Published in final edited form as:

J Chem Neuroanat. 2011 October ; 42(2): 111–117. doi:10.1016/j.jchemneu.2011.02.004.

Gender differences in NGF receptor expression in cholinergic nucleus basalis neurons during the progression of Alzheimer's disease

Scott E. Counts, PhD¹, Shaoli Che, MD, PhD^{2,3}, Stephen D. Ginsberg, PhD^{2,3,4}, and Elliott J. Mufson, PhD¹

¹ Department of Neurological Sciences, Rush University Medical Center, Chicago, Illinois, USA

² Center for Dementia Research, Nathan Kline Institute, New York University Langone School of Medicine, Orangeburg, New York, USA

³ Department of Psychiatry, New York University Langone School of Medicine, Orangeburg, New York, USA

⁴ Department of Physiology and Neuroscience, New York University Langone School of Medicine, Orangeburg, New York, USA

Abstract

The higher incidence rate of Alzheimer's disease (AD) in elderly women indicates that gender plays a role in AD pathogenesis. Evidence from clinical and pharmacologic studies, neuropathological examinations, and models of hormone replacement therapy suggest that cholinergic basal forebrain (CBF) cortical projection neurons within the nucleus basalis (NB), which mediate memory and attention and degenerate in AD, may be preferentially vulnerable in elderly women compared to men. CBF neurons depend on nerve growth factor (NGF) and their cognate receptors (trkA and p75^{NTR}) for their survival and maintenance. We recently demonstrated a shift in the balance of NGF and its receptors toward cell death mechanisms during the progression of AD. To address whether gender affects NGF signaling system expression within the CBF, we used single cell RNA amplification and custom microarray technologies to compare gene expression profiles of single cholinergic NB neurons in tissue specimens from male and female members of the Religious Orders Study who died with a clinical diagnosis of no cognitive impairment (NCI), mild cognitive impairment (MCI), or mild/moderate AD. p75^{NTR} expression within male cholinergic NB neurons was unchanged across clinical diagnosis, whereas p75^{NTR} mRNA levels in female NB neurons exhibited a ~40% reduction in AD compared to NCI. Male AD subjects displayed a ~45% reduction in trkA mRNA levels within NB neurons compared to NCI and MCI. In contrast, NB neuronal trkA expression in females was reduced ~50% in both MCI and AD compared to NCI. Reduced trkA mRNA levels were associated with poorer global cognitive performance and higher Braak scores in the female subjects. In addition, we found a female-selective reduction in GluR2 AMPA glutamate receptor subunit expression in NB neurons in AD. These data suggest that cholinergic NB neurons in females may be at greater risk for degeneration during the progression of AD and support the concept of gender-specific therapeutic interventions during the preclinical stages of the disease.

Address correspondence to: Scott E. Counts, PhD, Assistant Professor of Neurological Sciences, Rush University Medical Center, 1735 West Harrison Street Suite 300, Chicago IL 60612, Phone 312-563-3569, Fax 312-563-3571, scounts@rush.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Alzheimer's disease; cholinergic; gender; glutamate receptor; microarray; mild cognitive impairment; nerve growth factor; nucleus basalis

Introduction

The number of persons with AD is expected to rise dramatically in the coming decades as the “baby boomer” generation reaches the ages at greatest risk for the disease (Hebert et al., 2003; Wimo et al., 2011). Therefore, dementia research has increasingly focused on identifying neurobiologic risk factors as a long-term strategy to confront this looming crisis. To this end, epidemiological evidence shows that the female gender may be a significant risk factor for AD. Dementia prevalence studies consistently report that the prevalence of AD is greater in women than men, although this difference may reflect survival rates favoring women (Jorm et al., 1987; Ott et al., 1998). However, findings from the European Studies of Dementia (EURODEM) network show that the relative risk of AD for females, adjusting for age and education, is 1.54 compared to males (Launer et al., 1999). A more detailed EURODEM analysis of gender differences in the risk of AD estimated relative risk using the same adjustments as well as adjustments for age- and sex-specific prevalence of dementia and age- and sex-specific mortality rates (Andersen et al., 1999). This analysis demonstrated that the incidence rate of AD in men increased from 0.3 per thousand in the age group 65–69 to 16 per thousand in those 80–84 years of age. In contrast, the incidence rate of AD in women increased from 2 per thousand in the 65–69 age group to 28 per thousand in the 80–84 age group (Andersen et al., 1999). Hence, whereas greater longevity in women may favor a higher prevalence of AD in the aged population, carefully controlled epidemiological analysis shows that the incidence rate of AD is higher in aged women compared to men.

The neurobiological factors that influence the risk for AD in women have yet to be determined (Ott, 1999). Several studies reported no sex differences in the accumulation of senile plaques or neurofibrillary tangles (NFTs) in the hippocampus and neocortex of AD subjects (Miller et al., 1984; Sandberg et al., 2001). On the other hand, cholinergic basal forebrain (CBF) nucleus basalis (NB) neurons, a continuum of cholinergic cortical projection neurons that mediate memory and attentional processes (Baxter and Chiba, 1999) and degenerate in AD (Whitehouse et al., 1981), exhibit 35% more NFT-bearing neurons in females than males (Salehi et al., 1998). Furthermore, the number of NFT-bearing NB neurons correlated with Global Dementia Score in these subjects (Salehi et al., 1998). Interestingly, female AD patients performed significantly worse than male patients on language tests of semantic memory and verbal fluency (Buckwalter et al., 1996; Henderson and Buckwalter, 1994). In this regard, cholinergic modulation of language skills is supported by studies showing that elderly people using anticholinergic medication performed poorly on verbal fluency and semantic memory tests (Ancelin et al., 2006; Lechevallier-Michel et al., 2005), whereas nicotine improved language skills in AD patients (Parks et al., 1996) and the cholinergic modulator bifemelane improved performance on naming tests in subjects with fluent aphasia (Tanaka et al., 1997). Moreover, anticholinergic treatment of young women inhibited semantic memory and verbal fluency in a dose-dependent manner, suggesting cholinergic loss may be associated with the selective language impairments found in women with AD (Aarsland et al., 1994). Taken together, these clinical pathologic data offer a strong rationale for exploring gender-specific differences in the molecular signature of NB cholinergic neurons during the progression of AD.

