Prion protein and Aβ-related synaptic toxicity impairment

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Keywords: Alzheimer's disease; amyloid; prion protein; synaptic plasticity

DOI 10.1002/emmm.201000082

Received May 18, 2010
Revised June 17, 2010
Accepted June 21, 2010

→See accompanying Closeup by Benilova & de Strooper: http://dx.doi.org/10.1002/emmm.201000088

Alzheimer's disease (AD), the most common neurodegenerative disorder, goes along with extracellular amyloid-β (Aβ) deposits. The cognitive decline observed during AD progression correlates with damaged spines, dendrites and synapses in hippocampus and cortex. Numerous studies have shown that Aβ oligomers, both synthetic and derived from cultures and AD brains, potently impair synaptic structure and functions. The cellular prion protein (PrP^C) was proposed to mediate this effect. We report that ablation or overexpression of PrP^C had no effect on the impairment of hippocampal synaptic plasticity in a transgenic model of AD. These findings challenge the role of PrP^C as a mediator of Aβ toxicity.

INTRODUCTION

Alzheimer's disease (AD) is an age-dependent neurodegenerative disorder that culminates in cognitive decline with limited treatment options. Oligomeric amyloid-β (Aβ), derived from the β and γ cleavage of β-amyloid precursor protein (APP), may drive AD pathogenesis by activating ill-defined signalling pathways (Walsh et al, 2005). Several molecules have been suggested to trigger the latter (De Felice et al, 2009; Shankar et al, 2007; Snyder et al, 2005). The cellular prion protein (PrP^C) was reported to mediate the impairment of long-term potentiation (LTP) induced by synthetic Aβ oligomers in the hippocampal Schaffer collateral pathway (Lauren et al, 2009). Also, removal of PrP^C from mice carrying APPswe and PSen1ΔE9 transgenes rescued early death and memory impairment (Gimbel et al, 2010).

PrP^C is a membrane-anchored glycoprotein (Steele et al, 2007) crucial for axomyelinic integrity of peripheral nerves (Bremer et al, 2010). The remarkable finding that PrP^C mediates Aβ-related synaptic toxicity was taken to suggest that interference with PrP^C may represent a therapeutic option for AD (Lauren et al, 2009; Gimbel et al, 2010). However, upon intracerebral injection of synthetic Aβ oligomers, the absence of PrP^C did not prevent deficits in hippocampal dependent behavioural tests (Balducci et al, 2010).

In view of these conflicting reports, we reasoned that a better understanding of the impact of PrP^C onto AD may come from careful genetic analyses. Also, the utilization of a second, independent AD transgenic mouse model may help evaluating the universality of the observed phenomena. We therefore asked whether PrP^C would modulate the degradation of LTP in an *in vivo* model of AD. We crossed mice lacking (Büeler et al, 1992) or overexpressing membrane-anchored (Fischer et al, 1996) or secreted PrP (Chesebro et al, 2005) with $APPPS1^+$ mice coexpressing mutant APP ($APP^{KM670/671NL}$) and mutant presenilin-1 ($PS1^{L166P}$; Radde et al, 2006) which suffer from Aβ-dependent learning and memory deficits (Serneels et al, 2009; Table 1). We found that ablation or overexpression of PrP^C had no effect on the impairment of hippocampal synaptic plasticity in a transgenic model of AD. These findings challenge the role of PrP^C as a Aβ toxicity mediator.

RESULTS AND DISCUSSION

LTP impairment and APP processing are not altered in absence of the cellular prion protein

We crossed *Prnp*$^{o/o}$ mice lacking PrP^C (Büeler et al, 1992) with $APPPS1^+$ mice coexpressing mutant APP ($APP^{KM670/671NL}$) and

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Table 1. Genetically modified mice used in this study

Line	Description	Genetic modifications	Genetic background	References
APPPS1	Alzheimer's disease mouse model displaying Aβ42 cerebral amyloidosis	$APP^{KM670/671NL}$ transgene $PS1^{L166P}$ transgene (both on *Mmu*2)	C57BL/6	Radde et al (2006)
$Prnp^{o/o}$	Mouse lacking cellular prion protein	Introduction of a *neo* cassette replacing PrP codon 4–187 in the *Prnp* locus in *Mmu*2 ($Prnp^{o}$ allele)	C57BL/6 and 129/Sv	Büeler et al (1992)
tga20	Mouse overexpressing cellular prion protein	Introduction of a *neo* cassette replacing PrP codon 4–187 in the *Prnp* locus in *Mmu*2 ($Prnp^{o}$ allele) *Prnp* minigene on *Mmu*17	C57BL/6 and 129/Sv	Fischer et al (1996)
$tg44Prnp^{-/-}$	Mouse expressing GPI-anchorless prion protein	Introduction of a *neo* cassette into a *Kpn*I site following residue 93 of PrP in the *Prnp* locus in *Mmu*2 ($Prnp^{-}$ allele) Anchorless PrP transgene	C57BL/10 and 129/Ola	Chesebro et al (2005)

Mmu2 and *Mmu17*: *Mus musculus* chromosome 2 and 17, respectively; *neo*: neomycin phosphotransferase; $Prnp^{o}$ and $Prnp^{-}$ denote by convention the 'Zurich-I' and 'Edbg' knockout alleles of *Prnp*, respectively.

