Cell

Dendritic Function of Tau Mediates Amyloid-β Toxicity in Alzheimer's Disease Mouse Models

Lars M. Ittner,[1,6,*] Yazi D. Ke,[1,6] Fabien Delerue,[1] Mian Bi,[1] Amadeus Gladbach,[1] Janet van Eersel,[1] Heidrun Wölfing,[1] Billy C. Chieng,[2] MacDonald J. Christie,[2] Ian A. Napier,[2] Anne Eckert,[3] Matthias Staufenbiel,[4] Edna Hardeman,[5] and Jürgen Götz[1,*]

[1]Alzheimer's and Parkinson's Disease Laboratory
[2]Neuropharmacology Laboratory
Brain and Mind Research Institute, University of Sydney, Sydney NSW 2050, Australia
[3]Neurobiology Research Laboratory, Psychiatric University Clinic, University of Basel, Basel CH-4025, Switzerland
[4]Novartis Institutes for BioMedical Research, Basel CH-4002, Switzerland
[5]Neuromuscular and Regenerative Medicine Unit, University of New South Wales, Sydney NSW 2052, Australia
[6]These authors contributed equally to this work
*Correspondence: littner@med.usyd.edu.au (L.M.I.), jgoetz@med.usyd.edu.au (J.G.)
DOI 10.1016/j.cell.2010.06.036

SUMMARY

Alzheimer's disease (AD) is characterized by amyloid-β (Aβ) and tau deposition in brain. It has emerged that Aβ toxicity is tau dependent, although mechanistically this link remains unclear. Here, we show that tau, known as axonal protein, has a dendritic function in postsynaptic targeting of the Src kinase Fyn, a substrate of which is the NMDA receptor (NR). Missorting of tau in transgenic mice expressing truncated tau (Δtau) and absence of tau in *tau*$^{-/-}$ mice both disrupt postsynaptic targeting of Fyn. This uncouples NR-mediated excitotoxicity and hence mitigates Aβ toxicity. Δtau expression and *tau* deficiency prevent memory deficits and improve survival in Aβ-forming APP23 mice, a model of AD. These deficits are also fully rescued with a peptide that uncouples the Fyn-mediated interaction of NR and PSD-95 in vivo. Our findings suggest that this dendritic role of tau confers Aβ toxicity at the postsynapse with direct implications for pathogenesis and treatment of AD.

INTRODUCTION

Alzheimer's disease (AD) is characterized by two hallmark lesions, amyloid-β (Aβ) plaques and neurofibrillary tangles (NFTs) (Ballatore et al., 2007). Aβ is derived from the amyloid-β precursor protein (APP) by proteolytic cleavage (Haass et al., 1992; Selkoe, 1997). The major constituent of NFTs is tau, a microtubule (MT)-associated protein (Goedert et al., 1988). In the course of AD, tau becomes phosphorylated, forming aggregates that deposit as NFTs and neuropil threads (Geschwind, 2003). Tau can also form aggregates in the absence of an overt Aβ pathology, for example in frontotemporal dementia (FTD), where familial mutations have been identified in the tau-encoding *MAPT* gene (Ballatore et al., 2007). Evidence that tau pathology in AD is induced by Aβ comes from our previous observation that intracerebral Aβ injections exacerbate hyperphosphorylation of tau and NFT formation in transgenic mice that express FTD mutant P301L tau (Götz et al., 2001b). A similar finding was obtained by crossing transgenic mice with NFT and plaque pathologies (Lewis et al., 2001).

Aβ-plaque formation along with memory impairment and tau pathology with increased phosphorylation, in the absence of deposition and NFT formation, has been reproduced in several transgenic mouse lines that express human APP together with pathogenic mutations identified in familial AD (Götz and Ittner, 2008; Hsiao et al., 1996; Mucke et al., 2000; Sturchler-Pierrat et al., 1997). In one of these, PDAPP, *tau* deficiency (*tau*$^{-/-}$) was shown to rescue lethality and memory deficits by an unidentified mechanism (Roberson et al., 2007).

Tau is known as axonal protein that regulates MT stability and MT-dependent processes (Dixit et al., 2008; Drechsel et al., 1992; Lee et al., 1988), while Aβ likely exerts toxicity at the postsynapse (Selkoe, 2002; Shankar et al., 2008; Zhao et al., 2006). Although in AD, hyperphosphorylated tau accumulates in the somatodendritic compartment of neurons (Ballatore et al., 2007), given the spatial separation it remains unknown how tau is involved in mediating Aβ toxicity when AD is initiated.

Seizures characterize several APP transgenic strains (Minkeviciene et al., 2009; Palop et al., 2007; Palop and Mucke, 2009) and have been associated with AD; the extent of their contribution to pathology, however, remains to be established (Minkeviciene et al., 2009; Palop et al., 2007; Palop and Mucke, 2009). Excitotoxicity results from overactivation of N-methyl-D-aspartate (NMDA) receptors (NRs). Interestingly, tau reduction decreases susceptibility to excitotoxic seizures in vivo, which may explain the concomitant improvement of the PDAPP phenotype (Roberson et al., 2007). How tau prevents excitotoxic damage at a molecular level is not understood.

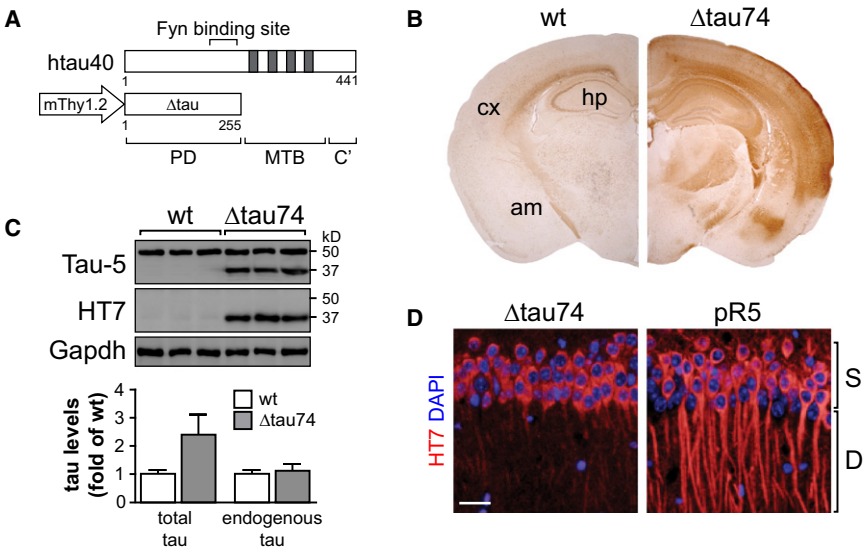

Figure 1. Truncated Tau Is Excluded from Dendrites in Δtau74 Mice

(A) The longest human tau isoform (htau40; 441 aa) is composed of an amino-terminal projection domain (PD), the microtubule-binding (MTB) domain with four repeats (gray boxes), and the carboxy-terminal tail (C′). Δtau transgenic mice express only the PD of tau under control of the neuronal mThy1.2 promoter. Δtau lacks the MTB domain and therefore the MT-binding and aggregation properties of full-length tau, but contains a Fyn binding site.

(B) Expression pattern of Δtau in Δtau74 brains. Immunohistochemistry (IHC) with a human tau-specific antibody (HT7; brown) reveals Δtau expression within several brain regions, including hippocampus (hp), cortex (cx), and amygdala (am).

(C) Western blotting of wild-type and Δtau74 hippocampal extracts reveals endogenous murine tau (50 kD) in all and Δtau (37 kD) only in transgenic samples. Quantification shows comparable levels of endogenous tau, while endogenous tau and Δtau levels add up to 2.4-fold increased total levels in Δtau74 compared to WT mice.

(D) IHC of the hippocampal CA1 region reveals that in Δtau74 mice, Δtau localizes to the soma (*S*) but is excluded from dendrites (*D*), whereas expression of P301L mutant full-length tau in pR5 mice results in a somato-dendritic localization of transgenic tau (HT7; reactive with Δtau and pR5 tau, but not endogenous tau, in red). The scale bar represents 50 μm.

Error bars represent the standard error. See also Figure S1.

Tau interacts via its amino-terminal projection domain (PD) with the kinase Fyn (Figure 1A) (Lee et al., 1998). Fyn phosphorylates the NR subunit 2 (NR2) to facilitate interaction of the NR complex with the postsynaptic density protein 95 (PSD-95) (Nakazawa et al., 2001; Rong et al., 2001; Tezuka et al., 1999), linking NRs to synaptic excitotoxic downstream signaling (Salter and Kalia, 2004). Disruption of the NR/PSD-95 interaction prevents excitotoxic damage in cultured neurons and a rat model of stroke, without affecting synaptic NMDA currents (Aarts et al., 2002). Reduction of Fyn in APP transgenic mice prevents Aβ toxicity, while overexpression enhances it (Chin et al., 2005; Chin et al., 2004).

To address how tau confers Aβ toxicity, we generated transgenic mice (Δtau74) that express only the amino-terminal projection domain (PD) of tau and crossed them with Aβ-forming APP23 and *tau*$^{-/-}$ mice. We found that tau has an important dendritic function, as in Δtau74 and *tau*$^{-/-}$ mice, postsynaptic Fyn localization is reduced, resulting in reduced NR phosphorylation, destabilized NR/PSD-95 interaction, and protection from excitotoxicity.

