Supporting Information

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SI Materials and Methods

Mice and Behavioral Testing. The derivation and characterization of the 3xTg-AD and the APP/tau mice have been described elsewhere (1, 2). The 3xTg-AD and the NonTg mice used in these studies were on a mixed C57Bl6/129 background. The mice were group-housed and kept on a 12-h light–12-h dark schedule. All animal procedures were performed in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals, and all appropriate measures were taken to minimize pain and discomfort in the experimental animals.

Spatial learning and memory were assessed using MWM testing, conducted in a circular tank of 1.5 m diameter located in a room with extra maze cues. The location of the platform (14 cm diameter) was kept constant for each mouse during training and was 1.5 cm beneath the surface of the water, which was maintained at 25 °C throughout the duration of testing. During 5 d of training, the mice underwent four trials per day alternated among four pseudorandom starting points. If a mouse failed to find the platform within 60 s, then it was guided to the platform by the researcher and kept there for 20 s. The intertrial interval was 25 s, during which each mouse was returned to its home cage. Probe trials were conducted 24 h after the final training trial. During the probe trials, the platform was removed, and mice were free to swim in the tank for 60 s. The training and probe trials were recorded by a video camera mounted on the ceiling, and data were analyzed using the EthoVisionXT tracking system (Noldus Information Technology).

Protein Extraction and Western Blot Analysis. Mice were killed by CO_2 asphyxiation, and their brains were extracted and cut in half sagittally. For immunohistochemical analysis, one half of the brain was drop-fixed in 4% paraformaldehyde in PBS for 48 h and then transferred in 0.02% sodium azide in PBS until being sliced. The other half of the brain was frozen in dry ice for biochemical analysis. Frozen brains were homogenized in a solution of tissue protein extraction reagent (T-PER; Pierce) containing 0.7 mg/mL of pepstatin A supplemented with a complete Mini Complete Protease Inhibitor tablet (Roche) and phosphatase inhibitors (Invitrogen). The homogenized mixes were briefly sonicated to shear the DNA and then centrifuged at 100,000 × *g* for 1 h at 4 °C. The supernatant was stored as the soluble fraction. The pellet was rehomogenized in 70% formic acid and centrifuged as described above, and the supernatant was stored as the insoluble fraction.

For Western blot analysis, proteins from the soluble fraction were resolved by 10% Bis-Tris SDS/PAGE (Invitrogen) under reducing conditions and then transferred to a nitrocellulose membrane. The membrane was incubated in a 5% solution of nonfat milk for 1 h at 20 °C. After overnight incubation at 4 °C with primary antibody, the blots were washed in Tween 20–TBS [0.02% Tween 20, 100 mM Tris (pH 7.5), and 150 nM NaCl] for 20 min and incubated at 20 °C with secondary antibody. The blots were then washed in Tween 20–TBS for 20 min, incubated for 5 min with Super Signal (Pierce), washed again, and exposed.

ELISA. Levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ were measured using a sensitive sandwich ELISA system. Protein from the soluble fraction (see above) was loaded directly onto ELISA plates. MaxiSorp immunoplates (Nalge Nunc) were coated with monoclonal antibody 20.1, a specific antibody against $A\beta_{1-16}$ (provided by Dr. David Cribbs, University of California Irvine) in coating buffer [0.1 M $NaCO_3$ (pH 9.6)], and blocked with 3% BSA. Synthetic Aβ standards (Bachem) were defibrillated by dissolving in hexafluoroisopropanol at 1 mg/mL, after which the hexafluoroisopropanol was evaporated with a stream of N_2. The defibrillated Aβ was then dissolved in DMSO at 1 mg/mL. Standards of both $A\beta_{1-40}$ and $A\beta_{1-42}$ were made in antigen capture buffer [20 mM NAH_2PO_4, 2 mM EDTA, 0.4 M NaCl, 0.5 g of CHAPS, and 1% BSA (pH 7.0)] and loaded onto ELISA plates in duplicate. Samples were then loaded in duplicate and incubated overnight at 4 °C. Plates were washed and probed with either HRP-conjugated anti-$A\beta_{35-40}$ (MM32-13.1.1, for $A\beta_{40}$) or anti-$A\beta_{35-42}$ (MM40-21.3.4, for $A\beta_{42}$) overnight at 4 °C. The chromagen was 3,3′,5,5′-tetramethylbenzidine, and the reaction was stopped with the addition of 30% O-phosphoric acid and read at 450 nm on a plate reader (Labsystems).

