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17. The 20 patients with Parkinson's disease achieved 3.7 categories (maximum = 6) on the Wisconsin Card Sorting Test (WCST) with an average of 19.2% perseverative errors, that is, errors that would have been correct responses in the previous phase of the test [R. K. Heaton, G. Chelune, J. Talley, G. Kay, G. Curtiss, *Wisconsin Card Sorting Test Manual* (Psychological Assessment Resources, Odessa, FL, 1993)]. Individuals from the Heaton *et al.* normative sample (n = 169, age = 50 to 79 years) achieved 4.6 ± 0.1 categories correct with 16.1 ± 0.7% perseverative errors.
18. The patients with frontal lobe lesions (six men and four women) averaged 68 years of age (range, 62 to 76 years) and 13.7 years of education. Six had sustained left frontal lesions, three had sustained right frontal lesions, and one had bilateral frontal lobe lesions. On the WCST (*17*), they achieved 3.6 categories and made 32.8% perseverative errors, marginally more than the Parkinson patients, $t(28) = 1.76$, $P = 0.09$. For reconstructions of the frontal lesions and examples of their impaired performance on other tests, see J. S. Janowsky, A. P Shimamura, M. Kritchevsky, L. R. Squire, *Behav. Neurosci.* **103**, 548 (1989) (patients JD, MD, JV); A. P. Shimamura, P. J. Jurica, J. A. Mangels, F. B. Gershberg, R. T. Knight, *J. Cognit. Neurosci.* **7**, 144 (1995) (patients EB, AL, MM, RM); J. A. Mangels, F. B. Gershberg, A. P. Shimamura, R. T. Knight, *Neuropsychology* **10**, 32 (1996) (patients OA, JD); L. L. Chao and R. T. Knight, *Neuroreport* **6**, 1605 (1995) (patient JC).
19. In addition, analysis of variance (severe PD patients compared with frontal patients and three 50-trial blocks: trials 1 through 50, 51 through 100, and 101 through 150) revealed a significant group x trial block interaction [$F(2,34) = 3.52$, $P < 0.04$]. The interaction resulted from the fact that the frontal patients, like the amnesic patients and controls, scored about the same overall in each of the three 50-trial blocks (frontals: 60.5, 56.5, and 66.1%; amnesics: 58.5, 61.2, and 59.2%; controls: 65.9, 67.3, and 66.1%). In contrast, the severely affected PD patients scored 47.8% correct (at chance) on trials 1 through 50, 58.2% on trials 51 through 100, and 61.1% on trials 101 through 150.
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21. Also see A. M. Owen *et al.*, *Brain* **116**, 1159 (1993); A. M. Owen *et al.*, *Neuropsychology* **9**, 126 (1995). Despite these differences between the effects on cognition of frontal lobe lesions and Parkinson's disease, the cognitive impairment in Parkinson's disease presumably arises from the effect that neostriatal lesions exert on the targets of the basal ganglia, which include frontal cortex.
22. The PD patients who performed poorest on the classification task (percent correct score during trials 1 through 50) also obtained the highest Hoehn and Yahr Scale scores of symptom severity (*9*) correlation coefficient, $r = -0.55$, $P < 0.02$). In contrast, in the case of the frontal patients, performance on the first 50 trials of the classification test was slightly and nonsignificantly better for those patients with the most severe frontal symptoms [measured by the number of categories achieved correctly on the WCST (*17*) and by the percent perseverative error score, $r = -0.14$ and $r = 0.22$, respectively]. Interestingly, for the PD patients, poor classification learning also correlated with frontal lobe symptoms ($r = 0.63$, $P < 0.01$ for categories; $r = -0.69$, $P < 0.01$ for perseverative errors), as would be expected if increasing frontal lobe dysfunction reflects the progression of the primary disease in the neostriatum. However, the overall pattern of correlations suggests that the neostriatal symptoms, not the frontal lobe symptoms, best predicted classification learning. This conclusion depends on the assumption that frontal lobe dysfunction in the PD patients was no more severe than in the patients with frontal lobe lesions. The neuropsychological findings (*17*, *18*) are consistent with this idea.
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24. Patients with Huntington's disease also did not learn the probabilistic classification task (B. J. Knowlton *et al.*, *Neuropsychology,* in press). However, it is difficult to isolate this deficit to the basal ganglia because of the dementia and widespread neuropathology associated with Huntington's disease.
25. We thank J. Zouzounis and J. Moore for research assistance, A. Shimamura for referral of patients with frontal lobe lesions, and C. Shultz for advice and referral of patients with Parkinson's disease. Supported by the Medical Research Service of the Department of Veterans Affairs, the National Institute of Mental Health (NIMH) (grant MH24600), and an NIMH postdoctoral fellowship (B.J.K.).

