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Dose-dependent, prion protein (PrP)-mediated facilitation of excitatory synaptic transmission in the mouse hippocampus

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Abstract Disruption of both alleles of the prion protein gene, *Prnp*, has been shown repeatedly to abolish the susceptibility of mice to developing prion diseases. However, conflicting results have been obtained from phenotypic analyses of prion protein (PrP)-deficient (*Prnp*^{0/0}) mice lines. To explore the possible neurophysiological properties associated with expression or absence of the normal isoform of the cellular prion protein (PrP^C), we used conventional in vitro extracellular field potential recordings in the hippocampal CA1 area of mice from two independently-derived *Prnp*^{0/0} strains. Basal synaptic transmission and a short-term form of synaptic plasticity were analysed in this study. Results were compared with animals carrying a wild-type mouse PrP transgene to investigate whether PrP expression levels influence glutamatergic synaptic transmission in the hippocampus. There was a clear correlation between excitatory synaptic transmission and PrP expression; i.e. the range of synaptic responses increased with the level of PrP^C expression. On the other hand, the probability of transmitter release, as assessed by paired-pulse facilitation, appeared unchanged. Interestingly, whereas the overall range for synaptic responses was still greater in older mice over-expressing PrP^C, this effect in these animals appeared to be due to better recruitment of fibres rather than facilitation of synaptic transmission per se. Taken together, these data are strong evidence for a functional role for PrP^C in modulating synaptic transmission.

Keywords Prion · Hippocampus · Brain slices · Input-output curves · Synaptic transmission

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Introduction

Prion diseases are fatal neurodegenerative illnesses that can present as genetic, sporadic or infectious disorders (Aguzzi and Weissmann 1997; Prusiner 1997). In their infectious forms, they are transmissible by experimental inoculation or dietary exposure, following prolonged incubation periods. Prion diseases include scrapie in sheep and goats, bovine spongiform encephalopathy and Creutzfeldt-Jakob disease in humans. It has been well established that the presence of normal cellular prion protein (PrP^C) is necessary for the development of the disease (Büeler et al. 1993; Manson et al. 1994; Moore et al. 1995; Prusiner et al. 1993). During the course of the disease, PrP^C undergoes post-translational modification to generate the abnormal, protease-resistant isoform of PrP (PrP^{Sc}) (reviewed by Prusiner et al. 1998).

While a wealth of data indicates that the PrP^{Sc} is the major, if not the sole, component required for the transmission and pathogenesis of prion diseases, the physiological role of PrP^C remains obscure. Elucidation of PrP^C function may shed some light on the pathogenesis of these diseases. The establishment of PrP-deficient mice has been disappointing in this regard. Although it was anticipated that PrP-deficient (*Prnp*^{0/0}) mice would exhibit a severe, and possibly embryonically lethal, phenotype, PrP-deficient animals obtained from four different lines are born and develop normally (Büeler et al. 1992; Manson et al. 1994; Moore et al. 1995; Prusiner et al. 1993). Mild late-onset peripheral nerve degeneration (Nishida et al. 1999) and Purkinje cell degeneration associated with progressive ataxia has been observed in two independently established *Prnp*^{0/0} lines (Moore 1997; Sakaguchi et al. 1995). However, the latter phenotype appears to be caused by a gene knock-out artefact, where deletion of the *Prnp* exon3 splice acceptor results in an intergenic splicing with up-regulation of a downstream PrP-related gene named *doppel* (*Dpl*, Moore et al. 1999). On the other hand, in two lines in which the *Prnp* open reading frames were partially replaced, defective sleep-wake cycles and altered circadian rhythms have

been reported (Tobler et al. 1996). A study on one of these two lines has suggested further that PrPC may be a copper-binding protein (Brown et al. 1997), in agreement with previous biochemical characterization of the PrP peptide and recombinant molecules (Hornshaw et al. 1995; Stöckel et al. 1998). Additionally, brain slices from these two Prnp^{0/0} lines reportedly show defective neurotransmission at γ -aminobutyric acid (GABA)-ergic synapses and diminished long-term potentiation (Collinge et al. 1994; Johnston et al. 1997; Manson et al. 1995; Whittington et al. 1995), although this result remains controversial for one of the Prnp^{0/0} lines (Herms et al. 1995; Lledo et al. 1996).

