J Physiol 586.21 (2008) pp 5119–5128

The Journal of Physiology

Spontaneous glutamatergic activity induces a BDNF-dependent potentiation of GABAergic synapses in the newborn rat hippocampus

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Spontaneous ongoing synaptic activity is thought to play an instructive role in the maturation of the neuronal circuits. However the type of synaptic activity involved and how this activity is translated into structural and functional changes is not fully understood. Here we show that ongoing glutamatergic synaptic activity triggers a long-lasting potentiation of γ-aminobutyric acid (GABA) mediated synaptic activity ($LLP_{GABA\text{-}A}$) in the developing rat hippocampus. $LLP_{GABA\text{-}A}$ induction requires (i) the activation of AMPA receptors and L-type voltage-dependent calcium channels, (ii) the release of endogenous brain-derived neurotrophic factor (BDNF), and (iii) the activation of postsynaptic tropomyosin-related kinase receptors B (TrkB). We found that spontaneous glutamatergic activity is required to maintain a high level of native BDNF in the newborn rat hippocampus and that application of exogenous BDNF induced $LLP_{GABA\text{-}A}$ in the absence of glutamatergic activity. These results suggest that ongoing glutamatergic synaptic activity plays a pivotal role in the functional maturation of hippocampal GABAergic synapses by means of a cascade involving BDNF release and downstream signalling through postsynaptic TrkB receptor activation.

(Received 20 June 2008; accepted after revision 2 September 2008; first published online 4 September 2008)
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Ongoing synaptic activity plays an important role in the construction and refinement of developing neuronal networks (Zhang & Poo, 2001). However the type of synaptic activity involved and how activity is translated into structural or functional changes are not fully understood. Neurotrophins, in particular brain-derived neurotrophic factor (BDNF), are attractive candidates. Neurotrophins are secreted proteins whose expression and release are up-regulated by neuronal activity (Lessmann *et al.* 2003). Moreover, impairing network activity produces structural and functional alterations that can be reverted by application of exogenous BDNF (McAllister *et al.* 1996; Rutherford *et al.* 1997; Marty *et al.* 2000; Seil & Drake-Baumann, 2000). These observations therefore suggest that activity-dependent secretion of BDNF can act as a regulatory mechanism of network development and plasticity. However, to date most of the studies investigating the effects of BDNF on synaptic development or efficacy have relied on non-physiological application of exogenous BDNF (Marty *et al.* 1996, 2000; MacLean Bolton *et al.* 2000; Jin *et al.* 2003) or on chronic alteration of the BDNF signalling pathway (Aguado *et al.* 2003; Ohba *et al.* 2005; Carmona *et al.* 2006). Although these studies have been extremely informative for the understanding of the BDNF actions, crucial questions remain to be addressed in terms of the physiological pattern of synaptic activity that triggers the release of BDNF, as well as the impact this release may have on network activity and development.

Several studies have shown that neuronal activity contributes to the formation of GABAergic synapses in the developing brain (Benevento *et al.* 1995; Kilman *et al.* 2002; Chattopadhyaya *et al.* 2004). Accordingly, in a previous study we reported that silencing neonatal rat hippocampus *in vitro* delayed the functional maturation of GABAergic synapses onto hippocampal CA3 pyramidal neurons (Colin-Le Brun *et al.* 2004). In the present work, we investigated the effect of synaptic activity recovery on GABAergic synaptic transmission. We show that a transient recovery from activity deprivation leads to a long-lasting potentiation of $GABA_A$ receptor-mediated synaptic transmission ($LLP_{GABA\text{-}A}$) in the developing

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DOI: 10.1113/jphysiol.2008.158550

rat hippocampus. $LLP_{GABA\text{-}A}$ induction requires (i) the activation of AMPA receptors and the voltage-dependent L-type calcium channel (VDCC), (ii) a postsynaptic rise in intracellular calcium concentration ($[Ca^{2+}]_i$), (iii) the release of endogenous brain derived neurotrophic factor (BDNF), and (iv) the activation postsynaptic tropomyosin-related kinase receptors B (TrkB).

Methods

Slices preparation

Experiments were performed on hippocampal slices obtained from Wistar rats, between postnatal days 1 and 6. All animal experiments have been carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Brains were removed from anaesthetized (350 mg kg^{-1} chloral hydrate I.P.) animals 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. Hippocampal slices (600 μm thick) were cut with a McIlwain tissue chopper and submerged in ACSF supplemented with 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX; 5 μM) and D(–)-2-amino-5-phosphonopentanoic acid (D-AP5; 40 μM) to reduce network-driven synaptic activity (Ben-Ari *et al.* 1989). Slices were then transferred to a submerged recording chamber and perfused with ACSF (3 ml min^{-1}) containing NBQX (5 μM) and D-AP5 (40 μM) at 34°C.

Electrophysiological recordings

Whole cell patch-clamp recordings of CA3 pyramidal neurons were performed with an Axopatch 200B amplifier (Axon Instruments, Union City, CA, USA). Microelectrodes (4–8 MΩ) were filled with the following solution (in mM): CsCl, 110; potassium gluconate, 30; Hepes, 10; EGTA, 1.1; $CaCl_2$, 0.1; MgATP, 4; and NaGTP, 0.3. With this solution, $GABA_A$ receptor-mediated responses reversed at 0 mV. In some experiments (Fig. 2), the intracellular solution contained (in mM): caesium gluconate, 120; CsCl, 14; Hepes, 10; EGTA, 1.1; $CaCl_2$, 0.1; Na_2GTP, 0.4; and MgATP, 4; pH 7.4. In these ionic conditions, $GABA_A$ currents reversed around −55 mV, while ionotropic glutamatergic currents reversed around +10 mV. Field potentials were recorded using a DAM80 Amplifier (World Precision Instruments, Sarasota, FL, USA) using a 1–3 Hz bandpass filter. Spontaneous synaptic activity was monitored and stored with Axoscope 8.1 (Axon Instruments). Series resistance (R_s), membrane capacitance (C_m) and input resistance (R_i) were determined by an online fitting analysis of the transient currents in response to a 5 mV pulse with Acquis 4.0 software (Bio-logic, Orsay, France). Criteria for accepting a recording included a resting potential < -55 mV, $R_i > 400$ MΩ and $R_s < 25$ MΩ.

