Epileptiform activity triggers long-term plasticity of $GABA_B$ receptor signalling in the developing rat hippocampus

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$GABA_B$ receptor ($GABA_BR$)-mediated presynaptic inhibition regulates neurotransmitter release from synaptic terminals. In the neonatal hippocampus, $GABA_BR$ activation reduces GABA release and terminates spontaneous network discharges called giant depolarizing potentials (GDPs). Blocking $GABA_BRs$ transforms GDPs into longer epileptiform discharges. Thus, $GABA_BR$-mediated presynaptic inhibition of GABA release (GABA auto-inhibition) controls both spontaneous network activity and excitability in the developing hippocampus. Here we show that extensive release of endogenous GABA during epileptiform activity impairs GABA auto-inhibition, but not $GABA_BR$-mediated inhibition of glutamate release, leading to hyperexcitability of the neonatal hippocampal network. Paired-pulse depression of GABA release (PPD) and heterosynaptic depression of glutamate release were used to monitor the efficacy of presynaptic $GABA_BR$-mediated inhibition in slices. PPD, but not heterosynaptic depression, was dramatically reduced after potassium (K^+)-induced ictal-like discharges (ILDs), suggesting a selective impairment of $GABA_BR$-dependent presynaptic inhibition of GABAergic terminals. Impairing GABA auto-inhibition induced a 44% increase in GDP width and the appearance of pathological network discharges. Preventing GABA-induced activation of $GABA_BRs$ during ILDs avoided PPD loss and most modifications of the network activity. In contrast, a partial block of $GABA_BRs$ induced network discharges strikingly similar to those observed after K^+-driven ILDs. Finally, neither loss of GABA auto-inhibition nor network hyperexcitability could be observed following synchronous release of endogenous GABA in physiological conditions (during GDPs at 1 Hz). Thus, epileptiform activity was instrumental to impair $GABA_BR$-dependent presynaptic inhibition of GABAergic terminals. In conclusion, our results indicate that endogenous GABA released during epileptiform activity can reduce GABA auto-inhibition and trigger pathological network discharges in the newborn rat hippocampus. Such functional impairment may play a role in acute post-seizure plasticity.

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The occurrence of seizures during development strongly increases the probability of developing chronic epilepsy at adult age (Holmes & Ben-Ari, 1998). This suggests that plastic changes occurring during and after seizures underlie subsequent remodelling. Ictal discharges are stereotyped epileptic events involving the synchronous activation of thousands of neurones in the cortex and hippocampus. Long-term changes associated with ictal activity have been thoroughly characterized in the developing brain, and include neuronal hyperexcitability (Villeneuve *et al.* 2000; Bender *et al.* 2003), glutamatergic axonal sprouting (Holmes *et al.* 1998; Bender *et al.* 2003) and modifications of ionotropic γ-aminobutyric acid (GABA)-receptor mediated synaptic transmission (Chen *et al.* 1999; Khalilov *et al.* 2003). Impaired $GABA_B$ receptor ($GABA_BR$)-mediated signalling has been observed after epileptic activity in the adult hippocampus (Mangan & Lothman, 1996; Wu & Leung, 1997; Haas *et al.* 1996; Chandler *et al.* 2003). In the developing hippocampus, however, it is not clear whether $GABA_BR$-mediated signalling is functional after seizures.

$GABA_BRs$ play a crucial role in controlling the physiological activity of the immature hippocampal network. $GABA_A$ currents, which mediate most of the inhibitory drive in the adult, are excitatory during the first postnatal week of life (Cherubini *et al.* 1991). Consequently, the synchronous activation of hippocampal interneurones results in excitatory network discharges

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called giant depolarizing potentials (GDPs) (Ben-Ari *et al.* 1989). In these peculiar conditions, $GABA_BR$ activation is essential to limit network excitability. Endogenous GABA released during GDPs activates $GABA_BRs$, thus reducing further GABA release from interneurones and leading to GDP termination (McLean *et al.* 1996). Consistent with this, the pharmacological block of $GABA_BRs$ progressively increases GDP width, transforming GDPs into epileptiform discharges (McLean *et al.* 1996; Tosetti *et al.* 2004). Similar effects can also be observed after the functional impairment of $GABA_BR$-mediated presynaptic inhibition of GABA release (GABA auto-inhibition) by a prolonged application of baclofen, a selective $GABA_BR$ agonist (Tosetti *et al.* 2004). This observation raises the interesting possibility that a sustained release of endogenous GABA may trigger a functional loss of GABA auto-inhibition, leading to hyperexcitability and pathological network activity. Ictal discharges, during which both GABA and glutamate are continuously released for several seconds (Velazquez & Carlen, 1999; Kohling *et al.* 2000; Rutecki *et al.* 1985), are one possible context for such loss to occur.

Here we report that extensive endogenous GABA release during ictal-like discharges impairs GABA auto-inhibition in the newborn rat hippocampus. Such impairment contributes to the network hyperexcitability observed following the epileptiform discharges. These data thus reveal a novel role of $GABA_BR$-mediated signalling in acute post-seizure plasticity of the developing hippocampus.

Methods

Tissue preparation

Brains were removed from anaesthetized (350 mg kg^{-1} chloral hydrate I.P.) male Wistar rats (postnatal day 2–5) and immersed into ice-cold (2–4°C) artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl, 126; KCl, 3.5; $CaCl_2$, 2; $MgCl_2$, 1.3; NaH_2PO_4, 1.2; $NaHCO_3$, 25; and glucose, 11; pH 7.4, when equilibrated with 95% O_2 and 5% CO_2. All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Intact hippocampal formations (IHFs) were dissected out as previously described (Khalilov *et al.* 1997), and incubated in oxygenated ACSF at room temperature. When needed, 600 μm slices were prepared from IHFs using a McIlwain tissue chopper. Both intact IHFs and slices were equilibrated at room temperature in oxygenated ACSF for at least 60 min prior to electrophysiological recordings.

Recording solutions

ACSF, the extracellular recording solution, was prepared from a 10× stock solution immediately before the experiment, equilibrated with 95% O_2 and 5% CO_2, heated at 34°C, and delivered to the recording chamber via a pressurized system at a flow rate of 3 ml min^{-1} (slices), or 10 ml min^{-1} (IHF).

For whole-cell recordings from hippocampal slices, intracellular solution contained (mM): 150 $CsCl_2$, 2 $MgCl_2$, 0.1 $CaCl_2$, 1 EGTA, 2.5 Na_2ATP, 0.4 Na_2-GTP, 1.5 Mg-ATP, 10 Hepes, pH = 7.25, 2QX314, osmolarity = 275 mosmol l^{-1}. In these ionic conditions, both ionotropic GABAergic and glutamatergic currents reversed around 0 mV. For patch-clamp recordings of the IHF, the intracellular solution contained (mM): 120 Cs-gluconate, 13.4 CsCl, 10 Hepes, 1.1 EGTA, 0.1 $CaCl_2$, 0.4 Na_2GTP, 4 MgATP, pH = 7.4. In these ionic conditions, $GABA_A$ currents reversed around −50 mV, while ionotropic glutamatergic currents reversed around +10 mV. For all patch-clamp experiments, 5,6-tetramethylrhodamine biocytin (rhodamine) was dissolved (0.5–1%) in the internal solution to allow *post hoc* visualization of the recorded neurones. For extracellular field recordings, pipettes were filled with ACSF.

Electrophysiological recordings

CA3 pyramidal neurones were recorded from either hippocampal slices or IHFs (Khalilov *et al.* 1997). Spontaneous and evoked synaptic activities were acquired with an Axopatch 2B patch-clamp amplifier (Axon Instruments, USA), filtered at 10 kHz, digitized using a Digidata 1200 A/D interface (Axon Instruments, USA) and stored on a Pentium II 450 MHz PC running Axoscope 8.1 (Axon Instruments, USA) for off-line analysis. Field potentials were recorded using a DAM80 Amplifier (World Precision Instruments, USA) with a 1–3 Hz band-pass filter. Capacitance, input and series resistances were measured online with Acquis Software (Biologic, France). Patch-clamp pipettes had a resistance of 3–5 MΩ, once filled with the internal recording solution. Series resistances ranged from 15 to 30 MΩ. Cells showing series resistances >30 MΩ were discarded. Field recordings were preformed with glass micropipettes of 10–20 MΩ resistance. To reserve the viability of the IHF preparation and avoid ischaemic artefacts, each IHF was recorded for no more than to 2 h.