To test whether differences in CBF molecular pathophysiology might contribute to the increased incidence of AD in women, we profiled gene expression patterns of single cholinergic NB neurons microaspirated *post mortem* from participants of the Religious Orders Study who died with a clinical diagnosis of no cognitive impairment (NCI), mild/moderate AD, or with mild cognitive impairment (MCI), a putative preclinical AD syndrome (Petersen, 2004). We focused on classes of genes which support cholinergic neuronal survival, particularly those mediating the actions of the cholinergic neurotrophin, nerve growth factor (NGF). In this regard, cholinergic NB neurons express two NGF receptors, the pan-neurotrophin receptor p75^{NTR} and the NGF-specific receptor tyrosine kinase TrkA, which mediate the binding, internalization, and retrograde transport of target derived NGF from the cortex to NB soma (Bothwell, 1995; Kaplan and Miller, 1997; Mufson and Kordower, 1999; Sobriela et al., 1994). These NGF receptor complexes activate signal transduction cascades that promote the survival and cholinergic phenotype of NB neurons (Kaplan and Stephens, 1994; Klesse and Parada, 1999; Lad et al., 2003; Sofroniew et al., 2001). Recently, we demonstrated a shift in the balance of NGF and its receptors toward cell death mechanisms during the progression of AD in a mixed-gender population of subjects (Counts and Mufson, 2005; Mufson et al., 2007). Hence, we specifically tested for gender differences in NGF receptor expression levels across clinical diagnostic groups ranging from no cognitive impairment to mild cognitive impairment to AD to gauge the level of neurotrophic support for these neurons in men and women during the onset of the disease.

Materials and Methods

Clinical and pathological evaluation

Details of the annual clinical and neuropsychological evaluation of the Religious Orders Study cohort have been published (Bennett et al., 2002; Mufson et al., 1999). Cognitive testing was performed under the auspices of a neuropsychologist and scores were available within the last year of death. A board-certified neurologist with expertise in the evaluation of the elderly made a clinical diagnosis for each participant based upon review of all clinical data and physical examination. Subjects were categorized as NCI ($n = 9$; 5 male, 4 female), MCI ($n = 9$, 4 male, 5 female), or AD ($n = 8$; 4 male, 4 female) (see Table I). The MCI population was defined as subjects who exhibited impaired neuropsychological test scores but did not meet the clinical criteria for AD recommended by the joint working group of the National Institute of Neurologic and Communicative Disorders and Stroke/AD and Related Disorders Association (NINCDS/ADRDA) (McKhann et al., 1984). These criteria are compatible with those used by experts in the field to describe subjects who are not cognitively normal but do not meet established criteria for dementia (Petersen et al., 2001).

At autopsy, brains were removed from the calvarium and cut into 0.5 cm thick slabs using a Plexiglas brain cutting apparatus. The slabs were hemisected and one hemisphere was immersion-fixed in a 4% paraformaldehyde solution in 0.1 M phosphate buffer (PB, pH 7.2), cryoprotected, and cut frozen into 40 μ m thick sections (Mufson et al., 2002). A neuropathologist blinded to clinical diagnosis performed the neuropathological evaluation. Cases were classified based on the NIA Reagan and CERAD criteria (Hyman and Trojanowski, 1997; Mirra et al., 1991) as well as Braak stage (Braak and Braak, 1991). Slabs from the opposite hemisphere were frozen at -80°C .

Immunohistochemistry

RNase-free precautions were used throughout the experimental procedures. The presence of intact RNA in the tissue sections was confirmed by acridine orange histofluorescence and bioanalysis (Agilent, Santa Clara, CA) (Che and Ginsberg, 2004; Ginsberg, 2005). Tissue

sections were processed using a monoclonal antibody raised against the human p75^{NTR} neurotrophin receptor, which colocalizes with approximately 95% of all CBF neurons within the human NB (Mufson et al., 1989). Tissue sections were incubated for 1 hour in a phosphate-buffered saline (PBS, pH 7.2) solution containing 0.3% Triton X-100, 3% normal horse serum (NHS) and 2% bovine serum albumin (BSA). Primary antibody (p75^{NTR}, 1:60,000; Neomarkers, Fremont, CA) was applied for 4 hours at ambient room temperature in PBS/0.4% Triton X-100, 1% NHS and 1% BSA. Sections were processed with the ABC kit (Vector Labs, Burlingame, CA) and developed with 2.5% nickel II sulfate, 0.05% 3' 3' diaminobenzidine (Sigma) and 0.005% hydrogen peroxide (Mufson et al., 2002). Immunostained tissue sections were slide mounted but not coverslipped and stored in PB at 4 °C.