RESULTS

Truncated Tau Is Excluded from Dendrites

Tau comprises an amino-terminal projection domain, an MT binding (MTB) domain that mediates interaction with MTs (Butner and Kirschner, 1991; Lee et al., 1988) and is essential for tau aggregation (Crowther et al., 1989; Ksiezak-Reding and Yen, 1991) and a carboxy-terminal tail region (Figure 1A). We generated truncated (Δtau) transgenic mice that express the projection domain of tau in neurons, intended to compete with functions of endogenous tau. Four phenotypically normal lines expressed Δtau throughout the brain (Figure 1B) at comparable levels, with line Δtau74 expressing the transgene at 1.4-fold higher levels than endogenous tau (Figure 1C). Expression of Δtau neither affected levels nor distribution of endogenous tau (Figure 1C and Figures S1A–S1C available online). Consistent with previous in vitro findings (Maas et al., 2000), Δtau localized to the cell membrane, as indicated by coimmunostaining with cadherin and subcellular fractionation of membranes (Figures S1D and S1E). In AD and also full-length P301L mutant tau transgenic pR5 mice, tau is hyperphosphorylated and redistributed into the somatodendritic compartment (Figure 1D) (Götz et al., 2001a). In contrast to full-length tau, Δtau, while in the soma, was virtually excluded from dendrites (Figure 1D). In pR5 mice, tau becomes progressively hyperphosphorylated and insoluble, and eventually the mice develop NFTs. Surprisingly, Δtau in Δtau74 mice is hardly phosphorylated at all (Figure S1F).

Postsynaptic Targeting of Fyn Is Tau Dependent

Different from full-length human tau in pR5 mice, in the absence of an MTB domain, Δtau fails to interact with MTs, as determined by MT precipitation from hippocampi (Figure 2A). However, Δtau contains motifs that mediate interaction with the Src kinase Fyn, as shown in vitro (Lee et al., 1998). Accordingly, Fyn can be coimmunoprecipitated with Δtau from Δtau74 hippocampi in vivo, using a human tau-specific antibody (HT7) (Figure 2B). Immunoprecipitation (IP) with tau-specific antibodies to epitopes not present on the Δtau construct reveals a significantly reduced interaction of Fyn with endogenous tau (Figure 2B). Likewise, IP with Fyn antibodies shows a reduced interaction with endogenous tau in Δtau74 mice (Figure 2B). Together, this suggests a dominant negative effect of Δtau on the normal interaction of Fyn and endogenous tau. A similar effect on the Fyn/Tau

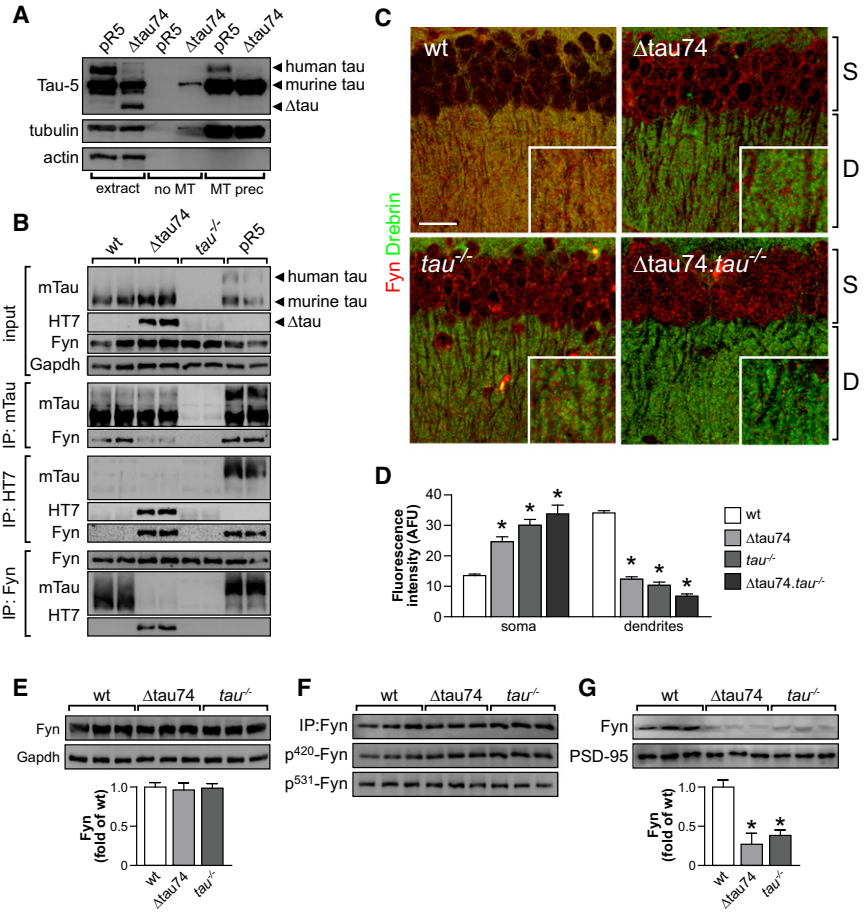

Figure 2. Δtau Impairs Tau-Dependent Dendritic Targeting of the Src Kinase Fyn

(A) Δtau from Δtau74 mice does not interact with microtubules. Endogenous murine tau, but not Δtau, precipitates with microtubules in extracts from Δtau74 mice. In contrast, both full-length human and endogenous murine tau precipitate with microtubules in extracts from pR5 mice.

(B) Expression of Δtau results in a 74% ± 6% (n = 8; *p < 0.01) reduced interaction of Fyn with endogenous murine tau (mtau) compared to the wild-type (wt), as revealed by coimmunoprecipitation (coIP) with antibodies to endogenous murine tau (mTau). In Δtau74 mice, Fyn instead coimmunoprecipitates with Δtau, as revealed by antibody HT7. Similarly, in pR5 mice, Fyn precipitates with full-length human tau (htau). CoIP with Fyn antibodies predominantly pulled down endogenous tau in WT, Δtau in Δtau74, and full-length human tau in pR5 mice. No precipitation was observed from *tau*$^{-/-}$ tissue.

(C) Fyn accumulates in cell bodies in Δtau74, *tau*$^{-/-}$, and Δtau74.*tau*$^{-/-}$ mice. While Fyn staining (red) colocalizes with dendritic drebrin (green) in WT CA1 neurons, Fyn staining is evident in the soma (*S*) and is reduced in the dendrites (*D*) of Δtau74 and *tau*$^{-/-}$ neurons. The insets show higher magnification of dendritic staining. The scale bar represents 50 μm.

(D) Quantification of fluorescence intensity of Fyn staining in cell bodies and dendrites shows accumulation of Fyn in cell bodies of Δtau74, *tau*$^{-/-}$, and Δtau74.*tau*$^{-/-}$ mice (n = 15, *p < 0.0001).

(E) Total Fyn levels are not reduced in Δtau74 and *tau*$^{-/-}$ mice. Western blots of hippocampal extracts from WT, Δtau74, and *tau*$^{-/-}$ brains show comparable levels of Fyn, normalized to Gapdh (n = 6).

(F) Phosphorylation of activating (Y420) and inactivating (Y531) sites of immunopurified Fyn from WT, Δtau74, and *tau*$^{-/-}$ brains is similar.

(G) Hippocampal synaptosomal preparations reveal reduced levels of Fyn in Δtau74 and *tau*$^{-/-}$ postsynapses compared to the WT (n = 6, *p < 0.005).

Error bars represent the standard error. See also Figure S2.

interaction was obtained by overexpression of full-length tau in pR5 mice (Figure 2B). Given the dendritic exclusion of Δtau in Δtau74 in contrast to full-length tau in pR5 mice (Figure 1D), we speculated that the aberrant Δtau/Fyn interaction might affect the normal intracellular distribution of Fyn. Immunohistochemistry showed that Fyn colocalized with drebrin in wild-type (WT) brain, consistent with postsynaptic targeting, while in Δtau74 brains it accumulated in the soma, an effect enhanced by crossing of Δtau and *tau*$^{-/-}$ (Figures 2C and 2D). Together with reduced dendritic Fyn staining, this suggests impaired postsynaptic targeting of Fyn. To determine the role of tau in dendritic localization of Fyn, we also analyzed *tau*$^{-/-}$ mice. Fyn also accumulated in the soma (Figures 2C and 2D), suggesting that postsynaptic targeting of Fyn is, at least in part, tau dependent. This is consistent with reduced localization of Fyn-DsRED in primary hippocampal neurons either from *tau*$^{-/-}$ mice or mice coexpressing Δtau (Figure S2). Interestingly, further truncation of Δtau shows that the Fyn-interactive motif, PXXP (Lee et al., 1998), is critical for Fyn localization.

Despite changes in the localization of Fyn, its total levels and activity were comparable in Δtau74, *tau*$^{-/-}$, and WT mice, as determined by total and phosphorylation site-specific anti-Fyn antibodies (Figures 2E and 2F). To quantify changes in the subcellular localization of Fyn, we prepared synaptosomes from WT, Δtau74, and *tau*$^{-/-}$ hippocampi. Consistent with the immunohistochemical findings of reduced postsynaptic targeting, levels of synaptic Fyn were reduced by 73% and 62% in Δtau74 and *tau*$^{-/-}$ mice, respectively, compared to WT controls (Figure 2G). Taken together, both the presence of Δtau and absence of endogenous tau impair synaptic localization of Fyn.

