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Lack of influence of prion protein gene expression on kainate-induced seizures in mice: studies using congenic, coisogenic and transgenic strains

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Abstract

Prion protein (PrP) is a glycosylphosphatidylinositol (GPI) anchored cell surface protein expressed by many cells, including those of the mammalian nervous system. At present the physiologic functions of PrP remain unclear. Deletion of *Prnp*, the gene encoding PrP in mice, has been shown to alter normal synaptic and electrophysiologic activities, indicating a potential role in seizure susceptibility. However, published efforts to link PrP with seizures, using both in vivo and in vitro models, are conflicting and difficult to interpret due to use of various mouse backgrounds and seizure induction techniques. Here we investigated the role of PrP in kainic acid (KA)-induced seizure sensitivity, using three types of mice. In contrast to previous published results, *Prnp*^{−/−} mice on the C57BL/10SnJ background had a significant decrease in KA-induced seizure susceptibility. In genetic complementation experiments using a PrP-expressing transgene, genes derived from strain 129/Ola, which flanked the *Prnp*^{−/−} locus in C57BL/10SnJ mice, rather than *Prnp* itself, appeared to account for this effect. Furthermore, using coisogenic 129/Ola mice differing only at *Prnp*, this difference was not reproduced when comparing PrP-negative and PrP-positive mice. In contrast, substrains of PrP-expressing C57BL mice, showed large variations in KA-induced seizure sensitivity. The magnitude of these differences in susceptibility was larger than that associated with the presence of the *Prnp* gene, suggesting extensive influence of genes other than *Prnp* on seizure sensitivity in this system.

Keywords

prion; PrP; kainic acid; seizures; *Prnp*; flanking genes; knockout mice

INTRODUCTION

Prion protein (PrP) is expressed in most cell types in all mammalian species, and is known to be essential for susceptibility to prion diseases (also known as transmissible spongiform encephalopathies or TSE diseases) (Bueler et al., 1992). The normal physiological role for PrP remains unclear, though many studies point to a role in neuronal function (Collinge et al., 1994, Aguzzi et al., 2008, Llorens and Del Rio, 2012). PrP null (*Prnp*^{−/−}) mice were

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previously found to be deficient in hippocampal spatial memory (Criado et al., 2005) and also showed reductions in paired pulse facilitation and long-term potentiation in the dentate gyrus (Collinge et al., 1994, Colling et al., 1996, Colling et al., 1997, Curtis et al., 2003, Criado et al., 2005). In addition, lack of PrP has been shown to affect normal physiology of the glutamatergic synapse (Khosravani et al., 2008, Pathmajeyan et al., 2011). Furthermore, deletion of PrP using a Cre-loxP mouse system resulted in reduction of afterhyperpolarization potentials, suggesting a direct role for PrP in the modulation of neuronal excitability (Mallucci et al., 2002). In summary, lack of PrP may have an influence on synaptic electrophysiology and/or epileptiform activity.

Seizure susceptibility of Prnp^{-/-} mice has previously been studied independently by 2 groups which found Prnp^{-/-} mice to be more susceptible than Prnp^{+/+} controls to kainic acid (KA)-induced seizures (Walz et al., 1999, Rangel et al., 2007). In contrast, using ex vivo hippocampal slices exposed to pentylentetrazol, bicuculline or zero-magnesium conditions, tissue from Prnp^{-/-} mice was less susceptible to induction of spontaneous epileptiform activity compared to tissue from Prnp^{+/+} controls (Ratte et al., 2011). Variations in protocols and/or the use of mice with different genetic backgrounds might explain these results. Indeed, susceptibility to induced seizures in mice is known to be influenced by multiple genes, many of which have not been identified but still might contribute to experimental studies of specific genes such as Prnp (Schauwecker, 2011).

