BDNF pro-peptide actions facilitate hippocampal LTD and are altered by the common BDNF polymorphism Val66Met

Toshiyuki Mizui[a,b,1], Yasuyuki Ishikawa[b,c,d,1], Haruko Kumanogoh[a,b,1], Maria Lume[e], Tomoya Matsumoto[b,f], Tomoko Hara[a,b], Shigeto Yamawaki[b,f], Masami Takahashi[b,g], Sadao Shiosaka[b,c], Chiaki Itami[h], Koichi Uegaki[a,b], Mart Saarma[e], and Masami Kojima[a,b,i,2]

[a]Biomedical Research Institute (BMD), National Institute of Advanced Industrial Science and Technology (AIST), Osaka 563-8577, Japan; [b]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Kawaguchi 332-0012, Japan; [c]Laboratory of Functional Neuroscience, Nara Institute of Science and Technology, Nara 630-0192, Japan; [d]Department of Systems Life Engineering Maebashi, Institute of Technology, Gunma 371-0816, Japan; [e]Institute of Biotechnology, University of Helsinki, FIN-00014, Helsinki, Finland; [f]Department of Psychiatry and Neurosciences, Graduate School of Biomedical & Health Sciences, Hiroshima University, Hiroshima 734-8551, Japan; [g]Department of Biochemistry, Kitasato University School of Medicine, Kanagawa 228-8555, Japan; [h]Department of Physiology, Faculty of Medicine, Saitama Medical University, Saitama 350-0495, Japan; and [i]Graduate School of Frontier Bioscience, Osaka University, Suita 565-0871, Japan

Edited by Mu-ming Poo, University of California, Berkeley, CA, and approved May 1, 2015 (received for review November 21, 2014)

Most growth factors are initially synthesized as precursor proteins and subsequently processed into their mature form by proteolytic cleavage, resulting in simultaneous removal of a pro-peptide. However, compared with that of mature form, the biological role of the pro-peptide is poorly understood. Here, we investigated the biological role of the pro-peptide of brain-derived neurotrophic factor (BDNF) and first showed that the pro-peptide is expressed and secreted in hippocampal tissues and cultures, respectively. Interestingly, we found that the BDNF pro-peptide directly facilitates hippocampal long-term depression (LTD), requiring the activation of GluN2B-containing NMDA receptors and the pan-neurotrophin receptor $p75^{NTR}$. The BDNF pro-peptide also enhances NMDA-induced α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor endocytosis, a mechanism crucial for LTD expression. Thus, the BDNF pro-peptide is involved in synaptic plasticity that regulates a mechanism responsible for promoting LTD. The well-known BDNF polymorphism valine for methionine at amino acid position 66 (Val66Met) affects human memory function. Here, the BDNF pro-peptide with Met mutation completely inhibits hippocampal LTD. These findings demonstrate functional roles for the BDNF pro-peptide and a naturally occurring human BDNF polymorphism in hippocampal synaptic depression.

growth factor | pro-peptide | synaptic plasticity | polymorphism | neuron

Growth factors control many cellular functions, including proliferation, differentiation, and cell migration. Most growth factors are initially synthesized as precursor proteins, which are processed into their mature forms by proteolytic cleavage within secretory pathways to exert their biological activities (1). In this study, we focused on brain-derived neurotrophic factor (BDNF) (2–4), which belongs to the neurotrophin family, along with proteins such as nerve growth factor (NGF), neurotrophin-3, and neurotrophin-4 (3, 4). BDNF promotes survival and differentiation of developing neurons, elicits synaptic transmission, and modulates synaptic plasticity in the adult brain (5–7).

As with other growth factors, BDNF is synthesized as a precursor protein, proBDNF (2). The 120 amino acids of the N-terminal fragment, the BDNF pro-peptide, is cleaved from proBDNF to produce biologically active mature BDNF (Fig. 1*A*). A recent report shows that the BDNF pro-peptide is highly expressed compared with proBDNF in the adult brain (8). It was also shown that the expression level of the BDNF pro-peptide increases during postnatal development and plateaus in adult mice (9), and the BDNF pro-peptide is released from neurons in an activity-dependent fashion (9). These reports raise the possibility that the BDNF pro-peptide may be present and function as a secreted protein. Based on these reports, we hypothesized that the BDNF pro-peptide exerts biological functions, beyond assisting in the folding of BDNF as a molecular chaperone (10). This hypothesis is also supported by a finding from our previous study examining an SNP in the *BDNF* gene, Val66Met, in which valine is replaced by methionine at codon 66 in the prodomain of human BDNF (11). In that study, we show that Val66Met mutation affects human memory retention and the activity-dependent secretion of BDNF, indicating that both the prodomain of proBDNF and the BDNF pro-peptide play functional roles. The primary sequence of the BDNF prodomain is conserved among species and differs significantly from that of other neurotrophins, indicating that the BDNF proregion, the pro-peptide, or both may have unique functions. Thus, as is the case for neuropeptides such as β-endorphin (12), the BDNF pro-peptide may serve as a bioactive molecule in neurons.

Recent accumulated evidence demonstrates that BDNF regulates synaptic plasticity and neuronal morphology in the adult

Significance

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that elicits biological effects on synaptic plasticity. BDNF is initially synthesized as precursor proBDNF, and then the BDNF pro-peptide is simultaneously produced from the precursor protein. However, the physiological functions of the pro-peptide are largely unknown. Here, we demonstrate that the BDNF pro-peptide is a facilitator of hippocampal long-term depression (LTD), requiring the activation of GluN2B-containing NMDA-type receptors and the pan-neurotrophin receptor $p75^{NTR}$. Second, a common BDNF polymorphism substitutes valine for methionine at amino acid position 66 (Val66Met) in the pro-peptide of BDNF and impairs memory function. Unexpectedly, the pro-peptide with Met mutation completely inhibits hippocampal LTD. These findings provide insights into the physiological role of the BDNF pro-peptide in the brain.

Author contributions: T. Mizui, Y.I., H.K., M.L., T. Matsumoto, S.Y., M.T., S.S., K.U., M.S., and M.K. designed research; T. Mizui, Y.I., H.K., M.L., T. Matsumoto, T.H., K.U., M.S., and M.K. performed research; T. Mizui, Y.I., H.K., M.L., T. Matsumoto, T.H., C.I., and K.U. contributed new reagents/analytic tools; T. Mizui, Y.I., H.K., M.L., T. Matsumoto, T.H., S.Y., M.T., S.S., K.U., M.S., and M.K. analyzed data; and T. Mizui, Y.I., H.K., M.L., T. Matsumoto, S.Y., M.T., S.S., C.I., K.U., M.S., and M.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

[1]T. Mizui, Y.I., and H.K. contributed equally to this work.

[2]To whom correspondence should be addressed. Email: m-kojima@aist.go.jp.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1422336112/-/DCSupplemental.

