of our agent against SHIV SF162. Recently, in the same animal model, the neutralizing antibody IgG1-b12 (*31*) gave partial protection against vaginal transmission of SHIV, also at concentrations vastly in excess of those needed in vitro. A small-molecule CCR5 inhibitor, highly potent in vitro, gave only minimal protection in the animal system used here, even as a virtually saturated solution (*32*). Cyanovirin partially inhibited vaginal transmission of a SHIV isolate that also targets CXCR4 but only at concentrations ~10,000 times those required for full inhibition in vitro (*33*). Possible explanations for these dose disparities include incomplete distribution, failure to penetrate to hypothetical submucosal target sites, nonspecific adsorption to mucosal surfaces, or degradation or inhibition by vaginal factors. But conceivably, the explanation might simply be that the progesterone treatment and the dose of SHIV that we and others use to ensure near-universal infection of control macaques [28 of 31 in this system (*28*, *31*, *32*)] constitute an extraordinary challenge. In the natural human setting, risk for acquisition of HIV infection after sexual exposure, although probably not uniform, is on average markedly lower than that shown by unprotected controls in this animal system (*34*). Given the discrepancy between in vitro and in vivo potency, we offer our findings as a proof of principle and as a direction for attempts to render the approach economically acceptable.

PSC-RANTES protected macaques from intravaginal challenge without detectable toxicity or histological changes. Consequently, further development of this and related compounds, either alone or in combination with other agents, and improvement of their formulation are reasonable subjects for further study as an approach to the prevention of sexual transmission of HIV.

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Supporting Online Material

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Materials and Methods
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Cleavage of proBDNF by tPA/Plasmin Is Essential for Long-Term Hippocampal Plasticity

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Long-term memory is thought to be mediated by protein synthesis–dependent, late-phase long-term potentiation (L-LTP). Two secretory proteins, tissue plasminogen activator (tPA) and brain-derived neurotrophic factor (BDNF), have been implicated in this process, but their relationship is unclear. Here we report that tPA, by activating the extracellular protease plasmin, converts the precursor proBDNF to the mature BDNF (mBDNF), and that such conversion is critical for L-LTP expression in mouse hippocampus. Moreover, application of mBDNF is sufficient to rescue L-LTP when protein synthesis is inhibited, which suggests that mBDNF is a key protein synthesis product for L-LTP expression.

Long-lasting changes in synaptic efficacy are thought to mediate long-term memory (*1*, *2*). A well-studied model system is the late phase of long-term potentiation (L-LTP) in the hippocampus. Unlike the early phase of LTP (E-LTP), L-LTP requires new protein synthesis and involves synaptic growth (*2*). A key molecule implicated in L-LTP is the secretory protein BDNF. Hippocampal slices from BDNF heterozygous ($BDNF^{+/-}$) mice fail to exhibit L-LTP (*3*). Inhibition of BDNF signaling by blocking its receptor TrkB with antibody to TrkB or with BDNF scavenger TrkB–immunoglobulin G also inhibits L-LTP (*4*, *5*). In the mammalian brain, BDNF is synthesized as a precursor called proBDNF, which is proteolytically cleaved to generate mature BDNF (mBDNF), the form of BDNF that binds to and activates TrkB (*6*, *7*). In cultured hippocampal neurons and in heterologous cells, proBDNF accounts for a substantial proportion of total BDNF secreted extracellularly (*8*–*11*). A recent study suggests that the precursor and mature forms of neurotrophins interact with very different receptor/signaling systems to induce opposing biological effects (*12*). If a similar principle could be applied to the hippocampus, extracellular cleavage of proBDNF at synapses may have profound implications for synaptic modulation (*7*).

One molecule that may play a role in the conversion of proBDNF to its mature form is the extracellular serine protease tPA. Several studies have implicated tPA in the expression of long-lasting forms of synaptic plasticity: Induction of L-LTP enhances the expression of tPA in the hippocampus (*13*), tPA can be secreted from neuronal growth cones and

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axonal terminals (*14*), and neuronal membrane depolarization also induces secretion of tPA into the extracellular space in the hippocampus in a Ca^{2+}-dependent manner (*15*). An inhibitor of tPA blocks L-LTP, whereas extracellular application of tPA results in L-LTP induced by a single tetanus, which otherwise only induces E-LTP (*16*). Mice lacking tPA exhibit a selective deficit in L-LTP expression without affecting E-LTP, and they also show impairment in context conditioning and in two-way active avoidance tests (*17–20*). Transgenic mice overexpressing tPA exhibit enhanced L-LTP and improved spatial learning (*21*).

