In addition to this, for five of the mutants studied, it was possible to perform an independent check of the $\Delta G_{\text{0}}^{0}$ value obtained by comparing the $\Delta G_{\text{0}}^{0}$ relative free energy difference determined from electrochemical measurements of the reaction center ($\Delta G_{\text{p-p}}^{0}$, Fig. 4 and table S1). In these mutants, only the P/P+ midpoint potential (and not the midpoint potentials of $S_1$) is the same for each mutant. This comparison has also been made between wild type and four of the mutants where it was possible to remove quinones without reaction center degradation ($M203GL(24), L168HE, L153HS$, and $L170ND$). All of these kinetic traces are also identical to within the noise of the measurements.

These results provide strong experimental evidence for the concept that the complex non-exponential charge separation kinetics in reaction centers largely reflect the time course of protein dynamics rather than the inherent electron transfer rates between two static states. This evidence supports the concept that protein movement plays a key role in the kinetics of the primary electron transfer reaction, as previously modeled in structurally based simulations of electron transfer in reaction centers (14, 15). Apparently, the observed kinetics are determined by protein conformational changes initiated by the light absorption event rather than a static barrier crossing between two potential surfaces. The dependence of electron transfer dynamics on protein movement lends a robustness to the electron transfer process that is likely very advantageous; changes in the local environment that alter the free energy of the charge-separated states (such as membrane potentials, for example) will only have minor effects on the time scale of electron transfer. It should be noted that the exact shape of $C_0(t)$ is not very critical, as explained in the Supporting Online Material (SOM) text.

Many potential treatments for Alzheimer’s disease target amyloid-β peptides (Aβ), which are widely presumed to cause the disease. The microtubule-associated protein tau is also involved in the disease, but it is unclear whether treatments aimed at tau could block Aβ-induced cognitive impairments. Here, we found that reducing endogenous tau levels prevented behavioral deficits in transgenic mice expressing human amyloid precursor protein, without altering their high Aβ levels. Tau reduction also protected both transgenic and nontransgenic mice against excitotoxicity. Thus, tau reduction can block Aβ- and excitotoxin-induced neuronal dysfunction and may represent an effective strategy for treating Alzheimer’s disease and related conditions.

**Reducing Endogenous Tau Ameliorates Amyloid β−Induced Deficits in an Alzheimer’s Disease Mouse Model**

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Many potential treatments for Alzheimer’s disease target amyloid-β peptides (Aβ), which are widely presumed to cause the disease. The microtubule-associated protein tau is also involved in the disease, but it is unclear whether treatments aimed at tau could block Aβ-induced cognitive impairments. Here, we found that reducing endogenous tau levels prevented behavioral deficits in transgenic mice expressing human amyloid precursor protein, without altering their high Aβ levels. Tau reduction also protected both transgenic and nontransgenic mice against excitotoxicity. Thus, tau reduction can block Aβ- and excitotoxin-induced neuronal dysfunction and may represent an effective strategy for treating Alzheimer’s disease and related conditions.

Deposits of amyloid-β peptide (Aβ) and tau are the pathological hallmarks of Alzheimer’s disease (AD). Treatments aimed at Aβ production, clearance, or aggregation are all in clinical trials. However, interest in tau as a target has been muted, partly because tau pathology seems to occur downstream of Aβ (4–6), making it uncertain whether tau-directed therapeutics would prevent Aβ-induced impairments. Also, tau is posttranslationally modified in
AD (5–8), and debate continues about which modifications should be targeted. Reducing overall tau levels might be an alternative approach (9). As tau haplotypes driving slightly higher tau expression increase AD risk (10), reducing tau levels might be protective. Therefore, we determined the effect of reducing endogenous tau expression on cognitive deficits in transgenic mice expressing human amyloid precursor protein (hAPP) with familial AD mutations that increase Aβ production.

We crossed hAPP mice (11) with Tau−/− mice (12) and examined hAPP mice with two (hAPP/Tau+/-), one (hAPP/Tau+/-), or no (hAPP/Tau−/−) endogenous tau alleles, compared with Tau+/-, Tau−/−, and Tau+−/− mice without hAPP (13). Tau reduction did not affect hippocampal hAPP expression, and conversely, hAPP did not affect hippocampal tau levels (fig. S1). The six genotypes showed no differences in weight, general health, basic reflexes, sensory responses, or gross motor function.