BDNF immunoassay

To measure the release of BDNF from hippocampal slices, 6 ml fractions of the medium were collected. The fractions were concentrated using the Amicon Ultra concentration devices (Millipore Corp., Ireland), with 200 μl final volume. The BDNF sandwich ELISA Kit (Chemicon International, Temecula, CA, USA) was used to quantify the amount of BDNF in the concentrated solutions following the manufacturer's protocol. Experiments and analysis were done blind.

Data acquisition and analysis

The spontaneous $GABA_A$ receptor-mediated synaptic activity was stored on-line on a personal computer and analysed off-line with Mini Analysis 6.0 software (Synaptosoft, Leonia, NJ, USA). The fact that no false events would be identified was confirmed by visual inspection for each experiment. To calculate the amplitude of spontaneous $GABA_A$ receptor-mediated postsynaptic currents ($sGABA_A$-PSCs), multiple overlapping events were discarded. For each recorded cell, the mean frequency of sGABA-PSCs within bins of 30 s was normalized to the mean frequency for the 10 min preceding activity recovery. The effective plasticity was quantified as the mean frequency or amplitude sGABA-PSCs between 20 and 30 min after activity recovery, normalized to the mean of the frequency and amplitude for the 10 min preceding activity recovery. When comparing cumulative distribution of $sGABA_A$-PSCs amplitude and frequency before and after activity recovery for a given cell, the Kolmogorov–Smirnov (K-S test) was used. For average data, statistical analysis was performed using Student's paired *t* test when comparing pre- and post-activity recovery values, and Student's unpaired *t* test when comparing post-giant depolarizing potential (GDP) values from control and treated slices.

Drugs

1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulphonamide (NBQX) and D-2-amino-5-phosphovaleric acid (D-AP-5) were from Tocris Cookson (Bristol, UK); k-252a (Calbiochem, San Diego, CA, USA); Thapsigargin (MP Biological, Solon, OH, USA); Nifedipine (Sigma, St Louis, MO, USA); TrkB-IgG and TrkC-IgG (R&D system, Minneapolis, MN, USA).

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Results

Transient recovery from activity-deprivation induces a calcium-dependent long-lasting enhancement of GABAergic synaptic activity

The aim of this study was to investigate the mechanisms underlying activity-dependent plasticity of GABAergic synapses. To this end, hippocampal slices were incubated for 5–8 h in medium containing NBQX (5 μM) and D-AP5 (40 μM). We have previously shown that incubation in the presence of the ionotropic glutamatergic receptors delayed the functional maturation of GABAergic synapses (Colin-Le Brun *et al.* 2004). Here, we investigate the effect of a transient recovery from activity deprivation.

Spontaneous $GABA_A$ receptor-mediated postsynaptic currents ($sGABA_A$-PSCs) were recorded at a holding potential of −70 mV in the presence of 5 μM NBQX and 40 μM D-AP5 (Fig. 1*A*). After 10 min of stable GABAergic transmission, NBQX and D-AP5 were washed out for 15 min to recover global network activity. Figure 1*A* illustrates a typical experiment in which activity recovery resulted in a long-lasting potentiation of GABAergic synaptic transmission ($LLP_{GABA\text{-}A}$), consisting of a persistent increase in both frequency (from 0.56 ± 0.02 Hz to 1.02 ± 0.09 Hz, $P < 0.05$, K-S test, Fig. 1*A*) and amplitude (from 38 ± 0.7 pA to 57 ± 4.6 pA, $P < 0.05$, K-S test, Fig. 1*A*) of $sGABA_A$-PSCs. A summary of 33 similar experiments showed an average $31 \pm 4\%$ (from 5.7 ± 0.9 to 7.3 ± 1.1 Hz) and $36 \pm 7\%$ (from 45 ± 6 to 59 ± 6 pA) increase in $sGABA_A$-PSC frequency and amplitude 30 min after restoration of synaptic activity ($P < 0.05$ for both, Student's *t* test, Fig. 1*B* and *C*). In three experiments, bicuculline (10 μM) was applied at the end of the experiments. All events were blocked, showing that they were mediated by $GABA_A$ receptor.

A postsynaptic rise in $[Ca^{2+}]_i$ is a common trigger for long-lasting changes in the strength of GABAergic synapses (Gaïarsa *et al.* 2002). We therefore investigated the contribution of postsynaptic Ca^{2+} in $LLP_{GABA\text{-}A}$ induction. Loading the recorded neuron with the calcium chelator BAPTA (10 mM) prevents the induction of $LLP_{GABA\text{-}A}$ (from 5.5 ± 1.3 to 4.7 ± 1 Hz and 42 ± 8 to