Single cell microaspiration, RNA amplification, and array hybridization

Immunopositive neurons from the anterior NB subfields were individually aspirated using a micromanipulator and microcontrolled vacuum source (Eppendorf, Westbury, NY) attached to a Nikon TE2000 inverted microscope (Fryer, Huntley, IL) (Counts et al., 2009; Ginsberg et al., 2010; Mufson et al., 2002). RNA amplification from NB neurons was performed using terminal continuation (TC) RNA amplification methodology (Che and Ginsberg, 2004; Ginsberg, 2005) (see <http://cdr.rfmh.org/pages/ginsberglabpage.html>). The final amplification step used cDNA made from individual neuronal mRNA as a template for *in vitro* transcription in the presence of T7 RNA polymerase and [³³P]-UTP. Radiolabeled TC RNA probes were then hybridized to custom-designed microarrays. Arrays were hybridized overnight at 42 °C in a rotisserie oven and washed sequentially in 2X SSC/0.1% SDS, 1X SSC/0.1% SDS and 0.5X SSC/0.1% SDS for 20 min each at 42°C (Counts et al., 2007; Ginsberg et al., 2010; Mufson et al., 2002). Arrays were placed in a phosphor screen for 24 hours and developed on a Storm phosphor imager (GE Healthcare, Piscataway, NJ).

Custom-designed microarray platforms and data collection

Array platforms consisted of 1µg of linearized cDNA purified from plasmid preparations adhered to high-density nitrocellulose (Hybond XL, GE Healthcare). Approximately 576 cDNAs were utilized on the current array platform. Hybridization signal intensity is quantified by subtracting background using empty vector (pBluescript). Expression of TC amplified RNA bound to each linearized cDNA was expressed as a ratio of the total hybridization signal intensity of the array (i.e., global normalization) (Ginsberg, 2005). Data analysis generated an expression profile of relative changes in mRNA levels among cholinergic NB neurons isolated from the different genders and clinical diagnostic groups. Each neuron was analyzed in triplicate via three independent probe amplifications and array hybridizations using the original neuronal cDNA pool as template.

Real time quantitative PCR (qPCR)

qPCR was performed on unfixed, microdissected frozen anterior NB ($n = 4-5$ per gender and diagnosis) using PCR primers specific for human trkA and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (SA Biosciences, Frederick, MD) (Counts et al., 2007; Ginsberg et al., 2010). Samples were loaded in triplicate on 96 well plates and analyzed using SYBR green reporter dye on an Opticon 2 DNA Engine (Bio-Rad, Hercules, CA). Standard curves and cycle threshold (Ct) were measured using standards obtained from human *post mortem* brain RNA. For graphical representation, Ct values were converted to signal intensity values using Easy Engine software (Bio-Rad).

Statistical analysis

Demographic variables (Table I) were compared among clinical diagnostic groups by Chi-square testing or the Kruskal–Wallis test with Dunn’s correction for pairwise comparisons. Expression levels of select mRNAs were clustered and displayed using a bioinformatics and graphics software package (GeneLinker Gold, Improved Outcomes, Kingston, ON) (Counts et al., 2007; Ginsberg et al., 2010; Mufson et al., 2002). A false discovery rate controlling procedure (Reiner et al., 2003) was used to reduce type I errors due to the large number of genes analyzed simultaneously. Relative changes in hybridization signal intensity of individual mRNAs and qPCR were analyzed by one-way ANOVA with *post hoc* Newman-Keuls analysis. The association between gene expression levels and clinical or neuropathological variables was evaluated via Spearman correlation (Counts et al., 2007). Level of significance was set at $p < 0.05$.

Results

Case demographics

Clinical and neuropathological characteristics of the 26 cases (9 NCI, 9 MCI, 8 mild/moderate AD) included in the microarray analysis are summarized in Table I. No significant differences were observed for age, gender, educational level, and *post mortem* interval (PMI) across the 3 groups. The ApoE4 allele was more frequent in AD compared to NCI and MCI cases. Distribution of Braak scores was significantly different across clinical conditions, with NCI cases having lower Braak staging than AD. NCI cases were classified as Braak stages I–II (44.5%) or III–IV (55.5%). The MCI cohort displayed Braak stages III–IV (77.8%) or V–VI (22.2%). AD cases were classified as Braak stages I–II (12.5%), III–IV (25%), or V–VI (62.5%) (Table I). NIA-Reagan criteria also significantly differentiated NCI subjects from AD cases. There were no within-group differences between male and female subjects.

Gender differences in NGF receptor gene regulation in cholinergic NB neurons in MCI subjects

A total of 96 neurons (~3–4/case) were analyzed by custom microarrays. p75^{NTR} mRNA expression was unchanged across clinical diagnosis in NB neurons derived from male CBF tissue (Fig. 1A). In contrast, p75^{NTR} mRNA levels in NB neurons from females exhibited an approximately 40% decrease in AD compared to NCI cases (Fig. 1B). In male subjects, NB neurons displayed a ~55% reduction in trkA mRNA levels in AD compared to NCI (Fig. 1A, right panel), whereas in female subjects trkA levels were reduced ~40% in MCI and ~65% in AD compared to NCI (Fig. 1B, right panel). These gender specific differences in NGF receptor expression across clinical groups were validated by qPCR using frozen samples of basal forebrain from the same cases (Fig. 2).

