deficits and for the early diagnosis of premature infants at risk of brain damage. If early arm movements have an important function for later reaching skills, then infants with signs of hypoactivity or spasticity of the arms should be monitored closely for retardation in the development of reaching, and possibly other perceptuo-motor skills too. In such cases, early intervention should concentrate on helping the baby to explore its arm and hand movements, both visually (*15*) and nonvisually (*16*).

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3. Infrared light–emitting diodes (LEDs) were fastened onto soft bands around the baby's wrists. The LEDs were viewed by an overhead Selspot camera, with optical axis vertical, from a distance of 1.5 m. The *x* axis in the camera's view was lined up perpendicular to the infant's body axis. The Selspot data were recorded on a computer at 62 frames per second. Strings were attached to the baby's wristbands. The strings passed over pulleys at the foot of the bed and could have weights attached to their ends. The weights used were 0%, 10%, and 25% of the estimated weight of the baby's arm (*17*). With weights added, the string exerted a pull on the baby's wrists approximately parallel to its body axis in the direction of its toes. Each infant was tested for a total of 12 min. The experiment comprised six blocks of four 30-s trials over which the three weights were randomly distributed. A total of 15 newborn babies were randomly assigned to one of the three experimental groups. In group 1 the babies could see the arm they were facing (seen ipsilateral arm, unseen contralateral arm). The babies in group 2 were prevented from seeing their arms directly, but were presented with a real-time video image of the arm they were not facing on a small video monitor placed beside the infant's head (unseen ipsilateral arm, seen contralateral arm). The babies in group 3 had occluders at the sides of the head ("blinkers"), which prevented them from seeing either arm while allowing them to see elsewhere (unseen ipsilateral arm, unseen contralateral arm).
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18. We are grateful to all the babies and parents for their enthusiastic participation. Supported by a grant from the Medical Research Council (UK).

23 August 1994; accepted 16 November 1994

Potentiation of Transmitter Release by Ciliary Neurotrophic Factor Requires Somatic Signaling

Ron Stoop and Mu-ming Poo*

Neurotrophic factors participate in the development and maintenance of the nervous system. Application of ciliary neurotrophic factor (CNTF), a protein that promotes survival of motor neurons, resulted in an immediate potentiation of spontaneous and impulse-evoked transmitter release at developing neuromuscular synapses in *Xenopus* cell cultures. When CNTF was applied at the synapse, the onset of the potentiation was slower than that produced by application at the cell body of the presynaptic neuron. The potentiation effect was abolished when the neurite shaft was severed from the cell body. Thus, transmitter secretion from the nerve terminals is under immediate somatic control and can be regulated by CNTF.

The differentiation and survival of neurons in the nervous system depend on the action of neurotrophic substances (*1*). Ciliary neurotrophic factor is a protein that promotes the differentiation or survival (or both) of a wide range of cell types in the vertebrate nervous system (*2*). Administration of CNTF to chick embryos reduces naturally occurring motor neuron death (*3*), and mice carrying a null mutation in the CNTF gene show progressive motor neuron atrophy and postnatal neuron loss (*4*). In addition to the long-term trophic effects of CNTF, we report here that CNTF also exerts acute regulatory actions on the synaptic function of developing neuromuscular synapses. The site of CNTF action was examined by local perfusion of CNTF to the synapse or the cell body of the presynaptic neuron in cell cultures. These studies demonstrated that rapid signaling with the cell body is required for the synaptic potentiation induced by CNTF. The secretory function of the presynaptic nerve terminal is thus under an immediate regulation by one or more factors from the cell body, and such somatic regulation can be modulated by CNTF.

Spontaneous synaptic currents (SSCs) were monitored by whole-cell, voltage-clamp recordings (*5*, *6*) from the postsynaptic myocyte in 1-day-old *Xenopus* nerve-muscle cultures (*7*). These currents are produced by spontaneous release of quantal packets of acetylcholine (ACh) from the presynaptic nerve terminal in the absence of action potentials (*8*). Addition of CNTF (final concentration 100 ng/ml) to the culture resulted in a gradual increase in the frequency of SSCs within 10 to 20 min (Fig. 1, A and B). The average SSC frequency (over 5-min periods) was determined at different times after CNTF application. A maximal response was obtained after 25 min of exposure to CNTF, when the SSC frequency had increased to an average level about five times its initial value (Fig. 1, A and B), although a minority of cells failed to respond to CNTF at this concentration (*9*). Unlike the frequency of the SSCs, the mean amplitude of the SSCs remained unchanged. At 25 to 30 min after CNTF addition, the mean amplitude was 93 ± 20% (SEM, n = 9) of that during the control period prior to CNTF addition, similar to that of the control cultures. No significant change was observed in the amplitude distribution (Fig. 1, C and D). The absence of any effect on the SSC amplitude suggests that the action of CNTF was predominantly on the probability of spontaneous quantal ACh release from the presynaptic nerve terminal, rather than on the size of ACh quanta or postsynaptic ACh sensitivity.

