NMDDA-Dependent Switch of proBDNF Actions on Developing GABAergic Synapses

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The brain-derived neurotrophic factor (BDNF) has emerged as an important messenger for activity-dependent development of neuronal network. Recent findings have suggested that a significant proportion of BDNF can be secreted as a precursor (proBDNF) and cleaved by extracellular proteases to yield the mature form. While the actions of proBDNF on maturation and plasticity of excitatory synapses have been studied, the effect of the precursor on developing GABAergic synapses remains largely unknown. Here, we show that regulated secretion of proBDNF exerts a bidirectional control of GABAergic synaptic activity with NMDA receptors driving the polarity of the plasticity. When NMDA receptors are activated during ongoing synaptic activity, regulated Ca2+-dependent secretion of proBDNF signals via p75NTR to depress GABAergic synaptic activity, while in the absence of NMDA receptors activation, secreted proBDNF induces a p75NTR-dependent potentiation of GABAergic synaptic activity. These results revealed a new function for proBDNF-p75NTR signaling in synaptic plasticity and a novel mechanism by which synaptic activity can modulate the development of GABAergic synaptic connections.

Keywords: BDNF, GABA, proneurotrophin, p75NTR, synaptic plasticity

Introduction

Appropriate wiring of neuronal networks depends on a tightly regulated sequence of events whereby neurogenesis and synaptogenesis are balanced by apoptosis and axonal pruning. Among the many factors essential in these developmental processes is the brain-derived neurotrophic factor (BDNF). BDNF is an activity-dependent secreted neurotrophin that mediates many aspects of brain development (Lu et al. 2005; Gottmann et al. 2009; Kuczewski et al. 2010). Studies of BDNF outcomes are challenging because of the complex interactions that take place between the different ligands and their receptors. Thus, like many peptide hormones or growth factors, BDNF is synthesized as a precursor (proBDNF) that is either converted intracellularly to mature BDNF (mBDNF) or secreted in an unprocessed form (Lee et al. 2001; Teng et al. 2005; Bergami et al. 2008; Nagappan et al. 2009; Yang, Siao, et al. 2009; but see Matsumoto et al. 2008). ProBDNF and mBDNF are assumed to elicit biological functions that often vary in diverse directions (Lu et al. 2005). For instance, mBDNF signals via the tropomyosin-related kinase receptor B (TrkB-R) to induce cell survival (Teng et al. 2005) or promote long-term potentiation (Korte et al. 1996; Patterson et al. 1996, 2001; Yang, Je, et al. 2009), while proBDNF binds to the p75 pan-neurotrophin receptor (p75NTR) to induce apoptosis (Teng et al. 2005) or facilitate long-term depression (Woo et al. 2005; Rosch et al. 2005; Yang, Je, et al. 2009).

The contribution of mBDNF-TrkB signaling pathway to the development and maturation of GABAergic synapses is well established and documented (Lessmann and Bridgada 2009; Kuczewski et al. 2010). p75NTR and proBDNF expression are developmentally regulated with the highest levels in the first postnatal weeks of life, at a time of synapse formation and elimination (Yang, Siao, et al. 2009; Barkowska et al. 2010). Whether and how proBDNF-p75NTR signaling affects developing GABAergic synapses is not known. The present study was aimed at determining the effects of proBDNF on developing GABAergic synapses. We show that regulated secretion of proBDNF can lead to either a long-lasting increase or decrease in GABAergic synaptic activity with NMDA receptors driving the switch from potentiation to depression. These results add new insights to the contribution of neurotrophins in activity-dependent refinement of synaptic connections.

Materials and Methods

Slices Preparation

Experiments were performed on hippocampal slices obtained from postnatal day-0 to -5 Wistar rats, as previously described (Kuczewski et al. 2008). All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Brains were removed and immersed into ice-cold (2-4 °C) artificial cerebrospinal fluid (ACSF) of the following composition (millimolar): NaCl, 126; KCl, 3.5; CaCl2, 2; MgCl2, 1.3; NaH2PO4, 1.2; NaHCO3, 25; and glucose, 11; pH 7.4, when equilibrated with 95% O2 and 5% CO2. Hippocampal slices (400 μm thick) were cut with a McIlwain tissue chopper and submerged in ACSF supplemented with NBQX (5μM) and D-AP5 (40μM) to reduce network-driven synaptic activity. In some experiments, slices were incubated with aprotinin, TrkB-IgG, TrkA-IgG, p75NTR-function-blocking antibody, or TAT-pep5 for at least 3 h before recording. Slices were then transferred to a submerged recording chamber and perfused with ACSF (3 mL/min) at 34 °C.