Immunohistochemistry. For immunohistochemical analysis, 30-μm-thick sections were obtained with vibratome slicing system (Leica Microsystems), and sections were stored at 4 °C in 0.02% sodium azide in PBS. To quench the endogenous peroxidase activity, free-floating sections were incubated for 30 min in H_2O_2. The appropriate primary antibody was applied, and sections were incubated overnight at 4 °C. Subsequently, sections were washed and incubated in the appropriate secondary antibody for 1 h at 20 °C. After a final wash for 20 min, sections were developed with diaminobenzidine substrate using an avidin-biotin-HRP system (Vector Laboratories).

Conditioned Medium. The 7PA2 cells were grown in DMEM with 10% FBS at 37 °C in a humidified environment (5% CO_2) in 75-cm^2 cell culture flasks until 90% confluent. Then the medium was replaced with 7 mL of serum-free DMEM for 18 h. The conditioned medium was then centrifuged to remove debris and frozen at −80 °C. The medium was concentrated using Amicon Ultra-15 centrifuge filters (Millipore) with a 3K molecular weight cutoff.

Statistical Analyses. Learning data were analyzed by two-way ANOVA. Specifically, the R statistical language was used with the NLME package to perform the mixed-model repeated-measures ANOVA. The following model formula was used for the fixed effects:

$$\text{escape latency} \sim \text{group} + \text{day} + \text{group} : \text{day},$$

where group is a categorical covariate with four levels (NonTg-sham, 3xTg-AD-sham, NonTg-CBP, and 3xTg-AD-CBP, with the first level used as a baseline against which the other three levels were compared in the posthoc test conducted with a Bonferroni correction); day is a numeric covariate; and group:day is the interaction term representing the effect of membership in a given experimental group on the slope of the escape latency over the five sessions. The random effect was animal ID, a categorical covariate distinguishing individual animals on which the repeated measures were performed. Probe trials were analyzed by one-way ANOVA followed by a post hoc Bonferroni test to determine individual differences in groups. The Student's *t* test was used when suitable.

1. Hock C, Heese K, Hulette C, Rosenberg C, Otten U (2000) Region-specific neurotrophin imbalances in Alzheimer disease: Decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. *Arch Neurol* 57:846–851.

2. Connor B, et al. (1997) Brain-derived neurotrophic factor is reduced in Alzheimer's disease. *Brain Res Mol Brain Res* 49:71–81.

