Brain-derived neurotrophic factor-dependent unmasking of "silent" synapses in the developing mouse barrel cortex

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Brain-derived neurotrophic factor (BDNF) is a critical modulator of central synaptic functions such as long-term potentiation in the hippocampal and visual cortex. Little is known, however, about its role in the development of excitatory glutamatergic synapses *in vivo*. We investigated the development of *N*-methyl-D-aspartate (NMDA) receptor (NMDAR)-only synapses (silent synapses) and found that silent synapses were prominent in acute thalamocortical brain slices from BDNF knockout mice even after the critical period. These synapses could be partially converted to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)-containing ones by adding back BDNF alone to the slice or fully converted to together with electric stimulation without affecting NMDAR transmission. Electric stimulation alone was ineffective under the BDNF knockout background. Postsynaptically applied TrkB kinase inhibitor or calcium-chelating reagent blocked this conversion. Furthermore, the AMPAR C-terminal peptides essential for interaction with PDZ proteins postsynaptically prevented the unmasking of silent synapses. These results suggest that endogenous BDNF and neuronal activity synergistically activate AMPAR trafficking into synaptic sites.

Neurotrophins had been identified as survival and neuronal differentiation factors in cell and tissue cultures (1) as well as regulatory factors for axonal and dendrite formation (2). Recently, they have been recognized as modulators for synaptic plasticity (3, 4). More direct evidence showed that locally applied brain-derived neurotrophic factor (BDNF) up-regulated postsynaptic Ca^{2+}-transients and synaptic potentiation at dendritic spines (5). However, the role of BDNF on the development of cortical excitatory and inhibitory synapses *in vivo* is uncertain. The existence of *N*-methyl-D-aspartate (NMDA) receptor (NMDAR)-only synapses (silent synapses) early in the critical period and the gradual conversion of these synapses to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (AMPAR)-containing ones have been reported and widely accepted as an important maturational step of glutamatergic synapses (6–8). Neuronal activity is supposed to regulate postsynaptic glutamate receptor trafficking, thus leading to the activation of AMPAR transmission (9). In the present study, we inquired about the role of endogenous BDNF on the development of "silent" synapses and provided evidence that BDNF is crucial for the maturation of AMPAR-mediated transmission in the developing mouse barrel cortex. We found that NMDAR-only synapses dominate in the initial phase of the critical period just after birth, and these synapses change to AMPAR-containing ones at the end of the critical period when examined in the acute thalamocortical brain slices under a whole-cell patch–clamp configuration. This change is mostly blocked in BDNF knockout mice but can be converted to be AMPAR-transmissible by adding back BDNF to the acute slices without affecting NMDAR transmission. Furthermore, the conversion required intact C-terminal amino acid residues of AMPAR subunits, suggesting that BDNF regulates one step of AMPAR trafficking into synaptic sites. Thus, our result showed another role of BDNF in the postsynaptic maturation of NMDAR-only synapses into AMPAR transmissible ones.

Materials and Methods

Animals. A line of BDNF-knockout mice generated in the C57BL/6 genetic background was bred in-house and genotyped (10). The experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of the National Institute of Neuroscience. All experiments using the knockout mice phenotype were analyzed under double-blind conditions with no knowledge of genotype, which was analyzed by someone who had not been informed about the result of phenotype analysis.

Electron Microscopy. Specimens were obtained from four mice at postnatal day (P)8. To avoid developmental heterogeneity among cortical barrels, we chose a particular barrel (C2) and layer four neurons in the C2 hollow region and prepared thin sections (1 μm) from the C2 region. Statistical analysis was performed by using the Mann–Whitney *U* test, and the data were assumed as significant when $P < 0.05$. More detailed information is in *Supporting Methods*, which is published as supporting information on the PNAS web site, www.pnas.org.

Preparation of Slices, Electrophysiology, and Data Analysis. Thalamocortical slices (400–500 μm thick) were prepared from neonatal mice (2–14 days old; the day of birth was defined as P0) by using a rotor slicer, and electrophysiological recording was carried out as described (11). More detailed information is provided in *Supporting Methods*.

Results

Subtle Difference of Synaptic Structure but No Difference of Synaptic Density Among BDNF Genotypes. BDNF regulates both axonal and dendritic morphology (12), thus it could establish neuronal connectivity and synaptic efficacy. In our previous observation during a critical period (10), BDNF and its receptor TrkB were transiently expressed in a barrel-hollow region where thalamic afferents make strong excitatory synapses on cortical neurons (13). BDNF knockout mice, however, did not show any anatomical abnormality examined by cytochrome oxidase and Nissl staining or fluorescent

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Abbreviations: BDNF, brain-derived neurotrophic factor; NMDA, *N*-methyl-D-aspartate; NMDAR, NMDA receptor; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPAR, AMPA receptor; P*n*, postnatal day *n*.