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A Requirement for Local Protein Synthesis in Neurotrophin-Induced Hippocampal Synaptic Plasticity

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Two neurotrophic factors, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), are able to produce a long-lasting enhancement of synaptic transmission in the hippocampus. Unlike other forms of plasticity, neurotrophin-induced plasticity exhibited an immediate requirement for protein synthesis. Plasticity in rat hippocampal slices in which the synaptic neuropil was isolated from the principal cell bodies also required early protein synthesis. Thus, the neurotrophins may stimulate the synthesis of proteins in either axonal or dendritic compartments, allowing synapses to exert local control over the complement of proteins expressed at individual synaptic sites.

The cellular changes that underlie both synaptic and behavioral plasticity are usually classified as either (i) short term, because they are based on the modification of preexisting proteins, or (ii) long term, because they require protein synthesis. For example, studies of synaptic plasticity in the hippocampus and in *Aplysia* have shown that, whereas the short-term phase (0 to 1 hour) of synaptic enhancement is not blocked by inhibitors of protein translation, the long-term phase (>1 hour) is [(*1*), but see (*2*)]. These cellular studies are paralleled by many studies of behavioral plasticity that also indicate that short-term memories are insensitive to inhibitors of protein synthesis (*3*). The neurotrophic factors BDNF and NT-3 can enhance synaptic efficacy (*4*), and we have now examined the temporal sensitivity of the neurotrophin-induced synaptic enhancement to inhibitors of protein synthesis.

Synaptic transmission was examined at the Schaffer collateral–CA1 pyramidal neuron synapse in adult rat hippocampal slices with the use of conventional extracellular recording techniques (*5*). In control experiments, extracellular application of BDNF (50 ng/ml) or NT-3 (50 ng/ml) elicited a robust enhancement of synaptic transmission (Fig. 1, A and B) (*4*) [mean percent of baseline: BDNF, 221.4 ± 16.4 (mean ± SEM, n = 7), $P < 0.005$; NT-3, 231.1 ± 19.5 (n = 8), $P < 0.005$]. Pretreatment with one of two protein synthesis inhibitors (*6*), either anisomycin (40 μM) or cycloheximide (40 μM), markedly attenuated the synaptic enhancement induced by either neurotrophin (Fig. 1, C through F and H) [mean percent of baseline: BDNF plus anisomycin, 134.2 ± 8.4 (n = 9), $P < 0.05$; BDNF plus cycloheximide, 138.7 ± 13.2 (n = 7), $P < 0.05$; NT-3 plus anisomycin, 130.1 ± 7.6 (n = 9), $P < 0.05$; NT-3 plus cycloheximide, 118.5 ± 14.0 (n = 7), not significant (NS)]. In contrast to previous studies of synaptic plasticity, the sensitivity to inhibitors of protein synthesis was evident within minutes of neurotrophin application (Fig. 1, C through F). Similar pretreatment of hippocampal slices with an inhibitor of prokaryotic protein synthesis, chloramphenicol (80 μM), did not significantly reduce the synaptic enhancement

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induced by either BDNF or NT-3 (Fig. 1H) [mean percent of baseline: BDNF plus chloramphenicol, 216.2 ± 6.7 (n = 5), P < 0.005; NT-3 plus chloramphenicol, 250.8 ± 32.6 (n = 5), P < 0.005].

This early requirement for protein synthesis is temporally inconsistent with the time necessary for somatic synthesis and transport of proteins to synaptic sites in pyramidal neurons (*7*). A potential source of protein synthesis closer to synaptic sites has been described in hippocampal pyramidal cells (*8*). To investigate whether the early requirement for protein synthesis resulted from synthesis independent of somatic protein translation machinery, we isolated the synaptic regions from either CA3 (Fig. 2, A and B) or CA1 (Fig. 3, A and B) cell bodies with the use of a microlesion (*9*). Previous studies have indicated that synaptic transmission and short-term plasticity can be recorded in similarly lesioned hippocampal slices (*10*). To control for general damage associated with the lesion, we conducted experiments in which a sham lesion was made in the dentate gyrus region of the hippocampal slice (9). The extracellular application of BDNF or NT-3 potentiated synaptic transmission in the sham-lesioned slices to a similar extent to that observed in unlesioned slices (Fig. 2, C and D) [mean percent of baseline: BDNF, 237.6 ± 35.5 (n = 7), P < 0.005; NT-3, 216.3 ± 27.0 (n = 6), P < 0.01].

