A Genetic Switch for Epilepsy in Adult Mice

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Premature death from seizures afflicts gene-targeted mice expressing the Q/R site-unedited glutamate receptor subunit GluR-B(Q) of AMPA receptors in central neurons. Early seizure-related death has now been circumvented by a genetic switch that restricts GluR-B(Q) expression to forebrain principal neurons from postnatal stages onward, prominently in hippocampus and striatum and less so in cortex and amygdala. When switched on, functional receptor incorporation of GluR-B(Q) could be demonstrated by imaging evoked AMPA channel-mediated spinous Ca2+ transients in CA1 pyramidal cells. Sustained GluR-B(Q) expression in adult mice led to smaller excitatory postsynaptic responses in the CA1 region with unchanged presynaptic fiber excitability. Notably, despite the smaller excitatory response, the CA1 cells exhibited a reduced population spike threshold, which might underlie the spontaneous manifestations of epilepsy, including myocloni and generalized seizures with limbic components, observed by synchronous video monitoring and electroencephalographic recordings. No neuropathological symptoms developed when GluR-B(Q) expression was restricted to only hippocampal neurons. Our results show that seizure susceptibility is triggered by GluR-B(Q) expression also in the adult brain and that circuit hyperexcitability is not an immediate consequence of GluR-B(Q) but requires yet unknown downstream events, likely to be induced by non-Hebbian plasticity from Ca2+-permeable AMPA channels in principal neurons.

Key words: temporal Cre regulation; RNA editing; spinous calcium transients; altered AMPA receptors; hippocampus; population spike threshold;

Introduction

In mice, a neurological syndrome with spontaneous and recurrent epileptic seizures starting at postnatal day 14 (P14) and ending with death at approximately P20–P25 can be induced by the inhibition of RNA editing at a single codon position for the AMPA glutamate receptor GluR-B (GluR2) (Brusa et al., 1995; Higuchi et al., 2000). Although presence and levels of unedited GluR-B correlate with the seizure attacks, the molecular and cellular mechanisms underlying the seizure susceptibility remain unknown, and contribution from disturbed development resulting in altered neuronal connectivity could not be excluded.

Among the four AMPA receptor subunits GluR-A to GluR-D (GluR1–GluR4), which constitute heterodimeric, AMPA receptor channels of tetrameric stoichiometry, the GluR-B subunit is of unique importance. GluR-B carries in its pore-forming domain an arginine (R) residue, resulting from RNA editing of a genomically encrypted glutamine (Q) codon (Sommer et al., 1991). This arginine residue is functionally dominant in heteromeric AMPA receptor subunit assemblies, because it determines gating kinetics, unitary channel conductance, voltage-independent gating, Ca2+ permeability, and even channel assembly (Burnashev et al., 1992; Geiger et al., 1995; Swanson et al., 1997; Feldmeyer et al., 1999; Greger et al., 2003). Because >99% of the GluR-B subunits are edited at this Q/R site (Sommer et al., 1991), most neurons express Q/R site-edited GluR-B and therefore contain AMPA receptors with low unitary channel conductance, low Ca2+ permeability, and voltage-independent gating. If editing of the Q codon is abolished by knock-out of the editing enzyme ADAR2 (adenosine deaminases that act on RNA) (Higuchi et al., 2000) or by removing the cis-acting exon complementarity (ECS) in intron 11 of the GluR-B gene (Brusa et al., 1995), a substantial portion of the AMPA channels switch from low to high Ca2+ permeability and exhibit a double-rectifying instead of linear current–voltage relationship. If this switch occurs already in the embryo, as it does in GluR-B(+/ΔECS) and ADAR2−/− mice, it ultimately engenders, by unknown downstream events, a severely compromised phenotype with growth retardation, spontaneous seizures, and premature death.

We have now used genetic regulation to induce the GluR-B(R)-to-GluR-B(Q) AMPA channel switch during juvenile development to investigate whether GluR-B(Q)-containing AMPA channels in adult mice contribute to the spontaneous seizures and decreased lifespan of GluR-B(Q) mice.
receptors when expressed postnatally will still cause an epileptic phenotype. For this, we used the expression-attenuated GluR-B<sup>B<sub>B</sub></sup> allele (Feldmeyer et al., 1999), which can be converted to the dominant-negative GluR-B<sup>B<sub>B</sub>ΔECS</sup> allele by Cre recombinase-mediated removal of the attenuating sequences engineered into intron 11 of the GluR-B<sup>B<sub>neo</sub></sup> allele. Thus, the AMPA receptor switch was tightly controlled by Cre expression. Furthermore, we restricted the GluR-B(R)-to-GluR-B(Q) switch to different principal neuron populations of the forebrain to delineate brain regions responsible for seizure activity. We could show that vulnerability to GluR-B(Q) is not confined to early postnatal life, because seizures were also triggered in the adult mouse from delayed GluR-B(Q) expression. The AMPA receptor switch was accompanied by increased excitability of hippocampal neurons, suggesting a dominant role in seizure induction by the hippocampus. Long-term electroencephalographic (EEG) and video analyses showed that the forebrain-specific AMPA receptor switch ultimately led to myoclonic jerks, followed by secondary generalized seizures. When GluR-B(Q) expression was restricted to only hippocampal neurons, all neuropathological symptoms except occasional spontaneous EEG activities were abolished, indicating that, in addition to hippocampus, other brain regions need to become hyperactive for generalized epileptic activity.

