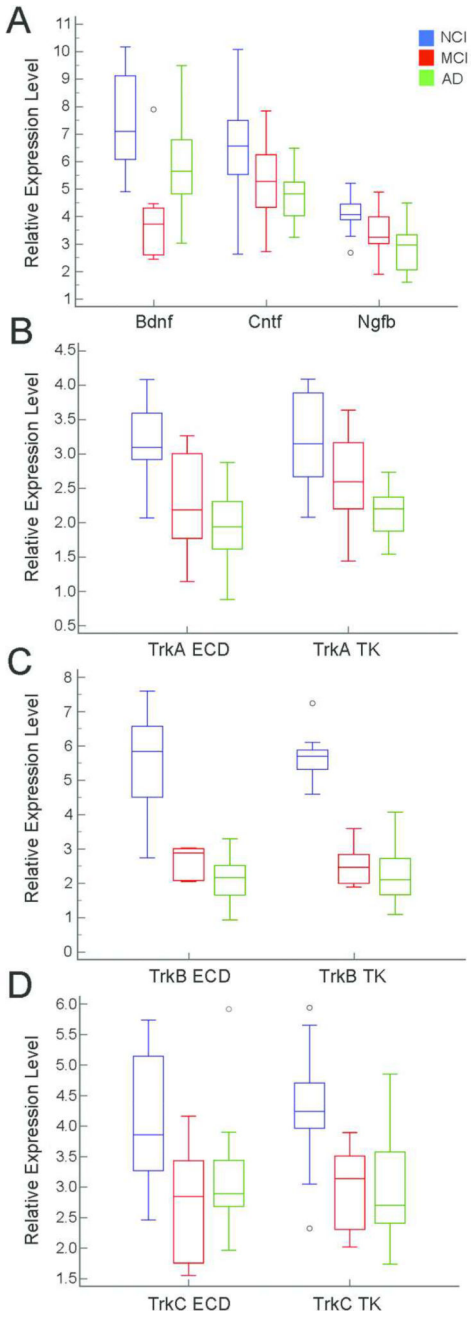

Figure 3.

Boxplots illustrating statistically significant clinical group differences within regional hippocampal dissections for neurotrophins *Bdnf*, *Cntf*, and *Ngfb* (**A**) and neurotrophin receptor transcripts *TrkA ECD* and *TrkA TK* (**B**), *TrkB ECD* and *TrkB TK* (**C**), and *TrkC ECD* and *TrkC TK* (**D**).

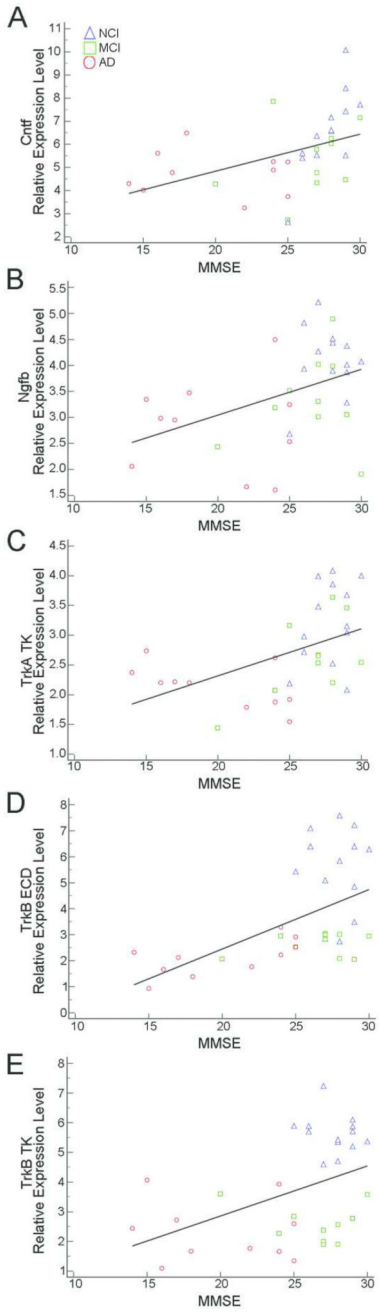

Figure 4.