Uncoupled NMDA Receptors and PSD-95 in Δtau and *tau*$^{-/-}$ Synapses

The postsynaptic NR subunit NR2b is a known substrate of Fyn (Nakazawa et al., 2001). NR2b phosphorylation at Y1472 strengthens the NR/PSD-95 interaction (Rong et al., 2001). In both Δtau74 and *tau*$^{-/-}$ mice, Y1472 phosphorylation is significantly reduced compared to the WT, while total levels of NR1, NR2a, and NR2b are unaffected (Figure 3A). To determine whether this affects the stability of NR/PSD-95 complexes, we performed coimmunoprecipitations (coIPs). Markedly less NR1, NR2a, and NR2b coimmunoprecipitated with PSD-95

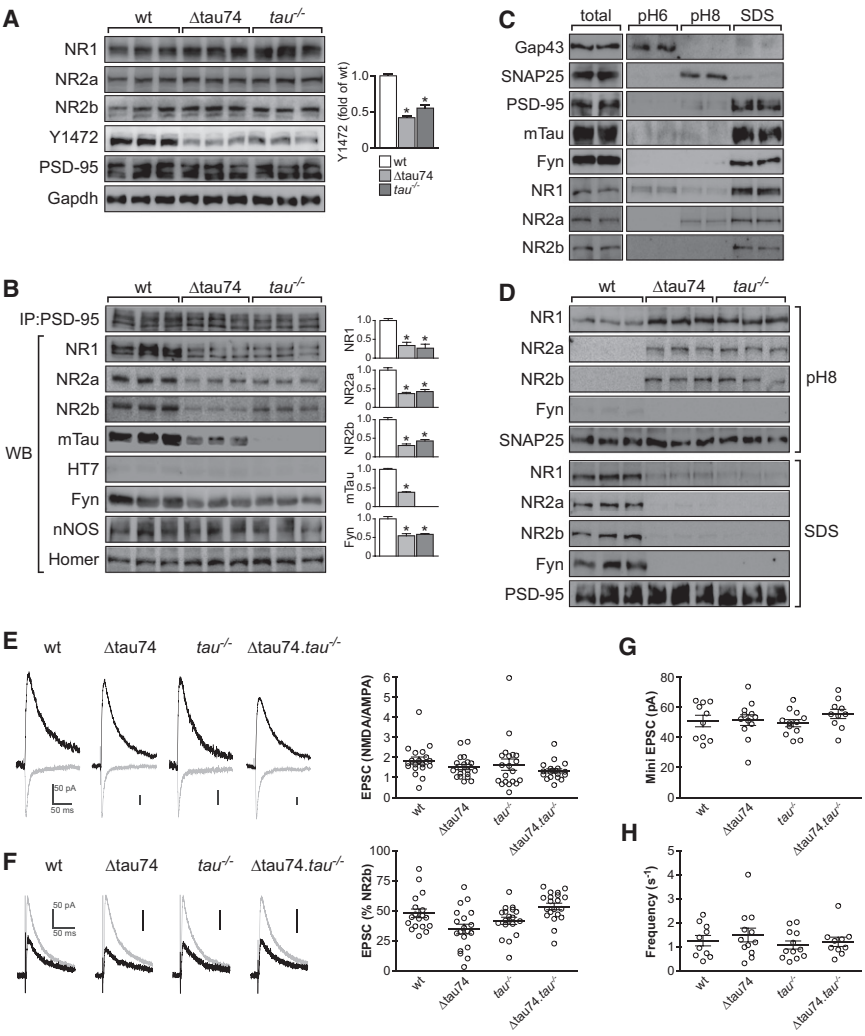

Figure 3. Destabilized NMDA Receptors in the Postsynaptic Density of Δtau74 and *tau*$^{-/-}$ Mice

(A) Levels of NR subunits NR1, NR2a and NR2b, and PSD-95 are comparable in extracts from WT, Δtau74, and *tau*$^{-/-}$ brains, whereas phosphorylation of NR2b at the Fyn site, Y1472, that is known to stabilize NR/PSD-95 complexes (Roche et al., 2001), is significantly reduced in Δtau74 and *tau*$^{-/-}$ than in the WT (n = 6, *p < 0.005).

(B) PSD-95 antibodies coimmunoprecipitate much less NR subunits NR1, NR2a, and NR2b from Δtau74 and *tau*$^{-/-}$ than from WT hippocampi. Similarly, coimmunoprecipitation (coIP) of Fyn with PSD-95 is reduced in Δtau74 and *tau*$^{-/-}$ compared to WT hippocampi, while that of nNOS and Homer was unaffected. Endogenous murine Tau (mTau) coprecipitates with PSD-95 from WT hippocampi, while much less mTau, but no Δtau (HT7), is recovered from Δtau74 hippocampi. mTau is absent in *tau*$^{-/-}$ coIPs, consistent with *tau* deficiency. (n = 3, *p < 0.005.)

(C) Sequential extraction of synaptosomes. Purified WT synaptosomes were further fractionated with buffers of increasing stringency (pH6 < pH8 < SDS), to purify proteins that are stably associated with the PSD (Phillips et al., 2001). Brain extracts (total) are loaded for comparison. NR subunits NR1, NR2a and NR2b, PSD-95, tau, and Fyn are purified in the SDS fraction, consistent with strong anchoring in the PSD. Soluble proteins, such as GAP43 and proteins that are not (such as SNAP25) or less stably associated with the PSD, are extracted with less stringent pH 6 and pH 8 buffers, respectively.

(D) SDS fractions from synaptosomes show that stable anchoring of NRs in the PSD is reduced in Δtau74 and *tau*$^{-/-}$ mice. While NRs are recovered in the SDS fraction of WT synaptosomes, they are primarily found in the pH 8 and hardly at all in SDS fractions from Δtau74 and *tau*$^{-/-}$ mice.

(E) Representative traces of AMPAR- (gray) and NR- (black) mediated components of electrically evoked (e) EPSCs in CA1 hippocampal neurons from WT, Δtau74, *tau*$^{-/-}$, and Δtau74.*tau*$^{-/-}$ mice (average of 12 sweeps per neuron) normalized with AMPAR-mediated component. Neurons were voltage clamped and held at +40 mV. AMPAR-mediated eEPSCs are inverted for clarity. There is no significant difference of NMDA/AMPA ratios between genotypes (n = 19–20).

(F) Representative traces of eEPSCs (average of 12 sweeps per neuron) separating total NMDAR-mediated and NR2b subunit-mediated components (black traces, total NR eEPSC minus component in CP-101,606 [5 μM]) normalized to the amplitude of the total NR eEPSC. Neurons were voltage clamped at +20 mV. NR2b EPSCs were obtained by subtraction of EPSCs generated in CP101 606 (5 μM) from total NR-mediated EPSCs, i.e., before CP applications. There is no significant difference in the percentage of NR2b component between genotypes (n = 18–20).

(G and H) Mean amplitude (G) and rate (s^{-1}) (H) of AMPAR-mediated mEPSCs (recorded in 1 μM TTX) were unaffected in Δtau74, *tau*$^{-/-}$, and Δtau74.*tau*$^{-/-}$ mice (n = 10–12).

Error bars represent the standard error. See also Figure S3.

from Δtau74 and *tau*$^{-/-}$ compared to WT extracts, consistent with a decreased interaction of NR and PSD-95 in both strains (Figure 3B). The PSD-95-interacting proteins Homer and nNOS, however, were coimmunoprecipitated to a similar extent from WT, Δtau74, and *tau*$^{-/-}$ brains, suggesting intact interactions. The NR/PSD-95 interaction facilitates stable anchoring of NRs in the postsynaptic density (PSD) (Roche et al., 2001). Therefore, we next extracted purified synaptosomes from WT, Δtau74, and *tau*$^{-/-}$ mice that show similar levels of NR subunits, but reduced NR2b phosphorylation at Y1472 (Figure S3A), using buffers of increasing stringency (Phillips et al., 2001). In line with a strong PSD association in WT synaptosomes, NR subunits were mostly found in the SDS fraction (Figure 3C and 3D). In contrast, they were markedly reduced in Δtau74 and *tau*$^{-/-}$, appearing instead in earlier fractions, suggestive of a weakened anchoring in the PSD (Figure 3D). Interestingly, endogenous tau that was enriched by synaptosome preparation, recovered in WT SDS fractions and coimmunoprecipitated with PSD-95 from WT, and to a lesser degree from Δtau74, brains (Figures 3B and 3C and Figure S1C).

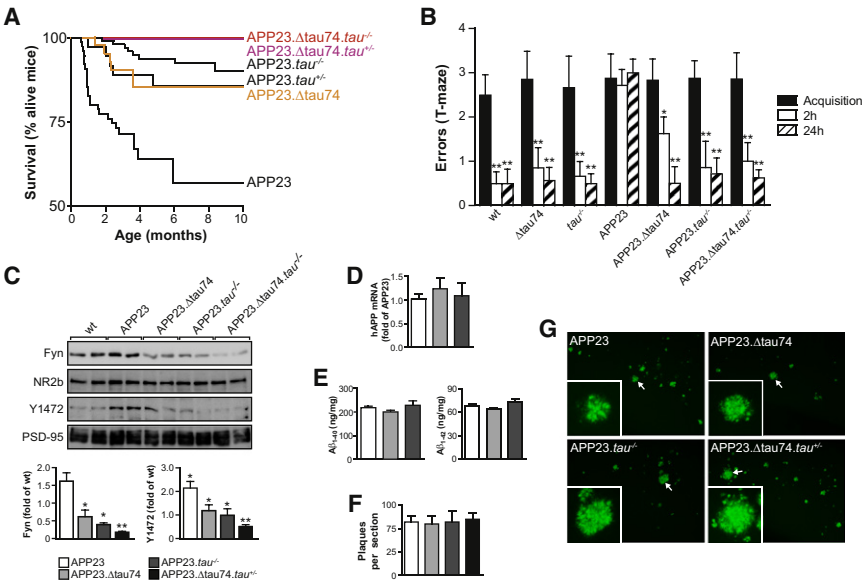

Figure 4. Δtau Expression Improves Memory and Ameliorates Premature Mortality of APP23 Mice

(A) APP^{swe} transgenic APP23 mice (n = 76) present with a pronounced premature mortality that is ameliorated by reducing tau levels in APP23.*tau*$^{+/-}$ (n = 41, p < 0.001) and even more in APP23.*tau*$^{-/-}$ mice (n = 108, p < 0.0001). Expression of Δtau improves the survival of APP23.Δtau74 mice (orange, n = 43, p < 0.01) similar to APP23.*tau*$^{+/-}$. Interestingly, combination of Δtau expression with tau reduction completely rescues APP23.Δtau74.-*tau*$^{+/-}$ (purple, n = 38, p < 0.0001) and APP23.Δtau74.*tau*$^{-/-}$ (red, n = 52, p < 0.0001) mice from lethality.