The effect of CNTF on impulse-evoked synaptic currents (ESCs) was also examined. Presynaptic neurons were stimulated at the cell body by an extracellular electrode to fire action potentials at a frequency of about 0.05 Hz, and postsynaptic recordings of ESCs were made at different times before and after addition of CNTF (100 ng/ml). An example of one recording and changes in the average ESC amplitude with time for five experiments are shown in Fig. 2. In control cultures not treated with CNTF, repetitive tests of evoked synaptic responses led to a gradual reduction of the mean ESC amplitude, an activity-dependent synaptic depression known to occur at these developing synapses (*10*). In contrast, a significant increase in the ESC amplitude was observed after 10 min in the presence of CNTF (*11*). Because no change was observed for the amplitude of SSCs, the increase in ESC amplitude is likely to result from an increased depolarization-evoked ACh release from the presynaptic nerve terminal (*12*), rather than an increased postsynaptic responsiveness.

To examine the site of CNTF actions on the neuron, we used a pair of perfusion

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micropipettes (*13*) to apply CNTF locally to either the synapse or the cell body of neurons that had synaptic sites at a distance of 150 to 200 μm from the cell body. The SSCs were recorded from the postsynaptic myocyte before and during the local perfusion. The perfusion provided a relatively localized flow across the cell, as illustrated by staining with a solution containing trypan blue (Fig. 3A). When local perfusion of CNTF (100 ng/ml) was applied to the cell body and to the synaptic region of two separate groups of cells in 1-day-old *Xenopus* cultures, we observed a gradual increase in the SSC frequency in both cases. At 25 to 30 min after the onset of the perfusion, the frequency was 4.24 ± 0.76 (SEM, n = 22) times the initial SSC frequency for cell body perfusion and 3.71 ± 0.76 (SEM, n = 23) times the initial SSC frequency for synapse perfusion. These values are not significantly different from that observed after addition of CNTF to the whole culture (5.13 ± 1.16, SEM, n = 11; $P > 0.05$, t test). However, the onset in the increase of SSC frequency induced by the cell body perfusion appeared earlier than that of the synapse perfusion (Fig. 3B). To ensure the localization of the perfusion flow, we performed perfusion of CNTF at a distance of 150 μm from both the cell body and the synapse. Over a perfusion period of 30 min, no increase in the SSC frequency was observed (Fig. 3B). We also examined the effect of local application of brain-derived neurotrophic factor (BDNF), a neurotrophin known to potentiate the synaptic activity at these neuromuscular synapses (*14*). After a 25-min perfusion of BDNF (100 ng/ml) at the synapse, a 2.43 ± 0.50 (SEM, n = 29) –fold increase in SSC frequency was observed. In contrast to the effect of CNTF, however, local perfusion of BDNF at the cell body resulted in no change in the SSC frequency (Fig. 3C).

In sensory neurons CNTF is transported in a retrograde fashion, and both sensory and motor neurons show increased transport of CNTF after nerve injury (*15*). The longer delay in the onset of CNTF-induced synaptic potentiation after synapse perfusion, as compared to that of cell body perfusion, may have been due to the time required for retrograde signaling to the cell body (*16*). This was tested by transection of the neurite shaft of a synapse near the cell body with a sharp micropipette. Immediately after the transection we observed a burst of spontaneous ACh secretion, as a result of Ca^{2+} influx at the injured site. After the burst of ACh release had subsided, CNTF or BDNF (100 ng/ml) was applied to the synapse now "cut loose" from the cell body. Application of CNTF for 35 min did not result in any significant increase in the SSC frequency (Fig. 4A), whereas similar application of BDNF resulted in a 2.44 ± 0.48 (SEM, n = 5) –fold increase in the SSC frequency (Fig. 4B), a value nearly identical to that found for the BDNF effect on intact synapses (Fig. 3C). In other experiments, BDNF was added to the same "cut-loose" synapse 30 min after the onset of CNTF treatment. Addition of BDNF resulted in a 2.70 ± 0.69 (SEM, n = 5) –fold increase in the SSC frequency, even though CNTF had failed to elicit any change at the same synapses (Fig. 4C).