Scatterplots demonstrating MMSE performance correlated with *Cntf* (**A**), *Ngfb* (**B**), *TrkA TK* (**C**), *TrkB ECD* (**D**), and *TrkB TK* (**E**) expression levels.

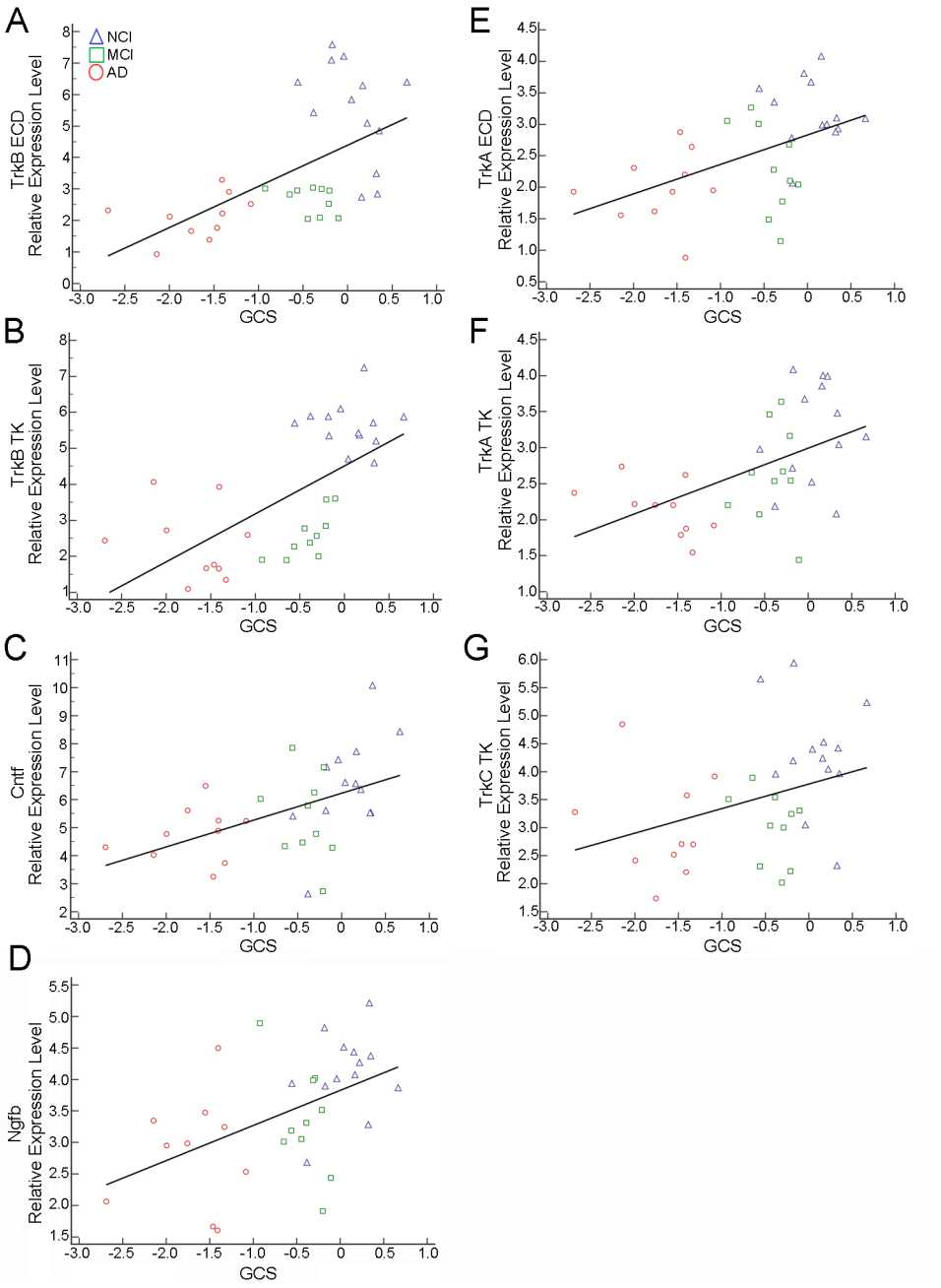

Figure 5.

Scatterplots showing GCS performance correlated strongly with *TrkB ECD* (**A**) and *TrkB TK* expression (**B**). Moderate correlations were found between GCS performance and *Cntf* (**C**), *Ngfb* (**D**), *TrkA ECD* (**E**), *TrkA TK* (**F**), and *TrkC TK* (**G**) expression levels.