Impairment of GABA auto-inhibition

In both hippocampal slices and IHFs, functional impairment of GABA auto-inhibition was induced via ictal-like discharge (ILD)-promoted sustained release of endogenous GABA (Fig. 1*A*). ILDs were triggered by the bath application (9.1 ± 0.9 min, range 8–10 min) of 8.5–10 mM K^+. When either 500 μM CGP35348 or 10 μM CNQX +40 μM APV were coapplied

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with high K^+, the cumulative discharge time was monitored to guarantee GABA release consistency among pharmacologically distinct induction phases. In high K^+, ILDs represented $41 \pm 3\%$ of the overall induction duration. In high K^+ + CGP35348, the ILD contribution was not significantly different ($39 \pm 2\%$, $P > 0.1$), although the presence of CGP35348 slightly shortened the ILD duration (high K^+: 90 ± 5 s, $n = 30$; high K^+ + CGP35348: 76 ± 3 s, $n = 12$, $P < 0.05$) and increased their frequency (high K^+: 4.1 ± 0.1 mHz; high K^+ + CGP35348: 5.6 ± 0.2 mHz; $P < 0.01$). In high K^+ + CNQX + APV, ILDs were not present, but GABA was still released via short, frequent bursts that constituted $63 \pm 4\%$ of the induction phase. When ILDs were induced by 500 μM CGP35348 alone, they represented $44 \pm 5\%$ of the induction phase.

Data analysis

All recordings were visualized using Axoclamp 8.1 software (Axon Instruments, USA), and analysed using the Acquis

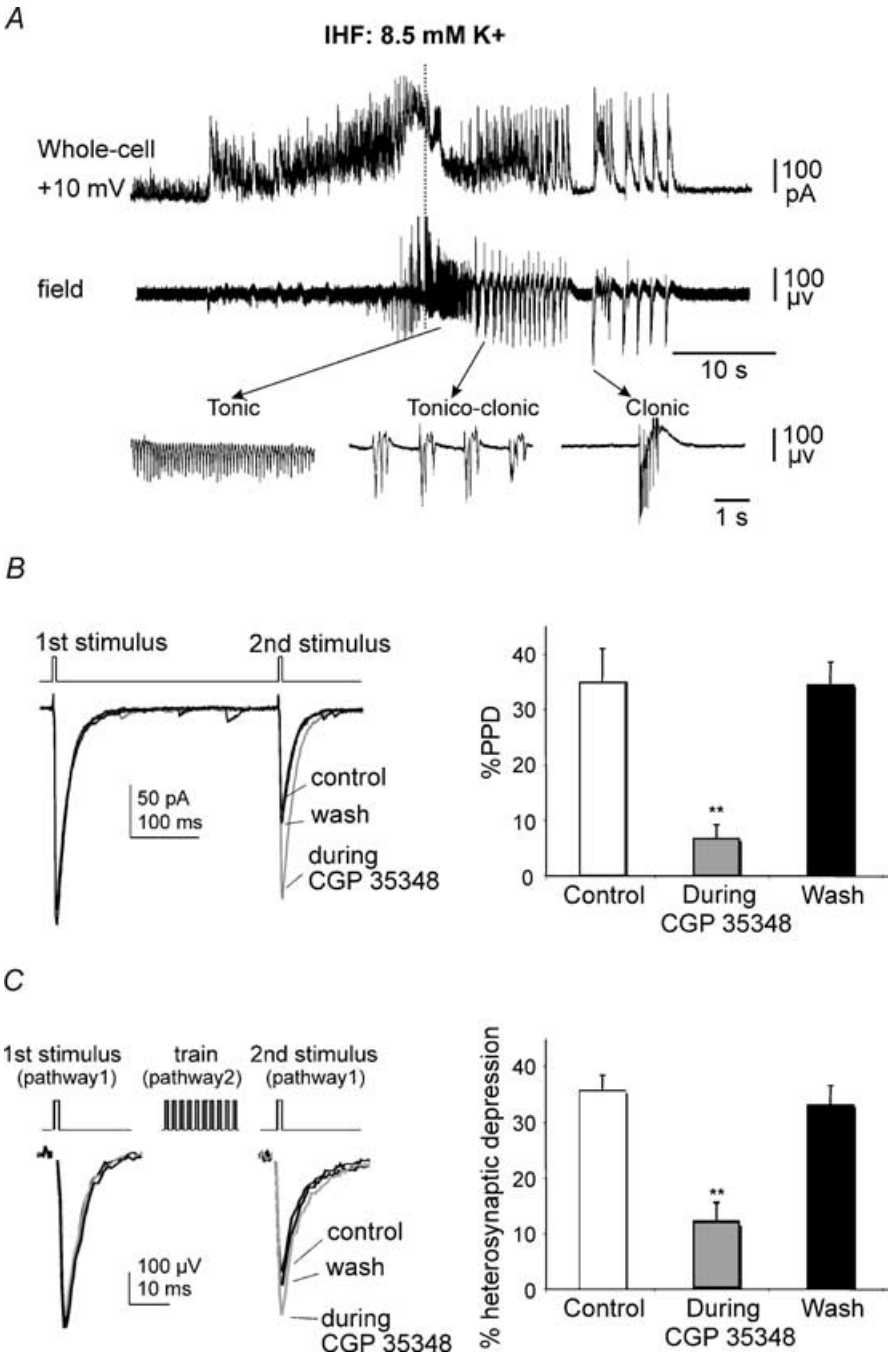

Figure 1. Induction and quantification of functional impairment of GABA auto-inhibition
A, paired whole-cell (top trace) and field (middle trace) recordings of an intact hippocampal formation (IHF) showing the GABAergic current underlying a K^+-induced ictal-like discharge (ILD). The GABAergic current was isolated using a combination of voltage (+10 mV) and ionic conditions (Cs-gluconate patch solution). Note the continuous release of endogenous GABA over several seconds. The tonic, tonico-clonic and clonic phases of the ILD are shown at an expanded time scale (bottom traces). *B*, left, whole-cell $GABA_A$ currents from a CA3 pyramidal neurone showing paired-pulse depression of GABA release (PPD) before (control), during (during CGP35348, grey trace) and after (wash) application of 500 μM CGP35348 in hippocampal slices. The stimulating protocol is shown above traces. Right, average PPD before (white bar), during (grey bar) and after (black bar) application of 500 μM CGP35348. Note that PPD is strongly reduced when $GABA_B$ receptors are blocked. $GABA_A$ currents were isolated in the presence of 10 μM CNQX and 40 μM APV *C*, left, glutamatergic field EPSPs from CA3 stratum radiatum showing heterosynaptic depression of glutamate release before (control), during (during CGP35348, grey trace) and after (wash) application of 500 μM CGP35348 in hippocampal slices. The stimulating protocol is shown above the traces. Right, average heterosynaptic depression before (white bar), during (dark grey bar) and after (black bar) application of 500 μM CGP35348. Error bars show standard errors. **Statistical difference with $P < 0.005$.

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(Biologic, France), Minianalysis (Synaptosoft, USA) and Igor software (Wavemetrics, USA).

Paired pulse depression (PPD) of GABAergic synapses impinging onto CA3 pyramidal neurones was measured using pairs of identical stimuli (4–15 V, 0.03 ms each) at 250–350 ms interval, delivered at a frequency of 0.03 Hz with a bipolar tungsten electrode placed in the CA3 stratum radiatum. The resulting pairs of postsynaptic $GABA_A$ currents ($GABA_A$-PSCs) were isolated in the presence of 10 μM CNQX and 40 μM D-APV. PPD was measured from the amplitude difference between the first and second $GABA_A$-PSCs, normalized to the amplitude of the first $GABA_A$-PSC. Average PPD values were calculated from 15 paired stimulations delivered at 0.03 Hz starting either 15 min before (control PPD) or 5 min after the different pharmacological treatments. For baclofen experiments, a 10 min ACSF wash was introduced between the end of the baclofen treatment and the restarting of the PPD stimulations, to allow a thorough washout of the drug. Cells showing differences in the amplitude of the first $GABA_A$-PSC before and after treatment were discarded.

Heterosynaptic depression of glutamatergic synapses impinging on CA3 pyramidal neurones was measured by stimulating two independent glutamatergic pathways via two stimulating electrodes positioned in the stratum radiatum on opposite sides of the recording electrode. The electrode position and stimulus intensity were optimized for maximal field EPSP (fEPSP) amplitude. Stimulated pathways were tested for independence by verifying the absence of cross-facilitation. To induce heterosynaptic depression, a single stimulus was given to the first pathway (unconditioned fEPSP). After 30 s, an identical stimulus was given to the same pathway, immediately (300 ms) preceded by a conditioning train (10 stimuli at 50 Hz) to the second pathway. This originated a conditioned fEPSP. The magnitude of heterosynaptic depression was calculated from the amplitude difference between the unconditioned and conditioned fEPSPs, normalized to the amplitude of the unconditioned fEPSP. All recordings were performed in the presence of 20 μM tiagabine, a selective blocker of GABA neuronal transporter 1.

To characterize the physiological activity of the hippocampal network, we measured the amplitude and duration of the GDPs that are spontaneously present in the untreated IHF. GDP duration was measured at 20% of GDP amplitude. To characterize the pathological network activity following the different pharmacological treatment, we measured the amplitude, duration and shape of abnormal events recorded extracellularly from the IHF. Event shape was quantified by the number of phases within each event, where one phase is defined as one change in the slope of the recorded trace. Amplitude and duration of abnormal events were normalized to the respective GDP values from the same cell. Frequency distributions of event amplitude, duration and shape were built for individual cells. These were then averaged to generate mean frequency histograms for each parameter.

Ionotropic GABA and glutamatergic currents underlying network events were isolated by means of their distinct reversal potentials while using Cs-gluconate internal solution. GABA and glutamate charge transfers for each event were then calculated from the areas of the GABAergic and glutamatergic currents underlying the event itself.