The reason underlying all these differences is still unclear but could be attributed to differences in biochemical conditions (Herms et al. 1999) or to differences in the knock-out construct. We therefore conducted a series of experiments to test the effect of age, PrP expression levels and the *Prnp* knock-out allele background. Wild-type and PrP-deficient mice, in which part of (Büeler et al. 1993) or the entire (Sakaguchi et al. 1995) *Prnp* open reading frame is excised, were used in combination with a wild-type mouse *PrP-A* transgene (Telling et al. 1996). Mice carrying the mouse *PrP-A* transgene on homozygous or hemizygous backgrounds were designated Tg(MoPrP-A) Prnp^{0/0} and Tg(MoPrP-A)Prnp^{0/+}, respectively.

To explore the possible neurophysiological changes related to the absence or presence of PrPC, we used conventional in vitro extracellular field potential recordings in the hippocampal CA1 area. Both basal synaptic transmission and a form of short-term synaptic plasticity were compared in wild-type, Prnp^{0/0} and Tg(MoPrP-A) mice at different ages. This analysis showed a dependence between PrPC expression and the range of synaptic responses, indicating that change in PrPC levels could influence hippocampal synaptic transmission. Finally, the study also demonstrated that with age, the absence of PrPC is accompanied by higher synaptic strength, suggesting the existence of some homeostatic mechanisms by which older hippocampal fibres devoid of PrPC tend to compensate for their smaller synaptic response range.

Materials and methods

Animal and slice preparation

For this study, two independently established PrPC-deficient mouse lines, designated Zrch Prnp^{0/0} (Büeler et al. 1992) and NgsK Prnp^{0/0} (Sakaguchi et al. 1995) were used. A previously established transgenic mouse line harbouring the wild-type *MoPrP-A* gene [Tg(MoPrP-A)B4053] was also used in combination with the Prnp^{+/+} (wild-type), Zrch Prnp^{0/0} or NgsK Prnp^{0/0} background introduced by breeding.

Animals were anaesthetized by i.p. injection of pentobarbitone sodium (100 μ l) and then decapitated. The brain was removed quickly and submerged in artificial cerebrospinal fluid (ACSF) at 4 °C for about 2 min. The ACSF contained (in mM): 124 NaCl, 3 KCl, 2 CaCl₂, 1.3 MgCl₂, 26 NaHCO₃, 1.25 NaH₂PO₄, 10 D-glu-

cose, and was equilibrated with 95% O₂/5% CO₂. During the cutting of the brain slices, NaCl in the standard ACSF was replaced by equiosmolar sucrose (256 mM) to prevent neurotoxicity. The brains were sectioned using a vibrating microslicer (Vibratome 1000, TPI, St Louis, Mo., USA). Transverse hippocampal slices (400 μ m thick) were allowed to recover for at least 1 h at 32 °C and then kept at room temperature. Slices were then transferred one at a time to a submersion recording chamber where they were superfused at room temperature with ACSF at about 2 ml/min. All drugs and salts were purchased from Sigma (Strasbourg, France).

Immunoblot analysis

Protein concentration of tissue homogenates was assessed by the bicinchoninic acid assay (Pierce, Rockford, IL, USA). Western blot analyses were performed as already described (Barry and Prusiner 1986) by using the RO73 polyclonal-PrP antiserum (Taraboulos et al. 1992) at 1:5000 dilution and developed with the enhanced chemiluminescent detection method (Amersham Life Science).

Electrophysiological recordings

Extracellular field recordings were made in brain slices from 6- to 18-month-old mice using glass electrodes containing 1 M NaCl (impedance 5–20 M Ω). Signals were amplified with a DAM-80 amplifier (WPI, Stevenage, UK). To evoke synaptic responses from CA1 region, stimuli (100 μ s, 0.05–0.1 Hz) were delivered through fine bipolar stainless steel electrodes placed in the stratum radiatum. Field responses were filtered at 1 kHz and digitized at 4 kHz on a TL-1 interface (Axon Instruments, Foster City, Calif., USA). Data were processed on- and off-line using custom-written software (Acquis1; Gérard Sadoc, U.N.I.C., Gif-sur-Yvette, France). Input-output relations of the field excitatory postsynaptic potentials (EPSPs) were constructed by delivering ascending series of stimulus intensities and curve fitting to a sigmoidal function:

$$S = \frac{S_{\max}}{1 + \exp \frac{E - E_{50}}{k}}$$

where S_{\max} is the maximal amplitude of the field EPSP slopes, E_{50} the stimulus intensity at which the amplitude of field EPSP slopes is half maximum and k the slope of the function. During the course of these experiments, use of animals groups was interleaved and, to ensure that measures of performance were unbiased, the genotype of the mice was coded and unknown to the tester during both experimental assessment and data analysis. The non-parametric Mann-Whitney U -test was used to determine the significance of differences between different groups, $P < 0.05$ was regarded as significant. For linear regression analysis, the confidence bands were also plotted and the regressions compared. Unless otherwise indicated, data are expressed as mean \pm SEM.