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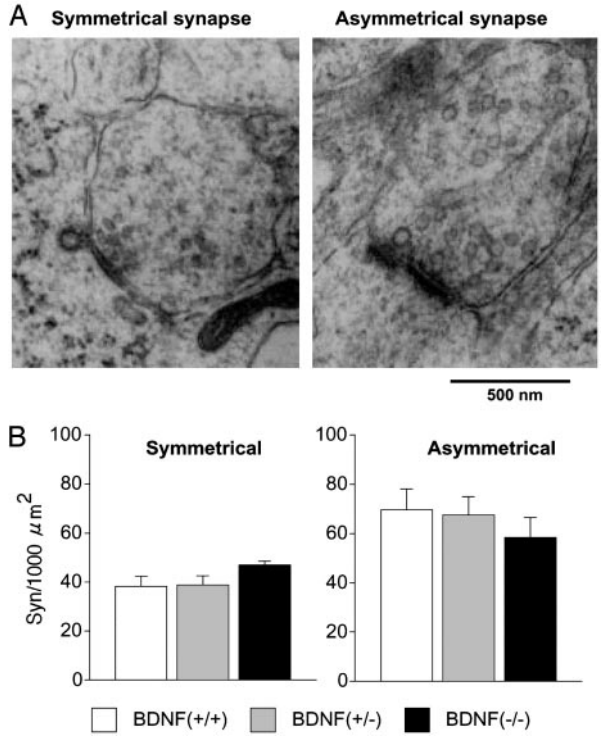

Fig. 1. Synaptic structure in the developing somatosensory cortex in the wild-type mouse at P8. (*A*) (*Left*) Symmetrical synapse. (*Right*) Asymmetrical synapse. (Bar = 500 nm.) (*B*) Comparison of synaptic density (number/1,000 μm^2) of symmetrical and asymmetrical synapses among three genotypes.

dye tracing of thalamocortical projections (10). To see more detailed structural abnormality in the knockout mouse, we carried out electron microscopic analysis of barrel neurons just after the critical period (P8). First we compared the synaptic density among BDNF genotypes. It has been shown that BDNF regulates the central synapse density (14). However, in the barrel cortex, no significant difference of synaptic density was observed, either symmetrical or asymmetrical, among genotypes (Fig. 1). Among asymmetrical synapses, ≈25% are attributed to glutamatergic thalamocortical synapses (13). Next, we measured the synaptic junction length and compared the result among genotypes. Both BDNF(+/−) and BDNF(−/−) showed smaller synaptic contact size compared with wild type (Table 1). In contrast, no significant difference was observed with asymmetrical synapses containing a low number of vesicles in the synapses (Table 1). In hippocampal neurons, the synaptic contact size positively correlated with the number of AMPAR in postsynaptic density (PSD) (15). Thus the shorter synaptic junction length in BDNF knockout mice may suggest less insertion of AMPAR in PSD.

Table 1. Synaptic junction lengths

	Synaptic vesicles (+)	Synaptic vesicles (−)
BDNF(+/+)	373.8 ± 8.4 (256)	290.4 ± 9.1 (146)
BDNF(+/−)	337.3 ± 7.4 (279)*	299.0 ± 8.8 (151)
BDNF(−/−)	343.0 ± 8.1 (228)*	308.5 ± 7.8 (165)

The difference in synaptic junction lengths among BDNF genotypes. The synaptic contact size was estimated as described in *Supporting Methods.* We defined the vesicle containing (+) and not containing (−) synapses according to the number (more than four and less than three, respectively) of synaptic vesicles appeared at the proximal region of postsynaptic density, as shown in Fig. 1*A*. The number in parentheses is the synaptic number examined. The data are shown as average length (nm) ± SEM. Significance was shown by the asterisk when $P < 0.05$ by using the Mann–Whitney *U* test.

Abundance of "Silent" Synapses in BDNF Knockout Mice Even After the Critical Period. Next we examined the normal development of cortical glutamatergic transmission by using acute thalamocortical slices under whole-cell patch recording conditions. At P4, the