Statistical analysis was performed with Kolmogorov–Smirnov (K–S), ANOVA or Student's *t*-tests, as appropriate. All values were expressed as mean ± standard error (S.E.M.). Differences were considered significant when $P < 0.05$.

Post hoc morphological characterization of recorded cells

After recordings, slices were fixated overnight at 4°C in 0.1 M phosphate buffer saline (PBS) containing 4% paraformaldehyde and 0.9% NaCl. Slices were then rinsed in PBS, mounted on gelatin-coated slides with an aqueous mounting medium (Gel Mount, Biomeda), and coverslipped. Rhodamine-filled cells were identified with an Olympus confocal microscope (Fluoview BX50WI) using a helium/neon laser as the excitation source (543 nm) and an emission filter band-pass (560 nm). Images were taken with a digital camera (ORCA-ER, Hamamatsu). Cells were classified as either pyramidal neurones or interneurones on the basis of their morphology and axonal projections. Electrophysiological data from interneurones were discarded.

Drug delivery

Drugs were diluted from stock solutions into freshly prepared ACSF immediately before the experiment. Drugs used in this study were: Baclofen (Sigma), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Tocris), D(−)2-amino-5-phosphovaleric acid (D-AP5; Tocris), P-3-aminopropyl-P-diethoxymethyl phosphoric acid (CGP35348, Novartis, Basel), tiagabine (Novo Nordisk, Denmark).

Results

Impairing $GABA_B$ receptor-mediated presynaptic inhibition

Our working hypothesis was that, during epileptiform activity, the sustained exposure of $GABA_B$ receptors ($GABA_B$Rs) to elevated quantities of endogenous GABA could impair $GABA_B$R-mediated presynaptic inhibition of GABA release (GABA auto-inhibition). Such functional loss would then increase hyperexcitability and induce

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spontaneous pathological discharges in the newborn rat hippocampus.

To test this idea, we first investigated whether ictal-like discharges (ILDs) released enough GABA to impair GABA auto-inhibition. ILDs were induced by application of 8.5–10 mM K^+ (9.1 ± 0.9 min, range 8–10 min) to either hippocampal slices or the intact hippocampal formation (IHF). We chose this acute model of epilepsy because elevation of $[K^+]_o$ occurs during seizures and contributes to their generation (Freund *et al.* 1990; Jensen & Yaari, 1997). High-K^+ induced interictal-like activity followed by ILDs lasting 10–20 s in slices, and 40–120 s in the IHF (Fig. 1*A*). ILDs always started with tonic oscillations at 10–20 Hz and ended with clonic bursts occurring at 1–2 Hz (Fig. 1*A*). In most cases, an intermediate phase of tonico-clonic bursts at variable frequency could also be observed (Fig. 1*A*). Paired extracellular field recordings from the stratum radiatum, and whole-cell recordings from CA3 pyramidal neurones demonstrated that GABA was continuously released over tens of seconds before and during ILDs in the IHF (Fig. 1*A*, upper trace). These are therefore ideal conditions for inducing a functional impairment of GABA auto-inhibition, since $GABA_B$ receptors are exposed to a sustained and intense activation during each discharge.

Paired-pulse depression and heterosynaptic depression as indices of efficacy of $GABA_B$R-mediated presynaptic inhibition

Paired-pulse depression at GABAergic synapses (PPD) (Davies *et al.* 1990) and heterosynaptic depression at glutamatergic synapses (Chandler *et al.* 2003) were used to monitor the efficacy of presynaptic $GABA_B$R-mediated inhibition in hippocampal slices.

Both PPD and heterosynaptic depression depended upon $GABA_B$ receptor activation, as they were severely reduced by the selective $GABA_B$R antagonist, CGP35348. As shown in Fig. 1*B*, average PPD was reduced from 35 ± 6% ($n = 6$) in control conditions to 7 ± 2% in the presence of 500 μM CGP35348 ($n = 6$, $P < 0.005$). Similarly, heterosynaptic depression was reduced from 35 ± 3% to 12 ± 3% in the presence of the $GABA_B$R antagonist (Fig. 1*C*). Both PPD and heterosynaptic depression were fully restored following CGP35348 washout. These results indicate that both PPD and heterosynaptic depression rely upon activation of presynaptic $GABA_B$ receptors in our experimental conditions. The functional impairment of presynaptic $GABA_B$R-mediated inhibition should therefore result in a reduction of both PPD and heterosynaptic depression.

In a previous work (Tosetti *et al.* 2004), we showed that, among the different $GABA_B$ receptor-mediated responses, only the presynaptic inhibition of GABA release was impaired after a prolonged application of baclofen. We confirmed this result by measuring PPD and heterosynaptic depression before and after a 5 min application of 100 μM baclofen, a $GABA_B$R agonist. While in the bath, baclofen induced a decrease in the probability of GABA release, resulting in a reduction of the first pulse amplitude (Fig. 2*A*, left panel), and a switch from depression to facilitation (control: 26 ± 5%; during baclofen: −28 ± 16%; $n = 6$, $P < 0.005$) (Fig. 2*A* control, during baclofen). Interestingly, PPD did not recover once baclofen was washed off, and it was still absent (−1 ± 2%) 20 min after removal of the agonist ($n = 6$, $P < 0.005$) (Fig. 2*A*, after baclofen). This could not be accounted for by an incomplete baclofen washout, since the amplitude of the first pulse recovered to pre-baclofen values (Fig. 2*A*, left panel) and the facilitation disappeared (Fig. 2*A*, right panel). These results therefore suggest a baclofen-induced loss of GABA auto-inhibition.

In contrast, heterosynaptic depression recovered to control values after baclofen washout (Fig. 2*B*, after baclofen). Heterosynaptic depression was 36 ± 2% in control conditions, 4 ± 4% during, and 51 ± 9% after baclofen application ($n = 6$, Fig. 2*B*). Baclofen therefore did not impair $GABA_B$Rs on glutamate fibres.

In conclusion, these results confirm that sustained activation of $GABA_B$Rs results in a selective decrease in the efficacy of presynaptic $GABA_B$R-mediated inhibition of GABA release. These results also show that PPD and heterosynaptic depression can be used as indices of functional impairment of $GABA_B$R-mediated presynaptic inhibition in conditions of conserved GABA uptake and release probability (Roepstorff & Lambert, 1994).

ILD-induced release of GABA impairs GABA auto-inhibition

We next investigated whether endogenous GABA released during ILDs could impair GABA auto-inhibition in hippocampal slices. Six ILDs were induced via an 8–10 min application of 10 mM K^+ (induction phase), as described above. We measured PPD and heterosynaptic depression before and following the induction phase, and found that only PPD was severely reduced with respect to control (Fig. 3). Average PPD was decreased from 34 ± 4% in control conditions to 8 ± 3% after K^+ treatment ($n = 8$; $P < 0.001$; Fig. 3*A*). It should be noted that the amount of residual PPD after K^+ treatment is consistent with the amount of $GABA_B$R-independent PPD measured in the presence of CGP35348. In contrast, average heterosynaptic depression did not significantly differ before (33 ± 3%; Fig. 3*B*, control) and after the induction phase (43 ± 9%, $n = 7$; Fig. 3*B*, after high K). Thus, in agreement with the results of the baclofen treatment, high-K^+ treatment selectively affected GABA auto-inhibition.

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Although a decreased PPD is consistent with the functional impairment of presynaptic $GABA_BR$ signalling, other factors may contribute to it. In fact, PPD depends also on GABA release probability and uptake (Roepstorff & Lambert, 1994). A decreased GABA release probability was unlikely since the amplitude of the $GABA_A$ postsynaptic current evoked by the first stimulus (1st GABA-PSC, see stimulating protocol in Fig. 1*B*) was preserved before and after high-K^+ treatment (Fig. 3*A*, left panel). In agreement with this, the coefficient of variation of the first GABA-PSC amplitude (CV_A) did not significantly differ before (0.13 ± 0.02) and after high-K^+ treatment (0.14 ± 0.03; $n = 8$, $P > 0.3$). Thus, the PPD loss cannot be accounted for by a decreased probability of GABA release. In control conditions, the CV_A of the second GABA-PSC was much higher than that of the first (0.41 ± 0.1, $P < 0.01$), in agreement with a reduced probability of release following $GABA_BR$ activation. However, such value decreased significantly after high-K^+ treatment (0.18 ± 0.04, $P < 0.01$), as expected after a loss of GABA auto-inhibition.

We next tested the possibility that PPD loss was due to ILDs reducing neuronal GABA uptake. PPD was measured in the presence of 20 μM tiagabine, a selective blocker of GABA neuronal transporter 1 (Andersen *et al.* 1993), both before and after ILDs. PPD in tiagabine was $48 \pm 2\%$ before and $8 \pm 4\%$ after high-K^+ treatment ($n = 9$; $P < 0.001$; data not shown). ILDs therefore significantly reduced PPD even in the absence of functional neuronal GABA transporters, ruling out an impaired neuronal GABA uptake as the cause for PPD loss.