Because of possible problems with control mice and Prnp^{-/-} flanking genes in the above-mentioned in vivo studies, as well as the conflicts in the conclusions of the in vivo and ex vivo experiments, we decided to re-examine sensitivity to KA-induced seizures using more appropriate controls for possible variations between Prnp^{+/+} versus Prnp^{-/-} mice. In the present work we used both congenic and coisogenic Prnp^{-/-} mice versus Prnp^{+/+} control mice. In our initial experiments using congenic mice on the C57BL/10SnJ background, by three criteria Prnp^{-/-} mice were less susceptible to KA-induced seizures, which conflicted with the published literature. To eliminate the effects of possible variations in genes flanking the Prnp^{-/-} locus, we tested KA-induced seizure sensitivity in Prnp^{-/-} and Prnp^{+/+} mice using coisogenic 129/Ola mice, but found minimal significant differences related to Prnp expression. We also used genetic complementation in a transgenic mouse line expressing the Prnp^{-/-} genotype with or without a transgene (tga20) expressing PrP. Failure to rescue the Prnp^{+/+} phenotype by the PrP transgene demonstrated that the altered phenotype in C57BL/10SnJ Prnp^{-/-} mice was not due to the lack of PrP expression, but rather was explained by the influence of non-Prnp genes flanking the Prnp^{-/-} locus.

EXPERIMENTAL PROCEDURES

Animals

All mice were housed at the Rocky Mountain Laboratories (RML) in an AAALAC-accredited facility and experimentation followed NIH RML Animal Care and Use Committee approved protocols (NIH/RML Protocol #2010-10). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Research Council. Mice were housed individually for at least 24 hours prior to experimentation in a temperature-controlled environment (22°C) under a 12 h light/dark cycle and allowed free access to food and water.

C57BL/10SnJ mice were originally obtained from Jackson Laboratories (Bar Harbor, Maine) and have been maintained at RML for several years as an inbred colony. C57BL/10J and C57BL/6J mice were obtained from Jackson Laboratories. C57BL/10Hsd mice were obtained from Harlan Sprague Dawley, Madison, WI.

C57BL10/SnJ-Prnp^{-/-} mice were created at RML by serially backcrossing 129/Ola-Prnp^{-/-} mice (Manson et al., 1994) to C57BL10/SnJ mice for nine generations (Chesebro et al., 2010). At each generation mice carrying the Prnp null allele were selected by PCR analysis (Chesebro et al., 2005) for further backcrossing. Following the ninth backcross mice hemizygous for the Prnp gene were interbred and homozygous Prnp null offspring were selected and bred to establish the current C57BL/10SnJ Prnp^{-/-} line. Analysis of 1449 SNPs (Taconic Laboratories) showed that a 47.4 Mb region flanking the Prnp gene on chromosome 2 was still of the original 129/Ola strain.

Transgenic tga20 mice homozygous for the “half-genomic” PrP DNA transgene and for the Zürich version of the Prnp^{-/-} gene (Fischer et al., 1996) were obtained from EMMA (Munich, Germany) on a mixed background of C57BL/6 and 129/S7. At RML these mice were backcrossed for 5 generations to C57BL/10SnJ-Prnp^{-/-} mice (Chesebro et al., 2010), which were homozygous for the Prnp^{-/-} gene developed in Edinburgh (Manson et al., 1994). At each backcross PCR was used to identify and select mice with the tga20 transgene and the Edinburgh-Prnp⁻ allele. At the fourth and fifth backcrosses tga20 transgene-hemizygous (TgPrP^{+/-}) and tga20 transgene-negative (TgPrP^{-/-}) littermates were identified by PCR and used for seizure experiments at the appropriate age. These mice were homozygous for the Edinburgh Prnp^{-/-} genotype and for the adjoining flanking genes from 129/Ola mice. Their background genes were 93.8–97.0% C57BL/10SnJ as determined by SNP analysis.

129/Ola Prnp^{-/-} and 129/Ola Prnp^{+/+} mice (Manson et al., 1994) were obtained from Dr. Jean Manson at the Roslyn Institute, Edinburgh, UK and bred and maintained at RML. These mice are co-isogenic as they are genetically identical to the parental strain with the exception of a genetic change at a single locus (Prnp). Due to breeding difficulties the 129/Ola-Prnp^{-/-} mice were bred to 129/Ola-Prnp^{+/+} mice to improve fertility and litter size. Hemizygous 129/Ola-Prnp^{+/-} mice from this breeding scheme were then bred to each other and weanlings were genotyped by PCR (Chesebro et al., 2005) to select Prnp^{-/-} and Prnp^{+/+} mice to be used for experimentation.

In order to assess the potential influence of gender, data was analyzed and presented separately for males and females. Because age is known to influence kainic acid susceptibility in mice (McCord et al., 2008), Prnp^{+/+} and Prnp^{-/-} adult mice of similar ages were compared.