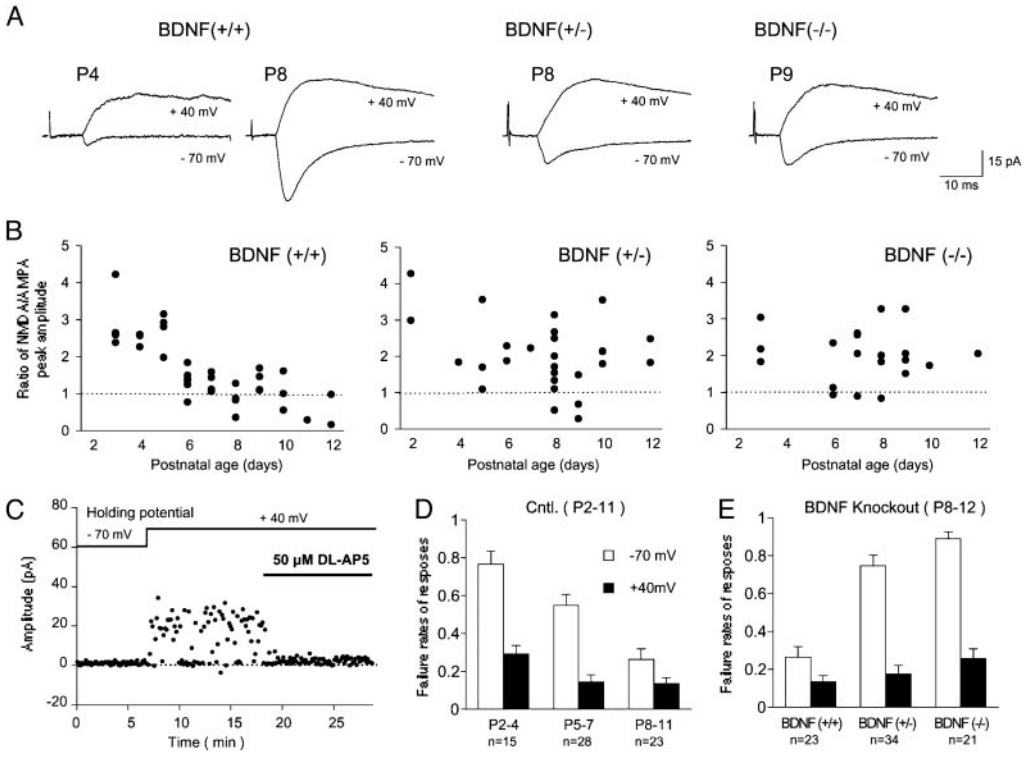

Fig. 2. Higher incidence of silent synapses in BDNF knockout mice compared with wild-type mice during postnatal development. (*A*) Developmental shift of the ratio of NMDAR- to AMPAR-mediated currents in wild-type mice and knockouts. Typical sweeps from P4, P8, or P9 mice are shown. (*B*) The ratio of NMDAR-mediated synaptic peak currents to AMPAR-mediated synaptic peak currents as a function of age among three genotypes. (*C*) An experiment demonstrating the existence of NMDAR-only synapses (P4). (*D*) Decrease in mean failure rates of glutamatergic EPSCs at −70 mV (open bars) and +40 mV (filled bars) holding potentials during postnatal development. Postnatal age (P) and number of experiments (*n*) are indicated below the bars. (*E*) More abundant NMDAR-only synapses in knockouts compared with wild-type mice at P8–P12. Open and filled columns show mean failure rates at −70 mV and 40 mV holding potential, respectively. For raw values, see Table 2.