Although tPA has been implicated in proteolytic degradation of several extracellular matrix proteins (*22–24*), the only well-defined, direct target of tPA is plasminogen. This inactive zymogen is converted to the active form, plasmin, through proteolytic cleavage by tPA (*25*). Plasminogen mRNA and protein in the hippocampus are exclusively expressed in neurons and primarily in the apical dendrites of pyramidal cells (*26*). Because in vitro experiments suggest that plasmin is capable of cleaving proBDNF to its mature form (*12*), we hypothesized that a major function of the tPA/plasmin system is to convert proBDNF to mBDNF at hippocampal synapses, and that such conversion is critical for the expression of L-LTP.

To determine the precise role of BDNF in long-term hippocampal plasticity, we recorded L-LTP in the CA1 area. We used a paradigm consisting of 12 bursts of theta burst stimulation (l-TBS), which is more reliable than the conventional four-tetanus protocol in revealing the L-LTP deficits in $BDNF^{+/-}$ mice (*4*). Similar to previous reports, we consistently observed a severe impairment in L-LTP induced by l-TBS in $BDNF^{+/-}$ mice [Fig. 1A, field excitatory postsynaptic potential (EPSP) slope measured 3 hours after application of tetanus: wild type, 170.1 ± 6.2; $BDNF^{+/-}$, 101.8 ± 8.6; $P < 0.001$].

To investigate whether mBDNF is sufficient for L-LTP, we used two additional approaches. First, we inhibited protein synthesis by a specific inhibitor, anisomycin (40 μM), and showed that L-LTP induced by l-TBS is dependent on protein synthesis. Perfusion of anisomycin throughout the recording effectively blocked L-LTP (Fig. 1B). Application of mBDNF (200 ng/ml) 2 to 3 min after l-TBS completely reversed the blockade of L-LTP by anisomycin (Fig. 1B, anisomycin, 101.3 ± 6.4; anisomycin + mBDNF, 153.4 ± 14.7; $P < 0.05$). Interestingly, the L-LTP deficit could no longer be rescued when mBDNF was applied 10 min after l-TBS, suggesting a critical period in which mBDNF acts (fig. S1). A similar rescuing effect of mBDNF was observed when emetine (20 μM) was used to inhibit protein synthesis (fig. S2). Perfusion of mBDNF to the slices did not alter the basal synaptic transmission over a 3-hour period, nor did it have an epileptic effect on hippocampal neurons (fig. S3). Second, we applied mBDNF to slices that received only three bursts of theta burst stimulation (s-TBS), which normally induces E-LTP but not L-LTP (Fig. 1C). Slices treated with mBDNF after application of s-TBS now exhibited bona fide L-LTP (Fig. 1C, s-TBS, 99.9 ± 10.2; s-TBS + mBDNF, 140.2 ± 10.4; $P < 0.05$).

To study the relationship between tPA and BDNF, we used tPA homozygous ($tPA^{-/-}$) mutant mice. Consistent with previous reports (*17*), we found that there was a severe impairment in L-LTP induced by l-TBS in hippocampal slices derived from the $tPA^{-/-}$