Administration of KA and analysis of behavioral response

Naturally derived kainic acid (KA) monohydrate, extracted from *Digenea simplex* (Sigma-Aldrich, K2389, Missouri) was dissolved in phosphate-buffered saline (pH 7.4) and administered intraperitoneally (i.p.) for induction of seizures. KA was stored at 4°C protected from light, and KA solutions were prepared fresh on the day of each experiment. In the course of these studies, five different lots of KA were used. Each lot of KA was assessed by comparing seizure induction in mice of the same wild type strain. No differences in seizure scores or doses required were noted, and results were pooled for the analyses presented.

Previous papers studying the effect of Prnp expression on sensitivity to KA-induced seizures used two different KA administration protocols. One group used a single intraperitoneal (i.p.) injection protocol at a dose of 10 mg/kg (Walz et al., 1999), and the other group used an injection protocol with 4 i.p. doses of 8mg/kg at 30 minute intervals (Rangel et al., 2007). In our initial experiments with the single dose protocol at various doses, we had difficulties reproducibly inducing stage 5 seizures in mice without inducing a high percent of death. However, the multiple injection protocol consistently induced a high incidence of stage 5

seizures with a minimum number of fatalities. Importantly, the use of this multiple injection protocol allowed for a direct comparison of our results with those of the previous study where Prnp expression and genetic backgrounds appeared to be accurately controlled (Rangel et al., 2007). Thus all strains of mice received 4 doses of KA (1mg/ml) at 8mg/kg at times 0, 30, 60 and 90 minutes.

After the first KA injection at time = 0 min, mice were placed back in their original cages and observed for changes in behavior and/or seizures over a 240 minute total observation period. Mice were removed for re-injection of KA at 30, 60, and 90 minutes respectively. Mice were scored using a modified Racine scale behavior score (Racine, 1972, Walz et al., 1999, McLin and Steward, 2006). Stage 1: immobility, facial clonus, staring and panting; stage 2: head nodding, tail rigidity; stage 3: myoclonic jerks, forelimb clonus, hunchback posture; stage 4: discrete rearing and falling seizures, continuous forelimb clonus; stage 5: repetitive rearing/falling or running/bouncing seizures. For each 5 minute interval for 48 intervals over 240 minutes of observation the highest seizure stage reached during the interval was recorded. Mice were euthanized if stage 5 was recorded for five consecutive time intervals. These guidelines were established in consultation with our Institutional Animal Care and Use Committee (IACUC). Observers were blind to mouse genotypes in all experiments. PBS control solution administered intraperitoneally to mice resulted in no detectable behavioral changes.

Data Analysis

The analysis of the seizures induced by KA focused on seizure stages 3, 4 and 5, as these were the most clinically severe stages. Data were analyzed and presented in three ways: (1) The percent of mice in each strain and gender group reaching seizure stage 3, 4 or 5. All the mice in each group, including those euthanized, were included in this analysis. Statistical differences between percentages were determined using Fishers Exact Analysis, and significant p values are presented in figure legends. (2) Average latency was average time in minutes to reach each seizure stage (S3, S4, and S5) for mice in each group. Only mice reaching each stage were included in calculating the average for that stage. (3) The relative amount of time spent displaying severe seizures was calculated for each mouse as the sum of the number of stage 3, 4 and 5 time-points. Mice euthanized due to prolonged stage 5 seizures were excluded from this comparison, and were noted in the figure legend. Statistical tests were done using Graph Pad software, and are indicated in figure legends.

RESULTS

KA seizure induction in PrP knockout mice versus wild-type mice on the C57BL/10SnJ background

To study the effect of PrP on sensitivity to KA-induced seizures, we injected mice intraperitoneally with four 8 mg/kg doses of KA at 30 minute intervals from time = 0 minutes to time = 90 minutes, and mice were evaluated over 48 successive five minute intervals for a total of 240 minutes. This multiple dose protocol was used by previous workers studying the influence of PrP on seizures (Rangel et al., 2007), and in preliminary experiments we found it to be more consistent than single dose protocols in inducing seizures in C57BL mice.

We used three types of data to determine seizure sensitivity. First, we calculated the percent of mice reaching seizure stage 5, 4 and 3. Next we analyzed the time required (i.e. latency) for each mouse to reach the stage in question. Lastly we measured the overall duration of severe seizure activity by calculating the total number of time-points where each mouse was at seizure stage 3, 4, or 5 during the entire 240 minute time of observation.