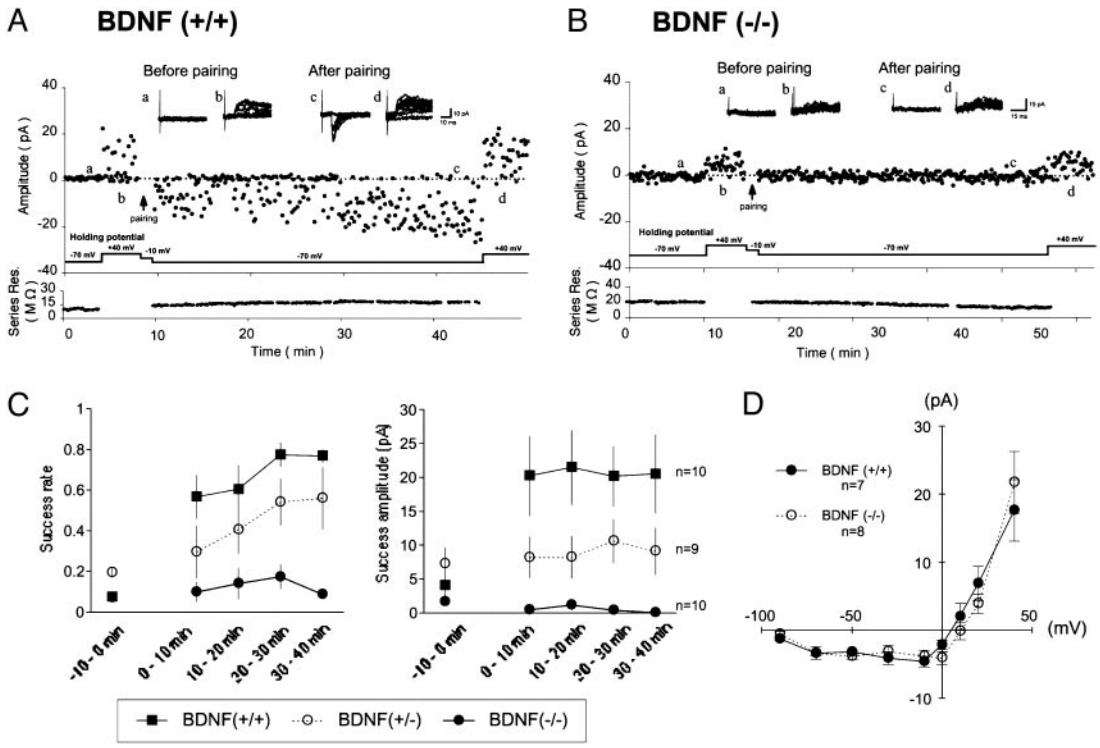

Fig. 3. BDNF requirement for conversion of NMDAR-only synapses. (*A*) A typical example of the conversion of NMDAR-only synapses by pairing stimulation. Black arrow indicates the timing of pairing stimulation. Series resistance (Series Res.) is plotted below. (*Inset*) Overlay of 15 baseline responses before (*a* and *b*) and 30 min after (*c* and *d*) pairing at −70 mV and at +40 mV, respectively. (*B*) The failure of conversion of NMDAR-only synapse in a P8 BDNF(−/−) mouse by pairing stimulation. (*C*) Summary of conversion of NMDAR-only synapses. (*Left*) Mean success rates at –70 mV holding potential before (−10 to 0 min) and after pairing at the specified time window. (*Right*) Mean success amplitude at −70 mV holding potential before and after pairing. For more detailed raw data, see Tables 2 (success rates) and 3 (success amplitudes). (*D*) Normal ratio of NMDAR-mediated currents to AMPAR-mediated currents in the BDNF(−/−) compared with the wild-type mouse.

NMDAR current was prominent, whereas the AMPAR current was minor (Fig. 2*A Left*). At P8, both NMDAR and AMPAR currents were observed. Thus, the relative contribution of NMDAR vs. AMPAR currents decreased during P2–P12 (Fig. 2*B Left*). In contrast, the relative NMDAR currents are still prominent in both BDNF(+/−) and BDNF(−/−) mice even at P8 (Fig. 2*A Center* and *Right*, and Fig. 2*B Center* and *Right*). To see whether this high ratio relates to the silent synapse, we measured the failure rate at minimum stimulation in which synaptic transmission shows failure (for more details, see *Supporting Methods*). A typical example of NMDAR-only transmission from P4 mice is shown in Fig. 2*C*. Synaptic transmission was not observed at −70 mV at all but was detected at +40 mV, which is sensitive to the NMDAR antagonist, AP5. Statistically, the failure rate of AMPAR was high (0.77 ± 0.07, $n = 15$) in P2–P4 and rapidly lowered below 0.26 and P8–P11 (Fig. 2*D*). However, the failure rate of NMDAR was low (0.29 ± 0.05, $n = 15$) even at P2–P4 and decreased below 0.13 at P8–P11 (Fig. 2*D*). This result suggests that NMDAR-only synapses are prominent at the beginning of the critical period (P2–P6), confirming previous observations by other researchers (16). However, the failure rate of AMPAR compared with that of NMDAR is selectively high even after P8 in BDNF knockout mice (Fig. 2*E*). These data suggest that silent synapses are prominent and not converted into active ones in the BDNF knockout mouse.

Impaired Unmasking of "Silent" Synapse in BDNF Knockout Mice. The activation of NMDAR current-only synapses into AMPAR current-containing ones could be induced by a pairing stimulation, as described in *Supporting Methods* (Fig. 3*A*). The failure rate of AMPAR was gradually decreased over 20 min after the pairing stimulation (see also summary data in Fig. 3*C*). The reason for this gradual increment is uncertain. Note that the failure rate as well as the success amplitude of NMDAR did not change before and after pairing stimulation (Fig. 4*C* and Table 2, which is published as supporting information on the PNAS web site). This result indicates that pairing stimulation selectively improved AMPAR transmission in NMDAR-only synapses. The same pairing stimulation, however, could not induce activation of silent synapses in the BDNF(−/−) mouse (Fig. 3*B*). The time courses of improvement of the success rate (one-failure rate) as well as success amplitude were summarized (Fig. 3*C*). In BDNF(+/−), a little improvement in success rate (Fig. 3*C* and Table 2), but not of success amplitude, was statistically significant (Fig. 3*C* and Table 3, which is published as supporting information on the PNAS web site). AP5 blocked the activation of silent synapses in wild-type slices (data not shown), suggesting that NMDAR function is essential for conversion. However, we did not find any difference in current–voltage plot of NMDAR between BDNF(+/+) and BDNF(−/−) mice (Fig. 3*D*). Therefore, the deficit of the activation of NMDAR-only synapses into AMPAR-containing ones should be caused by a signaling mechanism other than NMDAR transmission itself.

