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Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease

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Abstract

Profound neuronal dysfunction in the entorhinal cortex contributes to early loss of short-term memory in Alzheimer's disease^{1–3}. Here we show broad neuroprotective effects of entorhinal brain-derived neurotrophic factor (BDNF) administration in several animal models of Alzheimer's disease, with extension of therapeutic benefits into the degenerating hippocampus. In amyloid-transgenic mice, *BDNF* gene delivery, when administered after disease onset, reverses synapse loss, partially normalizes aberrant gene expression, improves cell signaling and restores learning and memory. These outcomes occur independently of effects on amyloid plaque load. In aged rats, BDNF infusion reverses cognitive decline, improves age-related perturbations in gene expression and restores cell signaling. In adult rats and primates, BDNF prevents lesion-induced death of entorhinal cortical neurons. In aged primates, BDNF reverses neuronal atrophy and ameliorates age-related cognitive

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AUTHOR CONTRIBUTIONS

A.H.N. contributed surgery, data collection, analysis and manuscript writing for all studies; D.A.M. contributed data collection, analysis and manuscript writing for aging studies; G.C. and D.G. contributed to array studies and analysis; S.T. contributed to *in vitro* A β toxicity studies; B.E.S., G.M.S. and E.K. contributed to A β *in vitro* toxicity studies and breeding and behavior of APP mice; L.W. contributed stereology of APP mice; A.B. generated gene expression vectors; A.K. and M.V.C. contributed western blot analysis in aged rats; J.M.C. contributed ELISA and biochemical analyses; E.R. contributed surgery of APP mice; E.M. contributed synaptophysin quantification and surgery in APP mice; A.A.C. contributed surgery and behavioral analysis in aged rats and manuscript writing; M.H.T. contributed experimental design, surgery in rats and primates, data analysis and manuscript writing.

COMPETING INTERESTS STATEMENT

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impairment. Collectively, these findings indicate that BDNF exerts substantial protective effects on crucial neuronal circuitry involved in Alzheimer's disease, acting through amyloid-independent mechanisms. BDNF therapeutic delivery merits exploration as a potential therapy for Alzheimer's disease.

The entorhinal cortex is a primary source of inputs to the hippocampus, exerting a key influence in learning and memory⁴. The most common age-related neurodegenerative disorder, Alzheimer's disease, affects the entorhinal cortex profoundly and early in the disease, contributing to cardinal symptoms of short-term memory loss^{1–3}. BDNF and its receptor, tyrosine receptor kinase B (TrkB), are widely expressed in the entorhinal cortex, and BDNF is anterogradely trafficked into the hippocampus⁵, where it is implicated in plasticity mechanisms hypothesized to underlie learning⁶. Furthermore, amounts of BDNF protein are reduced in the entorhinal cortex and hippocampus in Alzheimer's disease^{7–9}. We explored the hypothesis that therapeutic BDNF gene delivery to the entorhinal region can ameliorate entorhinal cortex and hippocampus degeneration in diverse models related to Alzheimer's disease.

We first examined whether BDNF influences neuronal degeneration in a transgenic mouse model directly related to Alzheimer's disease pathology in humans. J20 transgenic mice express the human amyloid precursor protein (APP) transgene bearing both the Swedish and the Indiana APP mutations¹⁰. Beginning at age 2–3 months, these mice show cortical plaques and progressive cell loss in the entorhinal cortex and show cognitive decline by 6–7 months of age^{10,11}. Lentiviral vectors constitutively expressing BDNF-GFP under control of the CMV- β -actin hybrid promoter (TG^{BDNF}, $n = 8$) or GFP alone (TG^{GFP}, $n = 8$) were injected into the entorhinal cortices of transgenic mice bilaterally at age 6 months, when neuropathological degeneration and cell loss are established^{10,11}. Age-matched wild-type (WT) littermates underwent sham surgery ($n = 4$) or injection of lentivirus expressing GFP (lenti-GFP) into the entorhinal cortex ($n = 4$). One month later, spatial memory was tested in the Morris water maze. The performance of sham-operated and GFP-injected WT mice did not differ, and these two groups were combined for subsequent statistical analysis (Fig. 1a). Over five successive daily blocks of trials, significant amelioration of spatial memory deficits occurred in TG^{BDNF} mice compared to TG^{GFP} mice ($P < 0.01$ by analysis of variance (ANOVA); $P < 0.05$ by *post hoc* Fisher's test comparing TG^{BDNF} to TG^{GFP}; Fig. 1a). The performance of TG^{BDNF} mice did not differ significantly from WT littermate controls (Fig. 1a). Transgenic mice were also tested on a fear-conditioning paradigm that measured both hippocampal-dependent (context) and hippocampal-independent (cue) learning (Fig. 1b and Supplementary Fig. 1a online). In the hippocampal-dependent aspect of the task, TG^{GFP} mice showed trends toward impaired performance compared to WT mice ($P < 0.05$ by ANOVA; $P = 0.12$ by *post hoc* Fisher's test comparing TG^{GFP} versus WT mice). TG^{BDNF} mice performed significantly better than TG^{GFP} mice on this task ($P < 0.05$; Fig. 1b). Hippocampal-independent fear conditioning was not impaired in either transgenic mouse group (Supplementary Fig. 1a), as expected. Weight loss, a potential consequence of disseminated growth factor spread in the central nervous system, did not occur (6.0% gain in TG^{BDNF} and 6.6% gain in TG^{GFP}).

