Brief Communications

Ablation of Cellular Prion Protein Does Not Ameliorate Abnormal Neural Network Activity or Cognitive Dysfunction in the J20 Line of Human Amyloid Precursor Protein Transgenic Mice

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Previous studies suggested that the cellular prion protein (PrP^c) plays a critical role in the pathogenesis of Alzheimer's disease (AD). Specifically, amyloid-β (Aβ) oligomers were proposed to cause synaptic and cognitive dysfunction by binding to PrP^c. To test this hypothesis, we crossed human amyloid precursor protein (hAPP) transgenic mice from line J20 onto a PrP^c-deficient background. Ablation of PrP^c did not prevent the premature mortality and abnormal neural network activity typically seen in hAPPJ20 mice. Furthermore, hAPPJ20 mice with or without PrP^c expression showed comparably robust abnormalities in learning and memory and in other behavioral domains at 6–8 months of age. Notably, these abnormalities are not refractory to therapeutic manipulations in general: they can be effectively prevented by interventions that prevent Aβ-dependent neuronal dysfunction also in other lines of hAPP transgenic mice. Thus, at least in this model, PrP^c is not an important mediator of Aβ-induced neurological impairments.

Introduction

Alzheimer's disease (AD) affects many millions of people and is on the rise worldwide (Thies and Bleiler, 2011). Amyloid-β (Aβ) peptides, released from the amyloid precursor protein (APP), are implicated in its pathogenesis. At high concentrations, Aβ peptides form diverse assemblies, among which Aβ oligomers may be the most pathogenic (Cheng et al., 2007; Shankar et al., 2008; Ashe and Zahs, 2010; Sakono and Zako, 2010). One of the most important unresolved questions is whether Aβ oligomers impair neuronal functions by interacting with specific cell surface receptors. Aβ oligomers bind a variety of receptors, including the receptor for advanced glycation end products (RAGE) (Origlia et al., 2009), α-7-nicotinic acetylcholine receptor (Dineley et al., 2002), cellular prion protein (PrP^c) (Laurén et al., 2009; Balducci et al., 2010), and Ephrin-type B2 receptor (EphB2) (Renner et al., 2010; Cissé et al., 2011). Determining which interaction most affects cognitive functions is an important objective.

PrP^c has been reported to mediate Aβ-induced deficits in hippocampal synaptic plasticity and cognitive impairments (Laurén et al., 2009; Gimbel et al., 2010). This membrane-anchored glycoprotein helps to maintain the brain's white matter and regulate its innate immune cells, responses to oxidative stress, and neurogenesis (Aguzzi et al., 2008). PrP^c ablation prevented deficits in long-term potentiation (LTP) elicited by Aβ oligomers in acute hippocampal slices (Laurén et al., 2009) and behavioral impairments in APPswe/PSenΔE9 transgenic mice (Gimbel et al., 2010). However, others could not reproduce the electrophysiological rescue (Calella et al., 2010; Kessels et al., 2010), and one study reported that acute intracerebroventricular injection of synthetic Aβ oligomers caused similar deficits in learning and memory in mice with or without PrP^c expression (Balducci et al., 2010).

Because the experimental design used in the latter study differs from the chronic exposure to Aβ oligomers that neurons experience in human APP (hAPP) transgenic mice and in AD, we set out to replicate the reported rescue of behavioral functions (Gimbel et al., 2010) in hAPPJ20 mice. hAPPJ20 mice have a robust phenotype with a range of AD-like alterations, including deficits in spatial and nonspatial learning and memory, behavioral abnormalities, synaptic impairments, changes in various synaptic activity-related proteins, amyloid plaques, dystrophic neurites, aberrant sprouting of axon terminals, astrocytosis, and microgliosis (Mucke et al., 2000; Cheng et al., 2007; Palop et al., 2007; Roberson et al., 2007, 2011; Meilandt et al., 2008; Harris et al., 2010; Cissé et al., 2011). We crossed hAPPJ20 mice onto a PrP^c-deficient background (Büeler et al., 1992) and compared hAPPJ20 mice with or without PrP^c expression at 6–8 months of age in a battery of behavioral tests and by video-EEG monitoring.