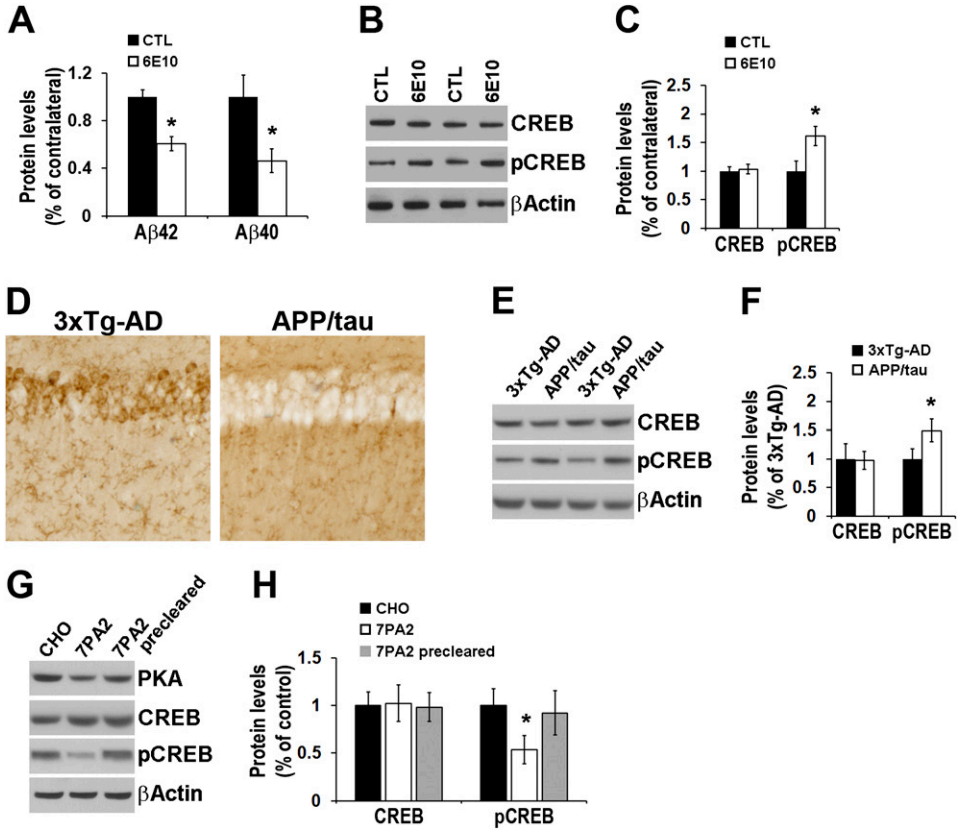

Fig. S1. Aβ accumulation decreases CREB signaling in vivo. (*A*) Aβ levels measured by sandwich ELISA in the hippocampi of 6-mo-old 3xTg-AD mice ($n = 6$) that received a single injection of 2 μg of 6E10 into the left hippocampi. The right uninjected hippocampi were used as internal controls. Mice were killed at 3 d after antibody delivery. The levels of $A\beta_{40}$ and $A\beta_{42}$ were significantly lower in the left 6E10-injected hippocampi compared with the right uninjected hippocampi. (*B*) Representative Western blots (probed with the indicated antibodies) of proteins extracted from the hippocampi of mice injected with 6E10 compared with the contralateral uninjected hippocampi. (*C*) Quantitative analyses of the blots showed that whereas CREB levels were similar between the ipsilateral 6E10-injected hippocampi and the contralateral uninjected hippocampi, the reduction in Aβ level was sufficient to significantly increase the pCREB level ($n = 6$). (*D*) Representative microphotograph of CA1-pyramidal neurons from the 3xTg-AD and APP/tau mice stained with an Aβ42 specific antibody. (*E*) Representative Western blots of proteins extracted from the hippocampi of 6-mo-old 3xTg-AD and APP/tau mice ($n = 6$/genotype) and probed with the indicated antibodies. (*F*) Quantitative analysis of the blots shows significantly higher pCREB levels, but not total CREB levels, in the hippocampi of the APP/tau mice compared with the 3xTg-AD mice. (*G*) Representative Western blots of proteins extracted from the hippocampi of 2-mo-old NonTg mice injected with CHO or 7PA2 conditioned medium, and with conditioned medium from 7PA2 cells depleted of Aβ by immunoprecipitation with 6E10 ($n = 6$/group). (*H*) Quantitative analysis of the blots shows significantly reduced pCREB levels, but not total CREB levels, after injection of 7PA2 conditioned medium. Data are presented as mean ± SEM. $^*P < 0.05$.