Immunolabeling confirmed high amounts of BDNF in the entorhinal cortices of TG^{BDNF} mice (Fig. 1c). Furthermore, substantial trafficking of BDNF into hippocampal regions CA1–3 and dentate gyrus occurred (Fig. 1d). BDNF induced significant recovery of synaptic markers in both entorhinal cortex and dentate gyrus of transgenic mice ($P < 0.05$; Fig. 1e,f). BDNF treatment also significantly increased extracellular signal-related kinase (Erk) phosphorylation, thereby augmenting cell signaling related to the functional state of neurons ($P < 0.001$; Fig. 1g,h). Notably, BDNF treatment initiated after disease onset affected neither neuronal number nor amyloid plaque density (Supplementary Fig. 1b–d), indicating that the

ameliorative effects of BDNF in this model are exerted independently of direct modulation of insoluble β -amyloid ($A\beta$; amyloid oligomers were not measured). Gene expression in the entorhinal cortex and hippocampus were compared with mouse whole-genome arrays in WT mice after sham surgery ($n = 3$), in WT mice receiving injections of lenti-GFP ($n = 2$), in TG^{GFP} mice ($n = 4$) and in TG^{BDNF} mice ($n = 4$) 4 weeks after gene delivery (Fig. 1i and Supplementary Fig. 2 and Supplementary Data 1 online). Overexpression of mutant APP perturbed normal patterns of entorhinal cortex and hippocampal gene expression, with dysregulation of canonical pathways related to oxidative stress and lipid metabolism, among other pathways (Supplementary Fig. 2 and Supplementary Data 1). Many of the genes whose expression changed in transgenic mice have been previously related to Alzheimer's disease and neurodegeneration (for example, *Mfge8* (ref. 12), *Galk2* (ref. 13) and *St13* (ref. 14), related to phagocytosis of $A\beta$ peptide¹², post-translational modifications of APP¹³ and heat shock protein interactions¹⁴, respectively). Notably, entorhinal *BDNF* gene delivery improved APP-related alterations in gene expression in 55% of dysregulated genes in transgenic mice (Fig. 1i, Supplementary Fig. 2 and Supplementary Data 1). The restorative effects of BDNF on array profiles were also observed in hippocampus (Fig. 1i and Supplementary Fig. 2). Thus, BDNF, acting independently of direct effects on insoluble $A\beta$, improved genetic, molecular, synaptic and behavioral parameters of APP-transgenic mice when treatment was initiated after disease onset.

We next tested whether BDNF would enhance the function of entorhinal cortex neurons in a second model of neuronal degeneration, aging (Fig. 2). Aging is accompanied by marked declines in cognitive function associated with the entorhinal cortex¹⁵; cell death is not a mechanism underlying this decline^{16,17}. Spatial learning and memory were assessed in 36 young (5-month-old) and 37 aged (24-month-old) male Fischer rats in the Morris water maze¹⁶. Eighty-one percent of aged rats showed baseline cognitive impairment and then received bilateral, 28-d infusions of either recombinant BDNF protein (120 ng per day per side) or vehicle (artificial cerebrospinal fluid) into the medial entorhinal cortex. BDNF infusions in aged cognitively impaired rats significantly improved spatial learning and memory on all three measures of water maze performance compared to vehicle-infused rats—distance and latency improved on test trials, and platform crossings increased on probe trials ($P < 0.05$ by ANOVA; $P < 0.05$ by *post hoc* Fisher's test comparing aged rats given BDNF to aged rats given vehicle; Fig. 2a–c). Before treatment, young and aged cognitively unimpaired rats concentrated searches for the escape platform in the quadrant normally containing the escape platform, whereas aged cognitively impaired rats had poorly focused search strategies (Fig. 2d). After BDNF treatment, aged cognitively impaired rats showed marked improvement in search strategy, with greater swimming around the platform location (Fig. 2d). Aged rats did not lose weight after BDNF infusion (3.1% gain in aged rats given BDNF, 2.7% gain in aged rats given vehicle). Functional impairment with aging was associated with significant reductions in entorhinal concentrations of phosphorylated Erk ($P < 0.05$ by ANOVA; $*P < 0.05$ by *post hoc* Fisher's test for aged cognitively impaired rats versus aged cognitively unimpaired rats and young rats); infusion of BDNF restored Erk phosphorylation to levels not differing from those in young and aged cognitively unimpaired rats (Fig. 2e,f). Alterations in the cell survival phosphoinositide-3 kinase–Akt pathway were not associated with age-related impairment in the water maze (Fig. 2e), an observation consistent with the fact that cell loss in the entorhinal cortex is not observed with aging^{16,17}. Deficiencies in canonical signaling through Erk–mitogen-activated protein kinase kinase, but not Akt, would be predicted in a model of cell dysfunction but not death⁶; our findings in aged rats are consistent with this prediction, as BDNF ameliorates age-related deficiencies in Erk without detectably perturbing Akt. Gene expression patterns were examined in additional aged rats 1 month after entorhinal cortex administration of BDNF or GFP lentivirus; viral injections of BDNF were used instead of protein infusions for gene array studies to avoid confounding effects of chronic low-level damage from continuous infusions ($n = 4$ aged rats given BDNF, $n = 4$ aged rats given GFP,

$n = 4$ control aged rats not receiving virus and $n = 4$ young rats; Fig. 2h). Aging was associated with altered patterns of gene expression compared to young rats in both the entorhinal cortex (61 probe sets) and hippocampus (181 probe sets) (Fig. 2h; $P < 0.005$ by Bayesian t -test, see also Supplementary Fig. 3 and Supplementary Data 2 online), consistent with previous reports¹⁸. Oxidative stress (as seen in the APP model) and lipid and glutathione functional pathways were altered (Supplementary Fig. 3). Entorhinal cortex BDNF delivery induced at least a 30% change toward normalization in 43% of entorhinal age-related genes and 42% of hippocampal genes ($P < 0.005$, Fig. 2h and Supplementary Data 2). Thus, entorhinal cortex BDNF administration compensates for genetic, molecular and behavioral parameters of age-related neural dysfunction.