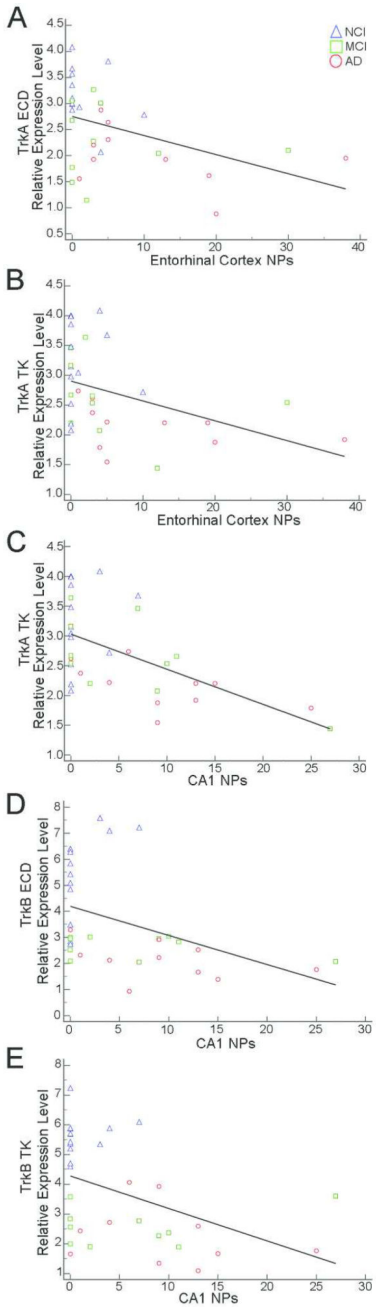

Figure 6.

Scatterplots illustrating negative correlations between entorhinal cortex NP burden and *TrkA ECD* (**A**) and *TrkA TK* (**B**) expression. Hippocampal CA1 NPs were negatively correlated with *TrkA TK* (**C**), *TrkB ECD* (**D**), and *TrkB TK* (**E**) expression levels.

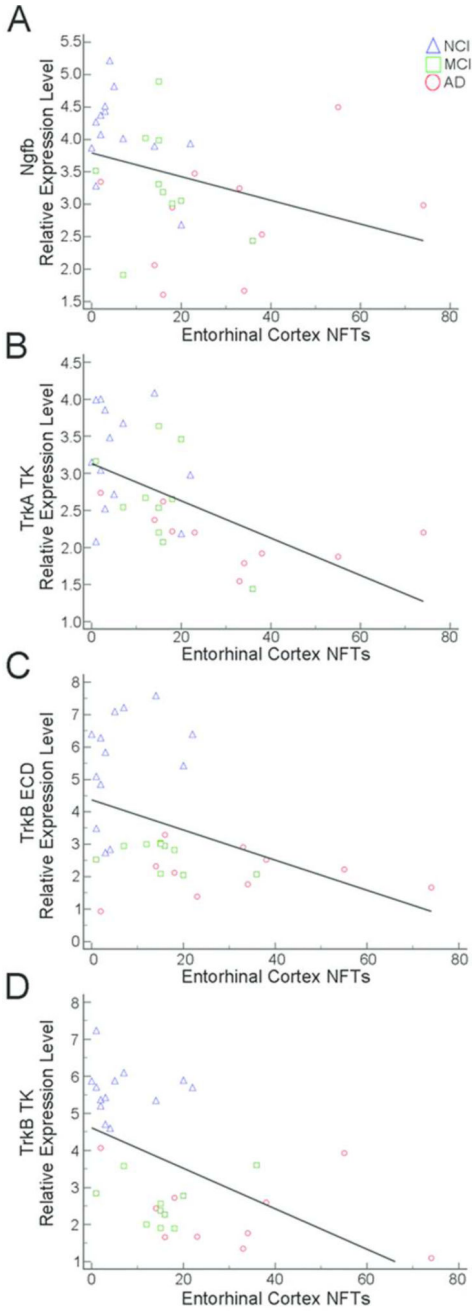

Figure 7.