(B) Improved memory acquisition of APP23.Δtau74, APP23.*tau*$^{-/-}$, and APP23.Δtau74.*tau*$^{-/-}$ compared to APP23 mice in the T maze, 2 and 24 hr after a five-trial acquisition, at 8 months of age. While WT, Δtau74, and *tau*$^{-/-}$ mice only make few errors during the trials, memory deficits of APP23 mice are obvious from the continuously high numbers of errors made during the entire test. In contrast, both APP23.Δtau74, APP23.*tau*$^{-/-}$, and APP23.Δtau74.*tau*$^{-/-}$ mice presented with WT-like numbers of errors (n = 8, *p < 0.05, **p < 0.01).

(C) In synaptosomal preparations obtained from 4-month-old APP23, both Fyn levels and NR2b phosphorylation at Y1472 are increased as compared to wild-type (wt) mice (n = 6, *p < 0.05). However, in synaptosomes from APP23.Δtau74 and APP23.*tau*$^{-/-}$, and even more in APP23.Δtau74.*tau*$^{-/-}$, both levels of Fyn and NR2b phosphorylation are significantly lower than in APP23 mice (n = 6, *p < 0.05, **p < 0.01). Representative western blots from three independent experiments are shown.

(D–G) Δtau expression and *tau* deficiency do not affect APP mRNA expression, Aβ levels, or plaque burden.

(D) Levels of APP mRNA are not altered in APP23 mice in the presence of Δtau or when tau is absent (*tau*$^{-/-}$).

(E) $A\beta_{1-40}$ and $A\beta_{1-42}$ levels are comparable in APP23, APP23.Δtau74, and APP23.*tau*$^{-/-}$ mice.

(F and G) Thioflavine S staining (green) reveals Aβ plaques (arrows; insets) at similar numbers (F) and with similar morphology (G) in APP23 mice, independent of coexpression of Δtau or tau reduction.

Error bars represent the standard error. See also Figure S4.

The organization of NRs within the PSD is important for coordinated signal transduction (Kim and Sheng, 2004). Hence, alterations of NRs in Δtau74 and *tau*$^{-/-}$ mice may affect synaptic currents. Therefore, we determined excitatory postsynaptic currents (ESPCs) in acute hippocampal slices from WT, Δtau74, *tau*$^{-/-}$, and Δtau74.*tau*$^{-/-}$ mice. In Δtau74, *tau*$^{-/-}$, and Δtau74.*tau*$^{-/-}$ mice, we found np significant changes in synaptic currents (Figure 3E). Similarly, no significant reduction emerged in the contribution of NR2b-containing NRs to ESPCs in Δtau74, *tau*$^{-/-}$, and Δtau74.*tau*$^{-/-}$ mice (Figure 3F). Baseline miniature amplitudes and frequency were also comparable (Figures 3G and 3H and Figures S3B–S3D). Taken together, these data indicate that both expression of Δtau or *tau* deficiency reduces the interaction of NRs with PSD-95 without affecting synaptic NR levels and currents.

Δtau Expression Prevents Premature Lethality and Memory Deficits in APP23 Mice

It has been shown previously that perturbing the interaction of NRs with PSD-95 had no effect on NR-mediated currents but reduced the resilience of neurons to NMDA-mediated excitotoxicity (Aarts et al., 2002). Interestingly, excitotoxicity has been proposed to contribute to Aβ toxicity in PDAPP mice, which was reduced when the mice were crossed onto a *tau*$^{-/-}$ background (Roberson et al., 2007). APP expression per se may contribute to toxicity in mice; however, primary disease-related effects are attributed to Aβ, as suggested by reverted deficits in Aβ-immunized APP models (Röskam et al., 2010) and absence of seizure-induced hippocampal remodeling in APP transgenic mice with low Aβ levels (Palop et al., 2007). Excitotoxicity has been linked to premature lethality in APP transgenic mice (Chishti et al., 2001; El Khoury et al., 2007; Leissring et al., 2003; Roberson et al., 2007). Hence, we speculated that in Δtau74 alterations in NR/PSD-95 interaction might similarly rescue the early lethality that characterizes APP^{swe} mutant APP23 mice (Figures S4A and S4B) (Sturchler-Pierrat et al., 1997). APP23 mice have high Aβ levels already at a very young age (Kuo et al., 2001; Van Dam et al., 2003), eventually forming plaques and presenting with neuronal loss and memory deficits (Calhoun et al., 1998; Kelly et al., 2003; Sturchler-Pierrat et al., 1997). When we crossed APP23 either with Δtau74 or *tau*$^{-/-}$ mice (Figure S4C), this caused both a significantly delayed onset of mortality and an improved overall survival (Figure 4A). Whereas any rescue (either on a *tau*$^{-/-}$ background or by expressing Δtau) was partial, expression of Δtau on a heterozygous or homozygous *tau*-deficient background rescued lethality completely, suggesting complementary beneficial effects of *tau* deficiency and Δtau expression on survival (Figure 4A). In contrast, crossing of APP23 mice with pR5 mice with an increased dendritic accumulation of tau (Figure 1D) (Götz et al.,

2001a), resulted in increased premature lethality, with no survival beyond 4 months of age (Figure S4D). Interestingly, both Fyn levels and Y1472 phosphorylation of NR2b are increased in pR5 synaptosomes (Figure S4E). Because of possible confounding effects of APP overexpression and Aβ formation in APP mutant mouse strains, we used also primary neurons treated with Aβ, in the absence of APP overexpression, as a model. In *tau*$^{-/-}$ and Δtau74-expressing neurons, acute Aβ toxicity was markedly reduced (Figure S2E). Interestingly, deletion of the Fyn-interacting motif, PXXP (Lee et al., 1998), from Δtau abrogated the protective effect.

We next determined whether Δtau expression or tau reduction also improves memory functions in APP23 mice. Memory deficits were both improved to WT levels in APP23.Δtau74 and APP23.*tau*$^{-/-}$ mice using the water T maze (Figure 4B). Consistent with the findings in Δtau and *tau*$^{-/-}$ mice, both synaptic Fyn levels and NR2b phosphorylation were reduced in APP23.Δtau74 and APP23.*tau*$^{-/-}$ and even more so in APP23.Δtau74.*tau*$^{-/-}$ synaptosomes, while they were increased in APP23 compared to WT brains (Figure 4C). Interestingly, in APP23 mice, neither Δtau expression nor *tau* reduction affected human *APP* messenger RNA (mRNA) levels (Figure 4D), Aβ levels (Figure 4E), or plaque burden (Figures 4F and 4G). Similarly, phosphorylation of endogenous tau was comparable in APP23 and APP23.Δtau74 mice (data not shown). Taken together, expression of Δtau in APP23 or crossing of APP23 with *tau*$^{-/-}$ mice reduces Fyn-mediated NR2b phosphorylation, attenuates premature mortality, and improves memory deficits without changing Aβ levels or plaque load.

Δtau Reduces Susceptibility to Excitotoxic Seizures

Aβ-induced aberrant excitatory neuronal activity may contribute to the deficits that characterize AD mouse models (Busche et al., 2008; Palop and Mucke, 2009). APP23 mice show spontaneous seizures (Lalonde et al., 2005), similar to other APP transgenic strains (Minkeviciene et al., 2009; Palop et al., 2007; Palop and Mucke, 2009). Hence, reduced mortality of APP23.Δtau74 and APP.*tau*$^{-/-}$ mice may be related to a reduced susceptibility to excitotoxic seizures. We therefore first induced convulsions in Δtau74, *tau*$^{-/-}$, Δtau74.*tau*$^{-/-}$, and WT mice using the γ-aminobutyrate (GABA) antagonist pentylenetetrazole (PTZ). Seizure severity was significantly reduced in Δtau74, *tau*$^{-/-}$, and Δtau74.*tau*$^{-/-}$ compared to the WT (Figure 5A), while the latency to develop severe convulsion increased (Figure 5B). Next, we induced seizures in APP23, APP23.Δtau74, APP23.*tau*$^{-/-}$, and APP23.Δtau74.*tau*$^{-/-}$ mice. APP23 mice presented with a reduced convulsion latency and showed the most severe seizure response, with the lowest survival rate (1/11) and all mice reaching *status epilepticus* (n = 11) (Figure 5C). However, when APP expression was combined with Δtau expression or *tau* deficiency, this significantly decreased seizure severity, reduced fatality, and increased convulsion latency (Figures 5C and 5D). The double mutant Δtau74.*tau*$^{-/-}$ prevented severe seizures better than Δtau74 or *tau*$^{-/-}$ alone, on both WT and APP23 backgrounds, in agreement with the survival data (Figure 4A). Interestingly, we found a similar degree of protection from PTZ-induced seizures as in Δtau74, *tau*$^{-/-}$, APP23.Δtau74, or APP23.tau$^{-/-}$ mice when we pretreated WT and APP23 mice,