The preceding models show the protective and restorative effects of BDNF in models of neuronal atrophy and synapse loss. Next, we determined whether BDNF can also prevent the death of entorhinal neurons. First, primary entorhinal cortex neurons from postnatal day 3 rats were exposed to the toxic A β 1–42 peptide *in vitro*, resulting in cell death within 24 h (Fig. 3a,b). BDNF treatment concurrent with A β 1–42 exposure prevented neuron death ($P < 0.05$; Fig. 3a,b). Nerve growth factor (NGF) had no effect (Fig. 3b; cortical neurons do not express the NGF receptor TrkA19). BDNF specifically prevented A β 1–42-induced cell death, because BDNF did not increase neuron number in naive entorhinal cultures (Supplementary Fig. 4 online). To determine whether BDNF also prevents entorhinal cortex neuronal death *in vivo*, we subjected rats to perforant path transections that typically induce death in 20% of neurons in layer II of the entorhinal cortex²⁰. Although the cell death in this model occurs independently of Alzheimer's disease-related A β toxicity and neurofibrillary degeneration, the final mechanisms of caspase activation and mitochondrial calcium release are common to apoptotic and necrotic cell death²¹. Indeed, growth factor-mediated prevention of neuronal loss in transection models has predicted protection from disease-specific mechanisms in other models^{22–25}. Rats received injections into the right entorhinal cortex of lentiviral vectors expressing either BDNF ($n = 8$ rats), GFP ($n = 6$) or NGF ($n = 8$, a negative control). Two weeks after the lesions were made, control GFP- and NGF-treated rats showed a significant 20% loss of stereologically quantified neurons in layer II of the entorhinal cortex compared to the contralateral side of the brain ($P < 0.05$ by ANOVA; $P < 0.05$ by *post hoc* Fisher's test comparing intact to lesioned control rats; Fig. 3c–e). BDNF prevented cell death (Fig. 3d; $P < 0.01$) and ameliorated cell atrophy (Fig. 3e; $P < 0.05$). BDNF also significantly increased expression of phospho-AKT, a marker of canonical cell survival pathways⁶, in entorhinal cortex neurons (Fig. 3f).

Next, we determined whether the restorative effects of BDNF are scalable to nonhuman primates. First, we established a nonhuman primate model of entorhinal cortex neuronal death. Radiofrequency lesions of the perforant path were performed bilaterally, and lentiviral vectors expressing BDNF were injected into the right entorhinal cortex ($n = 6$ monkeys). Lenti-GFP ($n = 4$ monkeys) or no virus ($n = 2$ monkeys) was injected into the left entorhinal cortex (Fig. 4a,b). Stereological quantification revealed that perforant path lesions without BDNF treatment resulted in the loss of $45.9 \pm 8.5\%$ of neurons in layer II of the entorhinal cortex in the lateral region relative to those in intact monkeys (Fig. 4a,b). BDNF significantly prevented lesion-induced entorhinal neuronal death ($P < 0.01$), maintaining $85.4 \pm 7.1\%$ of neurons on the lesioned side compared to intact subjects (Fig. 4b). (Because axonal regeneration and reinnervation of hippocampal targets does not occur in this model, no effort was made to assess cognitive performance after perforant path lesions.)

Finally, we examined the effects of *BDNF* gene delivery to the entorhinal cortex in aged monkeys (Fig. 4c–h). The aged primate is the best available model of age-related neurodegeneration in humans, showing cognitive decline with aging and reductions in neuronal function¹⁵. Extensive cell death does not occur in the aged primate entorhinal cortex with

aging^{17,26}, consistent with findings in aged rodents¹⁶. Aged monkeys ($n = 9$; seven males and two females, mean age = 24.5 ± 1.2 years) were preoperatively characterized on a visuospatial discrimination task sensitive to temporal lobe function^{27,28}. After learning the procedural component of the task (identification of a previously presented test stimulus on the touch screen after a delay)²⁷, the monkeys underwent testing on a two-choice version of the task (Fig. 4g), undergoing 20 trials per session over 20 preoperative test sessions. All aged monkeys showed significantly impaired visuospatial learning compared to young monkeys ($P < 0.01$; Fig. 4g,h). Aged monkeys then received bilateral injections of lentiviral vectors expressing either BDNF ($n = 4$) or GFP ($n = 5$) into four locations distributed over the rostral-to-caudal extent of the medial temporal cortex, targeting entorhinal cortex neurons projecting to the hippocampus (Fig. 4c,d). After treatment, monkeys were retested on the visuospatial discrimination task. BDNF-treated aged monkeys showed a significant improvement in performance compared to control aged monkeys by the tenth postoperative session ($P < 0.05$, Fig. 4h). Both BDNF-treated and control aged monkeys showed slight but nonsignificant reductions in weight over the experimental period (5.6% and 3.4% relative to preoperative baseline, respectively). Analysis of brain sections showed accurate targeting of lentiviral vectors to the entorhinal cortex and elevated BDNF protein distribution in the hippocampus, suggesting anterograde transport (Fig. 4c,d). Furthermore, mean entorhinal neuronal size significantly increased in BDNF-treated monkeys compared to monkeys receiving lenti-GFP ($P < 0.01$; Fig. 4e,f).