Rescue of the Impaired Unmasking of "Silent" Synapses by Exogenous BDNF. If BDNF had a direct role in the conversion of NMDAR current-only synapses into AMPAR current-containing ones, we could expect that the defect should be reversible on adding BDNF back to the acute slices from the knockout mice, which was the case. The acute slices had been preincubated with BDNF for at least 60 min, then BDNF was further included during recording. Thus applied BDNF (20 ng/ml) partially activated NMDAR-only synapses without pairing stimulation (Fig. 4 *A* and *C*). Meanwhile, pairing stimulation fully activated the silent synapses (Fig. 4 *B* and

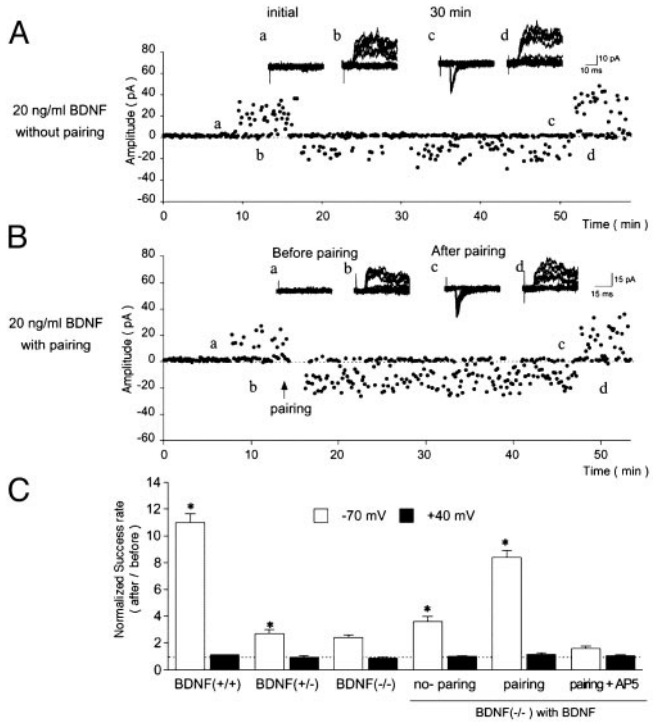

Fig. 4. Rescue of BDNF knockout mouse phenotype by exogenously added BDNF. (*A*) Time course of NMDAR-only synapses recording in the BDNF(−/−) mouse in the presence of exogenous BDNF without pairing stimulation. Slices were preincubated in solution containing 20 ng/ml BDNF before (for at least 1 h) and during experiments. (*Inset*) Overlay of 15 baseline responses, initial (*a*), at −70 mV and 30 min after returning to −70 mV (*c*), and at +40 mV (*b* and *d*), are presented. (*B*) Time course of conversion of NMDAR-only synapses in the BDNF(−/−) mouse with pairing stimulation in the presence of BDNF as described above. (*C*) A summary of rescue experiments showing the normalized success rate at −70 mV. The success rate at +40 mV did not change by pairing stimulation and is normalized as assuming the success rate before pairing to be 1. Only changes in the success rate at 20–30 min after pairing stimulation are presented. Raw values are presented in Tables 2 (success rates) and 3 (success amplitude).

C) and was blocked by AP5 (Fig. 4*C*), indicating that NMDAR-mediated Ca^{2+} transient is required for the BDNF effect. During the rescue experiment, NMDAR transmission did not change in terms of success rate (Fig. 4*C* and Table 2) as well as success amplitude (Table 3), suggesting that the effect of BDNF is selective on the AMPAR transmission at the postsynaptic site. It was reported that BDNF activated NMDAR through shifting the channel into the open state in the hippocampal culture (17). The critical step regulated by BDNF may be different depending on experimental conditions. A 20-min bath application of BDNF during recording was not effective, presumably because of the reluctant penetration of BDNF inside the slice (18). In our case, it needs at least a 60-min preincubation before recording (19).

AMPAR Trafficking as an Essential Step for Unmasking. AMPAR trafficking is supposed to be the molecular basis for the activation of silent synapses in hippocampus neurons (20, 21). To examine a similar possibility in the developing thalamocortical synapse, we postsynaptically applied C-terminal peptides of AMPAR subunits. The C-terminal residues of a long form of GluR1 form a group I PDZ ligand and are essential for interaction with the PDZ-domain regions of SAP97. The nine-residue peptide (GMPLGATGL) of a GluR1 C-terminal tail selectively blocked the activation of NMDAR-only synapses on pairing stimulation without affecting NMDAR transmission (Fig. 5 *A*, *E*, and *F*). In contrast, a mutated peptide (GMPLGAAGL) losing interaction with SAP97 did not block the conversion (Fig. 5 *B*, *E*, and *F*). Similarly, the 10-residue peptide (NVYGIESVKI) of a GluR2 C-terminal tail forms a group II PDZ ligand and is essential for interaction with ABP/GRIP. This peptide blocked conversion when applied postsynaptically (Fig. 5 *C*, *E*, and *F*). The conversion, however, was not blocked by a mutated peptide (NVYGIEAVKI) (Fig. 5 *D–F*), which lost ABP/GRIP binding but still binds with PICK1 (22). This result suggests that the interaction of GluR2R with ABP/GRIP is more important than that of GluR2 with PICK1 to recruit or stabilize the receptor in the synaptic site. The success amplitude of AMPAR was not affected by the mutated peptides (for raw values, see Table 3). The time course of the success amplitude with these peptides is also provided as Fig. 7, which is published as supporting information on the PNAS web site. Taken together, these results suggest that AMPAR subunit recruiting or stabilization into synaptic sites underlies a molecular mechanism for the conversion of NMDAR-only synapses into active ones. To examine any abnormal expression of glutamate receptors and their interacting proteins in knockout mice, we performed Western blotting analysis with a cell extract preparation of P8 barrel cortex using the following antibodies: NR1, NR2A, NR2B, NR2C, GluR1, GluR2/3, NSF, α-actinin, PSD95, SAP102, GRIP, and PICK1. We found no significant deficit (data not shown), consistent with the notion that delivery and/or interaction may be impaired in the absence of proper doses of BDNF.