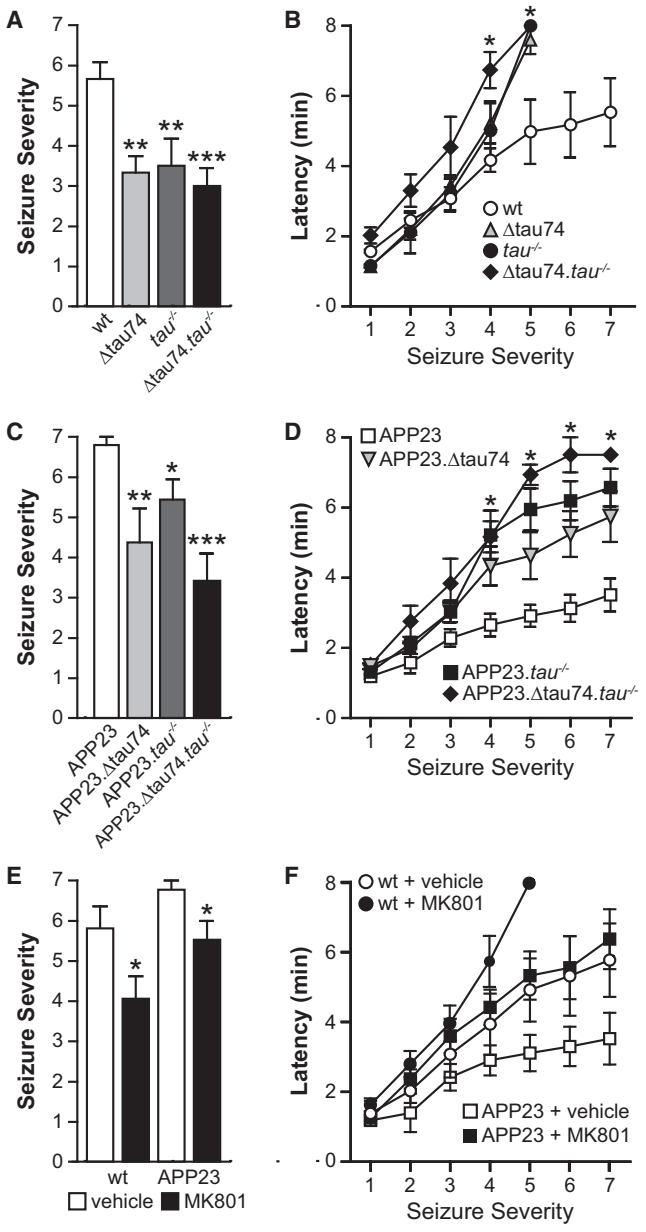

Figure 5. Δtau Expression Reduces Susceptibility to Excitotoxic Seizures

(A) When excitotoxic seizures were induced by i.p. injection of PTZ (50 mg/kg), mean seizure severity was significantly reduced in both Δtau74, *tau*$^{-/-}$, and Δtau74.*tau*$^{-/-}$ compared to WT mice (n = 10, **p < 0.01, ***p < 0.001).

(B) Similarly, the latency to more severe seizure stages is increased in Δtau74, *tau*$^{-/-}$, and Δtau74.*tau*$^{-/-}$ mice.

(C) In APP23 mice, PTZ-induced seizures are mostly lethal (10 of 11), whereas in APP23.Δtau74, APP23.*tau*$^{-/-}$, and APP23.Δtau74.*tau*$^{-/-}$ seizure severity is markedly reduced (n = 10, *p < 0.05, **p < 0.01, ***p < 0.001).

(D) APP23.Δtau74, APP23.*tau*$^{-/-}$, and APP23.Δtau74.*tau*$^{-/-}$ mice show an increased latency to more severe seizures compared to APP23 mice.

(E and F) Pretreatment of WT or APP23 mice with MK801 (0.1 mg/kg) reduced seizure severity (n = 8, p < 0.05) (E) and increased latency to more severe seizures (F).

Error bars represent the standard error.

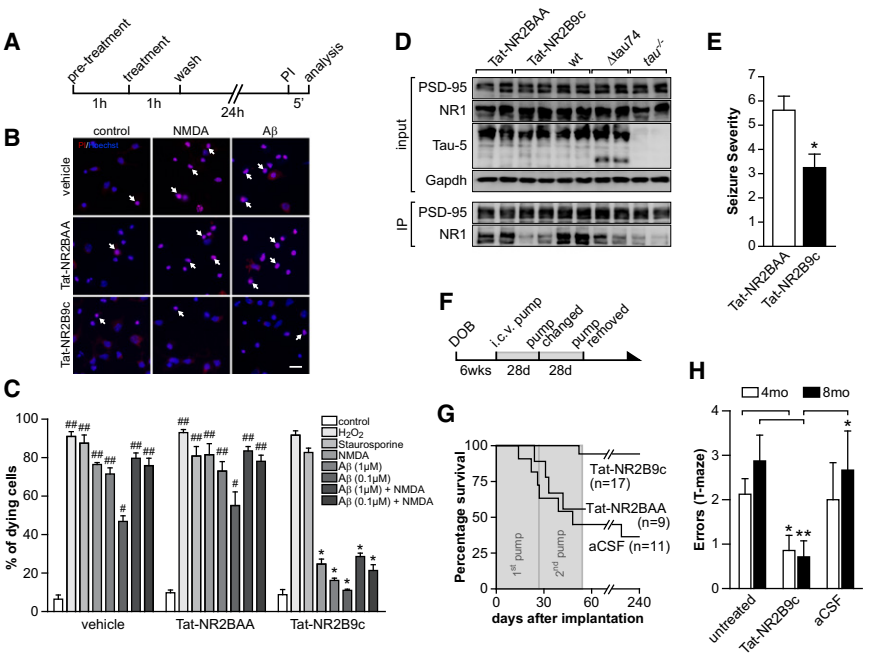

Figure 6. Peptide-Driven Uncoupling of the NR/PSD-95 Interaction Reduces Aβ Toxicity and Improves Survival and Memory of APP23 Mice

(A) Twenty-day-old primary cortical neurons were pretreated with 100 nM Tat-NR2B9c peptide, which disrupts the NR/PSD-95 interaction (Aarts et al., 2002), prior to treatment with the toxins NMDA, Aβ, H_2O_2, and staurosporine. Twenty-four hours after treatment, cell death was determined by propidium iodide (PI) uptake. Control cells were pretreated with vehicle or 100 nM Tat-NR2BAA (inactive peptide).

(B and C) Tat-NR2B9c (bottom row) significantly reduces toxicity of NMDA and Aβ to cortical neurons, as indicated by lower numbers of PI-positive cells (red; arrows) compared to pretreated vehicle (top row) or Tat-NR2BAA (middle row) controls. Nuclei were stained with Hoechst (blue). Treatment with H_2O_2, staurosporine, NMDA, Aβ, or NMDA/Aβ causes significant cell death (#p < 0.005, ##p < 0.0001), which for NMDA- and Aβ-treated neurons is reduced by pretreatment with Tat-NR2B9c, but not for H_2O_2- and staurosporine-treated neurons (*p < 0.05, **p < 0.01). One hundred cells each were counted in three independent experiments. The scale bar represents 25 μm.

(D) Immunoprecipitation (IP) with a PSD-95 antibody from hippocampus of WT mice that were i.c.v. infused with Tat-NR2B9c and Tat-NR2BAA for 1 week, and from untreated WT, Δtau74, and *tau*$^{-/-}$ mice. Less NRs were coimmunoprecipitated upon Tat-NR2B9c, but not Tat-NR2BAA treatment. The reduction was comparable to Δtau74 and *tau*$^{-/-}$ mice.

(E) One week of i.c.v. infusion of Tat-NR2B9c reduced PTZ-induced seizure severity significantly, compared to inactive Tat-NR2BAA (n = 10, *p < 0.01).

(F) APP23 mice treated with vehicle (artificial cerebrospinal fluid [aCSF]) alone or together with Tat-NR2B9c or Tat-NR2BAA, using osmotic mini pumps. DOB, date of birth.

(G) Survival of APP23 mice upon i.c.v. delivery of Tat-NR2B9c (n = 17) is markedly improved compared to vehicle (aCSF)-treated (n = 11, p < 0.01) and Tat-NR2BAA-treated (n = 9, p < 0.05) controls. Gray boxes indicate time of drug delivery from two consecutively implanted pumps.

(H) Tat-NR2B9c-treated APP23 mice show markedly improved memory functions at 4 and 8 months after initiating treatment, compared to aCSF-treated or age-matched untreated APP23 mice (n = 8, n = 4 for aCSF, *p < 0.05, **p < 0.01).

Error bars represent the standard error.

respectively, with the NR-antagonist MK801 (Figures 5E and 5F). Hence, reduced susceptibility to excitotoxicity is consistent with a reduced NR contribution and may contribute to reduced mortality in APP23 mice in the presence of Δtau or absence of endogenous tau.