Our EEG analyses and classification of seizures revealed similarities to human temporal lobe epilepsy. However, a role of Q/R site editing of AMPA or kainate receptors suggested by murine similarities to human temporal lobe epilepsy. However, a role of Q/R site editing of AMPA or kainate receptors suggested by murine similarities to human temporal lobe epilepsy. However, a role of Q/R site editing of AMPA or kainate receptors suggested by murine similarities to human temporal lobe epilepsy. However, a role of Q/R site editing of AMPA or kainate receptors suggested by murine similarities to human temporal lobe epilepsy. However, a role of Q/R site editing of AMPA or kainate receptors suggested by murine similarities to human temporal lobe epilepsy. However, a role of Q/R site editing of AMPA or kainate receptors suggested by murine similarities to human temporal lobe epilepsy. However, a role of Q/R site editing of AMPA or kainate receptors suggested by murine similarities to human temporal lobe epilepsy. However, a role of Q/R site editing of AMPA or kainate receptors suggested by murine similarities to human temporal lobe epilepsy. However, a role of Q/R site editing of AMPA or kainate receptors suggested by murine similarities to human temporal lobe epilepsy. However, a role of Q/R site editing of AMPA or kainate receptors suggested by murine similarities to human temporal lobe epilepsy.
isolated wires (0.17 mm in diameter) were implanted in the right dorsal hippocampus (anteroposterior, 2.0 mm; mediolateral, 1.5 mm; dorsoventral, 1.9 mm) with the bregma as reference (Franklin and Paxinos, 1997). The animals were also equipped with monopolar cortical electrodes made of tungsten rods (0.25 mm in diameter; Phymep, Paris, France) onto the left fronto-parietal cortex, and the right frontal cortex. Hippocampal and cortical electrodes were soldered on male pins (Far- nel, Paris, France) and secured onto the skull with acrylic dental cement. EGG activities were recorded with a computer-based digital acquisition system (Coherence; Deltamed, Paris, France) in animals freely moving in a Faraday cage. A referential setup was used in which cortical and hippocampal electrodes were referenced with an electrode placed over the cerebellum. This setup allowed visualizing the EEG recording in different derivations after the acquisition. Each animal was EEG monitored for several hours. After completion of the experiments, animals were injected with a lethal dose of Nembutal (100 mg/kg, i.p.). Brains were removed, frozen, and cut in 20 μm sections on a cryostat. Histological analysis was performed after cresyl violet staining to verify the location of the hippocampal and cortical electrodes.

**Two-photon imaging.** Acute transverse hippocampal slices (250 μm thick) were prepared from P28–P33 mice as described previously (Stuart et al., 1993). Whole-cell patch-clamp recordings were performed with an EPC-9 amplifier, filtered at 3 kHz, and digitized with 10 kHz. A galvanometer scanning unit (TCS NT; Leica) was adapted to an upright microscope (DMFLS; Leica) equipped with a 63x objective (harmonic components X apochromatic glass W63 × ultraviolet index; numerical aperture 0.9; Leica). For two-photon excitation (Denk et al., 1990), short pulses of 170–200 fs at 76 MHz from a titanium/sapphire laser (MIRA 900F; Coherent, Santa Clara, CA) pumped by a solid-state laser (Verdi 5W; Coherent) at 870–890 nm were used. Line-scan imaging was performed using both scan directions at highest magnification, resulting in a high-duty cycle with high temporal resolution (2.2 msec/line). External non-descanned detection behind the objective and the condenser was used for signal collection, yielding high signal collection efficiency. Transmission and epifluorescence signals were recorded by photomultiplier tubes (R6357; Hamamatsu Photonics, Herrsching, Germany) selected for high quantum efficiency and summed off-line. Simultaneously to fluorescence image acquisition, IR-scanning gradient contrast images were recorded. The transmitted IR laser light was imaged through a gradient-contrast tube (Rathenberg et al., 2003) onto a photomultiplier. Fluorescence line-scan images were analyzed using custom software macros based on the Leica confocal software. A line was scanned every 2.2 msec and two lines were averaged off-line, resulting in a temporal resolution of 4.4 msec. The fluorescence for each time point was averaged for regions of interest enclosing the spine examined. Stimulation protocols began 500 msec after the start of the line scan. Before stimulation, fluorescence was averaged for 100 msec to obtain the basal fluorescence \( F_0 \). A region distant to any indicator-containing structure was chosen to determine the background fluorescence \( F_{bg} \). Relative fluorescence changes were calculated as \( \Delta F/F(0) = (F(t) - F_0)/(F_0 - F_{bg}) \) and were fitted with a single exponential using a least-square fit routine (Igor; WaveMetrics, Lake Oswego, OR). This fit yielded the peak amplitude of the fluorescence increase and the decay time constant \( \tau \).