BDNF is produced in the entorhinal cortex throughout life and it undergoes anterograde trafficking into the hippocampus⁵. BDNF influences neuronal plasticity by facilitating neurotransmitter release²⁹, increasing vesicular docking, enhancing glutamate-evoked postsynaptic responses³⁰ and facilitating hippocampal and cortical long-term potentiation³¹. Notably, the concentrations of BDNF decline in the entorhinal cortex and hippocampus in Alzheimer's disease⁷⁻⁹, leading us to hypothesize that therapeutic application of BDNF would influence neurodegeneration. This study demonstrates the extensive trophic actions of BDNF on neurons of the entorhinal cortex, and, via anterograde transport, the hippocampus. BDNF administration in these models prevents cell death, reverses neuronal atrophy and ameliorates behavioral deficits. The mechanisms underlying these actions include normalization of gene expression patterns in models of disease and aging, augmentation of intracellular signaling related to the functional state of the cell and enhancement of synaptic marker expression. The therapeutic effects of BDNF extend across multiple animal models of Alzheimer's disease, including mutant amyloid mice, aged rats and adult perforant path-lesioned rats. Furthermore, these effects extend to primate models, which show amelioration of cell death, enhancement of cell size and improvement in age-related cognitive decline in response to BDNF. Taken together, these results establish a rationale for BDNF delivery to the entorhinal cortex as a means for treating entorhinal and hippocampal degeneration in Alzheimer's disease. This approach acts independently of direct modulation of amyloid, providing a key alternative to, and potential synergy with, amyloid-modifying treatments.

A lack of methods for effectively and safely delivering growth factors to the nervous system over sustained time periods has been a major factor limiting their translational testing³². Intraventricular infusions of growth factors typically fail to reach most neurons³³, and hence recent clinical trials used intraparenchymal infusions or gene delivery³⁴⁻³⁷. Our experiment demonstrated efficacy using both treatment methods. These approaches could be scaled up to deliver BDNF to a target the size of the human entorhinal cortex, $\sim 8 \text{ cm}^3$ in volume, thereby broadly affecting neuronal activity and function throughout the hippocampus as a consequence of BDNF transport⁵. The potency of BDNF in the preclinical models presented here, including its validation in primate models, provides a rationale for exploring clinical translation.

METHODS

Transgenic mice

BL/6 transgenic mice expressed mutant APP under control of the *Thy1* promoter¹⁰. We injected 2 μ l lentivirus expressing BDNF or enhanced GFP into two sites spanning the midportion of the medial entorhinal cortex. Four weeks later, we tested the mice for 5 consecutive d in the Morris water maze (four morning and four afternoon trials daily; probe testing was not done after the last test trial). We tested visual and motoric sufficiency in a subsequent cued version of the water maze (visible platform), resulting in the exclusion of one sham subject. One week later, we assessed contextual fear conditioning with a 20-s tone that terminated with a 3-s footpad shock (0.45 mA). Twenty-four hours later, we reexposed each mouse to the same chamber and recorded freezing over 3 min. Two hours later, we placed the mice into novel chambers (new shape, odor and color) and recorded freezing for 3 min before and during each of three conditioned stimulus tone presentations (cued conditioning). We tracked the movements by infrared monitors. See the Supplementary Methods online for a description of stereological analyses and immunolabeling procedures. Experimental manipulations and data analysis in this and all subsequent studies were conducted in a blinded fashion. The University of California–San Diego Institutional Animal Care and Use Committee (IACUC) gave approval for mouse studies.

Aged rats

We gave aged cognitively impaired rats (Harlan, National Institute of Aging colony) one of the following treatments: BDNF protein infusion ($n = 10$), vehicle (artificial cerebrospinal fluid) infusion ($n = 10$) or sham surgery ($n = 10$). Seven aged and cognitively unimpaired rats and 36 young rats did not receive infusions. We performed Morris water maze testing over four preoperative testing blocks, each consisting of six total trials (three trials per day, of a maximum duration of 90 s per trial). The last trial of each block consisted of a probe trial. Thirty of 37 aged rats were cognitively impaired, which we identified as inferior performance compared to the worst-scoring young rat on latency and distance measures during the fourth block. We then gave aged cognitively impaired rats continuous 4-week infusions (Alzet mini-pumps) of either 120 ng BDNF per day in artificial cerebrospinal fluid or artificial cerebrospinal fluid alone. Postoperative testing began 2 weeks later; we utilized four testing blocks of six trials per block. We used visible platform testing to assess visual and motoric adequacy, which showed no significant differences across groups (latency, $P = 0.68$; distance, $P = 0.35$). We performed western blotting for Erk, phosphorylated Erk (pErk), Akt and pAKT on entorhinal cortices from four BDNF-infused aged rats, four vehicle-infused aged rats, four nonoperated aged cognitively impaired rats, four nonoperated aged cognitively unimpaired rats and eight young rats. We normalized protein loading to Erk levels. Details of western blotting are in the Supplementary Methods. We used Affymetrix Mouse Genome 430_2 and Rat Genome 230_2 arrays per manufacturer instructions for microarray studies. We assessed quality by inter-array Pearson correlation and RNA degradation plotting. We performed contrast analysis of differential expression with the Linear Models of Microarray (LIMMA) software³⁸. We analyzed pathway and gene ontology with Ingenuity. The Veterans Administration San Diego Healthcare System IACUC provided approval for the aging rat study and the perforant path rat study.