Targeted Uncoupling of NR and PSD-95 Prevents Premature Death and Memory Seficits in APP23 Mice

Provided that disturbed NR/PSD-95 complexes with a reduced dendritic Fyn localization in Δtau74 and *tau*$^{-/-}$ mice contribute to improved memory functions and survival of APP23.Δtau74 and APP23.*tau*$^{-/-}$ mice, targeted perturbation of the NR/PSD-95 interaction, independent of tau or Fyn, should also decrease Aβ toxicity. Therefore, we treated primary cortical cultures with the Tat-NR2B9c peptide composed of carboxy-terminal amino acids of NR2b (including Y1472) fused to a HIV1-Tat peptide to achieve cell membrane permeability (Figure 6A). Tat-NR2B9c has been shown previously to protect from NMDA-induced excitotoxicity (Aarts et al., 2002; Kornau et al., 1995). As a negative control, we included Tat-NR2BAA in which critical amino acids were replaced by alanine (Aarts et al., 2002; Kornau et al., 1995). NMDA and Aβ both induced pronounced cell death, while a combined NMDA/Aβ treatment did not further increase cell death, consistent with shared signaling pathways mediating their toxicity (Figures 6B and 6C). Cell death induced by NMDA and Aβ, both separate and in combination, was significantly reduced by preincubation with Tat-NR2B9c, but not when induced by hydrogen peroxide or staurosporine (Figures 6B and 6C). Tat-NR2BAA had no protective effects. Hence, perturbing the NR/PSD-95 interaction with Tat-NR2B9c ameliorates Aβ-mediated toxicity in vitro.

Next, we tested in vivo whether APP23 mice would also benefit from treatment with Tat-NR2B9c. A single dose of this peptide has previously been shown to confer virtually complete protection from excitotoxic damage in a rat model of stroke (Aarts et al., 2002). First, we determined whether sufficient NR/PSD-95 uncoupling was achieved by intracerebroventricular (i.c.v.) Tat-NR2B9c treatment, using osmotic minipumps. We delivered either Tat-NR2B9c or Tat-NR2BAA for 1 week and then performed coIP with a PSD-95 antibody (Figure 6D). This revealed a reduced NR/PSD-95 interaction upon Tat-NR2B9c, but not Tat-NR2BAA, treatment. The level of reduction was similar to that found in Δtau74 and *tau*$^{-/-}$ brains (Figure 6D). Sufficient uptake of peptides by the brain was further confirmed by protection from PTZ-induced seizures by Tat-NR2b9c, but not Tat-NR2BAA (Figure 6E). Next, we implanted minipumps

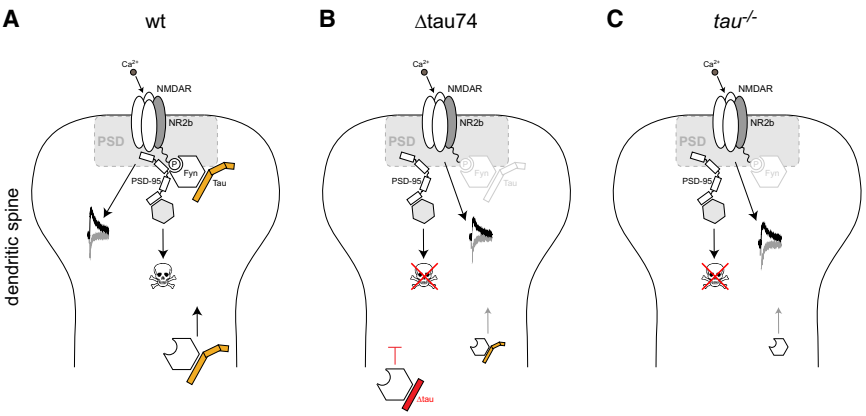

Figure 7. Simplified Scheme of the Proposed Mechanism Underlying Reduced Excitotoxicity in Δtau74 and *tau*$^{-/-}$ Mice Compared to the Wild-Type

(A) Postsynaptic NMDA receptors (NRs) are heteromeric complexes predominantly formed by subunits NR1, NR2A, and NR2B. The Src kinase Fyn localizes to the postsynapse in a tau-dependent manner and associates with the postsynaptic density (PSD; gray box), where it phosphorylates (P) the NR subunit NR2b at Y1472 in the extreme carboxy terminus. This phosphorylation facilitates the interaction of NRs with the scaffolding protein PSD-95. This interaction increases the stability of NRs within the PSD and couples NRs to excitotoxic downstream signaling (skull). NR-mediated currents (ESPC trace), however, do not depend on this NR/PSD-95 interaction. Whether tau is associated with the PSD via Fyn or another interaction partner remains to be elucidated.

(B) In Δtau74 mice, Δtau is excluded from entering dendrites. Since Fyn interacts with Δtau (red bar) in the cell body of neurons, it is therefore trapped and less localized to dendrites. Also, phosphorylation of NR2b and the interaction of NRs and PSD-95 are markedly reduced. Hence, excitotoxic downstream signaling is uncoupled from NRs and their stability within the PSD is reduced. As NR-mediated currents are not dependent on this interaction, they are not affected.

(C) As for Δtau74 mice, in *tau*$^{-/-}$ mice, tau-dependent localization of Fyn to the postsynapse is also markedly reduced. NR2b phosphorylation and the interaction of NRs and PSD-95 are decreased. Thus, excitotoxic downstream signaling is uncoupled from NRs, and their stability within the PSD is reduced. Again, NR-mediated currents are not affected.

into 6-week-old APP23 mice for i.c.v. delivery of artificial cerebrospinal fluid (aCSF), with and without Tat-NR2B9c or Tat-NR2BAA (Figure 6F). Mice in the aCSF and Tat-NR2BAA control groups died frequently (7 of 11 and 4 of 9, respectively), whereas only 1 of 17 mice died in the Tat-NR2B9c group (Figure 6E). Finally, we tested whether Tat-NR2B9c-treatment has long-term effects on memory in APP23 mice. The T maze revealed comparable memory deficits in age-matched aCSF-treated and untreated APP23 mice (Figure 6F). However, treatment with Tat-NR2B9c resulted in a significantly improved performance. Thus, perturbing NR/PSD-95 interaction is sufficient to prevent premature lethality and memory deficits in APP23 mice.

DISCUSSION

Dendritic Localization of Fyn Is Tau-Dependent

Our data reveal a dendritic function of the "axonal" protein tau, in targeting the kinase Fyn to the dendrite (Figure 7). We also found an association of tau with the PSD complex by using coIP, PSD purification, and immunohistochemistry with enhanced antigen retrieval. It is important to note that levels of tau in the dendritic compartment are much lower than in axons, suggesting that under physiological conditions a major function of tau is in axonal MT stabilization and regulation of MT-dependent processes (Dixit et al., 2008; Weingarten et al., 1975). Here, we show that the additional role of tau in dendrites becomes pivotal in disease, in particular in mediating early Aβ toxicity.

In both Δtau74 and *tau*$^{-/-}$ mice, dendritic targeting of Fyn is significantly reduced, as revealed by immunohistochemistry and synaptosomal purification and confirmed in primary neurons. In Δtau74 mice, this is due to a competition of Δtau with endogenous tau in the interaction with Fyn. Both the abundance of Δtau in the cell body and its exclusion from dendrites result in "trapping" of Fyn in the soma. *Tau*$^{-/-}$ mice, in comparison, show a similar accumulation of Fyn, suggesting that postsynaptic Fyn targeting requires tau. This difference in mediating aberrant sorting of Fyn between Δtau74 and *tau*$^{-/-}$ mice (Figure 7) may explain the additive effects on seizure susceptibility and survival in Δtau.*tau*$^{-/-}$ crosses. Reduced levels of postsynaptic Fyn in Δtau74 and *tau*$^{-/-}$ mice are associated with reduced phosphorylation of the Fyn-substrate NR2b at Y1472. Consistent with a critical role of Y1472 phosphorylation in facilitating the interaction of NRs with PSD-95 (Rong et al., 2001), this complex is reduced and destabilized in Δtau74 and *tau*$^{-/-}$ brains. Whether the Fyn-mediated stabilization of NR/PSD-95 complexes in the PSD under physiological conditions involves a direct interaction with tau and what the exact mechanism(s) of tau-mediated dendritic Fyn localization are remains to be established.

In a rat model of stroke, targeted disruption of the NR/PSD-95 interaction prevented excitotoxic damage and reduced the lesion size (Aarts et al., 2002). Consistent with this, reduced NR/PSD-95 complexes in Δtau74 and *tau*$^{-/-}$ mice were associated with a reduced susceptibility to excitotoxicity. Interestingly, NR-mediated currents were not affected in Δtau74 and *tau*$^{-/-}$ mice, which is in line with normal synaptic activity upon treatment with Tat-NR2B9c (Aarts et al., 2002). Normal NR-mediated currents in Δtau74 and *tau*$^{-/-}$ mice may be explained by reduced, but not totally depleted, synaptic Fyn in Δtau74 and *tau*$^{-/-}$ mice, comparable to the situation in heterozygous *fyn*-deficient mice that have no overt deficits (Yagi et al., 1993), while in homozygous *fyn*-deficient mice these are pronounced (Grant et al., 1992).