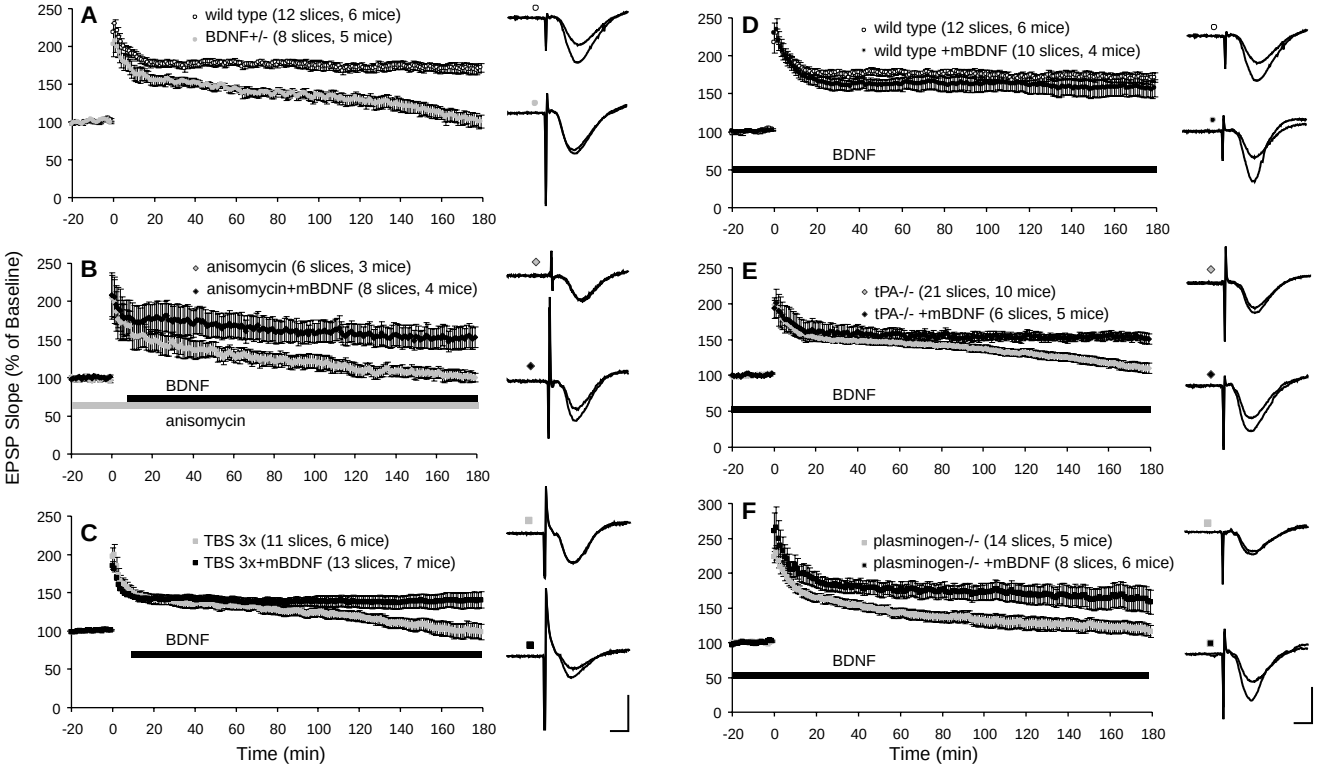

Fig. 1. mBDNF is both necessary and sufficient for L-LTP expression. Field EPSPs were recorded in the CA1 area of hippocampal slices derived from wild-type and various knockout mice. L-LTP was induced by l-TBS; E-LTP was induced by s-TBS. Application of various drugs is indicated by horizontal bars. Numbers of slices and mice used in each condition are indicated at the top of each plot. Superimposed sample traces before and 3 hours after l-TBS are shown at the right of each plot. Scales for these and all other traces: 4 mV (vertical), 4 ms (horizontal). (**A**) BDNF is required for the expression of L-LTP. Note that l-TBS failed to induce L-LTP in $BDNF^{+/-}$ mice. (**B**) mBDNF rescues the impairment of L-LTP caused by inhibition of protein synthesis. Hippocampal slices were treated with the protein synthesis inhibitor anisomycin (40 μM) throughout the entire experiments. mBDNF (200 ng/ml) was applied 5 min after l-TBS, as indicated by the black bar. (**C**) Application of mBDNF after s-TBS converts E-LTP to L-LTP. (**D**) mBDNF does not further enhance L-LTP in wild-type mice. (**E**) mBDNF rescues L-LTP in $tPA^{-/-}$ mice. (**F**) mBDNF rescues L-LTP in $plasminogen^{-/-}$ mice.

mice (Fig. 1E). Perfusion of mBDNF prevented the L-LTP impairment (Fig. 1E, tPA$^{-/-}$, 110.4 ± 6.8; tPA$^{-/-}$ + mBDNF, 151.7 ± 7.4; $P < 0.001$). mBDNF did not simply increase the magnitude of synaptic potentiation, because application of mBDNF to wild-type slices had no effect on L-LTP (Fig. 1D, l-TBS, 170.1 ± 6.2; l-TBS + mBDNF, 156.4 ± 11.5; $P = 0.286$). A direct target of tPA is plasminogen, which is proteolytically cleaved by tPA to form plasmin. We assessed whether plasmin is also involved in L-LTP and, if so, whether a downstream target of plasmin is BDNF. Plasminogen$^{-/-}$ mice also exhibited severe impairment in L-LTP (Fig. 1F). Application of mBDNF completely rescued the L-LTP deficit in these mice (Fig. 1F, plasminogen$^{-/-}$, 118.4 ± 8.6; plasminogen$^{-/-}$ + mBDNF, 161.2 ± 17.8; $P < 0.05$).