Analysis of the percent of mice reaching each seizure stage showed no significant differences between the percent of Prnp^{-/-} and Prnp^{+/+} mice of either sex to reach S5 (Fig. 1A), S4 (Fig. 1B) or S3 (Fig. 1C). However, female Prnp^{+/+} mice had significantly shorter latency times to S5 and S4 seizures compared to Prnp^{-/-} females (Figs. 1D, 1E, and 1F). In addition, male Prnp^{+/+} mice had a significantly increased number of time-points with severe seizure activity (S3, S4 or S5) compared to male Prnp^{-/-} mice, but female mice of these genotypes failed to show a significant difference (Fig. 1G). The differences in these parameters might be due to the difference in PrP expression in the Prnp^{+/+} and Prnp^{-/-} mice, but we could not exclude the possible influence of genes flanking the Prnp^{-/-} locus, which were derived from 129/Ola, the strain used to generate the Prnp^{-/-} mice.

KA seizure induction in mice positive or negative for expression of a PrP transgene

To evaluate the role of the genes flanking Prnp in our seizure susceptibility experiments, a genetic complementation experiment was done. C57BL/10SnJ mice with the Prnp^{-/-} genotype, plus the strain 129/Ola flanking genes adjacent to the Prnp^{-/-} allele, were serially backcrossed to tga20 mice expressing the “half-genomic” PrP transgene as described in the methods. Littermates positive or negative for the tga20 PrP transgene were identified after 4–5 backcrosses, and these mice were compared for sensitivity to KA induction of seizures. Since both types of mice had the Prnp^{-/-} genotype and also the flanking 129/Ola genes, they differed in the presence or absence of the PrP expressing transgene (designated by transgene genotypes TgPrP^{+/+} and TgPrP^{-/-}). Study of these mice should identify any possible contribution to seizure sensitivity by the strain 129/Ola genes flanking the Prnp^{-/-} allele.

In these experiments, no significant differences between TgPrP^{+/+} mice versus TgPrP^{-/-} mice of either sex were noted in the percent of mice reaching stages 5, 4 or 3 (Figs. 2A–C) or in the time of latency required to reach each of these stages (Figs. 2D–F). Furthermore, no difference was seen in the overall duration of seizures in TgPrP^{+/+} versus TgPrP^{-/-} mice (Fig. 2G). Thus, expression of a PrP transgene in these Prnp^{-/-} mice was not associated with significantly increased sensitivity to KA-induced seizures. Therefore, the effect seen previously in C57BL/10SnJ mice (Fig. 1G) was likely due to Prnp^{-/-} flanking genes rather than to Prnp itself.

Sensitivity to KA-induced seizures in coisogenic Prnp^{-/-} and Prnp^{+/+} mice on 129/Ola background

As a further investigation of the role of PrP in KA-induced seizures, Prnp^{-/-} and Prnp^{+/+} mice on the 129/Ola background were studied. These mice were constructed using 129/Ola embryonic stem cells and were bred exclusively to 129/Ola mice. Therefore, the genes flanking the Prnp^{-/-} and Prnp^{+/+} loci in these coisogenic mice were identical. Again no significant differences between Prnp^{+/+} and Prnp^{-/-} mice of either sex were noted in the percent of mice reaching stages 5, 4 or 3 (Figs. 3A–C) or in the time of latency required to reach each stage (Figs. 3D–F). However, by analysis of the overall duration of seizures male Prnp^{+/+} mice had a slightly reduced number of time-points at stages 3, 4 or 5 compared to Prnp^{-/-} mice (Fig. 3G). This difference was borderline significant ($p=0.0425$), and was in the opposite direction compared to the difference seen in C57BL/10SnJ mice (Fig. 1G).

Variable KA-induced seizure susceptibility in several substrains of C57BL PrP-expressing mice

To better understand the role of non-Prnp background genes in sensitivity to KA-induced seizures, we tested 4 substrains of closely related male C57BL mice. Comparison of the percent of mice reaching seizure stage 5 indicated that 10SnJ mice had a significantly lower percent reaching stage 5 compared to the other three strains (Fig. 4A). In addition, the

percent of mice requiring euthanasia for persistent stage 5 seizures was significantly lower for 10SnJ and 10J strains compared to the other two strains (Fig.4B). In contrast, the four strains showed no significant differences in the time of latency required to reach stage 5 (Fig.4C). In summary, by two of three measurements shown, male C57BL/10SnJ mice were less sensitive to KA-induced seizures than were the other three substrains. These four C57BL substrains had a common origin, but were maintained separately for many decades. Thus, the development of genetic diversity during the time they were bred separately is likely to explain the differences observed in their sensitivity to KA-induced seizures.

