Development/Plasticity/Repair

Brain-Derived Neurotrophic Factor Controls Activity-Dependent Maturation of CA1 Synapses by Downregulating Tonic Activation of Presynaptic Kainate Receptors

Marko Sallert,[1,2] Tomi Rantamäki,[1] Aino Vesikansa,[1,2] Heidi Anthoni,[1] Kirsi Harju,[3] Jari Yli-Kauhaluoma,[3] Tomi Taira,[1,2] Eero Castren,[1] and Sari E. Lauri[1,2]

[1]Neuroscience Center, [2]Division of Physiology, Department of Biological and Environmental Sciences, and [3]Division of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Helsinki, FI-00014 Helsinki, Finland

Immature hippocampal synapses express presynaptic kainate receptors (KARs), which tonically inhibit glutamate release. Presynaptic maturation involves activity-dependent downregulation of the tonic KAR activity and consequent increase in release probability; however, the molecular mechanisms underlying this developmental process are unknown. Here, we have investigated whether brain derived neurotrophic factor (BDNF), a secreted protein implicated in developmental plasticity in several areas of the brain, controls presynaptic maturation by regulating KARs.

Application of BDNF in neonate hippocampal slices resulted in increase in synaptic transmission that fully occluded the immature-type KAR activity in area CA1. Conversely, genetic ablation of BDNF was associated with delayed synaptic maturation and persistent presynaptic KAR activity, suggesting a role for endogenous BDNF in the developmental regulation of KAR function. In addition, our data suggests a critical role for BDNF TrkB signaling in fast activity-dependent regulation of KARs. Selective acute inhibition of TrkB receptors using a chemical–genetic approach prevented rapid change in synapse dynamics and loss of tonic KAR activity that is typically seen in response to induction of LTP at immature synapses.

Together, these data show that BDNF–TrkB-dependent maturation of glutamatergic synapses is tightly associated with a loss of endogenous KAR activity. The coordinated action of these two receptor mechanisms has immediate physiological relevance in controlling presynaptic efficacy and transmission dynamics at CA3–CA1 synapses at a stage of development when functional contact already exists but transmission is weak.

Introduction

Formation of functional synapses during development is dependent on endogenous electrical activity of the neuronal networks (Feller, 1999; Zhang and Poo, 2001; Lauri et al., 2003). Neuronal activity is thought to either reinforce or weaken the nascent neuronal connections by using synaptic mechanisms similar to those regulating synaptic efficacy in the adult brain, namely long-term potentiation (LTP) and long-term depression (LTD), and thereby control selection of synaptic inputs to a given neuron. It is well established that Hebbian-type plasticity mechanisms can control the targeting and insertion of glutamate receptors to the postsynaptic density already at very early stages of synapse development (Molnar and Isaac, 2002; Collingridge et al., 2004; Kerchner and Nicoll, 2008; Hanse et al., 2009). However, the corresponding presynaptic processes controlling activity-dependent maturation of glutamate release machinery are less well characterized.

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Correspondence should be addressed to Sari Lauri, Neuroscience Center and Department of Bio- and Environmental Sciences/Physiology, P.O. Box 65 (Viikinkaari 1), University of Helsinki, FI-00014 Helsinki, Finland. E-mail: sari.lauri@helsinki.fi.

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We recently described a novel mechanism by which endogenous activity of kainate-type glutamate receptors (KARs) regulates presynaptic function at immature hippocampal synapses (Lauri et al., 2005, 2006). The tonic KAR activity maintains a low glutamate release probability and renders the immature synapses in area CA1 to preferentially respond to high-frequency input, which represents the predominant mode of activity of CA3 neurons during early postnatal development (Palva et al., 2000). This immature-type, tonic KAR activity in CA3–CA1 is rapidly switched off in response to experimental induction of LTP and gradually lost in parallel with maturation of the circuitry (Lauri et al., 2005, 2006; Sallert et al., 2007). Similar activity-dependent and/or developmental regulation of KARs has also been described in other types of synapses (Kidd et al., 1999; Lauri et al., 2001; Park et al., 2006). However, the molecular signaling mechanisms controlling the switch from immature- to adult-type KAR function is not known.

Neurotrophic factors and in particular the brain derived neurotrophic factor (BDNF), represent attractive candidates coupling neuronal activity to presynaptic maturation. BDNF can be secreted in an activity-dependent manner and has been shown to promote quantal glutamate release (Poo, 2001; Tyler et al., 2002; Bramham and Messaoudi, 2005; Carvalho et al., 2008). The presynaptic action of BDNF supports induction of

LTP in juvenile (Figurov et al., 1996; Gottschalk et al., 1998; Xu et al., 2000; Zakharenko et al., 2003; Gärtner et al., 2006) and neonatal hippocampal slices (Mohajerani et al., 2007) as well as in hippocampal cultures (Shen et al., 2006). Interestingly, the extent of BDNF-induced potentiation of glutamatergic transmission is inversely proportional to the initial synaptic strength and preferentially occurs at immature contacts in cell culture (Lessmann and Heumann, 1998; Berninger et al., 1999; Shen et al., 2006) and is attenuated with age in hippocampal slices (Gottschalk et al., 1998; Kramár et al., 2004), similarly to what has been described for endogenously active KARs (Lauri et al., 2006).

We have here investigated the possibility that BDNF via its TrkB receptor controls presynaptic maturation by regulating KAR function in the hippocampus. Our results show that exogenous BDNF can readily downregulate tonic activity of KARs. Furthermore, data from two different transgenic mouse models supports a key role for endogenous BDNF/TrkB signaling in controlling presynaptic KAR function during development and in response to induction of LTP at immature synapses.

Materials and Methods

Animals. C57BL/6 wild-type (WT), BDNF null mutant (purchased from The Jackson Laboratory) (Ernfors et al., 1994), and *TrkB*^{F616A} knock-in mice (kindly provided by Dr. David Ginty, Johns Hopkins University, Baltimore, MD) (Chen et al., 2005) were used in the experiments. As reported previously, most homozygote mutants lacking BDNF die within 2 weeks after birth, and display severe developmental deficits, e.g., in the control of breathing (Ernfors et al., 1994; Erickson et al., 1996). In *TrkB*^{F616A} mice, the phenylalanine 616 at the ATP binding pocket site of TrkB is mutated to alanine. This mutation renders the receptor susceptible to inhibition by low concentrations of general kinase inhibitors, such as 1NMPP1. This mouse model has been used previously in both *in vitro* and *in vivo* settings to shut down ligand-dependent TrkB receptor activity (Chen et al., 2005; Huang et al., 2008).

Slice preparation. Acute hippocampal slices were prepared from wild-type, *TrkB*^{F616A}, and *BDNF*$^{-/-}$ mice at postnatal day 4 (P4) to P16. Mice were rapidly decapitated, and the brains were placed in ice-cold dissection solution containing the following (in mM): 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$, 26 $NaHCO_3$, 15 D-glucose, and 1 $CaCl_2$ (bubbled with 5% CO_2/95% O_2). Transverse hippocampal slices (400 μm thick) were cut with a vibratome (Vibratome) and placed in a recovery chamber, submerged in solution containing the following (in mM): 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 4 $MgSO_4$, 26 $NaHCO_3$, 15 D-glucose, and 2 $CaCl_2$ (5% CO_2/95% O_2, at room temperature). To prevent recurrent excitation, the CA3 region of the slices was cut away in experiments in which evoked EPSCs were recorded. The slices were used 1–5 h after cutting, except for the experiments in which BDNF was used. For these experiments, after a 30 min recovery time, the slices were placed into Millicell-CM membrane filter (Millipore) with 1 ml of artificial CSF (ACSF) containing (in mM): 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1 $MgSO_4$, 26 $NaHCO_3$, 15 D-glucose, and 2 $CaCl_2$ (5% CO_2/95% O_2 with or without BDNF at 250 ng/ml; provided by Peprotech). Before the slices were transferred into a CO_2 incubator (35°C under 5% CO_2 in air) for 2–3 h, a 10 μl droplet of BDNF final dilution or ACSF was applied on top of each slice. In some experiments, 1NMPP1 (20 nM) was applied 30 min before administration of BDNF.