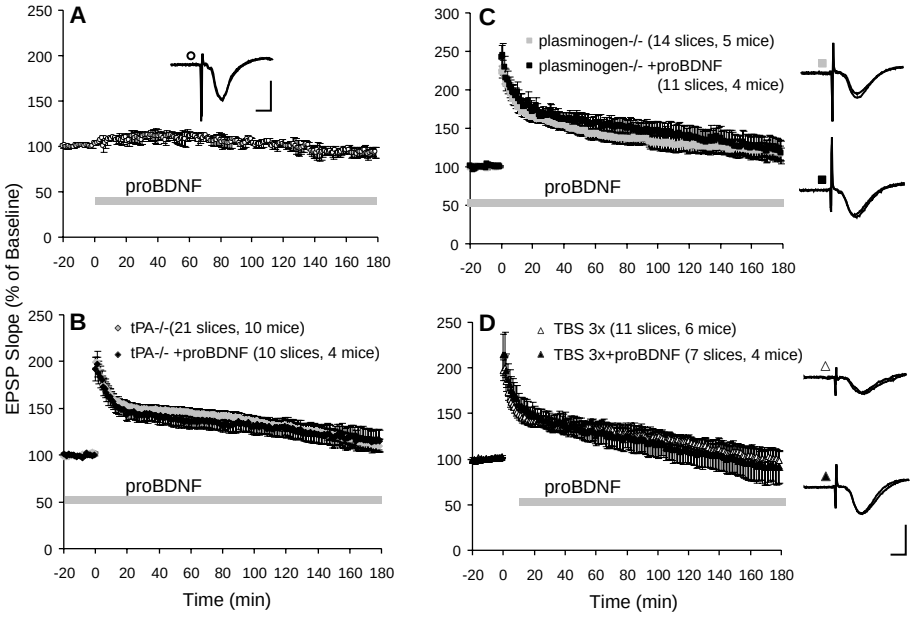

Fig. 2. Cleavage-resistant proBDNF does not mimic the role of mBDNF in L-LTP expression. (**A**) proBDNF has no effect on basal synaptic transmission ($n = 3$ slices; two mice). (**B**) proBDNF fails to rescue L-LTP in tPA$^{-/-}$ mice. (**C**) proBDNF fails to rescue L-LTP in plasminogen$^{-/-}$ mice. (**D**) proBDNF fails to convert E-LTP to L-LTP. s-TBS was applied to CA1 synapses of wild-type hippocampal slices. In (A) and (D), proBDNF (1 to 2 ng/ml) was perfused to the slices as indicated by the horizontal bars; in (B) and (C), proBDNF was applied to the slices for at least 60 min before the delivery of l-TBS.