TrkA mRNA levels in cholinergic NB neurons are associated with cognitive performance in female subjects

TrkA mRNA levels in NB neurons were plotted against the *ante mortem* performance of these subjects on the Mini Mental State Exam (MMSE), a measure of global cognitive function, as well as a Global Cognitive Score (GCS), a z score of the 19 tests comprising the Religious Orders neuropsychological test battery, measured within a year of death (Bennett et al., 2002; Mufson et al., 1999). Spearman rank correlations revealed that decreasing trkA mRNA levels correlate with poorer MMSE and GCS performance in females independent of clinical diagnosis, whereas males did not display an association between trkA mRNA levels and MMSE scores and GCS (Table 2). Moreover, there were positive associations between p75^{NTR} levels and both MMSE and GCS in female but not male subjects (Table 2).

A correlation analysis between NGF receptor expression and *post mortem* neuropathological diagnosis found no associations between p75^{NTR} mRNA levels and CERAD, Braak, or NIA-Reagan indices (not shown). On the other hand, we found an association between increasing Braak score and decreasing trkA mRNA levels in the female cases ($r = -0.65$, $p = 0.01$).

Glutamate receptor genes are differentially regulated in cholinergic NB neurons derived from male and female subjects with mild AD

A further analysis of the microarray data comparing the AMPA glutamate receptor transcripts GluR1 (NCBI/Unigene annotation GRIA1), GluR2 (GRIA2), GluR3 (GRIA3), and GluR4 (GRIA4) found a ~50% reduction in GluR1 transcript levels in NB neurons from both male and female subjects with AD (Fig. 3). Decreasing GluR1 expression levels were positively associated with poorer MMSE performance and lower GCS in both male and female subjects (Table 3). By contrast, GluR2 expression was selectively reduced ~50% in female subjects compared to males with AD (Fig 3). In females, GluR2 expression levels within NB neurons were associated with GCS; these correlations were not observed in male subjects (Table 3). GluR2 levels also displayed female-specific correlations with CERAD ($r = 0.73$, $p = 0.003$) and NIA-Reagan ($r = 0.69$, $p = 0.007$) diagnostic scores.

Discussion

In the present study, we investigated whether molecular pathogenic mechanisms for gender disparity in the risk for AD might be found within the cholinergic neurons of the NB. To this end, we found that trkA and p75^{NTR} NGF receptor gene expression was reduced to a greater extent in women compared to men during the progression of AD. Furthermore, we discovered a female-specific reduction of GluR2 expression in NB neurons in AD cases. Hence, while cholinergic NB neurons degenerate independent of gender in the later stages of AD, these data suggest that they may be preferentially vulnerable in women during the initial stages of the disease and hasten the onset of clinical symptoms.

With respect to NGF receptor expression, the most intriguing finding was that levels of trkA transcripts within NB neurons were reduced during the transition from NCI to MCI in females, but stable in males during this same prodromal phase of AD. This suggests that previous data showing a step-down reduction in trkA expression within NB neurons from NCI to MCI to AD in a mixed-gender population (Ginsberg et al., 2006) was driven by the female cases. In contrast to trkA, levels of p75^{NTR} mRNA in female NB neurons were unchanged in the transition from NCI to MCI. This is significant since NGF signaling through the TrkA receptor promotes survival of CBF neurons, yet recent studies have shown that the NGF precursor protein, proNGF, can elicit a pro-apoptotic signal through the p75^{NTR} receptor in the absence of TrkA via interactions with the co-receptor sortilin (Counts and Mufson, 2005; Mufson et al., 2007). Since proNGF levels are increased in cortical cholinergic projection fields of the NB in MCI (Peng et al., 2004), the observed shift in trkA:p75^{NTR} expression stoichiometry in female neurons during MCI may render these cells more vulnerable to degeneration by a relative lack of neurotrophic support and increased apoptotic signaling. Along these lines, reductions in neuronal trkA expression levels in the female subjects were positively associated with reduced *ante mortem* cognitive function as assessed by MMSE and GCS (Table 2), suggesting that differential changes in neurotrophic gene expression in CBF neurons may contribute to changes in the cognitive status of elderly women and potentially influence their higher AD incidence rate.

The mechanisms underlying this female-specific alteration in NB trkA receptor expression in preclinical AD are unclear. However, menopause and the concomitant loss of circulating estrogen is a critical physiological difference between men and women past the age of ~55,

and numerous rodent models of menopause show that estrogen regulates the cholinotrophic properties of NGF (Gibbs, 1998; Gibbs et al., 1998; McMillan et al., 1996; Toran-Allerand et al., 1999). For instance, separate groups found that *trkA* and choline acetyltransferase [ChAT, the synthetic enzyme for acetylcholine (ACh)] mRNA was reduced in NB neurons of ovariectomized female rats compared to age-matched controls, whereas estrogen replacement following ovariectomy restored *trkA* mRNA levels (Gibbs, 1998; McMillan et al., 1996). These data support the notion that estrogen facilitates cholinergic NB neuron responsiveness to NGF. Moreover, other putative neuroprotective effects of estrogen on cholinergic neurons include increased neuritogenesis (Dominguez et al., 2004) and the prevention of tau hyperphosphorylation (Alvarez-de-la-Rosa et al., 2005), an initial step in NFT formation. This latter observation is intriguing in light of increased NFT pathology within the NB in female AD subjects (Salehi et al., 1998) and the present finding of a female-specific association between lower NB *trkA* levels and a higher Braak stage (Table 3). Hence, given the current lifespan of ~75–80 years, the long term effects of estrogen loss on cholinergic NB neuron viability may contribute to a greater selective vulnerability of these cells in women as compared to men. In this study, only three of the female subjects (23%) received hormone replacement therapy *ante mortem* with no discernable effect on *trkA* expression. Therefore, whether hormone replacement therapy would influence NGF receptor expression during this interval remains an open question.