Synthetic compounds. 1-(1,1-Dimethylethyl)-3-(1-naphthalenylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (1NMPP1) was prepared from 5-amino-1-(1,1-dimethylethyl)-3-(1-naphthalenylmethyl)-1*H*-pyrazole-4-carbonitrile according to the literature (Hanefeld et al., 1996; Bishop et al., 1999) and was purified by SiO_2 column chromatography (eluent toluene/ethyl acetate, 3:1, R_f of 0.2). Yield was 1.02 g (43%), purity was >98% (contains ~1.5% CH_2Cl_2), and melting point was 175–176°C. ^{1}H nuclear magnetic resonance (NMR) (300 MHz, DMSO-d_6) δ (ppm) was as follows: 8.25 (s, 1H), 8.19–8.22 (m, 1H), 7.87–7.91 (m, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.50–7.59 (m, 2H), 7.39 (dd, J = 8.1 and 6.9 Hz, 1H), 7.20 (dd, J = 6.9 and 1.2 Hz, 1H), 4.92 (broad s, 2H), 4.75 (s, 2H), and 1.84 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) was as follows: 158.0, 155.0, 154.7, 140.9, 134.3, 134.2, 132.2, 129.2, 128.5, 126.9, 126.5, 126.1, 125.9, 123.8, 101.5, 60.4, 33.0, and 29.6. Fourier transform infrared values (KBr, cm^{-1}) were 3484, 3303, 1591, 1556, and 1247. Liquid chromatography–mass spectrometry measurement showed $[M+H]^+$ ion at the mass-to-charge ratio of 332. Elemental analysis for $C_{20}H_{21}N_5$ was as follows: calculated C, 72.48%; H, 6.39%; N, 21.13%; found C, 71.25%; H, 6.21%; N, 20.87%.

Western blotting. The time-dependent effect of BDNF on TrkB phosphorylation and the validity of TrkB^{F616A} mouse model (Chen et al., 2005) in slice experiments were confirmed by Western blot. After incubation, the slices were spun down on the bottom of Eppendorf tubes, ACSF was removed, and the sample was immediately frozen in N_2 and stored at −80°C before additional processing. Next, the sample was homogenized in 30 μl of cold NP++ lysis buffer (Rantamäki et al., 2007) and centrifuged (16,100 × *g*, 15 min, +4°C), and the supernatant was collected. An equal amount of protein (100 μg) was subjected to wheat germ agglutinin (WGA) (*Triticum vulgaris*; EY Laboratories) lectin precipitation (25 μl of 50% WGA/NP++ slurry; 2 h at +4°C), followed with washes with NP++ buffer. Next the samples were run in 7.5% SDS-PAGE under reducing conditions and blotted to a polyvinylidene difluoride membrane. TrkB receptor tyrosine phosphorylation was detected by using a polyclonal antibody directed against the phospholipase Cγ1 binding site of the receptor (Y816) (1:5000, overnight at +4°C) using a previously described protocol (Rantamäki et al., 2007).

Electrophysiology. Slices were transferred to a recording chamber in which they were kept submerged and continuously superfused (1 ml/min) with ACSF at 32°C. Whole-cell voltage-clamp recordings (holding potential, −70 mV) were performed from CA1 pyramidal neurons according to standard techniques using a MultiClamp 700B or Axoclamp 200A amplifier (Molecular Devices). Pipette resistance was 4–5 MΩ when filled with intracellular solution containing the following (in mM): 120 $CsMeSO_4$, 10 HEPES, 0.5 EGTA, 10 BAPTA, 4 Mg-ATP, 0.3 Na-GTP, 5 QX-314 [*N*-(2,6-dimethylphenylcarbamoylmethyl)triethylammonium chloride], and 8 NaCl (285 mOsm), pH 7.2. High-resistance electrodes (12–14 MΩ) [filled with 130 mM $CsMeSO_4$, 10 mM HEPES, 0.5 mM EGTA, 5 mM QX-314, 8 mM NaCl, 280 mOsm, pH 7.2, and amphotericin B at 300 μg/ml (Sigma)] were used during perforated patch-clamp recordings. Recordings were started when an access resistance was <120 MΩ. Series resistance was continuously monitored using a 50 ms, 5 mV hyperpolarizing pulse, and it was not allowed to change >20% during the course of the whole-cell and perforated patch experiments. Evoked EPSCs (eEPSCs) were elicited by Schaffer collateral pathway stimulation and recorded from CA1 pyramidal neurons in the presence of blockers of $GABA_A$, $GABA_B$, and NMDA receptors [100 μM picrotoxin (PiTX), 1 μM CGP55845 [(2*S*)-3-[(15)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl)(phenylmethyl)phosphinic acid], and 50 μM D-AP5, respectively], except for LTP experiments, in which only PiTX was included in the ACSF. Baseline stimulation frequency was 1/20 s for single shocks and 1/60 s for collection of the trains at 50 Hz, and the intensity was adjusted to the minimum strength eliciting a stable response (with the exception of experiments in which input–output data was collected). LTP was induced by pairing postsynaptic depolarization (−10 mV) to 10 brief high-frequency trains (five pulses at 50 Hz) of afferent activation. Experiments in which no LTP was induced (EPSC amplitude <120% of control, ~20% of inputs) were excluded from the analysis. Spontaneous miniature EPSCs (mEPSCs) were recorded in the presence of TTX (1 μM), D-AP-5 (50 μM), CGP55845 (1 μM), and PiTX (100 μM). Pharmacological compounds were from Tocris Biosciences, except for LY382884 (3*S*,4a*R*,6*S*,8a*R*)-6-(4-carboxyphenyl)methyl-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid (Eli Lilly & Co.).

Data analysis. WinLTP-program (Anderson and Collingridge, 2007) or pClamp software (Molecular Devices) were used for data acquisition. Evoked synaptic responses were analyzed using the WinLTP program. The amplitude of the evoked synaptic responses was measured as the peak relative to the average baseline level 2–8 ms before the stimulation. Synaptic facilitation was characterized by using short bursts of high-

frequency stimulation (five pulses at 50 Hz), and at least five consecutive trials were averaged for the analysis. Synapses were considered facilitatory when amplitude ratio of fifth/first EPSC was >1.5. mEPSCs were analyzed with the template search algorithm in the Clampfit 9.2 program (Molecular Devices). All the detected events were verified visually, and events with amplitude less than two times the baseline rms noise level were rejected. For time course plots, detected events were calculated in 120 s or 240 bins. Data are presented as percentage from baseline level before drug application or induction of LTP (100% indicates no change). All the pooled data are given as mean ± SEM for the number of cells indicated. Statistical significance was evaluated using Student's two-tailed *t* test or two-way ANOVA. $p < 0.05$ was considered as statistically significant.