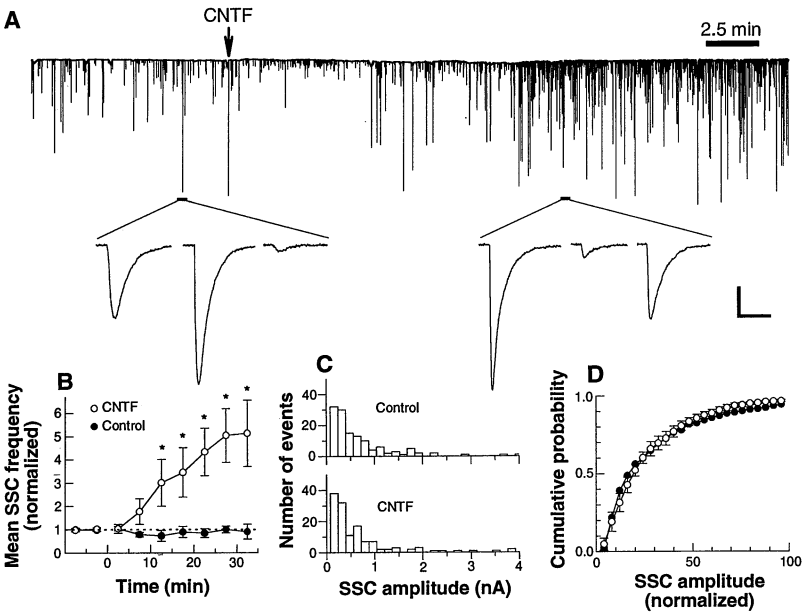

Fig. 1. Potentiation of spontaneous synaptic activity by CNTF at developing *Xenopus* neuromuscular synapses. (**A**) The continuous trace depicts the membrane current recorded from an innervated myocyte under voltage clamp [holding potential (V_h) = −70 mV] in a 1-day-old culture. Downward events are spontaneous synaptic currents (SSCs) and are induced by packets of ACh secreted from the presynaptic neuron. Arrow marks the onset of addition of CNTF to the culture (final concentration 100 ng/ml). Samples of SSCs are shown below at a higher time resolution. Scales, 0.5 nA and 10 ms. (**B**) The mean SSC frequency (over 5-min intervals) at various times after addition of CNTF (100 ng/ml) at time 0 (○) (n = 11). In control experiments culture medium containing no CNTF was added to the culture (●) (n = 5). Data were normalized to the mean SSC frequency of the same synapse prior to the addition of CNTF. Error bars represent the SEM. (*) Significantly different from the corresponding control values ($P < 0.05$, t test). (**C**) Histograms of amplitude distribution of SSCs observed during the 10-min period before (upper graph) and 25 to 30 min after (lower graph) CNTF treatment for the experiment shown in (A). (**D**) Composite graphs of SSC amplitude distribution for data obtained from 9 synapses. For each synapse, the amplitude of SSCs was normalized to the maximal amplitude that included 95% of all events. The cumulative probability refers to the fraction of total events with amplitudes smaller than a given amplitude before (●) and after (○) addition of CNTF (100 ng/ml). Data points represent mean values and error bars are the SEM.