Electrophysiological Recordings

Whole cell patch-clamp recordings of CA3 pyramidal neurons were performed with an Axopatch 200B amplifier (Axon Instruments). Microelectrodes (+8 MΩ) were filled with the following solution (mM): CsCl (110), K-glutonate (30), N2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (10); ethylene glycol-bis (β-aminoethyl ether)-N,N',N,N'-tetra-acetic acid (EGTA) (1.1), CaCl2 (0.1), MgATP (4), and NaGTP (0.3). In some experiments, MK801 (1 mM) was added to the pipette solution. Spontaneous synaptic activity was monitored and stored with Axoscope 8.1 (Axon Instruments). Series resistance was monitored in response to a 5 mV pulse during the recordings. Spontaneous GABA_B receptor-mediated postsynaptic currents (sGABA_B-PSCs) were recorded in NBQX and D-AP5. The protocol to induce GABAergic synaptic plasticity consists in the washing out of the glutamatergic antagonist during 15 min (Kuczewski et al. 2008). In some experiments, the extracellular potassium concentration was increased to 5 mM only during the washout period, to increase the secretion of BDNF, which is required for the induction of the...
GABAergic plasticity (Kuczewski et al. 2008). Because, activity recovery in 3.5 or 5 mM led to similar results (respectively, 38 ± 10 % (n = 18) and 30 ± 12 % (n = 8) increase in sGABA\_PSCs frequency), the data were pooled. On average, the frequency and amplitude of sGABA\_PSCs increased from 4.5 ± 0.4 to 5.7 ± 0.5 Hz (P = 0.0001) and 82 ± 12 to 100 ± 9 pA (P = 0.002) (n = 26, Fig. 1.A,C). In some experiments, the identity of the spontaneous PSCs was confirmed by exogenous application of gabazin (5 μM) at the end of the recording session. sGABA\_PSCs and series resistance (Rs) were analyzed off-line with Mini Analysis 6.0 software (Synapsoft). To generate the average sGABA\_PSCs, multiple overlapping events were discarded, and the remaining events were aligned on their rising phase. The effective plasticity was quantified as the mean frequency or amplitude sGABA\_PSCs between the 10-min preceding activity recovery and the 20-30 min after activity recovery using the Kolmogorov-Smirnov (K-S test). We used paired Student’s t-test to evaluate difference between groups.

**Immunohistochemistry**

Transverse hippocampal cryosections of paraformaldehyde-fixed brain from P4 rats were processed as previously described (Fiorentino et al. 2009), using primary antibodies chicken anti-proBDNF (1:1000, Chemicon MAB351) and rabbit anti-p75NTR (1:500; Abcam 8874). Cy3-conjugated secondary antibodies against chicken and rabbit IgG (1:1000; Chemicon) were used. Hippocampal sections were counterstained using the NeuroTrace green fluorescent Nissl stain (1:1000; Invitrogen). Sections were washed and coverslips mounted using Vectashield (Vector). Immunoreactivity was visualized using laser scanning confocal microscope (Zeiss LSM 510 Meta) with 10× objective.

**Phospho-CREB Activation**

Immunostaining against the nonphosphorylated and phosphorylated forms of CREB was performed on hippocampal slices, which underwent a procedure similar to that used for the electrophysiological study. Hippocampal slices were transferred to the submerged recording chamber and perfused with NBQX and D-AP5 (3 mL/min, at 34 °C) for 10 min. In a first series of experiments, NBQX and D-AP5 were washed out for 15 min in the presence or absence of aprotinin. In another series, mBDNF (10 ng/mL) or CR-proBDNF (10 ng/mL) was applied for 15 min in the presence of NBQX and D-AP5. Control

**Figure 1.** Aprotinin uncovers activity-dependent long-lasting depression in GABAergic synaptic activity. (A) Upper traces: representative recordings of sGABA\_PSCs illustrating the increase in events frequency after activity recovery in control ACSF. Average sGABA\_PSCs are shown at a higher time scale. The graph illustrates the time course of sGABA\_PSCs frequency modification in the same neuron. In this and following figures the frequency of sGABA\_PSCs is expressed as percentage of control prerecovery value and plotted against time (bin = 30 s). (B) Upper traces: representative recordings illustrating the increase in sGABA\_PSCs frequency after activity recovery in ACSF supplemented with aprotinin (3 μg/mL). The graph illustrates the time course of sGABA\_PSCs frequency modification in the same neuron (bins = 30 s). (C) Average time course of sGABA\_PSCs frequency before and after activity recovery in control ACSF (open symbol, n = 26) or with aprotinin (3 μg/mL, filled symbol, n = 17). (D) Percentage of frequency and amplitude changes 30 min after activity recovery in control ACSF (open symbol) or with aprotinin (filled symbol). Each symbol represents the result from one single cell.
experiments consist of slices maintained in NBQX and D-AP5 for 30 min. The slices were fixed overnight in 4% paraformaldehyde in 0.1 M phosphate buffer immediately after treatment. Cryostat-cut hippocampal sections (30 μm) were preincubated (1 h) in PBS-TritionX-100 (0.3%)-goat serum (3%) and co-incubated overnight at 4 °C with mouse anti-CREB (1:1000) and rabbit anti-phospho-CREB (pCREB, 1:1000) antibodies (Cell Signalling Technology Inc). Immunoreactivity for pCREB and CREB were detected with an Alexa 488-coupled (A488) rabbit secondary antibody (1:500; Fluoblot) and a Cy3-coupled mouse secondary antibody (1:500; Jackson ImmunoResearch Laboratories), respectively. Sections were washed and coverslips mounted using Vectashield (Vector) with the counterstained 4′,6-diamidino-2-phenylindole. Slices were imaged with a LSM 510 (Zeiss) confocal microscope using sequential dual channel recordings of A488 (pCREB) and Cy3 (CREB) fluorescence. The optical sections were digitized (1024 × 1024 pixels) and processed using Image J software. The pCREB to CREB intensity ratio was expressed as means value ratio of the pCREB-A488 staining intensity versus the CREB-Cy3 staining intensity. Average data are presented as percentage of control slices. We used unpaired Student’s t-test to evaluate difference between groups.