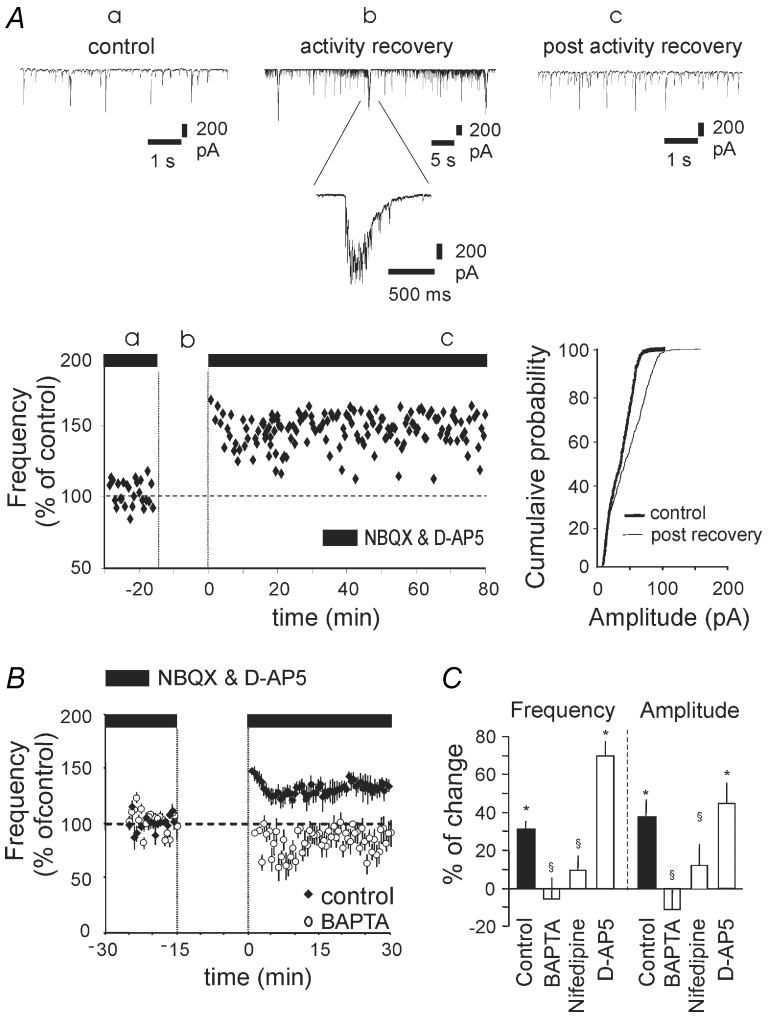

Figure 1. Synaptic activity recovery induced a Ca^{2+}-dependent long-lasting increase in GABAergic synaptic activity
A, upper, $sGABA_A$-PSCs (holding potential = −70 mV) before (left, *a*) and 30 min (right, *c*) after the activity recovery (middle, *b*). Middle, a single GDP recorded during activity recovery is shown at an expanded time scale. Lower right, graph showing the average time course of $sGABA_A$-PSC frequency. In this and following figures the frequency of $sGABA_A$-PSCs is expressed as a percentage of control pre-recovery value and plotted against time (bin = 30 s). Lower right, cumulative probability plot of $sGABA_A$-PSC amplitude before (control) and 20 min after activity recovery (post-recovery). *B*, summary plots showing the mean frequency of $sGABA_A$-PSCs before and after activity recovery in control (●, $n = 33$) and BAPTA-loaded cells (10 mM, ○, $n = 6$). *C*, bar plot showing the modification in $sGABA_A$-PSC frequency and amplitude induced by activity recovery in control (filled bars) and treated cells (open bars) (BAPTA 10 mM, $n = 6$; nifedipine 10 μM, $n = 12$; D-APV 40 μM, $n = 7$) (in this and following figures, $^*P < 0.05$ when compared to pre-recovery; $^{\S}P < 0.05$ when compared to control post-recovery).

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36 ± 5 pA, $n = 6$, $P > 0.05$ for both, Student's t test, Fig. 1*B* and *C*). We next investigated the possible contribution of VDCCs and NMDA receptors, two major sources of $[Ca^{2+}]_i$ implicated in many forms of GABAergic synaptic plasticity. $LLP_{GABA\text{-}A}$ was induced when synaptic activity was recovered in the presence of the NMDA receptor antagonist D-AP5 (from 3.2 ± 1.8 to 5.3 ± 1.6 Hz and 34 ± 5 to 47 ± 6 pA, $n = 7$, $P < 0.05$ for both, Student's t test, Fig. 1*C*), but not in the presence of nifedipine (10 μM), an L-type Ca^{2+} channel blocker (from 4.2 ± 0.9 to 4.7 ± 0.9 Hz and 57 ± 12 to 59 ± 8 pA, $n = 12$, $P > 0.05$ for both, Student's t test, Fig. 1*C*).

Overall, these experiments show that a transient recovery from network activity deprivation induces a persistent enhancement of GABAergic transmission requiring a postsynaptic rise in $[Ca^{2+}]_i$ and the activation of VDCCs.

Recovery of spontaneous glutamatergic synaptic activity is required to induce LLP_{GABA}

In normal ACSF, i.e. during NBQX and D-AP5 washout, synaptic activity consists of three broad classes of events: network-driven patterned activity, termed giant depolarizing potentials (GDPs), $sGABA_A$-PSCs and spontaneous glutamatergic postsynaptic currents (sGlu-PSCs) (Figs 1*A* and 2). Recent observations suggested a role of sGDPs in the induction of GABAergic and glutamatergic synaptic potentiation in the developing rat hippocampus (Kasyanov *et al.* 2004; Mohajerani *et al.* 2007). We therefore investigated the possible contribution of sGDPs in LLP_{GABA} induction. To this end, gabazine (GBZ), a specific $GABA_A$-receptor antagonist, was applied at a low concentration (0.1 μM) during the washout of NBQX and D-AP5. At this concentration, GBZ prevented the recovery of GDPs in 5 out of 6 slices. In theses slices,