In addition to gender differences in cholinotrophic support for NB neurons in early AD, we also found that expression of the AMPA-glutamate receptor GluR2 subunit was selectively reduced in these neurons in females compared to males during the transition from MCI to AD. GluR2 expression levels were also correlated with cognitive function as assessed by GCS (Table 3). The cholinergic NB neurons receive glutamatergic afferents, which presumably facilitate excitatory cortical cholinergic transmission through AMPA and other ionotropic glutamate receptor assemblies (Carnes et al., 1990; Zaborszky et al., 1997). On the other hand, overstimulation of these receptors can result in supraphysiological calcium (Ca^{2+}) levels in postsynaptic cholinergic neurons resulting in excitotoxic cell death (Ginsberg et al., 1999; Michel and Agid, 1995; Mufson et al., 2003). Significantly, AMPA GluRs containing the GluR2 subunit are Ca^{2+} -impermeable and the experimental down-regulation of GluR2 expression induces ischemic cell death (Liu et al., 2004). Therefore, the loss of GluR2 levels in female cholinergic NB cells may render them more vulnerable to Ca^{2+} -mediated excitotoxicity in the early stages of AD.

In summary, the current data suggest that neurotrophic and glutamatergic abnormalities within the basocortical cholinergic projection system in females play a key role in the selective vulnerability of these neurons during the onset of AD and therefore may increase the risk of cognitive dysfunction in women. Further studies using larger sample sizes and different study cohorts will be necessary to confirm these findings and potentially uncover other novel gender-specific differences in the molecular signature of NB neurons in AD. Although rodent studies modeling menopause suggest that estrogen replacement might restore cholinotrophic function in females, further studies will be required to gauge the relative contribution of menopause to cholinergic neurodegeneration. Nevertheless, the present report supports the notion that aged women in the initial stages of cognitive decline might especially benefit from neurotrophic (Houeland et al. 2010; Tuszynski et al., 2005) and/or novel gender-specific therapies.

Acknowledgments

We thank M. Nadeem for expert technical assistance. This work was supported by National Institute of Health grants AG30500, AG14449, AG16088, AG09466 AG10161, AG17617, NS48447 and the Illinois Department of Public Health.

Abbreviations

AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
MCI	mild cognitive impairment
NGF	nerve growth factor

References

- Aarsland D, Larsen JP, Reinvang I, Aasland AM. Effects of cholinergic blockade on language in healthy young women. Implications for the cholinergic hypothesis in dementia of the Alzheimer type. *Brain*. 1994; 117 (Pt 6):1377–1384. [PubMed: 7820573]
- Alvarez-de-la-Rosa M, Silva I, Nilsen J, Perez MM, Garcia-Segura LM, Avila J, Naftolin F. Estradiol prevents neural tau hyperphosphorylation characteristic of Alzheimer's disease. *Ann N Y Acad Sci*. 2005; 1052:210–224. [PubMed: 16024764]
- Ancelin ML, Artero S, Portet F, Dupuy AM, Touchon J, Ritchie K. Non-degenerative mild cognitive impairment in elderly people and use of anticholinergic drugs: Longitudinal cohort study. *BMJ*. 2006; 332:455–459. [PubMed: 16452102]
- Andersen K, Launer LJ, Dewey ME, Letenneur L, Ott A, Copeland JR, Dartigues JF, Kragh-Sorensen P, Baldereschi M, Brayne C, Lobo A, Martinez-Lage JM, Stijnen T, Hofman A. Gender differences in the incidence of AD and vascular dementia: The eurodem studies. EURODEM Incidence Research Group. *Neurology*. 1999; 53:1992–1997. [PubMed: 10599770]
- Baxter MG, Chiba AA. Cognitive functions of the basal forebrain. *Curr Opin Neurobiol*. 1999; 9:178–183. [PubMed: 10322180]
- Bennett DA, Wilson RS, Schneider JA, Evans DA, Beckett LA, Aggarwal NT, Barnes LL, Fox JH, Bach J. Natural history of mild cognitive impairment in older persons. *Neurology*. 2002; 59:198–205. [PubMed: 12136057]
- Bothwell M. Functional interactions of neurotrophins and neurotrophin receptors. *Annu Rev Neurosci*. 1995; 18:223–253. [PubMed: 7605062]
- Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol*. 1991; 82:239–259. [PubMed: 1759558]
- Buckwalter JG, Rizzo AA, McCleary R, Shankle R, Dick M, Henderson VW. Gender comparisons of cognitive performances among vascular dementia, Alzheimer disease, and older adults without dementia. *Arch Neurol*. 1996; 53:436–439. [PubMed: 8624219]
- Carnes KM, Fuller TA, Price JL. Sources of presumptive glutamatergic/aspartatergic afferents to the magnocellular basal forebrain in the rat. *J Comp Neurol*. 1990; 302:824–852. [PubMed: 1982006]
- Che S, Ginsberg SD. Amplification of RNA transcripts using terminal continuation. *Lab Invest*. 2004; 84:131–137. [PubMed: 14647400]
- Counts SE, He B, Che S, Ginsberg SD, Mufson EJ. Galanin fiber hyperinnervation preserves neuroprotective gene expression in cholinergic basal forebrain neurons in Alzheimer's disease. *J Alzheimers Dis*. 2009; 18:885–896. [PubMed: 19749437]
- Counts SE, He B, Che S, Ikonovic MD, DeKosky ST, Ginsberg SD, Mufson EJ. Alpha7 nicotinic receptor up-regulation in cholinergic basal forebrain neurons in Alzheimer disease. *Arch Neurol*. 2007; 64:1771–1776. [PubMed: 18071042]
- Counts SE, Mufson EJ. The role of nerve growth factor receptors in cholinergic basal forebrain degeneration in prodromal Alzheimer disease. *J Neuropathol Exp Neurol*. 2005; 64:263–272. [PubMed: 15835262]
- Dominguez R, Jalali C, de Lacalle S. Morphological effects of estrogen on cholinergic neurons in vitro involves activation of extracellular signal-regulated kinases. *J Neurosci*. 2004; 24:982–990. [PubMed: 14749443]
- Gibbs RB. Impairment of basal forebrain cholinergic neurons associated with aging and long-term loss of ovarian function. *Exp Neurol*. 1998; 151:289–302. [PubMed: 9628764]