To test learning and memory, we used the Morris water maze. In the cued version, mice learn to find the target platform using a conspicuous marker placed directly above it. At 4 to 7 months of age, Tau+−/− and Tau−/− mice learned quickly, but as expected (14, 15), hAPP/Tau−/− mice took longer to master this task (Fig. 1A; P < 0.001). In contrast, hAPP/Tau+−/− and hAPP/Tau−/− mice performed at control levels.

The more difficult hidden-platform version of the water maze demands spatial learning. Mice without hAPP learned this task over 3 days of training regardless of tau genotype, whereas hAPP/Tau+−/− mice showed no evidence of learning until days 4 and 5 (P < 0.001; Fig. 1B). Notably, hAPP/Tau+−/− mice were less impaired than hAPP/Tau+−/− mice (P < 0.02), and hAPP/Tau−/− mice did not differ from controls without hAPP (Fig. 1B). Probe trials, in which the platform was removed and mice were given 1 min to explore the pool, confirmed the beneficial effect of tau reduction (Fig. 1, C to E). In an initial probe trial 24 hours after 3 days of training, hAPP/Tau−/− mice had no apparent spatial memory of the platform location, crossing the target platform location no more than they crossed equivalent areas in nontarget quadrants (Fig. 1D). However, hAPP/Tau−/− mice, similar to mice without hAPP, did cross the target platform location more often (P < 0.01; Fig. 1D). After two additional days of training, hAPP/Tau−/− mice also had more target than nontarget crossings (P < 0.01), whereas hAPP/Tau−/− mice still showed no spatial learning and memory (Fig. 1E). Thus, the tau reduction gene dose-dependently ameliorates Aβ-dependent water maze learning and memory deficits.

In the probe trials 72 hours after completion of 5 days of hidden-platform training, target platform preference differed by genotype (target crossing by genotype interaction, P < 0.001). In post-hoc comparisons, all genotypes except hAPP/Tau+−/− and hAPP/Tau−/− exhibited a preference for the target location over equivalent areas in the other three quadrants (P < 0.05; **P < 0.01; ***P < 0.001). (E) Probe trial 72 hours after completion of 5 days of hidden-platform training. Target platform preference differed by genotype (target crossing by genotype interaction, P < 0.001; target crossing by hAPP by tau interaction, P < 0.05). In post-hoc comparisons, all genotypes except hAPP/Tau−/− exhibited a preference for the target location (**P < 0.01; ***P < 0.001). Error bars show SEM.

**Fig. 1. Tau reduction prevented water maze deficits in hAPP mice (n = 7 to 11 mice per genotype, age 4 to 7 months). (A) Cued platform learning curves. Day 0 indicates performance on the first trial, and subsequent points represent average of all daily trials. Performance differed by genotype (repeated measures analysis of variance (RMANOVA): P < 0.001; hAPP by tau interaction, P = 0.058). In post-hoc comparisons, only hAPP/Tau−/− differed from groups without hAPP (P < 0.001). (B) Hidden platform learning curves differed by genotype (RMANOVA: P < 0.001; hAPP by Tau interaction, P < 0.02). In post-hoc comparisons, hAPP/Tau+−/− differed from all groups without hAPP (P < 0.001), hAPP/Tau+−/− differed from groups without hAPP (P < 0.001; hAPP/Tau+−/− differed from groups without hAPP (P < 0.001). (C) Target and Others Platform crossings versus crossings of the equivalent area in the three other quadrants differed by genotype (target crossing by genotype interaction, P < 0.001). In post-hoc comparisons, all genotypes except hAPP/Tau+−/− and hAPP/Tau−/− exhibited a preference for the target location over equivalent areas in the other three quadrants (P < 0.05; **P < 0.01; ***P < 0.001). (D) Probe trial 72 hours after completion of 5 days of hidden-platform training. Target platform preference differed by genotype (target crossing by genotype interaction, P < 0.001; target crossing by hAPP by tau interaction, P < 0.05). In post-hoc comparisons, all genotypes except hAPP/Tau+−/− exhibited a preference for the target location (**P < 0.01; ***P < 0.001). Error bars show SEM.**
4 to 7 months (fig. S3) and 14 to 18 months (Fig. 3, A and B). We also found no effect of tau reduction on levels of Aβ in all genotypes; age 14 to 18 months). (Fig. S4). Thus, the beneficial effects of reducing tau were observed without detectable changes in Aβ burden, suggesting that tau reduction un couples Aβ from downstream pathogenic mechanisms.

