Seizures in the developing brain result in a long-lasting decrease in GABA\textsubscript{B} inhibitory postsynaptic currents in the rat hippocampus

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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\textsubscript{B} receptor antagonist CGP56999A in immature rats, decreased GABA\textsubscript{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\textsubscript{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\textsubscript{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.

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\textbf{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\textsubscript{B}-receptor-mediated inhibition together with enhanced \( h \) 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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{B}-receptor antagonist were inferred to cause a long-lasting decrease in GABA\textsubscript{B} receptor function, which may facilitate kindling epileptogenesis in adult rats (Leung et al., 2005; Vergnes et al., 1997).

The involvement of GABA\textsubscript{B} receptors in controlling seizures has been reported in various models of epilepsy. GABA\textsubscript{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\textsubscript{B1}-receptor polymorphism (G1465A) in humans is associated with temporal lobe epilepsy (Gambardella et al., 2003). A GABA\textsubscript{B1} 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\textsubscript{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) (Straesle et al., 2003). In addition, a decrease of postsynaptic GABA\textsubscript{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 seizures in the developing brain result in a long-lasting decrease in GABA\textsubscript{B} inhibitory postsynaptic currents in the rat hippocampus...
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 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_{2}PO_{4}/H_{2}O 1.25, MgSO_{4}/7H_{2}O 2, CaCl_{2}/6H_{2}O 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 (Piram FRG15; 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]etane-N,N′,N″-tetraacetic acid (BAPTA) 0.1, HEPES 10, Na_{2}GTP 0.2, Na_{2}ATP 2, and adjusted to pH 7.2 with KOH. In some experiments, bicuculline (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 break-in. 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, Bowdon, 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-phosphono-pentanoic 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_{B}-IPSCs was only observed at paired-pulse intervals of ≥ 25 ms (Davies et al., 1990), and the response of GABA_{B}-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 = 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 $-71.6 \pm 0.9$ mV (*n* = 12) and input resistance of $191.1 \pm 14.4$ 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 GABAB 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

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**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.
current (0.36 ± 0.05 nS (in DG neurons from CGP56999A-treated rats than control rats (Fig. 3A). Furthermore, the mean chord conductance 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, left; p > 0.05). In DG granule cells, GABA<sub>B</sub>-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<sub>B</sub>-IPSCs in CA1 and DG neurons

The slow GABA<sub>B</sub>-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<sub>B</sub>-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<sub>B</sub>-IPSC amplitudes. The reversal potential and mean chord conductance of GABA<sub>B</sub>-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<sub>B</sub>-IPSCs was obvious in the I-V graph of DG neurons (Fig. 3B), particularly in the control group. The reversal potential of the GABA<sub>B</sub>-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.

**Table 1**

| Current–voltage relation (I-V) of slow GABA<sub>B</sub>-IPSCs in CA1 and DG neurons |
|-----------------|-----------------|-----------------|
| **Control**     | **DG (21)**     | **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<sup>a</sup> | −75.8 ± 1.4<sup>a</sup> |
| Input resistance (MΩ) | 103.5 ± 2.9     | 121.3 ± 2.9     | 134.9 ± 4.4     | 134.9 ± 4.4     |
| Spike threshold (VF) | 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)<sup>a</sup> |
| SI 1 (ms)        | 46.0 ± 3.1 (11) | 25.8 ± 2.8 (17)  | 39.0 ± 5.2      | 14.1 ± 0.7 (14)<sup>a</sup> |
| SI 2 (normalized) n/a | 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 200-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. *p < 0.001; †p < 0.01; ‡p < 0.05; as compared with control group, unpaired t-test.

**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<sub>B</sub>-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<sup>+</sup> and Cl<sup>−</sup> permeabilities or an alteration in intracellular K<sup>+</sup> during patch recordings is suggested. While the RMP was influenced by the K<sup>+</sup> concentration in the whole-cell patch pipette, complete equilibration of pipette and intracellular K<sup>+</sup> was unlikely to occur during patch recordings of hippocampal neurons (Staley et al., 1992). The activity of Na<sup>+</sup>/K<sup>+</sup> pump and active K<sup>+</sup> co-transporters (K<sup>+</sup>-Cl<sup>−</sup> co-transporter) may alter the K<sup>+</sup> gradient and change the RMP; inhibition of Na<sup>+</sup>/K<sup>+</sup> 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<sup>+</sup> 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<sub>L</sub> 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<sub>L</sub> (data not shown) or I<sub>L</sub>-induced rebound depolarization.

**Alteration of GABA<sub>B</sub>-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<sub>B</sub>-IPSCs in DG. This is consistent with our previous findings that CGP56999A-induced early-life seizures resulted in a loss in the late, GABA<sub>B</sub>-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<sub>B</sub>-IPSCs after CGP56999A-induced seizures. A depolarizing shift in the GABA<sub>B</sub>-IPSC reversal potential and a reduction in GABA<sub>B</sub>-IPSC chord conductance in DG neurons of CGP56999A-treated rats are both responsible for the decrease of GABA<sub>B</sub>-IPSCs near the RMP. Since postsynaptic GABA<sub>B</sub>
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 CGP56999A-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. 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.
interneurons, while the in vivo GABA<sub>B</sub> receptor-mediated inhibition was activated by both feed-forward and feedback inhibition in an intact neuronal network. Thus, activation of GABA<sub>B</sub> 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<sub>B</sub> receptor function. Short-term (2 h) increase in GABA<sub>B</sub> receptor immunoreactivity was reported after the injection of GABA<sub>B</sub> antagonist phaclofen (Park et al., 2004). A long-term increase in GABA<sub>B</sub> receptor binding and increased cyclic AMP response to forskolin were found in the frontal cortex after 21-day repeated treatment with a GABA<sub>B</sub> antagonist CGP36742 (Pratt and Bowery, 1993). In addition, most expression and binding studies did not distinguish between presynaptic and postsynaptic GABA<sub>B</sub> receptors. While in general there may be no relation between expression and function of GABA<sub>B</sub> receptors (Francis et al., 1999), transient expression and long-lasting binding studies suggest an increase in GABA<sub>B</sub> receptor function after treatment with GABA<sub>B</sub> receptor antagonists. In contrast, the present study found a long-lasting decrease of GABA<sub>B</sub>-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<sub>B</sub> 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<sub>B</sub> receptor function. While both

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Fig. 3. Current–voltage (I-V) relation of slow GABA<sub>B</sub>-IPSCs in CA1 and DG neurons. Typical GABA<sub>B</sub>-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<sub>B</sub>-IPSCs. Filled circles indicate the stimulus artifacts. (A) No alterations in GABA<sub>B</sub>-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<sub>B</sub>-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<sub>B</sub>-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<sub>B</sub>-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.
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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