**Hippocampal field recordings and analyses.** Mice (P60–P90) were killed with halothane. The brain was removed, and transverse slices (400 μm) were cut from each hippocampus with a vibrisscop in artificial CSF (ACSF) (4°C, bubbled with 95% O₂–5% CO₂, pH 7.4) containing the following (in mM): 124 NaCl, 2 KCl, 1.25 KH₂PO₄, 2 MgSO₄, 1 CaCl₂, 26 NaHCO₃, and 12 glucose. Slices were placed in a humidified interface chamber at 28–32°C and perfused with ACSF containing 2 mM CaCl₂. Orthodromic synaptic stimuli (<290 μA, 0.1 Hz) were delivered through a tungsten electrode placed in the stratum radiatum. A glass electrode (filled with ACSF) was placed in the synaptic layer (stratum radiatum) and recorded the presynaptic volley and the field EPSP (fEPSP), whereas another electrode in the pyramidal cell body layer (stratum pyramidale) monitored the population spike. After obtaining stable responses, we stimulated the afferent fibers with increasing strength (increasing the stimulus duration in steps of 10 μsec from 0 to 150 μsec, five consecutive stimulations at each step). A similar approach was used to elicit paired-pulse responses (50 msec interstimulus interval, the two stimuli being equal in strength).

To assess synaptic excitability, we measured for different stimulation strengths the amplitude of the presynaptic volley and the amplitude of the fEPSP. In the pyramidal layer recording, the population spike amplitude was obtained by measuring the maximal negative deflection of the population spike onto a line drawn between the maximum prespike and postspike fEPSP positivity. To pool data from the paired-pulse experiments, we selected responses to stimulation strength just below the threshold for eliciting a population spike on the second fEPSP. Data are pooled across mice of the same genotype and are presented as mean ± SEM. Statistical significance was evaluated by Student’s t test.

**Results**

**Conditional Cre expression in forebrain**

The regional and temporal control of the AMPA receptor switch is central to our studies. Therefore, to optimize the Cre-mediated switch of AMPA receptors, we analyzed the Cre expression and activity patterns in different mouse lines carrying Cre transgenes (Fig. 1A). For this analysis, Cre indicator mice were used because the Cre-mediated AMPA receptor switch itself cannot be monitored by immunohistochemistry or in situ hybridization. In mouse line RosaR26, the Rosa26 locus harbors a lacZ gene silenced with a transcriptional terminator sequence (Soriano, 1999) (Fig. 1B). Cre-mediated removal of the floxed transcriptional terminator sequence activates lacZ gene expression, which can be easily monitored by X-gal staining.
Abundant lacZ gene expression could be induced in forebrain by Cre recombinase expressed from a transgenic α-CaMKII promoter fragment (Tgα-CaMKII-Cre (Mantamadiotis et al., 2002)), with striatum, cortex, hippocampus and olfactory bulb showing strong X-gal staining (Fig. 2A). An even more abundant Cre-induced lacZ expression was observed in mice expressing Cre controlled by α-CaMKII promoter-driven tTA (Tgα-CaMKII-tTA/lC1). X-gal staining was obtained from embryonic stages onward and increased in intensity postnatally in major forebrain areas that also showed Cre immunostaining throughout postnatal development (Fig. 2A) (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). However, in this genetic paradigm, the extent of Cre expression could be restricted by Dox. If Dox was applied from conception, no Cre activity was detectable in the brain (supplemental Fig. 1, available at www.jneurosci.org as supplemental material), concordant with previous results (Hasan et al., 2001; Schonig et al., 2002). If Dox was applied from conception until birth (P0) (Fig. 2A) (supplemental Fig. 1, available at www.jneurosci.org as supplemental material), the Cre-specific immunosignal in striatum of CA1 (Fig. 2A, Δ) increased over time, reflecting postnatal increase in tTA from the transgenic α-CaMKII promoter, but revealed slow Cre induction after Dox withdrawal (Fig. 2B).

To quantify the extent of Cre reactivation and to obtain cellular resolution of Cre activity, we evaluated the number of Cre-expressing neurons in striatum, in the CA1, CA3, and DG subfields of the dorsal hippocampus, and in somatosensory cortex of P30 Tgα-CaMKII-tTA/lC1/Rosa+/-R26R mice. Optical sections of single confocal scans were used to determine in single cell layers the proportion of Cre-positive neurons, identified as NeuN-immunopositive cells (Fig. 2B). In three Dox-naive mice, 80–90% of the neurons in CA1, DG, cortex, and striatum showed Cre expression, whereas only ~60% of the CA3 pyramidal cells were positive for Cre. In three mice suppressed by Dox during intrauterine development, high Cre/NeuN ratios were reached by P30 in striatal and CA1 neurons only. However, in CA3 pyramidal cells, DG cells, and neurons of the somatosensory cortex, Cre expression was induced in only a minor subset (Fig. 2C). Further aging of the mice and different time windows for Dox suppression did not significantly improve the Cre induction (data not shown).