Δtau and *tau*$^{-/-}$ Prevent Deficits of APP23 Mice

Excitotoxicity is increasingly recognized as a mechanism of how Aβ exerts toxicity in AD. Accordingly, we found that crossing

of Δtau74 and *tau*$^{-/-}$ mice, both characterized by reduced susceptibility to excitotoxicity, with Aβ-forming APP23 mice ameliorated premature mortality and memory deficits of APP23 mice. In contrast, early lethality was more pronounced in APP23 mice crossed with pR5. Similarly, *tau* deficiency or Δtau expression conferred protection from Aβ-induced toxicity in primary neuronal cultures. However, Aβ levels and plaque formation, as well as endogenous tau phosphorylation (in APP23.Δtau74), were comparable in APP23 mice, suggesting an alternative mechanism for protection. Interestingly, in APP23 mice we found both increased postsynaptic Fyn and Y1472 phosphorylation of NR2b that was completely reverted in APP23.Δtau74 and APP23.*tau*$^{-/-}$ mice. Further reduction of post-synaptic Fyn in APP23.Δtau74.*tau*$^{-/-}$ mice suggests additional tau-independent mechanisms in dendritic Fyn localization, which are partially competed with by Δtau. Consistent with a role for Fyn in Aβ pathology, Fyn transgenic mice present with seizures and premature mortality (Kojima et al., 1998). This is exacerbated in Fyn/APP^{mut} double-transgenic mice (Chin et al., 2004). Moreover, APP-associated mortality is reduced on a *fyn*$^{-/-}$ background (Chin et al., 2004). Hence, our findings in APP23.Δtau74 and APP23.*tau*$^{-/-}$ mice are consistent with previous data (Chin et al., 2005; Chin et al., 2004). Furthermore, they are in line with the recent observation that crossing of PDAPP mice onto a *tau*$^{-/-}$ background reverses Aβ-associated defects (Roberson et al., 2007).

Mechanistically, our data suggest that stable NR/PSD-95 complex formation is required for Aβ toxicity in APP23 mice. This is likely to contribute to disease together with other tau-dependent and -independent mechanisms of Aβ toxicity. In support of our findings, we used a tau/Fyn-independent approach to disrupt this interaction, by delivering the Tat-NR2B9c peptide to young APP23 mice. This peptide has been shown to protect from excitotoxicity in vitro and in vivo. We show specifically that perturbing the NR/PSD-95 interaction with the Tat-NR2B9c peptide improves survival and memory functions of APP23 mice. The data suggest that disruption of the NR/PSD-95 interaction is sufficient to prevent Aβ toxicity involving NR signaling. Remarkably, Tat-NR2B9c-treated APP23 mice survived long term, suggesting that treatment within a short therapeutic window is sufficient to prevent lethality.

In summary, we reveal a dendritic role for the "axonal" protein tau in postsynaptic targeting of Fyn. This involves interaction of Fyn with the tau projection domain (Lee et al., 1998). Accordingly, dominant negative effects of Δtau expression or *tau* deficiency result in reduced postsynaptic Fyn, decreased phosphorylation of its substrate NR2b and instability of NR/PSD-95 complexes (in Δtau74 and *tau*$^{-/-}$ mice). Importantly, this additional function of tau appears to be pivotal for mediating Aβ toxicity, in that premature lethality, memory deficits, and seizure susceptibility of APP23 mice were mitigated in APP23.Δtau74 and APP23.*tau*$^{-/-}$ mice. Hence, reduction of tau levels or targeting of tau-dependent mechanisms, such as the Fyn-mediated interaction of NRs and PSD-95, are suitable strategies in the treatment of AD and related disorders, highlighting tau as an attractive drug target, in addition to Aβ (Ashe, 2007).

EXPERIMENTAL PROCEDURES

Animals

APP23 and pR5 transgenic and *tau*$^{-/-}$ mice have been generated previously (Götz et al., 2001a; Sturchler-Pierrat et al., 1997; Tucker et al., 2001). The generation of Δtau74 mice is described in the Extended Experimental Procedures. Two- to three-month-old mice were analyzed in age- and sex-matched groups, unless stated otherwise. All animal experiments were approved by the Animal Ethics Committee of the University of Sydney.

Histology, Western Blotting, IP, and Synaptosome Preparation

Detailed protocols are provided in the Extended Experimental Procedures.

Electrophysiology

Electrophysiological recording were done in acute hippocampal slices obtained from 4- to 8-week-old wild-type, Δtau74, *tau*$^{-/-}$, and Δtau74.*tau*$^{-/-}$ mice as described in detail in the Extended Experimental Procedures.

Experimental Seizures

Seizures were induced by intraperitoneal (i.p.) injection of (50 mg/kg body weight) pentylenetetrazole (PTZ; Sigma) as described (Roberson et al., 2007). Where indicated, mice were injected i.p. with (0.1 mg/kg body weight) MK801 (Sigma) 30 min prior to PTZ administration. Mice were video monitored, and seizure severity was rated by an independent, blinded person, as follows: 0, no seizures; 1, immobility; 2, tail extension; 3, forelimb clonus; 4, generalized clonus; 5, bouncing seizures; 6, full extension; and 7, death.

i.c.v. Treatment with Osmotic Pumps

Six-week-old APP23 and WT mice were anesthetised with ketamine/xylazine, and i.c.v. delivery cannulas (Alzet; brain infusion kit #3 with one spacer) were implanted with a stereotaxic frame (KOPF Instruments) at the following coordinates according to the bregma: AP, −0.25 mm; ML, 1 mm; and DV, −2.5 mm. Osmotic mini pumps (Alzet; model #1004) were filled with aCSF (Alzet) with and without Tat-NR2B9c or Tat-NR2BAA peptide (750 μM) and equilibrated in 0.9% NaCl at 37°C for 48 hr. They were attached to the i.c.v. cannula tubing and subcutaneously implanted at the back. After 28 days, the pumps were replaced with a second batch of pumps via a small skin incision for another 28 days. Then, they were removed and the tubing was ligated.

Statistics

Statistics was done with the Prizm 4 software (GraphPad) with Student's t or two-way ANOVA test. Values are given as mean ± standard error.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures and four figures and can be found with this article online at doi:10.1016/j.cell.2010.06.036.

ACKNOWLEDGMENTS

The authors thank Yves-Alain Barde for providing *tau*$^{-/-}$ mice and him as well as Hanns Möhler and Nikolas Haass for helpful comments. This research was supported by grants from the University of Sydney, National Health and Medical Research Council, Australian Research Council, and Deutsche Forschungsgemeinschaft. J.G. is a Medical Foundation Fellow.

Received: December 14, 2009
Revised: April 6, 2010
Accepted: May 28, 2010
Published online: July 22, 2010

REFERENCES

Aarts, M., Liu, Y., Liu, L., Besshoh, S., Arundine, M., Gurd, J.W., Wang, Y.T., Salter, M.W., and Tymianski, M. (2002). Treatment of ischemic brain damage

by perturbing NMDA receptor- PSD-95 protein interactions. Science *298*, 846–850.

Ashe, K.H. (2007). A tale about tau. N. Engl. J. Med. *357*, 933–935.

Ballatore, C., Lee, V.M., and Trojanowski, J.Q. (2007). Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat. Rev. Neurosci. *8*, 663–672.

Busche, M.A., Eichhoff, G., Adelsberger, H., Abramowski, D., Wiederhold, K.H., Haass, C., Staufenbiel, M., Konnerth, A., and Garaschuk, O. (2008). Clusters of hyperactive neurons near amyloid plaques in a mouse model of Alzheimer's disease. Science *321*, 1686–1689.

Butner, K.A., and Kirschner, M.W. (1991). Tau protein binds to microtubules through a flexible array of distributed weak sites. J. Cell Biol. *115*, 717–730.

Calhoun, M.E., Wiederhold, K.H., Abramowski, D., Phinney, A.L., Probst, A., Sturchler-Pierrat, C., Staufenbiel, M., Sommer, B., and Jucker, M. (1998). Neuron loss in APP transgenic mice. Nature *395*, 755–756.

Chin, J., Palop, J.J., Yu, G.Q., Kojima, N., Masliah, E., and Mucke, L. (2004). Fyn kinase modulates synaptotoxicity, but not aberrant sprouting, in human amyloid precursor protein transgenic mice. J. Neurosci. *24*, 4692–4697.

Chin, J., Palop, J.J., Puoliväli, J., Massaro, C., Bien-Ly, N., Gerstein, H., Scearce-Levie, K., Masliah, E., and Mucke, L. (2005). Fyn kinase induces synaptic and cognitive impairments in a transgenic mouse model of Alzheimer's disease. J. Neurosci. *25*, 9694–9703.

Chishti, M.A., Yang, D.S., Janus, C., Phinney, A.L., Horne, P., Pearson, J., Strome, R., Zuker, N., Loukides, J., French, J., et al. (2001). Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. J. Biol. Chem. *276*, 21562–21570.

Crowther, T., Goedert, M., and Wischik, C.M. (1989). The repeat region of microtubule-associated protein tau forms part of the core of the paired helical filament of Alzheimer's disease. Ann. Med. *21*, 127–132.

Dixit, R., Ross, J.L., Goldman, Y.E., and Holzbaur, E.L. (2008). Differential regulation of dynein and kinesin motor proteins by tau. Science *319*, 1086–1089.

Drechsel, D.N., Hyman, A.A., Cobb, M.H., and Kirschner, M.W. (1992). Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. Mol. Biol. Cell *3*, 1141–1154.

El Khoury, J., Toft, M., Hickman, S.E., Means, T.K., Terada, K., Geula, C., and Luster, A.D. (2007). Ccr2 deficiency impairs microglial accumulation and accelerates progression of Alzheimer-like disease. Nat. Med. *13*, 432–438.