To address the possibility that the protein synthesis inhibitor–sensitive compartment resides in the presynaptic cell bodies, we isolated the CA3 cell bodies from the stratum radiatum (Fig. 2, A and B). In CA3-isolated slices, both neurotrophins continued to enhance synaptic strength (Fig. 2, C and D) [mean percent of baseline: BDNF, 230.7 ± 26.2 (n = 7), P < 0.001; NT-3, 211.33 ± 22.3 (n = 6), P <0.005]. In CA3-isolated slices pretreated with anisomycin, however, the enhancement produced by BDNF or NT-3 was markedly reduced (Fig. 2, C and D) [mean percent of baseline: BDNF plus anisomycin, 109.5 ± 8.3 (n = 5), NS; NT-3 plus anisomycin, 115.9 ± 15.7 (n = 5), NS]. This continued sensitivity to a protein synthesis inhibitor in the absence of CA3 somata indicated that the relevant protein translation machinery did not reside in the presynaptic cell bodies.

We next addressed the potential contribution of postsynaptic somatic protein synthesis by dissociating CA1 somata from the synaptic region in the stratum radiatum. The CA1 lesion was placed just beyond the apical boundary of the pyramidal cell layer (Fig. 3, A and B). We confirmed the complete dissociation of CA1 cell bodies from their dendrites by cresyl violet staining (Fig. 3B) and by placing a recording electrode in the cell body region and attempting to record a synaptic response to stimulation of the Schaffer collaterals. In no such instance did we detect any synaptic response. The remaining isolated neuronal cell bodies present in the synaptic neuropil were likely interneurons, which have been shown by immunocytochemistry and in situ hybridization to contain glutamic acid decarboxylase (*11*).

Excitatory synaptic responses recorded in slices with isolated CA1 somata still exhibited significant potentiation on exposure to either BDNF or NT-3 (Fig. 3, C and D) [mean percent of baseline: BDNF, 182.1 ±