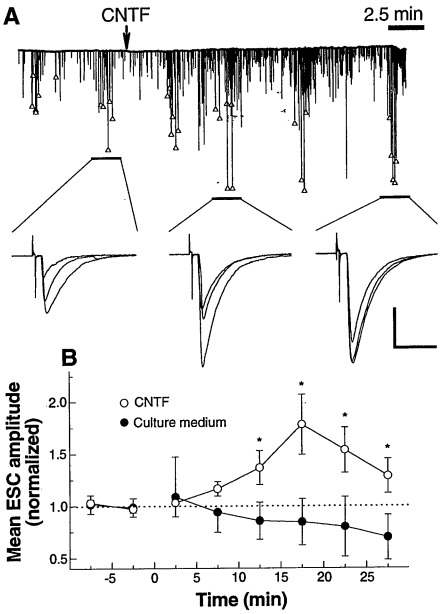

Fig. 2. Potentiation of evoked synaptic currents (ESCs) by CNTF. (**A**) The continuous trace depicts the membrane current recorded from an innervated myocyte under voltage-clamp (V_h = −70 mV) in a 1-day-old culture. ESCs were elicited at a low frequency at the time marked by small open triangles. The arrow marks the time of addition of CNTF (100 ng/ml) to the culture. Insets below depict samples of ESCs at a higher time resolution for the recording periods indicated. Scales, 1 nA and 10 ms. (**B**) Changes in ESC amplitudes after addition of CNTF (100 ng/ml) (○) at time 0. In control experiments (●), culture medium containing no CNTF was added to the culture. Mean ESC amplitudes at different times were normalized for each synapse by the mean ESC amplitude before addition of CNTF. Data are presented as the mean ± SEM (n = 5). (*) Significantly different from the corresponding control values ($P < 0.05$, t test).

An immediate consequence of severing the neurite is a surge of Ca^{2+} influx at the site of injury. It is possible that this Ca^{2+} influx specifically inhibits the CNTF effect, without affecting the action of BDNF. Two types of experiments were performed to test this idea. First, the surge of Ca^{2+} influx was mimicked by perfusion of intact synapses with 50 mM KCl for a period of 2 to 3 min. The KCl perfusion triggered a transient elevation of cytosolic Ca^{2+} similar to that induced by nerve transection (*17*), and the SSC frequency was also elevated to a comparable extent. When subsequently added to these KCl-treated synapses, CNTF remained as effective in potentiating spontaneous ACh release (Fig. 4D) as it was on untreated synapses (Fig. 1B). In the second set of experiments, the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) was lowered after neurite transection by removal of extracellular Ca^{2+} (*18*). Substitution with Ca^{2+}-free solution resulted in a reduction of the SSC frequency to a level comparable to or lower than the level before transection (Fig. 4E). No CNTF effect was found in any case examined. In parallel experiments on intact neurons, we showed that the removal of extracellular Ca^{2+} by itself did not interfere with the CNTF-induced increase in the SSC frequency (Fig. 4F). Thus, the absence of a CNTF effect on the "cut-loose" synapses was not due to a depletion of a releasable pool of synaptic vesicles or to a high $[Ca^{2+}]_i$ after transection, and the effect of CNTF did not require extracellular Ca^{2+}. The sensitivity of "cut-loose" synapses to BDNF shows that the transmitter secretion machinery remained susceptible to modulation after neurite transection. It appears that a specific factor required for the CNTF action at these synapses is lost after transection. Such a factor may either be constitutively supplied by the cell body or induced by CNTF.

It is not clear which step in the cascade of cellular events induced by CNTF is directly linked to the potentiation of transmitter secretion. The receptor complex for CNTF consists of an α subunit anchored to the plasma membrane by glycosyl-phosphatidylinositol linkage and two transmembrane proteins, gp130 and leukemia inhibitory factor receptor β subunit (*19*, *20*). The CNTF binding induces tyrosine phosphorylation of the Jak-Tyk family of tyrosine kinases (*21*) and phosphorylation of two transcription factors, p91 and acute-phase response factor, within 10 min after application (*22*). It also activates transcription of immediate-early primary response genes *tis*11 and c-*fos* within 15 to 30 min (*20*). In cultured sympathetic neurons, a rapid increase in the amount of diacylglycerol occurs within 5 min after CNTF treatment, which ultimately leads to activation of protein kinase C (PKC) and increased expression of choline acetyltransferase (*23*). Thus, both protein phosphorylation and gene activation may be induced by CNTF at the cell body. However, given the rapid onset (<10 min) of synaptic potentiation after somatic exposure to CNTF and the time required for the transport of regulatory signals along the length of the neurite (150 to 200 μm) (*16*), induction of gene tran-