Results

BDNF potentiates presynaptic function at immature CA3–CA1 synapses

BDNF has been shown previously to enhance the presynaptic efficacy at several synapses, including hippocampal glutamatergic terminals (Tyler et al., 2002). To test the effect of BDNF on transmission at immature CA1 synapses, hippocampal slices from 4- to 5-d-old mice were preincubated with BDNF (250 ng/ml) in parallel with control slices treated with vehicle only. BDNF treatment led to a robust phosphorylation of the TrkB neurotrophin receptor within 30 min, confirming that BDNF was biologically active in our conditions (Fig. 1*A*). Properties of glutamatergic transmission were analyzed after 2–3 h BDNF treatment by whole-cell patch-clamp recordings from CA1 pyramidal neurons. The average frequency of mEPSCs was clearly higher in BDNF-treated slices (18.1 ± 2.6 events/min; $n = 20$) compared with controls (10.2 ± 2.1 events/min; $n = 23$), whereas no differences in the amplitude (control, 17.4 ± 1.7 pA; BDNF, 18.9 ± 1.3 pA) or kinetics (decay time: control, 7.2 ± 0.4 ms and BDNF, 7.5 ± 0.4 ms; rise time: control, 1.7 ± 0.1 ms and BDNF, 1.7 ± 0.1 ms) of mEPSCs were detected (Fig. 1*B*,*C*). The efficacy and dynamic properties of transmission were further studied by recording evoked EPSCs. The average amplitude of the EPSC in response to afferent stimulation was higher in BDNF-treated slices ($n = 8$) compared with controls ($n = 9$; $p < 0.001$) (Fig. 1*D*,*E*), indicating that BDNF treatment enhanced synaptic efficacy. In addition, the threshold for evoking response was lower in BDNF-treated slices (5.8 ± 0.7 V; $n = 13$) compared with controls (8.3 ± 1.4 V; $n = 17$; $p < 0.05$). The synaptic responses to short bursts

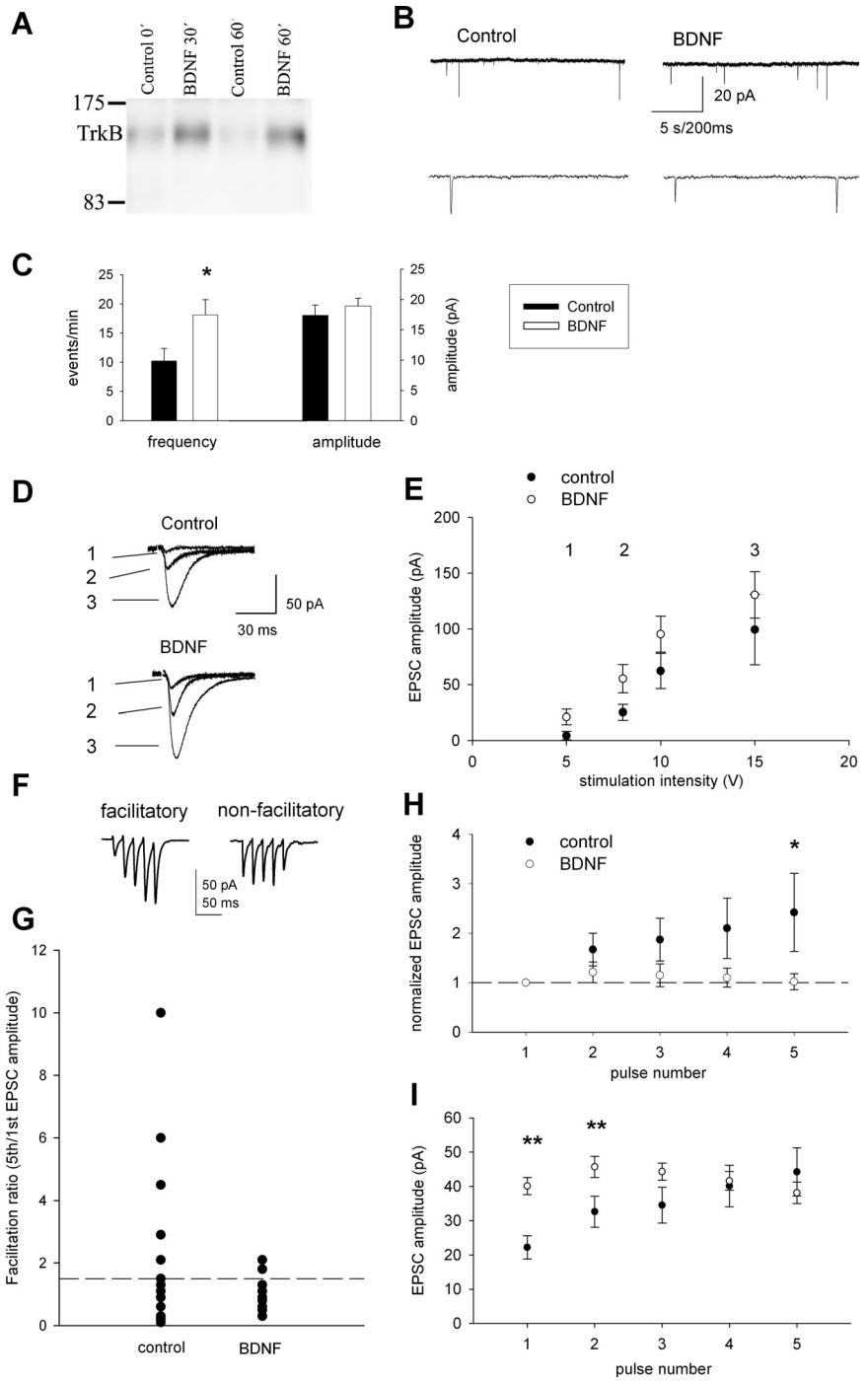

Figure 1. BDNF activates TrkB receptors and enhances presynaptic function in the area CA1 of newborn (P4–P5) mouse hippocampus. ***A***, A Western blot showing robust phosphorylation of TrkB receptors in acute hippocampal slices in response to BDNF treatment (250 ng/ml, 0–60 min) but not in response to 60 min incubation with vehicle alone. ***B***, Examples of recordings of mEPSCs in CA1 pyramidal neurons from control and BDNF-treated slices. ***C***, Pooled data showing higher frequency but no differences in the amplitude of mEPSCs in BDNF-treated slices compared with controls (control, $n = 23$; BDNF, $n = 20$). *$p < 0.05$. ***D***, Example traces of EPSCs evoked by three different stimulation voltages in control and BDNF-treated slices. ***E***, Input–output curves showing that BDNF pretreatment significantly increased the amplitude of EPSCs (control, $n = 9$; BDNF, $n = 8$). $p < 0.001$. ***F***, Example traces showing typical postsynaptic responses to short bursts of high-frequency afferent stimulation (5 pulses at 50 Hz) at immature CA3–CA1 synapses. Input was considered facilitatory when amplitude ratio of fifth/first EPSC was >1.5. ***G***, Synaptic facilitation ratio (fifth EPSC/first EPSC amplitude) at individual inputs in BDNF-treated and in control slices. BDNF pretreatment reduced the amount of facilitatory synapses compared with control (BDNF, 2 of 12 were facilitatory; in control, 6 of 13). ***H***, Averaged data on the effects of BDNF treatment on synaptic facilitation. The graph shows the amplitude of each EPSC in the train, normalized to the amplitude of the first EPSC in control versus BDNF-treated slices (control, $n = 13$; BDNF, $n = 12$). *$p < 0.05$. ***I***, BDNF reduces facilitation by increasing the amplitude of the first eEPSC. The graph shows the same data as in ***H*** without normalization. **$p < 0.01$.