In vitro β -amyloid toxicity studies

We cultured primary entorhinal cortex neurons from rats on postnatal day 3. We treated the cultured neurons with A β 1–42 peptide (5 or 10 μ M (gift from C. Glabe)) dissolved in DMSO. Control cultures received DMSO alone; scrambled A β had no cell toxic effect. We added BDNF or NGF (0, 0.5, 2.5 or 10 ng ml⁻¹) concurrently with A β , and we assessed cell viability 24 h later with the LIVE/DEAD Viability/Cytotoxicity Kit (Molecular Probes).

Rat perforant path lesions

We injected 5 μ l of lentivirus-BDNF-GFP (1.25×10^8 infectious units per ml) into three sites evenly distributed over the rostral-to-caudal extent of the right entorhinal cortex in adult Fischer rats (Harlan; $n = 8$); we injected lenti-GFP ($n = 6$) or lentivirus encoding NGF ($n = 8$) as controls. Four days later, we made right perforant path lesions. Two weeks later, we performed stereological counts of neurons in layer II of the medial entorhinal cortex.

Primate perforant path lesions

We gave six adult rhesus monkeys (California National Primate Research Center) perforant path lesions with a Radionics probe placed into the angular bundle, which we identified on preoperative magnetic resonance images. We then gave the monkeys injections of lentiviral vectors into the entorhinal cortex over four rostral-to-caudal sites expressing either BDNF ($n = 6$) on one side of the brain and GFP ($n = 4$) or no lentiviral injections ($n = 2$) on the opposite side of the brain. We injected 20 μ l of vector (5×10^8 infectious units per ml) per site at a rate of 1 μ l min^{-1} . One month later, we quantified the cells in layer II of the lateral entorhinal cortex on Nissl-stained sections with StereoInvestigator (Microbrightfield) software (optical sampling frame 10%, z axis inclusion-exclusion zone 5% at section top and bottom). The University of California–Davis IACUC gave approval for the primate studies.

Aged primates

Before gene transfer, we trained all monkeys to criterion performance on a computerized visuospatial learning task³⁹. Briefly, we showed the monkeys a stimulus, the computer screen became blank and, after a 2-s delay, three choice objects were presented. The monkey was required to choose the original object. 20 trials/session were presented over 20 consecutive sessions. Four aged monkeys then underwent lenti-BDNF injection into the entorhinal cortex at the same locations and doses used in the perforant path lesion study, and five monkeys received lenti-GFP. One month later, we reassessed cognitive performance daily over ten sessions. We compared each monkey's change in performance to the postoperative session 1 baseline. We quantified entorhinal cortex layer II neuronal size by stereological methods.

Statistical analyses

We used Student's t -tests to compare two groups, whereas we used analysis of variance with Fischer *post hoc* tests for multigroup comparisons. For gene array studies, we calculated a Bayesian estimate of differential expression after linear model fitting with a threshold for significance of $P < 0.005$ (Bayesian modified t -test). Graphs are expressed as means \pm s.e.m., unless noted otherwise.

Additional methods

Detailed methodology is described in Supplementary Methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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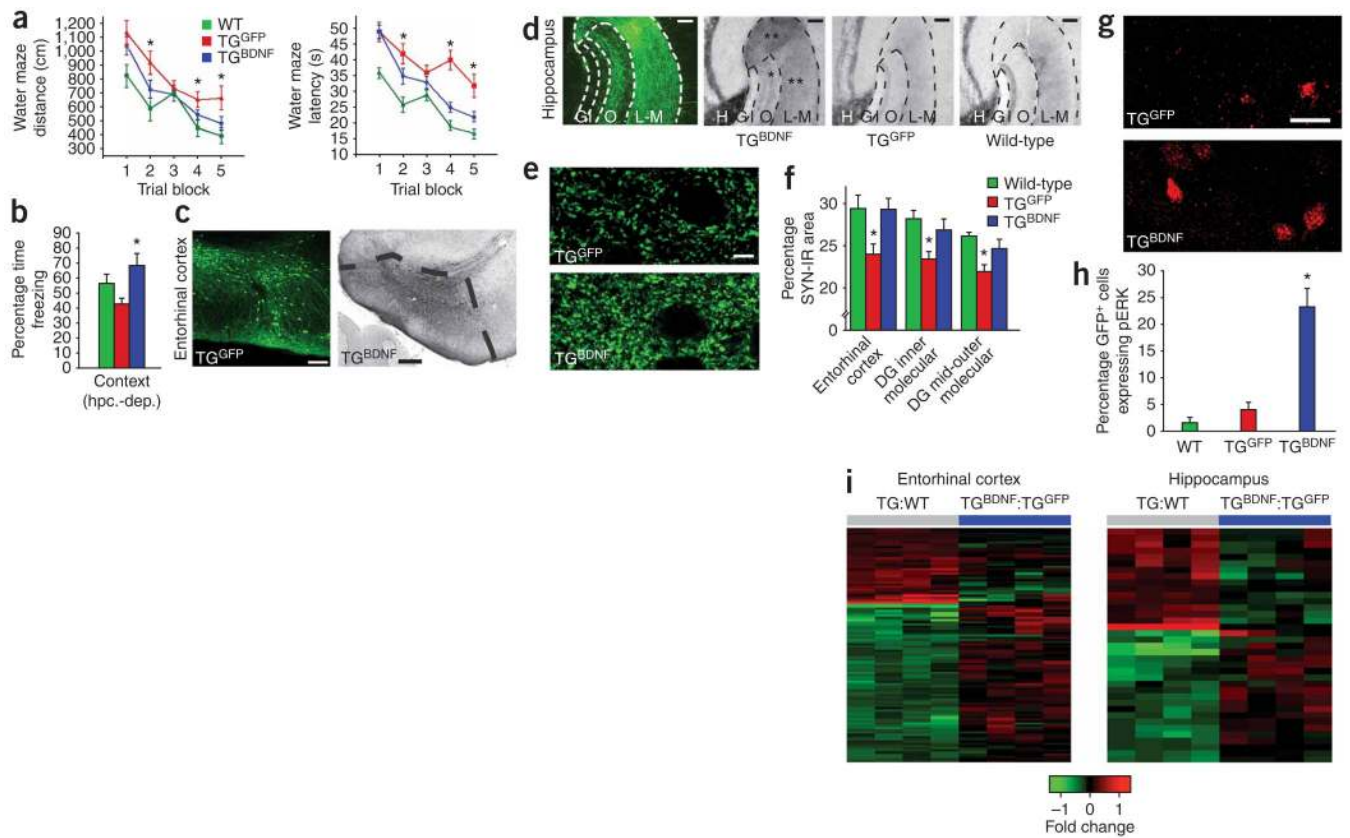