An even more restricted pattern of Cre activity was finally obtained, without taking recourse to Dox regulation, by introducing the neuron-restrictive silencer NRSE element of the NR2C promoter (Suchanek et al., 1997) into the α-CaMKII promoter fragment. When analyzing Cre activity on the Rosa26R locus in Tgα-CaMKII-tTA/lC1/Rosa+/-R26R mice, we observed a remarkably limited pattern of Cre activity, which appeared to be present selectively in hippocampal CA1 pyramidal and DG granule cells (Fig. 2A, bottom row).

**Lethal seizures by forebrain expression of GluR-B(Q)**

Next we determined whether a correlation existed between the Cre expression pattern of the different mouse lines and the neurological phenotype mediated by the Cre-induced GluR-B+/neo to GluR-BΔECS switch. GluR-B+/-/neo mice carrying the transgene of line Tgα-Cre4, which expresses Cre recombinase in forebrain from a transgenic α-CaMKII promoter fragment (Mantamadiotis et al., 2002), became seizure prone. These mice, although longer-lived than GluR-BΔECS mice (Brusa et al., 1995), also died prematurely from seizure attacks (Fig. 3), indicating that the AMPA receptor switch occurred in many brain regions early in development. Therefore, we used the transgenic LCI line in which Cre expression is stringently controlled by the Dox-dependent transcription factor tTA (Hasan et al., 2001; Schonig et al., 2002) (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). Double-transgenic Tgα-CaMKII-tTA/lC1 mice carrying a gene-targeted GluR-BΔECS allele (Brusa et al., 1995) died of seizures within 3 weeks of life (Fig. 3, -Dox), much earlier than the TgCre4/GluR-BΔECS mouse, because of earlier embryonic onset of Cre activity (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). However, they survived without signs of phenotypic impairments when given Dox in utero and during subsequent postnatal times (Fig. 3, +Dox) (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). Notably, most Tgα-CaMKII-tTA/lC1/GluR-BΔECS/+ mice.
mice (10 of 13) survived when Dox treated in utero until birth (P0) (Fig. 3, DoxP0), and the others died between P15 and P30. This postnatal period may be particularly sensitive for seizure-related death from the dominant-negative GluR-B^{ΔΔCS} allele, the generation of which is suppressed by the Dox exposure was confined to prenatal stages (filled squares), periodic mild seizure attacks were observed, but most mice survived. Prenatal Dox administration (open circles) prevented seizure-related death in less than half of the mice until P60. In Dox-naïve mice, therapeutic phenobarbital (Pb) administration (open stars) prolonged survival, and mice surviving P50 died from seizures within 10 d after drug removal. The number of mice (n) observed for each treatment and genotype is indicated on the right.

Q/R ratios for GluR-B CDNA were determined at P30 in mice, continuously given Dox, exposed to Dox during prenatal stages, or raised without Dox. Q/R ratios are <0.1% in wild type (Feldmeyer et al., 1999) but were 8.5 and 8.3% in two mice continuously on Dox, in good correspondence to the ratio determined previously for GluR-B^{neo} mice (Feldmeyer et al., 1999). The Q/R ratio increased to 14 ± 0.8% (mean ± SD; n = 4) for Tg^{α-CaMKII-itTA/LC1}GluR-B^{+/neo} mice, on Dox until P0. In two Dox-naïve mice, analyzed at P19 because they would not survive 3 weeks of age (Fig. 3) (see also below), the ratio further increased to 23%. This value is somewhat lower than the 25–28% determined for P15–P18 GluR-B^{+/ΔΔCS} mice (Brusa et al., 1995; Feldmeyer et al., 1999), probably reflecting the mosaic expression of Cre protein in Dox-naïve Tg^{α-CaMKII-itTA/LC1}GluR-B^{neo} mice (supplemental Fig. 1, available at www.jneurosci.org as supplemental material).