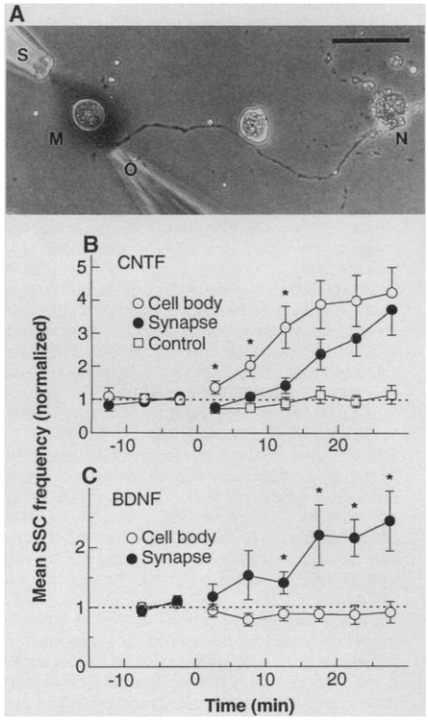

Fig. 3. Local perfusion of the cell body and the synapse with CNTF and BDNF. (**A**) Microscopic image of a *spherical Xenopus* myocyte (M) innervated by a cocultured spinal neuron (N). Local perfusion of a solution containing trypan blue was applied to the synapse, indicating the pattern of the perfusion flow during a typical experiment. O, outflow pipette; S, suction pipette. Bar, 50 μm. (**B**) Changes in the mean SSC frequency with time after the onset of CNTF perfusion (at t = 0 and 100 ng/ml) to either the cell body (n = 22) (○), the synapse (n = 23) (●), or a cell-free region at a distance 150 μm from both the cell body and the synapse (Control, n = 11) (□). The values for each synapse were normalized by the mean value prior to the CNTF perfusion. (*) Significantly different from the corresponding values for synapse perfusion ($P < 0.05$, t test). (**C**) Changes in the mean SSC frequency after local perfusion of BDNF (100 ng/ml) to either the cell body (n = 21) (○) or the synapse (n = 29) (●). (*) Significantly different from the corresponding values for cell body perfusion ($P < 0.05$, t test).

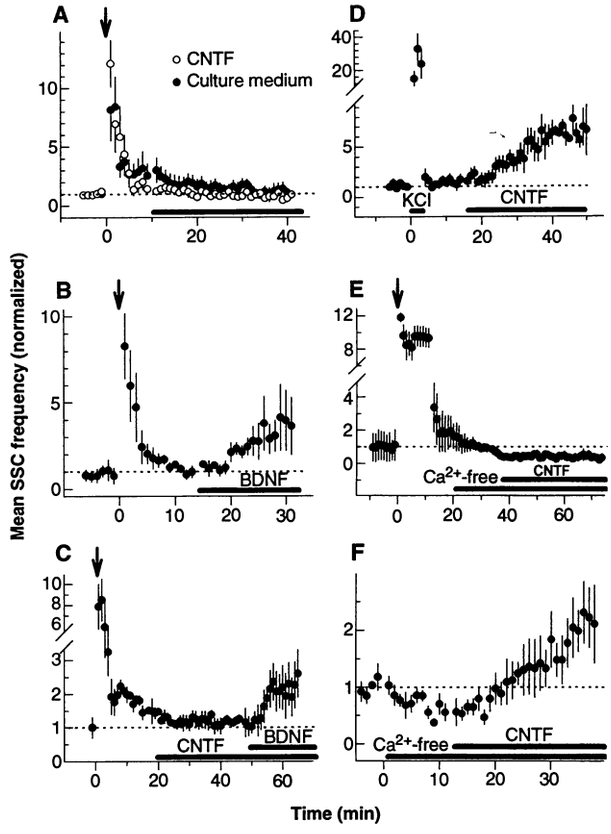

Fig. 4. Effects of CNTF and BDNF (both at 100 ng/ml) on "cut-loose" synapses and dependence of CNTF effect on Ca^{2+}. The SSC frequency was normalized for each synapse by the mean frequency prior to experimental treatments. All data are presented as mean ± SEM. (**A**) Changes in the mean SSC frequency with time. The synapses had been "cut loose" from their cell bodies by severing of the neurite at t = 0 (marked by arrow). The cells were treated by the addition of CNTF (○) (n = 5) or fresh culture medium (●) (n = 5) during the time marked by the horizontal bar below. (**B**) Same as in (A) except that BDNF was added to the culture instead of CNTF during the time marked by the bar (n = 5). (**C**) Same as in (B) except that CNTF was added 30 min before BDNF (n = 5). (**D**) Changes in the mean SSC frequency with time at intact synapses. The cells were perfused with a solution containing a high concentration of K^+ (50 mM) at t = 0 for a duration of 2 to 3 min, and CNTF was added subsequently (n = 6). (**E**) The same as in (B) except that the culture medium was later replaced with Ca^{2+}-free solution (*17*) before CNTF was added (n = 6). (**F**) Changes in the mean SSC frequency at intact synapses. The culture medium was replaced with Ca^{2+}-free solution at t = 0, and CNTF was added subsequently (n = 5).