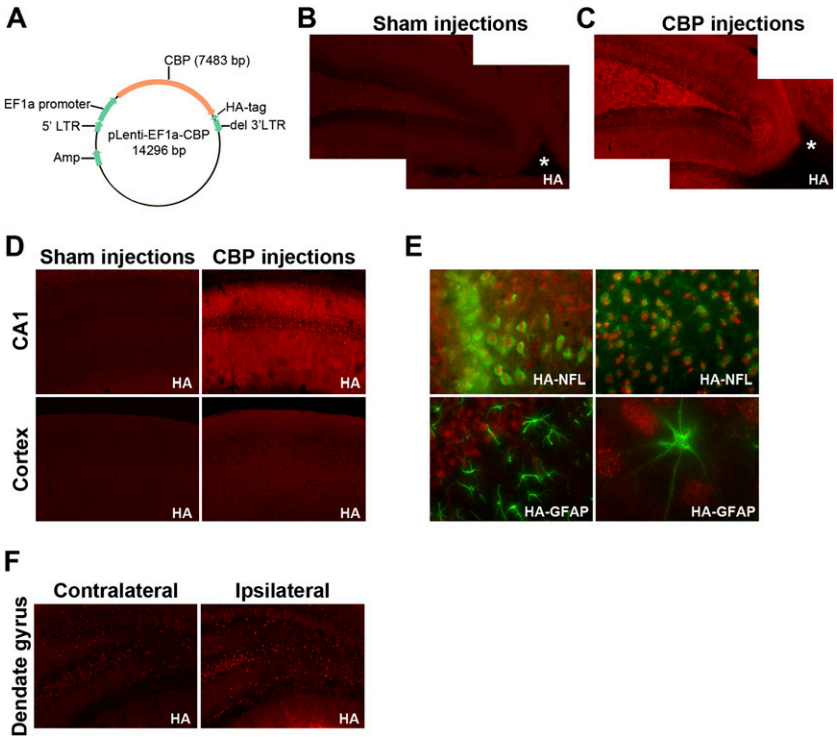

Fig. S2. Extent of viral diffusion. (*A*) Schematic representation of the plasmid used to generate the CBP-expressing lentivirus. The CBP gene was under the control of the neuronal-specific EF1a promoter. An HA tag was added at the 3′ end of the CBP gene. (*B* and *C*) Representative microphotographs depicting the dentate gyrus of sham-injected and CBP-injected mice, respectively. Sections were stained with an anti-HA antibody, revealing strong expression of the virus in this brain region. Notably, an asterisk (*) indicates the central ventricle. (*D*) Representative microphotographs of the CA1 pyramidal neurons (*Upper*) and the cortex (*Lower*) of sham- and CBP-injected 3xTg-AD mice. Sections were stained with an anti-HA antibody and indicate that the virus infected CA1 pyramidal neurons. In contrast, the virus did not spread to cortical regions. (*E*) Representative microphotographs from sham- and CBP-injected 3xTg-AD mice. (*Upper*) Staining with an anti-HA antibody (red) and an anti-neurofilament antibody (green) clearly shows the expression of the HA tag in neurons. (*Lower*) Staining with an anti-HA antibody (red) and an anti-GFAP antibody clearly shows that the HA tag was not expressed in astrocytes. (*F*) Representative microphotographs depicting the contralateral and ipsilateral (relative to the injection site) dentate gyrus of CBP-injected NonTg mice. Sections were stained with an anti-HA antibody and show that the virus infected neurons on both hemi-brains.

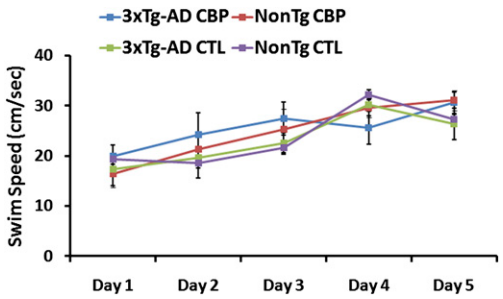

Fig. S3. Swimming speeds. Swimming speed was not significant across the four groups of mice, as determined by two-way ANOVA. Data are presented as mean ± SEM.