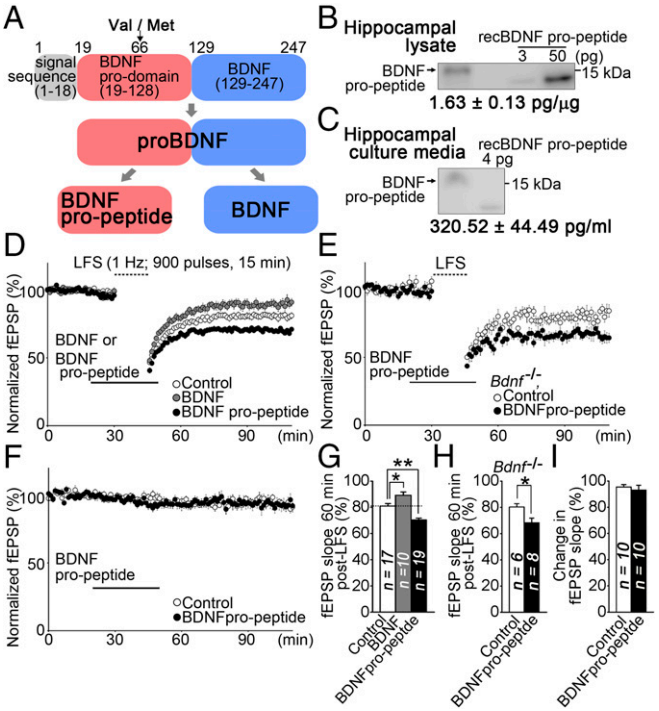

Fig. 1. BDNF pro-peptide is a novel facilitator of hippocampal LTD. (*A*) Schematic representation of the BDNF precursor, BDNF, and pro-peptide. The numbers of amino acids in the signal sequence, the BDNF pro-peptide (BDNF prodomain), and BDNF (mature BDNF) are indicated. The small black arrow designates the location of the Val66Met mutation. (*B*) Detection of the BDNF pro-peptide in hippocampal lysates. Experimental details of *B* and *C* are described in *SI Materials and Methods*. As a positive control, the nonglycosylated recombinant BDNF pro-peptide (rec BDNF pro-peptide) was loaded in these studies and in those shown in *C*. Before immunoblotting, transfer membranes were treated with 2.5% (vol/vol) glutaraldehyde as described in two recent reports (8, 9). Membranes were probed with a monoclonal antibody for the BDNF prodomain (mAb287). The estimated concentration of the BDNF pro-peptide in the hippocampal tissues from 3-wk-old animals is 1.63 ± 0.13 pg/μg total protein (n = 4 animals). (*C*) Detection of the BDNF pro-peptide in the media of cultured hippocampal neurons for 14 d in vitro. The estimated concentration of the BDNF pro-peptide in the supernatants of cultured hippocampal neurons is 320.52 ± 44.49 pg/mL (n = 8 independent cultures). (*D* and *E*) The effect of BDNF pro-peptide on hippocampal LTD. BDNF pro-peptide (10 ng/mL) was applied to hippocampal slices prepared from 3- to 4-wk-old *Bdnf*$^{-/-}$ mice. LTD was induced by low-frequency stimulation (LFS; 1 Hz; 900 pulses, 15 min) to the Schaffer collaterals. The periods of LFS and drug administration are indicated by the broken line and black bar, respectively. In *D*, BDNF (10 ng/mL) was applied for comparison. (*F*) BDNF pro-peptide does not affect basal synaptic transmission. (*G–I*) LTD measured 60 min after LFS application. In this and other panels (Figs. 2 and 4 *A* and *B*), bar graphs depict LTD measured 60 min after LFS application. In each graph, 100% corresponds to the pre-LFS baseline. *G–I* represent data from *D–F*, respectively. The number of slices used (n) is indicated on the corresponding bar in each graph. *P < 0.05; **P < 0.01; Student t test (two groups) or ANOVA with post hoc test (multiple groups). Error bars indicate SEM.

brain (5–7). Thus, BDNF is expressed in neurons of the CNS (2, 13), and BDNF stimulates the tyrosine kinase receptor TrkB, which activates downstream molecules that mediate its biological activities (4). A mutation at the phospholipase Cγ-docking site in the TrkB kinase domain impairs hippocampal long-term potentiation (LTP) (14).

By contrast, the modulation of long-term depression (LTD) by neurotrophins is not as well understood as that of LTP (6, 15). LTD is significantly impaired in KO mice deficient in the pan-neurotrophin receptor p75^{NTR} (15). The p75^{NTR} binds a variety of growth factors, including neurotrophins, and transmits biological signals (16, 17). However, blocking p75^{NTR} with antibodies does not inhibit induction of LTP (18). Recent reports showed that recombinant proBDNF elicited hippocampal LTD through p75^{NTR} (15) and that hippocampal slices prepared from a cleavage-resistant proBDNF knock-in mice enhanced LTD (19). However, less is known about the role of the BDNF pro-peptide in synaptic plasticity.

Here, we demonstrate that the BDNF pro-peptide itself facilitates NMDA receptor-mediated LTD and activates trafficking of AMPA receptors (AMPARs), a synaptic mechanism regulating LTD. Furthermore, both biological activities of this pro-peptide are markedly impaired by the well-known BDNF polymorphism Val66Met. This is the first report, to our knowledge, to demonstrate that the BDNF pro-peptide has biological roles that may be modulated by Val66Met polymorphism.

Results

BDNF Pro-Peptide Facilitates Hippocampal LTD. The BDNF pro-peptide is a portion of proBDNF (Fig. 1*A*). Recently, Dieni et al. demonstrated that the BDNF pro-peptide is endogenously detectable in hippocampal tissues and is located at presynaptic sites (8). More recently, Anastasia et al. reported that the expression of the BDNF pro-peptide increases during postnatal development and plateaus in adult mice and that it is secreted from neuronal cells in an activity-dependent manner (9). They also showed that the enzymatic removal of N-linked glycan moieties resulted in a reduction of the molecular mass of the endogenous BDNF pro-peptide from 15 to 12 kDa (9). In the present study, we detected the BDNF pro-peptide in hippocampal tissues of 3-wk-old WT mice (Fig. 1*B*) and in the supernatants of hippocampal neurons cultured for 14 d (Fig. 1*C*). Consistent with the report of Anastasia et al., the band of the endogenous BDNF pro-peptide was ∼15 kDa (Fig. 1 *B* and *C*), whereas that of the nonglycosylated recombinant BDNF pro-peptide (rec BDNF pro-peptide) was 12 kDa. Moreover, the concentration of the BDNF pro-peptide was determined using the method described by Anastasia et al. (9). The estimated concentrations of the BDNF pro-peptide in the hippocampal lysate and culture medium were 1.63 ± 0.13 pg/μg total protein (n = 4 mice) and 320.52 ± 44.49 pg/mL (n = 8 independent culture dishes), respectively. These results together suggest that the BDNF pro-peptide functions in postnatal stages and raise the possibility that the BDNF pro-peptide modulates synaptic plasticity in the brain.

To test this hypothesis, we chose a paradigm of LTD, which is modulated by BDNF and proBDNF (15, 20). In practice, we applied a sequence of low-frequency stimulation (LFS; 1 Hz, 900 pulses, 15 min) to Schaffer collaterals of hippocampal slices from juvenile mice and then measured field excitatory postsynaptic potential (fEPSP) slopes in the CA1, as previously described (15). The application of LFS led to robust LTD at the CA3–CA1 synapses (Fig. 1*D*, Control, and Fig. S1, Control). As reported previously (15, 20), BDNF treatment (10 ng/mL, 30 min) attenuated LTD (Fig. 1*D*, BDNF, and Fig. S1, BDNF). A statistical analysis revealed that the fEPSP slope values recorded 60 min after LFS stimulation were 80.9 ± 2.0% in the control group and 89.2 ± 2.5% in the BDNF-treated group (Fig. 1*G*; P < 0.05 relative to the control).

Next, we purified recombinant BDNF pro-peptide (Fig. S2, Val) and examined the effect of the pro-peptide on LTD. A 30-min treatment with the BDNF pro-peptide at a subnanomolar concentration (10 ng/mL; approximately 0.8 nM) significantly enhanced LTD in hippocampal slices (Fig. 1*D*, BDNF pro-peptide, and Fig. S1, BDNF pro-peptide). The mean fEPSP slope values recorded 60 min after the application of this protocol were 80.9 ± 2.0% in the control group and 70.4 ± 1.4% in BDNF pro-peptide–treated group (Fig. 1*G*; P < 0.01 relative to the control). Importantly, the application of BDNF pro-peptide (10 ng/mL, 30 min) enhanced LTD in *Bdnf*$^{-/-}$ hippocampal slices, which lack

endogenous expression of BDNF (Fig. 1 *E* and *H*; control, 80.3 ± 3.0%; BDNF pro-peptide, 68.2 ± 4.0%; $P < 0.05$ relative to the control), demonstrating that the BDNF pro-peptide is functional in vivo.

We next investigated whether the BDNF pro-peptide affected basal synaptic transmission in the absence of LFS. First, application of the BDNF pro-peptide had no effect on basal synaptic transmission elicited by test-pulse stimulation throughout the recording period, compared with nonapplied control (Fig. 1 *F* and *I*; control, 95.5 ± 1.8%; BDNF pro-peptide, 93.1 ± 3.6%; $P = 0.9$, between control and BDNF pro-peptide–treated groups). Second, we analyzed input-output (I/O) relationship of the Schaffer collaterals–CA1 pyramidal synapses and did not find significant difference in I/O relationship between before and after a treatment of the BDNF pro-peptide (Fig. S3*A*, Val-BDNF pro-peptide). Third, we tested a presynaptic form of short-term plasticity, paired-pulse facilitation (PPF). The analysis of PPF indicated that there was no significant change in the PPF ratio between before and after treatment of the BDNF pro-peptide (Fig. S3*B*, Val-BDNF pro-peptide).