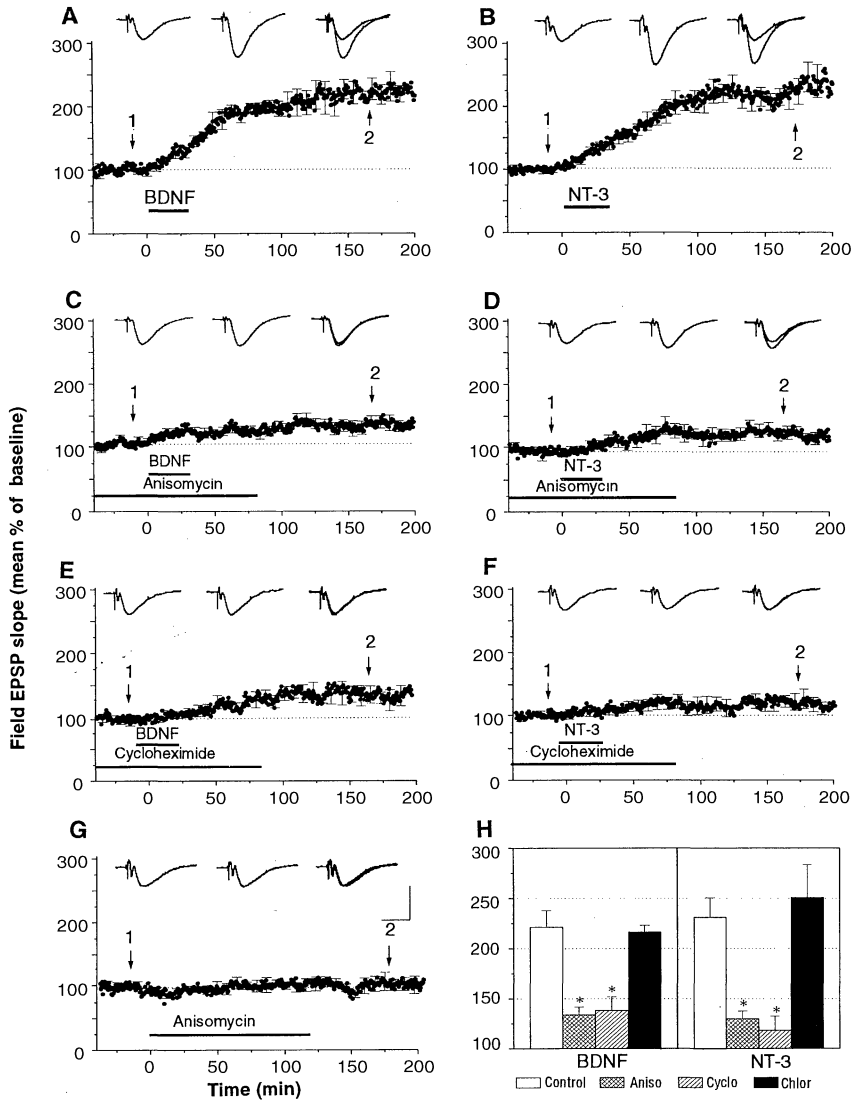

Fig. 1. Attenuation of neurotrophin-induced synaptic plasticity by inhibitors of protein synthesis. (**A** and **B**) Ensemble averages for control experiments in which application of BDNF (A) or NT-3 (B) (50 ng/ml) induced a rapid and persistent enhancement of synaptic strength. Mean field excitatory postsynaptic potential (EPSP) slope was 0.11 ± 0.01 mV/ms (mean ± SEM) before and 0.24 ± 0.02 mV/ms after BDNF, and 0.12 ± 0.01 mV/ms before and 0.27 ± 0.02 mV/ms after NT-3. Two representative field EPSPs and their superimposition are shown for the time points (arrows labeled 1 and 2) indicated. (**C** and **D**) Pretreatment of hippocampal slices with anisomycin attenuated the synaptic enhancement induced by BDNF (C) or NT-3 (D). Mean field EPSP slope was 0.13 ± 0.01 mV/ms before and 0.17 ± 0.02 mV/ms after BDNF, and 0.12 ± 0.01 mV/ms before and 0.16 ± 0.01 mV/ms after NT-3. (**E** and **F**) Pretreatment of hippocampal slices with cycloheximide attenuated the synaptic enhancement induced by BDNF (E) or NT-3 (F). Mean field EPSP slope was 0.13 ± 0.01 mV/ms before and 0.18 ± 0.02 mV/ms after BDNF, and 0.14 ± 0.01 mV/ms before and 0.16 ± 0.03 mV/ms after NT-3. (**G**) Control experiment demonstrating that anisomycin had no effect on basal synaptic strength. Similar results were obtained with cycloheximide. Mean field EPSP slope was 0.12 ± 0.01 mV/ms before and 0.13 ± 0.02 mV/ms after anisomycin, and 0.18 ± 0.01 mV/ms before and 0.20 ± 0.02 mV/ms after cycloheximide. Calibration bars [for (A) through (G)], 1 mV and 20 ms. (**H**) Summary of the percent enhancement of mean field EPSP slopes shown in (A) through (F) for control, anisomycin (Aniso), and cycloheximide (Cyclo), as well as for chloramphenicol (Chlor). *P < 0.05 versus control group.

12.7 (n = 8), $P < 0.005$; NT-3, 182.3 ± 9.5 (n = 6), $P < 0.005$]. The enhancement observed in these slices, however, was slightly, but significantly, smaller than that observed in sham-lesioned slices ($P < 0.05$). Preexposure of the CA1-isolated slices to anisomycin again attenuated the enhancement induced by either BDNF or NT-3 (Fig. 3, C and D) [mean percent of baseline: BDNF plus anisomycin, 119.7 ± 8.0 (n = 6), NS; NT-3 plus anisomycin, 119.6 ± 9.5 (n = 6), NS].

The results of the CA3 and CA1 isolation experiments indicate that neurotrophin-induced synaptic potentiation required protein synthesis at sites distinct from the pyramidal neuron cell bodies (*12*). To rule out the possibility that the site of protein synthesis changed systematically as a result of the lesioned area, we simultaneously isolated both pre- and postsynaptic cell bodies