Critical Role of Postsynaptic TrkB and Ca^{2+} Rise. Next we investigated a possible molecular mechanism of BDNF to regulate AMPAR trafficking into the synaptic site. First, we examined the site of BDNF action by postsynaptically applied K252b, a membrane-impermeable Trk tyrosine kinase inhibitor (ref. 23, but see also ref. 24). At 13 ng/ml, 29 nM, this inhibitor blocked the conversion of NMDAR-only synapses into AMPAR-transmissible ones without affecting NMDAR transmission (Fig. 6*A*). This result suggests that BDNF affects AMPAR trafficking through its postsynaptic receptor. BDNF elicited the postsynaptic Ca^{2+} transients (5, 25), leading to the induction of long-term potentiation (LTP). Thus, we examined a membrane-impermeable Ca^{2+}-chelating agent, 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetate or -tetraacetic acid (BAPTA). BAPTA postsynaptically blocked activation of the silent synapse, suggesting that postsynaptic transient Ca^{2+} is important for AMPAR trafficking. Next, we examined the role of PLC-γ, because among the possible downstream targets of the TrkB receptor, PLC-γ is reported to be essential for induction of LTP (26–28). A selective inhibitor of PLC-γ, U73122, but not the inactive counterpart, U73343, blocked activation (Fig. 6*B*). These results are consistent with the importance of Ca^{2+} transients for AMPAR trafficking. All experiments described above were carried out in the presence of a γ-aminobutyric acid (GABA) type A receptor ($GABA_A$ R) antagonist, bicuculline; thus, we could exclude the possible involvement of polysynaptic inhibition.

Discussion

In this study, we have presented evidence that endogenous BDNF is essential for unmasking "silent" synapses in the developing mouse barrel cortex. BDNF has been shown to regulate multiple steps to form a functional synapse. First, BDNF exerts morphological effect on both axons and dendrites, enhancing the surface area of these structures, thus increasing the number of potential contact sites (permissive effect) (12, 29). Second, BDNF more directly regulates synaptic efficacy through pre- and/or postsynaptic mechanisms (instructive effect) (12, 14, 30, 31). An intensive morphological study of hippocampal synaptogenesis in *trk*B(−/−) and *trk*C(−/−) mice revealed that the topographic patterns of hippocampal connections are normal, but the morphology of axons and dendrites as well as pre- and postsynaptic structures are reduced (14). In the developing barrel cortex, synaptic density is normal, but synaptic contact size is reduced in both BDNF(+/−) and BDNF(−/−) just