Thus, these electrophysiological data together suggest that the BDNF pro-peptide is a novel facilitator of hippocampal LTD.

The Role of p75^{NTR} in the BDNF Pro-Peptide–Enhanced LTD. We next sought to explore the mechanisms underlying BDNF pro-peptide–enhanced LTD. Because KO of the pan-neurotrophin receptor p75^{NTR} results in impaired hippocampal LTD (15, 21), we questioned whether p75^{NTR} plays a role in the facilitation of LTD by the BDNF pro-peptide. To address this question, we first investigated whether the REX antibody, a reagent that blocks the function of p75^{NTR}, affects pro-peptide–enhanced hippocampal LTD. Similar to previous reports (15, 18), REX (100 μg/mL) inhibited LFS-induced LTD (Fig. 2 *A* and *G*; control, 80.9 ± 2.0%; REX, 91.6 ± 2. 8%; $P < 0.05$ relative to the control). We also found that the BDNF pro-peptide failed to facilitate LTD in the presence of REX (Fig. 2 *A* and *G*; BDNF pro-peptide, 70.4 ± 1.4%; BDNF pro-peptide+ REX, 93.2 ± 5.6%; $P < 0.01$ between BDNF pro-peptide and BDNF pro-peptide + REX). This result was supported by another experiment using p75$^{NTR-/-}$ (*Ngfr*$^{-/-}$) animals, which lack the gene encoding p75^{NTR}. The treatment of *Ngfr*$^{-/-}$ hippocampal slices with BDNF pro-peptide did not facilitate LTD (Fig. 2 *B* and *H*; control, 83.6 ± 3.0%; BDNF pro-peptide, 84.4 ± 2.0%; $P = 0.8$ relative to the control). We also examined whether the BDNF pro-peptide required the BDNF receptor TrkB to facilitate hippocampal LTD. To address this, we used a pan-Trk receptor inhibitor K252a according to a previous report (20) and found that, in the presence of K252a (200 nM), the BDNF pro-peptide enhanced hippocampal LTD (Fig. 2 *C* and *I*; control, 80.9 ± 2.0%; BDNF pro-peptide, 70.4 ± 1.4%; K252a, 87.8 ± 3.1%; BDNF pro-peptide + K252a, 65.9 ± 3.0%; $P = 0.48$ between BDNF pro-peptide and BDNF pro-peptide + K252a). We further tested the effect of the BDNF pro-peptide on the activity of TrkB. To this end, we applied BDNF or its pro-peptide (10 ng/mL) to 3-wk cultured hippocampal neurons for 5 min and investigated the TrkB phosphorylation levels by Western blotting with anti–phospho-Trk antibody (pY490) as described previously (22). We showed that the BDNF pro-peptide had no apparent effect on TrkB phosphorylation (Fig. S4*A*; BDNF pro-peptide, p-TrkB and TrkB). However, as a positive control, BDNF treatment increased the phosphorylation levels of TrkB (Fig. S4*A*; BDNF, p-TrkB and TrkB). Moreover, to investigate the interaction of the BDNF pro-peptide with TrkB receptor, we carried out a BIAcore binding assay on the chip immobilized with recombinant TrkB-IgG fusion protein, corresponding to extracellular domain of TrkB receptor. The resultant data indicated that BDNF bound the extracellular domain of TrkB receptor, whereas the BDNF pro-peptide did not significantly bind (Fig. S4*B*). Thus, these data together show that the BDNF pro-peptide–enhanced hippocampal LTD is independent of TrkB receptor.

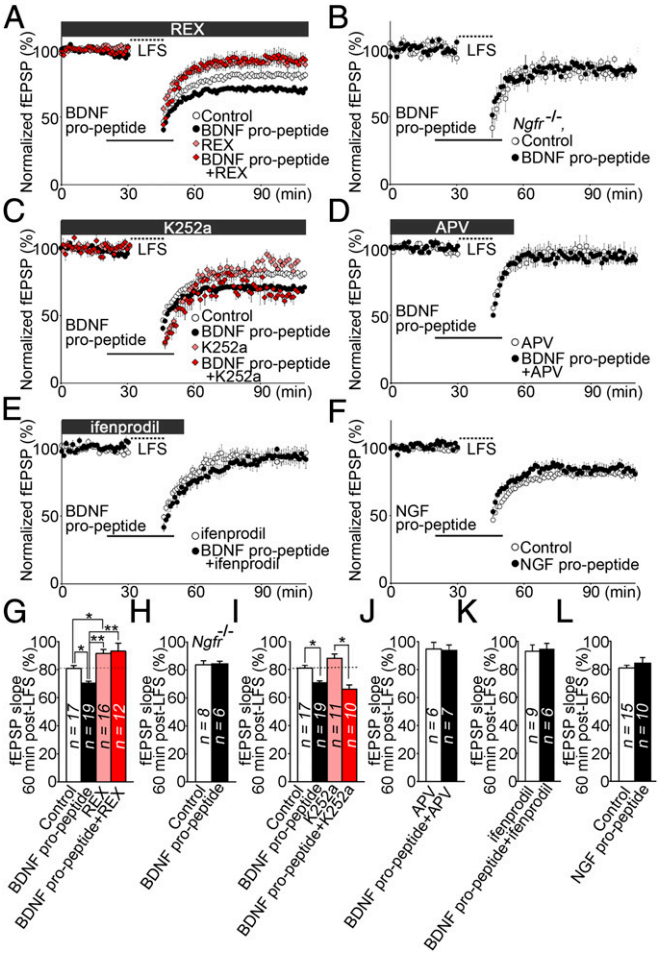

Fig. 2. Enhancement of hippocampal LTD by the BDNF pro-peptide is mediated by p75^{NTR} and GluN2B. Electrophysiological experiments and data analyses were performed as described in Fig. 1. Treatment with REX (100 μg/mL), K252a (200 nM), APV (25 μM), or ifenprodil (3 μM) over time was performed. Drug administration is indicated by the bold gray bar. The periods of LFS and BDNF pro-peptide (*A*–*E*) or NGF pro-peptide (*F*) administration are indicated by the broken line and black bar, respectively. BDNF pro-peptide was present at 10 ng/mL in all experiments. (*A*) The effect of REX. (*B*) No facilitation is observed in *Ngfr* $^{-/-}$ slices. (*C*) No effect of K252a on BDNF pro-peptide–enhanced LTD. (*D* and *E*) The effect of APV and ifenprodil on BDNF pro-peptide–enhanced hippocampal LTD. Note that treatment with APV (*D*) and ifenprodil (*E*) prevents BDNF pro-peptide–mediated facilitation of LTD. (*F*) No effect of the NGF pro-peptide. (*G*–*L*) LTD measured 60 min after LFS application. *G*–*L* represent data from *A*–*F*, respectively. The number of slices used (*n*) is indicated on the corresponding bar in each graph. *$P < 0.05$; **$P < 0.01$; Student *t* test (two groups) or ANOVA with post hoc test (multiple groups). Error bars indicate SEM.