Materials and Methods

hAPPJ20 transgenic and Prnp *knock-out mice*

Hemizygous transgenic and NTG mice were from line J20, which expresses an alternatively spliced hAPPJ20 minigene encoding hAPP695,

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hAPP751, and hAPP770 with the Swedish and Indiana familial AD mutations directed by the PDGF β-chain promoter (Rockenstein et al., 1995; Mucke et al., 2000). PrP^c-deficient *Prnp* knock-out mice (B6.129S7-Prnp^{tm1Cwe}/Orl) were acquired from the European Mutant Mouse Archive. hAPP mice and *Prnp*$^{-/-}$ mice were on a C57BL/6J background. Mice had access to food (Picolab Rodent Diet 20, Labdiet) and water *ad libitum*. For all experiments, groups were sex-balanced.

hAPP, C99, and Aβ measurements

For hAPP and C99 measurements, snap-frozen hippocampal samples were homogenized in PBS containing 0.5 mM EDTA, 1 mM DTT, 0.5% Triton, 0.1 M PMSF, Phosphatase Inhibitor Cocktails I and II (Sigma-Aldrich), and protease inhibitors (Roche). For Aβ measurements, the same lysates were diluted 1:2 with 7.5 M guanidine buffer. Total hAPP and C99 levels in hippocampal samples were assessed by Western blotting. The following antibodies were used: anti-hAPP (8E5, 1:1000; kindly provided by Elan Pharmaceuticals), anti-CTF (CT-15, 1:1000; kindly provided by Dr. Eddy Koo, University of California at San Diego, La Jolla, CA), and anti-tubulin (Sigma). $Aβ_{1-x}$ and $Aβ_{1-42}$ levels were quantified by ELISA as described previously (Johnson-Wood et al., 1997; Mucke et al., 2000) and expressed relative to total protein concentration determined by Bradford assay.

Behavioral tests

Elevated plus maze. The elevated plus maze has two open (without walls) and two enclosed (with walls) arms elevated 63 cm above the ground (Hamilton-Kinder). Mice were acclimated to the testing room under dim light for 1 h and tested as described previously (Harris et al., 2010; Cissé et al., 2011).

Open field. Spontaneous locomotor activity in an open field was measured as described previously (Harris et al., 2010; Cissé et al., 2011).

Novel object recognition. Mice were acclimated in the testing room for at least 1 h before testing. Testing was performed as described previously (Harris et al., 2010; Cissé et al., 2011) and the frequency of object interactions and time spent exploring each object were recorded with a video tracking system (Noldus) for analysis.

Morris water maze. The apparatus and training protocol have been described previously (Harris et al., 2010; Cissé et al., 2011).

EEG recordings

Mice were implanted for video-EEG monitoring after anesthesia with Avertin (tribromoethanol, 250 mg/kg, i.p.). Teflon-coated silver wire electrodes (0.125 mm diameter) soldered to a multichannel electrical connector were implanted into the subdural space over the left frontal cortex (coordinates relative to the bregma were M/L, ±1 mm; A/P, ±1 mm) and the left and right parietal cortex (M/L, ±2 mm, A/P, ±2 mm). The left frontal cortex electrode was a reference. EEG recordings were performed at least 7 d after surgery on freely moving mice in a recording chamber. EEG activity was recorded with the Harmonie software (version 5.0b) for 48 h. Epileptic spikes were automatically detected by Gotman spike and seizure detectors (Harmonie) and confirmed by inspection of EEG recordings.

