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Seizures in the developing brain result in a long-lasting decrease in $GABA_B$ inhibitory postsynaptic currents in the rat hippocampus

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ABSTRACT

Whether seizures in the developing brain cause long-term changes in the mature brain has been debated. We tested the hypothesis that a model of early-life seizures, induced by systemic injection of a $GABA_B$ receptor antagonist CGP56999A in immature rats, decreased $GABA_B$ receptor-mediated inhibitory postsynaptic currents (IPSCs) in the hippocampus of adolescent rats. Whole-cell recordings were made in CA1 pyramidal cells and dentate gyrus (DG) granule cells *in vitro*, 30–45 days after the rats had seizures induced by CGP56999A (1–1.5 mg/kg i.p.) or control saline injection on postnatal day 15. $GABA_B$ receptor-mediated IPSCs were reduced in DG neurons but not in CA1 neurons of early-life seizure rats as compared to controls. Additionally, hippocampal neurons of early-life seizure rats, as compared to those in control rats, showed a more depolarized resting membrane potential in both CA1 and DG, and a larger input resistance but reduced spike frequency adaptation in DG neurons. In conclusion, early-life seizures result in a long-lasting reduction in $GABA_B$ receptor-mediated transmission in DG principal neurons and depolarization in CA1 and DG principal neurons. These alterations are expected to increase seizure susceptibility in the adult brain.

Introduction

Seizures are common in children, and the question whether seizures in the developing brain cause long-term changes in neuronal excitability or seizure susceptibility is controversial. Although retrospective studies have shown an association between childhood seizures and temporal lobe epilepsy (Falconer et al., 1964; Maher and McLachlan, 1995), prospective studies did not support a causal relationship (Nelson and Ellenberg, 1978; Shinnar and Glauser, 2002).

Several models of early-life seizures result in long-lasting alterations in both neuronal excitability and seizure susceptibility. After a prolonged hyperthermic seizure in immature rats, the increase in $GABA_A$-receptor-mediated inhibition together with enhanced I_h currents (Chen et al., 1999; Chen et al., 2001) was suggested to contribute to an increased seizure susceptibility (Dube et al., 2006). On the other hand, early-life seizures induced by lithium-pilocarpine enhanced the type I benzodiazepine augmentation of, but not the baseline, $GABA_A$-receptor-mediated currents (Zhang et al., 2004). Fluorothyl-induced seizures in immature rats resulted in a loss of spike adaptation in CA1 neurons 2–3 weeks later (Villeneuve et al., 2000). In a recent model of early-life seizures, rat pups of postnatal day 15 were systemically injected with a $GABA_B$ receptor antagonist CGP56999A (Tsai et al., 2008), which induced repeated hippocampal and limbic seizures for several hours. Adult rats that had early-life CGP56999A-induced seizures showed facilitated hippocampal kindling. Interestingly, the late, presumed $GABA_B$ receptor-mediated paired-pulse inhibition in the adult hippocampus was decreased after early-life seizures (Tsai et al., 2008). Thus, the seizures induced by a $GABA_B$-receptor antagonist were inferred to cause a long-lasting decrease in $GABA_B$ receptor function, which may facilitate kindling epileptogenesis in adult rats (Leung et al., 2005; Vergnes et al., 1997).

The involvement of $GABA_B$ receptors in controlling seizures has been reported in various models of epilepsy. $GABA_B$ receptor-1 gene knockout mice developed spontaneous tonic-clonic seizures and died before reaching adulthood (Prosser et al., 2001; Schuler et al., 2001). $GABA_{B1}$-receptor polymorphism (G1465A) in humans is associated with temporal lobe epilepsy (Gambardella et al., 2003). A $GABA_B$ receptor antagonist induced clonic-tonic and partial seizures in adult rats (Leung et al., 2005; Vergnes et al., 1997). After kainic acid status epilepticus in adult rats, a decrease in mRNA for $GABA_B$ R1a, R1b and R2 subunits was observed in CA1 for up to 30 days (Furtinger et al., 2003), while its immunoreactivity was reduced in CA1 but increased in dentate gyrus (DG) (Straessle et al., 2003). In addition, a decrease of postsynaptic $GABA_B$ receptor-mediated inhibition in CA1 *in vitro* (Mangan and Lothman, 1996) or DG *in vivo* (Wasterlain et al., 1996) was observed after adult status epilepticus induced by continuous

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hippocampal stimulation, but not after partial hippocampal kindling (Liu and Leung, 2003). Presynaptic $GABA_B$ heteroreceptor efficacy was reduced after adult limbic kindling (Asprodini et al., 1992; Poon et al., 2006) and pilocarpine-induced status epilepticus (Chandler et al., 2003), and a decrease of presynaptic $GABA_B$ autoreceptor efficacy was reported after hippocampal kindling (Wu and Leung, 1997).

We are not aware of previous studies on direct recordings of the $GABA_B$ receptor-mediated postsynaptic potentials ($GABA_B$-IPSPs) or currents ($GABA_B$-IPSCs) after early-life seizures. We inferred, based on paired-pulse population spikes, a loss of the late $GABA_B$ receptor-mediated inhibition in the hippocampus *in vivo* after seizures in the immature rats, induced by hyperthermia (Tsai and Leung, 2006) or CGP56999A (Tsai et al., 2008). The initial purpose of the present study was to provide direct evidence of a loss of $GABA_B$ receptor-mediated inhibition after CGP56999A-induced early-life seizures. We hypothesized that CGP56999A-induced early-life seizures caused a long-lasting decrease in $GABA_B$-IPSCs recorded in CA1 and DG neurons in hippocampal slices of adolescent rats. The changes in basic intrinsic membrane properties of CA1 and DG neurons after early-life seizures were also investigated.

Material and methods

CGP56999A-induced seizures

All procedures were approved by the Animal Use Committee at the University of Western Ontario. Seizures were elicited by systemic CGP56999A injection in male Long–Evans rat pups (Charles River, Quebec, Canada) using a previously described paradigm (Tsai et al., 2008). Briefly, CGP56999A (1 mg/kg; a gift from W. Froestl of Novartis) was given intraperitoneally (i.p.) to rat pups of postnatal day 15. A supplementary dose of 0.5 mg/kg i.p. was given if no seizure was detected 3 h after the first injection. Littermate controls were injected with an equivalent volume of saline i.p. After injection, rat pups were reunited with their mother in their home cage, and behavioral seizures were monitored for at least 8 h. Previous electrical recordings indicated that most of the seizures originated in the hippocampus (Tsai et al., 2008), and rapidly progressed to convulsive seizures of various stages—stage 3 (forelimb clonus), stage 4 (bilateral forelimb clonus with rearing) and stage 5 (stage 4 seizure following by falling). More than 10 convulsive seizure episodes were detected in each CGP56999A-injected pup, with a peak frequency of more than 3 convulsive seizures per hour (Tsai et al., 2008). No paroxysmal behaviors were observed in the saline-injected pups.