DISCUSSION

In the present study, deletion of the mouse *Prnp* gene had a significant effect in decreasing the duration of KA-induced seizures in male C57BL/10SnJ-*Prnp*^{-/-} mice compared to male C57BL/10SnJ *Prnp*^{+/+} mice (Fig. 1G). Likewise, latency to S5 and S4 seizures was significantly shorter in the comparison of *Prnp*^{+/+} and *Prnp*^{-/-} females (Figs. 2D–F). However, no significant differences were seen between these two genotypes in the percent of mice reaching seizure stages S5, S4, or S3 (Figs. 1A–F). In addition to the inactivated *Prnp* locus, these *Prnp*^{-/-} mice have gene sequences flanking the *Prnp*⁻ locus, which were derived from the original 129/Ola embryo stem cells where the knockout was produced. Therefore, a complementation experiment was done where mice with the *Prnp*^{-/-} genotype and associated 129 flanking genes were bred to express a PrP DNA transgene on a different chromosome than *Prnp*. In this experiment the presence of the PrP transgene did not significantly alter sensitivity to KA-induced seizures (Figs. 2A–G). This result suggested that *Prnp*⁻ flanking genes rather than *Prnp* itself were likely responsible for the effect observed in C57BL/10SnJ mice (Fig.1G).

In additional studies 129/Ola mice with or without an intact *Prnp* locus were compared using the KA-induced seizure protocol. These mice did not have foreign genes flanking the *Prnp*^{-/-} locus because the original knock-out was generated using embryonic stem cells from 129/Ola mice and the cells with the inactivated *Prnp* were grafted into 129/Ola embryos. In this comparison no significant differences were noted between *Prnp*^{+/+} and *Prnp*^{-/-} mice in analysis of the percent of mice reaching seizure stages S5, S4, or S3 or in the time of latency prior to reaching these stages (Figs. 3A–F). However, *Prnp*^{-/-} mice did have an increase in seizure duration compared to *Prnp*^{+/+} mice (Fig. 3G). However, this change was of borderline statistical significance ($P=0.0425$), and was not seen in the other two parameters evaluated, i.e. percent of mice reaching each seizure stage and time of latency to each stage. Thus the difference between *Prnp*^{+/+} and *Prnp*^{-/-} mice on the 129/Ola background did not appear to be robust.

In a previous study, *Prnp*^{-/-} was associated with increased sensitivity to KA-induced seizures (Walz et al., 1999). In this paper, Zürich-*Prnp*^{-/-} mice on a background of mixed C57BL/6J and 129/Sv genes were more sensitive to KA-induced seizures compared with *Prnp*^{+/+} (C57BL/6J X 129/Sv)F1 mice used as controls (Bueler et al., 1992, Walz et al., 1999). Unfortunately, these F1 mice were not well-matched controls for the knockout mice used because the F1 mice were heterozygous for all genes, whereas the *Prnp*^{-/-} mice were mostly homozygous for C57BL/6 genes due to the previous backcrossing to this strain. In addition, any remaining 129 genes might have been homozygous or heterozygous depending on the number of intercrosses done to maintain the knockout line. Therefore, most of the background genes of these *Prnp*^{+/+} and ^{-/-} mice were not matched, making it difficult to draw accurate conclusions from these experiments.

Two additional studies also concluded that a lack of PrP correlated with higher susceptibility to KA-induced seizures (Rangel et al., 2007, Carulla et al., 2011). These studies used a