- Gibbs RB, Burke AM, Johnson DA. Estrogen replacement attenuates effects of scopolamine and lorazepam on memory acquisition and retention. *Horm Behav.* 1998; 34:112–25. [PubMed: 9799622]
- Ginsberg SD. RNA amplification strategies for small sample populations. *Methods.* 2005; 37:229–237. [PubMed: 16308152]
- Ginsberg SD, Alldred MJ, Counts SE, Cataldo AM, Neve RL, Jiang Y, Wu J, Chao MV, Mufson EJ, Nixon RA, Che S. Microarray analysis of hippocampal CA1 neurons implicates early endosomal dysfunction during Alzheimer's disease progression. *Biol Psychiatry.* 2010; 68:885–893. [PubMed: 20655510]
- Ginsberg SD, Che S, Wu J, Counts SE, Mufson EJ. Down regulation of trk but not p75^{ntr} gene expression in single cholinergic basal forebrain neurons mark the progression of Alzheimer's disease. *J Neurochem.* 2006; 97:475–487. [PubMed: 16539663]
- Ginsberg SD, Portera-Cailliau C, Martin LJ. Fimbria-fornix transection and excitotoxicity produce similar neurodegeneration in the septum. *Neuroscience.* 1999; 88:1059–1071. [PubMed: 10336121]
- Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the us population: Prevalence estimates using the 2000 census. *Arch Neurol.* 2003; 60:1119–1122. [PubMed: 12925369]
- Henderson VW, Buckwalter JG. Cognitive deficits of men and women with Alzheimer's disease. *Neurology.* 1994; 44:90–96. [PubMed: 8290098]
- Houeland G, Romani A, Marchetti C, Amato G, Capsoni S, Cattaneo A, Marie H. Transgenic mice with chronic NGF deprivation and Alzheimer's disease-like pathology display hippocampal region-specific impairments in short- and long-term plasticities. *J Neurosci.* 2010; 30:13089–13094. [PubMed: 20881126]
- Hyman BT, Trojanowski JQ. Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. *J Neuropathol Exp Neurol.* 1997; 56:1095–1097. [PubMed: 9329452]
- Jorm AF, Korten AE, Henderson AS. The prevalence of dementia: A quantitative integration of the literature. *Acta Psychiatr Scand.* 1987; 76:465–479. [PubMed: 3324647]
- Kaplan DR, Miller FD. Signal transduction by the neurotrophin receptors. *Curr Opin Cell Biol.* 1997; 9:213–221. [PubMed: 9069267]
- Kaplan DR, Stephens RM. Neurotrophin signal transduction by the trk receptor. *J Neurobiol.* 1994; 25:1404–1417. [PubMed: 7852994]
- Klesse LJ, Parada LF. Trks: Signal transduction and intracellular pathways. *Microsc Res Tech.* 1999; 45:210–216. [PubMed: 10383113]
- Lad SP, Neet KE, Mufson EJ. Nerve growth factor: Structure, function and therapeutic implications for Alzheimer's disease. *Curr Drug Target CNS Neurol Disord.* 2003; 2:315–334.
- Launer LJ, Andersen K, Dewey ME, Letenneur L, Ott A, Amaducci LA, Brayne C, Copeland JR, Dartigues JF, Kragh-Sorensen P, Lobo A, Martinez-Lage JM, Stijnen T, Hofman A. Rates and risk factors for dementia and Alzheimer's disease: Results from EURODEM pooled analyses. EURODEM Incidence Research Group and Work Groups. *European Studies of Dementia. Neurology.* 1999; 52:78–84. [PubMed: 9921852]
- Lechevallier-Michel N, Molimard M, Dartigues JF, Fabrigoule C, Fourier-Reglat A. Drugs with anticholinergic properties and cognitive performance in the elderly: Results from the PAQUID Study. *Br J Clin Pharmacol.* 2005; 59:143–151. [PubMed: 15676035]
- Liu S, Lau L, Wei J, Zhu D, Zou S, Sun HS, Fu Y, Liu F, Lu Y. Expression of ca(2+)-permeable AMPA receptor channels primes cell death in transient forebrain ischemia. *Neuron.* 2004; 43:43–55. [PubMed: 15233916]
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of alzheimer's disease: Report of the nincls-adrda work group under the auspices of department of health and human services task force on alzheimer's disease. *Neurology.* 1984; 34:939–944. [PubMed: 6610841]