Brain slices preparation and electrophysiological recordings

Thirty to forty-five days after treatment, brain slices were prepared from CGP56999A- and saline-treated rats, as described previously (Qu and Leung, 2008; Qu and Leung, 2009). Briefly, a rat was anesthetized with isoflurane and its brain quickly removed from skull and placed in cold (~2 °C), oxygenated artificial cerebrospinal fluid (aCSF). Brain slices of 450 μm thick were cut using a vibratome (Series 1000, Vibratome, St. Louis, MO) and incubated in oxygenated aCSF for at least 1 h at room temperature (~23 °C) before recording. The aCSF composition was (in mM): NaCl 124, KCl 2.4, $NaH_2PO_4 \cdot H_2O$ 1.25, $MgSO_4 \cdot 7H_2O$ 2, $CaCl_2 \cdot 6H_2O$ 2, $NaHCO_3$ 26, and glucose 10, and the pH was 7.4 after bubbling with carbogen (95% O_2 and 5% CO_2).

A single slice was transferred to the recording chamber and submerged in carbogen saturated aCSF (~23 °C) flowing at 3 ml/min. Whole-cell recordings were made at the CA1 pyramidal cell and DG granule cell layers, using the blind patch-clamp technique. Patch pipettes were pulled from borosilicate glass capillaries (Prism FLG15; 1.5 mm outer diameter, 1.2 mm inner diameter; Dagan, Minneapolis, MN) using a horizontal puller (Model P80, Sutter Instrument, Novato, CA). The resistance of the pipette was 5–6 MΩ when filled with an internal solution consisting of (in mM): K^+-gluconate 140, $MgCl_2$ 2, 1,2-bis[2-aminophenoxy]ethane-N,N,N′,N′-tetraacetic acid (BAPTA) 0.1, HEPES 10, Na_2GTP 0.2, Na_2ATP 2, and adjusted to pH 7.2 with KOH. In some experiments, biocytin (5 mM) was added to the internal solution for labeling the recorded cells. The series resistance was typically 7–14 MΩ immediately after whole-cell breakthrough. If the series resistance was ≥ 15 MΩ or changed by more than 10% during the experiment, the data were not included in the analysis. Membrane potentials and currents were recorded with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) and low-pass filtered at 2 kHz. Signals were digitized (12 bit) and stored at 10 kHz or 1 kHz using a custom-made program.

Resting membrane potential (RMP), input resistance and spikes were recorded for each neuron in the current clamp mode when perfused with normal aCSF. A neuron was included if the RMP was < −50 mV and the spike overshoot was >15 mV. RMP was determined after stabilization (within 4 min). A junction potential of 11 mV was subtracted from the membrane potential values with K^+-gluconate-based internal solution (Otis et al., 1993). Membrane responses to different hyper- and depolarizing currents of 200-ms duration were recorded; input resistance was determined by the steady-state response to a hyperpolarizing current of 0.1 nA (Qu et al., 2007). The spike height was measured from the baseline to the peak of an action potential evoked by a threshold depolarizing current (0.08−0.4 nA lasting 20 ms). Spike frequency adaptation (decrease of spike firing frequency during the 200-ms depolarization) was estimated by injecting a 200-ms depolarizing current of a magnitude twice the spike threshold current. Spike latency was the time at the peak of the first spike evoked by the latter depolarizing current.

Evoked $GABA_B$-IPSCs were recorded from CA1 pyramidal cells or DG granule cells in response to a bipolar concentric stimulating electrode (200 μm outer diameter; Frederic Haer, Bowdoin, ME) placed near the recording electrode (<0.5 mm) within CA1 stratum radiatum or DG stratum moleculare (Qu and Leung, 2008; Qu and Leung, 2009). The typical $GABA_B$-IPSC was evoked by a 300-Hz train of 10 pulses (0.1−0.5 mA; 0.2 ms duration) in the presence of 6,7 dinitroquinoxaline-2,3-dione (DNQX; 20 μM), (±)-2-amino-5-phosphonopentanoic acid (AP-5; 50 μM) and bicuculline (50 μM). The 10 stimulus pulses at 300 Hz spanned a duration of 30 ms. Presynaptic $GABA_B$ receptor involvement during the stimulus train is expected to be minimal because reliable paired-pulse depression of $GABA_A$-IPSCs was only observed at paired-pulse intervals of ≥25 ms (Davies et al., 1990), and the response of $GABA_A$-IPSCs to a 100-Hz train of stimuli was not affected by a $GABA_B$ receptor antagonist (Davies and Collingridge, 1993). All drugs were purchased from Sigma (St. Louis, MO) or Ascent Scientific (North Somerset, UK) except CGP56999A, which was a gift from Novartis (Basel, Switzerland).

Data analysis

The current–voltage relation (I-V) of evoked $GABA_B$-IPSCs was estimated by plotting the peak currents against the holding potentials (−40 to −120 mV, step 10 mV). Reversal potentials for $GABA_B$-IPSCs were determined by linearly fitting the I-V curve in the outward direction (R^2>0.90). Chord conductance (G) measures for evoked $GABA_B$-IPSCs were obtained at a holding potential (HP) of −50 mV using the formula G = $GABA_B$ current/(holding potential − E_{rev}), where E_{rev} is the reversal potential for $GABA_B$-IPSCs. Since $GABA_B$-IPSCs displayed inward rectification at the membrane potential of −50 mV in DG neurons, the conductance values were likely underestimated.

All values given below were mean ± standard error of the mean (SEM). Comparison between two groups used a Student's *t*-test. Multiple comparisons were made with a repeated measures analysis of variance (ANOVA), as appropriate. A significant ANOVA was followed by a post hoc *t*-test. Differences at $p < 0.05$ were considered to be significant.