NMDAR Activation Is Required for BDNF Pro-Peptide–Dependent Facilitation of Hippocampal LTD. *Ngfr*$^{-/-}$ mice exhibit a selective deficit in the NMDAR-dependent form of LTD (15), demonstrating that p75^{NTR} plays a unique role in this form of hippocampal synaptic plasticity. In the present study, we investigated whether the effect of the BDNF pro-peptide depended on the activation of NMDARs, i.e., we explored the mechanistic roles of the NMDAR and GluN2B subunit in BDNF pro-peptide-induced facilitation of hippocampal LTD. To this end, we treated slices by bath application of either a general NMDAR antagonist, DL-2-amino-5-phosphonovaleric acid (APV, 25 μM), or the noncompetitive, selective GluN2B-NMDAR antagonist ifenprodil

(3 μM) (15, 23). LFS-induced hippocampal LTD was completely blocked by APV, showing that the LTD paradigm used in this study is induced by activation of NMDARs (Fig. 2 *D* and *J*; APV, 94.7 ± 4.7%). Similarly, the application of ifenprodil, an antagonist of the GluN2B subunit that regulates LTD, also blocked hippocampal LTD (Fig. 2 *E* and *K*; ifenprodil, 92.9 ± 4.5%). We next investigated how the BDNF pro-peptide modulates LFS-induced LTD in hippocampal slices treated with APV or ifenprodil. Importantly, both inhibitors (i.e., APV and ifenprodil) completely blocked the BDNF pro-peptide–facilitated hippocampal LTD (Fig. 2 *D* and *J*; BDNF pro-peptide + APV, 94.7 ± 4.7%; $P = 0.9$ compared with the APV-treated group; Fig. 2 *E* and *K*; BDNF pro-peptide + ifenprodil, 94.2 ± 4.2%; $P = 0.9$ compared with the ifenprodil-treated group). Together, these findings demonstrate that the activation of GluN2B-containing NMDARs is required for BDNF pro-peptide–enhanced LTD.

We also tested whether the NGF pro-peptide, with a primary sequence appreciably different from that of the BDNF pro-peptide (Fig. S5*A*), modulates hippocampal LTD. Unlike the BDNF pro-peptide, the NGF pro-peptide (10 ng/mL; Fig. S5*B*, recombinant NGF pro-peptide) failed to facilitate hippocampal LTD (Fig. 2 *F* and *L*; control, 80.9 ± 2.0%; NGF pro-peptide, 84.3 ± 4.1%; $P = 0.3$ compared with the control), suggesting that the specific role of the BDNF pro-peptide in facilitating LTD depends on the structural information contained within the pro-peptide.

BDNF Pro-Peptide Enhances the NMDA-Dependent Reduction in the Level of AMPARs Expressed on the Cell Surface. Trafficking of the AMPAR GluA2 subunit is an important mechanism involved in LTD (24, 25). In mature hippocampal neurons, AMPARs occur predominantly as complexes containing GluA1/2 or GluA2/3 (26). Therefore, we investigated the effects of the BDNF pro-peptide on the surface expression of GluA2 in hippocampal neurons cultured for 3 weeks (27). The cultured hippocampal neurons were preincubated with BDNF pro-peptide for 30 min and then stimulated with the NMDAR agonist NMDA (100 μM) for 5 min. Surface labeling of the GluA2 subunit was performed as described in previous reports (28, 29). The treatment with NMDA (100 μM, 5 min) led to a significant reduction in the surface levels of GluA2, indicating that NMDA stimulation induces endocytosis of GluA2 in hippocampal neurons. The mean fluorescence intensity of GluA2 in the NMDA-treated group relative to that in controls was 0.73 ± 0.02 (Fig. 3 *A* and *B*; NMDA, $n = 33$ cells; $P < 0.01$ compared with the control).

The regulated trafficking of postsynaptic AMPARs is a crucial mechanism underlying activity-induced synaptic plasticity (30, 31). A 30-min pretreatment with the BDNF pro-peptide (10 ng/mL) promoted the NMDA-induced reduction of GluA2 receptors on the cell surface (Fig. 3 *A* and *B*; BDNF pro-peptide + NMDA, 0.64 ± 0.02, $P < 0.01$ relative to NMDA alone). We also found that application of the pro-peptide alone (10 ng/mL, 30 min) significantly decreased the surface level of GluA2 relative to control (Fig. 3 *A* and *B*; BDNF pro-peptide, 0.82 ± 0.02; $P < 0.01$ relative to the control), suggesting that the BDNF pro-peptide itself promotes AMPAR trafficking. Further statistical analysis indicated a significant difference in the surface levels of GluA2 between the two treated groups of the pro-peptide alone and the pro-peptide + NMDA ($P < 0.01$ relative to BDNF pro-peptide + NMDA), suggesting that the pro-peptide and NMDA function additively in this synaptic mechanism. However, this reduction was completely inhibited by the treatment with APV (100 μM), a specific antagonist of NMDA receptors (Fig. 3*C*; ANOVA, $P = 0.77$), indicating that the activation of the NMDAR is involved in the BDNF pro-peptide–induced enhancement of AMPAR endocytosis. These results together suggest that the BDNF pro-peptide facilitates hippocampal LTD by modulating AMPAR trafficking and may thus be a novel ligand to regulate neuronal synaptic plasticity.

BDNF Pro-Peptide Promotes NMDA Stimulation-Induced Internalization of AMPARs, and This Pro-Peptide Effect Is Implicated in the Activation of the $p75^{NTR}$ Receptor. Endocytosis of AMPARs is a crucial mechanism underlying activity-induced synaptic plasticity and LTD (30, 31). In the light of that finding, we next investigated whether the BDNF pro-peptide affects trafficking of a distinct AMPAR subunit, GluA1, in cultured hippocampal neurons (27). Using a procedure that allowed labeling of both surface and internalized GluA1 with different secondary antibodies (29), we found that a 5-min treatment with NMDA (100 μM) led to the internalization of GluA1, i.e., a significant increase was observed for the internalized GluA1 in the dendrites (Fig. 3 *D* and *E*; 100 μM NMDA; internalized); the mean ratio of the internalized to total GluA1 in the NMDA-treated group was 1.39 ± 0.02-fold higher than that in controls (Fig. 3 *D* and *E*; NMDA; $n = 30$ cells; $P < 0.01$ compared with the control). A 30-min pretreatment with the BDNF pro-peptide (10 ng/mL) further increased the NMDA-triggered endocytosis of GluA1 (Fig. 3 *D* and *E*; 10 ng/mL BDNF pro-peptide + 100 μM NMDA, arrows in the bottom row); under these conditions, the mean ratio of the internalized to total GluA1 was 1.74 ± 0.02-fold higher in the cells treated with BDNF pro-peptide + NMDA than that in control cells (Fig. 3 *D* and *E*; BDNF pro-peptide + NMDA; $n = 30$ cells; $P < 0.01$ compared with the control). Moreover, treatment with the BDNF pro-peptide alone (10 ng/mL, 30 min) promoted endocytosis of GluA1 (Fig. 3 *D* and *E*; BDNF pro-peptide; 1.39 ± 0.02; $n = 30$ cells; $P < 0.01$ relative to the control), and there was a significant difference in the internalized GluA1 between the group treated with the BDNF pro-peptide alone and the group treated with the pro-peptide + NMDA ($P < 0.01$).

Regarding the internalization of GluA2, an AMPAR subunit essential for LTD (24), a 5-min treatment with NMDA promoted internalization of GluA2 (Fig. 3 *F* and *G*; NMDA; 1.55 ± 0.03; $n = 30$ cells; $P < 0.01$ compared with the control). Pretreatment with the BDNF pro-peptide (10 ng/mL, 30 min) significantly increased the NMDA-triggered endocytosis of GluA2 (Fig. 3 *F* and *G*; 10 ng/mL BDNF pro-peptide + 100 μM NMDA, arrows in the bottom row). Thus, the mean ratio of the internalized to total GluA2 following the pretreatment was 1.70 ± 0.03-fold higher than that in control cells (Fig. 3 *F* and *G*; BDNF pro-peptide + NMDA; $n = 30$ cells; $P < 0.01$ compared with the control). In addition, as was observed for GluA1, GluA2 was internalized on stimulation with the pro-peptide alone (Fig. 3 *F* and *G*; BDNF pro-peptide, 1.36 ± 0.03; $n = 30$ cells; $P < 0.01$ relative to the control), and we observed a significant difference in the internalized levels of GluA2 between cells treated with the BDNF pro-peptide alone and those treated with the pro-peptide plus NMDA ($P < 0.01$). Thus, the BDNF pro-peptide decreases the surface expression of GluA2 and increases the NMDA-triggered internalization of GluA1 and GluA2, providing a plausible mechanistic explanation for the BDNF pro-peptide–induced facilitation of hippocampal LTD. Moreover, the trafficking of AMPARs in the chemical LTD is controlled additively by the BDNF pro-peptide and NMDA (Fig. 3 *B*, *E*, and *G*).