mutant presenilin-1 ($PS1^{L166P}$; Radde et al, 2006). The resulting mice did not display any early death independently of the *Prnp* genotype (data not shown). High-frequency stimulation (HFS) of Schaffer collateral CA1 synapses induced an increase in field excitatory postsynaptic potentials (fEPSP) reflecting LTP in both 4-month-old $Prnp^{+/+}$ and $Prnp^{o/o}$ mice (data not shown) as previously reported (Lledo et al, 1996). In contrast, age-matched $APPPS1^{+}Prnp^{+/+}$ ($n=6$), $APPPS1^{+}Prnp^{+/o}$ ($n=5$) and $APPPS1^{+}Prnp^{o/o}$ ($n=5$) all exhibited defective LTP after HFS (114.23 ± 9.61; 111.72 ± 9.64 and $105.51\pm12.23\%$, respectively; $p<0.001$; Fig 1A). The fEPSP slopes during the first 2 min were similar in $APPPS1^{+}Prnp^{+/+}$ and wild-type mice (124.1 ± 7.0 and $184.8\pm26.2\%$, respectively; $p>0.05$), indicating that immediate post-tetanic potentiation was not affected. Basal synaptic transmission as assessed by input–output curve analysis was normal in all mice (Fig 1B and C), confirming that the APPPS1 transgene induces a selective impairment in synaptic plasticity. In contrast to 4-month-old animals, robust LTP was induced in 2-month-old $APPPS1^{+}Prnp^{+/+}$ ($172.6\pm14.6\%$; $n=5$), $APPPS1^{+}Prnp^{+/o}$ ($168.9\pm14\%$; $n=5$) and $APPPS1^{+}Prnp^{o/o}$ mice ($204.4\pm15.9\%$; $n=4$) and was comparable to LTP in $Prnp^{+/o}$ ($174.6\pm7\%$; $n=5$; Fig 1D). We conclude that the LTP impairment was age related, appeared only in mice carrying the APPPS1 transgene after >2 months, and was independent of *Prnp* gene dosage.

Many genetic polymorphisms affect APP processing and Aβ levels (Lehman et al, 2003). The $APP^{KM670/671NL}$ and $PS1^{L166P}$ transgenes map to mouse chromosome 2 (*Mmu2*; Radde et al, 2006) along with *Prnp*, and are linked to a quantitative trait locus that modifies Aβ levels (Ryman et al, 2008). Furthermore, PrP^{C} itself was reported to directly interfere with APP catabolism (Parkin et al, 2007). Each of these factors, alone or in combination, may modulate the production of soluble $A\beta_{42}$, thereby indirectly affecting LTP impairment. However, we found that 2-month old gender-matched $APPPS1^{+}Prnp^{+/+}$ and $APPPS1^{+}Prnp^{o/o}$ mice displayed similar levels of APP catabolites (Fig S1A) and soluble $A\beta_{42}$ (Fig S1B). We conclude that the effects described here cannot be ascribed to any difference in APP generation or processing.

Evaluation of genetic confounders that might mask the impact of PrP^{C} on LTP in 4-month-old APPPS1 mice

A genome-wide screen of 192 polymorphic microsatellites revealed that $APPPS1^{+}Prnp^{o/o}$ mice contained significantly larger portions of 129/Sv-derived genome than $APPPS1^{+}Prnp^{+/+}$ mice (129/Sv-specific markers: average ± SEM: 60 ± 6.2 *vs.* 2 ± 0.4, respectively; $p<0.001$). This genetic constellation may be taken to suggest that the above intercrosses have inadvertently introduced genetic biases affecting LTP independently of Aβ levels (Gerlai, 2002). However, in subsequent intercrosses, the content in genome-wide 129/Sv-specific markers was 55.3 ± 3.9 *versus* 41.7 ± 3.2 ($n=7$ and 6, respectively; $p<0.05$), yet this statistically significant difference disappeared upon exclusion of markers on *Mmu2* (44.7 ± 3.8 *vs.* 38.0 ± 3.2, respectively; $p>0.05$). This indicates that the latter mice, although not inbred, were genetically similar except for the *Mmu2* genomic region that is closely linked to both *Prnp* and APPPS1 and does not desegregate easily from these loci by breeding. This genetic scenario may help explaining the differences in insoluble $A\beta_{42}$ levels seen in F2 $APPPS1^{+}$ mice with different *Prnp* genotypes generated by intercrosses of $APPPS1^{+}$ and $Prnp^{o/o}$ mice (Fig S2; Ryman et al, 2008).

Transgenic PrP^{C} overexpression disproves *Mmu2* bias and does not aggravate APPPS1-induced LTP impairment

To formally discriminate between PrP^{C}-dependent effect and potential confounders residing on *Mmu2*, we reintroduced PrP^{C} into $APPPS1^{+}Prnp^{o/o}$ mice via crosses to *tga20* mice (Fischer et al, 1996) that carry a *Prnp* minigene on *Mmu17* (Zabel et al, 2009) and overexpress PrP^{C} about fourfold (Fig S3). LTP was again affected in 4-month-old $APPPS1^{+}tga20^{tg/-}Prnp^{o/o}$ ($127.84\pm12.61\%$; $n=4$) and $APPPS1^{+}tga20^{-/-}Prnp^{o/o}$ littermates ($106.56\pm5.46\%$; $n=5$; $p=0.137$; Fig 2A). The genome-wide microsatellite patterns of these two groups of mice were indistinguishable even when *Mmu2* markers were included (129/Sv-specific markers: 61.0 ± 2.1 *vs.* 61.7 ± 3.9, respectively; $p>0.05$; Fig 2B), indicating that any contribution by genetic confounders to the phenotype is unlikely. To further explore the impact of supraphysiological levels on PrP^{C} in LTP, we analyzed $APPPS1^{+}tga20^{tg/-}Prnp^{+/o}$ which overexpress *ca.* sevenfold PrP^{C}