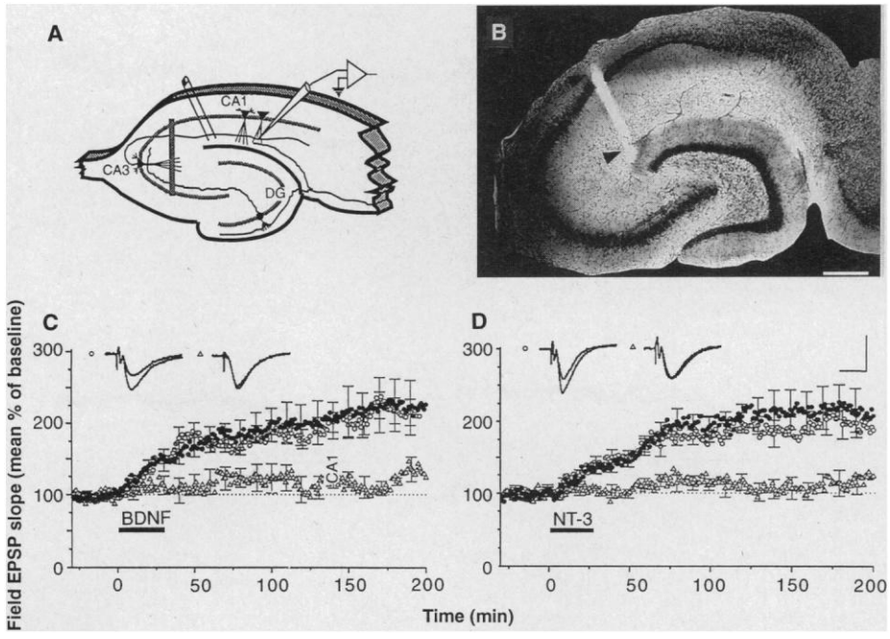

Fig. 2. Requirement of protein synthesis for neurotrophin-induced enhancement at synapses isolated from the presynaptic pyramidal cell somata. (**A**) Schematic representation of a hippocampal slice showing the placement of a microlesion to isolate CA3 cell bodies from their axons and the CA1 dendritic area, where electrophysiological recordings were made. DG, dentate gyrus. (**B**) Representative cresyl violet–stained hippocampal slice after the isolation of the CA3 cell somata from the synaptic region. Arrowheads indicate the site of the lesion. Scale bar, 550 μm. (**C** and **D**) Filled circles indicate the control enhancement obtained in sham-lesioned slices after the application of BDNF (C) or NT-3 (D). Mean field EPSP slope was 0.12 ± 0.01 mV/ms (mean ± SEM) before and 0.28 ± 0.04 mV/ms after BDNF, and 0.14 ± 0.01 mV/ms before and 0.29 ± 0.03 mV/ms after NT-3. Open circles indicate the enhancement obtained in CA3 somata–isolated slices. BDNF (C) and NT-3 (D) enhanced synaptic transmission to a similar extent as that observed in sham-lesioned slices. Mean field EPSP slope was 0.11 ± 0.01 mV/ms before and 0.25 ± 0.03 mV/ms after BDNF, and 0.13 ± 0.01 mV/ms before and 0.28 ± 0.02 mV/ms after NT-3. Open triangles show that pretreatment of the slices with anisomycin prevented the neurotrophin-induced plasticity at CA3 somata–isolated synapses. Mean field EPSP slope was 0.13 ± 0.02 mV/ms before and 0.14 ± 0.03 mV/ms after BDNF, and 0.16 ± 0.01 mV/ms before and 0.18 ± 0.03 mV/ms after NT-3. Superimposed representative EPSPs were recorded 5 min before and 3 hours after the application of neurotrophin. Calibration bars, 1 mV and 20 ms.