Because the $p75^{NTR}$ functional blocker REX attenuated BDNF pro-peptide–dependent LTD in hippocampal slices (Fig. 2*A*), we next examined the influence of REX on the pro-peptide-induced endocytosis of GluA2. Treatment with the BDNF pro-peptide alone (10 ng/mL, 30 min) promoted endocytosis of GluA2 (Fig. 3*H*; BDNF pro-peptide; 1.21 ± 0.03; $n = 30$ cells; $P < 0.01$ relative to the control). However, in the presence of REX, treatment with the BDNF pro-peptide did not affect GluA2 endocytosis (Fig. 3*H*; REX, 0.99 ± 0.01; BDNF pro-peptide + REX, 0.98 ± 0.02; $P = 0.29$ relative to the control), demonstrating that the $p75^{NTR}$ receptor is required for the BDNF pro-peptide–dependent dynamics of AMPARs.

The Common BDNF Polymorphism Val66Met Alters the Biological Activity of BDNF Pro-Peptide. To understand the physiological role of the BDNF pro-peptide, we investigated the effect of a

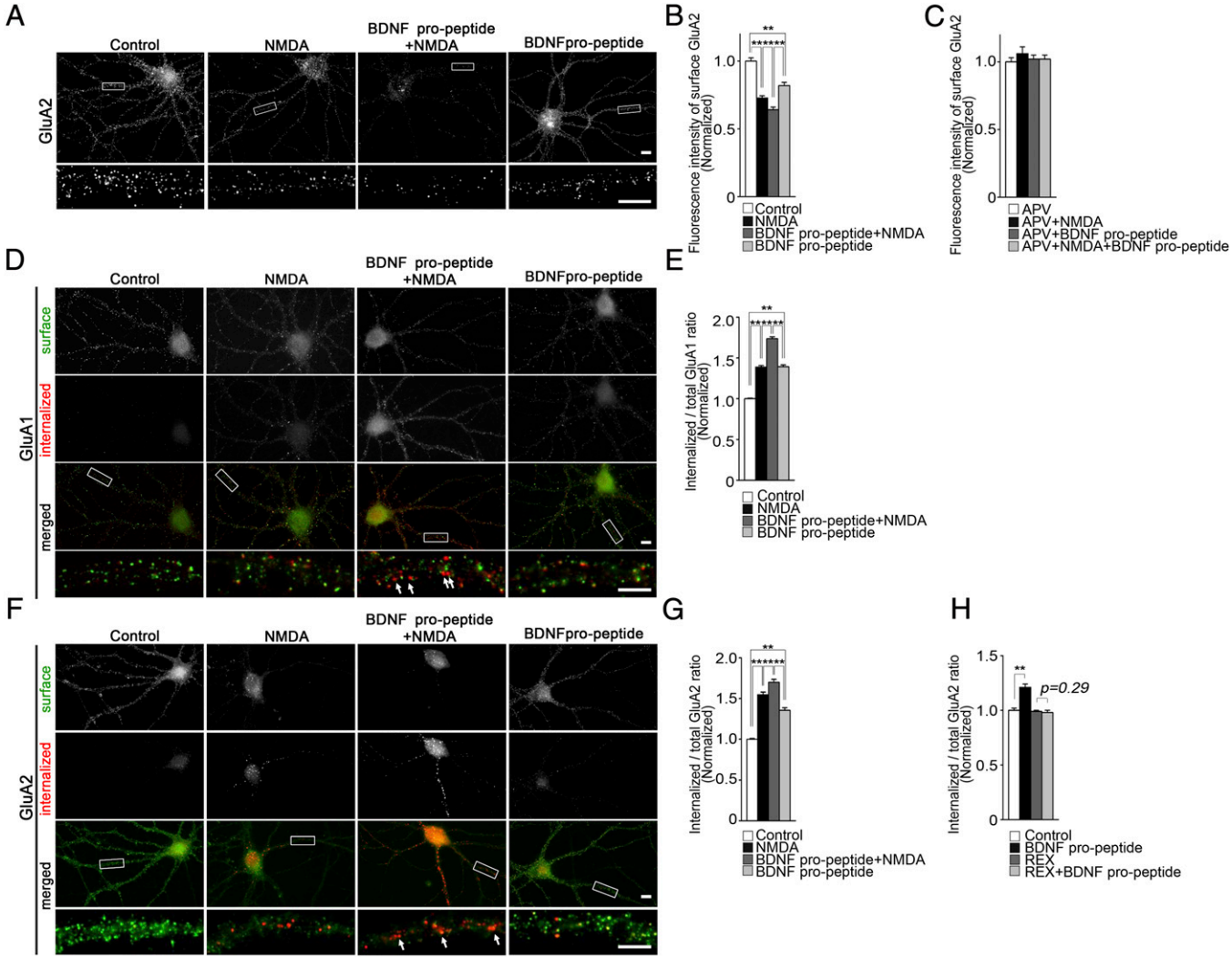

Fig. 3. Role of the BDNF pro-peptide on endocytosis and cell surface expression of AMPA receptors. (*A* and *B*) Effect of the BDNF pro-peptide on the NMDA-induced reduction of GluA2 on the cell surface. (*A*) Representative fluorescence images of surface GluA2. All boxed regions throughout this figure are magnified and shown in the rows below those with the boxes. BDNF pro-peptide promotes NMDA-triggered endocytosis of GluA2. (*B*) Quantitation of the intensity of surface GluA2. (*C*) Effect of APV on the NMDA and BDNF pro-peptide–induced reduction of cell surface GluA2. The reduction in surface GluA2 mediated by NMDA and the pro-peptide is completely blocked by APV. ANOVA, $P = 0.77$. (*D*) Representative fluorescence images of surface (green) and internalized (red) GluA1. Pretreatment with BDNF pro-peptide increases NMDA-triggered internalization of GluA1 signals (arrows in the bottom row). (*E*) Quantitation of GluA1 endocytosis. (Scale bars, 10 μm.) BDNF pro-peptide induces GluA1 endocytosis as efficiently as NMDA. (*F*) Representative fluorescence images of surface (green) and internalized (red) GluA2. BDNF pro-peptide enhances NMDA-triggered endocytosis of GluA2 (arrows in bottom row). (*G*) Quantitative data of GluA2 endocytosis. (Scale bars, 10 μm.) BDNF pro-peptide induces endocytosis of GluA2 as efficiently as that of GluA1. (*H*) Prevention of BDNF pro-peptide–induced endocytosis of GluA2 by REX, a functional inhibitor of $p75^{NTR}$. To examine the role of $p75^{NTR}$, cells were treated with or without BDNF pro-peptide (10 ng/mL) in the presence or absence of REX (100 μg/mL) for 30 min and fixed for labeling of GluA2. For *B*, *E*, *G*, and *H*, $^{**}P < 0.01$; ANOVA with post hoc tests; $n = 30$ independent cells from six coverslips, except $n = 33$ in *B*.

human BDNF polymorphism, Val66Met, on the biological action of the BDNF pro-peptide. To this end, we generated a recombinant pro-peptide containing a Met mutation (Fig. S2, Met) and investigated the effects of the mutant pro-peptide on hippocampal LTD. Notably, unlike the Val-BDNF pro-peptide, pretreatment with the Met-BDNF pro-peptide (10 ng/mL, 30 min) inhibited the LFS-induced hippocampal LTD (Fig. 4*A*, Met-pro-peptide, Fig. 4*B*, 60 min after LFS; control, 80.9 ± 2%; Met-BDNF pro-peptide, 97.1 ± 5.9%; $P < 0.01$ relative to the control; Met-BDNF pro-peptide). We next investigated whether the Met-BDNF pro-peptide affected basal synaptic transmission in the absence of LFS. Similarly to our earlier results (Fig. S3 *A* and *B*; Val-BDNF pro-peptide), neither the I/O relationship nor PPF ratio showed significant difference between before and after treatment of the Met-BDNF pro-peptide (Fig. S3 *A* and *B*, Met-BDNF pro-peptide).