Since neither GABA uptake nor the probability of GABA release were affected by ILDs, functional impairment of presynaptic $GABA_BR$ signalling is the most likely cause of high-K^+-induced PPD loss. To confirm such conclusion, we tested whether PPD loss required the activation of $GABA_BR$ by coapplying 500 μM CGP35348 during the high-K^+ induction phase. Interestingly, no PPD loss

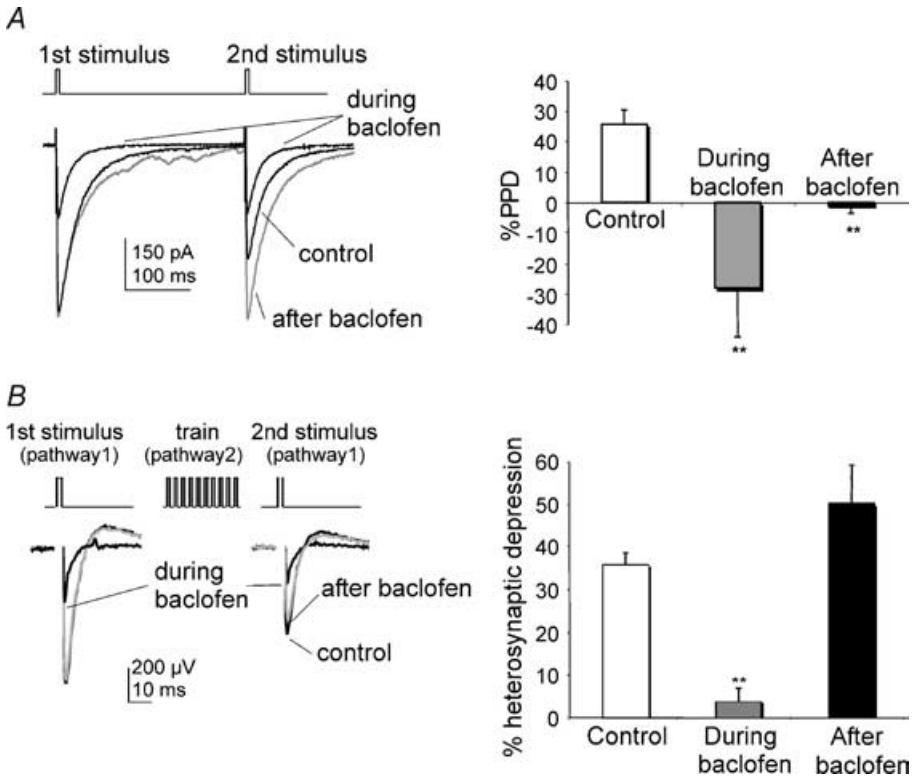

Figure 2. Selective impairment of GABA auto-inhibition by baclofen
A, left, whole-cell recordings showing PPD before (control), during (during baclofen) and after (after baclofen, dark grey trace) application of 100 μM baclofen in hippocampal slices. Right, average PPD before (white bar), during (black bar) and after (dark grey bar) a 5 min application of 100 μM baclofen. Note that PPD is lost following baclofen-induced functional impairment of the $GABA_BR$-mediated response. $GABA_A$ currents were isolated in the presence of 10 μM CNQX and 40 μM APV *B*, left, field recordings from CA3 stratum radiatum showing heterosynaptic depression of glutamate release before (control), during (during baclofen) and after (after baclofen, grey trace) application of 100 μM baclofen in hippocampal slices. Right, average heterosynaptic depression before (white bar), during (black bar) and after (grey bar) a 5 min application of 100 μM baclofen. Note that no functional loss of heterosynaptic depression could be recorded after baclofen treatment. Error bars show standard errors. **Statistical difference with $P < 0.005$.

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was observed after the high-K^+ + CGP35348 treatment (Fig. 4*A*). Average PPD was $27 \pm 2\%$, not significantly different from that measured in control conditions, $27 \pm 4\%$ ($P > 0.6$; $n = 9$). Thus, these results show that activation of $GABA_B$Rs by endogenous GABA is required to induce PPD loss.

We next investigated whether the pattern of $GABA_B$R activation played an important part in the impairment of GABA auto-inhibition. Co-applying 40 μM APV + 10 μM CNQX during the high-K^+ induction phase eliminated ILDs, leaving only shorter (500 ms) GABAergic bursts at 0.8 Hz (Fig. 4*B*). Such discharges, which are reminiscent of GDPs, release sufficient endogenous GABA to activate $GABA_B$Rs (McLean *et al.* 1996), but are probably too short to functionally impair $GABA_B$R-mediated signalling. Indeed, $GABA_B$Rs are functional in the presence of GDPs in physiological conditions (McLean *et al.* 1996). Interestingly, PPD was not significantly different before ($32 \pm 7\%$) and after K^+ + CNQX + APV ($42 \pm 12\%$; $n = 6$; $P > 0.05$; Fig. 4*C*). Thus, the intermittent activation of $GABA_B$Rs does not have any effect on PPD. These results show that it is not $GABA_B$R activation *per se*, but sustained $GABA_B$R activation that is responsible for decreasing PPD.

In conclusion, our results show that endogenous GABA released during epileptiform activity can induce the functional impairment of GABA auto-inhibition in the newborn hippocampus. This effect requires a sustained GABA release. Intermittent, short GABAergic discharges mimicking physiological GDPs are ineffective.

ILD-induced functional impairment of GABA auto-inhibition modifies network activity in hippocampal slices

Our next goal was to investigate the acute and long-term consequences of a reduced GABA auto-inhibition on the immature hippocampal network. GDPs, the giant depolarizing potentials, constitute the hallmark of early hippocampal network activity (Ben-Ari *et al.* 1997). In hippocampal slices, GDP duration was increased by $44 \pm 8\%$ following six high-K^+-induced ILDs (Fig. 5, after high K). Average GDP width (measured at 20% of GDP amplitude) increased from 300 ± 14 ms in control conditions to 432 ± 20 ms after ILDs ($n = 6$; $P < 0.01$). Such increase is consistent with a functional loss of $GABA_B$R-mediated presynaptic inhibition, since previous work demonstrated that $GABA_B$R activation is required for GDP termination (Tosetti *et al.* 2004).

We next confirmed that the increased GDP duration depended on $GABA_B$R activation by applying 500 μM CGP35348 during the high-K^+ induction phase. Such treatment indeed prevented the increase in GDP width

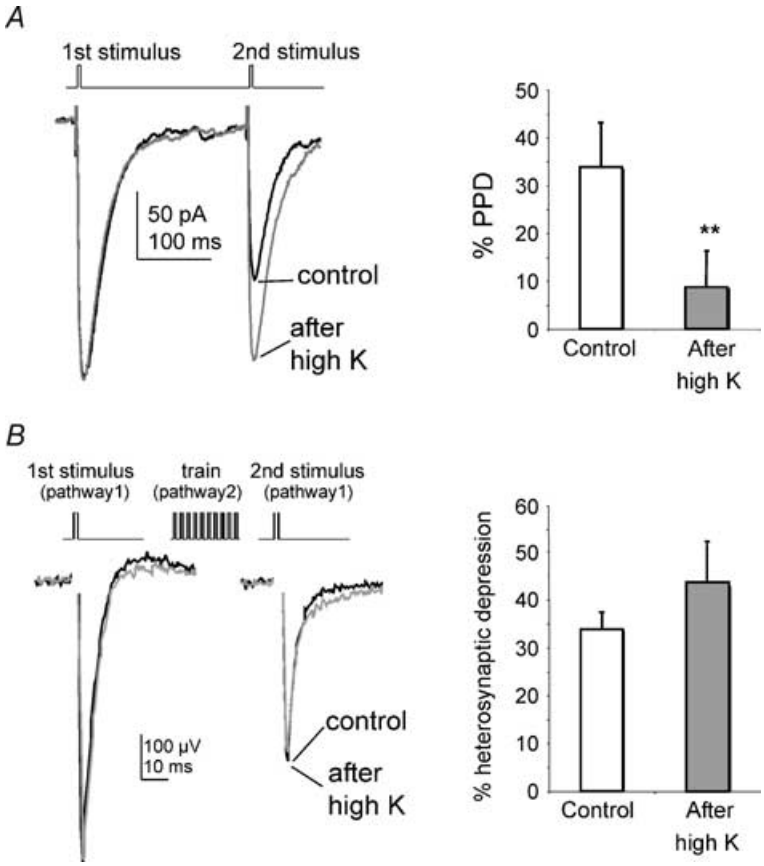

Figure 3. Selective impairment of GABA auto-inhibition by endogenous GABA
A, left, traces show PPD before (control) and after (after high K) high-K^+-induced ictal-like discharges (ILDs). $GABA_A$ currents were isolated in the presence of 10 μM CNQX and 40 μM APV. Right, average PPD before (control) and after ILDs (after high K). PPD is significantly reduced after ILDs. *B*, left, field recordings from CA3 stratum radiatum showing heterosynaptic depression of glutamate release before (control), and after ILDs (after high K) in hippocampal slices. Right, average heterosynaptic depression before (white bar), and after (grey bar) high-K^+ treatment. No functional loss of heterosynaptic depression could be recorded after K^+-induced ILDs. Error bars show standard errors. **Statistical difference with $P < 0.005$.