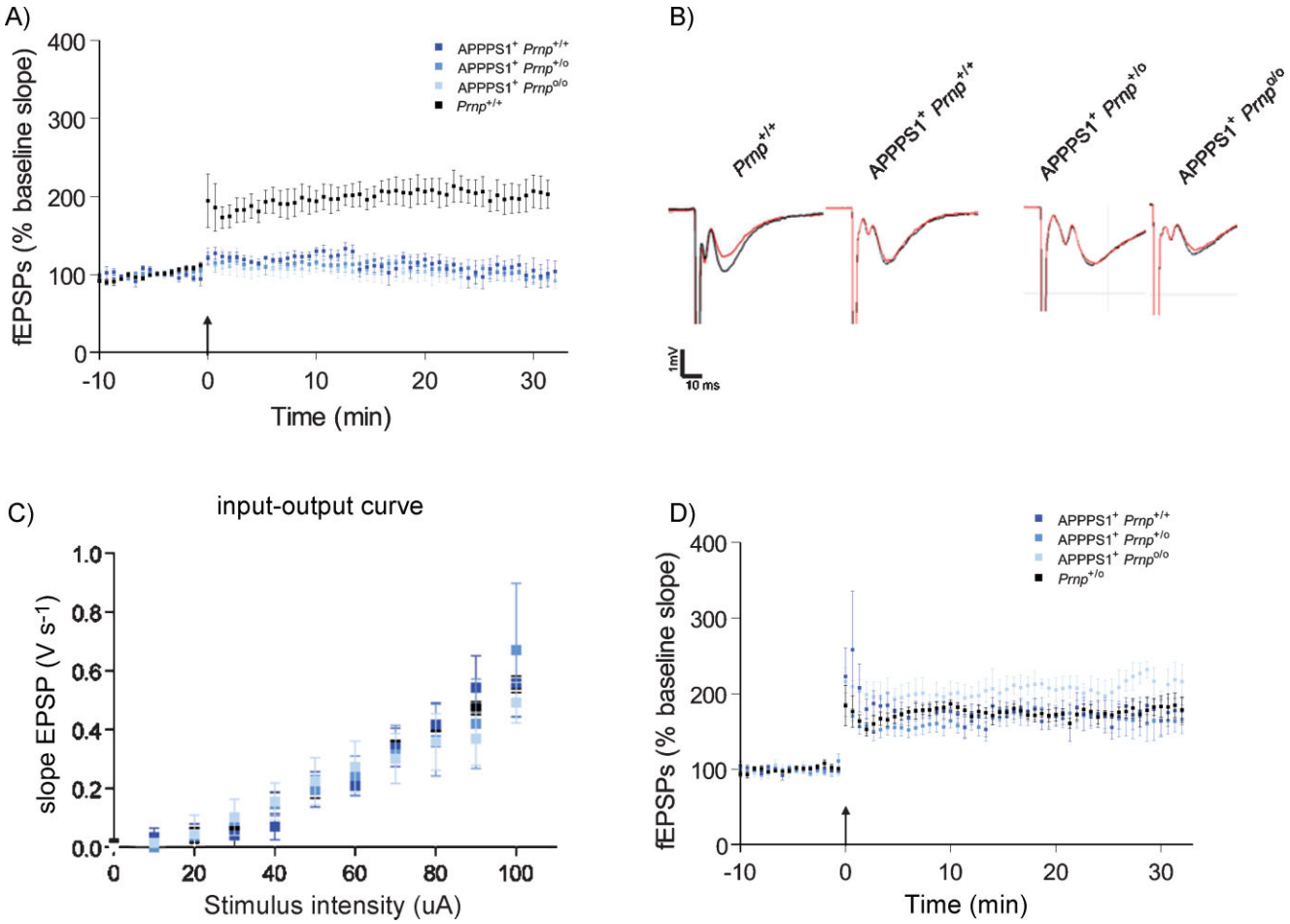

Figure 1. CA1 hippocampal LTP impairment in APPPS1$^+$ mice occurs at 4 months of age and is not regulated by PrP^C expression.

A. CA1 hippocampal LTP was induced in acute slices from 4-month-old *Prnp*$^{+/+}$ mice (black, $n = 7$), but was abolished in slices from age-matched APPPS1$^+$*Prnp*$^{+/+}$ (dark blue, $n = 6$), APPPS1$^+$*Prnp*$^{+/o}$ (blue, $n = 5$) and APPPS1$^+$*Prnp*$^{o/o}$ mice (light blue $n = 5$).

B. fEPSP traces before (red) and after (black) LTP induction. Calibration: 1 mV; 10 ms.

C. Input–output curves (stimulus intensity *vs.* fEPSP slope) indicative of normal basal synaptic transmission.

D. Unaffected LTP in slices derived from 2-month-old APPPS1$^+$*Prnp*$^{+/+}$ ($n = 5$), APPPS1$^+$*Prnp*$^{+/o}$ ($n = 5$), APPPS1$^+$*Prnp*$^{o/o}$ ($n = 4$) and *Prnp*$^{+/o}$ mice ($n = 5$). These results indicate that LTP impairment in APPPS1$^+$ mice was not a developmental defect, and occurred only after 2 months of age independently of *Prnp* gene dosage.

(Fig S3) and APPPS1$^+$*tga20*$^{-/-}$*Prnp*$^{+/o}$ littermates. These two groups of mice shared similar genomic microsatellite patterns (Fig 3A). At 4 months of age, LTP was significantly reduced in both APPPS1$^+$*tga20*$^{tg/-}$*Prnp*$^{+/o}$ and APPPS1$^+$*tga20*$^{-/-}$*Prnp*$^{+/o}$ littermates ($149.41 \pm 11.81\%$, $n = 6$ *vs.* $121.56 \pm 11.65\%$, respectively; $n = 4$; Fig 3B). Expression of the *tga20* allele showed a tendency towards improved LTP that was not statistically significant, without altering APP catabolites and soluble and insoluble $A\beta_{42}$ (Fig 3C and D). Therefore, PrP^C overexpression did not enhance Aβ-mediated LTP impairment; if anything, it may have marginally antagonized it.