The increased Q/R ratio of GluR-B should result in spinous Ca^{2+} influx through the functionally altered AMPA channels, which can be visualized by a transient increase in fluorescence of Ca^{2+}-sensitive dyes during two-photon laser excitation. Indeed, the maximal Ca^{2+} transient amplitude ([ΔF/F]_{max}) for Tg^{α-CaMKII-itTA/LC1}GluR-B^{−/−} mice at P30 ([ΔF/F]_{max} = 1.6 ± 0.8; n = 8 spines) than in age-matched GluR-B^{+/−} mice ([ΔF/F]_{max} = 0.6 ± 0.2; n = 9 spines; p < 0.01; one-sided, unpaired t test) and wild-type ([ΔF/F]_{max} = 0.8 ± 0.3; n = 9 spines; p < 0.05) mice (Fig. 4C, contr., D, left diagram). Bath application of the NMDA channel blocker AP-5 (25 μM) resulted on average in a reduction of the peak amplitude to 60 ± 16% (n = 6 spines) in Tg^{α-CaMKII-itTA/LC1}GluR-B^{−/−} mice, to 22 ± 13% (n = 6 spines) in GluR-B^{+/−} mice, and 6 ± 8% (n = 5 spines) in wild-type mice (Fig. 4C, AP5, D, middle diagram). Additional bath application of the AMPA receptor blocker 2,3-dihydroxy-6-nitro-7-sulfonyl-benzo[f]quinoxaline (NBQX) (10 μM; n = 4 spines) abolished the residual Ca^{2+} transient in Tg^{α-CaMKII-itTA/LC1}GluR-B^{−/−} mice, indicating that spinous Ca^{2+} influx indeed occurred via Ca^{2+}-permeable AMPA receptors (Fig. 4C, NBQX).

Notably, the decay time constant of spinous Ca^{2+} transients in Tg^{α-CaMKII-itTA/LC1}GluR-B^{+/−} mice (τ = 100 ± 30 msec; n = 8 spines) was significantly smaller than in GluR-B^{+/−} mice (τ = 200 ± 50 msec; n = 9 spines; p < 0.0005) and wild-type (τ = 180 ± 50 msec; n = 9 spines; p < 0.005) mice, which generated equal time-integrated Ca^{2+} influx in these genotypes (Fig. 3D, right diagram).

Thus, increased AMPA receptor-mediated Ca^{2+} entry can be detected in postsynaptic locations in GluR-B(Q)-expressing mice. However, a compensatory faster removal of free Ca^{2+} diminishes the potentially higher Ca^{2+} signal in these mice, strengthening the hypothesis that Ca^{2+} signaling by the modified AMPA channels is not a primary cause for seizures in GluR-B(Q)-expressing mice (Feldmeyer et al., 1999).

Increased synaptic excitability but reduced excitatory transmission in hippocampus by sustained GluR-B(Q) expression

To unmask excitability changes, which may be related to the epileptic phenotype ultimately resulting from sustained GluR-B(Q) expression, we recorded simultaneously in the apical dendritic and soma layers in the CA1 region of hippocampal slices (P60–P90 mice). We monitored the presynaptic fiber volley, the

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**Spinous AMPA channel-mediated Ca^{2+} transients in CA1 pyramidal cells**

To indicate altered AMPA receptor properties after the Cre-induced AMPA receptor switch, we monitored the Q/R codon ratio for GluR-B transcripts as a measure of Cre activity at the GluR-B\^{\text{neo}} locus in hippocampi of Tg^{α-CaMKII-itTA/LC1}GluR-B^{−/−} mice and recorded spinous Ca^{2+} transients in CA1 pyramidal cells (Fig. 4A, B).
Figure 4. Spineous Ca\(^{2+}\) transients mediated by altered AMPA channels. A, Two-photon excitation image of CA1 pyramidal neuron filled with Oregon Green BAPTA-1 from prenatally Dox-treated P30 Tg\(^{CaMKII-tTA/LC1/GluR-B^{+/neo}}\). B, Enlarged view of dendritic region indicated by white box in A. The fluorescence channel is overlaid with the infrared-scanning gradient contrast channel to show position of bipolar stimulation pipette close to dendrite. The white arrow indicates spine with clear Ca\(^{2+}\) transient after subthreshold synaptic stimulation. C, Somatic voltage recordings (\(V_m\), membrane potential) and fluorescence changes (\(\Delta F/F\)) in spine head indicated in B. Bath application of the NMDA receptor blocker AP-5 (25 \(\mu\)M) decreased Ca\(^{2+}\) influx (AP5), which could be abolished by additional application of the AMPA receptor blocker NBQX (10 \(\mu\)M, bottom trace). Ca\(^{2+}\) transients were fitted with single exponential (solid line), yielding the peak amplitude (\(\Delta F/F\)\(_{max}\)) and decay time. D, Left, Averaged peak amplitudes of Ca\(^{2+}\) transients. *p < 0.05 compared with wild type (wt) and neo. Middle, Reduction of peak Ca\(^{2+}\) transients amplitudes by bath application of AP-5. **p < 0.005 compared with wild type (wt) and neo. Right, Averaged timed-integrated spineous Ca\(^{2+}\), calculated by multiplication of peak amplitude with decay time. Values represent mean ± SD.

