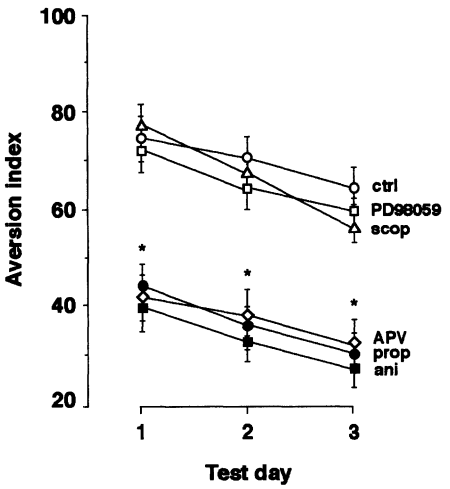

Fig. 4. Effect of the specific inhibitors on CTA to a familiar taste. Rats drank saccharin for 2 days, 10 min per day, instead of water. Three days later they were subjected to CTA training with saccharin as the conditioned stimulus (*14*). The drugs were microinjected into the IC 20 min before the exposure of the animals to the familiar taste in the CTA training session; ani (filled squares), anisomycin (100 μg); prop (filled circles), propranolol (20 μg); APV (diamonds), (10 μg); scop (triangles), scopolamine (50 μg); PD98059 (open squares), (8 ng); ctrl (open circles), control, vehicle only; $n = 10$ each.

depletion of catecholamines in brain, render classical conditioning of the nictitating membrane reflex in the rabbit resistant to extinction (*24*). The observation that β-receptors are obligatory for all the types of learning situations in our protocols is in accordance with the prominent role of these receptors in consolidation in the mammalian brain (*25*).

It has recently been reported that in fear conditioning in the amygdala, inhibition of protein synthesis immediately after retrieval extinguishes the fear behavior (*26*). We show here that in CTA, a similar treatment in the IC strengthens the trace rather than extinguishing it. The effect of protein synthesis inhibition in retrieval on the fate of the trace might hence be task- or region-dependent.

In conclusion, we show that extinction of long-term CTA memory is subserved by the same brain region that subserves the acquisition and consolidation of that same memory. Further, we identify in the IC essential core elements of the molecular machinery of learning, with other obligatory elements added according to the stimulus dimension and context. This means, among others, that the cortex honors at the molecular level the distinction between learning anew and learning the new.

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15. Male Wistar rats (~60 days old, 200 to 250 g) were caged individually at 22° ± 2°C. The behavioral protocol detailed in (*8*) was used. Sodium saccharin (0.1% w/v) was the conditioned stimulus, and LiCl ip (0.15 M, 2% body weight) was the toxicosis-inducing agent. Rats were pretrained to get their water ration once a day for 10 min from a pipette containing 10 ml of tap water. On the conditioning day, they were allowed to drink the saccharin solution instead of water for 10 min, and 40 min after the offset of the drinking period, they were injected with LiCl ip. Three days after training, the conditioned rats preferred water to saccharin at a ratio of 9:1 in a multiple-choice situation (three pipettes with 5 ml of saccharin each, three with water), whereas nonconditioned rats preferred saccharin to water. For extinction, the conditioned rats were presented once a day with the choice situation, for three to six consecutive days. The aversion index is {[ml of water/(ml of water + ml of saccharin)] × 100} consumed in the test.
16. Microinfusion into the IC was performed via chronically implanted cannulae. Rats were implanted bilaterally with a guide stainless steel cannula (23 gauge) aimed 1.0 mm above the gustatory cortex [anteroposterior +1.2 mm, lateral ±5.5 mm, ventral 5.5 mm relative to bregma, according to G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates* (Academic Press, New York, ed. 2, 1986)]. The cannulae were positioned in place with acrylic dental cement and secured with skull screws. A stylus was placed in the guide cannulae to prevent clogging. Animals were allowed 1 week to recuperate. The stylus was removed and a 28-gauge injection cannula, extending 1.0 mm from the tip of the guide cannula, was inserted. The injection cannula was connected via PE20 tubing to a microsyringe driven by a microinfusion pump. Solution (1 μl) was delivered per hemisphere over 1 min (Fig. 1A). The injection cannula was left in position before withdrawal for an additional 1 min to minimize dragging of the injectate along the injection track.
17. D. E. Berman, data not shown.
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Activity-Dependent Transfer of Brain-Derived Neurotrophic Factor to Postsynaptic Neurons

Keigo Kohara, Akihiko Kitamura, Mieko Morishima, Tadaharu Tsumoto*

Neurotrophins such as brain-derived neurotrophic factor (BDNF) are thought to be transferred from post- to presynaptic neurons and to be involved in the formation and plasticity of neural circuits. However, direct evidence for a transneuronal transfer of BDNF and its relation to neuronal activity remains elusive. We simultaneously injected complementary DNAs of green fluorescent protein (GFP)–tagged BDNF and red fluorescence protein into the nucleus of single neurons and visualized expression, localization, and transport of BDNF in living neurons. Fluorescent puncta representing BDNF moved in axons in the anterograde direction, though some moved retrogradely, and transferred to postsynaptic neurons in an activity-dependent manner.