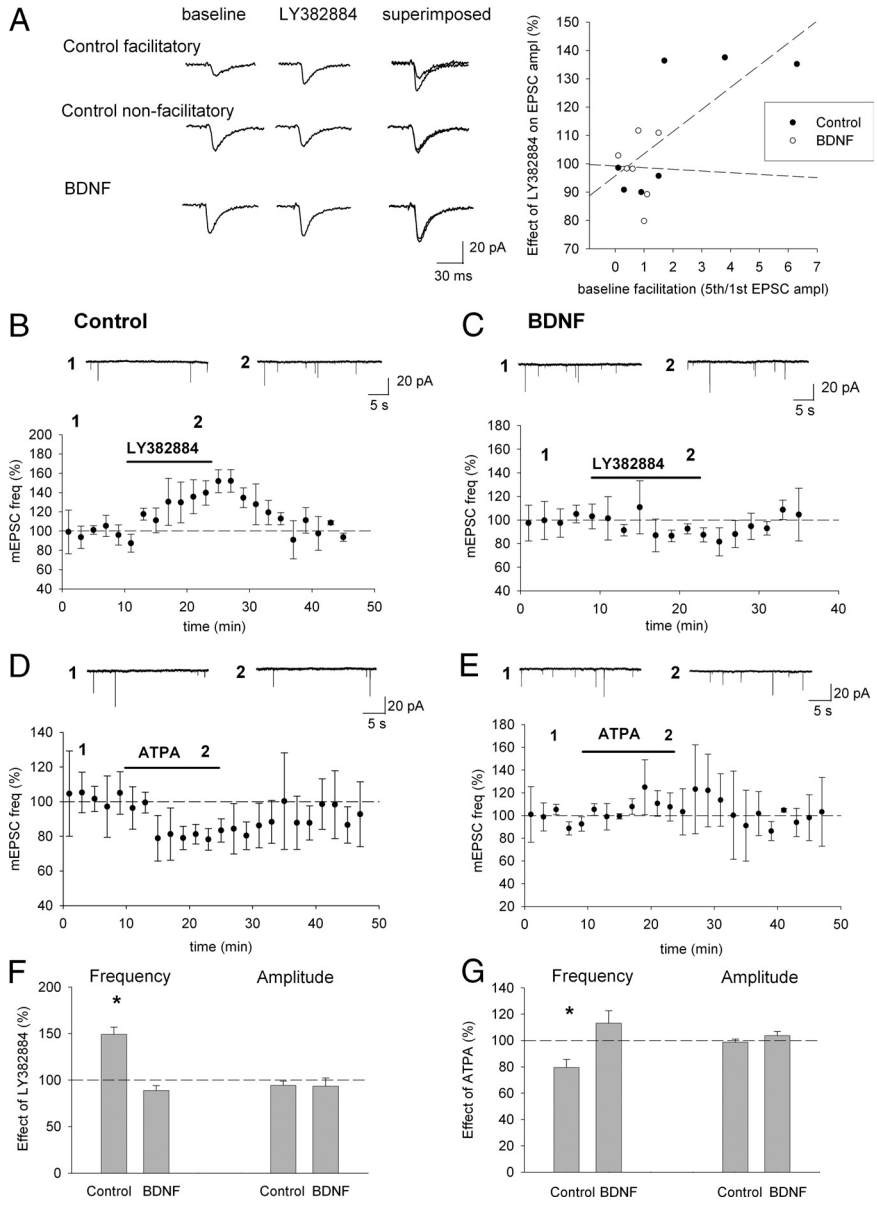

Figure 2. BDNF treatment fully abolishes immature-type KAR activity. ***A***, The effect of LY382884 on evoked EPSC amplitude in control ($n = 7$) and BDNF-treated slices ($n = 7$). Example traces show superimposed EPSCs recorded before and 10 min after application of LY382884, in a facilitatory control input and in nonfacilitatory inputs in control and BDNF-treated slices. The graph shows the effect of LY382884 on EPSC amplitude against the level of facilitation (fifth/first EPSC amplitude in response to 5 pulses at 50 Hz stimulation) at individual experiments. In BDNF-treated slices, no facilitatory inputs were observed. ***B***, Tonic KAR activity in the control slices. Example traces from the time points indicated and averaged data ($n = 6$) showing the effect of LY382884 (10 μM) on mEPSC frequency in control slices (P4–P5). mEPSCs were calculated in 120 s bins and normalized to the baseline level before application of LY382884. ***C***, Corresponding data for BDNF-treated slices ($n = 6$). ***D***, ***E***, Example traces and pooled data showing that ATPA (1 μM) decreases the frequency of mEPSCs in control slices ($n = 5$) but not in BDNF-treated slices ($n = 5$). ***F***, Summary data on the effect of LY382884 on mEPSC frequency and amplitude at control and BDNF-treated slices. *$p < 0.05$. ***G***, Summary data on the effect of ATPA on mEPSC frequency and amplitude at control and BDNF-treated slices. *$p < 0.05$.

(five pulses) of high-frequency (50 Hz) stimulation in the control slices displayed large heterogeneity in short-term plasticity (Fig. 1*F*,*G*), which is typical for immature CA3–CA1 synapses and reflects variability in the glutamate release probability within the synapse population (Hanse and Gustafsson, 2001; Lauri et al., 2006). Interestingly, after BDNF treatment, the facilitatory inputs representing the immature synapses with low initial release probability (P_r) were lost (Fig. 1*G*), and, on average, little or no short-term plasticity in response to 50 Hz stimulation was detected (average ratio of fifth/first EPSC was 242 ± 79 and 102 ± 16% in control and BDNF-treated slices) (Fig. 1*H*). The loss of facilitation was attributable to increase in the amplitude of the first EPSC after BDNF treatment (181 ± 17% compared with control) (Fig. 1*I*). Together, these data suggest that, similar to results obtained in cultured hippocampal neurons (Lessmann and Heumann, 1998), BDNF treatment in neonate CA1 leads to increase in glutamate release probability preferentially at developing synapses with initially low P_r.

BDNF downregulates immature type presynaptic KAR activity

To test whether BDNF was able to regulate KAR activity at CA3–CA1 synapses, we used KAR antagonist LY382884 (10 μM), which has been shown previously to potentiate transmission selectively at immature synapses that express tonically active presynaptic KARs (Lauri et al., 2006) and are characterized with a facilitatory response to 50 Hz stimulation.

As expected, in control slices, LY382884 potentiated transmission selectively at facilitatory inputs (EPSC amplitude, 136 ± 6% of control; $n = 3$) but had no effect on transmission at nonfacilitatory inputs (94 ± 2%; $n = 4$). After BDNF treatment, none of the tested inputs were facilitatory, and LY382884 had no detectable effects on EPSC amplitude (99 ± 4%; $n = 7$) (Fig. 2*A*).

Because LY382884 has no effect on transmission at mature synapses, the magnitude of the effect of LY388284 on mEPSC frequency can be used as a tool to assess the presence of immature-type, tonic KAR activity in a synapse population. At control slices, application of LY382884 increased the frequency of mEPSCs to 149 ± 8% ($n = 6$) of the baseline level without affecting their amplitude (93 ± 9%) (Fig. 2*B*,*F*). In BDNF-treated slices, however, LY382884 had no effect on mEPSCs or caused small reduction in their frequency (89 ± 5%; $n = 6$) (Fig. 2*C*,*F*). Because the baseline frequency of mEPSCs is higher after BDNF treatment, it is possible that the lack of effect of LY382884 is attributable to saturation of P_r rather than loss of tonic KARs activity. Therefore, we also tested the effect of KAR agonist (*RS*)-2-amino-3-(3-hydroxy-5- tertbutylisoxazol-4-yl)propanoic acid (ATPA), which at 1 μM concentration causes a small but significant depression of mEPSC frequency in the immature but not 2-week-old CA1 (Lauri et al., 2006; Sallert et al., 2007). Consistently, in the control slices, mEPSC frequency decreased to 79 ± 6% ($n = 5$) of control, with no associated changes in amplitude (99 ± 2%), in response to ATPA application (Fig. 2*D*,*G*). In BDNF-treated slices, this effect was completely lost, and, if anything, ATPA resulted in a slight increase in the frequency of mEPSCs (113 ± 9%;

$n = 5$) (Fig. 2*E*,*G*). Thus, in response to BDNF treatment, the immature-type presynaptic KAR activity is completely lost in parallel with the disappearance of inputs with a low glutamate release probability.