Results

Electrophysiological characteristics of CA1 and DG neurons

Whole-cell recordings were made from 21 CA1 pyramidal cells (saline-treated control, $n=12$ cells; CGP56999A-treated, $n=9$ cells) and 39 DG granule cells (control, $n=21$ cells; CGP56999A-treated, $n=18$ cells). CA1 pyramidal cells from control rats had RMP of -71.6 ± 0.9 mV ($n=12$) and input resistance of 191.1 ± 14.4 MΩ ($n=12$). Moreover, recorded CA1 neurons showed a depolarizing afterpotential (data not shown), characteristic of CA1 pyramidal cells, as was subsequently confirmed morphologically by labeling with biocytin. These intrinsic properties of CA1 pyramidal cells were consistent with previous studies (Liu and Leung, 2003; Spigelman et al., 1992; Spruston and Johnston, 1992; Surges et al., 2004). The RMP of CA1 pyramidal cells from CGP56999A-induced seizure rats was significantly depolarized compared with age-matched controls (Fig. 1A; Table 1; $p< 0.01$; unpaired *t*-test), whereas the input resistance did not differ between the two groups (Fig. 1A; Table 1; $p> 0.05$; unpaired *t*-test). In DG granule cells, cells recorded in slices from CGP56999A-treated rats showed a depolarized RMP (Fig. 1B; Table 1; $p< 0.001$; unpaired *t*-test) and an increased input resistance (Fig. 1B; Table 1; $p< 0.05$; unpaired *t*-test) as compared to controls.

Spike frequency adaptation was reduced in DG neurons but not in CA1 neurons of CGP56999A-treated rats as compared to controls. The interval between 1st and 2nd spikes during the depolarizing current injection (current intensity at twice the spike threshold) was significantly shorter in CGP56999A-treated rats ($n=14$) than control rats ($n=17$) (Fig. 1B; Table 1; $p< 0.05$; unpaired *t*-test). As a result of reduced spike frequency adaptation, the total number of spikes generated during the 200-ms depolarizing current was significantly higher in DG neurons of CGP56999A-treated rats as compared to controls (Table 1; $p< 0.001$). In both CA1 and DG neurons, there were no significant differences in the threshold, height, or latency of the spike between control and CGP56999A-treated rats (Table 1). In addition, no rebound depolarization or firing was observed in the two groups, either for CA1 or DG neurons (Fig. 1).

$GABA_B$-IPSCs in the CA1 and DG neurons

$GABA_B$-IPSCs were evoked by a short train of 10 pulses at 300 Hz and recorded at a HP of −50 mV in the presence of DNQX, AP-5 and bicuculline. When trains of pulses were applied, slow outward $GABA_B$-IPSCs were detected in the majority of CA1 and DG neurons. The train stimulation-induced $GABA_B$-IPSCs typically lasted longer than a second in duration, had a peak latency of approximately 300 ms after the stimulation train, and were abolished by CGP55845A (1 μM), a $GABA_B$ receptor antagonist (Fig. 2A). Moreover, the response of $GABA_B$-IPSCs increased to a maximum at a stimulus intensity of 0.4 mA (Fig. 2B) in both control and CGP56999A-treated groups. In CA1 pyramidal cells, the peak amplitude of $GABA_B$-IPSCs was not significantly different at the same stimulation intensity between

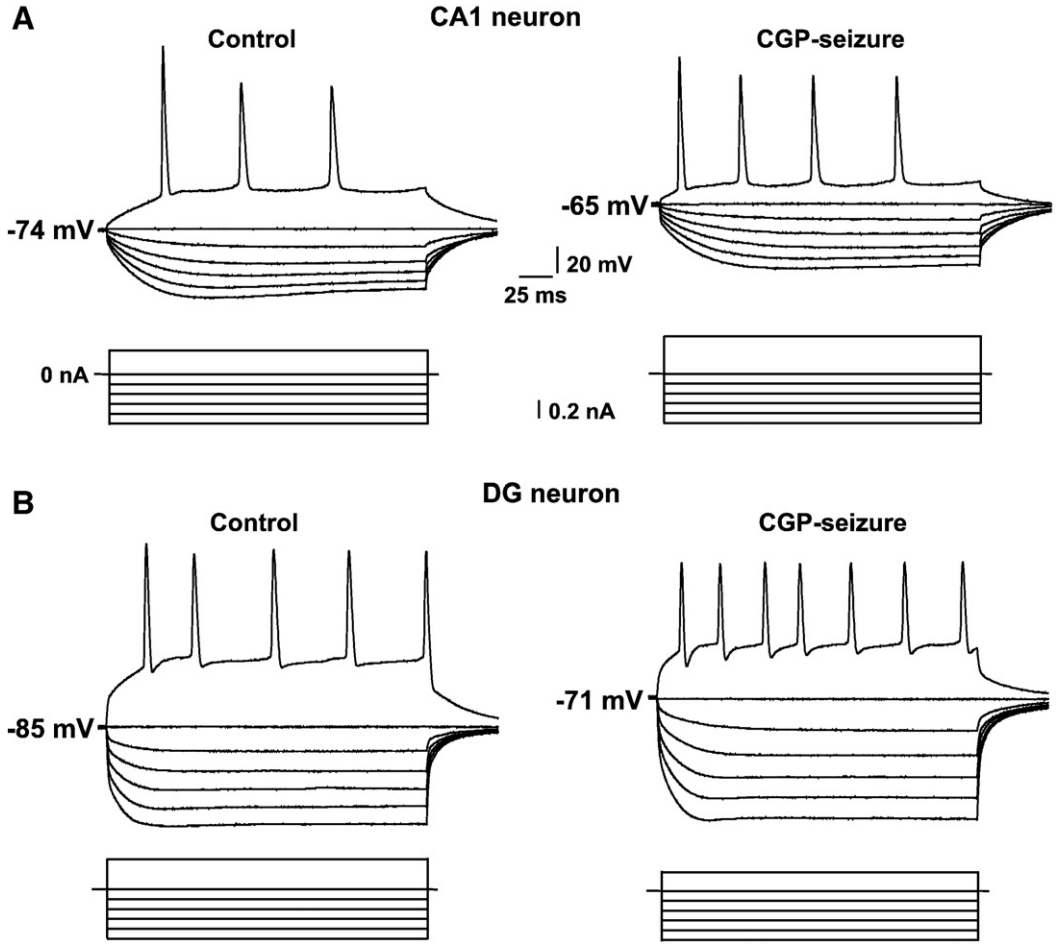

Fig. 1. Membrane responses to current injection in representative CA1 and DG neurons after saline-injection (Control) and early-life seizures induced by CGP56999A (CGP-seizure) on postnatal day 15. Currents of 200-ms duration were injected. Hyperpolarizing currents were −0.5, −0.4, −0.3, −0.2 and −0.1 nA and a single depolarizing current at 2× spike threshold evoked a train of action potentials. (A) CA1 neuron recording from a representative CGP-seizure rat shows a more depolarized resting membrane potential (−65 mV) than a control neuron (−74 mV); group data showed no significant changes in input resistance, spike adaptation were found between control and CGP-seizure groups. (B) A representative DG neuron shows a depolarized resting membrane potential, increased input resistance and reduced spike adaptation in CGP-seizure rats as compared to control rats.