Neuronal activity modifies the formation of neural circuits in developing cerebral cortex (*1–3*). Neurotrophins such as BDNF are attractive candidates for molecular signals that translate neuronal activity into such structural and functional changes in the cortex (*4–7*). Since the initial discovery of nerve growth factor, researchers have believed that neurotrophins are released or secreted from postsynaptic neurons or target cells (*4*, *8–10*). However, recent studies suggested that BDNF may be supplied from presynaptic axons (*11–18*). Because these studies detected BDNF mainly with immunocytochemistry after fixation, dynamics of BDNF trafficking was not analyzed in living neurons, and the crucial question of whether anterograde, transneuronal transfer of BDNF—if it exists—is related to neuronal activity re-

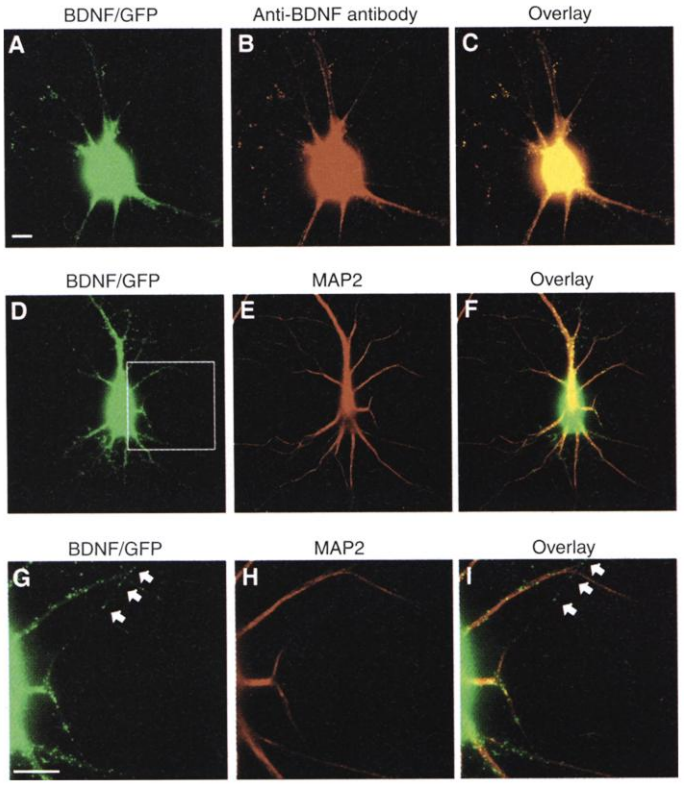

Fig. 1. Distribution of BDNF-GFP in cortical neurons and its localization in axon branches. (**A**) BDNF-GFP expressed in a neuron 24 hours after an injection of plasmid cDNA encoding BDNF-GFP into the nucleus. Bar in (A) indicates unit of measure for (A) through (F), 10 μm. (**B**) Immunocytochemical image of the neuron shown in (A), stained with antibody to BDNF (anti-BDNF antibody). (**C**) Superimposed image of (A) and (B). (**D**) Distribution of BDNF-GFP expressed in another neuron. (**E**) Immunocytochemical image of the neuron shown in (D) stained with antibody to MAP2. (**F**) Superimposed images of (D) and (E). (**G**, **H**, and **I**) Magnified image of the boxed area in (D), each of which corresponds to (D), (E), and (F), respectively. Arrows in (G) and (I) show clusters of BDNF-GFP–positive puncta in the axon branch. Bar in (G) indicates unit of measure for (G) through (I), 10 μm.