Lack of endogenous BDNF perturbs early functional maturation of CA3–CA1 synapses and prevents the developmental switch in KAR function

Given the strong effects of BDNF on presynaptic function and KAR activity at immature synapses, we next looked at the role of endogenous BDNF in the maturation of glutamatergic synapses using $BDNF^{-/-}$ mice. The average mEPSC frequency in CA1 pyramidal neurons in the $BDNF^{-/-}$ mice (4.5 ± 1.8 events/min; $n = 12$) was significantly less compared with WT controls (9.1 ± 2.8; $n = 11$) already at P4 (Fig. 3*A*,*B*). Both in the WT and $BDNF^{-/-}$, the average frequency of mEPSCs increased during the first postnatal week (59 and 53% increase from P4 to P7 for WT and $BDNF^{-/-}$, respectively); however, mEPSC frequency remained lower in the $BDNF^{-/-}$ mice throughout the 10 d of life (Fig. 3*A*,*B*). In contrast, no significant differences in the mean amplitude of mEPSCs were detected between the genotypes at any developmental stage tested (Fig. 3*A*,*C*).

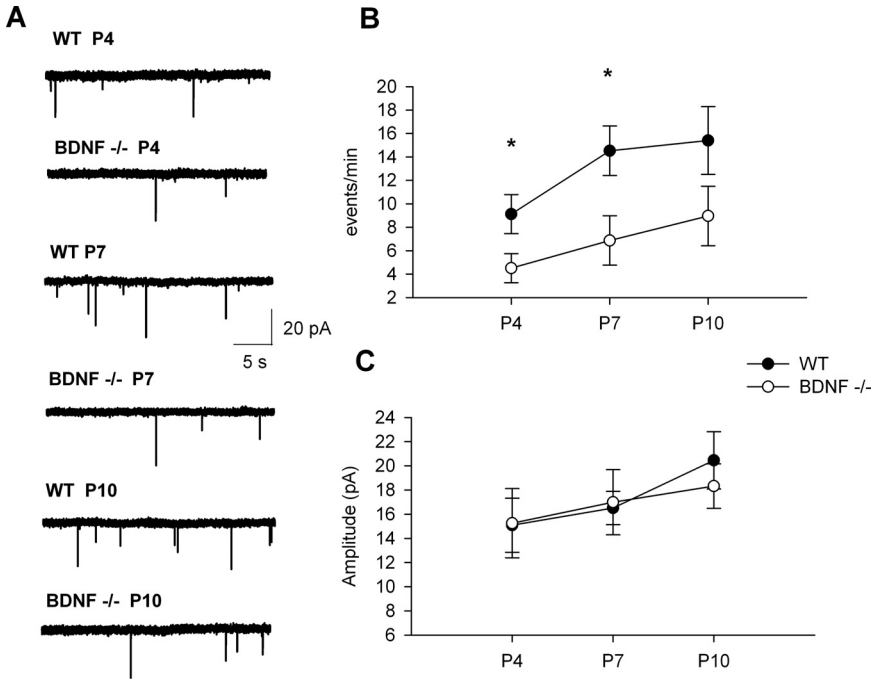

Figure 3. Perturbed development of glutamatergic input to CA1 pyramidal neurons in the absence of endogenous BDNF. ***A***, Example traces from recordings of mEPSCs from wild-type and $BDNF^{-/-}$ mice at different ages. ***B***, Pooled data showing that mEPSC frequency is significantly higher in wild-type than in $BDNF^{-/-}$ mice at P4 (WT, $n = 11$; $BDNF^{-/-}$, $n = 12$) and P7 (WT, $n = 12$; $BDNF^{-/-}$, $n = 11$). At P10, the difference in mEPSC frequency between the genotypes is not significant (WT, $n = 10$; $BDNF^{-/-}$, $n = 11$). *$p < 0.05$. ***C***, Analysis of mEPSC amplitudes from the same data as in ***B***. No significant differences were detected in amplitude between WT and $BDNF^{-/-}$ mice at any of the developmental stages studied.

We next studied the tonic KAR activity in the $BDNF^{-/-}$ mice by testing the sensitivity of transmission to KAR antagonist LY382884. In WT animals, the effect of LY382884 on mEPSC frequency was developmentally downregulated in parallel with maturation of the synapses, from 141 ± 16% ($n = 7$) at P4–P5 to 123 ± 9% ($n = 15$) at P7–P10 and to 97 ± 5% ($n = 5$) at P14–P16 (Fig. 4*A*,*B*,*C*,*F*). In contrast, in the $BDNF^{-/-}$ mice, the KAR activity at P7–P10 remained in the same level as in P4 (140 ± 12%, $n = 8$ at P4; 147 ± 11%, $n = 16$ at P7–P10) (Fig. 4*D*–*F*). Thus, presynaptic maturation and the developmental downregulation of KAR activity does not occur or is significantly delayed in the absence of BDNF.

Selective inhibition of TrkB signaling prevents rapid changes in synapse dynamics and in presynaptic KAR function in response to induction of LTP

A rapid change in both dynamic properties of transmission as well as in tonic KAR activity can be seen in response to pairing-induced LTP at the low P_r facilitatory synapses in the neonate CA1 (Lauri et al., 2006). This mechanism is thought to mimic the activity-dependent maturation process that gradually occurs in the synapse population during development. Hence, we went on to test whether BDNF signaling is involved in the presynaptic changes associated with LTP at immature synapses. Because the glutamatergic transmission was severely perturbed in the $BDNF^{-/-}$ mice, we here used another mouse model, $TrkB^{F616A}$, in which a single point mutation in the TrkB receptor renders it highly susceptible for inhibition by a chemical substance called 1NMPP1 (Chen et al., 2005). We first controlled that application of 1NMPP1 completely blocked BDNF-induced TrkB phosphorylation in hippocampal slices from P5 $TrkB^{F616A}$ mice, whereas a robust phosphorylation was seen in the absence of the drug (Fig. 5*A*).

Perforated patch-clamp recordings were made from CA1 pyramidal neurons to be able to reliably induce pairing LTP in the immature CA1. Similar to what has been shown before in neonate rat slices (Lauri et al., 2006), pairing of short bursts (five pulses at 50 Hz) of afferent stimulation to postsynaptic depolarization induced potentiation of EPSC amplitudes in the $TrkB^{F616A}$ mice (186 ± 10%; $n = 20$) (Fig. 5*B*). The level of potentiation 15–20 min after pairing in the presence of the $TrkB^{F616A}$ inhibitor 1NMPP1 was not significantly different from that seen under control conditions (1NMPP1; 185 ± 9%, $n = 22$) (Fig. 5*C*). Also, controls were made to ensure that application of 1NMPP1 had no effect on pairing-induced potentiation in WT mice (supplemental figure, available at www.jneurosci.org as supplemental material). These data indicate that TrkB signaling is not required for pairing-induced synaptic potentiation at immature CA1.

Under control conditions, pairing in the $TrkB^{F616A}$ mice was associated with a loss of sensitivity to KAR antagonist selectively at the inputs that were initially facilitatory. Thus, at naive inputs, LY382884 increased EPSC amplitude in a manner that correlates with the baseline level of facilitation, and this effect was lost after pairing. In contrast, application of LY382884 still enhanced EPSC amplitude at facilitatory inputs if pairing was done in the presence of 1NMPP1 (Fig. 5*D*,*E*).