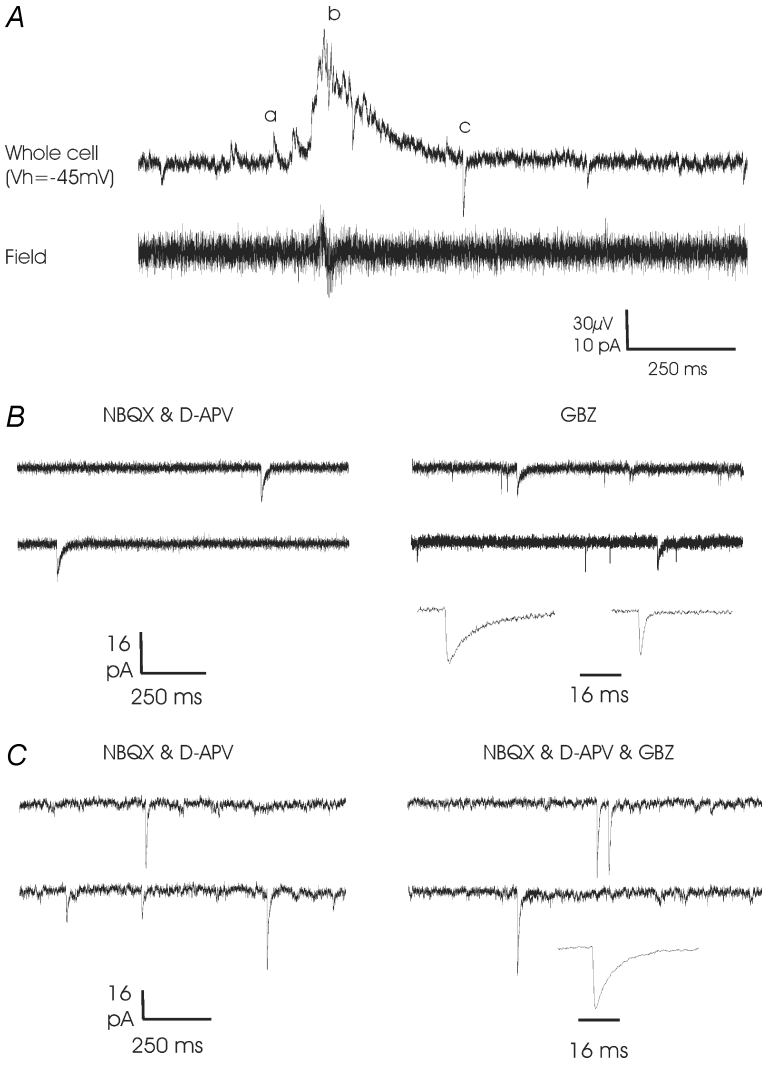

Figure 2. Spontaneous network-driven synaptic activity recovery
A, example of synaptic activity recorded during the washout of NBQX and D-AP5. Simultaneous whole cell (potassium methylsulphate pipette solution, the reversal GABA was set around −55 mV) and field recording in the CA3 rat hippocampal neuron. Spontaneous GABAergic (*a*, outward current) and glutamatergic (*c*, inward current) PSCs, as well as GDP (*b*) are shown. GDP is present in both whole-cell and field recordings. *B*, example of synaptic activity recorded during the washout of NBQX and D-AP5 in gabazine (GBZ, 0.1 μM) (CsCl pipette solution, the reversal GABA was set around −0 mV). Both fast, likely sGlu-PSCs, and slow, likely $sGABA_A$-PSCs, events were recorded. Averaged fast and slow synaptic events are shown at an expanded time scale. *C*, example of synaptic activity recorded in NBQX and D-AP5 and following a further application of gabazine (GBZ, 0.1 μM) (CsCl pipette solution). Only slow $sGABA_A$-PSCs were recorded. An averaged slow synaptic event is shown at an expanded time scale.

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LLP_{GABA} was induced despite the lack of GDP recovery (from 2.7 ± 1.6 to 4.1 ± 2.2 Hz and 39 ± 6 to 54 ± 8 pA, $n = 5$, $P < 0.05$ for both, Student's t test, Fig. 3*A* and *D*). Similar results were obtained with bicuculline (10 μM), which completely blocked the $sGABA_A$-PSCs (from 3.1 ± 1.3 to 5.2 ± 1.3 Hz and 42 ± 8 to 51 ± 6 pA, $n = 5$, $P < 0.05$ for both, Student's t test, Fig. 3*B* and *D*). These results therefore show that neither GDPs nor $sGABA_A$-PSCs are required to trigger LLP_{GABA}, and suggest that sGlu-PSCs play a pivotal role in LLP_{GABA} induction. This conclusion was confirmed by blocking AMPA receptor activation with NBQX (10 μM) during activity recovery in control conditions (Fig. 3*C*) or in the presence of GBZ (0.1 μM, Fig. 3*D*). In these conditions, LLP_{GABA} was not observed (from 5.9 ± 1.5 to 5.6 ± 1.1 Hz and 46 ± 7 to 43 ± 10 pA, $n = 6$, $P > 0.05$ for both, Student's t test).

Altogether, these data show that spontaneous glutamatergic synaptic activity is required to trigger LLP_{GABA}.

$LLP_{GABA\text{-}A}$ requires endogenous BDNF

In a previous study, we reported that BDNF is required for the induction of Ca^{2+}-dependent GABAergic long-term potentiation in the newborn rat hippocampus (Gubellini *et al.* 2005). We therefore investigated the possible contribution of BDNF in $LLP_{GABA\text{-}A}$ induction. k252a (200 nM), a membrane permeant inhibitor of protein tyrosine kinase coupled to Trk receptor family, bath applied during the entire recording session, prevents the induction of $LLP_{GABA\text{-}A}$ (from 4.2 ± 1.1 to 3.9 ± 0.9 Hz and 49 ± 6 to 51 ± 6 pA, $n = 13$, $P > 0.05$ for both, Student's t test, Fig. 4*A*, *E*). We next investigated the effect of neurotrophin scavengers (Shelton *et al.* 1995), consisting of the extracellular domain of the Trk receptors fused to a human IgG Fc domain (Trk-IgG). In slices preincubated for 5–8 h with TrkB-IgG (5 $\mu g\,ml^{-1}$), to sequester endogenous BDNF, activity recovery failed to induce $LLP_{GABA\text{-}A}$ (from 4.1 ± 1.1 to 3.9 ± 0.9 Hz and 43 ± 6 to 45 ± 8 pA, $n = 11$, $P > 0.05$ for both, Student's t test, Fig. 4*B* and *E*). As control, slices were preincubated with TrkC-IgG, a scavenger of neurotrophin 3 (NT3). In these slices $LLP_{GABA\text{-}A}$ was induced (from 4.5 ± 1.2 to 6.5 ± 1.1 Hz and 39 ± 5 to 62 ± 9 pA, $n = 11$, $P < 0.05$ for both, Student's t test, Fig. 4*B* and *E*). Thus, a release of endogenous TrkB ligands is necessary to trigger $LLP_{GABA\text{-}A}$. $LLP_{GABA\text{-}A}$ was also prevented when k252a (200 nM) was included in the patch pipette, showing that a postsynaptic protein tyrosine kinase activity is necessary to trigger $LLP_{GABA\text{-}A}$ (from 3.9 ± 0.8 to 4.0 ± 0.9 Hz and 46 ± 6 to 50 ± 5 pA, $n = 10$, $P > 0.05$ for both, Student's t test, Fig. 4*C* and *E*). Finally, to determine whether neurotrophin signalling is also required to maintain $LLP_{GABA\text{-}A}$, k252a was bath applied 15 min after $LLP_{GABA\text{-}A}$ induction (from 1.8 ± 0.9 to $3.2 \pm 1.$ Hz and 39 ± 4 to 51 ± 5 pA, $n = 4$, $P < 0.05$ for both, Student's t test, Fig. 4*D*). k252a did not reverse $LLP_{GABA\text{-}A}$ (from 3.2 ± 1.3 to 3.1 ± 1.2 Hz and 51 ± 11 to 52 ± 12 pA, $n = 4$, $P > 0.05$ for both, Student's t test, Fig. 4*D*).