Entorhinal cortex NFT burden was negatively correlated with *Ngfb* (**A**), *TrkA TK* (**B**), *TrkB ECD* (**C**), and *TrkB TK* (**D**) expression levels.

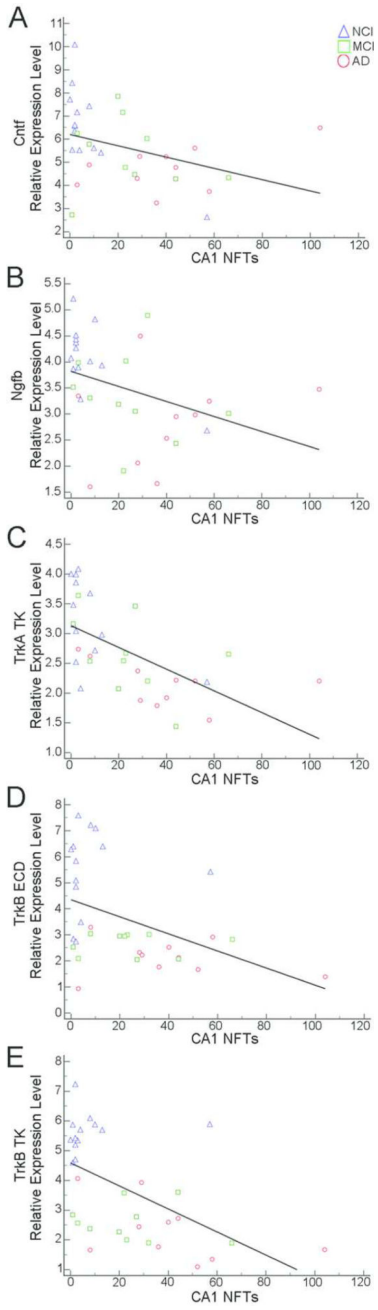

Figure 8.

Hippocampal CA1 NFTs correlated negatively with *Cntf* (**A**), *Ngfb* (**B**), *TrkB ECD* (**D**), and *TrkB TK* (**E**) expression levels while a strong negative correlation was found for *TrkA TK* (**C**) expression.