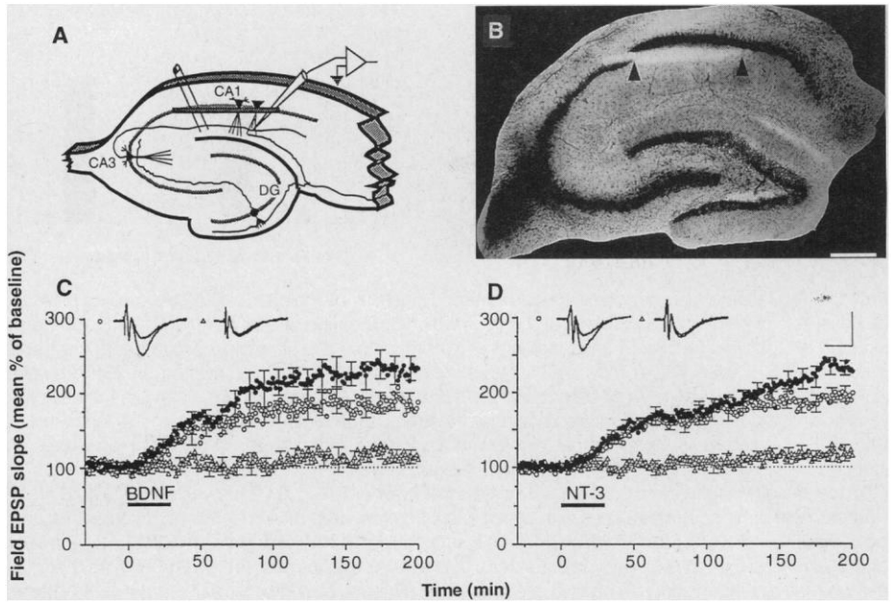

Fig. 3. Requirement of protein synthesis for neurotrophin-induced enhancement at synapses isolated from the postsynaptic pyramidal cell somata. (**A**) Schematic representation of a hippocampal slice showing the placement of a microlesion to isolate CA1 cell bodies from their dendrites, where electrophysiological recordings were made. (**B**) Representative cresyl violet–stained hippocampal slice after the isolation of the CA1 cell somata from the synaptic region. Arrowheads indicate the site of the lesion. Scale bar, 550 μm. (**C** and **D**) Filled circles indicate the control enhancement obtained in sham-lesioned slices after the application of BDNF (C) or NT-3 (D). Mean field EPSP slope was 0.11 ± 0.01 mV/ms (mean ± SEM) before and 0.26 ± 0.02 mV/ms after BDNF, and 0.13 ± 0.01 mV/ms before and 0.29 ± 0.01 mV/ms after NT-3. Open circles indicate the enhancement obtained in CA1 somata–isolated slices. BDNF (C) and NT-3 (D) enhanced synaptic transmission at synapses isolated from the postsynaptic cell bodies, although the magnitude of enhancement was slightly less than that observed in sham-lesioned slices. Mean field EPSP slope was 0.10 ± 0.01 mV/ms before and 0.17 ± 0.02 mV/ms after BDNF, and 0.10 ± 0.02 mV/ms before and 0.18 ± 0.03 mV/ms after NT-3. Open triangles show that pretreatment of the slices with anisomycin prevented the neurotrophin-induced plasticity at CA1 somata–isolated synapses. Mean field EPSP slope was 0.09 ± 0.01 mV/ms before and 0.11 ± 0.01 mV/ms after BDNF, and 0.10 ± 0.01 mV/ms before and 0.12 ± 0.01 mV/ms after NT-3. Superimposed representative EPSPs were recorded 5 min before and 3 hours after the application of neurotrophin. Calibration bars, 1 mV and 20 ms.

(Fig. 4A). In such slices, application of BDNF or NT-3 enhanced synaptic strength (Fig. 4, B and C) [mean percent of baseline: BDNF, 193.5 ± 6.7 (n = 8), $P < 0.001$; NT-3, 192.8 ± 12.4 (n = 6), $P < 0.001$]. Moreover, pretreatment of slices with anisomycin prevented the neurotrophin-induced enhancement (Fig. 4, B and C) [mean percent of baseline: BDNF plus anisomycin, 122.0 ± 7.0 (n = 6), NS; NT-3 plus anisomycin, 116.4 ± 9.1 (n = 5), NS]. The persistence of anisomycin sensitivity in slices isolated from both pre- and postsynaptic cell bodies indicates that the neurotrophins made use of protein synthesis machinery in the synaptic neuropil of the hippocampal slice.

The presumptive local site of protein synthesis could reside in axons or dendrites of pyramidal neurons, or in neighboring interneurons or glia. Although hippocampal interneurons may express TRK receptors (*13*), the synaptic enhancement induced by the neurotrophins does not require inhibitory transmission mediated by γ-aminobutyric acid type A ($GABA_A$) receptors (*14*). Thus, proteins synthesized in interneurons would presumably have to influence excitatory synaptic transmission by non–$GABA_A$ receptor–mediated diffusible signaling. Likewise, the potential involvement of glial protein synthesis would also require a diffusible signal. Astrocytes in the CA1 region, however, do not appear to express full-length TRKB or TRKC receptors (*15*), making glial participation unlikely. These data are most consistent with the hypothesis that the neurotrophins stimulate local protein synthesis within the pyramidal neurons themselves. The mRNAs encoding both TRKB and TRKC have been detected in both the presynaptic CA3 and the postsynaptic CA1 neurons (*16*). Although some mRNA species have been detected in axons (*17*), no protein synthesis has been detected in hippocampal axons (*18*). Thus, the most likely site of neurotrophin-induced protein synthesis is the dendrites of CA1 pyramidal neurons. Ultrastructural, in situ hybridization, and single-cell polymerase chain reaction techniques have revealed that both polyribosomes (*8*) and mRNAs (*19*) are present in CA1 dendrites, often associated with individual postsynaptic spines. Moreover, protein synthesis has been detected in synaptosomes (*20*), isolated axonal and dendritic fractions (*18*), and hippocampal slices exposed to synaptic stimulation and carbachol (*7*) or *N*-methyl-D-aspartate or nitric oxide (*21*).