**Elisa BDNF Immunodetection Assay**

The BDNF sandwich ELISA Kit (Chemicon International, Temecula, CA, USA) was used to quantify the amount of BDNF in hippocampal slices which underwent a procedure similar to that used for the electrophysiological study. Immediately after the treatment, the slices were weighed, and snap frozen in liquid nitrogen and stored at -80 °C. For extraction of BDNF, 20–30 vol/wt of extraction buffer consisting of 100 mM Tris/HCl pH 7.0, containing 1 M NaCl, 4 mM EDTA, 2% Triton X-100 and the protease inhibitors 10 μg/mL aprotinin, 10 μg/mL leupeptin and 17 μg/mL phenyl-methylsulfonyl fluoride (PMSF). The homogenates were centrifuged (14,000 g for 20 s). Supernatants were collected and analyzed with a commercial two-antibody sandwich ELISA (BDNF Emax Immunoassay system; Chemicon) according to the protocol of the manufacturer. The total protein content of each supernatant was measured with a Bradford protein assay. The BDNF level was expressed as the ratio of BDNF to the total soluble protein concentration. Final values were compared using an unpaired Student’s t-test.

**Relative Quantitative Expression of BDNF Transcripts**

The level of BDNF mRNA was quantified using Q-PCR (Roche LC 4PO) in intact hippocampi. Analysis of the hippocampi was done for BDNF paired with GAPDH mRNA as housekeeping gene. Standard curves for each gene were produced using mRNA extracted from hippocampi and diluted more than 300 times, demonstrating the linearity of the response over the range of values studied (r² = 0.998 for BDNF and r² = 0.999 for GAPDH). PCR was performed in replicates of 3 and resulting mean Ct values were plotted against standard curves to convert Ct data from the samples into concentrations. The relative concentration of BDNF mRNA was normalized to the concentration of GAPDH mRNA. The results are expressed as means ± standard error of mean. Final values were compared using an unpaired Student’s t-test.

**Drugs**

1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzo[fl]quinoxaline-7-sulfonamide (NBQX), Gabazine, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK801) and D-2-amino-5-phosphovaleric acid (D-AP5) (Tocris Cookson); k252a, TAT-pep5 (Calbiochem); Nifedipine, aprotinin (Sigma); p75NTR antibody (ab1554, Millipore); cleavage-resistant (CR) proBDNF, mBDNF (Alomone labs); TrkA-IgG, TrkB-IgG (R&D system).

**Results**

**Activity-Recovery Induces Plasticity of GABAergic Synaptic Activity**

Spontaneous GABAergic receptor-mediated postsynaptic currents (sGABAergicPSCs) were recorded from newborn rats CA3 pyramidal neurons in 5 μM NBQX and 40 μM D-AP5. After 10 min of stable GABAergic transmission, NBQX and D-AP5 were washed out for 15 min to recover global network activity. As already reported (Kuczewski et al. 2008), a transient activity recovery resulted in a long-lasting potentiation of GABAergic synaptic activity (LLP) consisting in a persistent increase in both frequency and amplitude of sGABAergicPSCs (Fig. 1A). A summary of 26 experiments showed that the frequency and amplitude of sGABAergicPSCs increased from 4.5 ± 0.4 to 5.7 ± 0.5 Hz (P = 0.0001) and 82 ± 12 to 100 ± 9 pA (P = 0.002), respectively (Fig. 1C,D). Recordings in the continuous presence of NBQX and D-AP5 showed no significant changes in sGABAergicPSCs frequency or amplitude (from 3.7 ± 1.6 to 3.9 ± 1.8 Hz and 74 ± 11 to 68 ± 8 pA, n = 5, P = 0.1, for both). Consistent with our previous study demonstrating the contribution of the mBDNF-TrkB receptor signaling pathway in LLP (Kuczewski et al. 2008), LLP was mimicked by bath applied mBDNF (see below and Fig. 4C) and prevented by k252a (200 nM) added to the patch pipette solution (from 5.1 ± 0.9 to 5.4 ± 0.9 Hz and 90 ± 10 to 97 ± 9 pA, n = 10, P = 0.1 for both, data not shown).

To determine whether proBDNF is secreted in the developing hippocampus and to investigate its effect on developing GABAergic synapses, we repeated the same protocol in the continuous presence of the cell-impermeable serine protease inhibitor aprotinin (3 μg/mL), to prevent the extracellular conversion of proBDNF into mBDNF (Teng et al. 2005). In aprotinin, synaptic activity recovery uncovered a long-lasting depression of sGABAergicPSCs frequency (LLDGABA-A). A typical experiment is illustrated in Figure 1B. A summary of 17 similar experiments showed that sGABAergicPSCs frequency was decreased by 31 ± 6% on average after activity-recovery in aprotinin (from 5.6 ± 0.4 to 4.1 ± 0.6 Hz, P = 0.0002, Fig. 1C,D). The kinetic properties of sGABAergicPSCs were not affected (the rise time change from 1.29 ± 0.09 to 1.21 ± 0.07 (P = 0.2) and the decay time from 8.6 ± 0.9 to 9.1 ± 0.7 (P = 0.4)). The effect on sGABAergicPSCs amplitude was however more complex. An increase (n = 6), a decrease (n = 7), or no significant effect (n = 3) on sGABAergicPSCs amplitude were observed (Fig. 1D). On average, a nonsignificant change of 4 ± 13% in sGABAergicPSCs amplitude was observed 30 min after activity recovery (n = 17, P = 0.2). It is worth noting that aprotinin had no effect per se on the frequency and amplitude of sGABAergicPSCs (from 2.2 ± 0.9 to 2.3 ± 0.9 Hz (P = 0.4) and 56 ± 6 to 72 ± 19 pA (P = 0.1, n = 5), 30 min after aprotinin application). Thus, a transient recovery from activity deprivation with the cell-impermeable serine protease inhibitor aprotinin results in a long-lasting depression of sGABAergicPSCs frequency (LLDgABA-A).