Results

Expression of PrPC in different strains

Figure 1 shows an immunoblot analysis of PrPC expressed in the different mouse lines used during the course of the present study. The figure depicts the increased levels of PrPC expression, from zero in Zrch Prnp^{0/0} to 8-fold over-expression (compared with wild-type) in Tg(MoPrP-A)/Prnp^{+/+}. All PrPC-expressing mice, regardless of their level of expression, displayed the same glycotype ratios. The most abundant glycoform

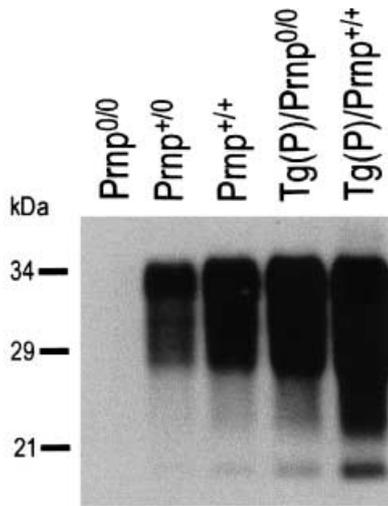


Fig. 1 Expression levels of normal cellular prion protein (PrP^C) expression in a PrP^C-deficient mouse strain, Zrch Prnp^{0/0}, and a transgenic line harbouring the wild-type *MoPrP-A* gene [Tg(MoPrP-A)B4053]. The lanes show Zrch Prnp^{0/0}, Prnp^{0/+}, Prnp^{+/+} and Tg(MoPrP)B4053 with both Zrch PrP-deficient and wild-type alleles (Telling et al. 1996)

was the fully glycosylated form, followed by the mono-glycosylated and the unglycosylated forms.

Basal synaptic transmission in the CA1 area of the hippocampus

To analyse basal synaptic transmission and a form of short-term plasticity, field potential recordings were used to assess the level of synaptic transmission in hippocampal slices. The basal level of synaptic transmission was studied at the Schäffer collateral-CA1 pyramidal cell synapses. The input-output relationships of the field excitatory post-synaptic potentials (EPSPs) were computed in standard external medium containing 100 μ M picrotoxin to measure the efficiency of excitatory synaptic transmission. We first analysed the stimulus/response curves obtained from 6-month-old Zrch Prnp^{0/0} mice. Figure 2 illustrates the relationship obtained in wild-type (Prnp^{+/+}) and Zrch Prnp^{0/0} with and without the *MoPrP-A* transgene. Both Tg(MoPrP-A)Prnp^{+/+} and Tg(MoPrP-A)Prnp^{0/0} exhibited greater field EPSPs than Prnp^{+/+} (Fig. 2A) or Prnp^{0/0} (Fig. 2B), respectively. Fitting sigmoidal curves to the pooled values from the same genotype yielded the two characteristic parameters S_{\max} and E_{50} . From the mean normalized curves given in Fig. 2A, E_{50} was 20.4 ± 3.8 V ($n=4$) and 18.5 ± 1.7 V ($n=6$) for Prnp^{+/+} and Tg(MoPrP-A)Prnp^{+/+} mice (Fig. 2A) respectively and 16.1 ± 2.2 V ($n=4$) and 12.3 ± 0.09 V ($n=5$) for Prnp^{0/0} and Tg(MoPrP-A)Prnp^{0/0} mice (Fig. 2B), respectively. Similarly, S_{\max} was 0.51 ± 0.05 V/s ($n=4$ Prnp^{+/+} slices) and 0.61 ± 0.08 V/s [$n=6$, Tg(MoPrP-A)Prnp^{+/+} slices] and for data given in Fig. 2B, 0.27 ± 0.05 V/s ($n=4$ Prnp^{0/0} slices) and 0.44 ± 0.07 V/s ($n=5$ Tg(MoPrP-A)Prnp^{0/0} slices). Interestingly, field responses were greater in all

