Sensitivity to *Jerky* Gene Dosage Underlies Epileptic Seizures in Mice

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Animals with one deleted *jerky* allele are more susceptible to chemically induced seizures than wild-type mice and display recurrent behavioral seizures. The phenotype of these hemizygotes is characterized by no apparent neurological symptoms other than recurrent seizures reminiscent of human idiopathic epilepsy. The *jerky* gene encodes a 60 kDa protein resembling a number of DNA-binding proteins. Here, we show that the *jerky* gene is expressed in all tissues examined, including brain, liver, lung, spleen, testis, and ovary, and study an apparent paradox of how an allelic deletion of the ubiquitously expressed *jerky* gene can lead to hyperexcitability and seizures but not to other symptoms. We demonstrate that jerky has a dosage-sensitive function (haploinsufficiency) in brain and that this sensitivity to reduced jerky dosage could explain the occurrence of seizures in hemizygotes. However, jerky has a nondosage-sensitive function as well, because the total absence of jerky in homozygotes results in abnormalities of somatic and sexual development. A number of idiopathic epilepsies are dominantly inherited, such as benign familial neonatal convulsions, juvenile myoclonic epilepsy, as well as benign epilepsy with centrotemporal spikes, and the pathomechanism of these epilepsies may be based on haploinsufficiency in the brain.

Key words: epilepsy; seizure; haploinsufficiency; gene dosage; jerky *gene; c-Fos;* in situ *hybridization; PTZ*

In humans, many types of epileptic seizures and epileptic conditions are recognized, presumably reflecting a number of pathophysiological mechanisms. Patients with idiopathic epilepsies show normal intelligence and no neurological symptoms other than seizures, and the disease is unassociated with specific structural pathology. The importance of research focusing on idiopathic epilepsies is that they account for at least 30% of all epilepsies, and most of them are genetic. Understanding the inheritance and pathophysiology of idiopathic epilepsies would greatly facilitate genetic counseling, prevention, and therapy.

Mapping of loci and identifying genes associated with idiopathic epilepsies are difficult for a variety of reasons, including complex trait, small family size, and heterogeneous phenotypes. Juvenile myoclonic epilepsy (JME) is a distinct subform of idiopathic generalized epilepsy with onset in adolescence. Linkage analysis suggests a 6 cM region as a candidate region for the JME gene on chromosome 6 (Durner et al., 1991; Greenberg and Delgado-Escueta, 1993; Liu et al., 1995). Another type of idiopathic epilepsy is benign familial neonatal convulsions (BFNC), which is a dominant disorder associated with markers on chromosome 20 (Leppert et al., 1989). Progressive myoclonus epilepsy (PME) is a heterogeneous group of rare genetic disorders that represents another type of idiopathic epilepsy. Recently, mutations in the cystatin B gene were identified as a cause for a subtype of PME. (Pennacchio et al., 1996). Levels of messenger RNA encoded by this gene were found to be decreased in cells from affected individuals. Cystatin B is a ubiquitously expressed protease inhibitor. It is not known how the near total absence of cystatin B in all cell types and tissues can lead to relatively specific symptoms such as epileptic seizures and ataxia.

Mouse models of genetic epilepsy could facilitate the discovery of pathophysiological processes leading to seizure induction. Recently, a number of knock-out mutant mice displaying seizures have been described (for review, see Noebels, 1996). We have recently generated a transgenic mouse strain called "jerky" that shows recurrent seizures with no detectable brain abnormalities or neurological symptoms other than seizures, resembling human idiopathic epilepsy (Toth et al., 1995). Molecular and genetic studies demonstrated that seizures were caused by insertion of a transgene into a novel cellular gene called "*jerky*" (*Jrk*), and therefore the transgenic mice are essentially knock-out animals for the *jerky* gene. Here, we studied the paradox that a deficiency in the ubiquitously expressed *jerky* gene causes a condition limited to epileptic seizures.

MATERIALS AND METHODS

Mice. C57BL/6J (C57) mice were obtained from Taconic (Germantown, NY). The transgenic mouse line with the *jerky* gene deletion was maintained on C57 background. The genotype of mice was determined by PCR from DNA samples prepared from tails, as described previously (Toth et al., 1995).

Seizure susceptibility measured by penthylenetetrazol (PTZ) test. Mice were injected with 30 or 50 mg/kg PTZ intraperitoneally, and seizure events were videotaped during an observation period of 20 min by one of the authors (J.G.). The videotapes were reviewed later by another investigator (C.H.), who was blinded to the genotype of the animal. Mice were scored for the sequence of four seizure behaviors produced by the drug (myoclonic jerks, clonic convulsions, tonic phase, and death). A human-guided computer-assisted scoring system was used to evaluate seizures. The timing of the seizure behaviors, as well as their duration after the PTZ injection, was recorded. The computer program contains a clock that was started at the time of injection of each animal as observed on the

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Table 1. Increased susceptibility of $jrk^{+/-}$ mice to PTZ-induced seizures

	30 mg/kg PTZ		50 mg/kg PTZ	
	Wt	$Jrk^{+/-}$	Wt	$Jrk^{+/-}$
Number of animals displaying clonus	4/10 (40%)	8/13 (62%)	11/12 (92%)	11/12 (92%)
Mean number of clonic events ±SEM/animal	0.5 ± 0.2	3.9 ± 1.2	1.5 ± 0.2	3.2 ± 1.1
Significance (median test)	<0.05		NS	
Death	0/10 (0%)	1/13 (7.7%)	1/12 (8.3%)	8/12 (66.7%)
Significance (Fisher's exact test)	NS		<0.0075	
Percent of time observed in clonus	0.6	4.7	2.2	9.5
Significance (χ^2)	<0.001		<0.0001	

NS, Not significant.