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(control: 295 ± 17 ms; after high K + CGP35348: 326 ± 23 ms; $n = 6$; $P > 0.2$) (Fig. 5, after high K^+ + CGP35348). These results indicate that $GABA_B$R activation is required to increase GDP duration, and support the idea that ILD-induced impairment of GABA auto-inhibition accounts for the majority of the observed increase in GDP width. An alternative approach to the same question was to induce ILDs via the application of 500 μM CGP35348 alone (without elevating extracellular K^+). In fact, by blocking $GABA_B$ receptors, saturating concentrations of CGP35348 transformed spontaneous GDPs into ILDs (McLean *et al.* 1996; Tosetti *et al.* 2004). This strategy had the dual advantage of generating a pattern of GABA release similar to that induced by high K^+ (see Methods) while preserving $GABA_B$ receptor function after the induction phase. CGP35348-induced ILDs did not affect GDP duration, which passed from 289 ± 27 ms to 316 ± 30 ms after treatment ($P > 0.1$; Fig. 5, after CGP35348).

We finally investigated the effects on GDP duration of a pattern of GABA release unable to impair GABA auto-inhibition. The short, frequent GABAergic bursts generated by the coapplication of high K^+ and 10 μM CNQX + 40 μM APV (see previous section and Fig. 4*B*) did not significantly increase GDP width, which was 312 ± 49 ms in control conditions and 335 ± 44 ms after treatment ($n = 8$; $P > 0.5$). Thus, $GABA_B$R activation by endogenous GABA could not *per se* increase GDP width, unless it was long enough to impair GABA auto-inhibition. It is therefore the ILD-induced decrease of GABA auto-inhibition that is responsible for increasing GDP duration.

To further support this conclusion, we tested the effect on GDP width of a 5 min application of 100 μM baclofen, a treatment known to impair GABA auto-inhibition (Fig. 2) (Tosetti *et al.* 2004). As expected, GDP duration after baclofen treatment was significantly increased by 50 ± 8%, from 275 ± 8 ms to 410 ± 18 ms ($n = 8$, $P < 0.001$). Interestingly, this increase is not significantly different from that measured after high-K^+ treatment ($P > 0.4$; Fig. 5*A*).

In conclusion, our results indicate that ILD-induced GABA release can increase GDP width and modify the spontaneous network activity by impairing

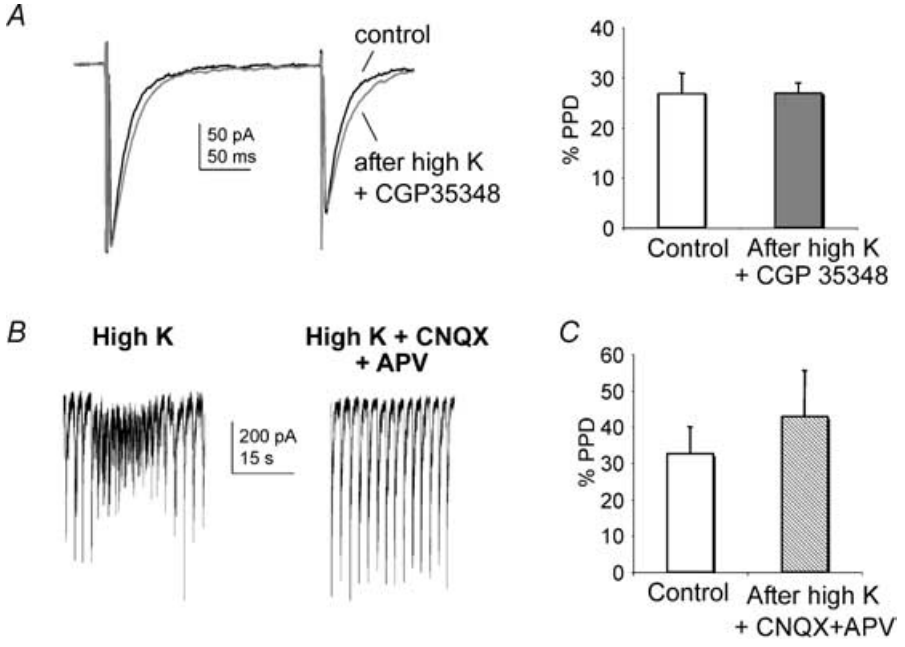

Figure 4. Sustained release of endogenous GABA impairs GABA auto-inhibition
A, left, whole-cell recordings of CA3 pyramidal neurone showing PPD before (control) and after 10 mM K^+ + 500 μM CGP35348 treatment. Preventing $GABA_B$ receptor activation during ILDs avoided PPD loss. Right, average PPD before (control) and after 10 mM K +500 μM CGP35348 treatment. $GABA_A$ currents were pharmacologically isolated using 10 μM CNQX + 40 μM APV. *B*, whole-cell recordings from two CA3 pyramidal neurones in hippocampal slices. ILDs are present in 10 mM K^+ (High K) but not in 10 mM K^+ +10 μM CNQX +40 μM APV (High K + CNQX + APV), where only short, GDP-like GABAergic discharges can be observed. Holding potential: −65 mV. *C*, average PPD before (Control) and after 10 mM K^+ + 10 μM CNQX + 40 μM APV (High K + CNQX + APV). Error bars show standard errors.

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$GABA_B$R-mediated GABA auto-inhibition in immature hippocampal slices.

ILD-induced impairment of GABA auto-inhibition modifies network activity in the intact hippocampal formation

Since hippocampal slices contain only a fraction of the hippocampal network, they may not be ideal to evaluate network activity. We therefore decided to further investigate the consequence of K^+-induced ILDs using the intact hippocampal formation (IHF), a preparation that preserves the hippocampal circuitry (Khalilov *et al.* 1997).

Network events occurring before and after high-K^+-induced ILDs were monitored using extracellular field recordings of CA3 stratum radiatum. Events were then classified according to their amplitude, duration and shape (quantified as number of phases, see Methods). We defined as abnormal all events that significantly differed from GDPs in at least two out of the three above parameters.

In control conditions, GDPs constituted the totality of network events ($n = 24$; Fig. 6*A*, Before high K^+) and could be described as stereotyped triphasic waveforms of $21 \pm 2\ \mu$V amplitude (range 8–43 μV; $n = 24$), and 484 ± 48 ms duration (range 131–692 ms). Following ILDs, however, GDPs accounted for only $26 \pm 4\%$ of total events ($n = 24$; Fig. 6*A*, After high K). The remaining $74 \pm 4\%$ of network activity was composed of abnormal events that were never recorded in control conditions. They could be divided into three major classes: short oscillations, long oscillations, and interictal-like events (Fig. 6*B*). Short oscillations were characterized by relatively small amplitude ($172 \pm 19\%$ of average GDP amplitude; range 35–405%; $n = 25$), intermediate duration ($424 \pm 53\%$ of average GDP duration; range 155–1161%) and intermediate number of phases (8.0 ± 0.8 phases; range 2–15; GDPs = 3 phases). Long oscillations not only displayed longer duration ($3907 \pm 797\%$ of average GDP duration, range 1097–9658%; $n = 10$), but also larger amplitude ($942 \pm 334\%$ of average GDP amplitude, range 320–3167%), and always more than 15 phases. Interictal-like events were mostly triphasic (average $= 3.2 \pm 0.2$, range 2–5; $n = 14$), with intermediate amplitude ($478 \pm 94\%$ of average GDP amplitude, range 103–1444%), and relatively short duration ($196 \pm 19\%$ of average GDP duration, range 35–438%). All three types of abnormal events were spontaneously present in the IHF up to at least 1 h 30 min after the induction phase.

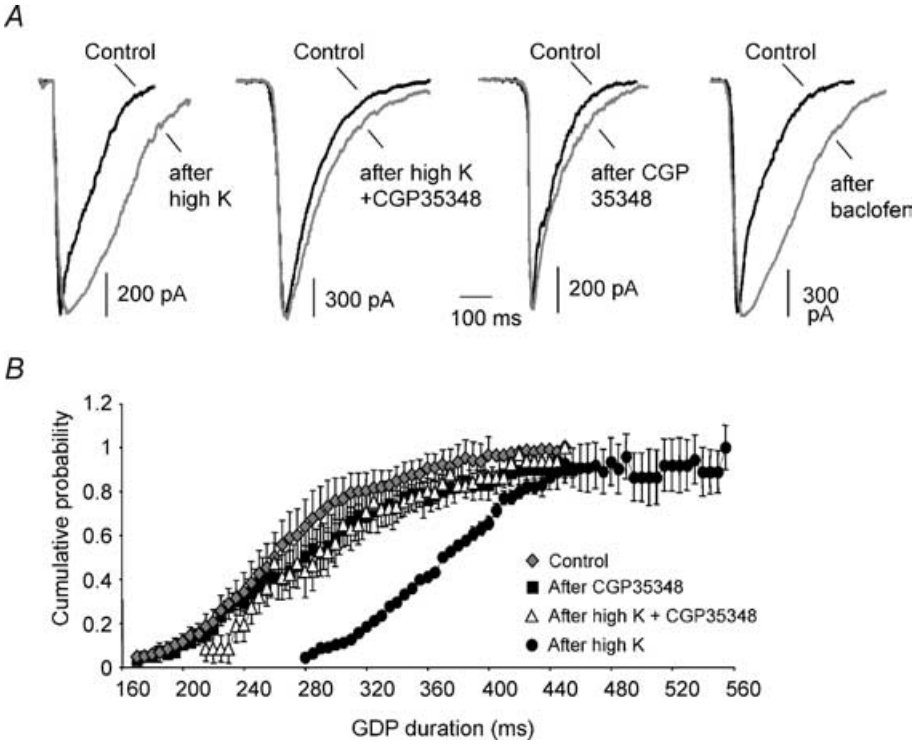

Figure 5. Impairing GABA auto-inhibition increases GDP width in hippocampal slices
A, voltage-clamp recordings showing GDP currents before (Control) and after the indicated treatments. Each trace is the mean of 15 events. Holding potential = −65 mV. *B*, cumulative distribution of GDP duration before (Control) and after the indicated treatments. Note the significant increase in GDP duration following high-K^+ treatment. All recordings are from CA3 pyramidal neurones in acute hippocampal slices. Error bars show standard errors.