Statistical analyses

Statistical analyses were performed with GraphPad Prism. Differences between two means were assessed by paired or unpaired *t* test. Differences among multiple means were assessed, as indicated, by one-way, two-way, or repeated-measures ANOVA, followed by Bonferroni's or Tukey's *post hoc* test. Error bars represent SEM. Differences between observed and expected values were assessed by one- or two-sided χ^2 test. Null hypotheses were rejected at the 0.05 level.

Results

PrP^c ablation does not prevent behavioral abnormalities in hAPPJ20 mice

Like other hAPP transgenic models, hAPPJ20 mice display a disinhibition-like phenotype in the elevated plus maze and hyperactivity in arenas, such as the open field (Chin et al., 2005; Kobayashi and Chen, 2005; Cheng et al., 2007; Roberson et al., 2007; Meilandt et al., 2009; Harris et al., 2010; Cissé et al., 2011). To determine whether PrP^c ablation prevents behavioral abnormalities, we generated hAPPJ20 mice with (*Prnp*$^{+/+}$) or without (*Prnp*$^{-/-}$) PrP^c and compared them in these paradigms at 6–8 months, when behavioral abnormalities are readily detectable in hAPPJ20 mice on the *Prnp*$^{+/+}$ background. hAPP/*Prnp*$^{+/+}$ and hAPP/*Prnp*$^{-/-}$ mice spent more time in the open arms of the elevated plus maze than *Prnp*$^{+/+}$ and *Prnp*$^{-/-}$ mice without hAPP (Fig. 1*A*), suggesting disinhibition or lower levels of anxiety. Ablation of PrP^c had no effect. It also had no significant effect on the hyperactivity of hAPP mice in the open field ($p = 0.132$ by unpaired, one-tailed Student's *t* test) (Fig. 1*B*).

PrP^c ablation does not prevent learning and memory deficits in hAPPJ20 mice

Like other hAPP transgenic mice, hAPPJ20 mice show deficits in spatial and nonspatial learning and memory (Cheng et al., 2007; Roberson et al., 2007, 2011; Meilandt et al., 2009; Harris et al., 2010; Cissé et al., 2011). Nonspatial learning and memory were assessed in the novel object recognition test. Unlike mice without hAPP, hAPP/*Prnp*$^{+/+}$ and hAPP/*Prnp*$^{-/-}$ mice did not prefer the novel over the familiar object (Fig. 1*C*), suggesting deficits in recognizing or remembering the familiar object. Ablation of PrP^c provided no benefit to hAPP mice.

Spatial learning and memory were tested in the Morris water maze. PrP^c ablation failed to prevent deficits hAPPJ20 mice typically show in this test and even tended to worsen them. In the cued-platform task, all mice learned similarly (data not shown). In the hidden-platform (spatial) version, both hAPP/*Prnp*$^{+/+}$ and hAPP/*Prnp*$^{-/-}$ mice showed significant learning deficits compared with *Prnp*$^{+/+}$ and *Prnp*$^{-/-}$ mice lacking hAPP (Fig. 2*A*). hAPP mice without PrP^c performed slightly worse than hAPP mice with PrP^c ($p < 0.05$ by linear contrast and Bonferroni test). Swim speeds during the hidden platform training were comparable among all groups (data not shown).

At 24 h after the last training, spatial memory retention was assessed in a probe trial. The platform was removed, and mice were given 60 s to explore the pool. Unlike mice without hAPP, hAPP/*Prnp*$^{+/+}$ and hAPP/*Prnp*$^{-/-}$ mice failed to spend significantly more time in the target quadrant (i.e., where the platform was) than in the other quadrants (Fig. 2*B*). hAPP/*Prnp*$^{+/+}$ and hAPP/*Prnp*$^{-/-}$ mice also failed to cross the original platform location significantly more often than corresponding locations in nontarget quadrants (Fig. 2*C*). Finally, hAPP/*Prnp*$^{+/+}$ and hAPP/*Prnp*$^{-/-}$ mice took longer to reach the original platform location than mice without hAPP (Fig. 2*D*). In all three outcome measures, hAPP/*Prnp*$^{-/-}$ mice tended to perform worse than hAPP/*Prnp*$^{+/+}$ mice, although these differences did not reach statistical significance.