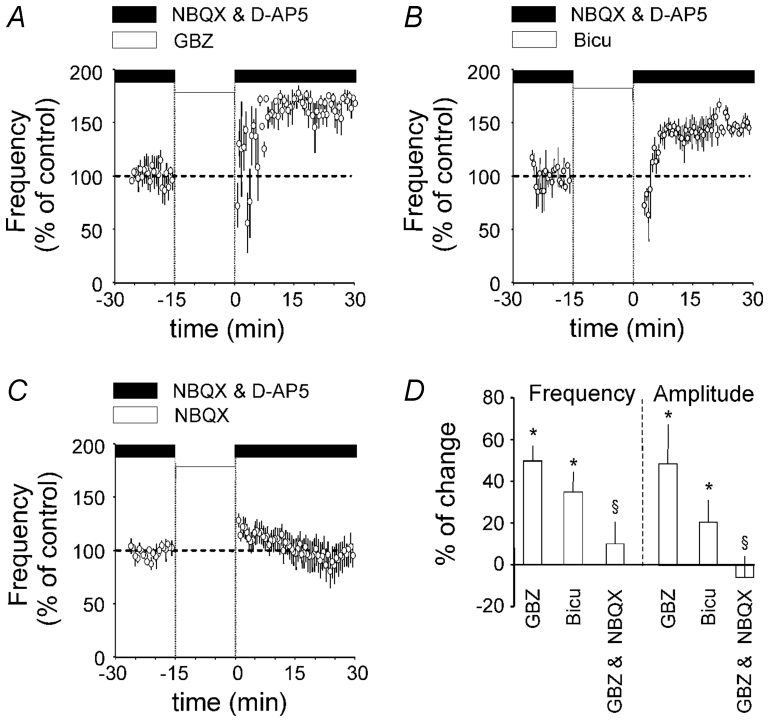

Figure 3. GDPs are not required to trigger $LLP_{GABA\text{-}A}$
A–C, average time course of $sGABA_A$-PSC frequency before and after activity recovery in the presence of gabazine (0.1 μM, $n = 5$; *A*), bicuculline (10 μM, $n = 5$; *B*), and NBQX (10 μM, $n = 10$; *C*). *D*, bar plot showing the modification in $sGABA_A$-PSC frequency and amplitude induced by activity recovery in the different conditions.

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Altogether, these data show that the activation of postsynaptic TrkB receptors by endogenous TrkB ligands is required to trigger $LLP_{GABA\text{-}A}$.

Exogenous BDNF induces a Ca^{2+}-dependent enhancement of GABAergic activity

We next investigated the relationship between BDNF and Ca^{2+}. To determine whether VDCC activation and calcium rise are downstream of TrkB receptors activation, we tested whether activation of these receptors with exogenous BDNF (5 ng ml^{-1}), during the period of activity recovery, bypasses the effect of calcium blockers and rescues $LLP_{GABA\text{-}A}$. As illustrated in Fig. 5*A*, exogenous BDNF completely rescued the deficit of $LLP_{GABA\text{-}A}$ induced by nifedipine (frequency and amplitude values of $sGABA_A$-PSCs increased, respectively, by $45 \pm 18\%$ (from 2.8 ± 1.1 to 4.4 ± 1.6 Hz) and $39 \pm 9\%$ (48 ± 2 to 67 ± 5 pA) 30 min after activity recovery in BDNF, $n = 5$, $P < 0.05$ for both, Student's t test), but had no effect on BAPTA loaded cells (frequency and amplitude values of $sGABA_A$-PSCs changed by $15 \pm 7\%$ (from 3.5 ± 0.8 to 4.1 ± 0.9 Hz) and $-5 \pm 6\%$ (from 54 ± 7 to 48 ± 6 pA) 30 min after activity recovery in BDNF, $n = 8$, $P > 0.05$ for both, Student's t test). One possible interpretation of these results is that during the recovery of synaptic activity, the activation of L-type VDCC leads to BDNF secretion, and that once released, BDNF triggers