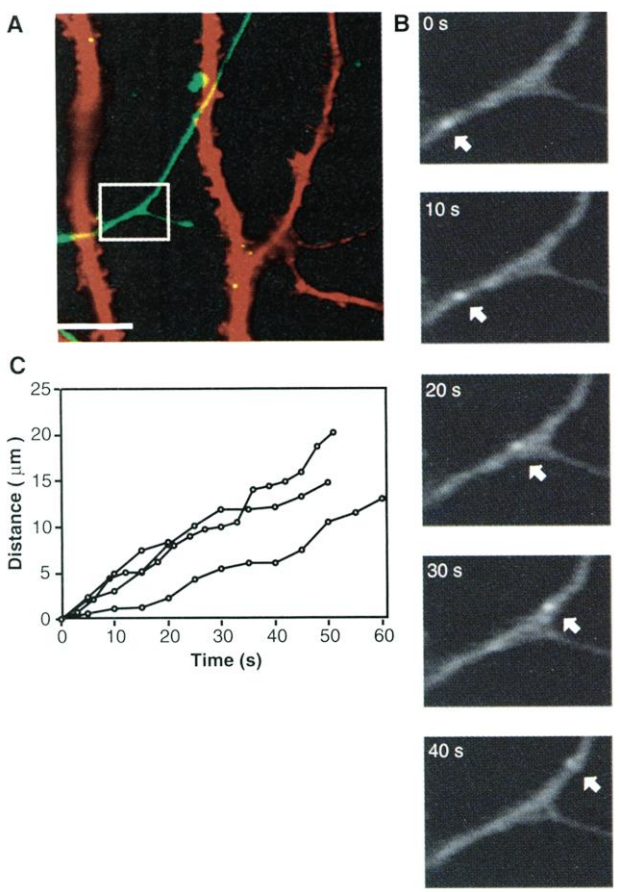

Fig. 2. Movement of BDNF-GFP in an axon of a living neuron. (**A**) Superimposed image of an axon that expressed BDNF-GFP (green) with dendrites of another neuron stained with antibody to MAP2 (red). Parent soma of the axon is outside this image in the left direction. The image in the white square is magnified and shown in (B). Bar, 10 μm. (**B**) Anterograde movement of BDNF-GFP in the axon. Each image was taken at time point indicated at top left. Arrows indicate the same puncta representing BDNF-GFP. (**C**) Location of BDNF-GFP puncta expressed as the distance from the starting point was plotted against time. The data from the same puncta were connected with a line.

mains unanswered. In this study, we directly injected cDNAs of GFP-tagged BDNF and another fluorescence protein into the nucleus of neurons, which expressed both kinds of fluorescence within 24 hours.

A plasmid encoding BDNF tagged with GFP (*19*, *20*) was injected into the nucleus of cultured cortical neurons (*21*) through a micropipette under visual control (*22*). GFP-tagged BDNF resulting from this plasmid was confirmed to be biologically active and mimicked the releasing characteristics of untagged BDNF (*20*). Twenty to 30 percent of the injected neurons expressed fluorescent signal (*23*). Punctuated fluorescent signals were seen as clusters in neurites, whereas in the soma the fluorescent signal appeared dense and diffuse (Fig. 1, A and D). This pattern of signal distribution was quite similar to that of endogenous BDNF (Web fig. 1, *24*), as reported previously (*14*, *20*, *25*). We tested whether the fluorescent signal detected in the plasmid-injected neurons reflected the presence of BDNF. Neurons that expressed fluorescent signals were stained immunocytochemically with antibody to BDNF (Fig. 1B). The distribution of BDNF was almost identical to that of GFP fluorescent signal. This was confirmed by superimposition of the two pictures, although in the periphery of neurites there was a small number of red dots that probably reflected the presence of endogenous BDNF (Fig. 1C). Plasmid-injected neurons were functionally normal because they generated action currents in response to depolarization of the soma and spontaneous synaptic currents (*26*), as seen in noninjected neurons.

In addition to the somatodendritic region, fluorescent signals of BDNF-GFP were also detected in neurites that seemed to be axon branches on the basis of their thin but constant caliber in the long distance (Fig. 1D and arrows in Fig. 1G). To confirm that these neurites were axons, the neurons were stained immunocytochemically with antibody against MAP2, which is known to exist in the somatodendritic region only (*27*). Neurites that contained the punctuated fluorescent signals (indicated by arrows in Fig. 1G) were spared from MAP2 staining (Fig. 1, E and H). This is seen in the superimposed pictures (Fig. 1, F and I). Such puncta arranged in line in MAP2-negative neurites were seen in all of the 24 neurons stained with antibody to MAP2. Four neurons were stained with antibody against tau, which is known to exist almost exclusively in axons (*27*). Fluorescent puncta were seen in tau-positive neurites in all of these neurons. These results suggest that

Division of Neurophysiology, Biomedical Research Center, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871 Japan, and CREST, Japan Science and Technology Corporation, 1-8, Honcho 4-chome, Kawaguchi, 332-0012, Japan.