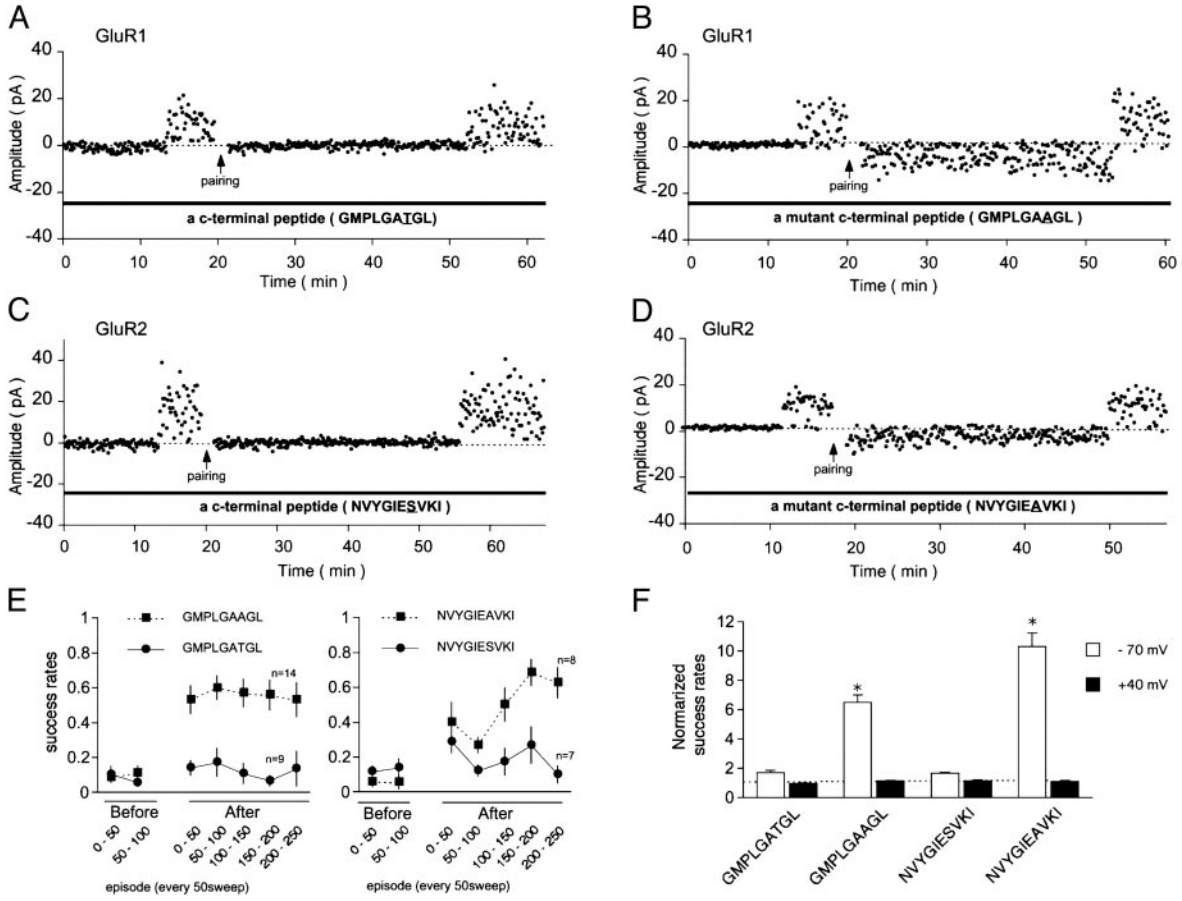

Fig. 5. The blocking of AMPAR transmission by postsynaptically applied C-terminal peptides of GluR1 and -R2 receptors. (*A* and *B*) An effect of a SAP97-binding GluR1 peptide. (*A*) A normal peptide (GMPLGATGL). (*B*) A mutated peptide (GMPLGA*A*GL) losing SAP97 binding activity. (*C* and *D*) Effect of a GRIP-binding GluR2 peptide. (*C*) A normal peptide (NVYGIESVKI). (*D*) A mutated peptide (NVYGIE*A*VKI) losing ABP/GRIP-binding activity. (*E*) Time course of changes in success rates at −70 mV after pairing in the presence of the C-terminal peptides of GluR1 (*Left*) and -R2 (*Right*). (*F*) Normalized success rates of steady-state synaptic AMPAR- (open bar) and NMDAR-mediated responses (filled bar) in neurons injected with the GluR1/GluR2 C-terminal peptides. Normalized success rates show changes in the success rate 20–30 min after pairing stimulation (see Table 2 for success rates, and see Table 3 and Fig. 7 for success amplitudes).

after the critical period (P8). Membrane properties of barrel neurons seemed normal among BDNF genotypes (data not shown). These results suggest that the unmasking of silent synapses by BDNF is instructive rather than permissive, which remains to be further clarified.

We found that activity and BDNF work synergistically to convert NMDAR-only synapses into AMPAR-containing ones. Neuronal activity is thought to locally secrete neurotrophins, which modulate pre- and/or postsynaptic efficacy (32–34). In our analysis, BDNF alone significantly, but not completely, rescued the defect of unmasking silent synapses in BDNF knockout mice in the absence of pairing electric stimulation. Thus, we propose that neuronal activity is required also for a mechanism other than BDNF secretion. Neuronal activity may be required for activation of NMDAR by coincident activation of pre- and postsynaptic sites. If this is so, we wonder why BDNF is required further in the conversion of silent synapses, because BDNF itself induces postsynaptic Ca^{2+} rise (5, 25). A prevailing idea is that the summation of Ca^{2+} concentration above the threshold level is essential for the induction of long-term potentiation (35). However, it should be clarified whether this is the case in the activation of silent synapses. Not only absolute concentration but also a speciotemporal pattern of intracellular Ca^{2+} transients may also be important (36, 37). Alternatively or together with NMDAR activation, neuronal activity may enhance the BDNF effect through other glutamate receptors. Metabotropic glutamate receptor is a candidate, because its function and downstream PLC-β1 are essential for cortical barrel development (38). The kainate receptor, having a relatively long-lasting depolarization, was found in developing thalamocortical synapses, and its activity was developmentally reduced during the critical period (39). Furthermore, the monoamine oxidase mutant (40) as well as the type I adenylate cyclase mutant mouse (41) showed a barrelless phenotype, and interactions between TrkB signaling and serotonin were suggested (42). Considering these findings, we speculate that modulators, including monoamine, catecholamine, and acetylcholine, are gating factors for BDNF potentiation in developing NMDAR-only synapses. In neuromuscular junctions, BDNF and activity synergistically potentiated presynaptic release (43) and the activity-dependent translocation of TrkB may be a molecular basis of this synergism (44). For insight into the possible contribution of presynaptic transmission in our system, we recorded miniature excitatory postsynaptic currents (EPSCs) just after pairing stimulation and compared the result between BDNF(+/+) and BDNF(−/−). Both mEPSC frequency and amplitude at –70 mV did not differ between BDNF(+/+) (n = 7) and BDNF(−/−) (n = 4) (0.13 ± 0.04 and 0.18 ± 0.07 Hz, and 18.4 ± 3.04 and 17.1 ± 4.34 pA, respectively) (For more detailed information, see Fig. 8, which is published as supporting information on the PNAS web site). However, we cannot at this moment exclude the possibility of improvement of presynaptic transmission during or after the conversion of silent synapses. BDNF alone significantly activates silent synapses (Fig. 4*A*). What might the mechanism be? BDNF could