videotape. The occurrence of the first seizure behavior, myoclonus, was recorded by touching a key on the computer keyboard. Myoclonus was defined as a single movement of the mouse that involved a downward motion of the head, combined with a single jerk of the body, and a brief upward extension of the tail. Exploratory or resting behavior was present before the myoclonus and was resumed after it occurred. All myoclonic events could be recorded in this manner by a designated computer input key, and their timing and frequency after the injection could be analyzed. Clonus was usually the second seizure behavior to occur chronologically after PTZ injection. It was defined as rapidly repetitive jerks of the mouse that involved the entire body such that the mouse would fall to the side. At times, the clonus was so forceful that the animal would be propelled around the cage. Clonus would begin out of resting or exploratory behavior and when it stopped, the animal would right itself and rest. The beginning of clonus was recorded by a designated computer input key as the onset of repetitive jerks. The offset of clonus was recorded by another designated computer input key as the moment the repetitive jerks stopped and the animal righted itself and rested or resumed exploratory behavior. In this manner, the timing of clonus after injection, as well as the duration and number of clonic seizures, could be determined accurately. Scoring clonus was less subjective and therefore more reliable than scoring myoclonus, but overall the two scores matched. Because the number of clonic events reflected susceptibility better and more reliably, only these data are included in Table 1 and Figure 1. Differences between groups of mice in the number of clonic events was determined by the Median test, used for analysis of groups of nonparametric data. The tonic stage of the seizure, when reached, was defined as a slow hindlimb extension, and its onset was recorded by another designated computer input key. In these mice, the tonic stage was invariably followed by death, the timing of which was recorded by another designated computer input key. The total observation time, if shortened from 20 min by death of the animal, could be determined accurately. Because males and females did not differ in seizure susceptibility, the data consist of measurements obtained from both groups that were represented by an approximately equal number of animals.

Scoring for behavioral seizures. Hemizygote and homozygote mice were scored for behavioral seizures from 1 month to 12 months of age by moving and opening the mouse cage once a day in the morning (between 10 AM and 12 PM) three times a week.

c-Fos immunoreactivity. The assay was performed as described previously (Weiser et al., 1993). Animals were anesthetized with Nembutal (150 mg/kg) and perfused transcardially with saline containing 0.5% sodium nitrate and 10 U/ml heparin sulfate, followed by cold 4% formaldehyde generated from paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.2. The brains were post-fixed in the same fixative for 2–4 hr and embedded in 30% sucrose overnight. Free-floating sections (40 μm), obtained on a freezing microtome, were washed for 30 min in 0.1 M sodium PBS and preincubated with 1% BSA and 0.2% Triton X-100 in 0.1 M PBS. Sections were washed in PBS containing 0.5% BSA and incubated overnight with c-Fos antiserum (Fos and related antigens) (1:8000; Genosys, Woodlands, TX). Sections were washed in PBS-BSA and incubated for 1 hr with biotinylated anti-sheep IgG (Vector Laboratories, Burlingame, CA). The tissue was washed and incubated for 1 hr with the avidin–biotin horseradish peroxidase complex according to Vector Elite kit instructions. The antigen was visualized by reaction with 3,3-diaminobenzidine tetrahydrochloride as a chromogen and 0.004% hydrogen peroxide for 6 min. Sections were mounted on gelatin-coated slides, dehydrated through graded ethanols, and coverslipped with Permount.

RT-PCR. Immediately after dissection, total RNA was prepared from tissues using TRIZOL Reagent (Life Technologies, Gaithersburg, MD) according to the manufacturer's protocol. Total RNA (9 μg) was incubated with 1 U of RNase-free DNase I (Life Technologies) in the presence of 20 U RNasin ribonuclease inhibitor (Promega, Madison, WI) to remove any remaining genomic DNA. The DNA-free RNA was reverse transcribed with MMLV reverse transcriptase (Life Technologies) according to the manufacturer's protocol using a primer specific to bases 687–670 of the *jerky* open reading frame (5′-CCGAGGGGAC-AAAAATATGAT-3′). Reverse transcribed cDNA was diluted, and an amount corresponding to 62.5, 31.25, 15.62, or 7.8 ng of total RNA template was used for PCR amplification. Primers complementary to bases 687–670 (sequence above) and 206–222 (5′-CTTCTACAAGCAGATGC-3′) were added to yield an expected 481 bp product. Additionally, 0.1 μl of [^{32}P]dATP (DuPont/New England Nuclear, Boston, MA) was added to each reaction to allow for quantitation. Reactions were amplified for 30 cycles (30 sec at 94°C, 30 sec at 55°C, 1 min at 72°C) and the resulting products resolved on a 1.25% agarose gel. Gels were dried and exposed to PhosphorImager screens (Molecular Dynamics, Sunnyvale, CA) for 1 hr. Bands corresponding to the 481 bp product were quantified in PhosphorImager units, which were graphed against the amount of RNA template used to prime PCR amplification. In some experiments, a 500 bp fragment of the neurofilament light gene was amplified as a control. Conditions were identical to those used for the amplification of the *jerky* gene-specific fragments, except that 22 cycles were used.

In situ *hybridization.* Brain specimens isolated from either 6- month-old female, C57 wild-type (wt), or *Jrk*−/− mice were fixed in paraformaldehyde and embedded in paraplast essentially as described (Zeller et al., 1987), with the exception that samples were cleared in Histoclear II (National Diagnostics, Atlanta, GA) instead of xylene. Histological sections (8 μm thick) were collected on slides pretreated with Vectabond Reagent (Vector Laboratories). Specimens were fixed onto the slides by heating at 42°C overnight followed by heating at 78°C for 2 hr. [^{35}S]-labeled sense and antisense riboprobes were synthesized from a 0.9 kb fragment of the jerky cDNA (corresponding to 767 bases from the coding and 133 bases from the 3′ noncoding region of the *jerky* gene). Near-adjacent sections from each specimen were hybridized to the probes. Hybridization and washing were conducted essentially as described in Hui and Joyner (1993), with the following minor modifications: (1) samples were deparaffined in histoclear II, and (2) proteinase K treatment was continued for 5 min. Tissue sections were coated with Kodak NTB2 emulsion and exposed for 15 d. The slides were developed in Kodak D19 developer and stained with eosin and hematoxylin.