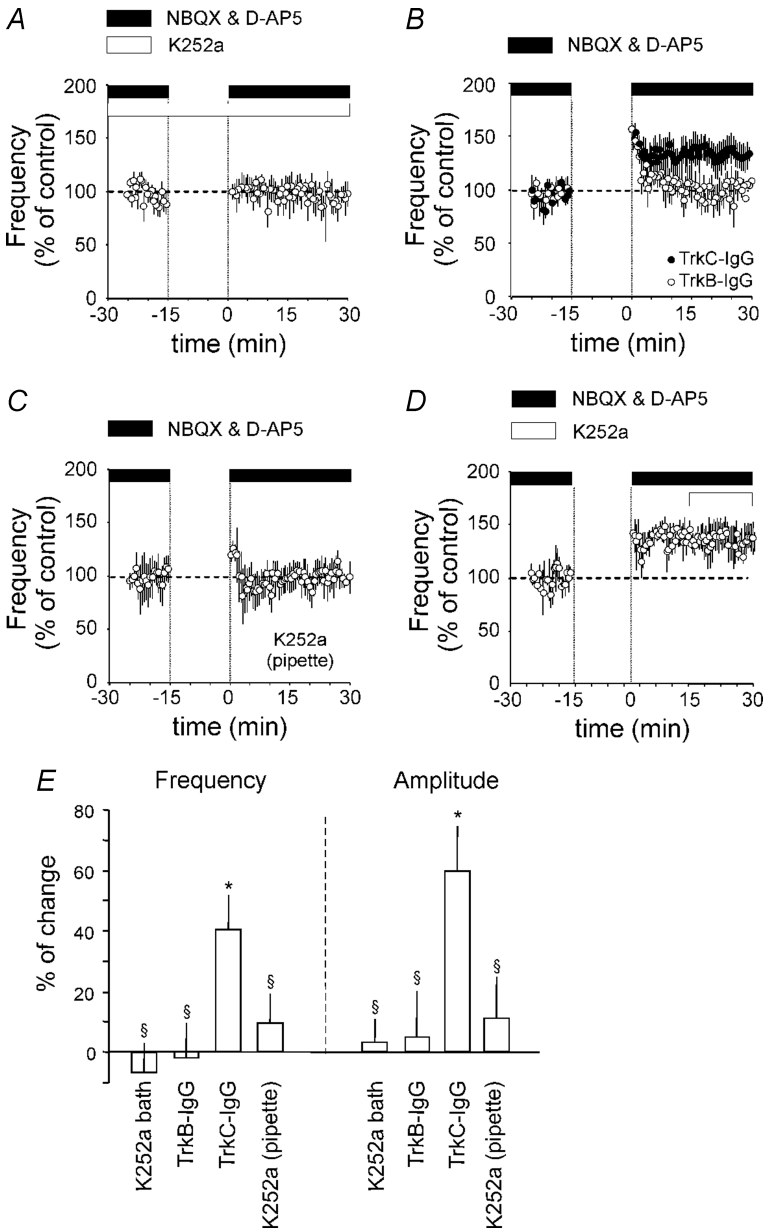

Figure 4. Requirement of postsynaptic BDNF-TrkB signalling activation for $LLP_{GABA\text{-}A}$
A, average time course of $sGABA_A$-PSC frequency before and after the synaptic activity recovery in the presence k252a (200 nM, $n = 13$). *B*, average time course of $sGABA_A$-PSC frequency before and after the activity recovery in TrkC-IgG (5 μg ml^{-1}, ●, $n = 11$) or in TrkB-IgG (5 μg ml^{-1}, ○, $n = 11$) preincubated slices. *C*, average time course of $sGABA_A$-PSC frequency before and after the activity recovery in k252a-loaded cells (200 nM, $n = 10$). *D*, average time course of $sGABA_A$-PSC frequency before and after the activity recovery. k252a (200 nM, grey bar, $n = 4$) was bath applied 15 min after $LLP_{GABA\text{-}A}$ induction. *E*, bar plot showing the modification in $sGABA_A$-PSC frequency and amplitude induced by activity recovery in the different conditions.

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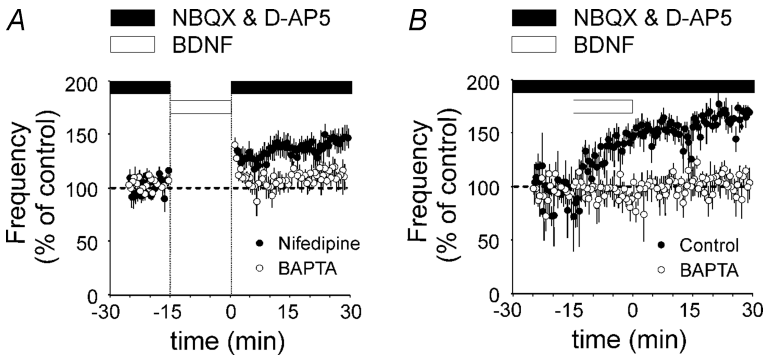

Figure 5. Requirement of TrkB receptor-mediated postsynaptic $[Ca^{2+}]_i$ rise for $LLP_{GABA\text{-}A}$
A, average time course of $sGABA_A$-PSC frequency before and after bath applied BDNF during activity recovery in the presence of nifedipine (10 μM, ●, $n = 5$) and BAPTA-loaded cells (10 mM, ○, $n = 8$). *B*, average time course of $sGABA_A$-PSC frequency before and after bath applied BDNF in the presence of NBQX (5 μM) and D-AP5 (40 μM) in control (●, $n = 5$) and BAPTA-loaded cells (10 mM, ○, $n = 6$).

$LLP_{GABA\text{-}A}$ through a Ca^{2+}-dependent, but L-type VDCC independent, postsynaptic signalling cascade. To test this hypothesis we investigate whether BDNF is able to induce $LLP_{GABA\text{-}A}$ in the presence of NBQX and D-AP5. As shown in Fig. 5*B* BDNF (5 ng ml^{-1}) induced a robust and long-lasting increase in both frequency (52 ± 10%, from 1.7 ± 0.7 to 2.9 ± 1 Hz) and amplitude (32 ± 11%, from 34 ± 6 to 48 ± 11 pA) of $sGABA_A$-PSCs that persisted for the duration of the recording session ($n = 5$, $P < 0.05$ for both, Student's *t* test). We next checked whether BDNF-dependent $LLP_{GABA\text{-}A}$ requires postsynaptic Ca^{2+}-dependent signalling cascade by loading the postsynaptic neuron with BAPTA. In this condition the modification of GABAergic synaptic activity induced by BDNF was not observed: frequency and amplitude were, respectively, -4 ± 8% (from 2.7 ± 0.9 to 3 ± 1.1 Hz) and 3 ± 9% (55 ± 6 to 58 ± 9 pA) of control 30 min after BDNF application ($n = 6$, $P > 0.05$ for both, Student's *t* test, Fig. 5*B*).

These results suggest that the activation of AMPA receptors trigger a VDCC-dependent secretion of BDNF that, in turn, induces $LLP_{GABA-A.}$ To challenge this hypothesis we determined the amount of BDNF released in the different experimental conditions using an ELISA BDNF immuno-detection assay. Ten hippocampal slices were incubated for 30 min in 30 ml of ACSF supplemented with NBQX and D-AP5. After, incubation, a 6 ml fraction of the medium was collected. The slices were then transferred for 30 min in 30 ml of ACSF alone or supplemented with nifedipine (10 μM), or GBZ (0.1 μM), or NBQX (10 μM), or AP5 (40 μM), to allow activity recovery. Another 6 ml fraction of the medium was then collected. As illustrated in the Fig. 6, exogenous BDNF level increased upon activity recovery in control ACSF, in the presence of GBZ ($n = 3$, $P < 0.05$, Student's *t* test) or AP5 ($n = 3$, $P < 0.05$, Student's *t* test), but not in the presence of nifedipine or NBQX ($n = 3$ for both, $P > 0.05$, Student's *t* test).