Next, we looked for abnormal forms of tau that might act as downstream effectors of Aβ in hAPP/Tau–/– mice. Major AD-related phosphorylation sites in human tau are conserved in murine tau, including those phosphorylated by proline-directed kinases, such as glycogen synthase kinase (GSK)–β and cdk5, or by microtubule affinity–regulating kinase (MARK). Changes in murine tau phosphorylation at these sites are easily detected, for example after brief hypothermia (20) (fig. S4). However, in hippocampal homogenates of 4- to 7-month-old hAPP/Tau–/– mice, we did not find changes in tau phosphorylation at proline-directed kinase sites, including Thr181, Ser202, Thr217, and Ser306/404, or at the primary site for MARK, Ser202 (fig. S5). Generation of neurotoxic tau fragments has also been implicated as a mechanism of Aβ toxicity (21). Tau-deficient primary neurons are resistant to Aβ-induced degeneration (3, 22), apparently because Aβ toxicity in vitro involves production of a 17-kD tau fragment (21). We confirmed the presence of a 17-kD tau fragment in lysates of Aβ-treated primary neurons, but found no abnormal tau proteolysis in hippocampal homogenates from hAPP mice (fig. S6), suggesting that the neuroprotective effects of tau reduction in the two systems are mechanistically different. The relative lack of modified tau also distinguishes our model from transgenic lines overexpressing tau with mutations that cause frontotemporal dementia, but not AD, in humans (2, 4, 23). In our study, reduction of endogenous, wild-type tau protected hAPP mice against Aβ-dependent cognitive impairments, and this did not involve the elimination of a large pool of tau with typical AD-associated modifications. Our experiments do not rule out the possibility that another type of tau fragment, not detected in our analysis, might also be involved.

Fig. 2. Tau reduction prevented behavioral abnormalities and premature mortality in hAPP mice. (A) Total arm entries during a 6-min exploration of the Y maze (n = 49 to 58 mice per genotype; age 4 to 7 months; ANOVA: genotype effect, P < 0.0001; hAPP by tau interaction, P < 0.0001; ***P < 0.001 versus groups without hAPP). (B) Percentage of time spent active during a 6-min exploration of a new cage (n = 7 to 14 mice per genotype; age 4 to 7 months; ANOVA: genotype effect, P < 0.01; hAPP by tau interaction, P < 0.05; *P < 0.05 versus groups without hAPP). (C) Total distance traveled in both open and closed arms during a 10-min exploration of the elevated plus maze (n = 49 to 59 mice per genotype; age 4 to 7 months; ANOVA: genotype effect, P < 0.0001; hAPP by tau interaction, P < 0.05; ***P < 0.001 versus groups without hAPP). (D) Total distance traveled during exploration of elevated plus maze (n = 6 to 13 mice per genotype; age 12 to 16 months; ANOVA: hAPP effect, P < 0.01; hAPP by tau interaction, P = 0.079; *P < 0.05 versus groups without hAPP). Error bars in (A) to (D) show SEM. (E) Kaplan-Meier survival curves showing effect of tau reduction on premature mortality in hAPP mice. All genotyped mice in the colony (n = 887) were included in the analysis. By log-rank comparison, only hAPP/Tau–/– mice differed from all other groups (P < 0.0005).