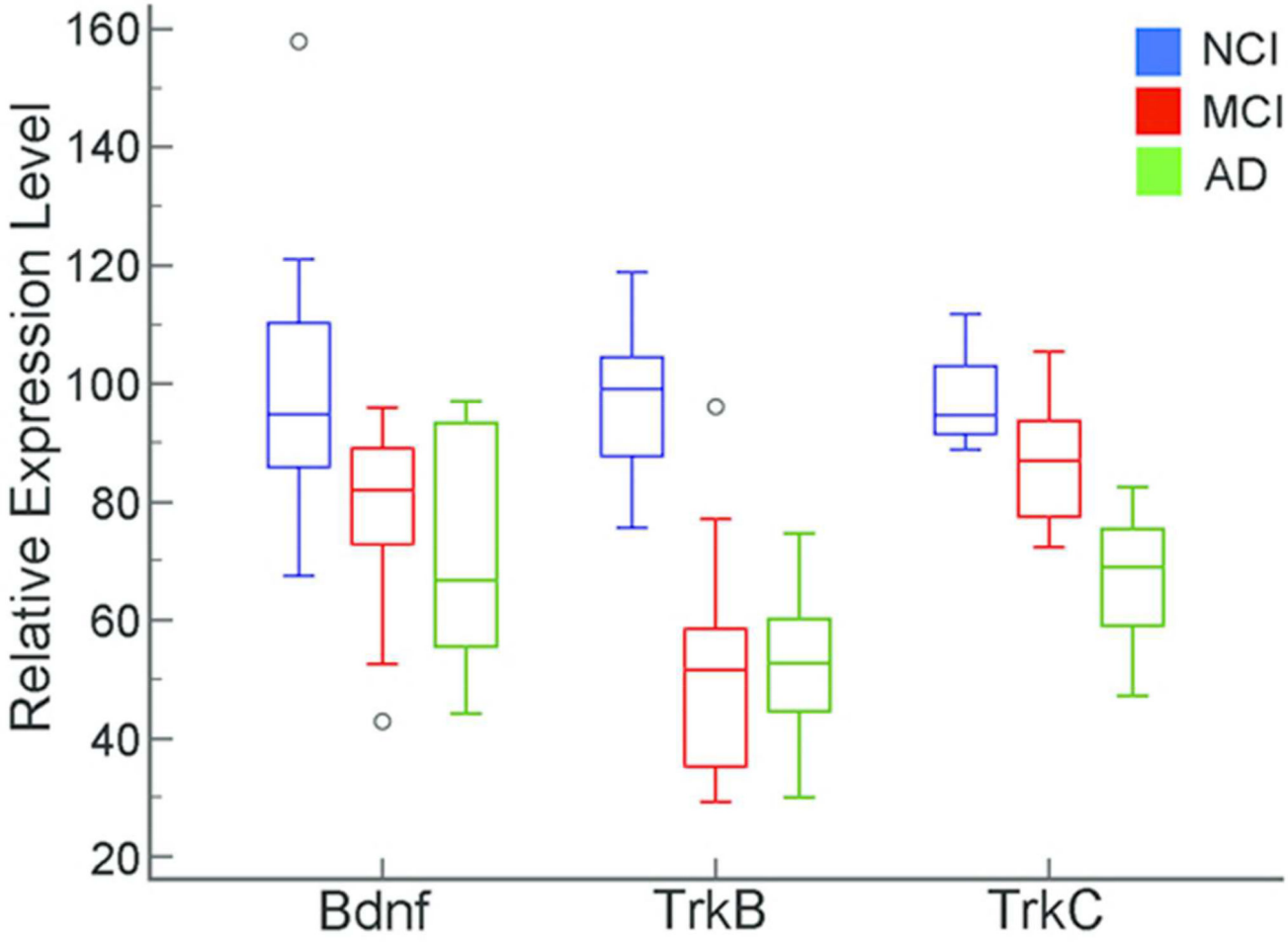

Figure 9.

qPCR validation of clinical group differences for *Bdnf*, *TrkB*, and *TrkC*. *Bdnf* was downregulated in MCI and AD compared to NCI. *TrkB* qPCR product levels were downregulated in MCI and AD compared to NCI. *TrkC* qPCR product levels were significantly downregulated in MCI and AD compared to NCI. Further, *TrkC* expression was less in AD than MCI, indicating progressive downregulation.

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Table 1

Demographic, cognitive, and neuropathological characteristics of cases used for CA1 neuron microarray analysis.

	NCI	MCI	AD	Total	p-value	Groupwise Comparisons
N	13	15	9	37	na	na
Gender (M/F)	7/6	6/9	2/7	15/22	0.33	na
APOE ε4 allele	1/12	7/8	8/1	16/21	<0.001	na
Age at Death (years)	82.95 ± 7.70	85.29 ± 4.51	86.84 ± 6.55	84.85 ± 6.29	0.68	na
Education (years)	17.46 ± 4.07	19.13 ± 2.17	17.56 ± 1.67	18.16 ± 2.94	0.19	na
MMSE	27.85 ± 1.57	26.80 ± 2.73	20.22 ± 4.06	25.57 ± 4.13	<0.001	NCI>MCI, NCI>AD
GCS	0.02 ± 0.27	−0.43 ± 0.25	−1.59 ± 0.37	−0.55 ± 0.69	<0.001	NCI>MCI>AD
Episodic Memory	0.25 ± 0.52	−0.5 8± 0.45	−2.09 ± 0.78	−0.66 ± 1.06	<0.001	NCI>MCI>AD
Semantic Memory	0.20 ± 0.45	−0.26 ± 0.52	−1.19 ± 0.54	−0.32 ± 0.73	<0.001	NCI>MCI>AD
Working Memory	−0.23 ± 0.47	−0.31 ± 0.45	−1.12 ± 0.53	−0.48 ± 0.59	<0.001	NCI>MCI, NCI>AD
Perceptual Speed	−0.37 ± 0.66	−0.59 ± 0.70	−1.83 ± 0.88	−0.81 ± 0.93	<0.001	NCI>MCI, NCI>AD
Visuospatial	−0.22 ± 0.39	−0.49 ± 0.68	−1.28 ± 0.75	−0.59 ± 0.73	<0.001	NCI>MCI, NCI>AD
PMI (hours)	7.45 ± 8.19	6.92 ± 4.01	7.57 ± 3.57	7.27 ± 5.62	0.45	na
Brain Weight (grams)	1245.77±170.39	1239.73±212.03	1123.75±152.59	1216.14±187.37	0.23	na
Braak Stage					<0.001	NCI<MCI, NCI<AD
I	3	2	0	5		
II	2	0	1	3		
III	3	2	0	5		
IV	5	6	2	13		
V	0	5	6	11		
CERAD Diagnosis					0.02	na