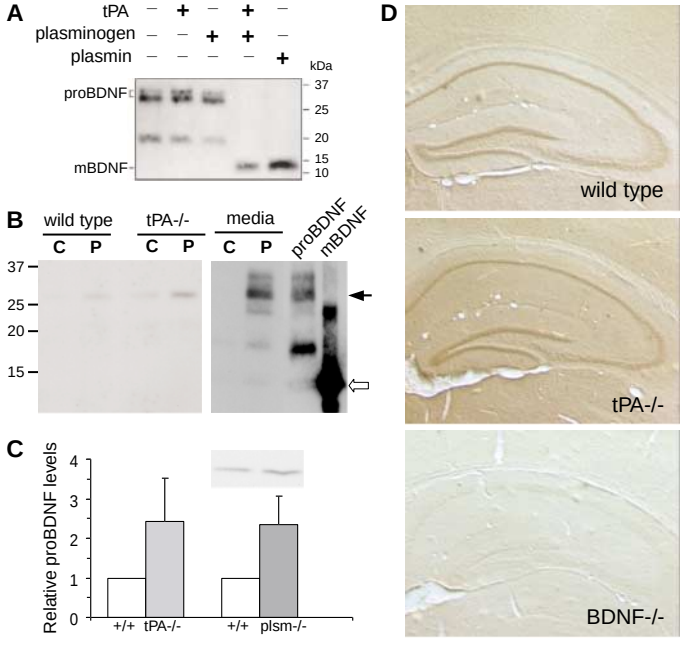

Fig. 3. Plasmin, but not tPA, converts proBDNF to mBDNF. (**A**) Protease cleavage of proBDNF in vitro. Recombinant proBDNF was incubated with tPA, plasmin, plasminogen, or plasminogen plus tPA as indicated. The cleaved products were probed on a Western blot with an antibody to mBDNF. Plasmin and plasminogen plus tPA, but not tPA alone, converted proBDNF to mBDNF. (**B**) Protease cleavage of proBDNF in the hippocampus. Left: Immunoprecipitation analysis shows that, relative to wild-type mice, substantially more proBDNF was detected in hippocampal tissues derived from tPA$^{-/-}$ mice (compare lanes 2 and 4). Right: Antibody specificity. Lysate (lane 7) and media from proBDNF-producing 293T cells (lanes 5 and 6) were immunoprecipitated with either control or specific antibody to proBDNF (366) followed by Western blotting with antibody to mBDNF. Recombinant mBDNF (20 ng) was included as a reference (lane 8). C, PBS coupled; P, proBDNF antibody coupled. (**C**) Summary of relative proBDNF levels in hippocampal tissues derived from tPA$^{-/-}$ and plasminogen$^{-/-}$ mice. (**D**) Protease cleavage of proBDNF in CA1 area. Immunohistochemistry was performed with an antibody specific for proBDNF. More proBDNF immunoreactivity was observed in hippocampal sections derived from tPA$^{-/-}$ mice as compared with those from wild-type mice. No immunoreactivity was detected in sections from BDNF$^{-/-}$ mice.

Plasmin is one of the few secreted extracellular proteases that effectively convert proBDNF to mBDNF through proteolytic cleavage in vitro (*12*). If the cleavage of proBDNF by plasmin was critical for BDNF regulation of L-LTP, a cleavage-resistant proBDNF should fail to rescue the L-LTP deficit seen in plasminogen$^{-/-}$ mice. This was indeed the case. Perfusion of cleavage-resistant proBDNF (1 to 2 ng/ml) was completely ineffective in rescuing the L-LTP deficit in plasminogen$^{-/-}$ mice (Fig. 2C, plasminogen$^{-/-}$, 118.4 ± 8.6; plasminogen$^{-/-}$ + proBDNF, 121.1 ± 14.4; $P = 0.866$). Perfusion of proBDNF also had no effect on basal synaptic transmission (Fig. 2A). Next, we tested the effect of proBDNF on tPA$^{-/-}$ slices. Again, proBDNF failed to rescue L-LTP in tPA mutants that are normally deficient in L-LTP (Fig. 2B, tPA$^{-/-}$, 110.4 ± 6.8; tPA$^{-/-}$ + proBDNF, 115.5 ± 11.4; $P = 0.689$). Finally, unlike mBDNF, application of proBDNF after s-TBS did not allow the expression of L-LTP (Fig. 2D, s-TBS, 99.9 ± 10.2; s-TBS + proBDNF, 91.2 ± 18.5; $P = 0.658$). To ensure that proBDNF at this concentration was biologically active, we performed two different types of assays. First, the same preparation of proBDNF at this concentration elicited a robust apoptosis in smooth muscle cells (*27*). Second, treatment of the slices with proBDNF (1 to 2 ng/ml) enhanced *N*-methyl-D-aspartate (NMDA) receptor–dependent long-term depression (LTD) (*28*).

To determine whether tPA and/or plasmin could directly cleave proBDNF, we performed biochemical analysis in vitro. Purified cleavable proBDNF was incubated with tPA (60 μg/ml), plasmin (20 μg/ml), plasminogen (18 μg/ml), or plasminogen plus tPA. The reaction products were subjected to Western blot with an antibody that detects both proBDNF and mature BDNF. Purified proBDNF exhibited a major doublet band of 30 kD as well as a minor band of 18 kD (Fig. 3A). Neither tPA nor plasminogen alone was capable of cleaving proBDNF in vitro. In contrast, treatment with plasmin converted virtually all proBDNF to mBDNF (Fig. 3A). Although tPA itself was unable to cleave proBDNF, tPA together with plasminogen

was as effective as plasmin in generating mBDNF; this finding suggests that tPA affects proBDNF cleavage indirectly by activating plasmin (Fig. 3A).