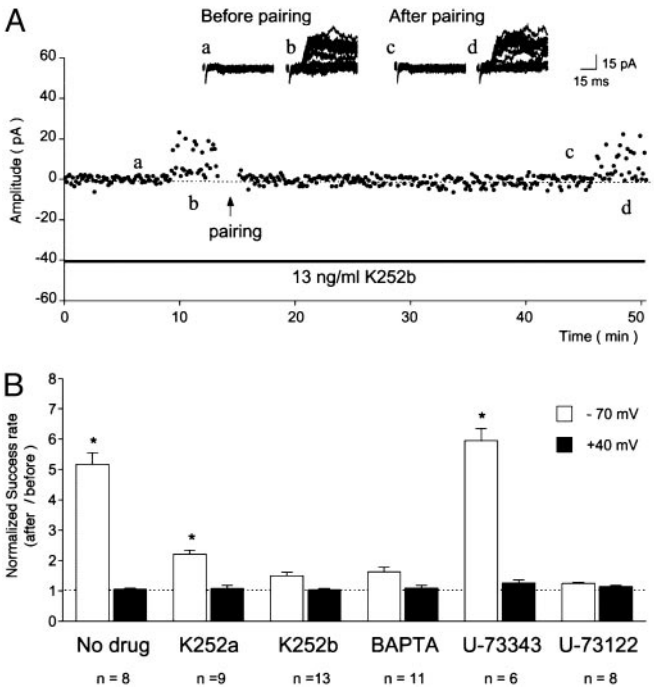

Fig. 6. Postsynaptic TrkB receptor kinase activity and calcium signaling-dependent conversion of NMDAR-only synapses. (*A*) K252b blocked the conversion of NMDAR-only synapses in the P5 wild-type mouse. Responses within a few minutes after forming the whole-cell configuration and after 10 min of loading K252b did not change. (*B*) Summary of the effect of various drugs on the conversion of NMDAR-only synapses in wild-type mice (P3–P6). The relative increment of the success rate is shown as assuming the success rate before pairing to be 1. Success rates were obtained 20–30 min after pairing. Raw values are presented in Tables 2 (success rates) and 3 (success amplitudes).

activate the Na(V)1.9 channel through TrkB kinase activity (45). Another possibility is the induction of Ca^{2+} influx through the activation of a nonselective cation channel (TRPC3) (46), which is an internal store-operated channel. TRPC3 protein is enriched in the central nervous system during embryonic day 18 and P20 in rat brain and colocalized with TrkB.

A molecular mechanism of AMPAR trafficking is now under intensive study in many laboratories, because the regulation of AMPAR-exocytotic and -endocytotic sorting (recycling) is a critical mechanism of synaptic potentiation (9, 12). Many molecules belonging to different functional categories have been studied, including PSD95, Stargazin, NSF-ATPase, ABP/GRIP, PICK1, and ubiquitin-dependent regulators. It is too early, however, to unify the regulatory mechanism of AMPAR recycling. It is of note that a rate-limiting step of AMPAR recycling may be different in depending on a neural subtype and a maturational stage of synapses. Spontaneous activity could drive GluR4-containing AMPAR into silent synapses in cultured hippocampal slices (47), and GluR2/3 replaces GluR4/1 in an activity-independent manner (47). In our case, both GluR1 and -R2 seem to be sorted into synaptic sites in an activity-dependent manner (Fig. 5), although the possibility of nonspecific blocking of PDZ-interacting factors by peptides is not excluded in our experiment (47). The development of excitatory synapses is differently regulated by BDNF in depending on the postsynaptic cell type (12, 48). Excitatory synapses on pyramidal neurons are negatively regulated, whereas inhibitory interneurons are positively regulated by BDNF in cortical cultures (30). In hippocampal cultures, BDNF strengthens excitation primarily by augmenting the amplitude of AMPAR-mediated miniature EPSCs but enhances inhibition by increasing the frequency of miniature inhibitory postsynaptic currents and increasing the size of γ-aminobutyratergic synaptic terminals (31). We found the dose-dependent effect of the BDNF gene in the unmasking efficiency of silent synapses (Fig. 3*C*), which may be related to the difference of the BDNF requirement among postsynaptic neuron subtypes. This point remains to be clarified further.

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