Table 1
Electrophysiological characteristics of CA1 and dentate gyrus (DG) neurons in control and CGP56999A-induced seizure rats.

	Control		CGP56999A seizure rats	
	CA1 (12)	DG (21)	CA1 (9)	DG (18)
Resting potential (mV)	−71.6 ± 0.9	−85.8 ± 1.0	−66.1 ± 1.4[b]	−75.8 ± 1.4[a]
Input resistance (MΩ)	191.1 ± 14.4	215.7 ± 12.5	178.0 ± 9.8	272.9 ± 18.5[c]
Spike height (mV)	108.6 ± 4.4 (10)	121.3 ± 2.9	103.5 ± 5.1	114.9 ± 4.4
Spike threshold (pA)	105.0 ± 12.1	127.6 ± 10.7 (17)	100.0 ± 12.9	103.6 ± 8.3 (16)
Spike latency (ms)	31.7 ± 3.0 (11)	18.8 ± 1.5 (17)	30.3 ± 3.2	18.7 ± 1.6 (14)
Number of spikes	3.2 ± 0.1 (11)	5.5 ± 0.2 (17)	3.8 ± 0.2	7.3 ± 0.2 (14)[a]
SI 1 (ms)	46.0 ± 3.1 (11)	25.8 ± 2.8 (17)	39.0 ± 5.2	14.1 ± 0.7 (14)[a]
SI 2 (normalized)	1.65 ± 0.09 (11)	1.62 ± 0.22 (17)	1.56 ± 0.13	1.57 ± 0.12 (14)
SI 3 (normalized)	n/a	1.81 ± 0.22 (17)	n/a	2.04 ± 0.12 (14)
SI 4 (normalized)	n/a	1.87 ± 0.25 (17)	n/a	2.40 ± 0.17 (14)

Values are mean ± SEM. The number of cells for each measure is the size of the group unless specified in parenthesis. Spike height was the peak amplitude of action potential evoked by a 20-ms depolarizing current. Spike threshold was the current required to evoke an action potential by a 200-ms depolarizing current pulse. The following are spike properties evoked by a 200-ms depolarizing current pulse at 2× spike threshold. Spike latency was the time of the peak of the first spike. The number of spikes during the 200-ms current was counted. Spike interval 1 (SI 1) is the interval between the 2nd and 1st spikes. SI 2 is the interval between the 3rd and 2nd spikes, SI 3 (4th and 3rd spikes), SI 4 (5th and 4th spikes); SI 2–4 intervals were normalized by SI 1. [a]$p < 0.001$; [b]$p < 0.01$; [c]$p < 0.05$; as compared with control group, unpaired *t*-test.

control ($n = 9$) and CGP56999A-treated rats ($n = 7$) (Fig. 2B, left; $p > 0.05$). In DG granule cells, $GABA_B$-IPSC peak amplitude of the CGP56999A-treated group ($n = 10$) was significantly smaller than that of the control group ($n = 9$) at a fixed stimulus intensity, from 0.1 to 0.5 mA (Fig. 2B, right; $p < 0.01$).

Current–voltage relation (I-V) of slow $GABA_B$-IPSCs in CA1 and DG neurons

The slow $GABA_B$-IPSCs evoked by supramaximal intensity (0.5 mA) at different holding potentials (−40 to −120 mV) were compared between control and CGP56999A-treated groups (Fig. 3). The I-V relation of slow $GABA_B$-IPSCs recorded from CA1 pyramidal cells in the two groups appeared linear, without large inward rectification, in the membrane potential range tested (Fig. 3A). The lack of an apparent inward rectification in CA1 pyramidal cells may be partly due to the small $GABA_B$-IPSC amplitudes. The reversal potential and mean chord conductance of $GABA_B$-IPSCs of CA1 neurons were not significantly different between control ($n = 9$) and CGP56999A-treated ($n = 7$) groups (Fig. 3A). In contrast to CA1 neurons, inward rectification of $GABA_B$-IPSCs was obvious in the I-V graph of DG neurons (Fig. 3B), particularly in the control group. The reversal potential of the $GABA_B$-IPSC was −75.5 ± 2.4 mV ($n = 10$) in CGP56999A-treated group, significantly more positive than −83.5 ± 3.9 mV ($n = 8$) in control group (Fig. 3C; $p < 0.05$; unpaired *t*-test). Furthermore, the mean chord conductance was significantly smaller in DG neurons from CGP56999A-treated rats than control rats (Fig. 3C; $p < 0.05$; unpaired *t*-test). The mean chord conductance was 0.36 ± 0.05 nS ($n = 10$) and 0.85 ± 0.15 nS ($n = 8$) in CGP56999A-treated and control groups, respectively.

Discussion

We demonstrated that CGP56999A-induced seizures in 15-day-old rats caused long-lasting changes in the electrophysiological properties of CA1 and DG neurons, including (1) a depolarization of RMPs of CA1 and DG neurons, (2) an increase in input resistance of DG neurons, (3) a reduction in the interval between 1st and 2nd spikes and the average spike frequency evoked by a depolarizing current in DG neurons, and (4) a decrease of $GABA_B$-IPSCs in DG neurons.

Changes of intrinsic membrane properties of hippocampal neurons

In the present study, we showed that the RMPs of CA1 and DG neurons were depolarized following early-life CGP56999A-induced seizures. The reason for the depolarization of the RMPs has not been further determined; a change in membrane Na^+ and Cl^- permeabilities or an alteration in intracellular K^+ during patch recordings is suggested. While the RMP was influenced by the K^+ concentration in the whole-cell patch pipette, complete equilibration of pipette and intracellular K^+ was unlikely to occur during patch recordings of hippocampal neurons (Staley et al., 1992). The activity of Na^+/K^+ pump and active K^+ co-transporters (K^+-Cl^- co-transporter) may alter the K^+ gradient and change the RMP; inhibition of Na^+/K^+ pump activity was reported in kainic acid-induced seizure rats (Vitezic et al., 2008). Depolarization of the RMP was also observed after hyperthermia-induced early-life seizures (Chen et al., 2001). In addition, DG granule cells in CGP56999A-induced seizure rats showed an increased membrane input resistance. The increased input resistance decreases the current required to activate the voltage-gated channels, including the voltage-gated Na^+ channels needed for spiking. Also, an increase in input resistance increases the space constant and allows a greater electrotonic current spread. No significant differences in spike threshold, latency or height were found between seizure and control groups, either for CA1 or DG neurons. However, DG neurons from CGP56999A-treated rats showed a shorter interval between 1st and 2nd spikes and more spikes evoked by a depolarizing current, suggesting an increased excitability and a reduced spike frequency adaptation. Increased spike output from DG neurons would facilitate seizure initiation and propagation to other areas of the hippocampus. Increased membrane excitability on account of reduced spike frequency adaptation has also been reported in other early-life seizure models (Villeneuve et al., 2000).