Furthermore, at the inputs that were initially facilitatory, pairing under control conditions was associated with a complete loss of synaptic facilitation (amplitude ratio of fifth/first EPSC; from 2.20 ± 0.13 to 0.98 ± 0.26; $n = 9$) and its sensitivity to LY382884 (Fig. 6*A*,*B*). No change in the synapse dynamics were seen in the nonfacilitatory inputs either after pairing or in response to

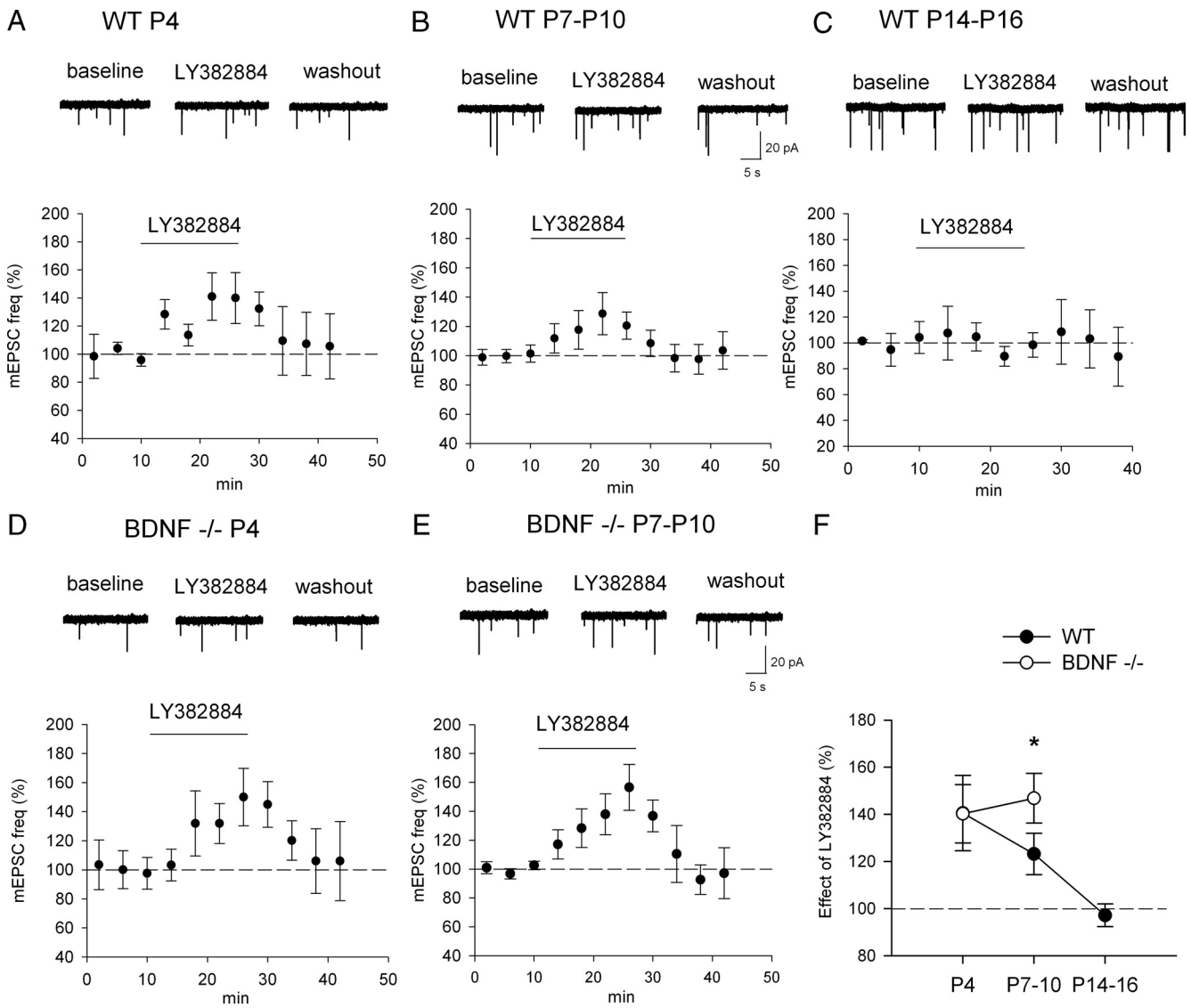

Figure 4. The immature-type, tonic KAR activity is persists during development in the absence of BDNF. ***A–C***, Example traces and averaged data on the effect of LY382884 on mEPSC frequency in WT mice at P4 ($n = 7$), P7–P10 ($n = 15$) (***B***), and P14–P16 ($n = 5$) (***C***). mEPSCs were calculated in 240 s bins. ***D***, ***E***, Corresponding data for $BDNF^{-/-}$ mice at P4 ($n = 8$) and P7–P10 ($n = 16$). ***F***, Summary graph showing the developmental profile of the effect of LY382884 on mEPSC frequency in WT and $BDNF^{-/-}$ mice. In the WT mice, the effect of LY382884 on mEPSC frequency is gradually downregulated during development and lost at P14. At $BDNF^{-/-}$ mice, the effect of LY382884 at P7 is retained at the same level as in P4. *$p < 0.05$ between the genotypes.

application of LY382884 (Fig. 6*A*,*B*). However, LTP induction in the presence of 1NMPP1 was not associated with changes in short-term plasticity; in contrast to control conditions, the facilitatory inputs remained facilitatory after pairing (from 2.28 ± 0.41 to 2.15 ± 0.42; $n = 9$), and this facilitation was completely blocked by application of LY382884 (from 2.15 ± 0.42 to 1.16 ± 0.23; $n = 9$) (Fig. 6*C*).

The loss of the tonic inhibition of glutamate release by KARs in response to pairing influences the level of LTP at the facilitatory inputs (Lauri et al., 2006). Consistently, pairing-induced potentiation at the subpopulation of facilitatory inputs was smaller in the presence of 1NMPP1 (179 ± 17%; $n = 10$) compared with control (192 ± 16%; $n = 11$; data not shown).

Thus, fast activity-dependent changes in synapse dynamics and in presynaptic KAR activity failed in the absence of TrkB signaling. These data strongly suggest that, although not necessary for pairing-induced synaptic potentiation per se, BDNF–TrkB signaling is critical for the presynaptic alterations, including downregulation of KAR activity, that are typically associated with LTP at immature synapses.

Discussion

Although the mechanisms by which BNDF–TrkB-initiated signaling influence presynaptic function have been widely studied, little is known about how these mechanisms interact with the function of other presynaptic receptors. Our data describe a previously unexplored mechanism by which BDNF–TrkB signaling controls presynaptic efficacy by regulating the function of kainate receptors. By detailed characterization of the developmental effects of both exogenous and endogenous BDNF on glutamatergic transmission in hippocampal slices, we show that BDNF-dependent synaptic maturation is tightly associated with a loss of endogenous activity of presynaptic KARs. Furthermore, our results show that the BDNF–TrkB signaling and the associated

change in presynaptic KAR function is critical in controlling fast changes in synapse dynamics that rapidly occur at immature synapses in response to induction LTP.