**Activation of p75NTR and Intracellular Ca²⁺ Rise Are Required for LLDgABA-A Induction**

We next asked whether p75NTR activation contributes to LLDgABA-A induction. We first performed immunostaining against proBDNF and p75NTR. Both proBDNF and p75NTR were distinguished in the CA1 and CA3 regions of the hippocampus and in the dentate gyrus of the newborn rats (Fig. 2A). The staining was prominent in pyramidal cell bodies and mild short processes extending from the somata to the stratum radiatum. The staining pattern was the same as that obtained by others (Yang, Siao, et al. 2009). To test the contribution of p75NTR in LLDgABA-A induction, hippocampal slices were incubated with.
a p75NTR function-blocking antibody (1/100) or with TAT-pep5 (2 μM), a cell-permeable inhibitor of p75NTR signaling (Yamashita and Tohyama 2003). In both cases, activity recovery failed to induce LLDGABA-A (Fig. 2B). The frequency and amplitude of sGABA_A-PSCs changed, respectively, from 3.7 ± 0.7 to 4.5 ± 1.2 Hz (n = 9, P = 0.2) and from 83 ± 8 to 101 ± 17 pA (n = 9, P = 0.06) in TAT-pep5-treated slices, and from 3.7 ± 0.8 to 4.9 ± 1.1 Hz (n = 7, P = 0.07) and from 87 ± 2 to 101 ± 14 pA (n = 7, P = 0.1) in p75NTR function-blocking antibody-treated slices. To determine the location of the p75NTR, TAT-pep5 (2 μM) was added in the pipette solution. LLDGABA_A was not observed in TAT-pep5 loaded neurons (Fig. 2C, from 2.1 ± 0.7 to 2.5 ± 1 Hz (n = 7, P = 0.12) and from 75 ± 7 to 90 ± 12 pA (n = 7, P = 0.2). Although we cannot completely exclude a diffusion of TAT-pep5 from postsynaptic neuron to presynaptic terminals, these observations suggest that the activation of postsynaptic p75NTR is required for LLDGABA_A.

We next challenged this conclusion and tested the hypothesis that regulated secretion of BDNF may contribute to LLDGABA_A. Because, L-type Ca2+ channels are required for postsynaptic secretion of BDNF and are instrumental for the induction of BDNF-dependent forms of GABAergic synaptic plasticity (Gubellini et al. 2005; Mohajerani et al. 2007; Kuczewski et al. 2008; Sivakumaran et al. 2009), we investigated the effect of the L-type Ca2+ channel blocker nifedipine. Nifedipine (10 μM) prevented the induction of LLDGABA_A (from 2.1 ± 0.4 to 2.2 ± 0.5 Hz, P = 0.2, Fig. 3A; from 69 ± 17 to 87 ± 10 pA, P = 0.09, n = 5, not shown). To determine whether L-type Ca2+ channels activation is responsible for proBDNF secretion, we tested whether the cleavable-resistant form of proBDNF (CR-proBDNF, 10 ng/mL) could bypass the effect of nifedipine. CR-proBDNF applied during activity recovery rescued LLDGABA_A in nifedipine (Fig. 3A, from 4.3 ± 0.9 to 3.4 ± 0.7 Hz, P = 0.04; from 90 ± 27 to 110 ± 24 pA, P = 0.2, n = 6, not shown). The rescue of LL DGABA_A by bath applied CR-proBDNF was not observed in p75NTR function-blocking antibody-treated slices. The frequency and amplitude of sGABA_A-PSCs changed, respectively, from 1.8 ± 0.3 to 2.1 ± 0.4 Hz (P = 0.3) and 90 ± 10 to 110 ± 20 pA (P = 0.2, n = 5, not shown) after the application of CR-proBDNF in p75NTR antibody-treated slices. These results therefore show that a Ca2+-dependent secretion of proBDNF is required to trigger p75NTR-LLDGABA_A.

To deepen the relationship between Ca2+ and LL DGABA_A induction, the Ca2+ chelator BAPTA was added into the pipette solution. LLDGABA_A induction was prevented in BAPTA-loaded neurons (Fig. 3B, from 5.3 ± 0.5 to 5.9 ± 1.2 Hz, P = 0.2 and from 79 ± 9 to 82 ± 13 pA, P = 0.4, n = 9). Although the average change does not reach significance, a long lasting potentiation of sGABA_A-PSCs was observed in 4 of the 9 BAPTA-loaded neurons recorded (from 4.1 ± 1.3 to 7.4 ± 2.9 Hz). This observation suggests that blocking intracellular Ca2+-dependent process may uncover a potentiation of GABAergic synaptic activity (see below).