Because the Val-BDNF pro-peptide enhanced NMDA-triggered endocytosis of GluA2 (Fig. 3*A*), we next investigated the effect of the Met-BDNF pro-peptide on this endocytosis. Unexpectedly, however, pretreatment with this mutant pro-peptide did not enhance the NMDA-induced decrease in the surface levels of GluA2. Moreover, the effect of the Met-BDNF pro-peptide was markedly less than that of the NMDA treatment alone (Fig. 4*C*; NMDA + Met-BDNF pro-peptide; 0.90 ± 0.02; $P < 0.01$ relative to the NMDA stimulation), and the effect of the Met-BDNF pro-peptide was diminished by APV (Fig. 4*D*; ANOVA, $P = 0.52$). These results suggest that differently from the Val-BDNF pro-peptide, the Met-BDNF pro-peptide does not enhance the NMDA-induced endocytosis of GluA2.

In our pharmacological study, we showed that the BDNF pro-peptide–induced hippocampal LTD required the activation of GluN2B (Fig. 2 *E* and *K*). Previously, it was demonstrated that proBDNF enhances the GluN2B-mediated LTD induction and synaptic currents (15). We thus examined the effects of the Val- and Met-BDNF pro-peptides on the expression levels of surface GluN2B in cultured hippocampal neurons. As a positive control, the Val-BDNF pro-peptide significantly increased the density of GluN2B clusters on the cell surface in a concentration-dependent manner (Fig. 4 *E* and *F*; Val-BDNF pro-peptide, 10 ng/mL Val-BDNF pro-peptide, $P < 0.05$; 100 ng/mL Val-BDNF pro-peptide, $P < 0.01$ relative to the control). However, applying the same concentrations of the Met-pro-peptide did not affect the density of GluN2B clusters (Fig. 4 *E* and *F*; Met-BDNF pro-peptide). These results provide a mechanistic explanation for the impairment of hippocampal LTD induced by the Met-BDNF pro-peptide.

Discussion

The present report demonstrates novel biological roles of the BDNF pro-peptide beyond that of assisting in the folding of BDNF (10). We found that the pro-peptide was a bioactive molecule that facilitated synaptic plasticity (LTD) by promoting the surface expression of GluN2B and the endocytosis of AMPAR, two crucial mechanisms for LTD expression. We also provided evidence demonstrating that these biological activities were specific to the Val-BDNF pro-peptide.

Within the present study, several lines of evidence indicate that the BDNF pro-peptide is a newly discovered facilitator of hippocampal LTD. Treatment with the BDNF pro-peptide for 30 min facilitated LTD in the hippocampus without affecting basal synaptic transmission. A subnanomolar concentration of the BDNF pro-peptide was sufficient to facilitate hippocampal LTD. Consistent with a previous report (32), LTD occurred normally in *Bdnf*$^{-/-}$ hippocampal slices, whereas application of the BDNF pro-peptide to the mutant slices facilitated LTD. These results demonstrated that the biological activity of the BDNF pro-peptide itself promoted LTD and that this effect of the pro-peptide was not mediated via an interaction with endogenous BDNF. Moreover, the pro-peptide of NGF, which has low sequence similarity to the BDNF pro-peptide (Fig. S5), did not facilitate LTD. Lastly, we found that the BDNF polymorphism Val66Met, which likely resulted in structural changes to the pro-peptide, led to the inhibition of hippocampal LTD. These results suggest that the biological action of the BDNF pro-peptide is based on its intrinsic structural information.

An important mechanism underlying LTD is the endocytosis and exocytosis of AMPARs at postsynaptic sites (33). Although the role of AMPAR trafficking in synaptic plasticity has been extensively studied (30, 31), the mechanisms underlying the modulation of AMPAR trafficking are not fully understood. In mature hippocampal neurons, AMPARs exist predominantly as complexes containing GluA1/2 or GluA2/3 (26), and the trafficking of GluA2 receptors is crucial for LTD (24, 25). In the present study, we explored the modulatory role of the BDNF pro-peptide during this trafficking by quantitating the AMPAR immunoreactivity in dissociated hippocampal neurons. Our investigation of AMPAR trafficking clarified several roles of the BDNF pro-peptide in this mechanism. First, in line with the electrophysiological data, a 30-min pretreatment with the Val-BDNF pro-peptide decreased the NMDA-triggered surface expression of GluA2 but increased the NMDA-triggered internalization of GluA1 and GluA2. These results provide a mechanistic explanation for the facilitation of hippocampal LTD mediated by the BDNF pro-peptide. Second, as with NMDA, the BDNF pro-peptide activated AMPAR trafficking. Thus, the effects of NMDA and the pro-peptide on AMPAR trafficking may be additive. Similarly, insulin and NMDA induce internalization of AMPARs using independent mechanisms (24). Therefore, both NMDA and growth factor signaling may be molecular mechanisms responsible for the induction/facilitation of LTD. Substantial evidence supports the idea that BDNF inhibits LTD (6) and that BDNF controls the surface expression of AMPARs on the plasma membrane by exocytosis (34). Thus, BDNF and its pro-peptide may exert opposing roles on the synaptic mechanisms underlying LTD and the trafficking of AMPARs.

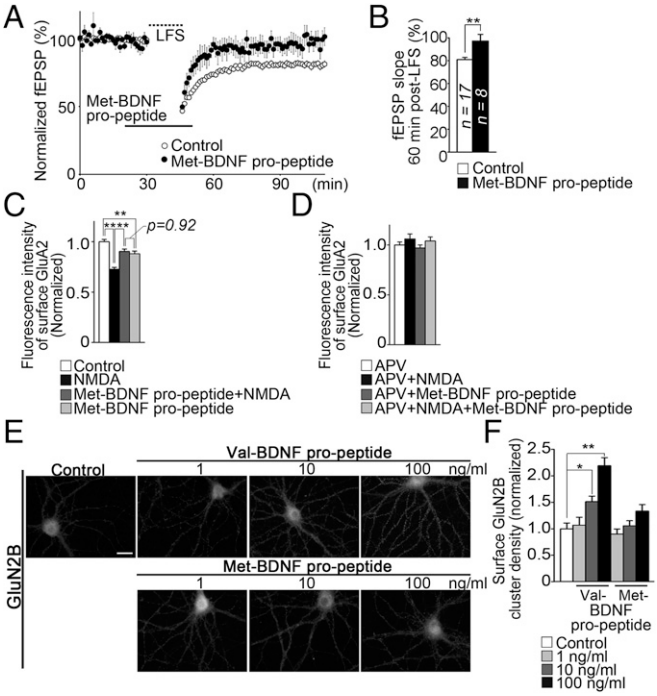

Fig. 4. Impact of the Val66Met polymorphism on BDNF pro-peptide–dependent synaptic depression. (*A* and *B*) The Met-BDNF pro-peptide inhibits LFS-induced hippocampal LTD. (*A*) LTD was induced by LFS (1 Hz; 900 pulses) to the Schaffer collaterals treated with the mutant BDNF pro-peptide (10 ng/mL, 30 min, Met-BDNF pro-peptide). (*B*) LTD measured 60 min after LFS application. $^{**}P < 0.01$; Student's *t* test. (*C*) The Met-BDNF pro-peptide prevents NMDA-induced reduction of cell surface AMPARs. To measure the surface expression levels of GluA2, neurons were preincubated with 10 ng/mL Met-BDNF pro-peptide for 30 min and then stimulated with NMDA (100 μM) for 5 min. Thirty minutes after NMDA treatment, cells were labeled with an antibody against GluA2 and analyzed under a fluorescence microscope. Bar graphs indicate surface intensity of GluA2. $^{*}P < 0.01$; ANOVA with post hoc test. $n = 33$ independent cells from six coverslips. All experiments, except for the treatment with Met-BDNF pro-peptide, were conducted similar to those described in Fig. 3*A*. (*D*) The effect of APV on the Met-BDNF pro-peptide–induced reduction of GluA2 on the cell surface. Note that the reduction of surface GluA2 by NMDA and the pro-peptide is completely blocked by APV. ANOVA, $P = 0.52$. (*E* and *F*) Effect of the Val- and Met-BDNF pro-peptide on surface expression of GluN2B. To examine the expression levels of cell surface GluN2B after the treatment with the indicated pro-peptide, cultured hippocampal neurons were incubated with the Val- or Met-BDNF pro-peptide (1, 10, or 100 ng/mL) for 30 min. The living cells were labeled with an anti-GluN2B (extracellular domain) antibody and analyzed under a fluorescence microscope. (*E*) Representative fluorescence images of surface GluN2B. (*F*) Quantitation of GluN2B cluster density. $^{*}P < 0.05$ $^{**}P < 0.01$; ANOVA with post hoc test. $n = 20$–26 independent cells from four coverslips.