Overexpression of a secreted PrP^C variant reduced the impairment of LTP in 4-month-old APPPS1 mice

We next asked whether a soluble version of PrP^C might intercept Aβ oligomers and interfere with synaptic toxicity. First we verified that interaction of PrP^C with Aβ species (Balducci et al, 2010; Lauren et al, 2009) can occur in the absence of PrP^C membrane anchoring. We therefore tested the binding properties of bacterially expressed recombinant full-length PrP (recPrP$_{23-230}$). We found that recPrP$_{23-230}$ bound low molecular weight $A\beta_{42}$ species, and that binding was reduced by monoclonal anti-PrP antibodies (Polymenidou et al, 2008) raised against its N-proximal region (Fig S4). Also, we found that a shortened variant of recPrP lacking the amino-proximal residues 23–121 (recPrP$_{121-230}$) did not bind $A\beta_{42}$ (Fig S4). These results confirm that PrP, even when produced in bacteria and therefore, lacking all eukaryotic post-translational modifications including the addition of a glycolipid anchor, can efficiently bind Aβ species.

We then crossed APPPS1$^+$*Prnp*$^{o/o}$ mice to mice expressing GPI-anchorless PrP (secPrP) which is secreted into body fluids of tg44*Prnp*$^{-/-}$ transgenic mice (Chesebro et al, 2005). The

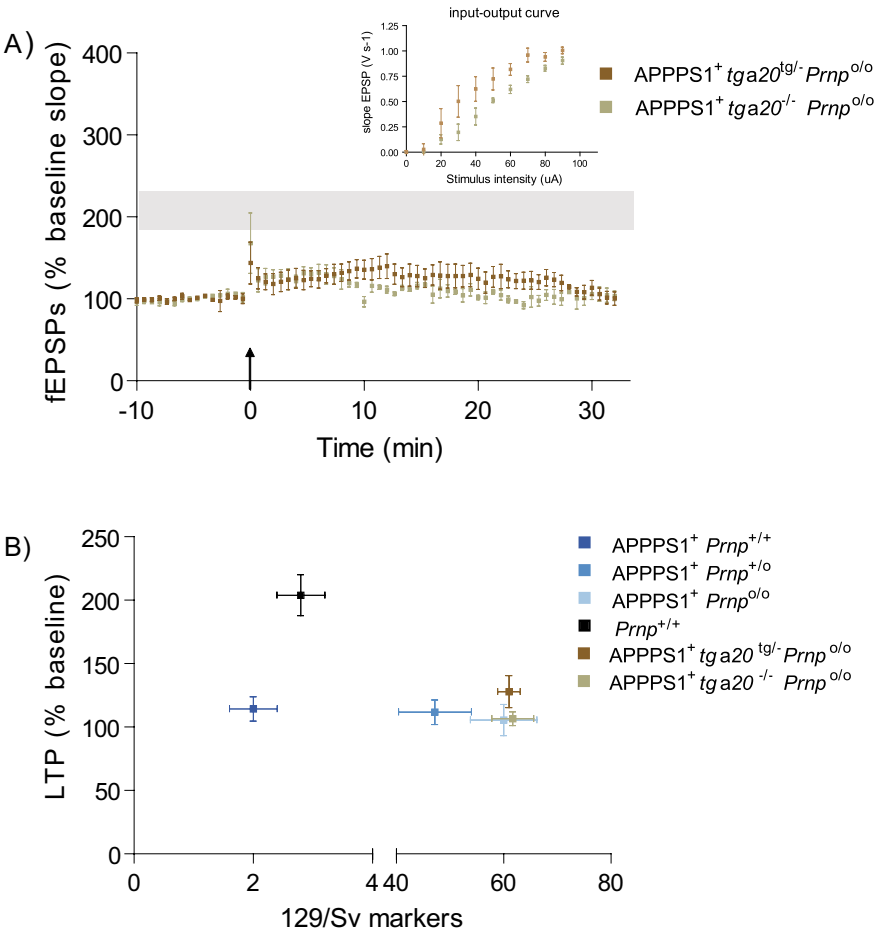

Figure 2. LTP in 4-month-old APPPS1$^+$ mice expressing a PrP^C transgene.

A. At 4 months of age, LTP was impaired in slices from both APPPS1$^+$*tga20*$^{tg/-}$*Prnp*$^{o/o}$ ($n=4$) and APPPS1$^+$*tga20*$^{-/-}$*Prnp*$^{o/o}$ ($n=5$) but not in *Prnp*$^{+/+}$ slices ($n=7$; LTP mean ± SEM from Fig 1A represented as grey ribbon). Basal synaptic transmission was normal as indicated by normal input–output curve (stimulus intensity *vs.* fEPSP slope).

B. Average fEPSP slopes (percentage of baseline) at 10–25 min post-LTP plotted against the average number of 129/Sv specific markers for mice depicted in panel A and Fig 1A. In all investigated paradigms, LTP suppression by the APPPS1 transgene was independent of the genetic background.

Prnp^o and *Prnp*$^-$ alleles refer to the 'Zurich-I' (Büeler et al, 1992) and 'Edbg' (Manson et al, 1994) gene ablation events. We measured LTP in hippocampal slices derived from 4-month-old APPPS1$^+$tg44$^{tg/-}$*Prnp*$^{-/o}$ ($n=7$) and APPPS1$^+$tg44$^{-/-}$*Prnp*$^{-/o}$ ($n=6$) littermates with comparable genomic microsatellite patterns (Fig 4A). Remarkably, secPrP significantly suppressed the APPPS1-related LTP impairment (151.5 ± 11 and 108.5 ± 7.5%, respectively; $p<0.05$, ANOVA and Tukey's multiple comparison test, see Fig 4B). The metabolism of APP and the levels of soluble and insoluble $A\beta_{42}$ did not appear to be altered by the tg44 transgene (Fig 4C and D), suggesting that secPrP exerted its beneficial effects interfering with the effectors of Aβ toxicity.