Next, we used semiquantitative immunoprecipitation analysis to examine whether the tPA/plasmin system affects proBDNF cleavage in vivo in the hippocampus. Hippocampal tissues from wild-type and $tPA^{-/-}$ mice were dissected. Equal amounts of hippocampal lysates (1.5 mg) were immunoprecipitated with beads coupled to either phosphate-buffered saline (PBS) or an antibody specific for proBDNF, followed by Western blot analysis with an antiserum to mBDNF. Relative to the wild-type control, increased levels of proBDNF in the hippocampi derived from $tPA^{-/-}$ mice were observed (Fig. 3B). Quantitative analysis showed a doubling of the amount of proBDNF in the $tPA^{-/-}$ tissues (Fig. 3C, $n = 3$ pairs of mice). A similar increase in the level of proBDNF was observed in hippocampi derived from $plasminogen^{-/-}$ mice (Fig. 3C, $n = 3$ pairs of mice). Next, to determine whether the proteolytic cleavage occurs in the CA1 region, we performed immunohistochemistry with an antibody specific for proBDNF. Side-by-side comparison revealed greater proBDNF immunoreactivity in the hippocampal sections from $tPA^{-/-}$ mice as compared to those from wild-type mice (Fig. 3D). As a negative control, sections from $BDNF^{-/-}$ mice did not exhibit any immunoreactivity (Fig. 3D). To ensure the reliability of the observation, we performed immunohistochemistry with a second, proBDNF-specific antibody. Again, more immunoreactivity was observed in $tPA^{-/-}$ sections (fig. S4). At a higher magnification, stronger proBDNF staining was seen in the apical dendrites of CA1 pyramidal neurons in $tPA^{-/-}$ mice (fig. S4, right panels). Similar results were obtained with three different pairs of $tPA^{+/+}$ and $tPA^{-/-}$ mice (Fig. 3D) (fig. S4).

Finally, we determined the sequential relationship among tPA, plasmin, and mBDNF in L-LTP expression. The expression and secretion of tPA in hippocampal neurons have been shown to be regulated by neuronal activity (*15*, *16*). Consistent with the extracellular action of tPA, application of tPA (500 ng/ml) to the $tPA^{-/-}$ slices completely reversed the L-LTP deficit (Fig. 4A, $tPA^{-/-}$, 110.4 ± 6.8; $tPA^{-/-}$ + tPA, 166.0 ± 8.1; $P < 0.001$). Exposure of the slices to plasmin for 1 hour (100 nM, from 30 min before to 30 min after l-TBS) also rescued L-LTP deficit in $tPA^{-/-}$ slices (Fig. 4B, $tPA^{-/-}$, 110.4 ± 6.8; $tPA^{-/-}$ + plasmin, 149.7 ± 5.0; $P < 0.001$). Shorter exposure was used to avoid damage of hippocampal neurons by plasmin (*29*). In contrast, application of tPA to $plasminogen^{-/-}$ slices had no effect (Fig. 4C, $plasminogen^{-/-}$, 118.4 ± 8.6; $plasminogen^{-/-}$ + tPA, 103.4 ± 9.4; $P = 0.297$). These results suggest that plasmin is downstream of tPA in controlling the expression of L-LTP. Similarly, mBDNF was able to rescue the L-LTP deficit in $tPA^{-/-}$ slices (Fig. 1E), but tPA failed to do the same in $BDNF^{+/-}$ slices (Fig. 4D, $BDNF^{+/-}$, 101.8 ± 8.6; $BDNF^{+/-}$ + tPA, 95.5 ± 10.9; $P = 0.658$); this result suggests that BDNF is downstream of tPA as well. Moreover, treatment of $plasminogen^{-/-}$ slices with mBDNF produced an excellent late-phase LTP (Fig. 1C), whereas treatment of $BDNF^{+/-}$ slices with plasmin did not rescue the L-LTP deficit (Fig. 4E, $BDNF^{+/-}$, 101.8 ± 8.6; $BDNF^{+/-}$ + plasmin, 108.3 ± 12.4; $P = 0.668$). Taken together, these results support the following model: tPA → plasmin → BDNF in controlling L-LTP.

Although conversion of precursor to mature neurotrophins by extracellular proteases has been shown in vitro (*12*), its physiological role in vivo remains to be established. Our results suggest that such conversion indeed occurs in the brain. We have identified tPA/plasmin as an endogenous extracellular enzyme system, expressed at the hippocampal synapses, that is capable of converting proBDNF to mBDNF. We have shown that tPA, through the activation of plasminogen, converts proBDNF to mBDNF in vitro, and that $tPA^{-/-}$ mice exhibit more proBDNF immunoreactivity in the hippocampus in vivo. Although direct evidence is missing that the tPA/plasmin system cleaves proBDNF extracellularly at the hippocampal synapses, several lines of evidence support this notion. First, most of