Because a growing body of clinical evidence indicates that the BDNF Val66Met polymorphism increases susceptibility to a variety of brain disorders (35), we explored the role of this genetic variation in the actions of the BDNF pro-peptide. The Val-BDNF pro-peptide facilitated hippocampal LTD, whereas the Met-BDNF pro-peptide markedly inhibited LFS-induced hippocampal LTD, demonstrating that this genetic variation altered the biological activity of the mutant pro-peptide.

We next examined the molecular mechanisms underlying the Met-BDNF pro-peptide–induced LTD inhibition. Notably, although

the Val-BDNF pro-peptide enhanced NMDA-induced endocytosis of GluA2, the Met-BDNF pro-peptide attenuated this endocytosis (Fig. 4*C*). This attenuation was diminished in the presence of APV, an antagonist of the NMDA receptor (Fig. 4*D*). Thus, the Met-BDNF pro-peptide may inhibit LFS-induced hippocampal LTD by attenuating the neuronal activity-dependent activation of NMDARs. Consequently, the Met-BDNF pro-peptide inhibited NMDA-induced endocytosis of GluA2, and this attenuation was diminished in the presence of the NMDA receptor antagonist APV (Fig. 4*D*), suggesting that the Met-BDNF pro-peptide modulates the neuronal activity-dependent endocytosis of GluA2. These results offer a plausible explanation for the Met-BDNF pro-peptide–induced impairment of hippocampal LTD.

Our previous study showed that BDNF Val66Met polymorphism affects human memory retention as well as the activity-dependent secretion of BDNF (11). A growing body of clinical evidence indicates that the BDNF Val66Met polymorphism increases susceptibility to a variety of brain disorders (35). Recently, it was reported that mice with the Val66Met mutation are defective in NMDAR-dependent plasticity in the hippocampus (36). Moreover, we showed that APV diminished the surface expression of GluA2 by the Val- and Met-BDNF pro-peptide at similar levels, suggesting that the Val- and Met-BDNF pro-peptide induced GluA2 endocytosis via NMDAR activation (Figs. 3*C* and 4*D*). Because the additive effect of NMDA and the Val-BDNF pro-peptide on the endocytosis of GluA2—a crucial mechanism for the expression of LTD—was reversed by this genetic variation, such mechanistic data may help explain how LTD is facilitated and inhibited by the Val- and Met-BDNF pro-peptide, respectively. These results together indicate that the Val66Met genetic variation may affect the regulation of NMDAR functions to ultimately impair NMDAR-dependent synaptic plasticity and provide new insights into the role of this BDNF polymorphism in synaptic plasticity, human brain function, and brain disorders.

Many previous reports have demonstrated the role of $p75^{NTR}$ in synaptic plasticity. First, deletion of the gene encoding $p75^{NTR}$ alters the expression of GluA2 and GluA3 (21). Second, the expression levels of GluN2B decrease in hippocampal tissues of $p75^{NTR}$ KO mice (15). Third, expression levels of $p75^{NTR}$ control dendritic complexity and spine morphology (37). Fourth, $p75^{NTR}$ colocalizes with PSD95, and proBDNF–dependent facilitation of hippocampal LTD requires the activation of $p75^{NTR}$ and GluN2B, a key molecule involved in induction of LTD (15). proBDNF reportedly decreases spine density via $p75^{NTR}$ (38). In the present study, the BDNF pro-peptide failed to enhance LTD in hippocampal slices from $p75^{NTR}$ KO mice, and the treatment with ifenprodil, an antagonist of the GluN2B subunit, prevented the BDNF pro-peptide–induced facilitation of LTD. These results indicate that the mechanism for the BDNF pro-peptide-elicited enhancement of hippocampal LTD is mediated through the activation of $p75^{NTR}$ and GluN2B. The abundance of postsynaptic GluA2 correlates with spine morphology (39), and the activity-dependent removal of postsynaptic GluA2 is a crucial mechanism for LTD expression (40). In the present study, the BDNF pro-peptide activated the endocytosis of GluA2, and the $p75^{NTR}$ antibody REX inhibited this cellular event. Thus, the present findings suggest a role for $p75^{NTR}$ in the BDNF pro-peptide–dependent synaptic depression.

Despite their differences in sequence and length, proBDNF and the pro-peptide both facilitated hippocampal LTD in hippocampal slices, raising the question of which one (proBDNF or BDNF pro-peptide) plays a greater role under physiological conditions. Recent reports address this issue. First, Yang et al. reported that proBDNF expression in hippocampi was highest during the second postnatal week (41). Recently, Dieni et al. demonstrated that the BDNF pro-peptide is present at much higher levels than proBDNF in adult hippocampal tissues and is stored in presynaptic dense-core vesicles in neurons of the adult brain (8). More recently, Anastasia et al. showed that cultured hippocampal neurons secrete the pro-peptide in a neuronal activity-dependent manner (9). They also showed that the enzymatic removal of N-linked glycans resulted in a reduction of the molecular mass of the endogenous BDNF pro-peptide from 15 to 12 kDa (9). In the present study, the concentration of the BDNF pro-peptide was determined using the method described by Anastasia et al. (9). In hippocampal tissue from 3-wk-old mice, the concentration of the pro-peptide was found to be comparable to that reported by Anastasia et al. (Fig. 1*B*). Moreover, it was previously reported that proBDNF enhanced hippocampal LTD (15), and, in the present study, we showed that the BDNF pro-peptide facilitated LTD. Thus, these findings all together suggest that proBDNF and the pro-peptide both enhance synaptic depression in the postnatal stage of brain.

A recent report indicates that many eukaryotic proteins exist in a disordered form under physiological conditions and fold into ordered structures only upon binding to their cellular targets (42). Experimental evidence for this theory was provided by a recent extensive report that investigated how intrinsically disordered proteins fold on binding to their targets (43). According to the structure prediction software, the BDNF prodomain is disordered (38). Nevertheless, we showed that the BDNF pro-peptide promoted hippocampal LTD, and Anastasia et al. showed that the application of Met-BDNF prodomain induced acute growth cone retraction (9). Thus, the BDNF pro-peptide may exert its biological activity in a manner as reported by Sugase et al. (43). Our findings and recent reports (8, 9) together suggest that the BDNF pro-peptide plays important biological roles beyond its traditional role in assisting the folding of BDNF (10), and, in light of this possibility, we propose a multiligand model in which neurotrophins, via their pro-peptides, exert numerous biological functions in the nervous system.

The present study suggests that the BDNF pro-peptide and BDNF have antagonistic functions on LTD, i.e., facilitation and blockade of LTD induction. Given the antagonistic actions of BDNF and its pro-peptide in hippocampal LTD, how these antagonistic peptides operate in physiological condition is a fundamental question. There were recent noteworthy reports to provide the mechanistic possibility. First, Guo et al. demonstrated the role of neuronal activity on the levels of BDNF-induced TrkB activation: whereas field stimulation with TBS (θ-burst stimulation) converted BDNF-induced TrkB phosphorylation from a transient to a sustained mode, another stimulation protocol used to induce the LTD paradigm did not (44). The understanding of such mechanism of $p75^{NTR}$, which involves the BDNF pro-peptide–dependent facilitation of hippocampal LTD, would be important to solve the question of how two antagonistic peptides (BDNF and its pro-peptide) operate as a whole. Second, activity-dependent secretion of BDNF is an important mechanism of BDNF-dependent synaptic plasticity (5), and it was reported that the BDNF pro-peptide is stored in presynaptic dense-core vesicles in brain neurons (8) and releasable in an activity-dependent manner (9). Given the pro-peptide modulates LTD, the mechanism for the pro-peptide secretion should be clarified in future studies.

The present study may provide new insights in the field of neuroscience, as well as cell biology, for understanding the general physiological roles and modes of action for the pro-peptides of growth factors.