kainic acid administration protocol similar to the one used in our current experiments. In these studies, *Prnp*^{-/-} mice on a mixed 129/Sv and C57BL/6J background were backcrossed several times to C57BL/6J before intercrossing, and littermates with *Prnp*^{+/+}, *Prnp*^{+/-} and *Prnp*^{-/-} genotypes were compared. However, in these experiments, 129 flanking genes remained linked to the *Prnp*^{-/-} locus, and no genetic experiments were done to exclude their influence. In addition to these flanking genes, two differences between these earlier studies and our own might have influenced the results: First, our experiments used mice with the Edinburgh *Prnp*^{-/-} gene whereas the earlier experiments used mice with the Zürich-*Prnp*^{-/-} genotype. The Zürich and Edinburgh constructs differ in the molecular structure of the altered *Prnp* gene, but both disrupt *Prnp* completely and prevent all PrP expression in the mice. Therefore, there is no reason to expect that these molecular differences within the *Prnp*^{-/-} genes would influence these seizure experiments. Second, the background genes of the mice used were from closely related, but different strains, C57BL10/SnJ in 2 of our experimental systems (Figs. 1 and 2) and C57BL/6J in the earlier studies (Rangel et al., 2007, Carulla et al., 2011).

Others have previously noted differences in seizure sensitivity independent of *Prnp* among a group of C57BL substrains; however, the number and identity of the genes responsible were not identified (McLin and Steward, 2006). In the present paper we also found strong effects of non-*Prnp* genes on sensitivity to KA-induced seizures. By two criteria, C57BL/10SnJ mice were more resistant to seizure induction than were C57BL/6, C57BL/10J and C57BL/10Hsd (Fig.4A and 4B). These results support the possibility that non-*Prnp* background gene differences might also influence detection of effects of PrP expression on susceptibility to KA-induced seizures.

Two previous studies using ex vivo or in vitro systems suggested that *Prnp*^{-/-} mice might be less sensitive to seizure induction. In Ratté et al, *Prnp*^{-/-} hippocampal slices were more resistant to spontaneous epileptiform activity than *Prnp*^{+/+} controls after exposure to pentylenetetrazol, bicuculline or zero-magnesium conditions (Ratte et al., 2011). This study used Zürich *Prnp*^{-/-} mice backcrossed on the FVB background and *Prnp*^{+/+} FBV controls. In a different study using cultured astrocytes from Edinburgh *Prnp*^{-/-} mice backcrossed to C57BL/10SnJ, *Prnp*^{-/-} astrocytes showed more rapid transport of glutamate than *Prnp*^{+/+} astrocytes, which correlated with increased protection against glutamate-induced excitotoxicity (Pathmajeyan et al., 2011). These data suggest that *Prnp*^{-/-} brain cells or tissue might be less sensitive to possible seizure inducing stimuli. However, both of these studies used *Prnp*^{-/-} mice with flanking genes from strain 129 mice, which might also have influenced the outcome.

Although the present experiments were successful in obtaining comparative seizure susceptibility for KA-induced seizures in a variety of *Prnp*^{+/+} and *Prnp*^{-/-} mouse strains, these data did not dissect the possible mechanisms responsible for these results. Any mechanisms in the brain which alter neuronal susceptibility to seizure development might be important. Furthermore, any variables capable of altering the time-course of KA absorption or degradation after i.p. injection might also influence these data. In addition, use of other seizure induction models involving drugs other than KA or involving other stimuli might possibly give different results on the influence of *Prnp*.

Hopefully other researchers attempting future experiments on the function of *Prnp* will exercise diligence in the use of *Prnp*^{-/-} mice. One solution is the use of knockout mice on a line 129 background where the positive and negative mice do not differ in genes flanking the *Prnp*^{-/-} locus. Similar coisogenic mice can now be made on the C57BL/6 background using embryo stem cells from this strain. In cases using knockout genes bred into mice other than the original strain 129 embryo stem cell donor, there will always be strain 129 flanking

genes adjacent to the knocked out gene regardless of how many backcrosses have been done, and one cannot distinguish whether the altered phenotype is due to the targeted knock-out gene or the 129 flanking genes. The best way to circumvent this problem is to do a genetic complementation or rescue experiment (Schauwecker, 2002). In the current paper, a PrP-encoding transgene, which lacks the flanking genes adjacent to the *Prnp*^{-/-} locus, was reintroduced into the *Prnp*^{-/-} mouse (Fig. 2A–G). In this way two groups of *Prnp*^{-/-} mice which differ only in whether they have the PrP-expressing transgene could be compared. Since the PrP transgene did not restore the *Prnp*^{+/+} phenotype, one can conclude that the phenotype of the knock-out was likely caused by adjacent flanking genes of strain 129 origin and not by the *Prnp* deletion itself.

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Highlights

PrP knockout partially reduces kainate seizure sensitivity in C57BL/10SnJ mice.

Reintroduction of PrP-transgene in knockout fails to restore original phenotype.