One possible interpretation of this result is that BAPTA prevents the Ca2+-dependent secretion of proBDNF leading to LLDGABA_A. Another interpretation is that LLDGABA_A induction requires a Ca2+-dependent, but L-type Ca2+ channel-independent, postsynaptic signaling cascade. To address this issue, we examine whether bath applied CR-proBDNF could rescue LLDGABA_A in BAPTA-loaded neurons. CR-proBDNF applied during the washout period had no significant effect on the frequency (Fig. 3B; from 4.5 ± 0.7 to 4.6 ± 0.8 Hz, P = 0.4, n = 6) and amplitude (from 58 ± 8 to 62 ± 8 pA, P = 0.2, n = 6, not shown) of sGABA_A-PSCs. Thus, in addition to the activation of downstream to p75NTR, a postsynaptic Ca2+-dependent signaling cascade is required to induce LLDGABA_A.
whether the activation of AMPA receptors is also required for the induction of LLDGABA-A, the AMPA receptor antagonist NBQX was applied during the washout period. LLDGABA-A was not observed in the presence of NBQX (5 μM) (Fig. 3C, from 3.3 ± 0.5 to 3.7 ± 0.7 Hz, \( P = 0.1, n = 8 \)). However, CR-proBDNF (10 ng/ml) applied during activity recovery rescued LLDGABA-A in NBQX (Fig. 3C, from 3.1 ± 0.4 to 2.5 ± 0.5 Hz, \( P = 0.007, \) from 103 ± 9 to 109 ± 13 pA, \( P = 0.3, n = 6 \)).

Overall, these experiments show that a transient recovery from activity deprivation in aprotinin induces a persistent depression of GABAergic synaptic activity by means of a cascade involving the activation of AMPA receptors, the secretion of proBDNF under the control of L-type Ca\(^{2+}\) channels and a postsynaptic Ca\(^{2+}\)-dependent and p75\(^{NTR}\)-dependent signaling cascade.

**Suppression of the mBDNF-TrkB Signaling Does Not Account for LLDGABA-A Induction**

Activation of p75\(^{NTR}\) has been reported to cause axonal degeneration (Singh et al. 2008) or cell death (Song et al. 2010), at least in part by suppressing Trk receptor-dependent signaling pathways. Because, LLDGABA-A induction requires the activation of TrkB receptors by endogenous BDNF (Kuczewski et al. 2008), we thought to address whether the mBDNF-TrkB receptor signaling is impaired in the presence of aprotinin. We first investigated whether aprotinin may have affected the production of BDNF using qRT-PCR and ELISA analysis. We found no significant differences in the BDNF mRNA (\( n = 4 \) intact hippocampi for each condition, \( P = 0.3 \)) and protein levels (\( n = 4 \) slices for each condition, \( P = 0.7 \)) between control and aprotinin-treated slices (Fig. 4A, B).

We next tested the effect of mBDNF on sGABA\(_A\)-PSCs. Bath applied mBDNF (10 ng/ml) induced a persistent enhancement of sGABA\(_A\)-PSCs frequency and amplitude in the absence (from 3.4 ± 0.9 to 4.3 ± 1.1 Hz, \( P = 0.005 \)) and from 68 ± 5 to 90 ± 8 pA, \( n = 8, P = 0.04 \)) and the presence (from 3.4 ± 1.2 to 4.4 ± 1.5 Hz, \( P = 0.4 \) ± 0.5 Hz, \( n = 7, P = 0.04 \)) of aprotinin (Fig. 4C). We also tested whether exogenous mBDNF (10 ng/ml) could rescue the deficit of LLDGABA-A in aprotinin. In control experiments, sGABA\(_A\)-PSCs frequency and amplitude decreased by, respectively, 25 ± 5% and 10 ± 12% (\( n = 5, 30 \) min after activity recovery in aprotinin (Fig. 4D). When, mBDNF was applied during the recovery period in aprotinin, the frequency and amplitude of sGABA\(_A\)-PSCs increased by, respectively, 13 ± 17% and 35 ± 14% (\( n = 6, \) Fig. 4D). Thus, mBDNF applied during the recovery period in aprotinin led to a significant enhancement of sGABA\(_A\)-PSCs frequency and amplitude when compared with activity recovery in aprotinin alone (\( P = 0.001, \) unpaired t-test).

These results therefore show that the mBDNF-TrkB signaling is not affected by aprotinin.

To confirm this observation, we used the phosphorylated form of the cAMP response element–binding protein (CREB) to monitor TrkB receptor activation by endogenous BDNF (Fiorentino et al. 2009). To normalize the results obtained from different slices, the pCREB to CREB ratio was quantified. Recovery of synaptic activity in control ACSF significantly increased the pCREB/CREB ratio, an effect prevented by TrkB-IgG, but not by TrkA-IgG (Fig. 5). The increase in pCREB/CREB ratio was not observed when activity recovered with aprotinin (Fig. 5). Aprotinin, however, did not abolish the response to exogenous mBDNF (Fig. 5), thus supporting the

In a previous study, we showed that AMPA receptors activation is required to maintain a high level of BDNF secretion through the activation of L-type Ca\(^{2+}\) channels during the washout period (Kuczewski et al. 2008). To determine...
During the recovery period, filled symbol, Aprotinin.

The conclusion that the mBDNF-TrkB signaling is not affected by aprotinin.

Overall, with the observation that the membrane permeable inhibitor of protein tyrosine kinase coupled to Trk receptor family, k252a, does not uncover LLD_{GABA_{A}} (Kuczewski et al. 2008), these data show that the activation of p75_{NTR}, but not the inactivation of the mBDNF-TrkB signaling, is required for LLD_{GABA_{A}} induction in aprotinin.