Despite decades of research, the cascade of events that originates with the aggregation of Aβ and leads up to cognitive impairment continues to be poorly understood. Many observations point to a crucial role of transmembrane signaling events triggered by aggregated Aβ. Several membrane proteins have been reported to bind soluble Aβ oligomers—thereby candidating as potential transducers of toxicity (Deane et al, 2004; De Felice et al, 2009; Shankar et al, 2007; Snyder et al, 2005; Yan et al, 1996). A great deal of excitement was generated by the recovery of PrP^C from an expression screen for soluble Aβ oligomer binders, particularly as synthetic soluble Aβ oligomers were found to damage hippocampal LTP in a PrP^C-dependent manner (Lauren et al, 2009) and impairment of spatial memory was rescued by genetic ablation of PrP in a mouse model of AD (Gimbel et al, 2010). However, the report that removal of PrP^C did not prevent the behavioural deficits caused by intracerebral injection of synthetic Aβ oligomers (Balducci et al, 2010) challenged the role of PrP^C as a crucial mediator of Aβ synaptotoxicity.

We crossed mice expressing human Aβ to mice lacking or overexpressing PrP^C or a soluble variant thereof to evaluate if the impact of PrP is persistent also in another AD mouse model which suffer from Aβ-dependent learning and memory deficits (Serneels et al, 2009). The latter experimental paradigm may more closely approximate the human disease than the previously published models (Balducci et al, 2010; Lauren et al, 2009) as exposure to Aβ species is chronic and uninterrupted over a protracted period, which is arguably more realistic than hyperacute exposure of brain tissue to Aβ. Furthermore, Aβ exists in AD brains as a vastly heterodisperse spectrum of assemblies ranging from monomers and dimers to oligomers and extremely large fibrillary aggregates, each one of which may partly contribute to the AD phenotype (Lesne et al, 2006; Shankar et al, 2008, 2009; Walsh et al, 2002). As the relative affinity of the various Aβ assemblies for PrP^C is not known in detail, transgenic mice expressing many such assemblies may

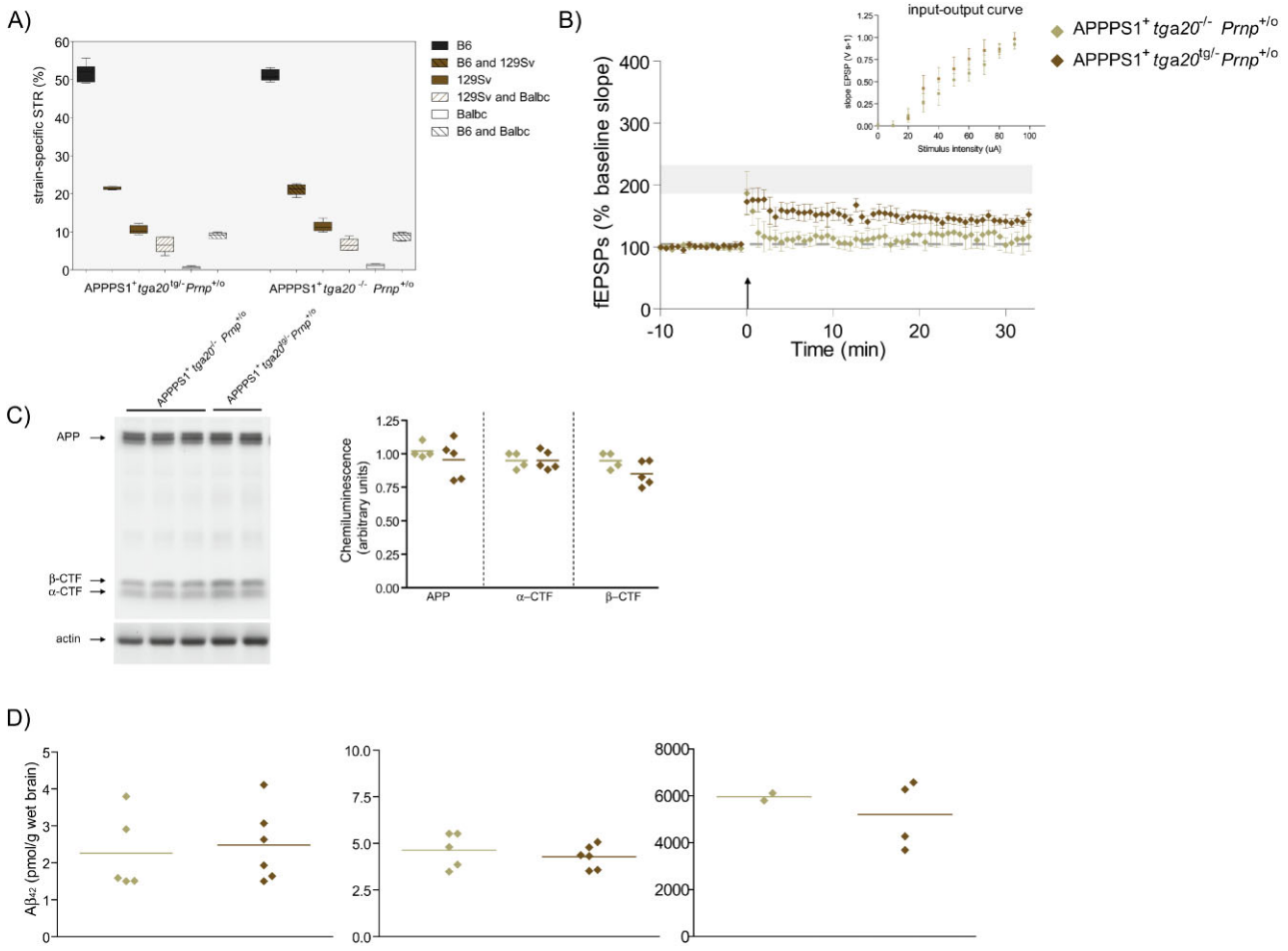

Figure 3. Analysis of 4-month-old APPPS1^{+} mice with supraphysiological levels of PrP^{C}.