	NCI	MCI	AD	Total	p-value	Groupwise Comparisons
No AD	7	1	0	8		
Possible AD	2	2	0	4		
Probable AD	2	5	3	10		
Definite AD	2	7	6	15		
NIA Reagan Diagnosis					0.01	na
Not AD	0	0	0	0		
Low Likelihood	9	4	1	14		
Intermediate	4	8	3	15		
High Likelihood	0	3	5	8		

Results are presented as the mean ± standard deviation.

Table 2

A. Diagnostic group differences for select neurotrophin and neurotrophin receptor genes within CA1 pyramidal neurons.

Transcript	p-value	Groupwise comparison
Gdnf	0.001	NCI>MCI, MCI<AD, AD=NCI
Bdnf	0.09	na
Cntf	0.87	na
Ngfb	0.002	NCI>MCI, MCI<AD, AD=NCI
Ntf3	0.68	na
Ntf4	0.003	NCI>MCI, MCI<AD, AD=NCI
$p75^{NTR}$	0.41	na
TrkA ECD	0.07	na
TrkA TK	0.30	na
TrkB ECD	<0.001	NCI>MCI, AD, AD=MCI
TrkB TK	<0.001	NCI>MCI, AD, AD=MCI
TrkC ECD	<0.001	NCI>MCI, AD, AD=MCI
TrkC TK	<0.001	NCI>MCI, AD, AD=MCI

B. Regression model for transcripts statistically different between groups within CA1 pyramidal neurons.

	Coefficient	Standard Error	p-value	Adjusted R^2	Model Form
Gdnf	0.62	0.18	0.002	0.37	Quadratic
Ngfb	3.28	0.98	0.002	0.34	Quadratic
Ntf4	1.20	0.42	0.008	0.13	Quadratic
TrkB ECD	1.02	0.24	<0.001	0.57	Quadratic
TrkB TK	−0.40	0.14	0.009	0.39	Linear
TrkC ECD	−0.43	0.11	<0.001	0.37	Linear
TrkC TK	−0.32	0.10	0.004	0.39	Linear

Regression models were adjusted for age at death, gender, education level, and APOE ε4 carrier status.

Table 3

Correlations of select neurotrophin and neurotrophin receptor mRNAs in CA1 pyramidal neurons with cognitive measures.

	Gdnf	*Bdnf*	*Cntf*	*Ngfb*	*Ntf3*	*Ntf4*	*p75^{NTR}*	*TrkA ECD*	*TrkA TK*	*TrkB ECD*	*TrkB TK*	*TrkC ECD*	*TrkC TK*
MMSE	0.01	0.10	0.03	−0.18	−0.11	−0.20	−0.03	0.36	0.15	0.23	0.32	0.31	0.44
GCS	0.17	0.26	0.01	0.11	0.05	−0.12	0.16	0.24	0.17	**0.55** *	**0.51** *	0.45	**0.50** *
Episodic Memory	0.18	0.26	0.10	0.07	−0.04	−0.10	0.06	0.24	0.15	0.45	0.42	0.39	**0.52** *
Semantic Memory	0.13	0.11	−0.02	0.17	0.31	−0.11	0.23	0.16	0.14	0.41	0.41	0.36	0.24
Working Memory	0.03	0.05	−0.002	−0.20	−0.12	−0.33	0.10	0.11	0.13	0.24	0.28	0.19	0.26
Perceptual Speed	−0.03	0.21	−0.06	0.10	0.28	−0.04	0.21	0.25	−0.01	0.24	0.26	0.18	0.19
Visuospatial	0.12	−0.20	−0.03	−0.09	−0.26	−0.16	−0.07	0.32	0.36	**0.48** *	0.35	**0.51** *	0.39

*Statistically significant correlations after adjusting for multiple comparisons; FDR α = 0.003.