Presynaptic development, BDNF, and KARs

The mechanisms by which BDNF regulates synaptic transmission depend on the type and developmental stage of the synapse and include direct and indirect effects on ion channel activity and on presynaptic release machinery (for review, see Poo, 2001; Tyler et al., 2002; Rose et al., 2004; Carvalho et al., 2008). At immature CA3–CA1 synapses, the predominant effect of BDNF application was to enhance glutamate release. Thus, in acute slices from 4- to 5-d-old mice, BDNF treatment resulted in a loss of synaptic facilitation as well as increase in the frequency, but no change in the amplitude or kinetics of mEPSCs. These functional changes can be fully explained by BDNF-induced increase in P_r at the developing synapses, a conclusion that is also supported by previous studies in the area CA1 (Tyler et al., 2001, 2006; Mohajerani et al., 2007). However, we cannot exclude the possibility that other mechanisms, for example, BDNF-dependent increase in the number of synapses, occur in parallel and contribute to the observed effects.

Glutamate release probability at central neurons can be rapidly and strongly regulated by presynaptic autoreceptors and heteroreceptors, including kainate receptors (Pinheiro and Mulle, 2008). At immature CA3–CA1 synapses, KAR activity tonically restrains presynaptic function (Lauri et al., 2006) and thus offers a potent target for regulation of synaptic efficacy. Interestingly, BDNF treatment fully occluded the mechanism by which KARs regulate mEPSCs at immature CA1 synapses. Therefore, BDNF acted on the undeveloped synapse population to mimic the developmental process of presynaptic maturation, in terms of increasing P_r and downregulating the KAR activity.

The link between BDNF, synaptic maturation, and KAR activity is further supported by delayed presynaptic development in BDNF knock-out mice. In the absence of BDNF, functional glutamatergic input to CA1 pyramidal neurons was severely impaired at all developmental stages tested during the first 2 postnatal weeks, observed as a lower mEPSC frequency in $BDNF^{-/-}$ mice compared with WT controls. In both genotypes, the frequency of mEPSCs significantly increased from P4 to P10, indicating that functional glutamatergic input to CA1 was developmentally strengthened also in the absence of BDNF. However, in contrast to wild types, the CA1 synapses in the $BDNF^{-/-}$ mice remained immature in terms of presynaptic KARs. Thus, in the absence of BDNF, the glutamatergic synapses failed to go through a process of presynaptic maturation that involves downregulation of the endogenous KAR activity.

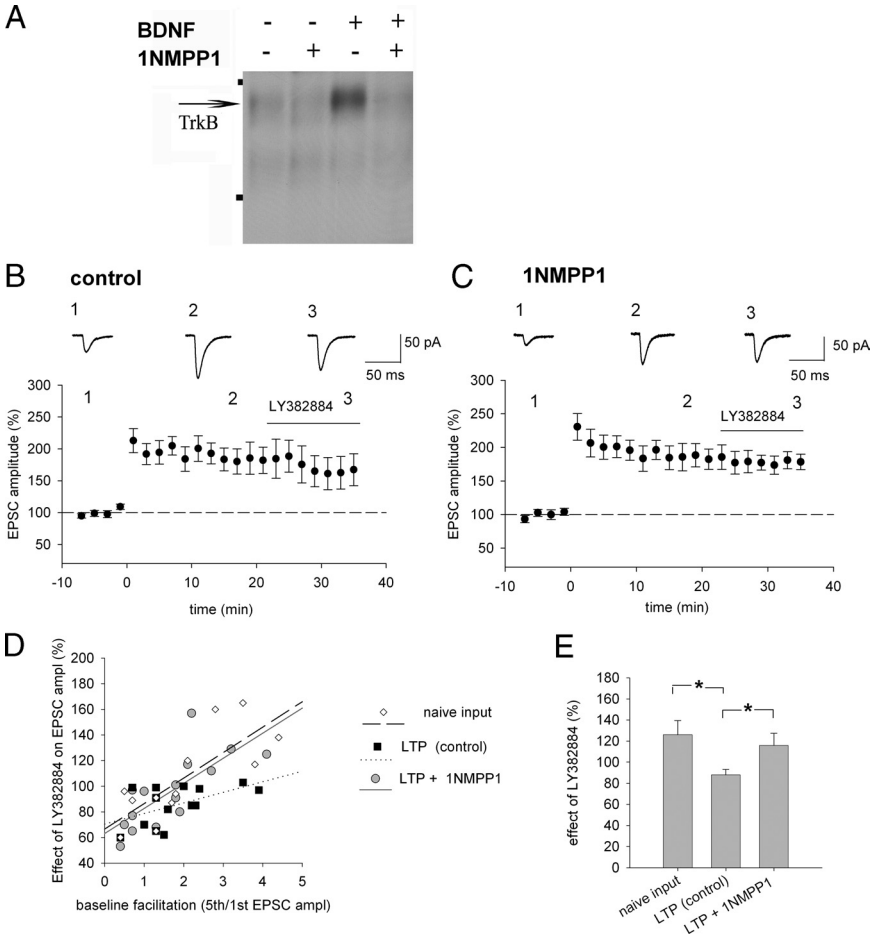

Figure 5. Acute inhibition of TrkB has no effect on induction of LTP but prevents the associated loss of tonic KAR activity. ***A***, Western blot showing that phosphorylation of TrkB receptors in response to BDNF treatment (250 ng/ml, 30 min) of acute hippocampal slices from the $TrkB^{F616A}$ mice (P5) is fully prevented by 1NMPP1. ***B***, Averaged data and example traces from perforated patch-clamp recordings, showing induction of LTP in response to pairing protocol in neonatal $TrkB^{F616A}$ mice ($n = 20$). LY382884 was applied 20 min after pairing to test tonic KAR activity. ***C***, Corresponding data from recordings in the presence of 1NMPP1 ($n = 22$). 1NMPP1 was added to the slices at least 30 min before pairing and was present throughout the recording. ***D***, A graph on the effect of LY382884 on first EPSC amplitude against the baseline (before pairing) level of facilitation in individual experiments. Positive correlation between the effect of LY382884 and the baseline facilitation is seen at naive inputs ($n = 12$) but not after LTP induction under control conditions [LTP (control), $n = 14$]. The correlation persists when LTP is induced in the presence of 1NMPP1 (LTP + 1NMPP1, $n = 15$). ***E***, Averaged data from the initially facilitatory inputs, showing that LY382884 increases EPSC amplitude at naive inputs ($n = 7$), and this effect is lost after LTP induction under control conditions [LTP (control), $n = 9$] but not when LTP is induced in the presence of 1NMPP1 (LTP + 1NMPP1, $n = 9$). $^*p < 0.05$.

Fast activity-dependent effects of TrkB signaling at immature synapses

The proposed mechanism to explain the presynaptic actions of BDNF in the context of Hebbian-type plasticity involves its activity-dependent secretion and binding to presynaptic TrkB receptors, which initiate downstream signaling pathways to modulate glutamate release (Poo, 2001; Tyler et al., 2002). To test whether such mechanism could be involved in the developmental maturation of CA1 synapses, we used a mouse model ($TrkB^{F616A}$) in which TrkB activity can be selectively inhibited by a chemical antagonist.