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Simultaneous patch-clamp and field recordings of the IHF were used to characterize the conductances underlying both GDPs and abnormal events (Fig. 6*B*). As previously reported, control GDPs were mainly driven by GABAergic currents (Ben-Ari *et al.* 1989; Khazipov *et al.* 1997). Their average GABA/glutamate charge transfer ratio was 16 ± 3 ($n = 10$), indicating that ionotropic glutamatergic currents accounted for only 6.25% of total GDP current. Abnormal waveforms, however, were driven by sizeable amounts of both GABAergic and glutamatergic currents. Their average GABA/glutamate charge transfer ratio was 2.9 ± 0.4 ($n = 11$), indicating that ionotropic glutamatergic currents represented approximately 34% of the total event current. Thus, GDPs and abnormal waveforms constitute distinct events.

In addition to releasing endogenous GABA, and impairing the $GABA_BR$-dependent response, high-K^+-induced ILDs also released large quantities of glutamate (Rutecki *et al.* 1985). Strong glutamate release could induce several forms of plasticity that could contribute to the observed changes in network activity (Ben-Ari & Gho, 1988). To identify which type of abnormal event depended upon the functional loss of GABA auto-inhibition, we prevented $GABA_BR$ activation during sustained GABA release with the application of 500 μM CGP35348. This resulted in the recovery of most of the spontaneous GDP activity (70 ± 8% of total events, $n = 12$; Fig. 6*A*, After high K + CGP). The remaining 30% of events consisted exclusively of interictal-like waveforms. These results suggest that a decreased GABA auto-inhibition is required for the appearance of both long and short oscillations, but not interictal-like events in the immature hippocampus.

Figure 7 shows frequency distributions of abnormal event properties (duration, shape, and amplitude) measured after high K^+ alone or high $K^+ + 500\ \mu$M CGP3534 ($n = 24$; for histogram values see Table 1). Superposed rectangles highlight properties whose frequency did not significantly differ in the presence or absence of $GABA_BR$ activation. Interestingly, the duration, shape, and amplitude values that were independent of $GABA_BR$ activation well matched the properties of interictal-like events. These results confirm that preserving $GABA_BR$-mediated signalling prevented the appearance of long and short oscillations.

If long and short oscillations are consequences of an impaired GABA auto-inhibition, treatments leading to a partial loss of function of $GABA_BRs$ should trigger similar discharges. To test such a hypothesis, we reduced $GABA_BR$-mediated signalling by applying non-saturating doses of CGP35348. Indeed, long and short oscillations strikingly similar to those recorded after high-K^+-induced ILD could be recorded during the application of 100 μM CGP35348 (Fig. 8*A*). CGP35348-driven events were mediated by both GABAergic and glutamatergic ionotropic currents (Fig. 8*A*). Their GABA/glutamate charge transfer ratio was 3.4 ± 0.8 ($n = 6$), a value not significantly different from that of K^+-induced oscillations. Even more striking, the type and frequency of events recorded in CGP35348 and after high-K^+-induced ILDs closely matched. After high-K^+ treatment, GDPs

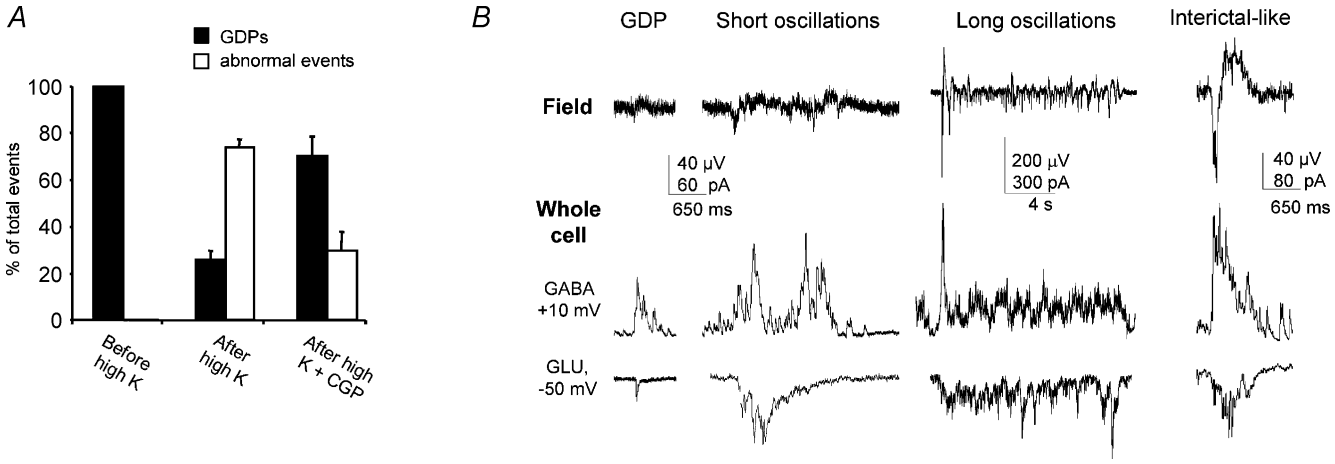

Figure 6. Impairing GABA auto-inhibition induces the appearance of abnormal discharges in the intact hippocampus

A, distribution of normal (GDPs) and abnormal events recorded from the intact hippocampal formation (IHF) in the presence (after high K) and absence (after high K + CGP) of $GABA_BR$ activation. *B*, extracellular (field) and intracellular (whole-cell) recordings of GDPs and abnormal events (short oscillations, long oscillations, interictal-like) present in the IHF after high-K^+ treatment. Glutamatergic and GABAergic currents were isolated at the indicated potentials by means of a Cs-gluconate patch solution. The field electrode was positioned in the stratum radiatum. Whole-cell currents were recorded from CA3 pyramidal neurones in the voltage-clamp configuration. Error bars show standard errors.

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accounted for $27 \pm 3\%$ of total events, short oscillations for $57 \pm 5\%$, and long oscillations for $5 \pm 2\%$ (Fig. 8*B*). Similarly, during $100\,\mu$M CGP35348, $32 \pm 3\%$ of the events were GDPs, $63 \pm 3\%$ were short oscillations, and $5 \pm 1\%$ were long oscillations (Fig. 8*B*). Interestingly, interictal-like events were not present during CGP application (Fig. 8*B*), in agreement with the idea of these events being a non-specific effect of ILDs rather then a consequence of impairing GABA auto-inhibition.

In conclusion, the partial block of $GABA_B$ receptors induces the appearance of abnormal events that are qualitatively and quantitatively similar to those recorded after high-K^+-induced ILDs. These results indicate that ILD-induced GABA release impairs GABA auto-inhibition and triggers the onset of abnormal short and long oscillations in the immature hippocampus.

Discussion

Our study focused on the functional consequences of acute epileptiform activity (ictal-like discharges, ILDs) on presynaptic $GABA_B$R-mediated signalling and network activity in the developing rat hippocampus. The central results were: (i) endogenous GABA release could impair $GABA_B$R-mediated presynaptic inhibition of GABAergic terminals (GABA auto-inhibition); (ii) ILDs, but not physiological GDPs, provided the sustained GABA release required to induce such impairment; (iii) the ILD-dependent loss of GABA auto-inhibition modified the spontaneous network activity and induced the onset of abnormal discharges, possibly contributing to post-seizure plasticity of the developing hippocampus.

Endogenous GABA impairs GABA auto-inhibition

We have demonstrated that high-K^+-induced epileptiform activity can significantly reduce paired pulse depression (PPD) in the CA3 region of the developing rat hippocampus. PPD loss occurred after as few as six ILDs. Interestingly, a similar decrease in PPD could be observed in the CA1 region and in dentate gyrus granule cells after kindling (Buhl *et al.* 1996; Wu & Leung, 1997).