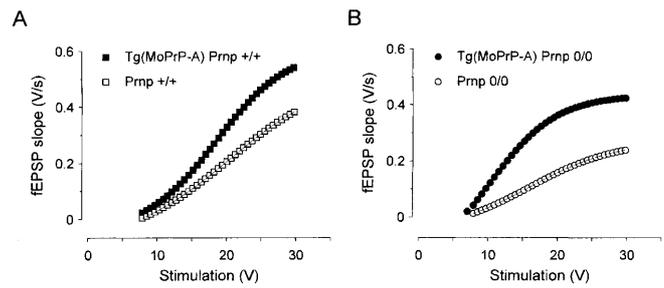


Fig. 2A, B Increased synaptic responses from CA1 hippocampal neurons in PrP^C-expressing animals. Field potential recordings from the CA1 region of 6-month-old mice expressing PrP^C or not (Zrch Prnp^{0/0}). Extracellular responses evoked by afferent stimulation delivering ascending series of stimulus intensities were recorded in the stratum radiatum to monitor field excitatory post-synaptic potentials (field EPSPs). The illustrations show the relationships between field EPSP slopes and stimulus intensity for Prnp^{+/+} (A) or Prnp^{0/0} animals (B) harbouring (filled symbols) the mouse *PrP-A* transgene or not (open symbols). The curves show the fit of the mean data collected from six slices for each of three Tg(MoPrP-A)Prnp^{+/+} and four Prnp^{+/+} mice (A) and from five slices from three Tg(MoPrP-A)Prnp^{0/0} and three Prnp^{0/0} mice (B)

genotypes than in the knock-out animals [S_{\max} : $P < 0.02$, $P < 0.05$, $P < 0.03$ for Prnp^{+/+}, Tg(MoPrP-A)Prnp^{0/0} and Tg(MoPrP-A)Prnp^{+/+} respectively]. Moreover, increasing the level of PrP expression increased also synaptic transmission compared with other animals (E_{50} : $P < 0.01$ and 0.02 respectively for Prnp^{+/+} and Tg(MoPrP-A)Prnp^{+/+} vs. Tg(MoPrP-A)Prnp^{0/0}). These results show that field EPSP responses are greater in presence of PrP^C. Taken together, these findings indicate that the range of excitatory synaptic potential responses in the stratum radiatum of the CA1 region is related to the level of PrP^C expression.

It is noteworthy that with short electrical stimulation applied in the Schäffer collateral, extracellular recordings reveal field potentials with two separable components: a fast, short component, reflecting action potentials in the activated axons (also called the fibre volley, indicated by the arrow in Fig. 3) and a slower component that could be abolished by removing external calcium or after application of the glutamate ionotropic receptor antagonist, kynureinate (not shown). This slow component represents the field EPSP. Thus, it is possible to use the amplitude of the presynaptic fibre volley to estimate the strength of afferent inputs and thereby compare the synaptic input/output values more directly from slice to slice. As shown in Fig. 3A and B, such normalized input-output functions show that the slope of field EPSP elicited by a given presynaptic fibre volley was larger in presence of the transgene (filled symbols) than in its absence (open symbols). Indeed, the slope of the input-output function from Tg(MoPrP-A)Prnp^{+/+} mice is significantly higher than the one obtained from Prnp^{+/+} mice (Fig. 3A; $P < 0.02$), with mean slopes of 1.14 ± 0.06 ms⁻¹ ($n=5$) and 0.91 ± 0.04 ms⁻¹ ($n=4$), respectively. Similarly, the slope of the normalized function from Tg(MoPrP-A)Prnp^{0/0} mice (1.13 ± 0.04 ms⁻¹; $n=4$) is higher com-

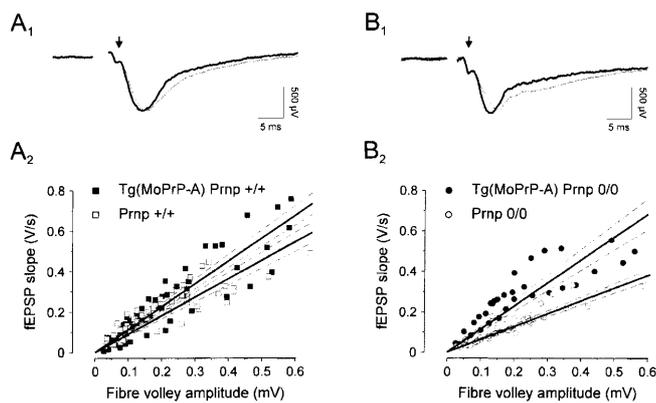


Fig. 3A, B Increased normalized input-output functions in PrPC-expressing animals. Both graphs show that synaptic transmission is stronger in 6-month old PrP-expressing animals than in wild-type (A) or Zrch Prnp^{0/0} animals (B). **A1** For an equal fibre volley amplitude (arrow), a larger field synaptic response was obtained in Tg(MoPrP-A)Prnp^{+/+} (thick line) than in Prnp^{+/+} (thin line) mice. Each trace is the mean of five responses. **A2** Mean slope of normalized functions from five experiments in Tg(MoPrP-A)Prnp^{+/+} (filled squares) and four experiments in Prnp^{+/+} (open squares) mice. **B1** Larger field responses are recorded in the presence of the transgene (thick line; traces are averages of five responses). **B2** Mean slope of normalized functions from four experiments in both Tg(MoPrP-A)Prnp^{0/0} (filled circles) and Prnp^{0/0} (open circles) mice. In **A2** and **B2**, the thick and the dotted lines represent mean and 95% confidence limits of the pooled data, respectively