RESULTS

Mice with one deleted jerky allele show increased seizure susceptibility

Handling-induced behavioral seizures have been described in mice hemizygous for the *jerky* gene (Toth et al., 1995). Seizures in *Jrk*+/− animals started with behavioral arrest, followed by facial twitching, then forelimb automatism. This progressed to rearing and falling with whole body jerking. This seizure behavior, when

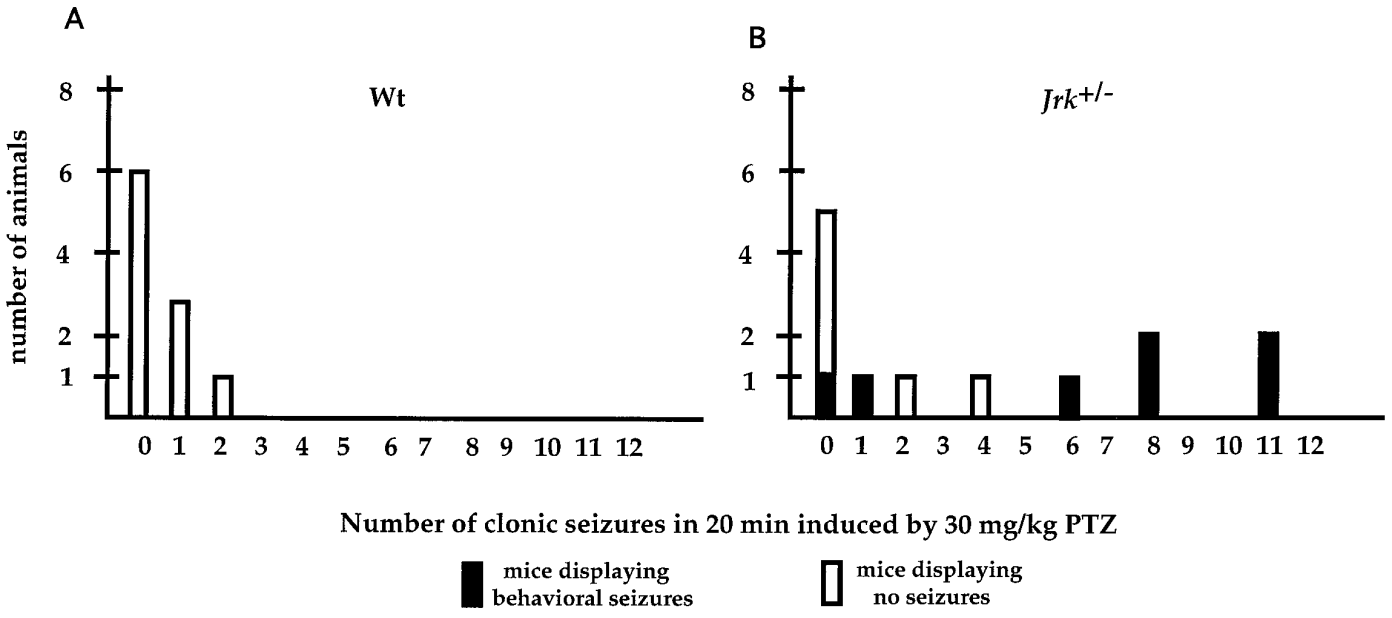

Figure 1. Distribution of wt and *Jrk*+/− mice based on the number of PTZ-induced seizures. Susceptibility to PTZ-induced seizures of *Jrk*+/− mice correlates with the animal's tendency to display handling-induced seizures.

combined with the electrophysiological data showing spiking in the hippocampus compared with other brain areas of jerky mouse (Toth et al., 1995), is most analogous to clinical partial seizures with secondary generalization. The predisposition of *Jrk*+/− mice to seizures could be demonstrated by their higher susceptibility to the seizure provoking-agent PTZ (Table 1). PTZ appears to decrease the potency of GABA-mediated inhibition in brain (Wilson and Escueta, 1974) and, depending on dosage, can produce myoclonic jerks, clonic convulsions, or tonic seizures in animals. A low dose of PTZ (30 mg/kg) caused an average of 0.5 ± 0.2 (mean ± SEM, $n = 10$) clonic convulsions in wt animals. As Table 1 shows, both the number of seizure-displaying animals and seizure events were increased in the group of PTZ-injected *Jrk*+/− mice, resulting in an average of 3.9 ± 1.2 (mean ± SEM, $n = 13$) convulsions (significant difference, $p < 0.05$). Also, 30 mg/kg PTZ caused tonic seizures that eventually led to death in 1 of the 13 *Jrk*+/− mice, whereas none of the 10 wt animals showed tonic seizures under identical conditions. The percent of time observed in clonus was also increased significantly in the group of *Jrk*+/− mice (4.7% vs control of 0.6%; $p < 0.001$). When the PTZ dose was increased to 50 mg/kg, the number of seizure-displaying animals was increased in both the wt and the *Jrk*+/− groups, and 8 of the 12 *Jrk*+/− but only 1 of the 12 wt mice died as a result of tonic seizure (significant difference, $p < 0.0075$). Because of the high early lethality of *Jrk*+/− mice to the 50 mg/kg PTZ dose, the average number of seizures in this group was less than that in the group injected with the 30 mg/kg dose. When seizure events were normalized for the time survived (percent of observation time in clonus, Table 1), *Jrk*+/− mice again showed a significantly increased susceptibility.