How does such functional loss occur? Our data indicate that a sustained release of endogenous GABA during ILDs induces a PPD loss that is the consequence of an impaired GABA auto-inhibition. To support this hypothesis, we showed that activation of $GABA_B$Rs during high-K^+-induced ILDs was required to induce the PPD loss since: (i) the application of the specific $GABA_B$R antagonist CGP35348 during the induction phase prevented it; and (ii) a 5 min baclofen application induced a similar PPD loss. We also proved that $GABA_B$R activation needed to be long lasting in order to induce the PPD loss. In fact, PPD was not reduced when the pattern of $GABA_B$R activation was changed from sustained (during ILDs) to intermittent (during GDP-like activity). Finally, we showed that other mechanisms potentially able to induce PPD loss, like a decreased probability of GABA release or an increased GABA uptake (Roepstorff & Lambert, 1994), were not involved. Altogether, these observations indicate that, in the newborn hippocampus, GABA released during

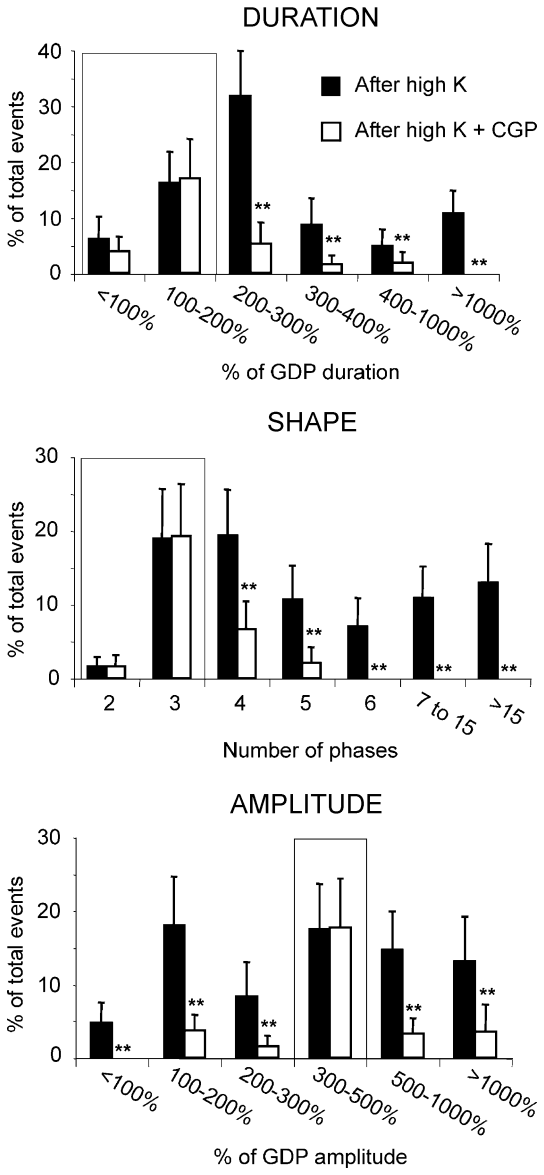

Figure 7. Properties of abnormal events
Frequency distributions of abnormal event properties (duration, amplitude and shape) in the presence (After high K) and absence (After high K + CGP) of sustained $GABA_B$R activation. Event amplitude and duration were normalized to control GDP values. The shape of events was expressed as a number of phases, where one phase corresponded to one slope change in the trace. Note that the only values that do not significantly differ in the presence or absence of $GABA_B$R activation perfectly describe the properties of interictal-like discharges. Error bars show standard errors. **Statistical difference with $P < 0.005$.

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Table 1. Properties of pathological events occurring after ILDs in the presence (after high K^+) and absence (after high K^+ + CGP) of impairment of GABA auto-inhibition

	After high K^+ (%)	After high K + CGP (%)
Duration		
<100%	6.0 ± 4.1	3.9 ± 2.7
100–200%	16.1 ± 5.5	16.9 ± 7.1
200–300%	31.7 ± 8.0	5.2 ± 3.8
300–400%	8.7 ± 4.7	1.6 ± 1.6
400–1000%	4.9 ± 3.0	1.9 ± 1.9
>1000%	10.7 ± 0.1	—
Amplitude		
<100%	4.6 ± 2.8	—
100–200%	18.0 ± 6.6	3.6 ± 2.2
200–300%	8.3 ± 4.7	1.5 ± 1.5
300–500%	17.4 ± 6.2	17.6 ± 6.8
500–1000%	14.6 ± 5.2	3.1 ± 2.1
>1000%	13.0 ± 6.0	3.6 ± 3.6
Phases		
2	1.6 ± 1.2	1.6 ± 1.6
3	18.9 ± 6.8	19.2 ± 7.0
4	19.3 ± 6.2	6.6 ± 3.8
5	10.6 ± 4.6	2.1 ± 2.1
6	7.0 ± 3.9	—
7–15	10.8 ± 4.2	—
>15	13.0 ± 5.3	—

Event amplitude and duration were normalized to average GDP amplitude and duration from the same cell.

ILDs impairs presynaptic $GABA_B$R-mediated inhibition, leading to PPD loss.

The mechanism leading to the functional impairment of presynaptic $GABA_B$R-mediated inhibition is presently unknown. The simplest explanation is possibly that the release of endogenous GABA is sufficient to induce a conformational change of the $GABA_B$ receptor, leading to a desensitized state insensitive to the agonist. For example, the impaired GABA auto-inhibition could result from uncoupling to the effectors via agonist-induced dephosphorylation of the $GABA_B$ receptors (Couve *et al.* 2002). Alternatively, it could result from phosphorylation by G-protein receptor kinases (GRKs) which, through the binding of β-arrestins, target the G-protein-coupled receptors for endocytosis (Carman & Benovic, 1998; Ferguson *et al.* 1998; Tsao & von Zastrow, 2000). Independently of the mechanism involved, the present study provides the first demonstration that endogenous GABA can impair $GABA_B$R-mediated inhibition, and reveals one functional context where this occurs: during epileptiform activity.

Functional consequences of ILD-induced impairment of GABA auto-inhibition

We have shown that the ILD-induced functional loss of GABA auto-inhibition increased the duration of physiological GDPs and triggered pathological network oscillations in the intact hippocampus. In fact, procedures that prevented such impairment (coapplication of CGP35348 or CNQX + APV during the high-K^+ induction phase) also prevented alterations of network activity. Furthermore, procedures that induced (bath application of baclofen) or simply mimicked impairment (non-saturating concentrations of CGP35348) triggered similar abnormal oscillations and increased GDP duration.

These results are consistent with previous studies reporting the fundamental role of $GABA_B$Rs in preventing excessive hippocampal excitability during development. In fact, during the first postnatal week, the synchronous activation of GABAergic interneurones triggers depolarizing $GABA_A$R-mediated currents, and contributes to spontaneous network discharges called giant depolarizing potentials (GDPs) (Cherubini *et al.* 1991). However, once released, GABA also acts on presynaptic $GABA_B$Rs, limiting further GABA release, and effectively leading to GDP termination (McLean *et al.* 1996). Impairing $GABA_B$R function at this age prevents GDP termination, transforming GDPs into long epileptiform discharges (Tosetti *et al.* 2004). In agreement with these findings, our present data show that ILDs impair GABA auto-inhibition and decrease the inhibitory control exerted by endogenous GABA on the network activity. As a direct consequence, the hippocampal network develops a situation of hyperexcitability, revealed by the presence of longer GDPs, and abnormal long and short oscillations.

In the hippocampus, $GABA_B$Rs are ubiquitously located at both postsynaptic and presynaptic sites on both GABAergic and glutamatergic neurones. Thus, ILDs can potentially impair the responses of distinct pools of $GABA_B$Rs, producing complex effects on the hippocampal network. The present results show that $GABA_B$Rs on glutamatergic terminals, in contrast to those on GABAergic terminals, are not impaired following high-potassium exposure.

We have previously shown that bath application of saturating concentration of baclofen does not impair postsynaptic $GABA_B$Rs on pyramidal neurones (Tosetti *et al.* 2004). Considering the low number of ILDs used to induce such functional impairment, we think it unlikely that the postsynaptic $GABA_B$R-mediated control of excitability was affected in the present study. However, a recent study (Wetherington & Lambert, 2002) reported that both pre- and postsynaptic $GABA_B$Rs on glutamatergic neurones desensitized when exposed to GABA agonists for longer periods (2–96 h). It is thus possible that a fully developed status epilepticus, allowing $GABA_B$Rs to be exposed for longer and to higher concentration of endogenous GABA, could affect additional pools of $GABA_B$R-mediated responses. We however, predict that impairing the postsynaptic $GABA_B$R-mediated inhibition would contribute

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to aggravating hyperexcitability and favour the emergence of pathological activity.