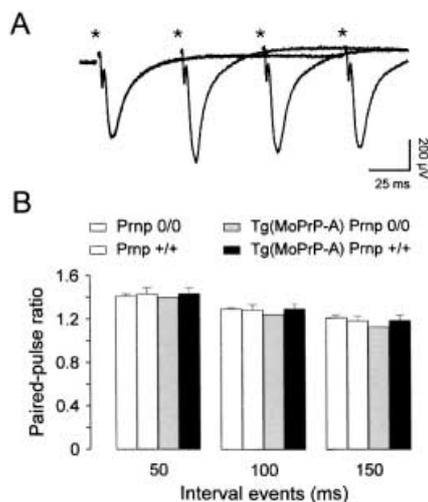


Fig. 4A, B Paired-pulse facilitation is not influenced by PrPC expression. Paired-pulse facilitation was measured on 6-month-old wild-type, Zrch Prnp^{0/0} and Tg(MoPrP-A) mice. **A** Representative traces (average of five traces) in which two consecutive fields EPSPs were elicited with different time intervals are superimposed. Stars indicate the stimulation time. **B** The columns represent the mean ratio between the second and the first field EPSP (paired-pulse ratio) measured for three different interstimulus intervals

pared than that from Prnp^{0/0} animals ($0.63 \pm 0.02 \text{ ms}^{-1}$; $n=4$; $P < 0.005$) (Fig. 3B).

This first set of experiments indicates that synaptic transmission is strengthened in mice expressing PrPC. To investigate whether this could result from an increase in

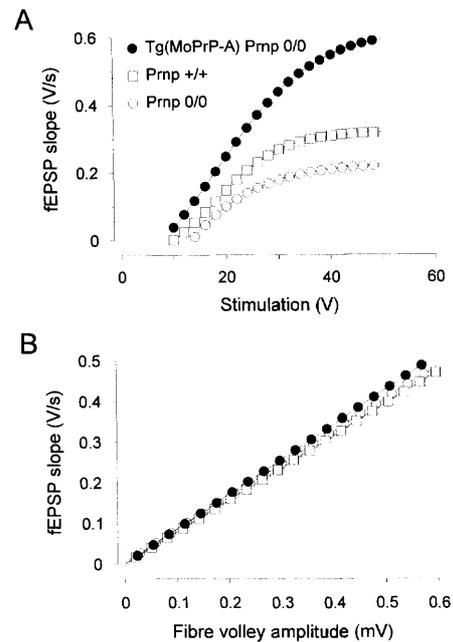


Fig. 5A, B Enhanced synaptic response ranges of PrPC-expressing CA1 hippocampal neurons from old animals is not accompanied by a change in synaptic strength. **A** Input-output curves showing field EPSP slopes as a function of stimulus intensity in 10- to 14-month-old Zrch Prnp^{0/0}, wild-type and MoPrP-A over-expressing mice. The EPSP slope is higher at any given intensity in animals expressing the Prnp transgene (filled symbols) than in its absence (open symbols). Curves show the fit of the mean data from four Prnp^{+/+} slices, ten Tg(MoPrP-A)Prnp^{0/0} slices and six Prnp^{0/0} slices. **B** When the input-output functions were normalized with respect to fibre volley amplitude, the field synaptic responses were identical in Tg(MoPrP-A)Prnp^{0/0} ($n=9$), Prnp^{0/0} ($n=5$) and Prnp^{+/+} ($n=4$) animals

the probability of transmitter release (pr), we assessed the degree of paired-pulse facilitation (PPF) for the different groups of mice. PPF is a form of short-term plasticity that depends on presynaptic properties and is inversely correlated with pr (for review, see Zucker, 1989). As previously described by others, facilitation decreased when interval events increased from 50 to 150 ms. Figure 4 shows no changes in PPF across animal groups over an interpulse interval range of 50–150 ms ($P > 0.4$). For example, when measured at 50-ms intervals, the mean facilitation ratio was 1.41 ± 0.02 ($n=4$ slices) from three Prnp^{0/0} mice; 1.43 ± 0.05 ($n=5$ slices) from four Tg(MoPrP-A)Prnp^{+/+} mice and 1.43 ± 0.06 ($n=5$ slices) from three Prnp^{+/+} mice. This suggests that PrPC does not influence short-term plasticity as measured by a paired-pulse protocol.