We have published previously that only 50% of *Jrk*+/− mice show handling-induced seizures (Toth et al., 1995). This raised the question of how the increased PTZ sensitivity of *Jrk*+/− mice is correlated with their vulnerability to handling-induced seizures. Figure 1 shows the distribution of wt and *Jrk*+/− mice (Table 1) based on the number of PTZ-induced seizures. Controls showed no or few (1–2) seizures using a 30 mg/kg dose of PTZ (Fig. 1*A*). Seizure response of *Jrk*+/− mice to 30 mg/kg PTZ was variable, but ~50% displayed more than three seizures during the 20 min observation period (Fig. 1*B*). Susceptibility to PTZ-induced seizures clearly correlated with the animal's tendency to display handling-induced seizures ($p < 0.005$, Kendall's rank correlation). Animals experiencing more clonus in the PTZ test tended to show behavioral seizures as well, whereas animals responding to PTZ with less clonus generally did not show seizures (Fig. 1*B*). Because of the high lethality of *Jrk*+/− mice to the 50 mg/kg PTZ dose, a similar analysis could not be performed in this group. To exclude genetic heterogeneity as a variable in these experiments, all mice were maintained on the C57BL/6J (C57) inbred background. Therefore, the variability in seizure susceptibility of *Jrk*+/− mice was probably attributable to developmental/environmental effects.

Hippocampal c-Fos immunoreactivity in hemizygote jerky mice after seizures

Electrophysiological recordings showed the presence of interictal spikes in the hippocampus of jerky mice (Toth et al., 1995), and we asked whether the hippocampus is also involved during behavioral seizures. Because activation of the immediate-early gene *c-fos* is known to be associated with neuronal activation after seizures (Morgan et al., 1987; Shehab, 1992), the levels of c-Fos immunoreactivity were visualized by immunohistochemistry. Indeed, c-Fos immunoreactivity was highly induced bilaterally in the granular cells of the dentate gyrus of the hippocampus and to a lower extent in the hippocampal CA pyramidal cells and the amygdala 2 hr after seizure (Fig. 2*A*,*C*,*E*,*G*). Because the amygdala and hippocampus are bilaterally interconnected, their simultaneous activation was not surprising. Neither hippocampus nor amygdala was activated in wt animals handled as *Jrk*+/− mice (Fig. 2*B*,*D*,*F*,*H*). In the cortical regions, especially in the cingulate and temporal cortices, only moderate immunoreactivity was found in seizure mice, which could be the consequence of secondary generalization or handling-induced stress (Ceccatelli et al., 1989; Smith et al., 1992). *Jrk*+/− animals selected for this exper-

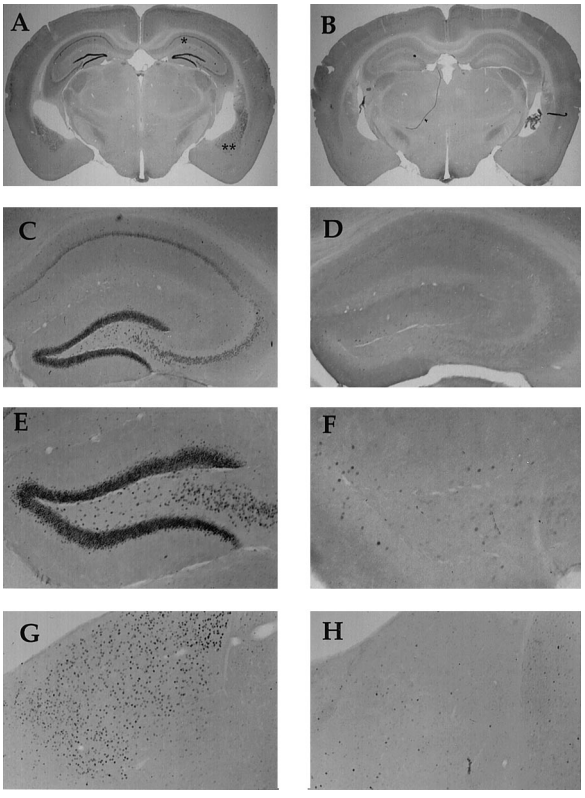

Figure 2. c-Fos immunoreactivity in a *Jrk*+/− (*A*, *C*, *E*, *G*) and wt (*B*, *D*, *F*, *H*) mouse (6 months old) 2 hr after handling. *A*, *B*, Coronal sections at the level of amygdala; *C*, *D*, hippocampus magnified (2×) from regions in *A* and *B*, indicated by an *asterisk*; *E*, *F*, dentate gyrus magnified (5×) from regions in *A* and *B*, indicated by an *asterisk*; *G*, *H*, amygdala magnified (5×) from regions in *A* and *B*, indicated by a *double asterisk* in *A*.

iment showed hindlimb myoclonus and head nodding but not generalized seizures, indicating a limited spreading of neuronal firing to the cortex. However, the cortical c-Fos activation in seizure mice was attributable, at least in part, to stress, because c-Fos was activated in handled (Fig. 2*B*) but not in undisturbed wt mice (data not shown). A moderate activation was also seen in subcortical nuclei such as the septal, paraventricular, and hypothalamic nuclei, as well as the paraventricular nuclei of the thalamus in handled wt animals, and this activation pattern was superimposed on the robust seizure-induced pattern in *Jrk*+/− mice. Based on the electrophysiological and immunohistochemical data, we concluded that *Jrk*+/− mice have partial seizures that involve the hippocampus and amygdala.

Ubiquitous expression of jerky

The hippocampal-amygdala localization of neuronal activation raised the possibility that these regions express jerky exclusively. However, RT-PCR experiments demonstrated the presence of jerky mRNA throughout the CNS including frontal cortex, brainstem, hippocampus, thalamus, and olfactory bulb (Fig. 3*A*). Outside of the CNS, jerky is also expressed in all adult peripheral tissues surveyed thus far (Fig. 3*B*). Among these tissues, the highest level of jerky was found in the testis.