**ProBDNF-p75_{NTR} Signaling Potentiates GABAergic Synaptic Activity in the Absence of NMDA Receptor Activation**

Since most forms of synaptic plasticity require the activation of NMDA receptors, we investigated the effect of D-AP5 on LLD_{GABA_{A}} induction. We found that activity recovery in D-AP5 and aprotinin led to a long-lasting increase in sGABA_{A}-PSCs frequency and amplitude. The Figure 6A illustrates a typical experiment. A summary of 17 similar experiments showed a significant increase in both frequency (from $2.8 \pm 0.9$ to $4.3 \pm 1.5$ Hz, $n = 17$, $P = 0.01$) and amplitude (from $83 \pm 10$ to $120 \pm 21$ pA, $n = 17$, $P = 0.02$) of sGABA_{A}-PSCs (Fig. 6B, C), with no changes in rise time (from $1.32 \pm 0.09$ to $1.35 \pm 0.1$, $P = 0.4$) and decay time (from $8.5 \pm 0.4$ to $9.2 \pm 0.6$, $P = 0.2$). To determine whether postsynaptic NMDA receptors are involved, the NMDA channel blockers MK801 was added into the pipette solution. On average, the frequency and amplitude of sGABA_{A}-PSCs increased respectively from $4.9 \pm 1.2$ to $5.4 \pm 1.1$ Hz ($P = 0.3$) and from $69 \pm 18$ to $79 \pm 20$ Hz ($P = 0.4$, $n = 9$, data not shown). However, even though the modification was not significant on average, a long-lasting potentiation of sGABA_{A}-PSCs frequency in 5 of the 9 MK801-loaded neurons (from $3.5 \pm 1.3$ to $5.3 \pm 1.6$ Hz, $P = 0.01$, $n = 5$). This result shows that the activation of postsynaptic NMDA receptors is required to induce LLD_{GABA_{A}} and suggests that blockade of these receptors could uncover a long-lasting potentiation of GABAergic synaptic activity.

The enhancement of GABAergic activity induced by activity-recovery in the presence of D-AP5 was not observed in slices preincubated with TAT-pep5 (Fig. 6D, from $3.3 \pm 0.7$ to $3.3 \pm 0.8$ Hz and $85 \pm 11$ to $96 \pm 16$ pA, $n = 7$, $P = 0.1$) or with the p75_{NTR} function-blocking antibody (Fig. 6D, from $2.8 \pm 0.6$ to $3.1 \pm 0.6$ Hz, $P = 0.06$ and $85 \pm 14$ to $87 \pm 10$ pA, $P = 0.2$, $n = 6$). The induction of GABAergic synaptic potentiation was also prevented by intracellular infusion of TAT-pep5 (2 μM) (Fig. 6E, from $2.4 \pm 0.8$ to $2.2 \pm 0.6$ Hz, $P = 0.3$ and $81 \pm 9$ to $85 \pm 8$ pA, $P = 0.2$, $n = 10$). This form of plasticity will be referred to hereafter as p75_{NTR}-LLPGABA-A.

To determine whether regulated secretion of BDNF is required for p75_{NTR}-LLPGABA-A induction, we investigated the effect of the L-type Ca_{2+} channel blocker nifedipine. Nifedipine prevented the induction of p75_{NTR}-LLPGABA-A (Fig. 7A, from $3.1 \pm 0.8$ to $3.2 \pm 0.9$ Hz, $P = 0.3$, from $77 \pm 14$ to $84 \pm 14$ pA, $P = 0.4$, $n = 7$). Moreover, CR-proBDNF applied during activity recovery in D-AP5 and nifedipine rescued the long-lasting potentiation of sGABA_{A}-PSCs (Fig. 7A, from $1.9 \pm 0.5$ to $2.4 \pm 0.4$ Hz, from $73 \pm 5$ to $94 \pm 9$ pA, $P = 0.04$, $n = 8$), an effect prevented by the p75_{NTR} function-blocking antibody (from $2.6 \pm 0.1$ to $2.3 \pm 0.6$ Hz, from $94 \pm 14$ to $115 \pm 20$ pA, $P = 0.1$, $n = 8$, data not shown). Thus, regulated secretion of proBDNF is required to induce p75_{NTR}-LLPGABA-A. Next, we investigated whether the induction of p75_{NTR}-LLPGABA-A requires a postsynaptic Ca_{2+}-dependent signaling cascade by loading the postsynaptic neuron with BAPTA. A long-lasting potentiation of sGABA_{A}-PSCs frequency (Fig. 7B, from $2.0 \pm 0.7$ to...
2.5 ± 0.6 Hz, \( P = 0.05, n = 6 \) and amplitude (from 41 ± 4 to 56 ± 7 pA, \( P = 0.02, n = 6 \), data not shown) were however observed in BAPTA-loaded neurons following activity recovery in aprotinin and D-AP5.

As stated above, aprotinin had no effect per se on the frequency and amplitude of sGABAergic PSCs recorded in the presence of D-AP5 and NBQX (from 2.2 ± 0.9 to 2.3 ± 0.9 Hz, \( P = 0.4 \) and 56 ± 6 to 72 ± 9 pA, \( P = 0.1, n = 5 \)), indicating that p75NTR-LLPGABA-A induction required the activation of AMPA receptors during the recovery of synaptic activity. We therefore asked whether bath applied CR-proBDNF could induce p75NTR-LLPGABA-A in the presence of NBQX and D-AP5. Bath applied CR-proBDNF (10 ng/mL) induced a long-lasting potentiation of GABAergic synaptic activity (Fig. 7C, from 3.1 ± 0.7 to 4.2 ± 0.8 Hz, \( P = 0.004 \), from 83 ± 13 to 109 ± 813 pA, \( P = 0.001, n = 14 \)). We used pCREB to determine whether TrkB-Rs were activated in these conditions and found that neither activity recovery in aprotinin and D-AP5 nor bath applied CR-proBDNF in NBQX and D-AP5 increases the pCREB/CREB ratio (Fig. 5).