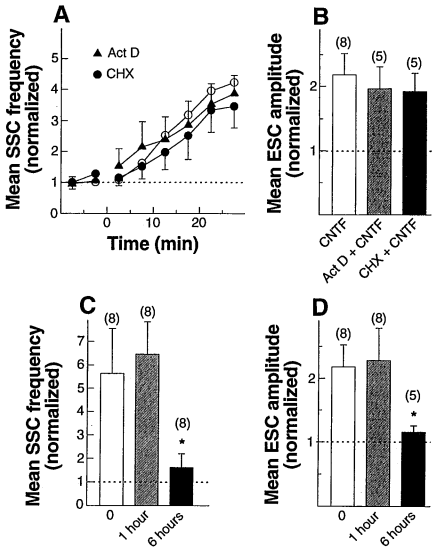

Fig. 5. Effects of RNA and protein synthesis inhibitors and the reversibility of the CNTF effect. (**A**) The cultures were preincubated in actinomycin D (25 μg/ml) (Act D, Sigma) or cycloheximide (10 μg/ml) (CHX, Sigma) for 1 hour. In the continued presence of the drug, the mean SSC frequency was then measured for a 10-min control period and for 30 min after the addition of CNTF (100 ng/ml). The values were determined for 5-min periods and normalized by the control value of the same synapse before the CNTF treatment. The values for cultures treated with Act D (▲) (n = 9) and CHX (●) (n = 8) were not significantly different ($P > 0.05$, t test) from that of parallel control cultures (○) (n = 13), which were treated with CNTF but not with any inhibitor, at all time points. Error bars represent the SEM and those associated with control cultures are omitted for clarity. (**B**) Similar experiments as that in (A) except that the mean ESC amplitude was determined. The values shown are the mean ESC amplitudes observed 30 min after the addition of CNTF, normalized by the control ESC amplitude of the same synapse before the addition of CNTF. Error bars represent the SEM, and the number of synapses examined is shown in the parentheses. No significant difference between the drug-treated and the control cultures was found ($P > 0.05$, t test). (**C** and **D**) The mean SSC frequency and ESC amplitude were determined immediately (0 hours), 1 hour, and 6 hours after a 30-min treatment with CNTF (100 ng/ml). The cells were thoroughly washed and incubated with fresh culture medium after the CNTF treatment. Data were normalized as described in (A) and (B). (*) Significant difference was observed between the groups at 0 and 6 hours ($P > 0.05$, t test).

scription or new protein synthesis seems unlikely to be involved in the initial action of CNTF. Indeed, we found that inhibition of RNA synthesis with actinomycin D (25 μg/ml) or inhibition of protein synthesis with cycloheximide (10 μg/ml) (*24*) had no effect on the rapid potentiation of spontaneous and evoked ACh secretion induced by CNTF (Fig. 5, A and B). Thus, the most likely somatic action of CNTF is a post-translational modification—for example, tyrosine or PKC-dependent phosphorylations—of preexisting components in the cell body. Such action appears to last for more than 1 hour after a 30-min exposure to CNTF (Fig. 5, C and D). In comparison, the synaptic potentiation by a similar treatment with neurotrophin 3 (NT-3) was found to disappear within 30 min after removal of NT-3 (*14*).

Myelinating Schwann cells have been shown to abundantly express CNTF in their cytoplasm (*25*). Our results suggest that CNTF secreted from these cells at the presynaptic nerve terminal, along the axon, or at the cell body could all potentiate transmitter secretion at developing or regenerating synapses. The more rapid onset of synaptic potentiation after CNTF perfusion at the cell body as compared to that at the synapse could be accounted for by a higher number of CNTF receptors at the cell body, by the additional time required for retrograde signaling to the cell body after CNTF binding at the nerve terminal, or by both. A number of molecules may serve as retrograde signals, including CNTF and its receptor complex, as well as cytoplasmic second messengers and kinases. Subsequent cellular events triggered by these signals at the cell body may in turn exert a regulatory control of the synthesis, packaging, or secretion of transmitters at the nerve terminal. The rapidity of CNTF-induced synaptic potentiation points to the efficiency of such somatic control of secretory functions.