A more refined analysis of brain-specific expression of *jerky* was performed by *in situ* hybridization. Jerky mRNA appeared to be present in many brain regions of wt animals. Hybridization was stronger in the granule cells of the dentate gyrus and the pyramidal cells of the CA region (Fig. 3*C*, *top*). However, this could be attributable, at least in part, to the high density of neurons in these regions. The hybridization signal was less intense in the neocortex (Fig. 3*D*, *top*). The amygdala, a site of strong c-Fos activation in seizure, did not contain a significant amount of mRNA. As expected, there was no detectable level of jerky transcript in either the hippocampus or the neocortex of *Jrk*−/− mice (Fig. 3*C*,*D*, *bottom*). A sense probe in wt mouse brain also resulted in no specific hybridization (data not shown).

Table 2. Individual mRNA levels of seizure displaying (S), nonseizure (NS) hemizygotes, and wt littermates at age of 8 months

Animal		MSF[a]	jerky mRNA % of control	NF-L mRNA[b] % of control
Wt 1		0	80	102
Wt 2		0	120	98
	Average		100	100
NS 1		0	23.8	92
NS 2		0	29.4	126
	Average		26.3	109
S 1		0.8	25	112
S 2		0.6	34.5	120
	Average		29	116

[a]MSF, Mean seizure frequency; animals were tested five times for handling induced seizures, every 3 d in a 2 week period.

[b]NF-L, Neurofilament-light used as a control.

Hemizygosity suggested a 50% reduction in jerky expression. Quantitative RT-PCR experiments demonstrated a somewhat lower than expected jerky mRNA level (24–35% of wt) in the brains of hemizygotes (Table 2). Although the penetrance of the phenotype in hemizygotes is incomplete, seizure displaying and nonseizure animals showed a comparable reduction in total brain jerky mRNA levels (Table 2). A reduced jerky mRNA level (30–40% of wt) was also found in the peripheral tissues such as liver, testes, spleen, heart, lung, intestine, and kidney of hemizygotes (data not shown). Furthermore, different brain regions of hemizygotes showed an equally reduced jerky mRNA level (brainstem, 25%; cerebellum, 26%; hippocampus, 35%; hypothalamus, 40%; frontal cortex, 40% of wt). Taken together, jerky is expressed ubiquitously, and hemizygotes have a reduced level of jerky mRNA level in all tissues and brain regions surveyed.

Somatic and sexual abnormalities in jerky homozygotes

The lack of symptoms in organs other than brain raised the possibility that jerky function is dispensable in most tissues and reduced jerky expression results only brain-specific abnormalities. However, this is not likely the case, because the absence of jerky in homozygotes leads to the emergence of somatic and sexual abnormalities. Although *Jrk*−/− mice were born with normal weight, a few days later they started to show a reduced growth rate, resulting in an ~25% deficit in adult weight compared with wt mice (Fig. 4*A*). Low weight was accompanied by proportionate dwarfism in homozygotes (Fig. 4*B*). In humans, short stature is often associated with skeletal dysplasia, and *Jrk*−/− mice displayed a hunchback posture suggesting kyphosis (Fig. 4*B*). However, skeletal preparations of *Jrk*−/− mice did not reveal bone abnormalities (data not shown). The second characteristic symptom of *Jrk*−/− mice was their shortened life span, although some of them reached the age of 4 months or more (Fig. 4*C*). Because the onset of seizures in *Jrk*−/− mice was not markedly different from that of the *Jrk*+/− mice (see above), many of the *Jrk*−/−