The fiber excitability appeared unchanged by GluR-B(Q) expression, judging from the similar stimulation strengths necessary to elicit fiber volleys with different amplitudes in the two genotypes (Fig. 5B, left diagram). Furthermore, comparison between the two genotypes revealed no statistical difference in the stimulation strength or the fiber volley amplitude necessary to elicit a just-above-threshold population spike (\(p = 0.73\) and \(p = 0.35\), respectively). However, the evoked fEPSPs were significantly lowered by 24, 29, and 30% for presynaptic fiber volleys at 0.5, 1.0, and 1.5 mV in GluR-B(Q)-expressing mice (Fig. 5B, right diagram), which indicates that excitatory synaptic transmission is smaller in GluR-B(Q)-expressing mice than wild-type mice. This reduced synaptic transmission seems to be caused by a weaker response of the postsynaptic cell because paired-pulse facilitation (Fig. 5C) and the excitability of the presynaptic fibers (Fig. 5B, left) were not altered. However, despite the reduced excitatory synaptic transmission in GluR-B(Q)-expressing mice, the threshold for generating a population spike was lowered by 25%. Similarly, the fEPSP size necessary to elicit a population spike of 2 mV was 22% smaller than in wild type (EPSP spike potentiation) (Fig. 5D). Increased excitability was further indicated by the multiple population spikes during the paired-pulse facilitation experiments (Fig. 5A, bottom traces). Notably, multiple spikes were only observed in response to the second stimulus and occurred in 10 of 35 experiments in slices of GluR-B(Q)-expressing mice but only in 1 of 47 of wild type.

Thus, despite the smaller excitatory response, the CA1 cells exhibited a reduced population spike threshold, which might underlie the spontaneous manifestations of epilepsy.

Seizure phenotypes depend on regional expression of altered AMPA receptors

Depth-electrode and single-channel epidural long-term EEG recording combined with video monitoring (5–14 d) were used for detailed analysis of seizure activity in adult mice. Tg\(^{CaMKII-tTA/LC1/GluR-B^{+/neo}}\) mice given Dox until birth and surviving until P30 had mild seizure attacks on average every 4 d. A similar frequency of seizures was recorded in GluR-B(Q)-expressing Tg\(^{Cre+/GluR-B^{+/neo}}\) mice. Most attacks occurred at night, during the active diurnal phase. Seizure relapses monitored in two Dox-naïve Tg\(^{Cre+/GluR-B^{+/neo}}\) mice after phenobarbital withdrawal occurred in clusters at night, were very severe, and eventually lethal.

In general, the EEG analysis showed interictal EEG patterns consisting of spikes, spike series, polyspikes, and 8–10 Hz rhythmic sharp activity (Fig. 6A), which were rarely accompanied by behavioral abnormalities. Spike series usually appeared 1 or 2 d before seizure attacks, and individual spikes consistently increased in number, days before seizures, in both mice given Dox prenatally (Fig. 6B, LC1/neo DoxP0) and Dox-naïve mice after phenobarbital treatment (Fig. 6B, LC1/neo + Pb). However, the number of spikes did not correlate with seizure severity. Mice previously on phenobarbital showed fewer spikes per day but had more severe and eventually lethal seizure attacks than prenatally Dox-treated mice. Intercital spikes were also observed in GluR-B\(^{+/neo}\) mice, and, in one of these mice, the number of spikes
surged for 1 d without seizure incidence (Fig. 6B, neo). In GluR-B<sup>−/−neo</sup> mice, the GluR-B<sup>−/−neo</sup> allele is incompletely silenced (Feldmeyer et al., 1999), and the low levels of GluR-B(Q) from this allele seem to be sufficient for the appearance of interictal EEG patterns.

Without exception, all seizures in GluR-B(Q)-expressing mice started with a 1-min-long series of jerks that coincided with spikes in the electroencephalogram and hence justified the diagnosis of myoclonic jerks (Fig. 7A, B, 1). Jerks were more severe in Dox-naive mice that had undergone previous phenobarbital treatment. In these mice, bursts of jerks corresponded to groups of polyspikes (1–3 sec duration) compared with single myoclonic jerks (spikes ≤500 msec duration) in mice treated prenatally with Dox. Beside the different severity, jerks or groups of jerks occurred on average every 8 sec at a remarkably stable frequency of 0.1–0.2 Hz (Fig. 7A, B, 1). Myoclonic jerks in both Dox-naive and prenatally Dox-treated mice switched into seizure attacks with signs for generalized seizures [EEG, 4–7 Hz (Fig. 7A, B, 2)] and tonic [9–15 Hz (Fig. 7A, B, 3)] and focal seizures with oral automatisms [4–9 Hz (Fig. 7A, B, 4)] and hypomotor fits [7–10 Hz (Fig. 7B, 5)]. Oral automatisms included chewing and repetitive tongue flicking, and hypomotor activity consisted of extensive running fits.

Notably, when GluR-B(Q) expression was restricted to hippocampus (Tg<sup>itTA/</sup>Lc1<sup>GluR<sub>B</sub>/neo</sup> mice; Fig. 1B) (see also Fig. 2A, bottom panel), the mice still displayed rhythmic spike discharge (5–8 Hz activity) but showed no overt seizure activity in simultaneous video monitoring (Fig. 7C). This may indicate failure of synchronous hippocampal activity to spread to other brain regions to elicit seizure symptoms.