*To whom correspondence should be addressed. E-mail: ttsumoto@nphys.med.osaka-u.ac.jp

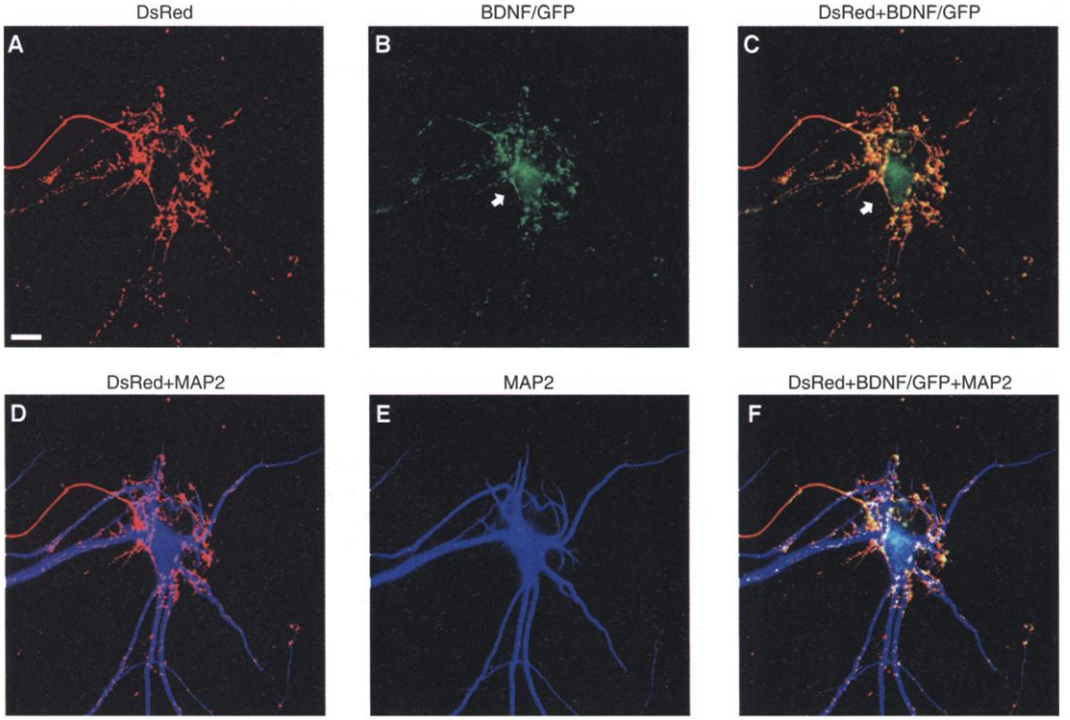

Fig. 3. Transfer of BDNF-GFP to a postsynaptic neuron. (**A**) Horizontally running axon and its terminals containing DsRed. Its parent soma is outside this frame. Bar, 10 μm. (**B**) BDNF-GFP image of the same frame as in (A), visualized by antibody to GFP. (**C**) Superimposition of (A) and (B). Arrows in (B) and (C) indicate the soma of the postsynaptic neuron that is not seen in (A). (**D**) Superimposition of (A) and another image visualized by antibody to MAP2 (blue). (**E**) MAP2 image of the same frame as the others, stained by antibody to MAP2. (**F**) Superimposed image of (C) and (E).

BDNF exists not only in the somatodendritic region of neurons but also in the axon and its branches.

Fluorescent signals of BDNF moved quickly in axons of living neurons in the anterograde as well as retrograde directions. As shown in Fig. 2A, a long and thin neurite was spared from the staining with antibody to MAP2, indicating that it was an axon of a neuron whose soma was outside the frame of this picture. In this axon, a bright fluorescent spot was detected and was found to move in the anterograde direction toward the axon terminal (Fig. 2B; see also time-lapse Web movie 1, *24*) (*28*). The movement velocity was 0.2 μm/s in this case. A few, other fluorescent puncta were seen to move in the retrograde direction and to pass by anterogradely moving puncta (see the second case in Web movie 1, *24*). A similar movement of fluorescent puncta was observed also in dendrites of plasmid-injected neurons, but an exact quantification of movement was not feasible in dendrites because of complicated arbolizations. In Fig. 2C, we plotted against time the locations of four puncta that moved in the anterograde direction in three neurons. The mean velocity of the movement was 0.3 ± 0.1 (SEM) μm/s. This value is comparable to the reported value (mean ± SD, 0.69 ± 0.33 μm/s) of anterograde transport of a synaptic vesicle protein, synaptophysin, in axons of mouse dorsal root ganglion cells (*29*).