- McMillan PJ, Singer CA, Dorsa DM. The effects of ovariectomy and estrogen replacement on trka and choline acetyltransferase mrna expression in the basal forebrain of the adult female sprague-dawley rat. *J Neurosci*. 1996; 16:1860–1865. [PubMed: 8774454]
- Michel PP, Agid Y. Death of septal cholinergic neurons produced by chronic exposure to glutamate is prevented by the noncompetitive nmda receptor/channel antagonist, mk-801: Role of nerve growth factor and nitric oxide. *J Neurosci Res*. 1995; 40:764–775. [PubMed: 7629890]
- Miller FD, Hicks SP, D'Amato CJ, Landis JR. A descriptive study of neuritic plaques and neurofibrillary tangles in an autopsy population. *Am J Epidemiol*. 1984; 120:331–341. [PubMed: 6332530]
- Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology*. 1991; 41:479–486. [PubMed: 2011243]
- Mufson EJ, Bothwell M, Hersh LB, Kordower JH. Nerve growth factor receptor immunoreactive profiles in the normal, aged human basal forebrain: Colocalization with cholinergic neurons. *J Comp Neurol*. 1989; 285:196–217. [PubMed: 2547849]
- Mufson EJ, Chen EY, Cochran EJ, Beckett LA, Bennett DA, Kordower JH. Entorhinal cortex beta-amyloid load in individuals with mild cognitive impairment. *Exp Neurol*. 1999; 158:469–490. [PubMed: 10415154]
- Mufson EJ, Counts SE, Fahnstock M, Ginsberg SD. Cholinergic molecular substrates of mild cognitive impairment in the elderly. *Curr Alzheimer Res*. 2007; 4:340–350. [PubMed: 17908035]
- Mufson EJ, Counts SE, Ginsberg SD. Gene expression profiles of cholinergic nucleus basalis neurons in Alzheimer's disease. *Neurochem Res*. 2002; 27:1035–1048. [PubMed: 12462403]
- Mufson EJ, Ginsberg SD, Ikonovic MD, DeKosky ST. Human cholinergic basal forebrain: Chemoanatomy and neurologic dysfunction. *J Chem Neuroanat*. 2003; 26:233–242. [PubMed: 14729126]
- Ott A, Breteler MM, van Harskamp F, Stijnen T, Hofman A. Incidence and risk of dementia. The Rotterdam Study. *Am J Epidemiol*. 1998; 147:574–580. [PubMed: 9521184]
- Ott BR. Cognition and behavior in patients with Alzheimer's disease. *J Genet Specif Med*. 1999; 2:63–69. [PubMed: 11252854]
- Parks RW, Becker RE, Rippey RF, Gilbert DG, Matthews JR, Kabatay E, Young CS, Vohs C, Danz V, Keim P, Collins GT, Zigler SS, Urycki PG. Increased regional cerebral glucose metabolism and semantic memory performance in Alzheimer's disease: A pilot double blind transdermal nicotine positron emission tomography study. *Neuropsychol Rev*. 1996; 6:61–79. [PubMed: 8976498]
- Peng S, Wu J, Mufson EJ, Fahnstock M. Increased prongf levels in subjects with mild cognitive impairment and mild alzheimer's disease. *J Neuropathol Exp Neurol*. 2004; 63:641–649. [PubMed: 15217092]
- Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med*. 2004; 256:183–194. [PubMed: 15324362]
- Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, Ritchie K, Rosser M, Thal L, Winblad B. Current concepts in mild cognitive impairment. *Arch Neurol*. 2001; 58:1985–1992. [PubMed: 11735772]
- Reiner A, Yekutieli D, Benjamini Y. Identifying differentially expressed genes using false discovery rate controlling procedures. *Bioinformatics*. 2003; 19:368–375. [PubMed: 12584122]
- Salehi A, Gonzalez Martinez V, Swaab DF. A sex difference and no effect of apoE type on the amount of cytoskeletal alterations in the nucleus basalis of Meynert in Alzheimer's disease. *Neurobiol Aging*. 1998; 19:505–510. [PubMed: 10192208]
- Sandberg G, Stewart W, Smialek J, Troncoso JC. The prevalence of the neuropathological lesions of Alzheimer's disease is independent of race and gender. *Neurobiol Aging*. 2001; 22:169–175. [PubMed: 11182466]
- Sobreviela T, Clary DO, Reichardt LF, Brandabur MM, Kordower JH, Mufson EJ. TrkA-immunoreactive profiles in the central nervous system: Colocalization with neurons containing p75 nerve growth factor receptor, choline acetyltransferase, and serotonin. *J Comp Neurol*. 1994; 350:587–611. [PubMed: 7890832]

- Sofroniew MV, Howe CL, Mobley WC. Nerve growth factor signaling, neuroprotection, and neural repair. *Annu Rev Neurosci.* 2001; 24:1217–1281. [PubMed: 11520933]
- Tanaka Y, Miyazaki M, Albert ML. Effects of increased cholinergic activity on naming in aphasia. *Lancet.* 1997; 350:116–117. [PubMed: 9228973]
- Toran-Allerand CD, Singh M, Setalo G Jr. Novel mechanisms of estrogen action in the brain: New players in an old story. *Front Neuroendocrinol.* 1999; 20:97–121. [PubMed: 10328986]
- Tuszynski MH, Thal L, Pay M, Salmon DP, UHS, Bakay R, Patel P, Blesch A, Vahlsing HL, Ho G, Tong G, Potkin SG, Fallon J, Hansen L, Mufson EJ, Kordower JH, Gall C, Conner J. A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nat Med.* 2005; 11:551–555. [PubMed: 15852017]
- Whitehouse PJ, Price DL, Clark AW, Coyle JT, DeLong MR. Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann Neurol.* 1981; 10:122–126. [PubMed: 7283399]
- Wimo A, Jonsson L, Gustavsson A, McDaid D, Ersek K, Georges J, Gulacsi L, Karpati K, Kenigsberg P, Valtonen H. The economic impact of dementia in Europe in 2008-cost estimates from the Eurocode Project. *Int J Geriatr Psychiatry.* 2011 in press.
- Zaborszky L, Gaykema RP, Swanson DJ, Cullinan WE. Cortical input to the basal forebrain. *Neuroscience.* 1997; 79:1051–1078. [PubMed: 9219967]

Figure 1.