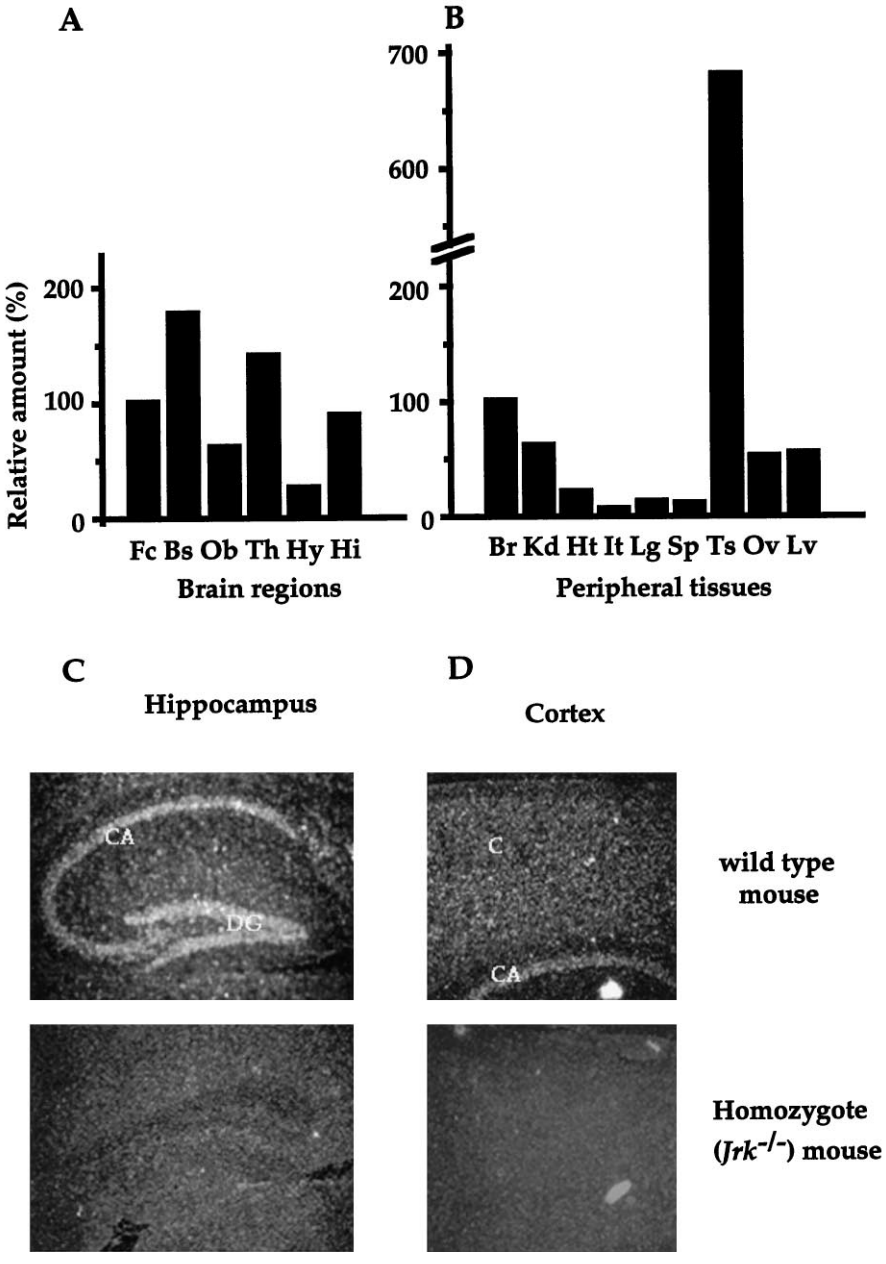

Figure 3. Tissue-specific expression of jerky determined by quantitative RT-PCR (*A*, *B*) and *in situ* hybridization (*C*, *D*). *A*, Relative levels of jerky mRNA in brain regions (*Fc*, frontal cortex; *Bs*, brainstem; *Ob*, olfactory bulb; *Th*, thalamus; *Hy*, hypothalamus; *Hi*, hippocampus). Level of Fc is taken as 100%. *B*, Relative levels of jerky mRNA in peripheral tissues compared with the level in brain (*Br*, brain; *Kd*, kidney; *Ht*, heart; *In*, intestine; *Lg*, lung; *Sp*, spleen; *Ts*, testes; *Ov*, ovary; *Lv*, liver). *C*, Hippocampus of a wt (*top*) and homozygote (*Jrk*−/−) mouse (*bottom*) hybridized with a jerky antisense probe. *D*, Cortex of a wt (*top*) and homozygote mouse (*bottom*) hybridized with a jerky antisense probe (*CA*, CA region of the hippocampus; *DG*, dentate gyrus of the hippocampus; *C*, cortex).

mice died before seizure age. The third symptom observed in *Jrk*−/− mice was male sterility. Mating 8 week old *Jrk*−/− males with wt C57 females over several months never resulted in pregnancy. On the other hand, *Jrk*−/− females occasionally became pregnant from wt C57 males, but either were unable to deliver and died close to term or delivered and were unable to nurse. The male sterility in *Jrk*−/− mice raised the possibility that jerky is essential for the proper development or function of the testes. Involvement of jerky in these processes is supported by its high level of expression in the testes (Fig. 3*B*). On the other hand, jerky is not essential for female sexual development, because *Jrk*−/− females are fertile. It is probably not coincidental that the level of jerky in ovary is relatively low (Fig. 3*B*).

Interestingly, severity of the seizure phenotype was not at all or only marginally increased in *Jrk* mice compared with *Jrk*+/− mice. Analysis of *Jrk*−/− mice demonstrated that the penetrance of behavioral seizures was incomplete (71%, $n = 7$) and not very different than the 51% penetrance found in *Jrk*+/− animals ($n = 51$). Moreover, the onset of seizures in *Jrk*−/− mice (day 143 in average, $n = 4$) was similar to that measured in *Jrk*+/− animals (day 156 in average, $n = 7$).

These results indicated a threshold in dosage sensitivity of the brain; once the threshold was reached, seizures occurred and additional reductions in jerky levels did not lead to a more severe seizure phenotype.

DISCUSSION

Hemizygote jerky mice display seizures but not other neurological abnormalities, and the phenotype is unassociated with specific structural pathology, which is reminiscent of human idiopathic epilepsy. Understanding the genetics of idiopathic epilepsies is difficult for a variety of reasons, including complex trait, incomplete penetrance, and heterogeneous phenotypes. Based on the similarity of the phenotype to the human condition, *Jrk*+/− mice

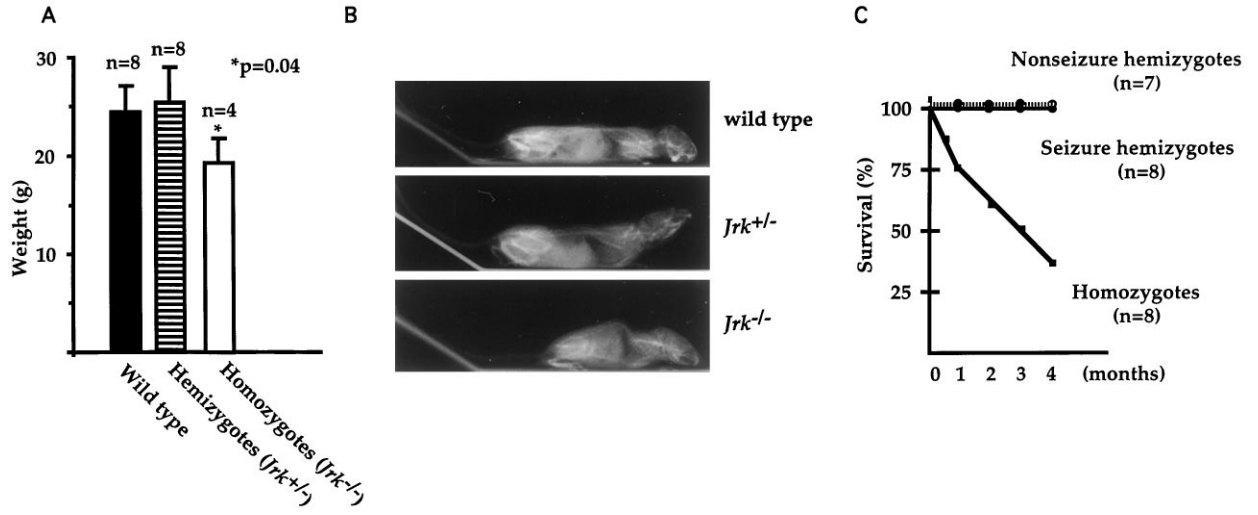

Figure 4. *Jrk*−/− animals, in addition to behavioral seizures, display a complex phenotype including abnormal somatic and sexual development. *A*, Reduced weight of male *Jrk*−/− at age of 4 months, compared with age- and sex-matched *Jrk*+/− and wt mice. The two female homozygotes analyzed showed a similar reduction in weight, compared with age- and sex-matched wt and hemizygotes (data not shown). *B*, Proportionate dwarfism of *Jrk*−/− animals (*bottom*), in comparison with *Jrk*+/− (*middle*) and wt mice (*top*) demonstrated by x-ray. *C*, Reduced survival of *Jrk*−/− animals. Approximately 25% of homozygotes reached seizure age.