Geschwind, D.H. (2003). Tau phosphorylation, tangles, and neurodegeneration: the chicken or the egg? Neuron *40*, 457–460.

Goedert, M., Wischik, C.M., Crowther, R.A., Walker, J.E., and Klug, A. (1988). Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau. Proc. Natl. Acad. Sci. USA *85*, 4051–4055.

Götz, J., and Ittner, L.M. (2008). Animal models of Alzheimer's disease and frontotemporal dementia. Nat. Rev. Neurosci. *9*, 532–544.

Götz, J., Chen, F., Barmettler, R., and Nitsch, R.M. (2001a). Tau filament formation in transgenic mice expressing P301L tau. J. Biol. Chem. *276*, 529–534.

Götz, J., Chen, F., van Dorpe, J., and Nitsch, R.M. (2001b). Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. Science *293*, 1491–1495.

Grant, S.G., O'Dell, T.J., Karl, K.A., Stein, P.L., Soriano, P., and Kandel, E.R. (1992). Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. Science *258*, 1903–1910.

Haass, C., Schlossmacher, M.G., Hung, A.Y., Vigo-Pelfrey, C., Mellon, A., Ostaszewski, B.L., Lieberburg, I., Koo, E.H., Schenk, D., Teplow, D.B., et al. (1992). Amyloid beta-peptide is produced by cultured cells during normal metabolism. Nature *359*, 322–325.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., and Cole, G. (1996). Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science *274*, 99–102.

Kelly, P.H., Bondolfi, L., Hunziker, D., Schlecht, H.P., Carver, K., Maguire, E., Abramowski, D., Wiederhold, K.H., Sturchler-Pierrat, C., Jucker, M., et al. (2003). Progressive age-related impairment of cognitive behavior in APP23 transgenic mice. Neurobiol. Aging *24*, 365–378.

Kim, E., and Sheng, M. (2004). PDZ domain proteins of synapses. Nat. Rev. Neurosci. *5*, 771–781.

Kojima, N., Ishibashi, H., Obata, K., and Kandel, E.R. (1998). Higher seizure susceptibility and enhanced tyrosine phosphorylation of N-methyl-D-aspartate receptor subunit 2B in fyn transgenic mice. Learn. Mem. *5*, 429–445.

Kornau, H.C., Schenker, L.T., Kennedy, M.B., and Seeburg, P.H. (1995). Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. Science *269*, 1737–1740.

Ksiezak-Reding, H., and Yen, S.H. (1991). Structural stability of paired helical filaments requires microtubule-binding domains of tau: a model for self-association. Neuron *6*, 717–728.

Kuo, Y.M., Beach, T.G., Sue, L.I., Scott, S., Layne, K.J., Kokjohn, T.A., Kalback, W.M., Luehrs, D.C., Vishnivetskaya, T.A., Abramowski, D., et al. (2001). The evolution of A beta peptide burden in the APP23 transgenic mice: implications for A beta deposition in Alzheimer disease. Mol. Med. *7*, 609–618.

Lalonde, R., Dumont, M., Staufenbiel, M., and Strazielle, C. (2005). Neurobehavioral characterization of APP23 transgenic mice with the SHIRPA primary screen. Behav. Brain Res. *157*, 91–98.

Lee, G., Cowan, N., and Kirschner, M. (1988). The primary structure and heterogeneity of tau protein from mouse brain. Science *239*, 285–288.

Lee, G., Newman, S.T., Gard, D.L., Band, H., and Panchamoorthy, G. (1998). Tau interacts with src-family non-receptor tyrosine kinases. J. Cell Sci. *111*, 3167–3177.

Leissring, M.A., Farris, W., Chang, A.Y., Walsh, D.M., Wu, X., Sun, X., Frosch, M.P., and Selkoe, D.J. (2003). Enhanced proteolysis of beta-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. Neuron *40*, 1087–1093.

Lewis, J., Dickson, D.W., Lin, W.L., Chisholm, L., Corral, A., Jones, G., Yen, S.H., Sahara, N., Skipper, L., Yager, D., et al. (2001). Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science *293*, 1487–1491.

Maas, T., Eidenmüller, J., and Brandt, R. (2000). Interaction of tau with the neural membrane cortex is regulated by phosphorylation at sites that are modified in paired helical filaments. J. Biol. Chem. *275*, 15733–15740.

Minkeviciene, R., Rheims, S., Dobszay, M.B., Zilberter, M., Hartikainen, J., Fülöp, L., Penke, B., Zilberter, Y., Harkany, T., Pitkänen, A., and Tanila, H. (2009). Amyloid beta-induced neuronal hyperexcitability triggers progressive epilepsy. J. Neurosci. *29*, 3453–3462.

Mucke, L., Masliah, E., Yu, G.Q., Mallory, M., Rockenstein, E.M., Tatsuno, G., Hu, K., Kholodenko, D., Johnson-Wood, K., and McConlogue, L. (2000). High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J. Neurosci. *20*, 4050–4058.

Nakazawa, T., Komai, S., Tezuka, T., Hisatsune, C., Umemori, H., Semba, K., Mishina, M., Manabe, T., and Yamamoto, T. (2001). Characterization of Fyn-mediated tyrosine phosphorylation sites on GluR epsilon 2 (NR2B) subunit of the N-methyl-D-aspartate receptor. J. Biol. Chem. *276*, 693–699.

Palop, J.J., and Mucke, L. (2009). Epilepsy and cognitive impairments in Alzheimer disease. Arch. Neurol. *66*, 435–440.

Palop, J.J., Chin, J., Roberson, E.D., Wang, J., Thwin, M.T., Bien-Ly, N., Yoo, J., Ho, K.O., Yu, G.Q., Kreitzer, A., et al. (2007). Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron *55*, 697–711.

Phillips, G.R., Huang, J.K., Wang, Y., Tanaka, H., Shapiro, L., Zhang, W., Shan, W.S., Arndt, K., Frank, M., Gordon, R.E., et al. (2001). The presynaptic particle

web: ultrastructure, composition, dissolution, and reconstitution. Neuron *32*, 63–77.

Roberson, E.D., Scearce-Levie, K., Palop, J.J., Yan, F., Cheng, I.H., Wu, T., Gerstein, H., Yu, G.Q., and Mucke, L. (2007). Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. Science *316*, 750–754.

Roche, K.W., Standley, S., McCallum, J., Dune Ly, C., Ehlers, M.D., and Wenthold, R.J. (2001). Molecular determinants of NMDA receptor internalization. Nat. Neurosci. *4*, 794–802.

Rong, Y., Lu, X., Bernard, A., Khrestchatisky, M., and Baudry, M. (2001). Tyrosine phosphorylation of ionotropic glutamate receptors by Fyn or Src differentially modulates their susceptibility to calpain and enhances their binding to spectrin and PSD-95. J. Neurochem. *79*, 382–390.

Röskam, S., Neff, F., Schwarting, R., Bacher, M., and Dodel, R. (2010). APP transgenic mice: the effect of active and passive immunotherapy in cognitive tasks. Neurosci. Biobehav. Rev. *34*, 487–499.

Salter, M.W., and Kalia, L.V. (2004). Src kinases: a hub for NMDA receptor regulation. Nat. Rev. Neurosci. *5*, 317–328.

Selkoe, D.J. (1997). Alzheimer's disease: genotypes, phenotypes, and treatments. Science *275*, 630–631.

Selkoe, D.J. (2002). Alzheimer's disease is a synaptic failure. Science *298*, 789–791.

Shankar, G.M., Li, S., Mehta, T.H., Garcia-Munoz, A., Shepardson, N.E., Smith, I., Brett, F.M., Farrell, M.A., Rowan, M.J., Lemere, C.A., et al. (2008). Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat. Med. *14*, 837–842.

Sturchler-Pierrat, C., Abramowski, D., Duke, M., Wiederhold, K.H., Mistl, C., Rothacher, S., Ledermann, B., Bürki, K., Frey, P., Paganetti, P.A., et al. (1997). Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. Proc. Natl. Acad. Sci. USA *94*, 13287–13292.

Tezuka, T., Umemori, H., Akiyama, T., Nakanishi, S., and Yamamoto, T. (1999). PSD-95 promotes Fyn-mediated tyrosine phosphorylation of the N-methyl-D-aspartate receptor subunit NR2A. Proc. Natl. Acad. Sci. USA *96*, 435–440.

Tucker, K.L., Meyer, M., and Barde, Y.A. (2001). Neurotrophins are required for nerve growth during development. Nat. Neurosci. *4*, 29–37.

Van Dam, D., D'Hooge, R., Staufenbiel, M., Van Ginneken, C., Van Meir, F., and De Deyn, P.P. (2003). Age-dependent cognitive decline in the APP23 model precedes amyloid deposition. Eur. J. Neurosci. *17*, 388–396.

Weingarten, M.D., Lockwood, A.H., Hwo, S.Y., and Kirschner, M.W. (1975). A protein factor essential for microtubule assembly. Proc. Natl. Acad. Sci. USA *72*, 1858–1862.

Yagi, T., Aizawa, S., Tokunaga, T., Shigetani, Y., Takeda, N., and Ikawa, Y. (1993). A role for Fyn tyrosine kinase in the suckling behaviour of neonatal mice. Nature *366*, 742–745.

Zhao, L., Ma, Q.L., Calon, F., Harris-White, M.E., Yang, F., Lim, G.P., Morihara, T., Ubeda, O.J., Ambegaokar, S., Hansen, J.E., et al. (2006). Role of p21-activated kinase pathway defects in the cognitive deficits of Alzheimer disease. Nat. Neurosci. *9*, 234–242.