Together, these results demonstrate that synaptic transmission is strengthened by PrPC expression, apparently dose dependently, and that these changes occur without change in pr . As Zrch Prnp^{0/0} animals present some mild progressive neurodegenerative changes, we tested whether this PrPC-mediated effect could be influenced by age, using older animals from the same groups (10–14 months old). As for the young animals, input-output relations of excitatory synaptic transmission were

computed to measure the efficiency of basal synaptic transmission. From the mean normalized curves (Fig. 5A), S_{\max} was 0.23 ± 0.04 V/s ($n=6$) and 0.32 ± 0.06 V/s ($n=4$) for $Prnp^{0/0}$ and $Prnp^{+/+}$ mice respectively and 0.62 ± 0.09 V/s ($n=10$) for $Tg(MoPrP-A)Prnp^{0/0}$ mice. Similarly, E_{50} was 17.4 ± 2.8 V ($n=6$) and 18.2 ± 1.6 V ($n=4$) for $Prnp^{0/0}$ and $Prnp^{+/+}$ mice respectively and 23.6 ± 3.3 V ($n=10$) for $Tg(MoPrP-A)Prnp^{0/0}$ animals. Again, the level of PrP expression correlated with the increase in synaptic strength [S_{\max} : $P < 0.003$ and 0.03 respectively for $Prnp^{0/0}$ and $Prnp^{+/+}$ vs. $Tg(MoPrP-A)Prnp^{0/0}$]. However, in contrast to the younger animals (Fig. 3), the slope of the normalized input-output functions was similar between all different genotypes (Fig. 5B; $P > 0.05$) with a mean slope of 0.80 ± 0.21 ms⁻¹ ($n=5$), 0.79 ± 0.13 ms⁻¹ ($n=4$) and 0.85 ± 0.19 ms⁻¹ ($n=9$) for $Prnp^{0/0}$, $Prnp^{+/+}$ and $Tg(MoPrP-A)Prnp^{0/0}$ mice, respectively. Therefore, contrary to young animals, the increase in the range of synaptic responses seen in older animals expressing high levels of PrP^C does not depend on a modification of the synaptic transmission efficacy.

As the phenotype differs among different PrP-deficient lines (Nishida et al. 1999; Sakaguchi et al. 1996), we examined whether some of these effects could be influenced by the *Prnp*^{0/0} allele used. For this purpose, 16- to 18-month-old *Ngsk Prnp*^{0/0} mice were analysed for their synaptic responses in the presence or absence of the *MoPrP-A* transgene. Similar to the *Zrch PrP*-deficient animals, *Ngsk Prnp*^{0/0} mice showed smaller field EPSP responses than *Tg(MoPrP-A)Ngsk Prnp*^{0/0} (Fig. 6A). From the pooled data, S_{\max} was 0.32 ± 0.04 V/s ($n=5$) and 0.46 ± 0.03 V/s ($n=5$) for *Prnp*^{0/0} and *Tg(MoPrP-A) Ngsk Prnp*^{0/0} mice respectively ($P < 0.03$); E_{50} was 17.7 ± 2.6 V ($n=5$) and 11.3 ± 0.8 V ($n=5$) for *Prnp*^{0/0} and *Tg(MoPrP-A) Ngsk Prnp*^{0/0} mice ($P < 0.03$), respectively. Thus, expression of PrP^C appears to increase the range of the synaptic strength independently of the *Prnp*^{0/0} allele used [$P < 0.03$ and 0.04 for S_{\max} and E_{50} respectively for *Prnp*^{0/0} vs. *Tg(MoPrP-A) Ngsk Prnp*^{0/0}, data not shown]. However, as shown in Fig. 6B, when field responses corresponding to the same fibre volley amplitude were superimposed, the accompanying synaptic response was larger in *Prnp*^{0/0} than in *Tg(MoPrP-A) Ngsk Prnp*^{0/0} mice. Measurements of the field EPSP slope for a fibre volley amplitude of 100 μ V indicated a significant difference between *Prnp*^{0/0} (mean slope 1.98 ± 0.04 ms⁻¹; $n=5$ slices from 4 mice) and *Tg(MoPrP-A)Prnp*^{0/0} (mean slope 1.42 ± 0.09 ms⁻¹; $n=7$ slices from 4 mice; $P < 0.0005$); this was true for all values of fibre volley amplitude (see Fig. 6B). Thus, synaptic transmission seems to be more efficient for a given number of axons activated in old *Ngsk Prnp*^{0/0} mice, whereas it is more difficult to recruit axonal fibres in these animals. Similar to the *Zrch* transgenic groups, no changes in PPF could be seen between these animal groups over an interpulse interval of 50–150 ms (data not shown). For example, when measured at 50 ms interval, the mean facilitation ratio was 1.36 ± 0.07 ($n=5$ slices) from three *Prnp*^{0/0} mice and 1.37 ± 0.15 ($n=5$ slices) from three *Tg(MoPrP-A)Prnp*^{0/0}