Then we asked whether BDNF in axons moves transsynaptically to postsynaptic neurons. We simultaneously injected two kinds of plasmid into the nucleus: a plasmid containing GFP-tagged BDNF and one containing DsRed

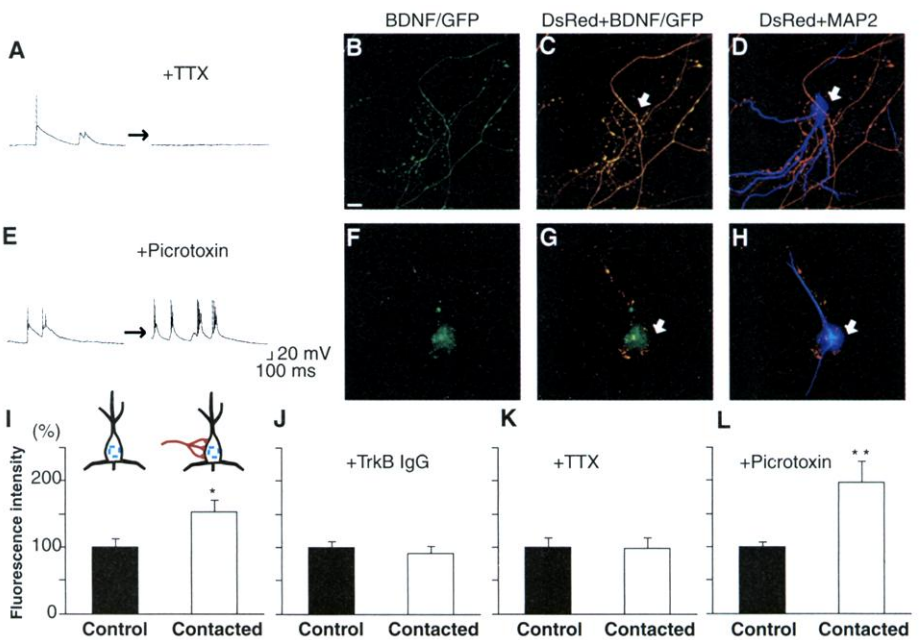

Fig. 4. Activity-dependent transfer of BDNF-GFP to postsynaptic neurons. (**A**) Representative records from a neuron before (left) and 5 min after (right) TTX treatment. (**B**) BDNF-GFP image of neurons treated with TTX for 48 hours and visualized by antibody to GFP. Bar, 10 μm. (**C**) Superimposition of (B) and DsRed image of the same frame. Arrow indicates the soma of the postsynaptic neuron, which was not visible in this image. (**D**) Superimposition of DsRed image (red) and MAP2 image (blue). Arrow indicates the soma of the postsynaptic neuron. (**E**) Representative records from a neuron before (left) and 5 min after (right) picrotoxin treatment. (**F**) BDNF-GFP image of neurons treated with picrotoxin for 48 hours and visualized by antibody to GFP. (**G**) Superimposition of (F) with DsRed image of the same frame. Arrow indicates the soma of the postsynaptic neuron. (**H**) Superimposition of DsRed image (red) and MAP2 image (blue). (**I**) Mean fluorescence intensity of BDNF-GFP in neuronal somata that did not contact DsRed-positive terminals (solid) and contacted such terminals (open). Vertical bars indicate SEM. Asterisk indicates statistical significance of the difference from the control at $P < 0.02$ (t test). (**J**, **K**, and **L**) Mean fluorescence intensity of BDNF-GFP in somata of neurons treated with TrkB-IgG, TTX, and picrotoxin, respectively. Double asterisks in (L) indicate statistical significance of the difference from the control at $P < 0.01$ (t test). Other conventions are the same as in (I).

fluorescent protein. An expression of these two kinds of protein in a single neuron was detected by changing the wavelength of the fluorescent excitation. DsRed was expressed together with BDNF-GFP in soma and neurites of plasmid-injected neurons (Fig. 3). As visualized with DsRed, an axon of a neuron whose soma was outside this frame terminated, so as to form many punctuated spots surrounding the soma of another, unstained neuron (Fig. 3A). A superimposed image of the DsRed with an image of the staining with antibody to MAP2 showed that the DsRed-positive puncta represented terminals of the axon surrounding the soma of the postsynaptic neuron (Fig. 3D). Such punctuated spots were confirmed to form presynaptic sites by immunocytochemical staining of a synaptic vesicle protein, synapsin I, in part of the experiments (*30*). In neurons illustrated in Fig. 3, the distribution of BDNF-GFP shown in Fig. 3B was almost identical to that of DsRed, except that the soma of the postsynaptic neuron had the fluorescent signal of BDNF-GFP only (arrow). The superimposed image in Fig. 3C shows that the soma of the postsynaptic neuron remained green, indicating the existence of BDNF-GFP in the postsynaptic neuron. Figure 3F confirms that the soma of the postsynaptic neuron contained the BDNF-GFP signal. These results suggest that BDNF was transferred from the presynaptic axon to the postsynaptic neuron because only the presynaptic neuron received plasmid injection. To quantify these findings, neurons were stained immnunocytochemically with antibody to GFP, and the fluorescence intensity was measured in somata adjacent to DsRed-positive axon terminals and in other somata that did not make contact with those terminals as a control (see insets of Fig. 4I) (*31*). Because the somata of control neurons had some background fluorescence, the intensity of this fluorescence was expressed as 100% and that in the former somata was normalized to this value. The mean fluorescence intensity of seven neuronal somata, which were contacted by DsRed-positive axon terminals, was 153 ± 18% of that of another seven control somata (Fig. 4I). Next, we addressed the question of whether the transfer of BDNF-GFP is mediated through BDNF receptors, TrkB (*4*). We stained neurons with antibody to TrkB and found that almost all the neurons including postsynaptic neurons contained TrkB. Then, we tested if a blocker of TrkB function, TrkB–immunoglobulin G (IgG) (*32*), can affect the transfer of BDNF-GFP. In all of the five neurons treated with TrkB-IgG, we did not see any visible signal of BDNF-GFP in postsynaptic neurons. The mean intensity of fluorescence in the somata contacted by DsRed-positive terminals was 92 ± 10% of control (Fig. 4J). This value was significantly [analysis of variance (ANOVA), $P < 0.05$] smaller than that without TrkB-IgG, suggesting that the transfer of BDNF-GFP was mediated by TrkB.