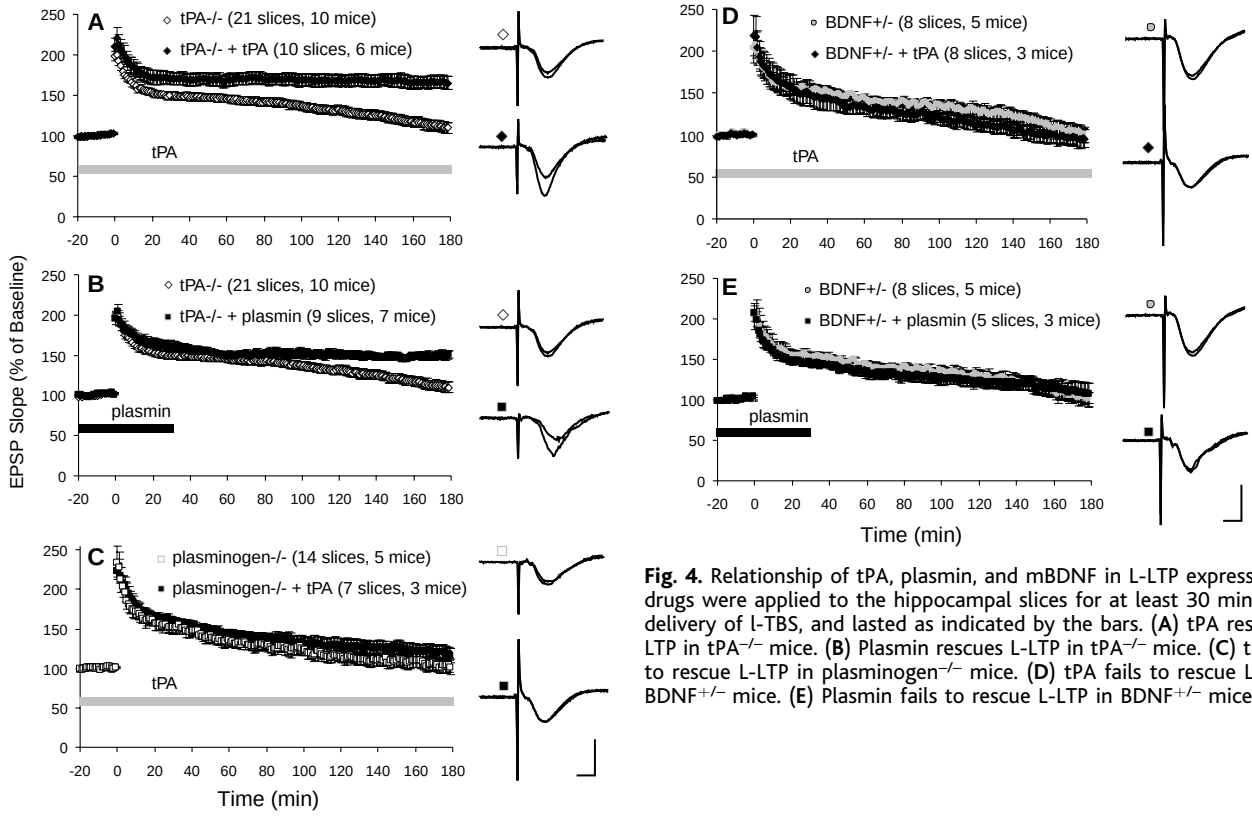

Fig. 4. Relationship of tPA, plasmin, and mBDNF in L-LTP expression. All drugs were applied to the hippocampal slices for at least 30 min before delivery of l-TBS, and lasted as indicated by the bars. (**A**) tPA rescues L-LTP in $tPA^{-/-}$ mice. (**B**) Plasmin rescues L-LTP in $tPA^{-/-}$ mice. (**C**) tPA fails to rescue L-LTP in $plasminogen^{-/-}$ mice. (**D**) tPA fails to rescue L-LTP in $BDNF^{+/-}$ mice. (**E**) Plasmin fails to rescue L-LTP in $BDNF^{+/-}$ mice.

the BDNF secreted by hippocampal neurons appears to be in the precursor form (*8*–*11*). Second, the secretion of proBDNF is activity dependent (*9*). Moreover, both tPA and plasmin are expressed and secreted at hippocampal synapses (*15*, *16*, *26*). It is conceivable that application of l-TBS induces the secretion of proBDNF or the activation of the tPA/plasmin system (or both) at the CA1 synapses, and that subsequent conversion of proBDNF to mBDNF plays an essential role in L-LTP expression.