A persistent enhancement of I_h has been suggested to contribute to the increased seizure susceptibility in the hyperthermia model of febrile seizures (Chen et al., 2001). However, in the present study, we did not find a difference between neurons of seizure and control rats in I_h (data not shown) or I_h-induced rebound depolarization.

Alteration of $GABA_B$-IPSCs in CA1 and DG neurons

We have provided direct and original evidence that CGP56999A-induced early-life seizures caused a decrease in the $GABA_B$-IPSCs in DG. This is consistent with our previous findings that CGP56999A-induced early-life seizures resulted in a loss in the late, $GABA_B$-receptor antagonist-dependent, paired-pulse inhibition of population spikes in urethane-anesthetized rats *in vivo* (Tsai et al., 2008). Several mechanisms may contribute to the decrease of $GABA_B$-IPSCs after CGP56999A-induced seizures. A depolarizing shift in the $GABA_B$-IPSC reversal potential and a reduction in $GABA_B$-IPSC chord conductance in DG neurons of CGP56999A-treated rats are both responsible for the decrease of $GABA_B$-IPSCs near the RMP. Since postsynaptic $GABA_B$

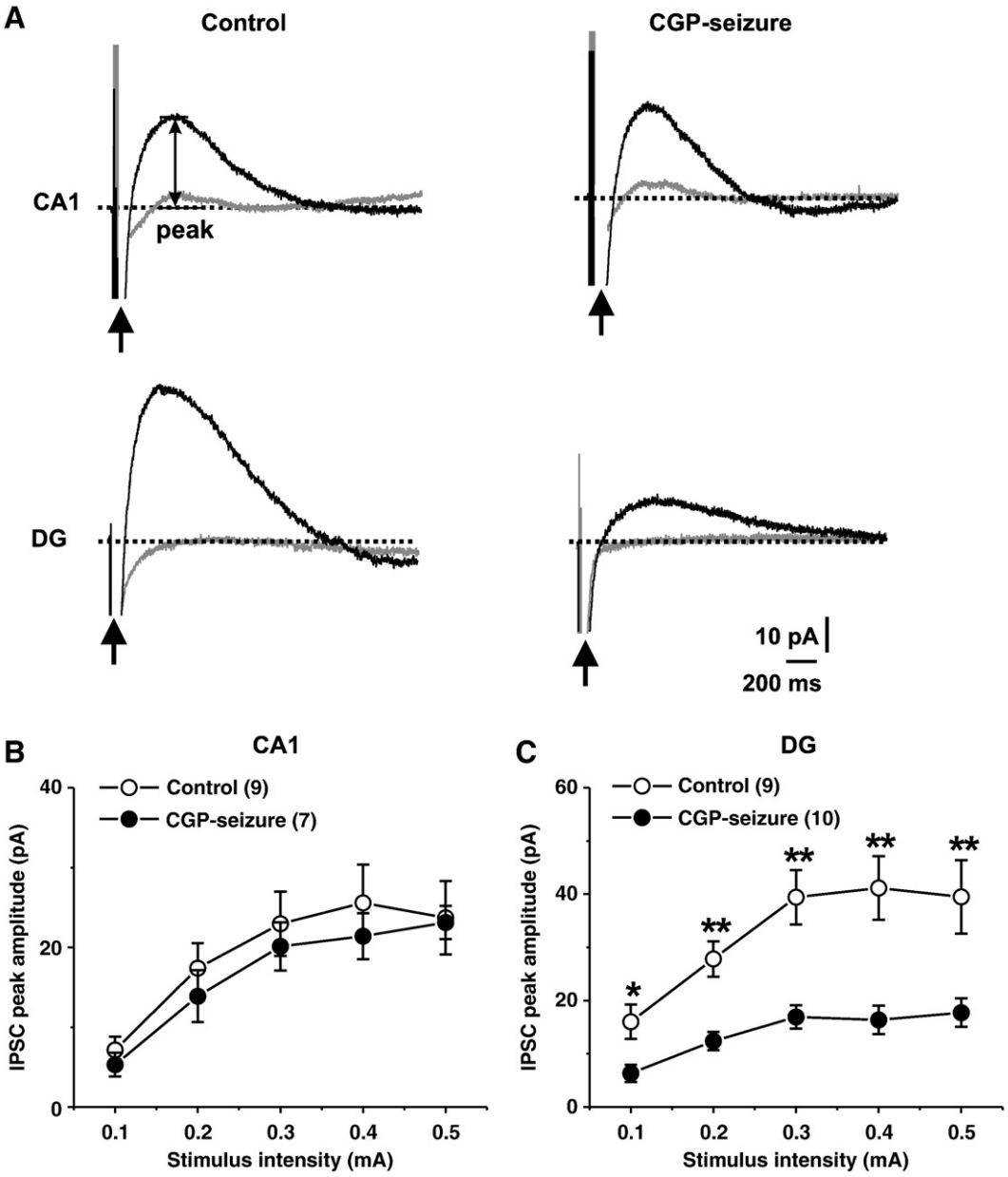

Fig. 2. Electrical stimulation-evoked $GABA_B$ receptor-mediated postsynaptic currents ($GABA_B$-IPSCs) were decreased in DG but not in CA1 neurons following CGP56999A-induced seizures (CGP-seizure). (A) Representative evoked $GABA_B$-IPSCs were recorded in CA1 and DG neurons, 30 days after control and CGP-seizure treatment. Examples of $GABA_B$-IPSCs on each graph were evoked by a 300 Hz train of 10 pulses at the stimulation intensity of 0.5 mA and recorded at a holding potential (HP) of −50 mV in voltage clamp mode (black traces). Evoked $GABA_B$-IPSCs were blocked by 1 μM CGP55845A (grey traces). The arrow before the evoked $GABA_B$-IPSCs indicates the stimulation artifact (truncated). (B) Summary plot of data obtained from recordings similar to those in panel A. The evoked peak $GABA_B$-IPSC amplitude was significantly different between control and CGP-seizure rats in the DG but not in CA1; number of neurons in brackets. $^{**}p < 0.01$, $^{*}p < 0.05$; post hoc *t*-test after a significant two-way (group × intensity) repeated-measures ANOVA.