Application of anisomycin had no detectable effect on basal synaptic transmission (Fig. 1G) or short-term synaptic plasticity (*22*). Basal levels of protein synthesis in hippocampal dendrites are low and completely blocked by chloramphenicol, but are not affected by anisomycin or cycloheximide (*7*). Moreover, anisomycin did not affect the abundance of the TRKB protein or its phosphorylation induced by BDNF (*23*). These observations argue against the interpretation that anisomycin reduces the amounts of locally synthesized proteins that are necessary for signal transduction by BDNF or NT-3.

Our data suggest that BDNF and NT-3 stimulate the local synthesis of proteins that are required for the induction of synaptic enhancement (*24*). In the hippocampus, neurotrophins stimulate TRK phosphorylation (*25*) and increase intracellular Ca^{2+} concentrations (*26*); these same signaling events may be coupled to protein kinase activities (*27*) to stimulate protein synthesis. The newly synthesized proteins may act locally to enhance postsynaptic responsiveness or may communicate with the presynaptic terminal to increase neurotransmitter release (*4*, *28*). During developmental and adult plasticity, the regulated release (*29*) of neurotrophins and consequent stimulation of local protein synthesis may permit the site-specific modification of synaptic transmission (*4*) and structure (*30*).

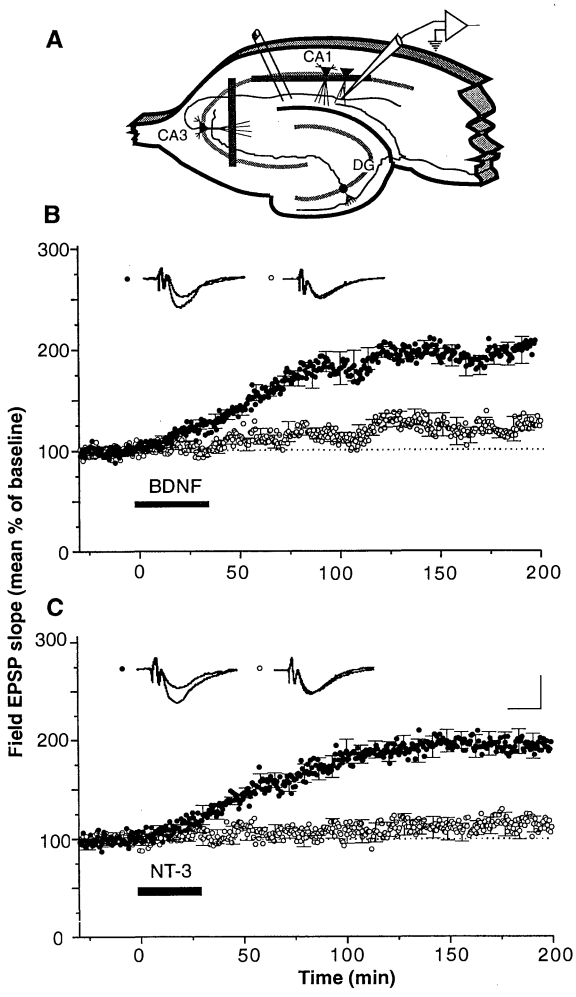

Fig. 4. Requirement of protein synthesis for neurotrophin-induced enhancement at synapses isolated from both pre- and postsynaptic pyramidal cell somata. (**A**) Schematic representation of a hippocampal slice showing the placement of two microlesions to isolate the pre- and postsynaptic cell bodies from the synaptic region of the slice, where electrophysiological recordings were made. (**B** and **C**) Filled circles indicate enhancement of synaptic transmission by BDNF (B) or NT-3 (C) at synapses isolated from both pre- and postsynaptic cell bodies. Mean field EPSP slope was 0.09 ± 0.01 mV/ms (mean ± SEM) before and 0.17 ± 0.01 mV/ms after BDNF, and 0.11 ± 0.01 mV/ms before and 0.19 ± 0.01 mV/ms after NT-3. Open circles show that the neurotrophin-induced plasticity in synaptically isolated slices required protein synthesis. Mean field EPSP slope was 0.13 ± 0.01 mV/ms before and 0.14 ± 0.01 mV/ms after BDNF, and 0.11 ± 0.01 mV/ms before and 0.12 ± 0.01 mV/ms after NT-3. Superimposed representative EPSP traces were recorded from control and anisomycin-treated slices 5 min before and 3 hours after the application of neurotrophin. Calibration bars, 1 mV and 20 ms.

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5. Hippocampal slices (thickness, 500 μm) were pre-

pared from young adult male Sprague-Dawley rats. Slices were submerged in a stream (flow rate, 210 ml/hour) of artificial cerebrospinal fluid (ACSF) (119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 11 mM glucose) maintained at room temperature (22° to 25°C) and gassed with 95% O_2 and 5% CO_2. The initial slope (1 to 2 ms) of field excitatory postsynaptic potentials (EPSPs) evoked by stimulation of the Schaffer collateral–commissural afferents (once every 15 s) was measured in the stratum radiatum at a depth of 100 to 150 μm below the slice surface. The application and storage of the neurotrophic factors were as previously described (*4*). Anisomycin and