A. Percentage of strain-specific microsatellites in APPPS1^{+}*tga20*$^{tg/-}$*Prnp*$^{+/o}$ ($n = 6$) and APPPS1^{+}*tga20*$^{-/-}$*Prnp*$^{+/o}$ ($n = 4$) mice is displayed by box plot. No significant difference in the genetic background of the two mouse strains was detected (Mann–Whitney *U*-test, two-tailed, $p > 0.05$).

B. At 4 months of age, slices of both APPPS1^{+}*tga20*$^{tg/-}$*Prnp*$^{+/o}$ ($n = 6$) and APPPS1^{+}*tga20*$^{-/-}$*Prnp*$^{+/o}$ mice ($n = 4$) displayed reduced LTP when compared to *Prnp*$^{+/+}$ mice ($n = 7$); LTP mean ± SEM from Fig 1A represented as grey ribbon. Basal synaptic transmission was normal as indicated by normal input–output curve (stimulus intensity *vs.* fEPSP slope). All error bars: standard errors of the mean.

C. APP expression and processing by secretases were similar in 4-month-old APPPS1^{+} *tga20*$^{tg/-}$*Prnp*$^{+/o}$ and APPPS1^{+}*tga20*$^{-/-}$*Prnp*$^{+/o}$ mice. Left panel: representative SDS–PAGE followed by immunoblotting using an APP C-terminal antibody detecting full-length APP and αβ-CTF; actin was used as loading control. Right panel: quantitation of chemiluminescence for APP, α-CTF and β-CTF.

D. TRIS-soluble (left panel), detergent-soluble (middle panel) and insoluble (right panel) human $A\beta_{42}$ levels as assessed by ELISA. Each symbol denotes one individual mouse.

reveal phenomena that might go unrecognized in simpler systems, such as application of defined synthetic Aβ oligomers.

On the other hand, the genetic crosses described in our study and in previous work (Gimbel et al, 2010) may suffer from limitations. PrP^{C} was reported to regulate β-secretase cleavage (Parkin et al, 2007), and overexpression may interfere with APP metabolism and Aβ levels, thereby indirectly affecting LTP impairment. Indeed, careful genetic quality control revealed a mouse-strain dependent effect on insoluble $A\beta_{42}$ levels—a phenomenon that should be taken into account while interpreting results from mouse AD models. However, all mice analyzed in this study displayed similar levels of APP catabolites and $A\beta_{42}$ independently of *Prnp* gene dosage.

We also considered the possibility that potential confounders residing on *Mmu2* might have introduced alterations of the experimental evaluation (Steele et al, 2007), a problem which remains unsolved in the study by Gimbel et al. However, in our paradigm, genome-wide microsatellite analyses and expression of PrP^{C} from the *tga20* minigene on chromosome *Mmu17* disproved any *Mmu2* bias.

Additionally, one might argue that the exceedingly rapid amyloid pathology of APPPS1 mice used in our study leads to irreversible synaptic damage that is independent of Aβ oligomers and, consequently, of PrP^{C}. However, the original report (Radde et al, 2006) and our observations indicate that immunohistochemically and biophysically recognizable