receptors indirectly gated inward rectifying K^+ channels, the alteration in $GABA_B$-IPSC reversal potential suggests that a change in intracellular K^+ concentration occurred in hippocampal neurons after CGP56999A-induced early-life seizures. We inferred that the intracellular K^+ concentration was not the same as that in the pipette solution, but was maintained actively by the Na^+/K^+ pump and other transporters. $GABA_B$ receptors were also dense in the dendrites, which were less influenced by K^+ leak from the pipette at the soma. The reduction in $GABA_B$-IPSC chord conductance may be due to a reduction in $GABA_B$ receptor number and sensitivity in the postsynaptic membrane. In addition, spillover of GABA appears to be a critical factor for the activation of postsynaptic $GABA_B$ receptors (Isaacson et al., 1993; Scanziani, 2000), but whether GABA release and uptake are altered after CGP56999A-induced seizures remains to be studied. The decrease of $GABA_B$-IPSCs may not be caused by a reduction in the number of neurons in DG since no overall cell loss was found in DG or hilus after CGP569999A-induced early-life seizures, but specific labeling of GABAergic neurons was not done (Tsai et al., 2008).

In contrast to DG, we did not find evidence in support of our hypothesis that $GABA_B$-IPSCs in CA1 neurons were decreased after CGP56999A-induced early-life seizures. There were no significant differences between early-life seizure and control rats in the peak amplitude, reversal potential or chord conductance of the $GABA_B$-IPSCs in CA1. There are several possible reasons why the *in vitro* results may differ from the inferred loss of a late paired-pulse inhibition in CA1 *in vivo* following early-life seizures (Tsai et al., 2008). The most likely reason was that the *in vitro* $GABA_B$-IPSCs were monosynaptically activated by direct stimulation of the GABAergic

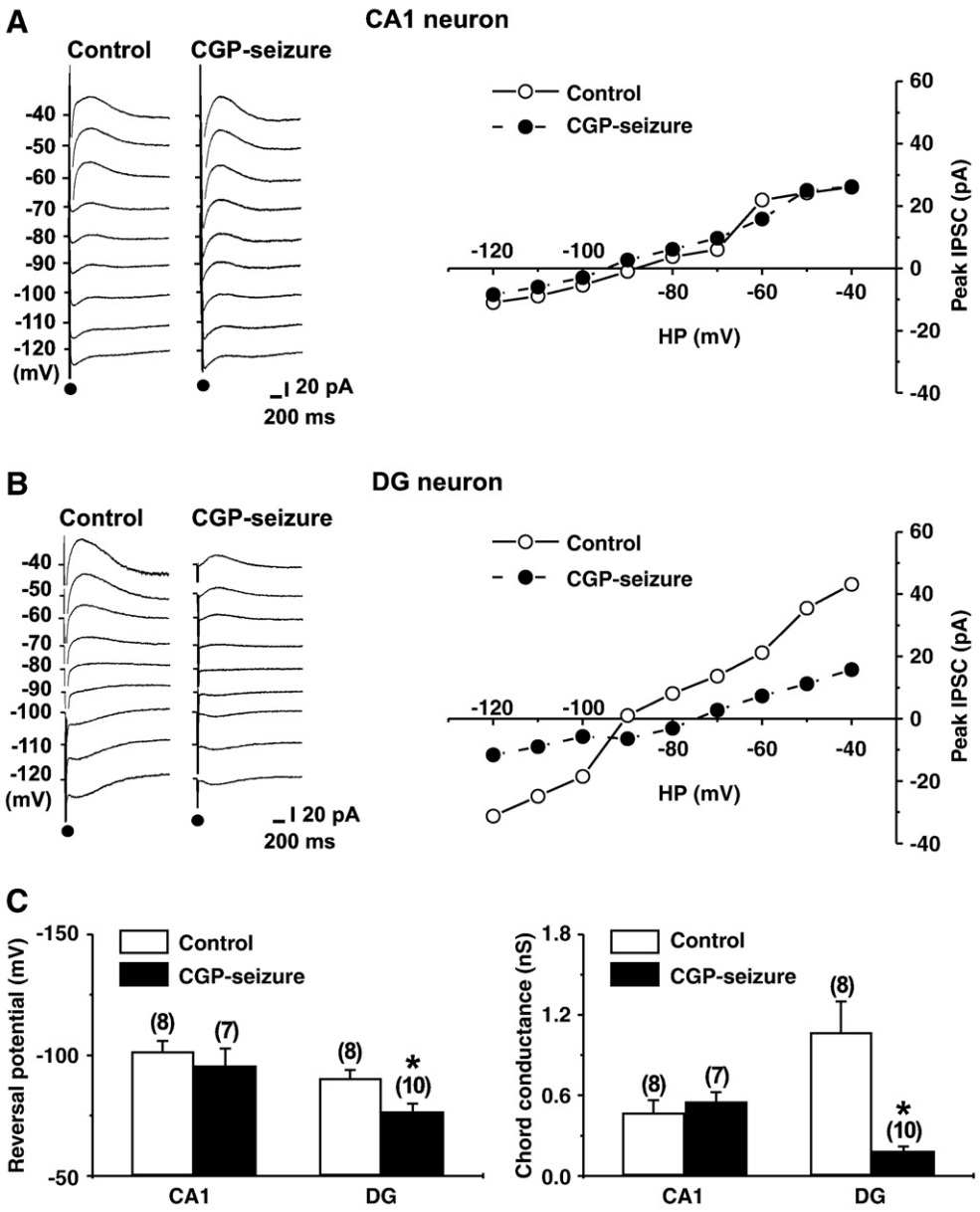

Fig. 3. Current–voltage (I-V) relation of slow $GABA_B$-IPSCs in CA1 and DG neurons. Typical $GABA_B$-IPSCs (average of 3 sweeps) recorded at different holding potentials from −40 to −120 mV, are shown for representative neurons of control and CGP56999A-induced seizure (CGP-seizure) rats. A 300 Hz train of 10 pulses at the stimulation intensity of 0.5 mA were used to evoke $GABA_B$-IPSCs. Filled circles indicate the stimulus artifacts. (A) No alterations in $GABA_B$-IPSC reversal potential and chord conductance of CA1 neurons were observed between the two groups. (B) A depolarizing shift of the reversal potential of $GABA_B$-IPSC was observed in DG neurons from CGP-seizure rats, as compared to control rats. (C) Summary graphs show a significant depolarizing shift in the reversal potential of $GABA_B$-IPSCs in DG neurons, but not in CA1 neurons, when the CGP-seizure group was compared to the controls. In addition, the mean chord conductance of $GABA_B$-IPSCs was significantly reduced in DG neurons but not in CA1 neurons, when the CGP-seizure group was compared to the control group. Number of neurons in brackets. *$p < 0.05$, unpaired t-test.