PrP^c ablation does not change hippocampal levels of hAPP or Aβ in hAPPJ20 mice

We compared hippocampal Aβ levels by ELISA and hippocampal levels of hAPP and hAPP C-terminal fragment C99 by Western blots in 8- to 13-month-old hAPP/*Prnp*$^{+/+}$ and hAPP/*Prnp*$^{-/-}$ mice (n = 11–13 per genotype, mean ± SEM, relative levels): $Aβ_{1-x}$ (148 ± 23 vs 100 ± 26), $Aβ_{1-42}$ (108 ± 16 vs 78 ± 19), $Aβ_{1-42}/Aβ_{1-x}$ ratio (0.67 ± 0.08 vs 0.81 ± 0.05), hAPP (4895 ± 498 vs 5007 ± 488), and C99 (8310 ± 1171 vs 6212 ± 989). None of these differences was statistically significant by unpaired *t* test.

PrP^c ablation worsens premature mortality in hAPPJ20 mice

Like other hAPP transgenic lines, hAPPJ20 mice exhibit early mortality, which may be caused by epileptic seizures (Chin et al.,

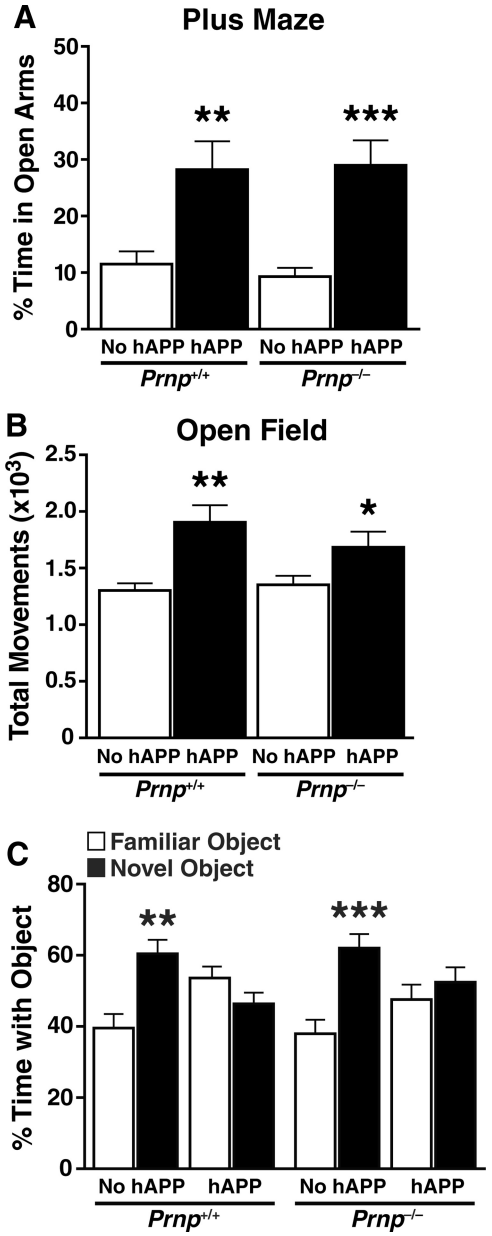

Figure 1. Ablation of PrP^c in hAPPJ20 mice does not prevent their behavioral abnormalities in the elevated plus maze, open field, and object recognition test. ***A–C***, Six- to eight-month-old *Prnp* $^{+/+}$ and *Prnp* $^{-/-}$ mice with or without hAPP ($n = 10–15$ mice per genotype) were tested in the indicated behavioral paradigms. ***A***, In the elevated plus maze, mice with hAPP spent more time in the open arms than mice without hAPP. hAPP/*Prnp* $^{+/+}$ mice and hAPP/*Prnp* $^{-/-}$ mice showed a similar level of disinhibition. Two-way ANOVA revealed a significant effect of hAPP ($p < 0.0001$), but not of *Prnp* ($p = 0.748$), and no interaction between hAPP and *Prnp* ($p = 0.607$). ***B***, In the open field, mice with hAPP were hyperactive compared to mice without hAPP. The subtle difference between hAPP/*Prnp* $^{+/+}$ mice and hAPP/*Prnp* $^{-/-}$ mice was not significant even by one-tailed *t* test ($p = 0.132$). Two-way ANOVA revealed a significant effect of hAPP ($p < 0.0001$), but not of *Prnp* ($p = 0.445$), and no interaction between hAPP and *Prnp* ($p = 0.223$). ***C***, Mice were analyzed in the novel object recognition test. Unlike littermates without hAPP, hAPPJ20/*Prnp* $^{+/+}$ mice and hAPPJ20/*Prnp* $^{-/-}$ mice failed to spend more time with the novel than with the familiar object in test sessions. Two-way ANOVA of the average ratios of time spent with the novel versus the familiar object revealed