Expression profiling of p75^{NTR} and trkA NGF receptors within male and female cholinergic NB neurons during the progression of AD. A, C) p75^{NTR} mRNA levels measured in single NB neurons microaspirated from male (A) and female (C) subjects with an *ante mortem* diagnosis of NCI, MCI, or mild/moderate AD. B, D) trkA mRNA levels measured in single NB neurons microaspirated from male (A) and female (C) subjects with NCI, MCI, or AD. *, $p < 0.05$ vs. MCI; **, $p < 0.01$ vs. NCI; ***, $p < 0.001$ vs. NCI.

Figure 2. qPCR validation of the microarray analysis demonstrating down regulation of trkA expression. A, B) trkA mRNA levels within the anterior NB in male (A) and female (B) subjects. *, $p < 0.05$ and **, $p < 0.01$ vs. NCI.

Figure 3.

Expression profiling of AMPA glutamate receptor subunits within male and female cholinergic NB neurons during the progression of AD. A) Representative array hybridization data for GluR1 (GRIA1), GluR2 (GRIA2), GluR3 (GRIA3), and GluR4 (GRIA4) AMPA receptor transcripts from NB neurons of male (M) and female (F) NCI, MCI, and AD subjects. B, C) Heatmaps show relative hybridization signal intensities for GRIA1-4 mRNAs in cholinergic NB neurons from male (B) and female (C) subjects (white to black = decreasing mRNA levels).*, $p < 0.05$ vs. NCI, MCI.

Table 1

Case demographics

	Clinical Diagnosis				Group comparison	Pair-wise comparisons [*]
	NCI (n = 9)	MCI (n = 9)	AD (n = 8)	Total (n = 26)		
Age (yrs) mean ± sd	80.0 ± 9.9	82.2 ± 4.3	86.0 ± 4.9	82.7 ± 7.1	p = 0.11 ^a	--
% males	5 (55%)	4 (44%)	4 (50%)	13 (42%)	p = 0.9 ^b	--
Education (years)	17.7 ± 5.3	18.8 ± 2.3	16.2 ± 4.4	17.7 ± 4.1	p = 0.35 ^a	--
PMI (hours)	7.2 ± 4.2	7.8 ± 4.7	6.6 ± 3.5	7.3 ± 4.1	p = 0.78 ^a	--
MMSE	27.6 ± 1.7	26.6 ± 2.8	20 ± 4.5	25 ± 4.5	p = 0.001 ^a	(NCI, MCI) > AD
GCS	0.0 ± 0.3	-0.4 ± 0.2	-1.7 ± 0.5	-0.6 ± 0.7	p < 0.001 ^a	(NCI, MCI) > AD
ApoEε4 allele	1 (11%)	3 (33%)	5 (63%)	9 (35%)	p = 0.08 ^b	--
Braak score						
0	0	0	0	0	p = 0.006 ^a	NCI < AD
I/II	4	0	1	5		
III/IV	5	7	2	15		
V/VI	0	2	5	7		
NIA Reagan diagnosis						
No	0	0	0	0	p = 0.022 ^a	NCI < AD
Low	5	2	1	8		
Intermediate	4	7	3	14		
High	0	0	4	4		

^a Kruskal-Wallis test^b Chi-square test^{*} Dunn's correction

Table 2

Spearman correlations between NGF receptor transcript levels in NB neurons and cognitive performance

gender, NB transcript	MMSE	GCS
male <i>p75NTR</i>	$r = 0.43, p = 0.15$	$r = 0.43, p = 0.11$
male <i>trkA</i>	$r = \mathbf{0.57}, p = \mathbf{0.02}$	$r = 0.45, p = 0.08$
female <i>p75NTR</i>	$r = \mathbf{0.66}, p = \mathbf{0.007}$	$r = \mathbf{0.69}, p = \mathbf{0.01}$
female <i>trkA</i>	$r = \mathbf{0.72}, p = \mathbf{0.006}$	$r = \mathbf{0.75}, p = \mathbf{0.005}$

Table 3

Spearman correlations between AMPA GluR subunit transcript levels in NB neurons and cognitive performance.

gender, test	GRIA1	GRIA2	GRIA3	GRIA4
male MMSE	$r = 0.81, p = 0.001$	$r = 0.48, p = 0.15$	$r = 0.47, p = 0.15$	$r = -0.63, p = 0.11$
male GCS	$r = 0.76, p = 0.008$	$r = 0.47, p = 0.15$	$r = 0.28, p = 0.4$	$r = -0.53, p = 0.15$
female MMSE	$r = 0.79, p = 0.004$	$r = 0.5, p < 0.1$	$r = 0.22, p = 0.46$	$r = -0.03, p = 0.9$
female GCS	$r = 0.57, p = 0.03$	$r = 0.58, p = 0.03$	$r = 0.28, p = 0.4$	$r = -0.01, p = 0.96$