interneurons, while the *in vivo* $GABA_B$ receptor-mediated inhibition was activated by both feed-forward and feedback inhibition in an intact neuronal network. Thus, activation of $GABA_B$ receptor-mediated inhibition by the interneuronal network may be decreased in early-life seizure rats *in vivo* but synaptic excitation of the interneurons was blocked by glutamatergic antagonists *in vitro*. In addition, while a physiological temperature (37 °C) was maintained *in vivo*, measurement of the late inhibition *in vivo* does not control for membrane depolarization changes.

We did not address the issue whether a single CGP56999A injection in immature rats, without inducing seizures, may induce alterations in $GABA_B$ receptor function. Short-term (2 h) increase in $GABA_B$ receptor immunoreactivity was reported after the injection of $GABA_B$ antagonist phaclofen (Park et al., 2004). A long-term increase in $GABA_B$ receptor binding and increased cyclic AMP response to forskolin were found in the frontal cortex after 21-day repeated treatment with a $GABA_B$ antagonist CGP36742 (Pratt and Bowery, 1993). In addition, most expression and binding studies did not distinguish between presynaptic and postsynaptic $GABA_B$ receptors. While in general there may be no relation between expression and function of $GABA_B$ receptors (Francis et al., 1999), transient expression and long-lasting binding studies suggest an *increase* in $GABA_B$ receptor function after treatment with $GABA_B$ receptor antagonists. In contrast, the present study found a long-lasting *decrease* of $GABA_B$-IPSCs, which is not expected after a single injection of CGP56999A. The fact that both repeated hyperthermia-induced seizures and CGP56999A-induced seizures resulted in loss of $GABA_B$ receptor-mediated paired-pulse inhibition *in vivo* also suggests that seizure but not CGP56999A exposure in the immature rats was responsible for the decrease of $GABA_B$ receptor function. While both

hyperthermia and CGP56999A induced seizures that involved the hippocampus in immature rats, hyperthermia induced myoclonus, tonic postures and hindlimb kicking more often than the typical limbic seizures induced by CGP56999A.

In conclusion, this study indicates that early-life seizures induced by CGP56999A increase the excitability of CA1 and DG neurons as evidenced by the depolarized RMPs and a reduction in spike frequency adaptation in mature DG neurons. In addition, early-life seizures induce a long-lasting decrease of $GABA_B$ receptor-mediated synaptic transmission, which is expected to contribute to the increased seizure susceptibility and epileptogenesis throughout adult life.

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References

Asprodini, E.K., Rainnie, D.G., Shinnick-Gallagher, P., 1992. Epileptogenesis reduces the sensitivity of presynaptic gamma-aminobutyric acidB receptors on glutamatergic afferents in the amygdala. J. Pharmacol. Exp. Ther. 262, 1011–1021.

Chandler, K.E., Princivalle, A.P., Fabian-Fine, R., Bowery, N.G., Kullmann, D.M., Walker, M.C., 2003. Plasticity of GABA(B) receptor-mediated heterosynaptic interactions at mossy fibers after status epilepticus. J. Neurosci. 23, 11382–11391.

Chen, K., Baram, T.Z., Soltesz, I., 1999. Febrile seizures in the developing brain result in persistent modification of neuronal excitability in limbic circuits. Nat. Med. 5, 888–894.

Chen, K., Aradi, I., Thon, N., Eghbal-Ahmadi, M., Baram, T.Z., Soltesz, I., 2001. Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. Nat. Med. 7, 331–337.

Davies, C.H., Collingridge, G.L., 1993. The physiological regulation of synaptic inhibition by GABAB autoreceptors in rat hippocampus. J. Physiol. 472, 245–265.

Davies, C.H., Davies, S.N., Collingridge, G.L., 1990. Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. J. Physiol. 424, 513–531.

Dube, C., Richichi, C., Bender, R.A., Chung, G., Litt, B., Baram, T.Z., 2006. Temporal lobe epilepsy after experimental prolonged febrile seizures: prospective analysis. Brain 129, 911–922.

Falconer, M.A., Serafetinides, E.A., Corsellis, J.A., 1964. Etiology and pathogenesis of temporal lobe epilepsy. Arch. Neurol. 10, 233–248.

Francis, J., Zhang, Y., Ho, W., Wallace, M.C., Zhang, L., Eubanks, J.H., 1999. Decreased hippocampal expression, but not functionality, of GABA(B) receptors after transient cerebral ischemia in rats. J. Neurochem. 72, 87–94.

Furtinger, S., Bettler, B., Sperk, G., 2003. Altered expression of GABAB receptors in the hippocampus after kainic-acid-induced seizures in rats. Brain Res. Mol. Brain Res. 113, 107–115.

Gambardella, A., Manna, I., Labate, A., Chifari, R., La Russa, A., Serra, P., Cittadella, R., Bonavita, S., Andreoli, V., LePiane, E., Sasanelli, F., Di Costanzo, A., Zappia, M., Tedeschi, G., Aguglia, U., Quattrone, A., 2003. GABA(B) receptor 1 polymorphism (G1465A) is associated with temporal lobe epilepsy. Neurology 60, 560–563.

Isaacson, J.S., Solis, J.M., Nicoll, R.A., 1993. Local and diffuse synaptic actions of GABA in the hippocampus. Neuron 10, 165–175.

Leung, L.S., Canning, K.J., Shen, B., 2005. Hippocampal afterdischarges after GABA(B)-receptor blockade in the freely moving rat. Epilepsia 46, 203–216.

Liu, X., Leung, L.S., 2003. Partial hippocampal kindling increases GABAB receptor-mediated postsynaptic currents in CA1 pyramidal cells. Epilepsy Res. 57, 33–47.

Maher, J., McLachlan, R.S., 1995. Febrile convulsions. Is seizure duration the most important predictor of temporal lobe epilepsy. Brain 118 (Pt 6), 1521–1528.