Lastly, we asked whether the transfer of BDNF-GFP to postsynaptic neurons is modified by activity of neurons. Tetrodotoxin (TTX) was applied to neurons at a concentration of 1 μM, which abolished their spontaneous activities (Fig. 4A) (*33*). After TTX treatment, the soma of the neuron adjacent to the DsRed-positive terminals did not show any GFP signal (Fig. 4C). In seven neurons tested, the mean intensity of fluorescence in neuronal somata adjacent to DsRed-positive axon terminals was 98 ± 15% of control (Fig. 4K). This value was significantly (ANOVA, $P < 0.05$) smaller than that without any drug. These results indicate that the transfer of BDNF was dependent on neuronal activity. To further confirm this, we applied picrotoxin, a $GABA_A$ (γ-aminobutyric acid) receptor antagonist, at 50 μM, which increased the discharge rate of neurons under observation (Fig. 4E). After picrotoxin treatment the neuronal soma adjacent to DsRed-positive axon terminals had a strong green signal (Fig. 4G), indicating that BDNF-GFP was transferred markedly to the postsynaptic neuron. In seven neurons thus tested, the mean intensity of fluorescence in somata adjacent to DsRed-positive terminals was 197 ± 32% of that of the control (Fig. 4L).

To our knowledge, the direct injection of plasmid cDNA into the nucleus has not successfully been applied to neurons in the central nervous system, probably because of its technical difficulty. However, in the present study we found that it is applicable to cultured cortical neurons and has notable advantages over conventional methods such as viral vectors. The expression of BDNF-GFP and DsRed after direct intranuclear injection was very rapid. Usually the fluorescent signal emerged in the whole dendritic field within 24 hours after injection. Furthermore, only the neuron that has received the intranuclear injection of plasmid is expected to express the signal. Therefore, other neurons in which fluorescence signal of BDNF-GFP is detected must have received the tagged BDNF through transneuronal transfer. On the basis of such a simple but direct logic, we could demonstrate that BDNF was transferred to other neurons. This transfer occurred in the anterograde direction, because we detected BDNF-GFP in the soma of postsynaptic neurons that were surrounded by DsRed-positive axon terminals. There is a possibility that axon terminals of the postsynaptic neuron might have contacted the soma of the plasmid-injected neuron and BDNF-GFP might have been transported retrogradely to the soma of the postsynaptic neuron. This possibility seems unlikely, however, because we did not detect any fluorescent signal in axons originating from postsynaptic neurons, despite the fact that we could clearly see axons of presynaptic neurons that expressed DsRed and BDNF-GFP.

The present results are inconsistent with the traditional view that neurotrophins are released or secreted from postsynaptic neurons or target cells. Previous reports also suggested anterograde transport of BDNF, but they were based on indirect evidence in specific nervous systems: structures that lacked mRNA for BDNF production or changes in phenotype or receptor activity in target neurons after activation of presynaptic afferents in the cortico-striatal or noradrenergic projection system (*11–18*). In a study using radio-iodinated neurotrophins, only exogenously applied NT-3 was suggested to be transported anterogradely in the developing retino-tectal pathway of the chick (*34*). In none of these studies was the dynamics of BDNF observed in living neurons.