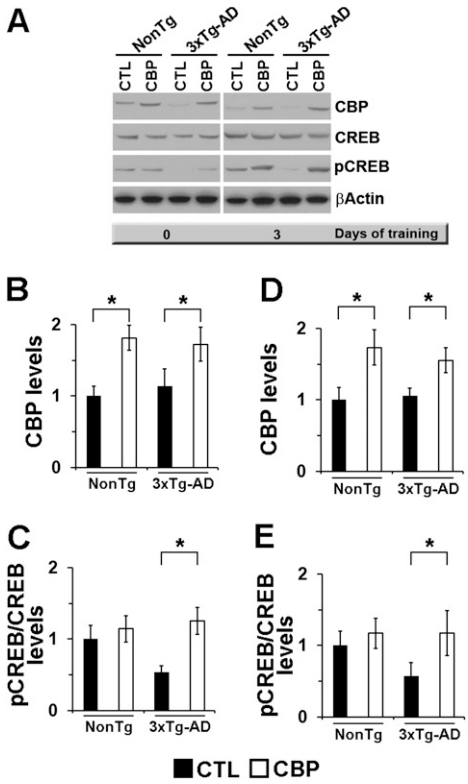

Fig. S4. CBP gene delivery restores pCREB levels. (*A*) Representative Western blots of proteins extracted from the hippocampi of CBP- and sham-injected 3xTg-AD and NonTg mice (n = 6/group) at baseline (7 d after the viral injection and without training in the MWM) and after 3 d of training in the MWM. Blots were probed with the indicated antibodies. (*B–E*) Quantitative analysis of the blots shows that at baseline (*B*) and after 3 d of training (*D*), CBP levels were significantly increased in the 3xTg-AD and NonTg mice that received the virus. In contrast, at both time points, pCREB levels were significantly increased in the hippocampi of the CBP-injected 3xTg-AD mice compared with sham-injected 3xTg-AD mice, but not in the NonTg mice. Data are presented as fold changes over sham-injected NonTg mice and represent mean ± SEM. $*P < 0.05$.

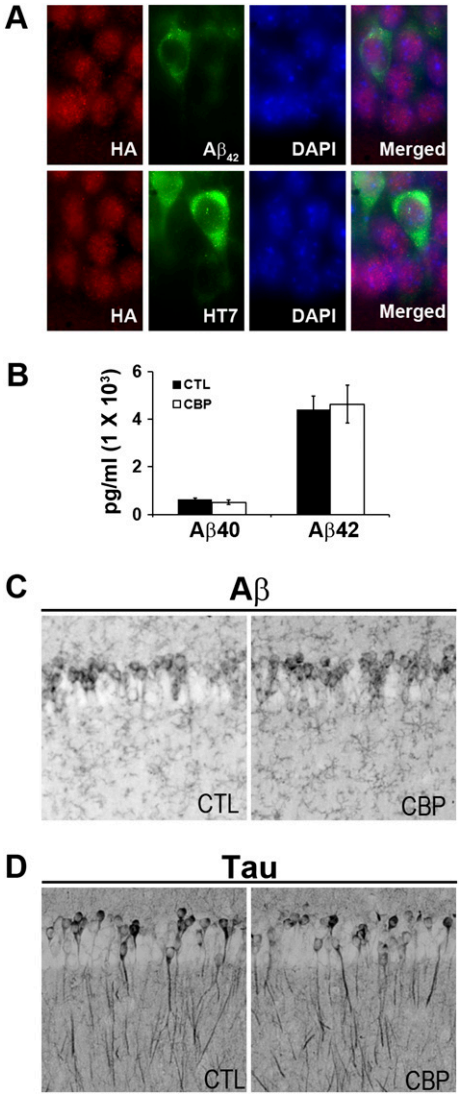

Fig. S5. CBP gene delivery does not alter Aβ and tau pathology. (*A*) Representative microphotographs of CA1 pyramidal neurons from CBP-injected 3xTg-AD mice. Sections were stained with the indicated antibodies and show that the HA tag is expressed in $A\beta_{42}$- and tau- bearing neurons. (*B*) Aβ levels were measured in the hippocampi of injected mice by sandwich ELISA and indicated that CBP gene delivery did not alter Aβ levels. (*C* and *D*) Representative microphotographs of CA1 pyramidal neurons of CBP- and sham-injected 3xTg-AD mice. The sections were immunostained with the reported antibodies and show that CBP gene delivery did not alter Aβ and tau immunoreactivity in the hippocampus. Data are presented as mean ± SEM.