Figure 1. BDNF effects in APP-transgenic mice

(a) *BDNF* gene delivery effect on spatial memory in the Morris water maze in mutant APP-transgenic mice (TG) on distance (left) and latency (right) measures ($P < 0.01$ by ANOVA, $*P < 0.05$ by *post hoc* Fisher's test, comparing TG^{BDNF} to TG^{GFP}). (b) BDNF effect on hippocampal-dependent (hpc.-dep.) contextual fear conditioning ($P < 0.05$). (c) Lentiviral gene delivery effect on gene expression (GFP reporter, left) and dense BDNF immunolabeling (black reaction product, right) in the entorhinal cortex of TG^{BDNF} mice. (d) Axon terminals of entorhinal cortex neurons projecting into the hippocampus identified by GFP immunolabeling after entorhinal lenti-GFP injection in APP-transgenic mice (left panel, TG^{GFP}); injection of lenti-BDNF into entorhinal neurons increases BDNF expression in the hippocampal dentate gyrus (TG^{BDNF}), stratum lacunosum-moleculare (L–M) (**), and outer molecular layer (O, *), compared to TG^{GFP} controls and WT mice. H, hilar region; G, granule cell layer; I, inner molecular layer (BDNF immunolabel). (e) Synaptophysin labeling in entorhinal cortex of TG^{BDNF} mice compared to TG^{GFP} mice. (f) Quantification of the effect of entorhinal cortex delivery of lenti-BDNF on synaptophysin immunoreactivity (SYN-IR) in both entorhinal cortex and hippocampus of APP-transgenic mice ($P < 0.01$ by ANOVA, $*P < 0.05$ by *post hoc* Fisher's test comparing TG^{BDNF} and TG^{GFP}). (g, h) pErk immunolabeling in entorhinal cortex of TG^{BDNF} mice compared to TG^{GFP} mice ($P < 0.001$ by ANOVA, $P < 0.001$ by *post hoc* Fisher's test comparing TG^{BDNF} to TG^{GFP} or WT). (i) Heat maps depicting fold changes of APP-related genes before and after treatment with BDNF. 107 probe sets are differentially expressed in entorhinal cortex of TG mice compared to WT (TG:WT), and 37 probe sets are differentially expressed in hippocampus (gray bar columns, $P < 0.005$ by Bayesian *t*-test, see Supplementary Data 1). Upregulated genes are shown in red, and downregulated genes are shown in green; color intensity corresponds to fold change in expression (Supplementary Fig. 2 and Supplementary Data 1). BDNF treatment in APP-transgenic mice (blue columns) shifts gene expression toward wild type patterns in entorhinal

cortex and hippocampus. Genes are clustered by similarity; individual columns represent array samples from individual mice compared to expression in WT mice. Error bars indicate means \pm s.e.m. Scale bars: **c**, 100 μm (left) and 200 μm (right); **d**, 50 μm ; **e**, 10 μm ; **g**, 10 μm .