BDNF and tPA are perhaps the two best-characterized secretory molecules involved in L-LTP and long-term memory. Our study provides a mechanistic link between these two seemingly independent molecule systems in L-LTP expression. We have also worked out the sequential relationship among tPA, plasmin, and mBDNF. Thus, the extracellular protease tPA cleaves plasminogen to generate plasmin, which in turn converts the precursor proBDNF to mBDNF. A new mechanism that regulates late-phase LTP could be the activity-dependent extracellular cleavage of proBDNF. Given that proBDNF preferentially activates pan neurotrophin receptor p75NTR over TrkB receptor, it is also possible that the inhibition of proteolytic conversion of proBDNF may lead to a negative regulation of hippocampal plasticity.

mBDNF applied after the delivery of l-TBS appears to be sufficient to maintain L-LTP in slices incubated in the protein synthesis inhibitor anisomycin (40 μM) during the entire course of recording (Fig. 1B). At this concentration of anisomycin, all protein synthesis is completely blocked. Consistent with this finding, perfusion of mBDNF after the application of the E-LTP–inducing s-TBS converts E-LTP to L-LTP (Fig. 1C).

It has long been believed that L-LTP and long-term memory requires new protein synthesis, but the specific product(s) mediating the long-term changes is not known. Our results imply that mBDNF is a key protein synthesis product, if not the only one, needed to carry on all the necessary functions for long-term modification of hippocampal synapses. L-LTP involves long-lasting changes in the structure and function of the hippocampal synapses. BDNF elicits long-lasting enhancement of synaptic transmission (*30*, *31*), promotes dendritic arborization (*32*), and stimulates the growth of dendritic spines (*33*). Activity-dependent BDNF synthesis has been shown with the use of L-LTP–inducing tetanus (*34*). The key function of the L-LTP–inducing tetanus could be to induce the synthesis and/or processing of proBDNF, and the newly generated mBDNF would then be responsible for eliciting all the structural and functional changes underlying L-LTP at the CA1 synapses.

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27. The ability of proBDNF to induce apoptosis in p75-expressing vascular smooth muscle cells was assessed as described (*12*) using terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling (TUNEL) detection. Treatment of cells with proBDNF (2 ng/ml) resulted in 11.2 ± 1.8% TUNEL$^+$ cells, whereas the vehicle-treated cells exhibited only 2.1 ± 1.1% TUNEL$^+$ cells.
28. Although application of proBDNF failed to affect basal transmission and L-LTP, it facilitated NMDA receptor–dependent LTD in the hippocampus. In 8-week-old, wild-type hippocampal slices, LTD induced by low-frequency stimulation (LFS; 1 Hz, 15 min) was significantly higher (29.5 ± 2.4%, $n = 11$) in slices treated with proBDNF as compared to untreated slices (8.0 ± 6.6%, $n = 10$) ($P < 0.01$).
29. Plasmin is known to degrade a variety of substrates, including the extracellular matrix proteins laminin, fibrin, and fibronectin; such degradation leads to cell death. In our own work, we found that plasmin caused detrimental effects to L-LTP in wild-type slices if treated more than 2 hours. In contrast, a short-term exposure was without effect. Thus, to avoid the damaging effect of long-term plasmin exposure, we used a protocol that treated the slices for only 60 min. As indicated in Fig. 4C, this short-term treatment was sufficient to rescue the L-LTP defects.
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Supporting Online Material
www.sciencemag.org/cgi/content/full/306/5695/487/DC1
Materials and Methods
Figs. S1 to S4
References

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Molecular Architecture of the KvAP Voltage-Dependent K$^+$ Channel in a Lipid Bilayer

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We have analyzed the local structure and dynamics of the prokaryotic voltage-dependent K$^+$ channel (KvAP) at 0 millivolts, using site-directed spin labeling and electron paramagnetic resonance spectroscopy. We show that the S4 segment is located at the protein/lipid interface, with most of its charges protected from the lipid environment. Structurally, S4 is highly dynamic and is separated into two short helices by a flexible linker. Accessibility and dynamics data indicate that the S1 segment is surrounded by other parts of the protein. We propose that S1 is at the contact interface between the voltage-sensing and pore domains. These results establish the general principles of voltage-dependent channel structure in a biological membrane.

Voltage-dependent channels are composed of two functionally linked but structurally independent domains (*1*–*4*). The pore domain is responsible for ion selectivity and contains the channel gate, whereas a voltage-sensing domain (segments S1 to S4) alters the conformation of the gate in response to changes in transmembrane voltage. Crystal structures of