Fig. 3. Tau reduction did not change Aβ plaque deposition, neuritic dystrophy, or aberrant sprouting. (A) Thioflavin-S staining of hippocampal amyloid plaques in hAPP mice. Percentage of hippocampal area covered by plaques was normalized to the mean value in hAPP/Tau–/– mice (n = 6 to 11 mice per genotype; age 14 to 18 months). (B) Immunostaining of hippocampal Aβ deposits in hAPP mice. Percentage of hippocampal area covered by plaques was normalized to the mean value in hAPP/Tau–/– mice (n = 6 to 11 mice per genotype; age 14 to 18 months). (C) Double-labeling of hippocampus for dystrophic neurites (antibody 8ES, red) and amyloid plaques (thioflavin-S, green) in hAPP mice aged 14 to 18 months, with quantification of dystrophic neurites expressed as percentage of thioflavin-S–positive plaques with surrounding neuritic dystrophy (n = 9 to 11 mice per genotype). (D) GAP43 immunostaining of aberrant axonal sprouting in the molecular layer of the dentate gyrus (oml, outer molecular layer; mml, middle molecular layer; lml, inner molecular layer; dgc, dentate granule cells). Bracket highlights GAP43-positive sprouting in the outer molecular layer of hAPP mice. Sprouting was quantified by densitometry and normalized to the mean value in Tau–/– mice (n = 7 to 14 mice per genotype; age 4 to 7 months; ***P < 0.001 versus groups without hAPP). Error bars show SEM.
of tau modification, or a small pool of modified tau in a restricted subcellular compartment or cellular population, could play a role downstream of Aβ.

To begin addressing this possibility, we stained brain sections from Tau+/− and hAPP+/− mice with phospho-tau antibodies. We saw little difference overall between Tau+/− and hAPP+/− mice in phospho-tau immunoreactivity, but we did observe scattered phospho-tau–positive punctae in dystrophic neurites surrounding amyloid plaques (fig. S7). We thus wondered whether the benefits of tau reduction in hAPP mice could relate to prevention of neuritic dystrophy, which may contribute to AD-related cognitive decline (24). Despite the differences in their behavior, hAPP+/−, hAPP+/+−, and hAPP−/− mice had similar amounts of neuritic dystrophy (Fig. 3C). Thus, tau is not required for the formation of plaque-associated dystrophic neurites. Given that tau reduction prevented behavioral deficits but not neuritic dystrophy, these may represent parallel, rather than causally linked, disease manifestations, or tau reduction may act downstream of neuritic dystrophy.

Tau has a well-characterized role in axonal outgrowth (12), so we tested whether tau reduction prevented the aberrant sprouting of hippocampal axons observed in AD (25) and hAPP mice (18). Similar degrees of sprouting were observed, regardless of tau genotype (Fig. 3D). Thus, although tau reduction affected important outcome measures related to Aβ–induced neuronal dysfunction, not all effects of Aβ were blocked.

Excitotoxicity is implicated in the pathogenesis of AD (26, 27). Consistent with the increased incidence of seizures in AD patients (28), TgCRND8 hAPP mice are more susceptible to the γ-amino butyric acid type A (GABA_A) receptor antagonist pentylentetrazole (PTZ) (29). Using a similar paradigm, we found that hAPP+/− mice were also abnormally sensitive to PTZ, with 20% suffering fatal status epilepticus at a dose that was not lethal to mice without hAPP (P < 0.05). Tau reduction prevented this effect, as no hAPP+/− or hAPP+/− mice died. Seizures in hAPP+/+− and hAPP–/− mice were less severe and occurred at longer latencies than in hAPP+/− mice (P < 0.01; Fig. 4, A and B).

Tau reduction also increased resistance to PTZ in hAPP-nontransgenic mice, lowering seizure severity and delaying seizure onset (P < 0.01; Fig. 4, A and C). To confirm that tau reduction could reduce aberrant neuronal overexcitability, we challenged mice with excitotoxic doses of the glutamate receptor antagonist kainate. As expected, intraperitoneal injection of kainate dose-dependently induced seizures in Tau−/− mice (Fig. 4D). In contrast, Tau+/− and Tau−/− mice were resistant to kainate across a range of doses (P < 0.05; Fig. 4D). Thus, tau modulates sensitivity to excitotoxins and may be involved in regulating neuronal activity. The excitoprotective effect of tau reduction in mice without hAPP is more likely related to a physiological function of tau than to the removal of a pathological form of the protein. Sensitization of neurons to Aβ by physiological forms of tau could explain why tau reduction is effective in hAPP+/− mice despite their lack of obvious tau modifications.