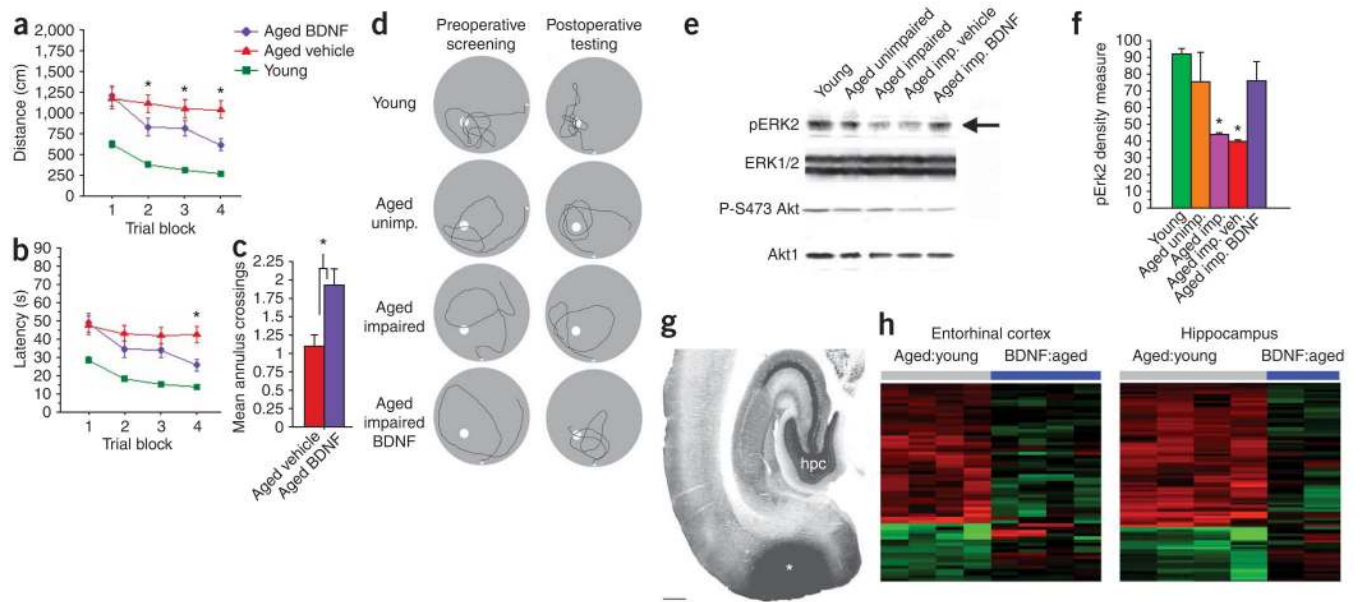


Figure 2. BDNF effects in aged rats

(a, b) Effect of BDNF infusion into entorhinal cortex of aged rats on spatial memory in Morris water maze determined by distance (a) and latency (b) measures ($P < 0.05$ by ANOVA, $*P < 0.05$ by *post hoc* Fisher's test comparing BDNF-treated to aged controls). (c) BDNF effects on search strategy in water maze after platform removal (probe trials) ($P < 0.001$). (d) Representative samples of search strategy during probe trials in individual rats. (e) Western blot of Erk phosphorylation and Akt phosphorylation (on Ser473, P-S473Akt) in entorhinal cortex of aged cognitively impaired rats compared to young and aged cognitively unimpaired rats; arrow indicates effect of BDNF treatment. Protein loading was standardized to Erk1/2, an abundant cellular protein. (f) Quantification of western blots ($P < 0.05$ by ANOVA, $*P < 0.05$ by *post hoc* Fisher's test comparing aged cognitively impaired rats and aged cognitively impaired rats treated with vehicle to all other groups). (g) BDNF immunolabeling showing accurate targeting of infusion to entorhinal cortex (*). hpc, hippocampus. Scale bar, 1 mm. (h) Heat maps depicting fold changes of aging-related genes before and after treatment with BDNF. Genes and samples are clustered by similarity; individual columns represent array samples from individual rats compared to young rats.