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Table 4

Demographic, cognitive, and neuropathological characteristics of cases used for regional hippocampal microarray analysis.

	NCI	MCI	AD	Total	p-value	Groupwise Comparisons
N	13	10	10	33	na	na
Gender (M/F)	7/6	4/6	4/6	15/18	0.74	na
APOE ε4 allele	2/11	4/6	7/3	13/20	0.03	na
Age at Death (years)	80.30 ± 9.13	82.96 ± 4.62	85.80 ± 4.61	82.78 ± 6.97	0.18	na
Education (years)	17.77 ± 4.51	18.90 ± 2.38	16.30 ± 3.89	17.67 ± 3.81	0.27	na
MMSE	27.77 ± 1.48	26.50 ± 2.88	20.00 ± 4.42	25.03 ± 4.50	<0.001	NCI>MCI, NCI>AD
GCS	0.07 ± 0.33	−0.41 ± 0.25	−1.68 ± 0.47	−0.61 ± 0.83	<0.001	NCI>MCI>AD
Episodic Memory	0.25 ± 0.56	−0.60 ± 0.46	−2.30 ± 0.84	−0.78 ± 1.24	<0.001	NCI>MCI>AD
Semantic Memory	0.14 ± 0.55	−0.10 ± 0.28	−1.24 ± 0.62	−0.35 ± 0.78	<0.001	NCI>MCI, NCI>AD
Working Memory	−0.09 ± 0.57	−0.40 ± 0.47	−1.06 ± 0.55	−0.48 ± 0.66	<0.001	NCI>MCI, NCI>AD
Perceptual Speed	−0.33 ± 0.56	−0.43 ± 0.45	−2.01 ± 0.95	−0.87 ± 1.01	<0.001	NCI>MCI, NCI>AD
Visuospatial	0.02 ± 0.36	−0.57 ± 0.76	−1.28 ± 0.68	−0.55 ± 0.80	<0.001	NCI>MCI>AD
PMI (hours)	11.05 ± 10.59	7.73 ± 4.77	5.98 ± 3.40	8.51 ± 7.52	0.54	na
Brain Weight (grams)	1291.15±161.38	1255.70±201.58	1155.56±135.56	1241.94±172.81	0.14	na
Braak Stage					<0.001	NCI<MCI, NCI<AD
I	3	1	0	4		
II	4	0	1	5		
III	2	1	0	3		
IV	4	6	3	13		
V	0	2	6	8		
CERAD Diagnosis					0.05	na

	NCI	MCI	AD	Total	p-value	Groupwise Comparisons
No AD	6	2	0	8		
Possible AD	2	2	0	4		
Probable AD	4	2	4	10		
Definite AD	1	4	6	11		
NIA Reagan Diagnosis					<0.001	na
Not AD	0	0	0	0		
Low Likelihood	9	4	1	14		
Intermediate	4	6	4	14		
High Likelihood	0	0	5	5		

Results are presented as the mean ± standard deviation.

Table 5

A. Diagnostic group differences for select neurotrophin and neurotrophin receptor genes within the hippocampus.

Transcript	p-value	Groupwise comparison
Gdnf	0.08	na
Bdnf	0.001	NCI>MCI, MCI<AD, AD=NCI
Cntf	0.02	NCI>MCI, AD, AD=MCI
Ngfb	0.005	NCI>MCI, AD, AD=MCI
Ntf3	0.56	na
Ntf4	0.37	na
Ntf5	0.47	na
$p75^{NTR}$	0.54	na
TrkA ECD	0.001	NCI>MCI, AD, AD=MCI
TrkA TK	0.004	NCI>AD
TrkB ECD	<0.001	NCI>MCI, AD, AD=MCI
TrkB TK	<0.001	NCI>MCI, AD, AD=MCI
TrkC ECD	0.03	NCI>MCI, AD, AD=MCI
TrkC TK	0.001	NCI>MCI, AD, AD=MCI