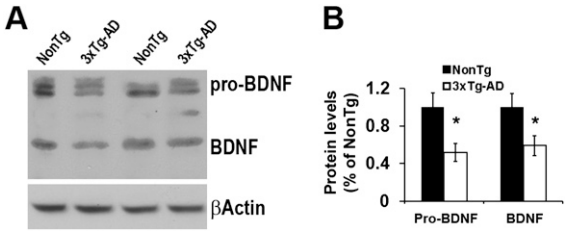

Fig. S6. BDNF levels are lower in the hippocampi of the 3xTg-AD mice. (*A*) Representative Western blots of proteins extracted from the hippocampi of 6-mo-old 3xTg-AD and NonTg mice (n = 6/genotype) and probed with an anti-BDNF antibody. (*B*) Quantitative analysis of the blots shows significantly lower pro-BDNF and BDNF levels in the hippocampi of the 3xTg-AD mice compared with NonTg mice. Data are presented as mean ± SEM. *$P < 0.05$.

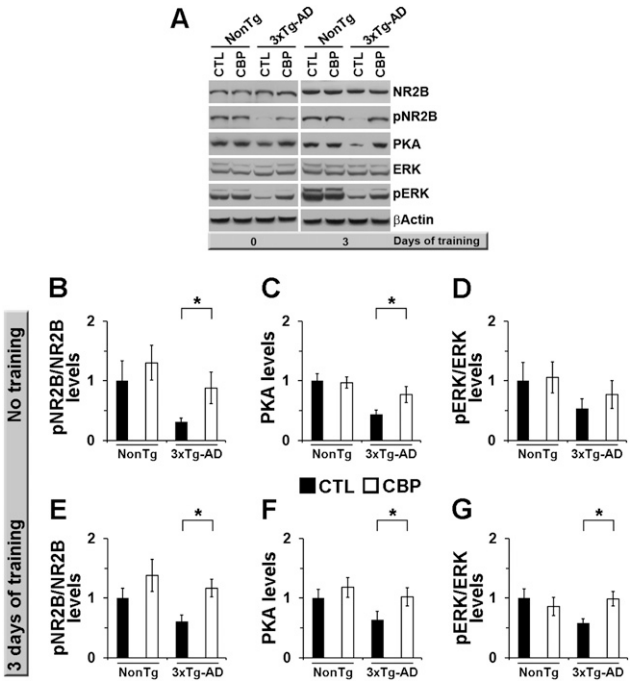

Fig. S7. CBP gene delivery restores NDMA signaling. (*A*) Representative Western blots of proteins extracted from the hippocampi of CBP- and sham-injected 3xTg-AD and NonTg mice (n = 6/group) at baseline (7 d after the viral injection and without training in the MWM) and after 3 d of training. Blots were probed with the indicated antibodies. (*B* and *C*) Quantitative analysis of the blots shows significantly higher baseline levels of pNR2B and PKA in the CBP-injected 3xTg-AD mice compared with the sham-injected 3xTg-AD mice. In contrast, no differences were seen between the sham-injected and CBP-injected NonTg mice. (*D*) At baseline, ERK phosphorylation was not altered in the CBP-injected mice. (*E*–*G*) The levels of pNR2B, PKA, and pERK were significantly increased in the CBP-injected 3xTg-AD mice compared with the sham-injected 3xTg-AD mice after 3 d of training. In contrast, no differences were found between sham- and CBP-injected NonTg mice. Data are presented as fold changes over sham-injected NonTg mice and represent mean ± SEM. $^*P < 0.05$.