represent an excellent model to study the genetic basis of epilepsies. We specifically sought to determine how the *jerky* gene, which is widely expressed, can cause a condition limited to epileptic seizures in hemizygotes. The complex phenotype of homozygote animals indicates that jerky plays an important role in somatic and sexual development. However, these functions are not impaired in hemizygotes, because the expression of the nondeleted allele provides a sufficient amount of protein to maintain function in most tissues. The only apparent consequence of jerky deficit in hemizygotes is hyperexcitability and seizures (Fig. 5). Electrophysiological recordings (Toth et al., 1995) and c-Fos immunoreactivity studies (Fig. 2) indicated the involvement of the hippocampus in both interictal and ictal events and raised the possibility of functional jerky insufficiency in this brain region. However, it is also possible that the hippocampus is secondarily activated, and the seizure focus is located elsewhere in the brain. It is known that the hippocampus is prone to excessive firing, mostly because CA3 pyramidal cells are interconnected through excitatory connections and excitation can spread very efficiently in the network (Traub and Miles, 1991). We speculate that the jerky level in the hippocampus or other brain regions has to be maintained at a certain level and that even a moderate reduction in jerky can lead to abnormal neuronal function. Alternatively, maintaining an appropriate level of jerky during development may be critical, and if the level of jerky is suboptimal during development, abnormalities occur resulting in seizures.

What is the function of jerky and how could a reduction in its function lead to neuronal hyperexcitability? The structure of the jerky protein and its biochemical properties indicate that it binds DNA (Toth et al., 1995). As Figure 6 shows, jerky is similar both to human and mouse centromere-binding protein B (CENP-B) (Toth et al., 1995) and to the fission yeast autonomously replicating sequence-binding protein 1 (Abp1) (Murakami et al., 1996). The similarity is especially high at the N-terminal portions of the

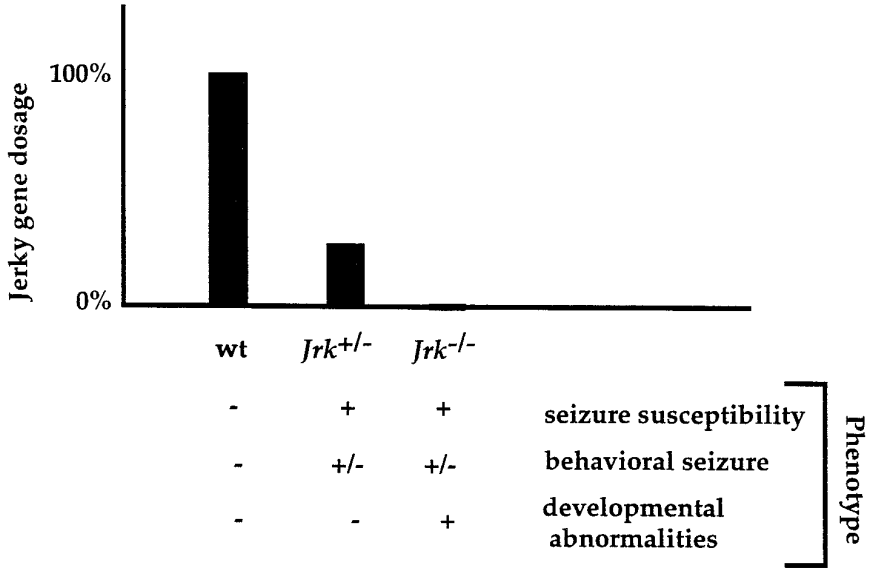

Figure 5. Correlation between dosage of jerky and disease phenotype. Because of the sensitivity of the brain to reduced jerky dosage, hemizygote mice display epileptic seizures. Other tissues do not show this dosage sensitivity, therefore, hemizygotes show no symptoms other than hyperexcitability and seizures. However, jerky is indispensable for proper somatic and sexual development indicated by the complex phenotype of homozygotes. Plus symbol (+) for seizure susceptibility indicates increased sensitivity to PTZ-induced seizures; +/− for behavioral seizures represents partial penetrance of seizures; and + for developmental abnormalities refers to disturbance in the somatic and sexual development.