B. Regression model for transcripts statistically significant between groups within the hippocampus.

	Coefficient	Standard Error	p-value	Adjusted R^2	Model Form
Bdnf	2.84	0.71	<0.001	0.36	Quadratic
Cntf	−1.09	0.37	0.007	0.19	Linear
Ngfb	−0.84	0.19	<0.001	0.36	Linear
TrkA ECD	−0.50	0.16	0.003	0.38	Linear
TrkA TK	−0.46	0.15	0.006	0.34	Linear
TrkB ECD	1.16	0.46	0.02	0.61	Quadratic
TrkB TK	1.41	0.32	<0.001	0.79	Quadratic
TrkC ECD	1.03	0.41	0.02	0.22	Quadratic
TrkC TK	−0.64	0.23	0.01	0.25	Linear

Regression models were adjusted for age at death, gender, education level, and APOE ε4 carrier status

Table 6

Correlations of select neurotrophins and neurotrophin receptor mRNAs in the hippocampus with cognitive measures.

	Gdnf	*Bdnf*	*Cntf*	*Ngfb*	*Ntf3*	*Ntf4*	*Ntf5*	*p75^{NTR}*	*TrkA ECD*	*TrkA TK*	*TrkB ECD*	*TrkB TK*	*TrkC ECD*	*TrkC TK*
MMSE	−0.07	0.12	**0.60** *	**0.43** *	−0.11	0.19	0.07	0.29	0.38	**0.56** *	**0.59** *	**0.47** *	0.25	0.30
GCS	0.06	0.23	**0.54** *	**0.52** *	−0.01	0.23	0.27	0.18	**0.51** *	**0.52** *	**0.62** *	**0.70** *	0.27	**0.42** *
Episodic Memory	0.11	0.34	0.46	**0.54** *	−0.05	0.16	0.15	0.17	**0.55** *	**0.61** *	**0.57** *	**0.67** *	0.32	**0.43** *
Semantic Memory	0.19	0.03	**0.55** *	0.34	−0.32	0.11	0.28	0.24	**0.44** *	**0.53** *	**0.43** *	**0.48** *	0.32	0.18
Working Memory	−0.21	0.08	0.27	0.20	0.17	0.28	0.32	−0.11	0.16	0.22	0.38	**0.43** *	0.17	0.19
Perceptual Speed	−0.12	0.05	0.38	0.32	0.07	0.17	0.13	0.25	0.37	0.32	**0.40** *	**0.43** *	0.17	0.20
Visuospatial	−0.05	0.19	**0.67** *	**0.44** *	0.11	0.10	0.24	0.18	0.37	0.28	**0.71** *	**0.50** *	0.10	0.29

*Statistically significant after adjusting for multiple comparisons; FDR α = 0.02.

Table 7

Correlation of select hippocampal neurotrophin and neurotrophin receptor mRNAs with NPs and NFTs.

	Gdnf	*Bdnf*	*Cntf*	*Ngfb*	*Ntf3*	*Ntf4*	*Ntf5*	*p75^{NTR}*	*TrkA ECD*	*TrkA TK*	*TrkB ECD*	*TrkB TK*	*TrkC ECD*	*TrkC TK*
Entorhinal cortex NPs	0.00	−0.17	−0.03	−0.42	0.08	−0.22	−0.14	0.16	**−0.46** *	**−0.49** *	−0.33	−0.38	−0.05	−0.32
Hippocampal CA1 NPs	−0.03	−0.08	−0.27	−0.38	0.12	−0.18	−0.18	−0.20	−0.35	**−0.56** *	**−0.52** *	**−0.50** *	−0.06	−0.26
Entorhinal cortex DPs	−0.22	−0.04	−0.09	−0.38	−0.20	−0.10	−0.04	−0.08	−0.20	−0.14	−0.28	−0.28	0.02	−0.28
Hippocampal CA1 DPs	−0.17	−0.00	−0.10	−0.22	−0.24	−0.10	−0.08	−0.23	−0.34	−0.20	−0.32	−0.21	0.08	−0.05
Entorhinal cortex NFTs	−0.28	−0.22	−0.41	**−0.47** *	0.25	−0.02	−0.16	−0.20	−0.37	**−0.64** *	**−0.48** *	**−0.57** *	−0.10	−0.35
Hippocampal CA1 NFTs	−0.38	−0.26	**−0.49** *	**−0.54** *	0.31	−0.18	−0.24	−0.09	−0.30	**−0.73** *	**−0.45** *	**−0.56** *	−0.13	−0.41

*Statistically significant after adjusting for multiple comparisons; FDR α = 0.008.