a significant effect of hAPP ($p < 0.005$), but not of *Prnp* ($p = 0.322$), and no interaction between hAPP and *Prnp* ($p = 0.561$). *$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$ versus mice without hAPP of the same *Prnp* genotype (Tukey test) (***A***, ***B***) or versus familiar object (paired, two-tailed *t* test) (***C***). Data represent means ± SEM.

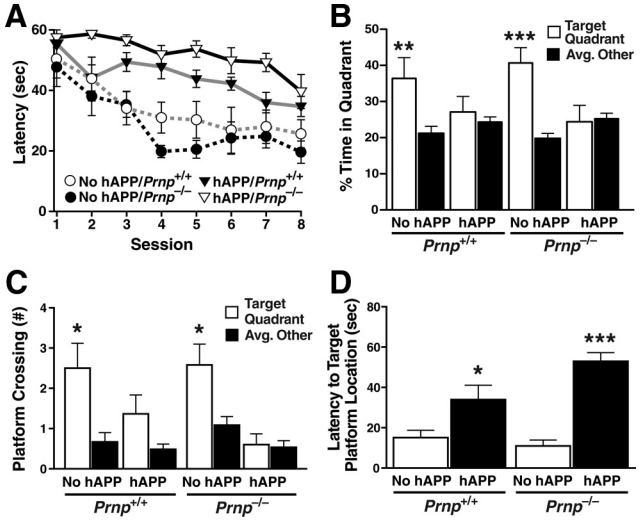

Figure 2. PrP^c ablation in hAPPJ20 mice does not prevent deficits in spatial learning and memory. Six- to eight-month-old *Prnp* $^{+/+}$ and *Prnp* $^{-/-}$ mice with or without hAPP ($n = 10–15$ mice per genotype) were trained in the Morris water maze for 4 d. Time (latency) and distance swum (data not shown) before reaching the platform were recorded. A probe trial (platform removed) was conducted 24 h after the last training. ***A***, hAPP/*Prnp* $^{+/+}$ and hAPP/*Prnp* $^{-/-}$ mice learned this task more poorly than mice without hAPP. Two-way repeated-measures ANOVA revealed a significant effect of hAPP ($p < 0.0001$), but not of *Prnp* ($p = 0.0613$), and no interaction between hAPP and *Prnp* ($p = 0.632$). ***B***, ***C***, During the probe trial, mice without hAPP, but not mice with hAPP, favored the target quadrant (***B***) and crossed the target location more often than corresponding places in nontarget quadrants (***C***). Two-way ANOVA of these data revealed a significant effect of hAPP (***B***, $p < 0.05$; ***C***, $p < 0.005$), but not of *Prnp* (***B***, $p = 0.8691$; ***C***, $p = 0.647$), and no interaction between hAPP and *Prnp* (***B***, $p = 0.4620$; ***C***, $p = 0.281$). ***D***, During the probe trial, mice with hAPP took longer to reach the target location than mice without hAPP. Two-way ANOVA revealed a significant effect of hAPP ($p < 0.0001$), but not of *Prnp* ($p = 0.238$), and no interaction between hAPP and *Prnp* ($p = 0.483$). One-way ANOVA and Bonferroni test revealed a significant difference between hAPP/*Prnp* $^{+/+}$ and hAPP/*Prnp* $^{-/-}$ mice ($p < 0.05$). *$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$ versus nontarget quadrants by paired, two-way *t* test (***B***, ***C***) or versus mice without hAPP of the same *Prnp* genotype by Tukey test (***D***). Data represent means ± SEM.