Neurotrophins such as BDNF are suggested to be involved in activity-dependent neural plasticity and, thus, implicitly supposed to be released in an activity-dependent manner (*2–7*). However, there has been no direct evidence for this, although BDNF assayed with the methods of immunoprecipitation or enzyme immunoassay in the perfusion medium of cultured neurons or tissue slices was reported to increase by high K^+- or electrical stimulation–induced activation (*35–37*). The results presented here indicate that the transneuronal transfer of BDNF is dependent on neuronal activity. We have also demonstrated that the transfer of BDNF-GFP is not due to general transneuronal movement of protein molecules because DsRed, which was co-expressed with BDNF-GFP, was not detected in the postsynaptic neurons. This suggests that BDNF is released or secreted through presynaptic secretion mechanisms. The existence of BDNF associated with secretory vesicles was suggested previously (*14*), but these results did not show actual movement of BDNF in living neurons. Thus, the co-expression of two fluorescent proteins in this study has made it possible to directly observe the activity-dependent, transneuronal transfer of BDNF.

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21. Neonatal mice (C57BL/6, postnatal day 0-1) were anesthetized with ketamine (>30 mg/kg, intraperitoneally), and then killed by cervical dislocation. The experimental procedures met the regulation of the Animal Care Committee of Osaka University Graduate School of Medicine. Neurons were cultured at low density by using conventional methods (*38*). A piece of visual cortex was removed from neonatal mice, enzymatically dissociated with papain (20 U/ml), and triturated with a fire-polished glass pipette. Neurons were plated on previously prepared glial feeder layer, and were grown in a solution based on Neurobasal A Medium (Gibco, Rockville, MD) supplemented with 5% B27 (Gibco). All experiments were carried out 14 to 24 days after plating.
22. The cDNA of mouse BDNF tagged with GFP at the COOH-terminus was provided by M. Kojima (*20*). Glass micropipettes were filled with TE buffer (pH 8.0), which contained cDNA of BDNF-GFP (0.5 to 1 μg/μl) or both cDNAs of BDNF-GFP (0.5 μg/μl) and DsRed (0.5 μg/μl, pDsred-N1; Clontech, Palo Alto, CA). Cultured neurons were placed on the stage of an inverted epifluorescence microscope (TE300; Nikon, Tokyo, Japan), and cDNAs were injected into the nucleus of a neuron through a micropipette under visual control, using a micromanipulator (MMO-202ND; Narishige, Tokyo, Japan).
23. Neurons that expressed BDNF-GFP and DsRed signals were observed with a 40×, 1.3 NA oil immersion objective (Nikon) attached to the inverted epifluorescence microscope. The fluorescence of GFP, DsRed, aminomethylcoumarin (AMCA), and Cy5, excited by light at the wavelength of 480, 530, 350, and 650 nm, respectively, was measured using a cooled charge-coupled device (CCD) camera (C4742-95; Hamamatsu Photonics, Hamamatsu, Japan). This system consisted of 1024 pixels by 1024 pixels, each of which corresponded to 0.17 μm by 0.17 μm with the 40× objective. Data were further analyzed with an Aquacosmos system (Hamamatsu Photonics). Neurons were fixed with 4% paraformaldehyde (Sigma, St. Louis, MO) and 4% sucrose in Dulbecco's phosphate-buffered saline (PBS) for 20 or 40 min at room temperature. The cells were incubated with PBS containing 0.2% Triton-X (Sigma) for 1 min and were blocked by 10% donkey serum in PBS for 1 hour at 37°C. Then, monoclonal antibody to MAP2 (1:250, Sigma), monoclonal antibody to tau (1:150; Cedarlane, Hornby, Ontario, Canada), polyclonal antibody to BDNF (2 μg/ml, provided by R. Katoh-Semba), polyclonal antibody to synapsin I (1:500; Chemicon, Temecula, CA) or polyclonal antibody to TrkB (1:100; Santa Cruz, Santa Cruz, CA) was applied for 2 hours at 37°C. Synapsin I, TrkB and endogenous BDNF were visualized by secondary antibody to rabbit conjugated with Alexa 546 (1:2000; Molecular Probe, Eugene, OR) or Cy5 (1:200; Chemicon). MAP2 and tau were visualized by secondary antibody to mouse conjugated with Alexa 546 or AMCA (1:100, Chemicon).
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26. In three neurons, synaptic and action currents were recorded through a whole-cell patch electrode (resistance, 3 to 5 megohm) in a voltage-clamp mode with a patch-clamp amplifier (Axoclamp 2B; Axon Instruments, Foster, CA) 48 hours after the intranuclear injection of cDNAs. The signal was digitized at 10 kHz and filtered at 2 kHz. Recordings were carried out at room temperature. The perfusing solution, unless otherwise noted, contained the following: NaCl, 120 mM; KCl, 4 mM; $CaCl_2$, 2 mM; $MgSO_4$, 1 mM; Hepees, 10 mM; glucose, 10 mM(pH 7.4). Osmolality was adjusted to 250 to 255 mOsm by adding sucrose when necessary. The electrode solution was as follows: K-gluconate, 110 mM; Hepes, 10 mM; KCl, 10 mM; EGTA, 0.5 mM; MgATP, 5 mM; and Na_2GTP, 1 mM.
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28. Time-lapse recordings were carried out 16 to 24 hours after transfection and sequential images were acquired with a cooled CCD camera at room temperature. Exposure time was 1 or 2 s, lapse of time was 3 or 5 s, and the total time was 10 to 20 min. The solution for the time-lapse recordings contained the following: NaCl, 120 mM; KCl, 4 mM; KH_2PO_4, 1.2 mM; $CaCl_2$, 2 mM; $MgSO_4$, 1 mM; Hepes, 20 mM; glucose, 30 mM (pH 7.4).
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30. Localization of synapsin I in DsRed-positive puncta representing presynaptic sites was visualized in 10 neurons with immunocytochemistry, as mentioned (*23*).
31. A quantitative assessment of fluorescence intensity was carried out 48 hours after the intranuclear injection of cDNA. BDNF-GFP was visualized by polyclonal antibody to GFP (1:1000, Molecular Probe) or monoclonal antibody to GFP (1:500, Molecular Probe) and secondary antibody to rabbit or mouse conjugated with Cy5, to avoid autofluorescence of neurons and glia at 480 nm. The fluorescence intensity was measured on a square window (30 pixels by 30 pixels) placed on a soma which contacted DsRed-positive axon terminals, and the mean fluorescence intensity of 900 pixels was calculated. As control, another soma that did not contact DsRed-positive terminals was randomly selected from the same culture dish, and the intensity of background fluorescence was calculated as above.
32. TrkB-IgG fusion protein (Genentech, San Francisco, CA) was applied to neurons after transfection through the medium at 40 μg/ml, which is known to block function of TrkB ligands (*39*).
33. TTX (Wako Pure Chemicals, Osaka, Japan) and picrotoxin (Tocris Cookson, Bristol, UK) were applied to neurons through the perfusion medium at the concentration 50 and 1 μM, respectively. To confirm that these drugs actually affect activities of neurons, synaptic and action potentials were observed for 5 min each before and after the application. In some experiments, picrotoxin or TTX was applied to neurons for 48 hours after the intranuclear injection of cDNAs. Synaptic and action potentials were recorded in the current-clamp mode in the same way as described (*26*) except for the clamp mode.
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Interference by Huntingtin and Atrophin-1 with CBP-Mediated Transcription Leading to Cellular Toxicity