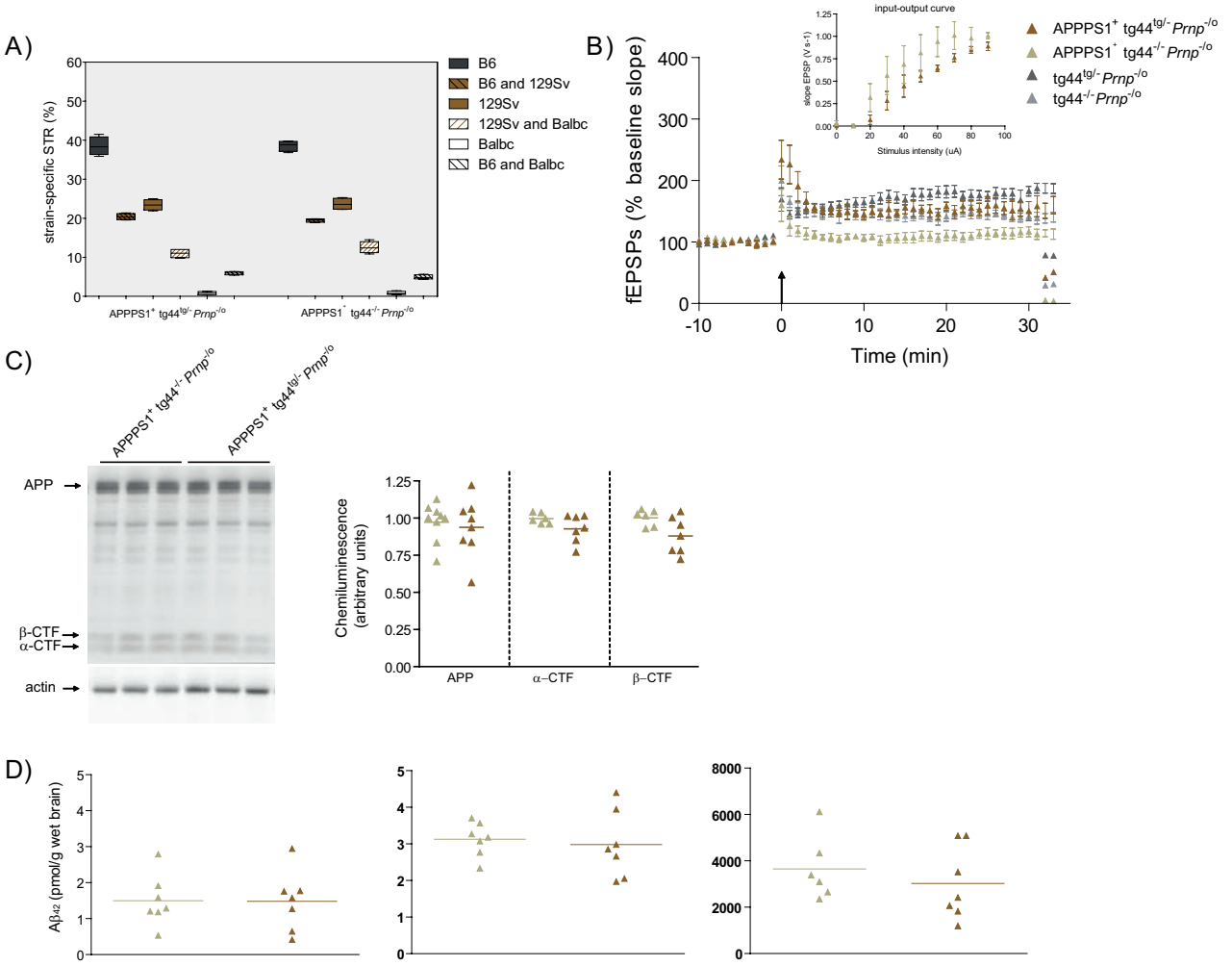

Figure 4. Anchorless soluble PrP^C reduces hippocampal LTP impairment in APPPS1$^+$ mice.

A. Percentage of strain-specific microsatellites in APPPS1$^+$tg44$^{tg/-}$*Prnp*$^{-/o}$ ($n = 5$) and APPPS1$^+$tg44$^{-/-}$*Prnp*$^{-/o}$ ($n = 5$) mice is displayed by box plot. No significant difference in the genetic background was detected (Mann–Whitney *U*-test, two-tailed, $p > 0.05$).

B. LTP was induced in slices prepared from 4-month-old tg44$^{tg/-}$*Prnp*$^{-/o}$ ($n = 5$) and tg44$^{-/-}$*Prnp*$^{-/o}$ ($n = 7$) mice, but was impaired in slices from APPPS1$^+$tg44$^{-/-}$*Prnp*$^{-/o}$ mice ($n = 6$) and partially rescued in APPPS1$^+$tg44$^{tg/-}$*Prnp*$^{-/o}$ ($n = 7$) mice. Basal synaptic transmission was normal as indicated by normal input–output curve (stimulus intensity *vs.* fEPSP slope). All mice were compound heterozygotes for the 'Zurich-I' (*Prnp*^{o}) and the 'Edbg' (*Prnp*$^-$) knockout alleles of *Prnp*.

C. APP expression and processing by secretases were similar in APPPS1$^+$tg44$^{tg/-}$*Prnp*$^{-/o}$ and APPPS1$^+$tg44$^{-/-}$*Prnp*$^{-/o}$ mice at 4 months of age. Left panel: representative SDS–PAGE followed by immunoblotting using an APP C-terminal antibody detecting full-length APP and C-terminal fragments (αβ-CTF); actin was used as loading control. Right panel: quantitation of chemiluminescence revealed no difference in APP, α-CTF and β-CTF between the two groups.

D. TRIS-soluble (left panel), detergent-soluble (middle panel) and insoluble (right panel) human Aβ$_{42}$ levels as assessed by ELISA. Each symbol denotes one individual mouse.

amyloid deposition does not occur in APPPS1 hippocampi before 4–5 months of age (Fig S5). Therefore, at the time of our analysis, there was no massive amyloid deposition in the hippocampus. Furthermore, the rescue of LTP impairment by secPrP negates the possibility that an overly aggressive amyloid pathology precludes the evaluation of the role of PrP^C in these mice.

The combined weight of all these results favours the conclusion that, however enticing, the hypothesis of PrP^C being a crucial mediator of Aβ synaptotoxicity might be not universal.

MATERIALS AND METHODS

Mice

To remove the prion protein locus (*Prnp*), *Prnp*$^{o/o}$ mice (Büeler et al, 1992) were crossed with APPPS1 mice (Radde et al, 2006). APPPS1$^+$*Prnp*$^{o/o}$ or APPPS1 mice were then crossed with *tga20*$^{tg/-}$*Prnp*$^{o/o}$ (Fischer et al, 1996) or tg44$^{tg/-}$*Prnp*$^{-/-}$ mice (Chesebro et al, 2005) to generate the different APPPS1$^+$ and APPPS1$^-$ littermate control mice (Table 1 and Fig S6). The genetic pattern of mouse strains was determined with a panel of 192 polymorphic microsatellites as

The paper explained

PROBLEM:

Alzheimer's disease (AD), the most common neurodegenerative disorder, culminates in cognitive decline with limited treatment options. Aggregated Aβ, possibly in the form of oligomers, accumulates in the brain of affected individuals and may drive AD pathogenesis by activating ill-defined signaling pathways. The PrP^C was reported to mediate the impairment of LTP induced by synthetic Aβ oligomers and removal of PrP^C from an AD mouse model rescued early death and memory deficit. In another study, however, the absence of PrP^C did not prevent deficits in hippocampal dependent behavioural tests caused by intracerebral injection of Aβ oligomers. To investigate the universality of the observed phenomena, we asked whether PrP^C modulates LTP in a second independent AD mouse model.

RESULTS:

We crossed mice lacking or over-expressing PrP^C with $APPPS1^+$ mice coexpressing mutant APP and mutant presenilin-1, which suffer from Aβ-dependent learning and memory deficits. We found defective LTP in $APPPS1^+$ mice at 4 months of age. Ablation or overexpression of PrP^C had no effect on this impairment of hippocampal synaptic plasticity.

IMPACT:

The results reported here suggest that PrP^C may not be a universal mediator of Aβ synaptotoxicity. Additional work is required to refine our understanding of the interaction between PrP^C and Aβ and establish whether PrP^C is a viable target for pharmaceutical interventions in AD.

described (Bremer et al, 2010). All mice were maintained under specific pathogen-free conditions. Housing and experimental protocols were in accordance with the Swiss Animal Welfare Law and in compliance with the regulations of the Cantonal Veterinary Office, Zurich.