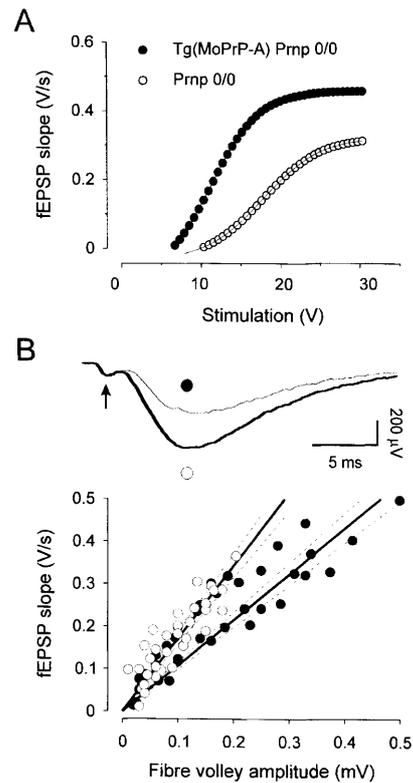


Fig. 6A, B Increased field EPSP responses in hippocampal CA1 neurons expressing PrP^C in 18-month-old *Ngsk Prnp* mice. **A** The field EPSP slope at a given stimulus intensity is higher for animals expressing the *Prnp* transgene (filled symbols) than in its absence (open symbols). The curves are fits of the mean data from five slices from three *Tg(MoPrP-A)Prnp*^{0/0} and five slices from three *Prnp*^{0/0} mice. **B Inset**: for equal fibre volley amplitude (arrow), a larger field synaptic response was obtained in *Prnp*^{0/0} mice. Sample recordings from *Prnp*^{0/0} and *Tg(MoPrP-A)Prnp*^{0/0} mice (each trace is an average of five responses). **Lower panel**: normalized field input-output relationship for *Prnp*^{0/0} (open symbols; five slices from four mice) and *Tg(MoPrP-A)Prnp*^{0/0} (filled symbols; seven slices from four mice) animals

mice, indicating that the modification of synaptic transmission efficacy was not accompanied by a change in the probability of transmitter release.

Discussion

In this study, we compared both the range and strength of excitatory synaptic transmission in the CA1 region of the hippocampus, using different mouse lines expressing various levels of PrP^C. The range of excitatory synaptic responses correlated with the level of PrP^C expression, independently of the *Prnp*^{0/0} mouse line used (*Zrch* vs. *Ngsk* lines) and the age of the animals used. On the other hand, the synaptic strength, as measured by normalizing the input-output curves, did not correlate with PrP^C levels, as opposite effects were found in different age groups. In view of these results, it may be concluded that, in contrast to young animals (6 months), older animals (12–14 months) are less able to respond to electri-

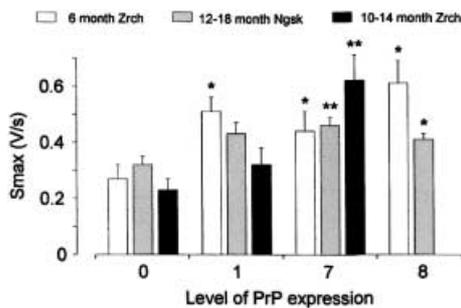


Fig. 7 Increased synaptic responses of CA1 hippocampal neurons in PrPC-expressing animals. Maximal field potentials (S_{max}) recorded in PrPC-deficient mice or mice expressing PrPC at various levels (1- to 8-fold that of wild-type FVB animals). Experimental groups were obtained from the breeding of two different $Prnp^{0/0}$ lines (Zrch or Ngsk) with Tg(MoPrP-A) mice and analysed at 6–18 months of age. * $P < 0.05$; ** $P < 0.01$ vs. control $Prnp^{0/0}$ animals from the same line

cal stimulation at low levels of PrPC. On the other hand, responses originating from older fibres had a higher synaptic strength. In all of these groups, neither the age nor the PrPC levels seemed to affect the probability of glutamate release as assessed with the paired-pulse protocol.