Altogether, these results show that AMPA/KA-PSCs are required to maintain a high level of BDNF secretion through the activation of L-type VDCCs and that a postsynaptic $[Ca^{2+}]_i$ rise downstream of TrkB receptor activation is required to trigger $LLP_{GABA\text{-}A}$.

Discussion

The major finding of this study is that spontaneous AMPA/KA-PSCs trigger a Ca^{2+}-dependent long-lasting potentiation of GABAergic synaptic activity in the newborn rat hippocampus. This potentiation required the release of endogenous BDNF, which plays an instructive role through the activation of postsynaptic TrkB receptors. A Ca^{2+}-dependent long-term increase of GABAergic

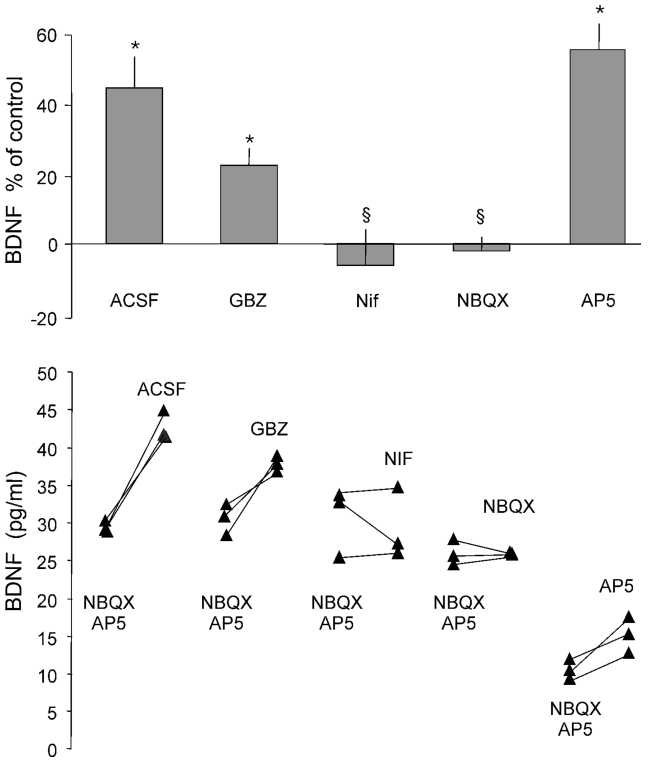

Figure 6. Spontaneous glutamatergic synaptic activity induced BDNF release
Mean percentage changes in the level of native BDNF measured in hippocampal slices during synaptic activity recovery in different conditions. Ten hippocampal slices were incubated for 30 min in NBQX (5 μM) and D-AP5 (40 μM) and then transferred in control ACSF (ACSF) or in ACSF containing Nifedipine (Nif, 10 μM), gabazine (GBZ, 0.1 μM), NBQX (10 μM) or AP5 (40 μM). $n = 3$ for each (*$P < 0.05$ when compared to control (NBQX and D-AP5); §$P < 0.05$ when compared to control recovery in ACSF).

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synaptic efficacy can be induced in the developing rat hippocampus following tetanic stimulation of GABAergic fibres (McLean *et al.* 1996), postsynaptic firing of CA3 pyramidal neurons (Caillard *et al.* 1999) or pairing of GDPs with presynaptic stimulation of mossy fibres (Kasyanov *et al.* 2004). The present study shows that ongoing AMPA/KA-PSCs can induce a BDNF- and Ca^{2+}-dependent long-lasting potentiation of GABAergic synaptic transmission ($LLP_{GABA\text{-}A}$). In the developing rat hippocampus the GABAergic mossy fibres are potentiated by pairing sGDPs with presynaptic stimulation (Kasyanov *et al.* 2004). In the present study, sGDPs were not involved since $LLP_{GABA\text{-}A}$ was observed in the presence of a low concentration of gabazine, which blocks GDPs but not AMPA/KA-PSCs. Therefore at least two different forms of GABAergic plasticity are produced by spontaneous ongoing activity in the developing hippocampus. The first one relies on the associative activation of mossy fibre and GDPs and might contribute to the fine reorganization of mossy fibre connections. The second one relies on glutamatergic activity and might be important for the development of a correct balance between glutamatergic and GABAergic synaptic transmission in the CA3 circuitry.

Using ELISA BDNF immuno-detection we show that BDNF secretion is up-regulated during the recovery of AMPA/KA-PSCs. Because GABAergic interneurons do not produce neurotrophins themselves (Ernfors *et al.* 1990; Gorba & Wahle, 1999), BDNF is likely to be provided by the CA3 pyramidal neurons (Brigadski *et al.* 2005) or by mossy fibres arising from dentate gyrus granular cells (Danzer & McNamara, 2004). Whether dendritic and/or axonal release of BDNF (Kohara *et al.* 2001; Brigadski *et al.* 2005; Kuczewski *et al.* 2008) contribute to the induction of $LLP_{GABA\text{-}A}$ remains to be determined. However, previous studies have reported that dendritic release of BDNF is blocked by the L-type Ca^{2+} channel blocker nifedipine (Kolarow *et al.* 2007) while axonal BDNF release is prevented by N-type Ca^{2+} channel blocker (Balkowiec & Katz, 2002; Wang *et al.* 2002). In the present study, nifedipine prevents $LLP_{GABA\text{-}A}$ and the increase in BDNF level during activity recovery suggesting a contribution of BDNF dendritic release. Therefore, along with the observation that exogenous BDNF potentiated GABAergic synapses and rescued the inhibitory effect of nifedipine, these results support the notion that BDNF released during AMPA/KA-PSCs acts as a target-derived and instructive messenger for $LLP_{GABA\text{-}A}$ induction.