Overall, these data show that regulated secretion of proBDNF can signal through p75NTR to either depress or enhance GABAergic synaptic activity (p75NTR-LLGABA-A and p75NTR-LLPGABA-A), with NMDA receptors driving the polarity of the plasticity.

**Discussion**

The major conclusion of the present study is that, in the presence of the serine protease inhibitor aprotinin, spontaneous glutamatergic activity can lead to a p75NTR-dependent long-lasting potentiation or depression of GABAergic synaptic activity in the newborn rat hippocampus. Both forms of synaptic plasticity required a Ca\(^{2+}\)-dependent secretion of proBDNF that plays an instructive role through the activation of postsynaptic p75NTR. The present study also shows that the NMDA receptors drive the switch from potentiation to depression in GABAergic activity (Fig. 8).

**ProBDNF-p75 Signaling Induces a Bidirectional Control of GABAergic Synaptic Activity in the Developing Rat Hippocampus**

BDNF is synthesized as precursor that undergoes proteolytic cleavage to generate the mature form. Whether proBDNF is secreted has been debated (Matsumoto et al. 2008; Yang, Siao, et al. 2009). In the present study, we provided evidences suggesting that a significant proportion of BDNF is secreted as a precursor in the newborn rat hippocampus and signals via p75NTR to induce a bidirectional control of GABAergic synaptic activity. Thus, we found that activity recovery with the cell-impermeable serine-protease inhibitor aprotinin—which among different protease inhibits plasmin activity (Teng et al.
can either depress or enhance the level of GABAergic synaptic activity. Both forms of plasticity rely on proBDNF-p75NTR signaling because they were prevented by blockers of the p75NTR signaling and rescued by bath applied CR-proBDNF, under conditions that prevented the regulated postsynaptic secretion of BDNF (i.e., with nifedipine or NBQX; Lessmann et al. 2003; Kuczewski et al. 2008; Matsuda et al. 2009). Furthermore, the experiments showing that bath applied CR-proBDNF leads to a p75NTR-potentiation or depression of GABAergic synaptic activity when applied, respectively, in the absence (Fig. 3A,C) or the presence (Fig. 7A,C) of the NMDA receptor antagonist D-AP5, clearly exemplified the
bidirectional action of proBDNF-p75NTR signaling on GABAergic synaptic activity. Thus, activity-dependent activation of NMDA receptors can switch the action of the proBDNF-p75NTR pathway on GABAergic synaptic activity from potentiation (p75NTR-LLDGABA-A) to depression (p75NTR-LLPGABA-A).

### Regulated Secretion of BDNF Is Required to Induce GABAergic Synaptic Plasticity

BDNF can be secreted from both the axon and dendrite in a regulated (Ca\(^{2+}\)-dependent) and constitutive (Ca\(^{2+}\)-independent) manner (Lessmann and Brigadski 2009). Previous studies have reported that dendritic release of BDNF is prevented by L-type Ca\(^{2+}\) channel blockers (Kolarow et al. 2007; Matsuda et al. 2009), while axonal BDNF release is prevented by N-type Ca\(^{2+}\) channel blockers (Balkowiec and Katz 2002; Wang et al. 2002; Matsuda et al. 2009). In the present study, the L-type Ca\(^{2+}\) channel blocker nifedipine prevents the induction of both p75NTR-LLDGABA-A and p75NTR-LLPGABA-A, an effect rescued by exogenous application of CR-proBDNF. Along with the finding that GABAergic interneurons do not produce neurotrophins themselves (Ernfors et al. 1990; Gorba and Wahle 1999), this result suggests that a dendritic secretion of proBDNF from the CA3 pyramidal neurons is required to induce p75NTR-LLDGABA-A and p75NTR-LLPGABA-A (Fig. 8). Even if L-type Ca\(^{2+}\) channels are unlikely to be activated in the recorded neuron (the potential was clamped at -70 mV), L-type Ca\(^{2+}\) channel located on the neighboring cells are activated during activity recovery and will contribute to the secretion of BDNF leading to GABAergic synaptic plasticity (Kuczewski et al. 2008). We further show that the AMPA receptor antagonist NBQX prevents the induction of both forms of plasticity and that exogenous application of CR-proBDNF rescued the inhibitory effect of NBQX. These observations suggest that the activation of AMPA receptors during activity recovery is required to trigger the secretion of proBDNF and subsequent GABAergic synaptic plasticity. In agreement with this hypothesis, using ELISA BDNF immunodetection, we have previously shown that BDNF secretion is upregulated during the recovery of AMPA receptors mediated synaptic activity in the developing rat hippocampus (Kuczewski et al. 2008). Moreover, a dendritic AMPA receptor-dependent secretion of BDNF-GFP has been observed following presynaptic tetanic stimulation of glutamatergic terminals in neuronal cultures (Hartmann et al. 2001). Overall, these observations support the notion that a regulated dendritic secretion of proBDNF plays an instructive role in the induction of p75NTR-LLDGABA-A and p75NTR-LLPGABA-A in the developing rat hippocampus (Fig. 8).