In contrast to many previous reports on juvenile/adult animals (for review, see Poo, 2001; Bramham and Messaoudi, 2005) (but see Huang et al., 2008), inhibition of TrkB signaling had no significant effect on the average level of potentiation within first

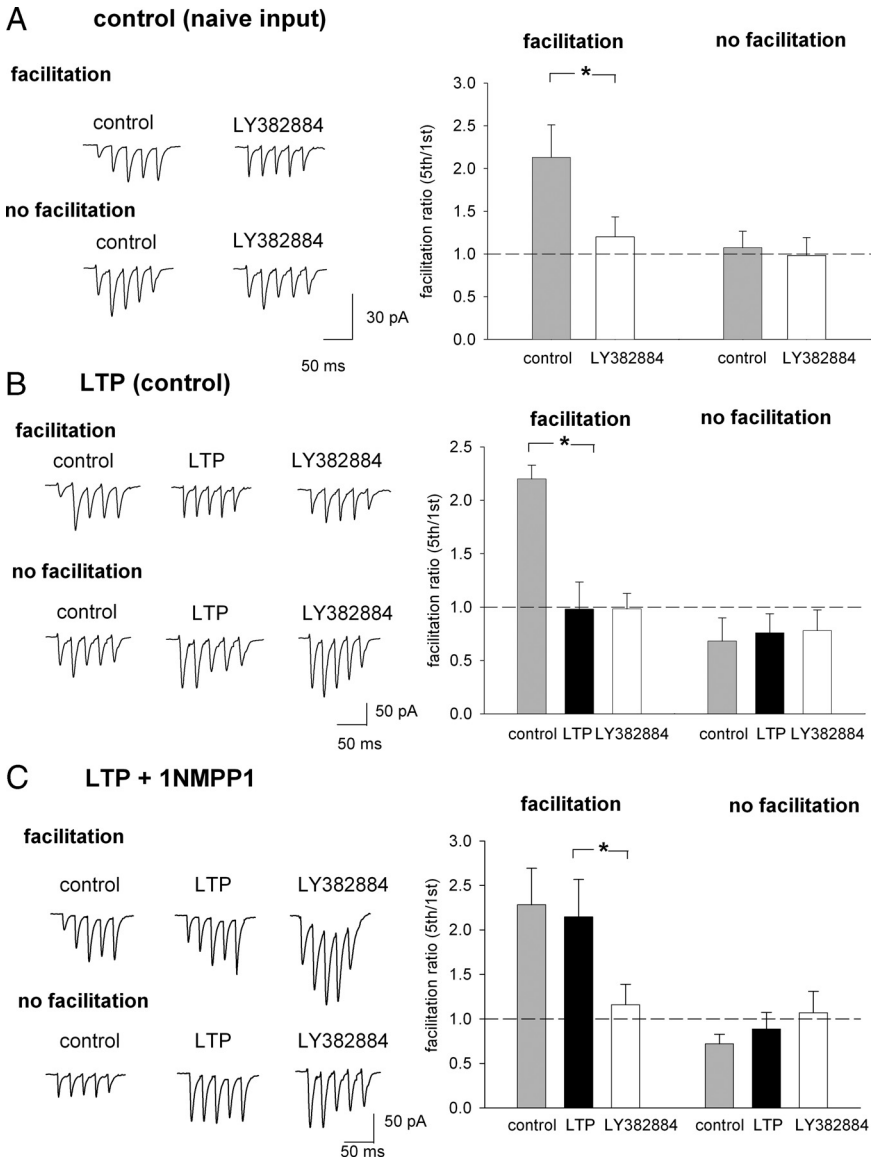

Figure 6. Acute inhibition of TrkB signaling prevents rapid changes in synapse dynamics and KAR activity in response to induction of LTP in the TrkB^{F616A} mice (P4–P5). ***A***, Example traces and pooled data showing the effect of LY382884 on EPSCs evoked at 50 Hz at naive inputs. LY382884 blocks synaptic facilitation and increases the amplitude of first EPSC at inputs that are initially facilitatory ($n = 7$) but has no effect on transmission at nonfacilitatory synapses ($n = 5$). ***B***, Representative traces and pooled data showing the effect of pairing-induced LTP and consequent application of LY382884 on EPSCs, evoked by 50 Hz afferent stimulation. LTP is associated with a complete loss of synaptic facilitation, and LY382884 has no effect on the synaptic facilitation after LTP induction (facilitatory, $n = 9$; nonfacilitatory, $n = 6$). *$p < 0.05$. ***C***, Corresponding data for experiments in which LTP was induced in the presence of 1NMPP1. On average, no change in synaptic facilitation was seen when LTP was induced in the presence of TrkB inhibiting 1NMPP1. Furthermore, the facilitation after LTP induction remained sensitive to inhibition by LY38824 (facilitatory, $n = 9$; nonfacilitatory, $n = 7$). *$p < 0.05$.

20 min after pairing at immature CA3–CA1 synapses. However, acute inhibition of TrkB completely blocked the presynaptic changes, including decrease in frequency-dependent facilitation of EPSCs as well as loss of endogenous KAR activity, that are associated with LTP at immature synapses with initially low P_r (Lauri et al., 2006). This suggests that, in young tissue, TrkB is not necessary for postsynaptic mechanisms underlying induction and early expression of pairing-induced synaptic potentiation, involving insertion and modified function of AMPA receptors at the postsynaptic density (Molnar and Isaac, 2002; Kerchner and Nicoll, 2008; Hanse et al., 2009). Rather, BDNF/TrkB appears critical for presynaptic plasticity at a stage of development when functional contact already exists but transmission is weak.

Interestingly, BDNF/TrkB signaling has been selectively associated with presynaptic LTP that can be induced by strong stimulation protocols at mature/juvenile CA1 (Zakharenko et al., 2003). At this developmental stage, weak stimulation protocols of LTP induction, such as used in the present study, do not produce obvious presynaptic changes at CA3–CA1 synapses (Zakharenko et al., 2001) (M. Sallert, T. Taira, and S. Lauri, unpublished data). This suggests that the mechanism for rapid BDNF/TrkB-dependent presynaptic potentiation is prominent during early development, downregulated in parallel with maturation of the circuitry, but recapitulated in adult/juvenile synapses in response to certain strong stimulation patterns.

Mechanism coupling BDNF–TrkB activation to KARs

Regulation of KAR function in response to patterned activity inducing LTP or LTD has been shown in several areas of the brain. Thus, in addition to immature CA1, plasticity-associated changes in the function of synaptic KARs has been observed at the hippocampal mossy fibers (Lauri et al., 2001)–thalamocortical connections (Kidd and Isaac, 1999) and in the perirhinal cortex (Park et al., 2006). In cell culture, surface expression of KAR subunits is regulated by pharmacological activation of glutamate receptors, including KAR itself (Cho et al., 2003; Martin and Henley, 2004; Martin et al., 2007, 2008; Rivera et al., 2007). Both the surface expression as well as the postsynaptic KAR function is regulated by PKC (Cho et al., 2003; Hirbec et al., 2003; Martin and Henley, 2004; Park et al., 2006; Rivera et al., 2007). However, the corresponding presynaptic mechanisms are not known.

At the immature CA3–CA1 synapses, the activity-dependent changes in the synapse dynamics as well as in the KAR activity occur in the timescale of minutes (15–20 min) after pairing. The temporal window suggests that these TrkB-dependent changes are mediated by local presynaptic signaling cascades. BDNF binding to the TrkB receptor initiates distinct kinase pathways that could lead to parallel or sequential regulation of vesicle release machinery (Tyler et al., 2002) and KAR activity to efficiently enhance P_r at immature synapses. Long-term effects of BDNF involve changes in gene expression and are associated with structural alterations at presynaptic terminals (Tartaglia et al., 2001; Tyler and Pozzo-Miller, 2001; Bramham and Messaoudi, 2005); whether these processes involve additional changes in KAR targeting, function, and/or

expression similar to what has been shown for postsynaptic AMPA- and NMDA-type glutamate receptors (Carvalho et al., 2008) is not known.

Conclusions

In conclusion, our data indicate that BDNF–TrkB signaling represents a potent mechanism that can couple neuronal activity to regulation of KAR function. This mechanism has immediate physiological relevance in controlling presynaptic maturation of CA3–CA1 synapses during early postnatal development. Furthermore, our data sheds light on how distinct activity-dependent signals regulate each other to coordinate the effects of neuronal activity on presynaptic development.

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