Frederick C. Nucifora Jr.,[1,2] Masayuki Sasaki,[3] Matthew F. Peters,[1] Hui Huang,[3] Jillian K. Cooper,[1] Mitsunori Yamada,[7] Hitoshi Takahashi,[7] Shoji Tsuji,[7] Juan Troncoso,[6] Valina L. Dawson,[2,3,4,5] Ted M. Dawson,[2,3,4]* Christopher A. Ross[1,2,4]*

Expanded polyglutamine repeats have been proposed to cause neuronal degeneration in Huntington's disease (HD) and related disorders, through abnormal interactions with other proteins containing short polyglutamine tracts such as the transcriptional coactivator CREB binding protein, CBP. We found that CBP was depleted from its normal nuclear location and was present in polyglutamine aggregates in HD cell culture models, HD transgenic mice, and human HD postmortem brain. Expanded polyglutamine repeats specifically interfere with CBP-activated gene transcription, and overexpression of CBP rescued polyglutamine-induced neuronal toxicity. Thus, polyglutamine-mediated interference with CBP-regulated gene transcription may constitute a genetic gain of function, underlying the pathogenesis of polyglutamine disorders.

Huntington's disease (HD) and dentatorubral and pallidoluysian atrophy (DRPLA) are neurodegenerative disorders caused by polyglutamine expansions in the huntingtin and atrophin-1 proteins, respectively (*1–4*). Huntingtin with expanded polyglutamine aggregates in vitro and forms neuronal intranuclear and cytoplasmic inclusions in mice and in HD patients, although the inclusions themselves are not directly toxic (*5–14*). The mechanisms of polyglutamine pathogenesis remain uncertain. One hypothesis suggests that the expanded polyglutamine alters protein conformation, resulting in aberrant protein interactions (*15–17*), including interactions of the expanded polyglutamine with cellular proteins containing short polyglutamine stretches. CREB binding protein (CBP) is a coactivator for CREB-mediated transcription (*18*) and contains a 15 (mouse) or an 18 (human) glutamine stretch. CREB-mediated gene transcription promotes cell survival, and CBP is a major mediator of survival signals in mature neurons (*19–21*). CBP has been found in