### Receptors Location and Underlying Mechanisms

Pre- and or postsynaptic TrkB receptor activation has been reported to up or down regulate the function of GABA receptors, to increase or decrease the number or activity of GABAergic terminals and to modulate the excitability of GABAergic interneurons (Gottmann et al. 2009). The effects of p75NTR activation on the GABAergic circuitry have been less documented (Gascon et al. 2005; Salama-Cohen et al. 2006; Lin et al. 2007). In the present study, TAT-pep5 added in the recording solution prevents the induction of both p75NTR-LLDGABA-A and p75NTR-LLPGABA-A. Although we cannot completely exclude retrograde diffusion of TAT-pep5 from the postsynaptic neuron to the presynaptic terminals, this result suggests that the p75NTR are located on the CA3 pyramidal neurons. This conclusion is supported by previous studies showing that GABAergic interneurons are not equipped with...
postsynaptic p75NTR in the adult hippocampus (Dougherty and Milner 1999; Holm et al. 2009). We also showed that both activity recovery and exogenous application of CR-proBDNF failed to induce LLDPABA in BAPTA-loaded cells. This observation suggests that, in addition to the L type-dependent secretion of proBDNF, a postsynaptic Ca2+ dependent signaling is required to induce LLDPABA. We can only speculate on 3 possible relationships between postsynaptic p75NTR activation and postsynaptic [Ca2+]i: either p75NTR activation induces a rise in [Ca2+]i leading to LLDPABA, or a basal level of [Ca2+]i, is required to allow p75NTR-dependent induction of LLDPABA, or a concomitant activation of postsynaptic p75NTR and Ca2+ influx through NMDA channels triggers LLDPABA. Based on the observation that postsynaptic loading of BAPTA and MK801 led to same outcomes on GABAergic synaptic activity, we may suggest that Ca2+ entering through postsynaptic NMDA receptors is required for the induction of LLDPABA (Fig. 8). Moreover, a potentiation of GABAergic synaptic activity was observed in about 50% of the BAPTA- or MK801-loaded neurons, an effect consistent with the action of D-AP5. This observation may suggest that postsynaptic NMDA receptors and subsequent Ca2+ influx through these channels may regulate the direction of the GABAergic plasticity, although we cannot completely exclude the contribution of NMDA receptors located on other sites (either presynaptic or postsynaptic on neighboring pyramidal cells) in this process.

The downstream signaling pathways involved as well as the mechanisms underlying the expression of GABAergic synaptic plasticity are at present still to be determined. Activation of p75NTR has been reported to cause axonal degeneration (Singh et al. 2008) or cell death (Song et al. 2010), at least in part by suppressing Trk receptor-dependent signaling pathways. However, the observations that proBDNF, applied during the recovery period, could potentiate GABAergic synaptic activity (Fig. 4D), and phosphorylate CREB (Fig. 5) in aprotinin shows that antagonistic interactions between p75NTR and TrkB-Rs are not required for LLDPABA induction. p75NTR form multimeric complexes with different proteins and coreceptors providing a wide range of biological actions depending on the cellular context (Lu et al. 2005). For instance, both promoting (Brann et al. 1999; Gascon et al. 2005) and inhibiting (Yamashita et al. 2002; Zagrebelsky et al. 2005) effects of p75NTR activation have been reported on neurite outgrowth. The modifications of GABAergic synaptic activity observed in the present study could have resulted from modifications in the firing rate of GABAergic interneurons, in the number of functional synaptic connections, or in the probability of GABA release. To date, p75NTR activation has been reported to enhance the dendritic complexity of cultured GABAergic neurons derived from the subventricular zone (Gascon et al. 2005), to increase the number of GABAergic terminals in hippocampal cultures (Salama-Cohen et al. 2006) and to promote the expression of GABAergic neuronal phenotype in the basal forebrain (Lin et al. 2007). Interestingly, in the former study, p75NTR were not located on GABAergic interneurons but on cholinergic neurons, suggesting a noncell autonomous regulation of GABAergic neuron development (Lin et al. 2007). p75NTR activation has also been reported to result in long-lasting depression of synaptic strength coupled with synapse elimination at the neuromuscular junction (Yang, Je, et al. 2009) and CA1-Schaffer collaterals junction (Egashira et al. 2010). Further studies will be required to determine whether similar phenomenon occurs at the GABAergic terminals in the developing rat hippocampus.

**Conclusion**

The present study shows that regulated secretion of proBDNF can exert a bidirectional control of GABAergic synaptic activity in the developing hippocampus. The formation of neuronal circuit is dependent upon spontaneous or sensory-driven synaptic activities determining, for instance, which connections are maintained and which are eliminated. Regulated secretion of BDNF contributes to activity-dependent neuronal network wiring. The general thought is that BDNF signals via TrkB receptors to strengthen synaptic connections and
promote network development. Because, the extracellular conversion of proBDNF can be modulated physiological conditions depending on the level and/or pattern of synaptic activity generated by the neuronal network (Nagappan et al. 2009), our results suggest that p75NTR activation may also fine-tune GABAergic connectivity under physiological conditions, underscoring the importance of determining the mBDNF to proBDNF ratio in different in vivo situations.

Funding
The National Institute of Health and Medical Research (INSERM); the National Center for Scientific Research (CNRS); and the National Agency for Research (ANR; grants R0690AS 2006-2010 and R07066AS 2008-2011).

Notes
We wish to thank Drs L. Aniksztejn, C. Rivera, and I. Medina for critical reading of an earlier version of the manuscript. Conflict of Interest: None declared.

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