The hippocampus plays a dominant role in seizure episodes

Additional evidence for the hippocampus as location of seizure onset was obtained by use of depth electrodes. Two electrodes were placed into the right hippocampus and two electrodes were placed in deeper layers of parietal cortex of mice expressing GluR-B(Q) after birth (<i>n</i> = 3; P80–P100) (mice 1, 3, two electrodes in left cortex; mouse 2, one electrode in each side of the parietal cortex) (for electrode positions, see supplemental Fig. 2, available at www.jneurosci.org as supplemental material).

For each mouse undergoing seizures, 100 randomly chosen interictal spikes were analyzed for occurrence in hippocampal and/or cortical electrode recordings and relative temporal onset. In all three mice, most interictal spikes (86, 77, and 94%) occurred only in the hippocampal recording or showed hippocampal onset and propagated subsequently to the neocortical electrode recording (Fig. 7D). Moreover, the recorded amplitudes were always larger in hippocampus than cortex. Propagation time from hippocampal to cortical electrode recording was impressively constant at 13 msec in mice 1 and 3 and 11 msec in mouse 2. The remaining 14% of spikes in mouse 1 occurred in only one of the unilateral cortical recordings. For mouse 2, 18% of spikes showed simultaneous onset in cortical and hippocampal recordings, and 5% occurred in only one of the cortical recordings. The remaining 6% of spikes in mouse 3 were again of simultaneous onset in cortical and hippocampal recordings.

As observed in the simultaneous epidural one-channel EEG and video recordings, multiple depth-electrode EEG recordings revealed spike series indicative of myoclonic jerks. In one myoclonic series preceding a seizure episode, spikes occurred simultaneously in hippocampal and cortical recordings, whereas in another series, most hippocampal spikes preceded cortically recorded spikes (data not shown). The myoclonic spike series preceding both seizure attacks shifted to a 2 Hz spike pattern with leading hippocampal activity, followed by a low-amplitude, fast-activity pattern of ~40 Hz superimposed on basic α-activity predominantly in hippocampus. The generalized seizure evolved into a 9–10 Hz pattern and subsequently in high-amplitude spike and polyspike activities that were again pronounced in the hippocampus (Fig. 7D).

Collectively, these data strongly suggest that seizure activity in our model is triggered in hippocampal circuits, in agreement with the high GluR-B(Q) expression in CA1 pyramidal cells relative to other brain regions (supplemental Fig. 1, available at www.jneurosci.org as supplemental material).
The hippocampal origin of the seizure attacks mediated by GluR-B(Q) finds support in mice that express GluR-B(Q) exclusively in the postnatal hippocampus. This selective expression was obtained by physically linking the α-CaMKII promoter driving tTA to the neuronal silencer of the NR2C gene of NMDA receptors, which suppresses NR2C gene expression in brain areas other than cerebellum, hippocampus, and some cortical neurons (Suchanek et al., 1997). GluR-B(Q) expression, now even in the absence of Dox confined to hippocampal CA1 pyramidal and DG granule cells, never resulted in clinical seizure symptoms at adult stages. The rhythmic paroxysmal discharge in the electroencephalogram was, however, maintained, indicating that hippocampal expression of functionally switched AMPA receptors is sufficient to generate epileptic activity. Lack of GluR-B(Q) expression in additional brain areas may prevent spread of epileptic excitation to other limbic lobe and motor cortex structures in this clinically mute mouse.

Compatible with the EEG recordings, the hippocampal slices from GluR-B(Q)-expressing forebrains showed increased synaptic excitability, as judged by a lower threshold for population spikes in the CA1 pyramidal cell layer. The reduced spike threshold and the EPSP spike potentiation, previously described as features of LTP (Andersen et al., 1980; Abraham et al., 1987; Chavez-Noriega et al., 1990; Staff and Spruston, 2003; Frick et al., 2004), may thus also feature in epileptiform circuits. We could not yet demonstrate a reduced spike threshold in single GluR-B(Q)-expressing CA1 pyramidal cells by current injection or evoked EPSPs. We surmise that downstream consequences of GluR-B(Q) expression underlying the enhanced excitability include altered intrinsic neuronal excitability from, for example, acquired dendritic channelopathy (Bernard et al., 2004) and/or alterations in the relative contribution of evoked excitation–inhibition (for review, see Zhang and Linden, 2003). Indeed, a significant reduction in the number of somatostatin-positive interneurons in hippocampal stratum oriens was revealed in a study on the cellular consequences of long-term GluR-B(Q) expression (D. Shimshek, Y. Geng, H. Krestel, P. H. Seeburg, and R. Sprengel, unpublished observations), which could, in part, explain the increased excitability. Because the genetic switch to GluR-B(Q) implemented here by use of the α-CaMKII promoter does not operate in GABAergic interneurons in which this promoter is silent, the reduction in interneuron number may well result from recurrent seizure activity, as reported in other seizure models (Cossart et al., 2001). Thus, the persistent increase in circuit excitability by long-term GluR-B(Q) expression may primarily derive from increased intrinsic neuronal excitability by as yet unknown molecular changes in principal neurons and may be accelerated by seizure-induced death of interneurons.