2004; Palop et al., 2007; Roberson et al., 2007, 2011; Meilandt et al., 2009). *Prnp* $^{+/+}$, *Prnp* $^{-/-}$, hAPP/*Prnp* $^{+/+}$ and hAPP/*Prnp* $^{-/-}$ mice were born at comparable proportions (Fig. 3*A*). However, from birth to weaning (P27), more hAPP mice tended to die than mice without hAPP, independently of *Prnp* genotype (Fig. 3*B*). By 8 months of age, 8% of hAPP/*Prnp* $^{+/+}$ mice died (Fig. 3*C*). Surprisingly, PrP^c ablation worsened early mortality in hAPPJ20 mice, resulting in a 22% death rate in hAPP/*Prnp* $^{-/-}$ mice (Fig. 3*C*). During the observation period, only one death occurred in *Prnp* $^{+/+}$ or *Prnp* $^{-/-}$ mice lacking hAPP.

PrP^c ablation does not prevent spontaneous epileptiform activity in hAPPJ20 mice

EEG recordings from the neocortex and hippocampus of hAPPJ20 mice revealed spontaneous epileptiform activity (Palop et al., 2007), and similar findings have been obtained in other lines of hAPP mice (Minkeviciene et al., 2009; Roberson et al., 2011). Because *Prnp* $^{-/-}$ mice were reported as more resistant to proconvulsant drugs than *Prnp* $^{+/+}$ mice (Ratté et al., 2011), we examined whether PrP^c ablation makes hAPPJ20 mice more resistant to Aβ-induced epileptiform activity. *Prnp* $^{+/+}$ and *Prnp* $^{-/-}$ mice with or without hAPP were compared by 48 h video and EEG recordings at 6–8 months of age. Bilateral recording electrodes over the parietal cortex revealed very few or no spikes in mice without hAPP (Fig. 4*A*) and an average of 7 spikes per hour in hAPPJ20/*Prnp* $^{+/+}$ mice (Fig. 4*B*). Remarkably, PrP^c