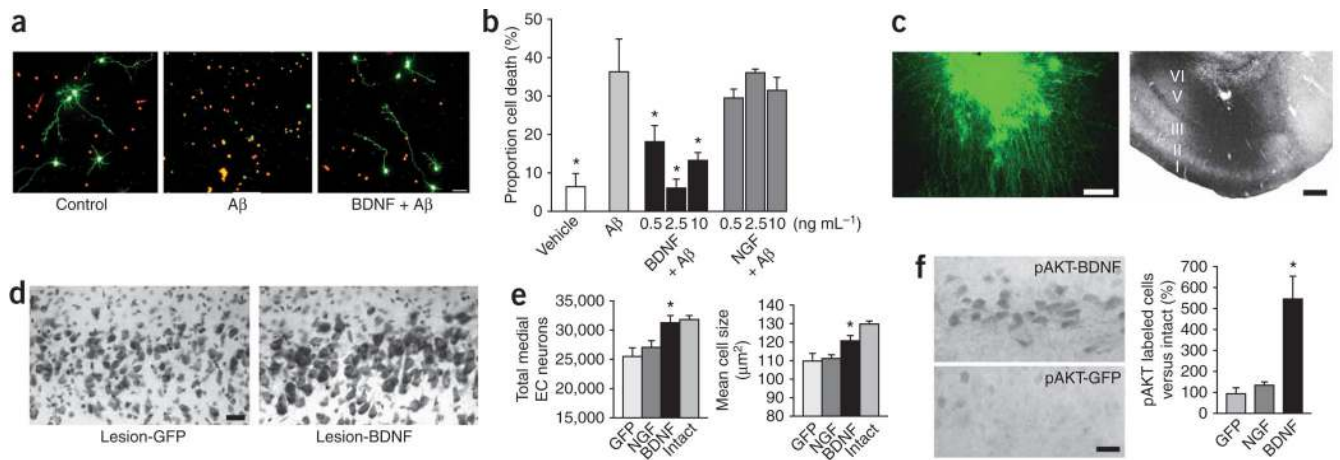


Figure 3. BDNF effects on cell survival

(a) Fluorescent images of living cells (green, calcein label) and dead cells (red, ethidium homodimer-1 label) in cultures of postnatal day 3 entorhinal cortex neurons in control condition (no A β exposure), after addition of A β 1–42 peptide (A β), and after addition of A β peptide plus 2.5 ng ml⁻¹ BDNF. Scale bar, 50 μm . (b) Quantification of A β 42-induced cell death after 24 h *in vitro*. $P < 0.05$ by ANOVA, * indicates the difference between the vehicle- and BDNF-treated groups and the A β group, $P < 0.001$ by Fisher's *post hoc* test. (c) Perforant path lesion model: GFP immunolabeling in entorhinal cortex at site of lenti-GFP vector injection (left) and BDNF protein production (region of dark immunolabeling) in the same region (right). I–VI indicates cortical laminae. Scale bar, 150 μm . (d,e) Perforant path lesion causes significant loss of Nissl-stained neurons in layer II of the entorhinal cortex (EC) in GFP-injected controls ($P < 0.01$ versus intact). Nissl stain of layer II entorhinal cortex after perforant path lesions in GFP-injected controls (Lesion-GFP) and BDNF-treated rats (Lesion-BDNF) is shown in d. Stereological quantification of cell number and size after perforant path lesions is shown in e (left, $P < 0.05$ by ANOVA, * $P < 0.01$ BDNF versus GFP and NGF by Fisher's *post hoc* test; right, $P < 0.01$ by ANOVA, * $P < 0.05$ BDNF versus GFP and NGF by *post hoc* Fisher's test). Scale bar, 25 μm . (f) pAKT label in entorhinal cortex of treated versus control perforant path-lesioned rats, with stereological quantification at right. Scale bar, 10 μm (* $P < 0.001$).

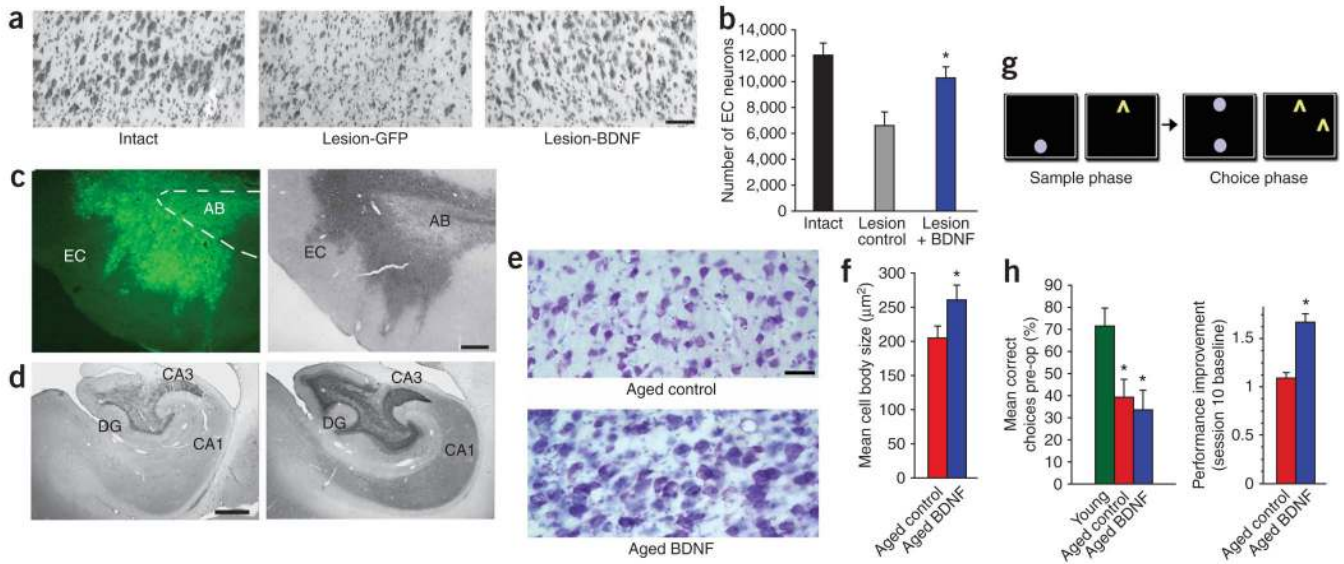


Figure 4. BDNF effects in primates

(a) Nissl stain of layer II entorhinal cortex neuronal death induced by perforant path lesion and neuroprotection by lenti-BDNF injection. Scale bar, 65 µm. (b) Stereological quantification showing loss of layer II entorhinal cortex neurons after perforant path lesions and protection of neurons by injection of lenti-BDNF ($P < 0.01$ by ANOVA, $*P < 0.01$ by Fisher's *post hoc* test compared to lesion controls). (c) GFP immunolabeling in entorhinal cortex (EC, left) and BDNF protein expression (right, BDNF immunolabel) following injection of lenti-BDNF vector in aged, cognitively impaired monkeys. AB, angular bundle (dashed lines). Scale bar, 75 µm. (d) BDNF immunolabeling in hippocampal dentate gyrus (DG) and hippocampal regions CA3 and CA1 in control aged subjects injected with lenti-GFP in entorhinal cortex (left); BDNF increases after lenti-BDNF injection (right). Scale bar, 250 µm. (e) Nissl stain showing hypertrophy of neurons in aged monkey entorhinal cortex after BDNF gene delivery. Scale bar, 35 µm. (f) Quantification of neuronal size in aged monkeys ($*P < 0.01$ by *t*-test). (g) Visuospatial discrimination task used to test cognitive function in aged monkeys²⁷ (details in text). (h) Quantification of preoperative choice accuracy in aged and young monkeys before treatment (left; $P < 0.01$ by ANOVA, $*P < 0.01$ both aged groups compared to young) and proportional improvement in performance after lenti-GFP or lenti-BDNF gene delivery to entorhinal cortex (right; $*P < 0.05$, two-tailed *t*-test).