PrP knockout in coisogenic 129 mice slightly increases kainate seizure sensitivity.

Fig. 1. Comparison of KA-induced seizure sensitivity in C57BL/10SnJ Prnp^{+/+} and C57BL/10SnJ Prnp^{-/-} mice

(A–C) Percent of mice reaching seizure stage S5, S4 and S3 respectively. Prnp^{+/+} and Prnp^{-/-} mice were not significantly different by Fisher's exact analysis. Numbers (n) of mice per group are shown on panel A. (D–F) Mean latency times required to reach each seizure stage (i.e. from time=0 to onset of seizure stages S3, S4 or S5). Bars show standard deviations. Female Prnp^{+/+} mice had significantly shorter latency times to S5 (*p=0.048) and S4 (*p=0.040), but not S3 seizures. Male Prnp^{+/+} and Prnp^{-/-} mice did not differ significantly. Analysis was done by two-tailed Mann-Whitney test. (G) Male C57BL/10SnJ background Prnp^{+/+} mice had significantly more time-points at stages S3, S4 and S5

compared to $\text{Prnp}^{-/-}$ mice (***, $p=0.0006$). Mice requiring euthanasia were excluded from this analysis (2 female $\text{Prnp}^{+/+}$, 1 male $\text{Prnp}^{-/-}$ and 1 female $\text{Prnp}^{-/-}$). Statistics were done by a two-tailed Mann-Whitney test.

Fig. 2. Comparison of KA-induced seizure sensitivity in C57BL/10SnJ Prnp^{-/-} mice with (TgPrP^{+/-}) or without (TgPrP^{-/-}) expression of a PrP transgene (A–C) Percent of mice reaching seizure stage S5, S4 and S3 respectively. TgPrP^{+/-} and TgPrP^{-/-} mice were not significantly different by Fisher's exact analysis. Numbers (n) of mice per group are shown on panel A. (D–F) Mean latency times required to reach each seizure stage (i.e. from time=0 to onset of seizure stages S3, S4 or S5). Bars show standard deviations. TgPrP^{+/-} and TgPrP^{-/-} mice did not differ significantly by two-tailed Mann-Whitney. (G) TgPrP^{+/-} mice were not significantly different from TgPrP^{-/-} mice in the number of time-points at stages S3, S4 and S5. Mice requiring euthanasia were excluded from this analysis (4 male TgPrP^{+/-}, 2 female TgPrP^{+/-} and 1 female TgPrP^{-/-}). Statistics were done by a two-tailed Mann-Whitney test.

Fig. 3. Comparison of KA-induced seizure sensitivity in 129/Ola Prnp^{+/+} and 129/Ola Prnp^{-/-} mice

(A–C) Percent of mice reaching seizure stage S5, S4 and S3 respectively. Prnp^{+/+} and Prnp^{-/-} mice were not significantly different by Fisher's exact analysis. Numbers (n) of mice per group are shown. (D–F) Mean latency times required to reach each seizure stage (i.e. from time=0 to onset of seizure stages S3, S4 or S5). Bars show standard deviations. Prnp^{+/+} and Prnp^{-/-} mice did not differ significantly by two-tailed Mann-Whitney. (G) Male coisogenic 129/Ola Prnp^{+/+} mice had a borderline significantly shorter duration of seizure compared to Prnp^{-/-} mice (*, p=0.0425). Thus these results were opposite to the results

seen in Fig. 1G. No mice in these groups required euthanasia. Statistics were done by a two-tailed Mann-Whitney test.

Fig. 4. Comparison of KA-induced seizure sensitivity in four C57BL mouse strains

Statistical differences were determined using Fisher's two-tailed exact analysis (***, $p < 0.0001$; **, $p = 0.004-0.007$; *, $p = 0.02-0.04$). (A) Percent of mice reaching seizure stage S5. 10SnJ mice were significantly different from 6J, 10Hsd and 10J. Numbers (n) of mice per group are shown. (B) Percent of mice meeting criteria for euthanasia (see methods) prior to completion of the 240 min experiment. 10SnJ were significantly different from 6J, and 10Hsd and 10J were significantly different from both 10Hsd and 6J. (C) Mean latency times (and standard deviations) required to reach stage S5. No significant differences were noted among the 4 strains by 1way-ANOVA (Kruskall-Wallis with Dunn's Multiple Comparison test).