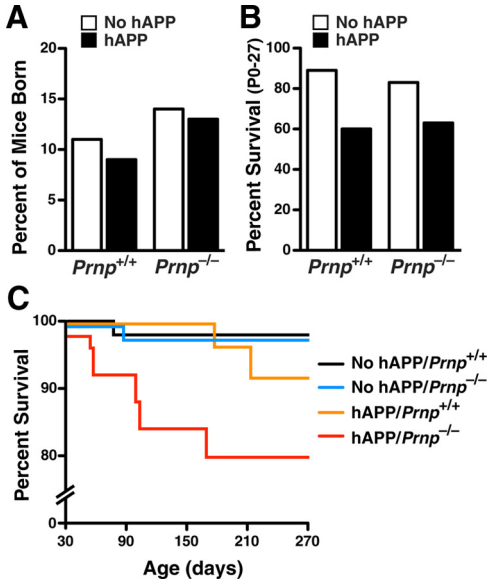

Figure 3. Ablation of PrP^c worsens early mortality in hAPPJ20 mice. Death and survival were monitored in a total of 169 mice. ***A***, Percentage of mice born by genotype. The difference between the number of mice obtained and expected for each genotype, based on Mendelian principles of inheritance (12.5%), was not significant ($\chi^2 = 0.72$; $p = 0.86$). ***B***, Premature death reported from P0 to P27. Mortality tended to be higher in hAPP than NTG mice (one-sided $\chi^2 = 1.9$, $p = 0.07$), independently of their *Prnp* genotype (two-sided $\chi^2 = 0.28$, $p = 0.59$). ***C***, Kaplan–Meier survival plot shows more mice with hAPP died prematurely than mice without hAPP after weaning ($p < 0.01$, Mantel–Cox test). Premature mortality was greater in hAPP/*Prnp* $^{-/-}$ mice than in hAPP/*Prnp* $^{+/+}$ mice ($p < 0.001$, Mantel–Cox test).

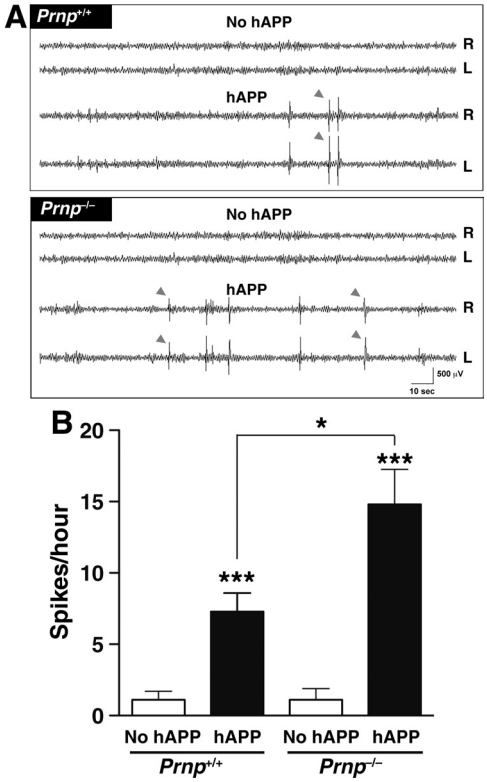

Figure 4. Ablation of PrP^c worsens spontaneous epileptiform activity in hAPPJ20 mice. EEG was recorded over both parietal cortices for 48 h in 6- to 8-month-old mice ($n = 3–6$ mice per genotype). ***A***, In contrast to mice lacking hAPP, hAPP/*Prnp* $^{+/+}$ and hAPP/*Prnp* $^{-/-}$ mice showed frequent epileptiform spikes (see gray arrowheads). L, Left; R, right. ***B***, Quantification of epileptiform spikes. Two-way ANOVA revealed effects of hAPP ($p < 0.0001$) and *Prnp* ($p = 0.03$), and an interaction between hAPP and *Prnp* ($p = 0.036$). $^*p < 0.01$, $^{***}p < 0.0001$ versus mice without hAPP or as indicated by bracket (Bonferroni *post hoc* test). Values are means ± SEM.

ablation actually doubled the epileptiform activity recorded in hAPPJ20 mice (Fig. 4*B*). No seizures occurred during the recording period in any of the groups.

Discussion

PrP^c ablation did not prevent neuronal network dysfunction and behavioral abnormalities in hAPPJ20 transgenic mice. In fact, it doubled the early mortality rate and epileptiform activity in this line. The resulting selection process and survival of mice with relative resistance against the synergistic effects of hAPP/Aβ overexpression and PrP^c ablation might have diminished our ability to detect an exacerbating effect of PrP^c ablation on hAPP/Aβ-dependent behavioral abnormalities. Subtle signs of such an effect were evident in the probe trial of the Morris water maze test.