Electrophysiology

Hippocampal slice preparation from male mice and fEPSPs recordings in the CA1 region were as described (Knobloch et al, 2007). The LTP induction protocol was considered successful, and entered in the analysis, only if a stable baseline for at least 10 min was achieved. To generate input–output curves, slices were prepared as above and stimulated every 20 s with increasing intensity (from 0.0 to 0.1 mA in 0.01 mA increments) using a total of 10 stimuli. For comparing groups, potentiation of fEPSP slopes during the interval 10–25 min post-tetanus was evaluated. Data points were normalized to the mean baseline value and expressed as mean ± SEM All numbers in brackets indicate analyzed mice; 2–3 slices were typically analyzed for each mouse.

Tissue preparation

Brain fractionation was performed as described (Shankar et al, 2008) with modifications. Briefly, snap frozen forebrains were homogenized in ice-cold tris buffered saline (TBS), after centrifugation at $100{,}000 \times g$ for 1 h the supernatant (called soluble fraction) was used to determine soluble $A\beta_{42}$. The pellet was homogenized in phosphate buffered saline plus 0.5% 4-nonylphenyl-polyethylene glycol (NP40S), 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and spun at $16{,}000 \times g$ for 30 min. The resultant supernatant was used to quantify APP, α-C terminal fragment (CTF) and β-CTF and the remaining pellet was solubilized in 70% formic acid and insoluble $A\beta_{42}$ was measured after tris(hydroxymethyl)aminomethane (TRIS)-base neutralization.

Quantification of $A\beta_{42}$ and PrP^C

Levels of $A\beta_{42}$ were assessed by sandwich enzyme-linked immunosorbent assay (ELISA; hAmyloid $A\beta_{42}$, The Genetics Company) according to manufacturer's instructions. PrP^C concentration was determined by sandwich ELISA as described (Polymenidou et al, 2008).

Immunoblotting

To determine APP and CTFs levels, 20 μg of proteins were separated by electrophoresis on a 4–12% polyacrylamide gel. Primary antibodies were: anti-APP C-terminal (Sigma) recognizing both mouse and human APP and CTFs; anti-actin (Chemicon). Protein bands were detected by adding SuperSignal West Pico Chemiluminescent Substrate (Pierce) and exposing the blot in a Stella detector (Raytest). Chemiluminescence quantification was performed by TINA software.

In vitro binding assay

Binding of synthetic human $A\beta_{42}$ (Bachem AG) to immobilized recombinant PrP (Zahn et al, 1997) was analyzed by ELISA. Recombinant PrP ($recPrP_{23-231}$ or $recPrP_{121-231}$) was immobilized overnight at 4°C on 96-well microtiter plates. Varying concentrations of synthetic human $A\beta_{42}$ were added to wells and incubated for 1 h. Bound proteins were detected by incubation with 6E10 antibody (Covance) followed by horseradish peroxidase-conjugated antimouse IgG_1. Absorbance was measured at 450 nm. For Western blot analysis various concentrations of $A\beta_{42}$ were incubated in the same conditions, followed by Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and blotting with 6E10 antibody. Binding of human $A\beta_{42}$ (25 nM) to $recPrP_{23-231}$ was assessed also in presence of decadic dilutions (100, 10 and 1 nM) of anti-PrP antibodies (Polymenidou et al, 2008).

Histological analyses

Brains were removed and fixed in 4% formaldehyde in phosphate buffered saline, pH 7.5, paraffin embedded and cut into 2–4 μm sections. Sections were stained with hematoxylin–eosin (HE) or antibodies against glial fibrillary acidic protein (GFAP) (DAKO), ionized calcium binding adapter molecule 1 (Iba1; WAKO) and Aβ (4G8; Signet).

Statistical analyses

Statistical significance was determined according to one-way ANOVA followed by Tukey's post-test for multiple comparison, unpaired Student's *t*-test and Mann–Whitney test using Prism software (GraphPad Software). Error bars in the graphs and numbers following the ± sign denote standard errors of the mean unless otherwise indicated.

Author contributions

A.M.C. designed the study, organized and maintained the mouse colony, performed biochemical and histologic analyses, analyzed the data and cowrote the paper; M.F. performed electrophysiology experiments, analyzed the data and cowrote the paper; M.N. helped in organizing and maintaining the mouse colony, performed genetic analyses, analyzed the data and cowrote the paper; O.M. performed electrophysiology experiments and analyzed the data; R.M. performed biochemical experiments; J.F. performed biochemical experiments; I.M.M. supervised electrophysiology experiments, analyzed the data and wrote the paper; A.A. designed and coordinated the study, supervised biochemical, genetic and histologic analyses, analyzed the data and wrote the paper.

Acknowledgements

We thank Dr M. Jucker for APPPS1 mice, Dr B. Chesebro and Dr M.B.A. Oldstone for tg44 mice, Dr S. Hornemann for providing advice on protein purification, P. Schwarz and M. Delic for technical assistance, M. Bieri and N. Wey for software development and Dr F.D. Heitz for helpful comments on the manuscript. This work was supported by grants of the European Union, the Swiss National Research Foundation, the Novartis Foundation, the National Center for Competence in Research 'Neural Plasticity and Repair' and an Advanced Investigator Grant of the European Research Council to A.A. A.M.C. is partly supported by the 'Alzheimer und Depression Fonds der SAMW'. M.N. is partly supported by an investigator fellowship of Collegio Ghislieri, Pavia, Italy.

Supporting information is available at EMBO Molecular Medicine Online.

The authors declare that they have no conflict of interest.

For more information

Accompanying Closeup:
http://dx.doi.org/10.1002/emmm.201000088

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