cycloheximide were maintained as 40 mM stock solutions in ethanol at 4°C. The final concentration (0.1%) of ethanol to which the slices were exposed had no detectable effect on synaptic transmission. All experiments with protein synthesis inhibitors were paired with a same-day control experiment in which BDNF or NT-3 potentiated synaptic transmission. Inhibitors were applied at least 30 min before the addition of neurotrophin. BDNF and NT-3 potentiated synaptic strength at least 30% in 84.4 and 83.7% of control experiments, respectively. The percent of baseline measurements indicated in the text were obtained 170 to 180 min after the application of neurotrophin, unless otherwise noted. Ensemble average plots represent group means of each EPSP slope, for all experiments, aligned with respect to the time of neurotrophin application. Statistical significance was assessed by paired *t* tests or one-way analysis of variance; a *P* value of < 0.05 was considered statistically significant.

6. In vitro assays of protein synthesis inhibition in hippocampal slices were performed basically as previously described (*2*). Slices were individually maintained in 200 μl of ACSF in a 24-well tissue culture dish for 1 hour at room temperature. Either anisomycin (40 μM) or cycloheximide (40 μM) was added to each well, and, after 30 min, [^{3}H]leucine (20 μCi/ml) was introduced and the slice was incubated for an additional hour. The inhibitor and [^{3}H]leucine were washed out with ice-cold ACSF and the slices were homogenized. Protein synthesis was measured by incorporation of [^{3}H]leucine into trichloroacetic acid–precipitable material. When compared to controls, anisomycin and cycloheximide inhibited protein synthesis by 71.9 ± 5.2 and 71.2 ± 6.0%, respectively ($n = 4$ for each inhibitor).
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CRINKLY4: A TNFR-Like Receptor Kinase Involved in Maize Epidermal Differentiation

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The maize *crinkly4* (*cr4*) mutation affects leaf epidermis differentiation such that cell size and morphology are altered, and surface functions are compromised, allowing graft-like fusions between organs. In the seed, loss of *cr4* inhibits aleurone formation in a pattern that reflects the normal progression of differentiation over the developing endosperm surface. The *cr4* gene was isolated by transposon tagging and found to encode a putative receptor kinase. The extracellular domain contains a cysteine-rich region similar to the ligand binding domain in mammalian tumor necrosis factor receptors (TNFRs) and seven copies of a previously unknown 39–amino acid repeat. The results suggest a role for *cr4* in a differentiation signal.

The surface of plant organs is defined by a specialized epidermal cell layer. The leaf epidermis has essential functions in development, gas exchange, water retention, and defense against pathogens. In grass seeds, the endosperm contains an epidermis-like layer called aleurone, which is an important source of hydrolytic enzymes required for remobilization of stored starch and protein during germination. The recessive *cr4* mutation of maize affects the differentiation of both epidermis and aleurone. It was identified in a line containing *Mutator* transposable elements and mapped to chromosome 10S (*1*).

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