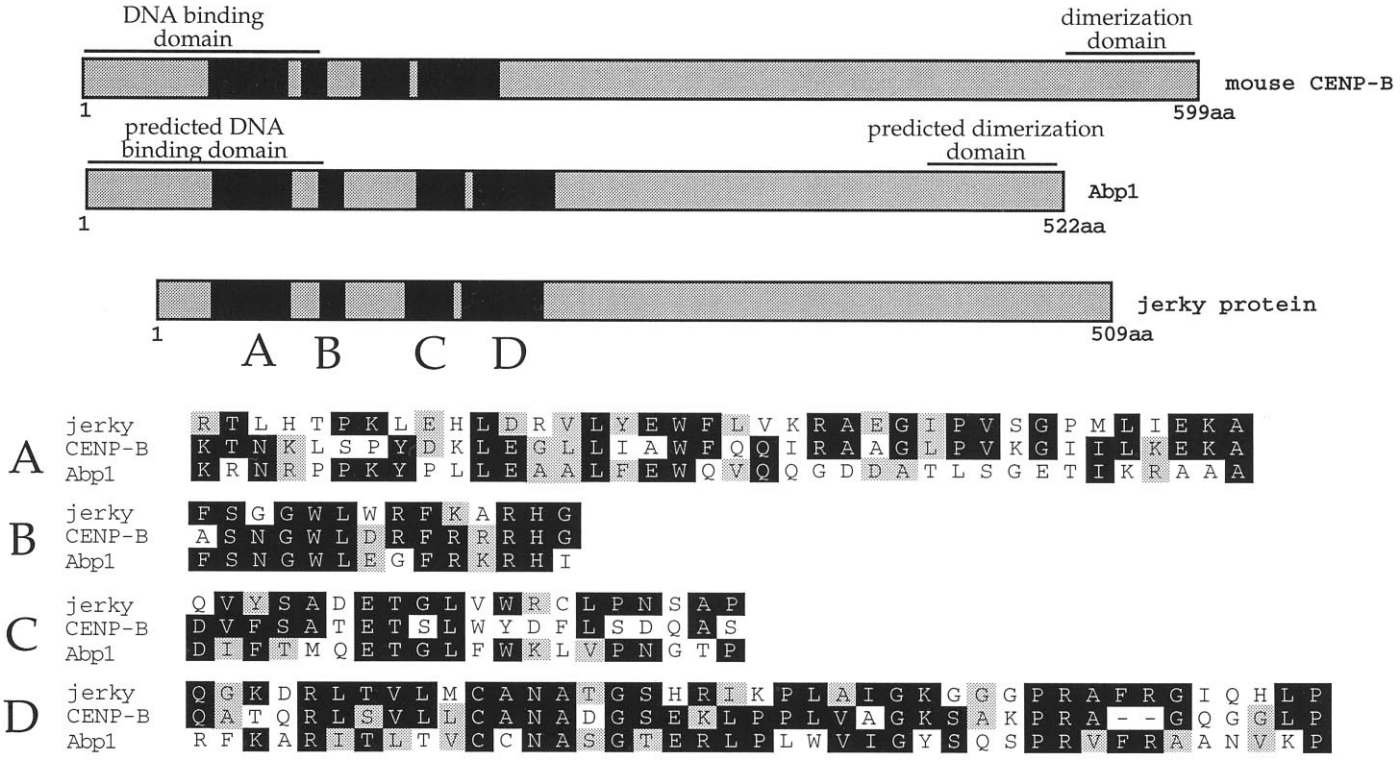

Figure 6. Similarities in the protein sequences of CENP-B, jerky, and Abp1 BP-1. The *top* of the figure represents schematically the positions of four highly homologous domains (*A–D*) within the three proteins. The *bottom* of the figure displays the aligned sequences of these four domains (*A–D*). *White letters on black background* and *black letters on shaded background* represent identical and similar residues, respectively.

three proteins (on average, 58% identity and 72% similarity). This region is absolutely required for binding of CENP-B to the 17 base pair CENP-B box sequence of α-satellite DNA (Pluta et al., 1992). It has also been reported that the N-terminal portion of Abp1 is essential for binding to the 11 base pair autonomously replicating sequence consensus sequence (Murakami et al., 1996). Jerky also binds DNA, but it is neither α-satellite DNA nor the ARS consensus sequence (G. Donovan and M. Toth, unpublished results) indicating that although the three proteins share a similar DNA-binding domain, their sequence recognition is quite different. Another similarity among CENP-B, Abp1, and jerky is that they are involved in chromatin assembly and function. CENP-B and Abp1 play a role in assembly of the kinetochore and replication complexes, respectively (Cooke et al., 1990; Murakami et al., 1996). Jerky seems to be attached to the nuclear matrix and might be involved in chromatin functions such as replication and transcription (Donovan and Toth, unpublished results). Although the exact nature of jerky deficiency-induced cellular defect is not known, it is likely that jerky is part of a complex in which subunit stoichiometry is important for function. Neurons may be particularly sensitive to even a modest reduction in this function. Based on the similarity of the seizure susceptibility of *Jrk*+/− and *Jrk*−/− mice, it is possible that a partial reduction in dosage almost completely disrupts this function in neurons. In contrast, cells in other tissues show malfunction only when jerky is completely absent.

Haploinsufficiency has been described for several genes coding for transcriptional regulators, receptors, and signal transduction molecules, such as the ribosomal protein homologs RPS4X and RPS4Y (Zinn et al., 1991), the WD40 motifs containing LIS-1 and TUPLE-1 (Reiner et al., 1993; Tommerup, 1993), the nuclear protein CREB-binding protein (Petrij et al., 1995), and the homeobox protein PAX6 (Ton et al., 1991). The common feature of these proteins is their involvement in the assembly of intermolecular complexes that probably have an exact stoichiometry. Haploinsufficiency of these genes can lead to diseases. RPS4X and RPS4Y have been implicated in Turner syndrome (Watanabe et al., 1993). LIS-1 and TUPLE-1 are believed to be involved in Miller–Dieker lissencephaly and CATCH-22 syndrome, respectively (Halford et al., 1993; Reiner et al., 1993). Patients heterozygous for a mutation in the gene encoding the CREB-binding protein suffer from Rubinstein Taybi syndrome (Petrij et al., 1995). Finally, it is likely that a certain level of PAX6 is necessary for optimal regulation of homeobox genes and that insufficiency leads to aniridia, a disease characterized by blindness (Ton et al., 1991).

Although gene dosage sensitivity is only one of the several mechanisms that can underlie dominant inheritance (Wilkie, 1994), it is reasonable to think that the pathomechanism of certain idiopathic epilepsies is based on brain-specific haploinsufficiency. Several idiopathic epilepsies with a dominant inheritance pattern are known, including BFNC, JME, benign epilepsy with centrotemporal spikes, and benign adult familial myoclonus epilepsy (Leppert et al., 1989; Liu et al., 1995; Scheffer et al., 1995; Kuwano et al., 1996). Identification of the genes associated with these disorders will allow for the determination of their dosage sensitivity in specific brain regions.

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