It may seem contradictory that the effects of high-potassium exposure on PPD (in slices) and network activity (in the IHF) are mimicked by different concentrations of CGP35348 (500 *versus* 100 μM). This can be explained knowing that: (i) all (pre- and post-synaptic) $GABA_B$ receptors participate in the control of network-driven synaptic activity, including those that do not desensitize following high-K^+-induced ILDs; GABA-PPD, however, is mediated mostly by $GABA_B$Rs on GABAergic terminals; (ii) CGP35348 blocks all $GABA_B$ receptors within the network, while high K^+ only impairs GABA auto-inhibition. Since high K^+ impairs only one group of $GABA_B$Rs out of the three controlling the network, a partial block of $GABA_B$ receptors (using non-saturating concentrations of CGP35348) best mimics the high-K^+ effects on the network activity. Higher concentrations of the $GABA_B$ antagonist lead to a more dramatic phenotype (i.e. ILDs). In the case of GABA-PPD, however, high K^+ impairs precisely the receptor group that is solely responsible for the phenomenon. Thus, an almost complete block of $GABA_B$Rs on GABAergic terminals (using 500 μM CGP35348) is required to mimic the consequences of high-K^+ treatment on GABA-PPD.

Although it is difficult to precisely mimic high-K^+ effects on the network activity using a $GABA_B$ antagonist, we believe CGP experiments are helpful as they demonstrate that both sustained activation and partial blockade of the $GABA_B$ receptors lead to the same

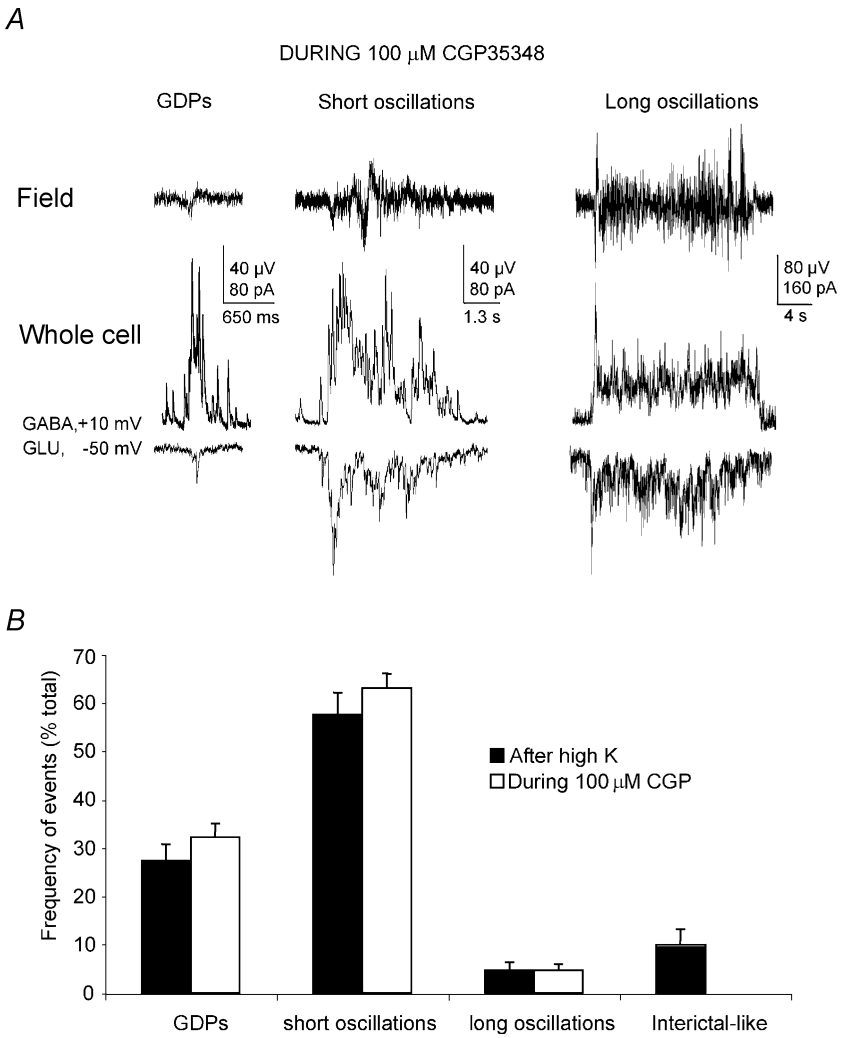

Figure 8. Partial pharmacological block of $GABA_B$Rs induces short and long oscillations
A, extracellular (field) and intracellular (whole cell) recordings of GDPs, long and short oscillations present in an IHF during a non-saturating application of CGP35348 (100 μM). Recording conditions were the same as in Fig. 4*B*. *B*, frequency distributions of normal (GDPs) and abnormal events recorded from an IHF during 100 μM CGP35348 (open bars), and after high-K^+ treatment (filled bars). Note that the distributions are not significantly different, with the exception of interictal-like discharges, which are absent during CGP treatment. Error bars show standard errors.

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consequences on network activity. This observation therefore strengthens the hypothesis that the abnormal activity observed after high-K^+ exposure is due to a 'desensitization-like' impairment of $GABA_BR$-mediated inhibition.

Impairment of GABA auto-inhibition as a possible ictogenic mechanism

Our data demonstrate that ILDs induce the loss of $GABA_BR$-mediated control of GABA release in the neonatal hippocampus, thus triggering network hyperexcitability and the appearance of spontaneous pathological discharges. Among the many seizures-induced alterations that characterize the epileptic tissue, some can underlie or facilitate the emergence of epileptiform activity, while others can be seen as compensatory mechanisms to prevent seizures aggravation (Morimoto *et al.* 2004). Whether $GABA_BR$ activation is protective or pro-convulsive is still controversial, with effects varying greatly depending on the age, brain region and type of epileptiform activity (Engel., 1995; Bettler *et al.* 1998; Sutor & Luhmann, 1998; Motalli *et al.* 1999; Bettler *et al.* 2004; Tosetti *et al.* 2004). The protective role of $GABA_BRs$ during development has been confirmed by the severe epileptic phenotype of $GABA_{B1}$-deficient mice (Prosser *et al.* 2001; Schuler *et al.* 2001). In the adult, however, the activation of $GABA_BRs$ is generally considered pro-convulsive because it limits the inhibitory action of $GABA_ARs$ (Engel, 1995). It may thus appear contradictory to evoke a loss of presynaptic inhibition of GABA release as a pro-convulsive effect. It should be considered, though, that in both adult (Kohling *et al.* 2000; Fujiwara-Tsukamoto *et al.* 2003) and neonatal rat hippocampus (Dzhala & Staley, 2003), $GABA_A$ receptor activation contributes to the emergence of epileptiform activity. In the neonates, $GABA_A$ receptors are excitatory in physiological conditions (Cherubini *et al.* 1991). In the adult system, $GABA_A$ receptors are usually inhibitory, but the intracellular accumulation of Cl^- ions during sustained $GABA_AR$ activation (i.e. ILDs) can switch $GABA_AR$-mediated synaptic responses from hyperpolarizing to depolarizing, favouring network hyperexcitability (Staley *et al.* 1995; Kaila *et al.* 1997). Thus, the impairment of GABA auto-inhibition is likely to favour the emergence of pathological activity in both immature and adult animals. Several plastic changes occurring after seizures facilitate the onset of chronic epileptic activity (Morimoto *et al.* 2004). Our data provide, however, the first evidence that $GABA_BRs$ may contribute to post-seizure plasticity and remodelling.

It is possible that seizure-induced loss of GABA auto-inhibition contributes to the long-term hyperexcitability observed in epileptic tissue. Several studies reported a functional loss of $GABA_BR$-mediated inhibition (Haas *et al.* 1996; Mangan & Lothman, 1996; Wu & Leung, 1997; Chandler *et al.* 2003) and a decrease in the number of $GABA_BRs$ after intense epileptic activity in adult models of chronic epilepsy (Kokaia & Kokaia, 2001; Chandler *et al.* 2003; Straessle *et al.* 2003). However, the mechanisms underlying the long-term impairment of $GABA_BR$-mediated inhibition are not known. Our data raise the interesting possibility that seizure-induced GABA release contributes to the reduced number of $GABA_BRs$ observed in chronic epileptic tissue. Indeed, the activity-dependent impairment of most G-protein-coupled receptors is mediated by phosphorylation followed by receptor internalization (Tsao & von Zastrow, 2000). It is tempting to speculate that a prolonged $GABA_BR$ activation, initially induced by ictal activity and later maintained by pathological discharges, leads to receptor internalization and a long-term decrease in the number of functional $GABA_BRs$.

Our present results indicate that the functional loss of GABA auto-inhibition is a consequence of ILDs. However, a sustained release of endogenous GABA always precedes the actual development of the ILD, as is well documented in Fig. 1*A*. It is possible, although speculative, that functional impairment of $GABA_BR$-mediated GABA auto-inhibition occurs during such release. If this is the case, the reduced control of GABA release might not be simply a consequence of ILDs, but could play a role in the generation of pathological discharges.

Conclusion

In the neonatal hippocampus, during physiological activity, i.e. GDPs, $GABA_BR$ signalling is recruited to limit the release of excitatory GABA. Here we show that, during epileptiform activity, an excessive release of GABA impairs such a protective mechanism, leading to aggravation of hyperexcitability and appearance of spontaneous pathological discharges. The ILD-dependent impairment of GABA auto-inhibition may thus contribute to the pro-convulsive plastic changes that characterize chronic epileptic tissues.

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