Our findings raise the possibility that tau reduction could protect against AD and other neurological conditions associated with excitotoxicity. Of course, the therapeutic implications of our findings must be interpreted with caution. First, there are differences between the mouse model and AD, including the absence of substantial neuronal loss or neurofibrillary pathology in hAPP mice. The contribution of these abnormalities to AD-related cognitive impairment, relative to the role of reversible Aβ–induced neuronal dysfunction that is modeled in hAPP mice, remains to be determined (30). Second, microdeletions of chromosome 17q21 encompassing the tau gene are associated with learning disabilities in humans (31), although abnormalities in these individuals may relate to insufficient of other genes in the region, such as the corticotropin-releasing hormone receptor gene, which is implicated in neuropsychiatric disease (32). We found no adverse effects of tau reduction on health or cognition in mice, and the evidence that even partial tau reduction robustly protected mice from Aβ excitoxins highlights its potential benefits.

References and Notes
13. Materials and methods are available as supporting material on Science Online.
Regulation of NF-κB Activation in T Cells via Association of the Adapter Proteins ADAP and CARMA1

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The adapter protein ADAP regulates T lymphocyte adhesion and activation. We present evidence for a previously unrecognized function for ADAP in regulating T cell receptor (TCR)–mediated activation of the transcription factor NF-κB. Stimulation of ADAP-deficient mouse T cells with antibodies to CD3 and CD28 resulted in impaired nuclear translocation of NF-κB, a reduced DNA binding, and delayed degradation and decreased phosphorylation of IκB (inhibitor of NF-κB). TCR-stimulated assembly of the CARMA1–BCL-10–MALT1 complex was substantially impaired in the absence of ADAP. We further identified a region of ADAP that is required for association with the CARMA1 adapter and NF-κB activation but is not required for ADAP-dependent regulation of adhesion. These findings provide new insights into ADAP function and the mechanism by which CARMA1 regulates NF-κB activation in T cells.

Membrane localization of PKCθ was similar in ADAP+/- and ADAP−/− T cells upon stimulation with antibodies to CD3 and CD28 (Fig. 1, A and B). Stimulated ADAP+/− and ADAP−/− T cells also showed similar levels of PKCθ phosphorylation (Fig. 1C). Thus, ADAP is not required for TCR signaling events leading to and including PKCθ activation. Because PKCθ regulates NF-κB activation downstream of the TCR (11, 13), we next examined NF-κB signaling in ADAP−/− T cells. Image scanning flow cytometry (14, 15) (fig. S1) revealed a striking defect in p65 nuclear translocation after stimulation of ADAP−/− lymph node T cells (Fig. 2, A and B) or CD4 T cells (fig. S2) by CD3 and CD28 (CD3/CD28). In contrast, no impairment in NF-κB activation was detected after stimulation with tumor necrosis factor-α (TNF-α), which activates NF-κB independently of the TCR. These results were confirmed with electrophoretic mobility shift assays (Fig. 2C). ADAP−/− T cells also displayed defective NF-κB translocation after treatment with phorbol 12-myristate 13-acetate (PMA), which activates PKC (Fig. 2, A and B).

Fig. 1. TCR-dependent membrane localization and activation of PKCθ in ADAP−/− T cells. (A) Localization of PKCθ (bottom) in ADAP+/+ and ADAP−/− T cells to the contact site with beads coated with antibodies to CD3 and CD28. Differential interference contrast (DIC) images are shown in top panels. (B) Quantification of PKCθ localization. T cell–bead conjugates (minimum 90 per group) were scored for PKCθ polarization from two independent experiments. Graph shows the average percent of T cell–bead conjugates with polarized PKCθ (+SD). (C) Phosphorylation of PKCθ after CD3/CD28 stimulation of ADAP+/+ and ADAP−/− T cells for the indicated time points was assessed by Western blotting of whole-cell lysates with antibody to phosphorylated PKCθ (Thr538) (top panels). Blots were also probed with antibody to β-actin (bottom panels).

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Articles and Methods
Figs. S1 to S7

Supporting Online Material
www.sciencemag.org/cgi/content/full/316/5825/750/DC1

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26 February 2007; accepted 27 March 2007
10.1126/science.1141736