Although the molecular events causing the CNS dysfunctions by GluR-B(Q) remain to be resolved, our study documents that...
expression of the Q/R site-unedited form of the GluR-B subunit is sufficient to produce over time pathological synchronized firing in neuronal populations. Efficient RNA editing in the pore-forming membrane loop M2 of GluR-B can thus be viewed as a critical safeguard against epileptic network activity. This may imply a signal for retention in endoplasmic reticulum, ensuring the generation of functional heteromeric receptors (Greger et al., 2003). The GluR-B(Q) form would override this assembly checkpoint, and GluR-B(Q) containing channels might exhibit altered synaptic trafficking properties (Shi et al., 2001).

Second, the arginine in the pore loop confers to AMPA channels insensitivity to polyamines and reduced Ca\(^{2+}\) permeability and unitary conductance (Burnashev et al., 1992; Swanson et al., 1997). The increased Ca\(^{2+}\)-permeability of AMPA channels in CA1 cells expressing GluR-B(Q), previously determined in nucleated patches (Brusa et al., 1995; Feldmeyer et al., 1999), was demonstrated by AMPA channel-mediated spinous Ca\(^{2+}\) transients in the present study. The altered AMPA channel properties in our conditional mouse model were recorded at P30, weeks before seizures became manifest. Thus, circuit hyperexcitability appears not to be an immediate consequence of GluR-B(Q) expression but to require additional molecular and cellular events. Third, the synaptically localized GluR-B(Q)-containing channels can induce NMDA receptor independent (“non-Hebbian”) plasticity (Feldmeyer et al., 1999), which may trigger the postulated additional plasticity (Feldmeyer et al., 1999), that is, AMPA receptor-mediated conductance in CA1 pyramidal cells (Feldmeyer et al., 1999). However, analysis of synaptic responses reveals that the increased somatic AMPA conductance is not paralleled by a corresponding synaptic increase. Rather, we observed reduced excitatory synaptic transmission,judging from the significantly smaller fEPSPs elicited by prevolleys in GluR-B(Q)-expressing mice. This could be confirmed by recording from single CA1 pyramidal cells after stimulation of Schaffer collaterals in acute slices derived from P40 mice. Wild-type cells showed higher EPSC amplitudes than mutant cells [mean ± SEM of stimulation intensity (in pA/V): wild type, 14.2 ± 1.9 (n = 6); mutant, 8.6 ± 1.1 (n = 10); p = 0.03] (B. Schupp, D. Shimshek, P. H. Seeburg, R. Sprengel, and G. Kohr, unpublished observations), with AMPA currents exhibiting pronounced rectification in the mutant, as expected from the GluR-B(Q) expression. We were unable to detect a presynaptic contribution to the reduced excitatory transmission, as judged from unchanged excitability of presynaptic fibers and unaltered paired-pulse facilitation. Postsynaptic AMPA receptor channel function may be downregulated by sustained GluR-B(Q) expres-
sion, perhaps by non-Hebbian synaptic plasticity (Feldmeyer et al., 1999) or by reduced GluR-B gene transcription after seizures (Huang et al., 2002).

It should be noted that the AMPA channel-mediated Ca$^{2+}$ influx in principal neurons cannot be the sole determinant for triggering the epileptic phenotype of mice expressing GluR-B(Q), because mice with genetically induced expression of AMPA receptors in their maximally Ca$^{2+}$-permeable form, by either global ablation of GluR-B (Jia et al., 1996) or GluR-B depletion in forebrain principal neurons (Krestel, Shimshek, Sprengel, and Seeburg, unpublished observations), do not manifest this phenotype. Hence, the presence of GluR-B in AMPA receptors appears to be necessary to bring about circuit hyperexcitability. This is best explained by the function of GluR-B in AMPA receptor trafficking. According to studies in virus-infected brain slices, GluR-B is important for stabilizing AMPA receptors for normal synaptic transmission (Shi et al., 2001). In the absence of GluR-B, synaptic AMPA receptors are reduced in number, as can be deduced from the decreased synaptic transmission in GluR-B-deficient mice (Jia et al., 1996; Meng et al., 2003). Thus, Ca$^{2+}$ signaling by AMPA channels without GluR-B differs from that of AMPA channels with GluR-B(Q), implicating GluR-B itself (in its unedited form) as a critical determinant in generating epileptiform activity. The investigation of downstream mechanisms after GluR-B(Q) expression, aided by use of the genetically controlled mouse model described here, should ultimately help in elucidating the molecular and cellular changes responsible for the epileptic phenotype.

References