The first observable effect linked to PrPC expression was found in the input-output functions, in which S_{max} increased with increasing PrPC expression. Interestingly, this finding was common to all lines examined, revealing a trend upward by the maximum amplitude of synaptic responses as the level of expression of PrPC increased (Fig. 7). These changes were accompanied by a reduction in the effective stimulus intensity required to evoke field responses near threshold levels. The high stimulus intensity required to evoke field EPSPs at threshold values with low levels of PrPC could result from a reduction in the number of fibres and/or their excitability. Similarly, the reduction of field potential responses induced by low levels of PrPC could be explained by a marked cell loss from the CA1 area, as there are fewer cells contributing to extracellular current.

To assess the strength of excitatory synaptic transmission directly and independently of fibre recruitment by the stimulating electrodes, input-output curves were normalized with respect to the fibre volley amplitude. This normalization clearly demonstrated that in 6-month-old animals, maximal field potential responses in presence of high levels of PrPC result from a more robust synaptic transmission. These data agree with previous studies localizing PrPC at the neuropil (Taraboulos et al. 1992), together with the presynaptic vesicle protein synaptophysin (Fournier et al. 1995), suggesting that PrP may be involved in the regulation of synaptic vesicle release. This hypothesis has been supported further by recent observations using biochemical, immunohistochemical and electrophysiological approaches confirming the synaptic localization of PrPC and its ability to modulate synaptic transmission (Herms et al. 1999).

PrPC-mediated effects on synaptic strength appeared to be modulated by age. Whereas high levels of PrPC increased the excitatory synaptic transmission in 6-month-old transgenic mice, no effect could be seen in older animals (10–14 months old) from the same mouse background (Zrch). Thus, in the absence (or low level) of PrPC, the range of synaptic response amplitudes was considerably reduced, but the synaptic strength was increased with age, from 6- to 12-month-old animals. This age-dependent effect could reflect the presence of some plasticity in the hippocampal synaptic transmission, induced in a state where low levels of PrPC are expressed. This mechanism, by which synaptic transmission is strengthened to compensate for the small amplitude in synaptic responses, could be exploited to restore functionality to compromised neuronal networks, as previously reported for other systems (for a recent review, see Turrigiano 1999). The reduction in amplitude of evoked synaptic responses could result from a loss of fibres, as neurodegenerative changes have now been associated with both the Zrch $Prnp^{0/0}$ and Ngsk $Prnp^{0/0}$ lines (Nishida et al. 1999).

Differences observed between the Zrch and Ngsk backgrounds could also be explained by the expression of a PrP-related protein, designated Dpl and encoded by the *Prnd* locus situated 16 kbp downstream from *Prnp* (Moore et al. 1999). This protein, present at very low levels in the normal brain, is up-regulated in Ngsk and Rcm0 $Prnp^{0/0}$ lines and may be responsible for the degenerative cerebellar changes (Purkinje cell loss) observed in these lines (Moore 1997; Sakaguchi et al. 1996). The up-regulation appears to result from a gene-targeting artefact, as the deletion of the *Prnp* exon3 splice acceptor may favour the formation of aberrant intergenic splicing events between *Prnp* and *Prnd*. Whether *Dpl* participates in spontaneous or prion-induced neurodegenerative process remains to be verified, but the conservation of structural motifs between PrP and Dpl suggests that these two proteins may interact somewhat (dimerization, heterodimerization) or compete for a common ligand (Moore et al. 1999). In this context, the over-expression of Dpl in the Ngsk $Prnp^{0/0}$ mice could conceivably antagonize some of the normal functions performed by PrPC. So far, we do not have any clear interpretations of these results but, in the absence of PrPC, a specific loss of functional fibres characterized by a low synaptic strength could explain our findings. We do not know if this results from axon degeneration, anatomical change in the location of the fibres and/or changes of their excitability. More experiments are needed to clarify this issue.

Field recordings from all transgenic mice also have shown a normal PPF, suggesting that the mechanisms of transmitter release, at least during the low-frequency stimulation we used (i.e. 0.05 Hz) are largely unaffected by the level of PrPC expression at all ages (i.e. 6- to 12- and 18-month-old mice). These results suggest that, whatever the role of PrPC might be in vivo, its influence on synaptic transmission may be modulated by its ex-

pression level. The use of the conditional PrP^C expression system (Tremblay et al. 1998) should allow us to test this hypothesis by studying electrophysiological parameters discussed above, over the time course of repression and induction of PrP.

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