These results differ from studies showing that PrP^c ablation prevents Aβ-induced LTP deficits in acute hippocampal slices (Laurén et al., 2009) and diminishes early mortality and impairments in spatial learning and memory in APPswe/PSenΔE9 transgenic mice (Gimbel et al., 2010). However, our findings agree with others showing that PrP^c ablation does not affect Aβ-induced synaptic depression, reduction in spine density, and LTP deficits in acute hippocampal slices (Kessels et al., 2010), behavioral impairments from intracerebroventricular injection of Aβ (Balducci et al., 2010), and impairments of hippocampal synaptic plasticity (Calella et al., 2010).

hAPPJ20 mice and other hAPP lines show epileptiform activity (Palop and Mucke, 2010; Roberson et al., 2011). Genetic manipulations that prevent or counteract aberrant excitatory neuronal activity (e.g., ablation of Fyn, group IVA phospholipase A2, or tau) reduce premature mortality and/or cognitive deficits in hAPP-J20 mice (Chin et al., 2004, 2005; Roberson et al., 2007, 2011; Sanchez-Mejia et al., 2008). Interestingly, PrP^c has also been implicated in epilepsy (Walz et al., 2002). However, PrP^c ablation actually increased premature mortality and did not affect cognitive deficits in hAPPJ20 mice, making it unlikely that it protects against Aβ-induced seizure activity, which is closely linked to cognitive impairments in these mice (Palop et al., 2007; Palop and Mucke, 2010; Roberson et al., 2011). Indeed, PrP^c ablation enhanced spontaneous epileptiform activity in hAPPJ20 mice, consistent with the increased susceptibility of *Prnp* $^{-/-}$ mice to kainate-induced seizures (Walz et al., 1999; Rangel et al., 2007).

We found that PrP^c ablation doubled premature mortality in hAPPJ20 mice but had no effect on mice without hAPP, suggesting a specific interaction between hAPP/Aβ- and PrP^c-dependent mechanisms. The lower seizure threshold of *Prnp* $^{-/-}$ mice might partially explain the increased early mortality of hAPPJ20/*Prnp* $^{-/-}$ mice. These results are in stark contrast to the increased survival of PrP^c-deficient APPswe/PSenΔE9 mice, which were also on the C57BL/6J background (Gimbel et al., 2010).

The different effects of PrP^c ablation on Aβ-induced deficits observed by different investigators might stem from differences in experimental protocols, hAPP lines and *Prnp* $^{-/-}$ strains. For example, if levels of pathogenic Aβ oligomers in relevant brain regions were higher in hAPPJ20 mice than in APPswe/PSenΔE9 mice, beneficial effects of PrP^c ablation in hAPPJ20 mice might have been obscured. However, if PrP^c were crucial in Aβ-induced cognitive deficits, its ablation should prevent those deficits in hAPPJ20 mice as did ablation of group IVA phospholipase A_2 (Sanchez-Mejia et al., 2008) or tau (Roberson et al., 2007, 2011). Even partial reduction of these two molecules in hemizygous knock-out mice significantly ameliorated the deficits in

hAPPJ20 mice (Roberson et al., 2007, 2011; Sanchez-Mejia et al., 2008). Thus, Aβ-induced cognitive deficits in hAPPJ20 mice are not so severe that they simply cannot be overcome by any intervention. In fact, neuronal and cognitive dysfunction in these mice could be reversed effectively by normalizing neuronal expression of EphB2, an alternative receptor for Aβ oligomers (Cissé et al., 2011).

Because Aβ binds to many molecules (Dineley et al., 2002; Laurén et al., 2009; Origlia et al., 2009; Balducci et al., 2010; Renner et al., 2010; Cissé et al., 2011), it is critical to determine which of these interactions have the greatest functional impact. Interactions of Aβ oligomers with the receptor for advanced glycation end products (Origlia et al., 2009) and with EphB2 (Cissé et al., 2011) have striking effects on neuronal and cognitive functions and, we suspect, may be more promising targets for therapeutic interventions in AD than PrP^c.

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