Materials and Methods

Rats and mice were maintained according to the guidelines of the National Institute of Advanced Industrial Science and Technology and the Nara Institute of Science and Technology. All experiments were approved by the Institutional Animal Care and Use Committees of these two organizations. Full methods, including reagents, production of recombinant proteins, SDS/PAGE and immunoblotting analysis, hippocampal slice preparation, electrophysiology, hippocampal cell cultures, quantitative analysis of AMPAR

trafficking and GluN2B on the cell surface, fluorescence microscopy, and statistics are described in *SI Materials and Methods*.

ACKNOWLEDGMENTS. Recombinant human BDNF was kindly provided by Sumitomo Pharmaceuticals. We thank Dr. L. F. Reichardt (University of California) for kindly providing the $p75^{NTR}$-blocking REX antibody. We thank Drs. Teiichi Furuichi (RIKEN) and Kazuyuki Kiyosue (AIST) for helpful discussion. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (Elucidation of neural network function in the brain) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (40344171) (to M.K.); Japan Science and Technology Agency; and Core Research for Evolutional Science and Technology (T. Mizui, H.K., T.H., K.U., M.K., T. Matsumoto, S.Y., M.T., Y.I., and S.S.).

1. Alberts B, Wilson JH, Hunt T (2008) *Molecular Biology of the cell* (Garland Science, New York), 5th Ed.
2. Leibrock J, et al. (1989) Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* 341(6238):149–152.
3. Bibel M, Barde YA (2000) Neurotrophins: Key regulators of cell fate and cell shape in the vertebrate nervous system. *Genes Dev* 14(23):2919–2937.
4. Reichardt LF (2006) Neurotrophin-regulated signalling pathways. *Philos Trans R Soc Lond B Biol Sci* 361(1473):1545–1564.
5. Park H, Poo MM (2013) Neurotrophin regulation of neural circuit development and function. *Nat Rev Neurosci* 14(1):7–23.
6. Lu B, Pang PT, Woo NH (2005) The yin and yang of neurotrophin action. *Nat Rev Neurosci* 6(8):603–614.
7. Lessmann V, Brigadski T (2009) Mechanisms, locations, and kinetics of synaptic BDNF secretion: An update. *Neurosci Res* 65(1):11–22.
8. Dieni S, et al. (2012) BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. *J Cell Biol* 196(6):775–788.
9. Anastasia A, et al. (2013) Val66Met polymorphism of BDNF alters prodomain structure to induce neuronal growth cone retraction. *Nat Commun* 4:2490.
10. Kolbeck R, Jungbluth S, Barde YA (1994) Characterisation of neurotrophin dimers and monomers. *Eur J Biochem* 225(3):995–1003.
11. Egan MF, et al. (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112(2): 257–269.
12. Jessop DS (1999) Review: Central non-glucocorticoid inhibitors of the hypothalamo-pituitary-adrenal axis. *J Endocrinol* 160(2):169–180.
13. Jones KR, Reichardt LF (1990) Molecular cloning of a human gene that is a member of the nerve growth factor family. *Proc Natl Acad Sci USA* 87(20):8060–8064.
14. Minichiello L, et al. (1999) Essential role for TrkB receptors in hippocampus-mediated learning. *Neuron* 24(2):401–414.
15. Woo NH, et al. (2005) Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. *Nat Neurosci* 8(8):1069–1077.
16. Hempstead BL (2002) The many faces of p75NTR. *Curr Opin Neurobiol* 12(3):260–267.
17. Chao MV (2003) Neurotrophins and their receptors: A convergence point for many signalling pathways. *Nat Rev Neurosci* 4(4):299–309.
18. Xu B, et al. (2000) The role of brain-derived neurotrophic factor receptors in the mature hippocampus: Modulation of long-term potentiation through a presynaptic mechanism involving TrkB. *J Neurosci* 20(18):6888–6897.
19. Yang J, et al. (2014) proBDNF negatively regulates neuronal remodeling, synaptic transmission, and synaptic plasticity in hippocampus. *Cell Reports* 7(3):796–806.
20. Akaneya Y, Tsumoto T, Hatanaka H (1996) Brain-derived neurotrophic factor blocks long-term depression in rat visual cortex. *J Neurophysiol* 76(6):4198–4201.
21. Rösch H, Schweigreiter R, Bonhoeffer T, Barde YA, Korte M (2005) The neurotrophin receptor p75NTR modulates long-term depression and regulates the expression of AMPA receptor subunits in the hippocampus. *Proc Natl Acad Sci USA* 102(20):7362–7367.
22. Suzuki S, et al. (2007) Brain-derived neurotrophic factor regulates cholesterol metabolism for synapse development. *J Neurosci* 27(24):6417–6427.
23. Massey PV, et al. (2004) Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. *J Neurosci* 24(36):7821–7828.
24. Beattie EC, et al. (2000) Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. *Nat Neurosci* 3(12):1291–1300.
25. Araki Y, Lin DT, Huganir RL (2010) Plasma membrane insertion of the AMPA receptor GluA2 subunit is regulated by NSF binding and Q/R editing of the ion pore. *Proc Natl Acad Sci USA* 107(24):11080–11085.
26. Wenthold RJ, Petralia RS, Blahos J II, Niedzielski AS (1996) Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. *J Neurosci* 16(6):1982–1989.
27. Takahashi H, et al. (2003) Drebrin-dependent actin clustering in dendritic filopodia governs synaptic targeting of postsynaptic density-95 and dendritic spine morphogenesis. *J Neurosci* 23(16):6586–6595.
28. Lu W, et al. (2001) Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. *Neuron* 29(1):243–254.
29. Bhattacharyya S, Biou V, Xu W, Schlüter O, Malenka RC (2009) A critical role for PSD-95/AKAP interactions in endocytosis of synaptic AMPA receptors. *Nat Neurosci* 12(2):172–181.
30. Südhof TC, Malenka RC (2008) Understanding synapses: Past, present, and future. *Neuron* 60(3):469–476.
31. Kessels HW, Malinow R (2009) Synaptic AMPA receptor plasticity and behavior. *Neuron* 61(3):340–350.
32. Matsumoto T, et al. (2008) Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. *Nat Neurosci* 11(2):131–133.
33. Shepherd JD, Huganir RL (2007) The cell biology of synaptic plasticity: AMPA receptor trafficking. *Annu Rev Cell Dev Biol* 23:613–643.
34. Narisawa-Saito M, et al. (2002) Brain-derived neurotrophic factor regulates surface expression of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors by enhancing the N-ethylmaleimide-sensitive factor/GluR2 interaction in developing neocortical neurons. *J Biol Chem* 277(43):40901–40910.
35. Bath KG, Lee FS (2006) Variant BDNF (Val66Met) impact on brain structure and function. *Cogn Affect Behav Neurosci* 6(1):79–85.
36. Ninan I, et al. (2010) The BDNF Val66Met polymorphism impairs NMDA receptor-dependent synaptic plasticity in the hippocampus. *J Neurosci* 30(26):8866–8870.
37. Zagrebelsky M, et al. (2005) The p75 neurotrophin receptor negatively modulates dendrite complexity and spine density in hippocampal neurons. *J Neurosci* 25(43): 9989–9999.
38. Koshimizu H, et al. (2009) Multiple functions of precursor BDNF to CNS neurons: Negative regulation of neurite growth, spine formation and cell survival. *Mol Brain* 2(1):27.
39. Passafaro M, Nakagawa T, Sala C, Sheng M (2003) Induction of dendritic spines by an extracellular domain of AMPA receptor subunit GluR2. *Nature* 424(6949):677–681.
40. Brown TC, Tran IC, Backos DS, Esteban JA (2005) NMDA receptor-dependent activation of the small GTPase Rab5 drives the removal of synaptic AMPA receptors during hippocampal LTD. *Neuron* 45(1):81–94.
41. Yang J, et al. (2009) Neuronal release of proBDNF. *Nat Neurosci* 12(2):113–115.
42. Wright PE, Dyson HJ (1999) Intrinsically unstructured proteins: Re-assessing the protein structure-function paradigm. *J Mol Biol* 293(2):321–331.
43. Sugase K, Dyson HJ, Wright PE (2007) Mechanism of coupled folding and binding of an intrinsically disordered protein. *Nature* 447(7147):1021–1025.
44. Guo W, Ji Y, Wang S, Sun Y, Lu B (2014) Neuronal activity alters BDNF-TrkB signaling kinetics and downstream functions. *J Cell Sci* 127(Pt 10):2249–2260.