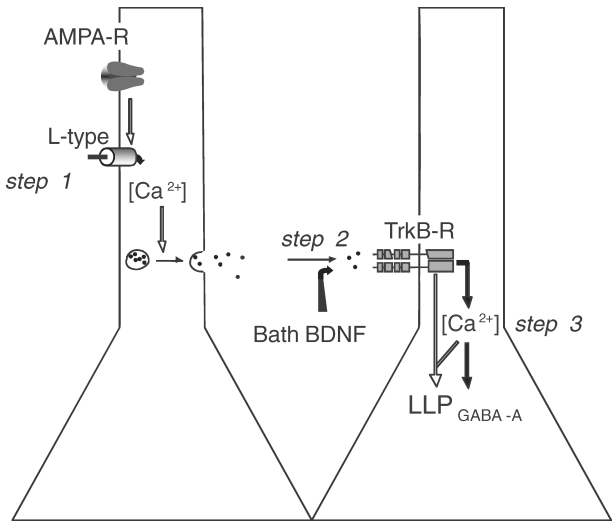

Figure 7. Paracrine action of BDNF triggers $LLP_{GABA\text{-}A}$
Schematic representation of the steps leading to $LLP_{GABA\text{-}A}$. Step 1 is prevented by nifedipine and NBQX, step 2 by TrkB-IgG, K252a, and step 3 by postsynaptic injection of BAPTA through the recording electrode. Bath applied BDNF can initiate step 2.

A dendritic AMPA- and NMDA-dependent release of BDNF-GFP evoked by presynaptic tetanic stimulation of glutamatergic terminals can be induced in neuronal cultures (Hartmann *et al.* 2001). In agreement with a previous study (Mohajerani *et al.* 2007), we show that during ongoing synaptic activity activation of AMPA/KA receptors, but not NMDA receptors, is required to trigger a BDNF-dependent synaptic plasticity in the developing rat hippocampus. A glutamatergic receptor-independent dendritic release of BDNF can also be triggered by prolonged membrane depolarization of sufficient amplitude (Magby *et al.* 2006; Kuczewski *et al.* 2008). Thus, the stimulation conditions used critically determine the contribution of glutamatergic receptors for BDNF secretion. We previously reported that the depolarization mediated by the activation of ionotropic glutamatergic receptors during ongoing synaptic activity does not directly lead to BDNF secretion in neuronal cultures, but that instead back-propagating action potentials (b-APs) are required (Kuczewski *et al.* 2008). Whether b-APs similarly contribute to dendritic BDNF secretion in hippocampal slices, or whether AMPA/KA receptor mediated depolarization alone is sufficient to trigger VDCC activation and subsequent BDNF secretion remained to be determined.

Exogenous BDNF failed to rescue $LLP_{GABA\text{-}A}$ in BAPTA-loaded cells. This observation suggests that, in addition to the VDCC-dependent release of BDNF, a postsynaptic Ca^{2+}-dependent signalling cascade downstream to TrkB receptor activation is required to induce $LLP_{GABA\text{-}A}$. Accordingly, as reported in other studies (Tanaka *et al.* 1997; Cheng & Yeh, 2005*a*,*b*), we found that postsynaptic injection of BAPTA also prevents exogenous BDNF-induced $LLP_{GABA\text{-}A}$. With the observation that postsynaptic injection of k252a prevents $LLP_{GABA\text{-}A}$, these results support a postsynaptic action of BDNF. This does not, however, exclude the possibility that postsynaptic TrkB receptor activation might trigger the release of a retrograde messenger acting on the

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presynaptic site. Thus, a recent study reported that pairing presynaptic Schaffer collaterals with GDPs induced a BDNF-dependent potentiation of hippocampal glutamatergic synapses. Although this potentiation is expressed presynaptically, its induction involved postsynaptic mechanisms including a $[Ca^{2+}]_i$ rise and the phosphorylation of ERK induced by the activation of postsynaptic TrkB receptors (Mohajerani *et al.* 2007).

Based on our present data, the steps leading to $LLP_{GABA\text{-}A}$ can be summarized as followed (Fig. 7). CA3 pyramidal cells release BDNF in response to the activation of L-type Ca^{2+} channels during AMPA/KA-PSCs (step 1). Once released, BDNF diffuses to bind on post-synaptic TrkB receptors (step 2), and induces $LLP_{GABA\text{-}A}$ through a postsynaptic Ca^{2+}-dependent process (step 3). The relationship between postsynaptic TrkB receptor activation and postsynaptic $[Ca^{2+}]_i$ is presently unknown. Two hypotheses can be put forward: either TrkB receptor activation induces a rise in $[Ca^{2+}]$ leading to $LLP_{GABA\text{-}A}$ (black arrow) or a basal level of $[Ca^{2+}]$ is required to allow TrkB-dependent induction of $LLP_{GABA\text{-}A}$ (white arrow). If a $[Ca^{2+}]$ rise is required, possible candidates include release of Ca^{2+} from internal stores (Canossa *et al.* 1997; Li *et al.* 1998) or activation of transient receptor potential canonical (TRPC) channels (Li *et al.* 1999), which have been reported to mediate several biological effects induced by BDNF (Jia *et al.* 2007; Amaral *et al.* 2007).

In conclusion, we have shown that BDNF, released during spontaneous AMPA/KA-PSCs, trigger a long-lasting potentiation of GABAergic synaptic transmission in the newborn rat hippocampus. Impairing synaptic activity has been reported to produce structural and functional alterations that can be reverted by application of exogenous BDNF (McAllister *et al.* 1996; Rutherford *et al.* 1997; Marty *et al.* 2000; Seil & Drake-Baumann, 2000). Together with previous data showing that GDPs induce a BDNF-dependent LTP of glutamatergic synapses in the developing hippocampus (Mohajerani *et al.* 2007), these results point to a complex scenario in which synaptic activity and BDNF can modulate all aspects of hippocampal circuit formation.

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Acknowledgements

This work was supported by INSERM, CNRS and ANR (Agence Nationale pour la Recherche). N.K. was the recipient of a FRM (Fondation pour la Recherche Médicale) fellowship and ANR grant (ANR-06-Neuro-017-01). We wish to thank Drs I. Medina, R. Khazipov and M. Colonnese for helpful comments and critical reading of the manuscript.

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