Mangan, P.S., Lothman, E.W., 1996. Profound disturbances of pre- and postsynaptic GABAB-receptor-mediated processes in region CA1 in a chronic model of temporal lobe epilepsy. J. Neurophysiol. 76, 1282–1296.

Nelson, K.B., Ellenberg, J.H., 1978. Prognosis in children with febrile seizures. Pediatrics 61, 720–727.

Otis, T.S., De Koninck, Y., Mody, I., 1993. Characterization of synaptically elicited GABAB responses using patch-clamp recordings in rat hippocampal slices. J. Physiol. 463, 391–407.

Park, S.K., An, S.J., Hwang, I.K., Kim, D.W., Jung, J.Y., Won, M.H., Choi, S.Y., Kwon, O.S., Jeong, Y.G., Kang, T.C., 2004. Altered GABAB receptor immunoreactivity in the gerbil hippocampus induced by baclofen and phaclofen, not seizure activity. Neurosci. Res. 49, 405–416.

Poon, N., Kloosterman, F., Wu, C., Leung, L.S., 2006. Presynaptic GABA(B) receptors on glutamatergic terminals of CA1 pyramidal cells decrease in efficacy after partial hippocampal kindling. Synapse 59, 125–134.

Pratt, G.D., Bowery, N.G., 1993. Repeated administration of desipramine and a GABAB receptor antagonist, CGP 36742, discretely up-regulates GABAB receptor binding sites in rat frontal cortex. Br. J. Pharmacol. 110, 724–735.

Prosser, H.M., Gill, C.H., Hirst, W.D., Grau, E., Robbins, M., Calver, A., Soffin, E.M., Farmer, C.E., Lanneau, C., Gray, J., Schenck, E., Warmerdam, B.S., Clapham, C., Reavill, C., Rogers, D.C., Stean, T., Upton, N., Humphreys, K., Randall, A., Geppert, M., Davies, C. H., Pangalos, M.N., 2001. Epileptogenesis and enhanced prepulse inhibition in GABA(B1)-deficient mice. Mol. Cell. Neurosci. 17, 1059–1070.

Qu, L., Leung, L.S., 2008. Mechanisms of hyperthermia-induced depression of GABAergic synaptic transmission in the immature rat hippocampus. J. Neurochem. 106, 2158–2169.

Qu, L., Leung, L.S., 2009. Effects of temperature elevation on neuronal inhibition in hippocampal neurons of immature and mature rats. J. Neurosci. Res. 87, 2773–2785.

Qu, L., Liu, X., Wu, C., Leung, L.S., 2007. Hyperthermia decreases GABAergic synaptic transmission in hippocampal neurons of immature rats. Neurobiol. Dis. 27, 320–327.

Scanziani, M., 2000. GABA spillover activates postsynaptic GABA(B) receptors to control rhythmic hippocampal activity. Neuron 25, 673–681.

Schuler, V., Luscher, C., Blanchet, C., Klix, N., Sansig, G., Klebs, K., Schmutz, M., Heid, J., Gentry, C., Urban, L., Fox, A., Spooren, W., Jaton, A.L., Vigouret, J., Pozza, M., Kelly, P. H., Mosbacher, J., Froestl, W., Kaslin, E., Korn, R., Bischoff, S., Kaupmann, K., van der Putten, H., Bettler, B., 2001. Epilepsy, hyperalgesia, impaired memory, and loss of pre-and postsynaptic GABA(B) responses in mice lacking GABA(B(1)). Neuron 31, 47–58.

Shinnar, S., Glauser, T.A., 2002. Febrile seizures. J. Child Neurol. 17 (Suppl. 1), S44–S52.

Spigelman, I., Zhang, L., Carlen, P.L., 1992. Patch-clamp study of postnatal development of CA1 neurons in rat hippocampal slices: membrane excitability and K+ currents. J. Neurophysiol. 68, 55–69.

Spruston, N., Johnston, D., 1992. Perforated patch-clamp analysis of the passive membrane properties of three classes of hippocampal neurons. J. Neurophysiol. 67, 508–529.

Staley, K.J., Otis, T.S., Mody, I., 1992. Membrane properties of dentate gyrus granule cells: comparison of sharp microelectrode and whole-cell recordings. J. Neurophysiol. 67, 1346–1358.

Straessle, A., Loup, F., Arabadzisz, D., Ohning, G.V., Fritschy, J.M., 2003. Rapid and long-term alterations of hippocampal GABAB receptors in a mouse model of temporal lobe epilepsy. Eur. J. Neurosci. 18, 2213–2226.

Surges, R., Freiman, T.M., Feuerstein, T.J., 2004. Input resistance is voltage dependent due to activation of Ih channels in rat CA1 pyramidal cells. J. Neurosci. Res. 76, 475–480.

Tsai, M.L., Leung, L.S., 2006. Decrease of hippocampal GABA B receptor-mediated inhibition after hyperthermia-induced seizures in immature rats. Epilepsia 47, 277–287.

Tsai, M.L., Shen, B., Leung, L.S., 2008. Seizures induced by GABAB-receptor blockade in early-life induced long-term GABA(B) receptor hypofunction and kindling facilitation. Epilepsy Res. 79, 187–200.

Vergnes, M., Boehrer, A., Simler, S., Bernasconi, R., Marescaux, C., 1997. Opposite effects of GABAB receptor antagonists on absences and convulsive seizures. Eur. J. Pharmacol. 332, 245–255.

Villeneuve, N., Ben-Ari, Y., Holmes, G.L., Gaiarsa, J.L., 2000. Neonatal seizures induced persistent changes in intrinsic properties of CA1 rat hippocampal cells. Ann. Neurol. 47, 729–738.

Vitezic, D., Pelcic, J.M., Zupan, G., Vitezic, M., Ljubicic, D., Simonic, A., 2008. Na^+, K^+-ATPase activity in the brain of the rats with kainic acid-induced seizures: influence of lamotrigine. Psychiatr. Danub. 20, 269–276.

Wasterlain, C.G., Shirasaka, Y., Mazarati, A.M., Spigelman, I., 1996. Chronic epilepsy with damage restricted to the hippocampus: possible mechanisms. Epilepsy Res. 26, 255–265.

Wu, C., Leung, L.S., 1997. Partial hippocampal kindling decreases efficacy of presynaptic GABAB autoreceptors in CA1. J. Neurosci. 17, 9261–9269.

Zhang, G., Raol, Y.H., Hsu, F.C., Coulter, D.A., Brooks-Kayal, A.R., 2004. Effects of status epilepticus on hippocampal GABAA receptors are age-dependent. Neuroscience 125, 299–303.