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7. Preparation of *Xenopus* nerve muscle cultures was as described [N. C. Spitzer and J. C. Lamborghini, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1641 (1976); N. Tabti and M.-m. Poo, in *Culturing Nerve Cells*, G. Banker and K. Goslin, Eds. (MIT Press, Cambridge, MA, 1991), pp. 137–154]. The cells were plated on clean glass cover slips and were used for experiments after a 24 hour incubation at room temperature (20° to 22°C). The culture medium consisted of (v/v) 50% Leibovitz's medium (L-15, Gibco), 1% fetal bovine serum (Gibco), and 49% Ringer's solution [115 mM NaCl, 2 mM $CaCl_2$, 2.5 mM KCl, 10 mM Hepes (pH 7.3)]. *Escherichia coli*–derived rat CNTF was provided by Regeneron Inc. and has been shown to be active in E8 chicken dissociated CG neuron survival assays [P. Masiakowski *et al.*, *J. Neurochem.* **57**, 1003 (1991)].
8. The frequency and the amplitude of SSCs was not affected by the presence of tetrodotoxin [Z. Xie and M.-m. Poo, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7069 (1986)], which blocks action potentials in these cultured cells. These spontaneous currents are physiologically relevant, because they are known to accelerate the development of cross-striation in the postsynaptic muscle cells [Y. Kidokoro and M. Saito, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1978 (1988)].
9. The large variability of the data was due to the absence of a CNTF effect in a minority of cells recorded. Similar variability was also noted in the potentiation of these *Xenopus* synapses by NT-3 and BDNF (*14*). There is likely to be a heterogeneity in the types of cholinergic neurons in these cultures. Based on their ability to induce ACh receptor clusters and to elicit synaptic responses in the contacted muscle cells, M. W. Cohen *et al.* [*J. Neurosci.* **7**, 2849 (1987)] have estimated that 70 to 85% of the neurons in these *Xenopus* cultures are motor neurons.
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11. The increase in ESC amplitude cannot be accounted for by an increased overlap of spontaneous and evoked synaptic currents. In the case shown in Fig. 2A, even for the highest SSC frequency of 0.6/s observed after the CNTF treatment, the probability of superposition of the peaks of ESCs and SSCs is less than 0.006, assuming a 10-ms duration for the SSCs.
12. The amplitude of SSCs (quantal size) varies greatly in these developing neuromuscular synapses, with an amplitude distribution skewed toward smaller amplitudes. The lack of a defined quantal unit renders quantal analysis of transmitter secretion difficult and presumptive (*6*).
13. Local perfusion of CNTF was created by means of two micropipettes, one for the suction (inner diameter 10 to 12 μm) and one for the outflow of perfusion medium (inner diameter 2 to 3 μm). The pipettes were connected by fluid-filled tubings to separate fluid reservoirs. We adjusted the suction or outflow pressure through the micropipettes by changing the level of the fluid reservoirs relative to that of the culture dish. We tested the flow pattern before the experiment by using medium containing trypan blue, and monitored the flow pattern during the experiment by observing the movement of small cell debris in the culture.
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16. Retrograde transport may take place by diffusion or active transport along the neurite. The rate of diffusion along the neurite was estimated by the equation $x^2 = 2Dt$, where x is the average neurite length, D is the diffusion coefficient, and t is the average time required for the diffusion. For a globular protein of molecular size 60 kD [for example, bovine serum albumin (BSA)], the diffusion coefficient in these *Xenopus* neurites was found to be 67.7 ± 2.9 $\mu m^2/s$ [S. Popov and M.-m. Poo, *J. Neurosci.* **12**, 77 (1992)]. Thus, the average time required for diffusion

of a cytosolic protein 60 kD in size over a distance of 150 to 200 μm will be about 3 to 5 min. Simple diffusional signaling between the synapse and cell body could thus account for the delay in the potentiation observed in the present study. Active retrograde transport is known to take place at a rate of about 30 to 180 μm/min [R. D. Allen, J. Metuzals, I. Tasaki, S. T. Brady, S. P. Gilbert, *Science* **218**, 1127 (1985)], which would allow more rapid signaling than diffusion.
17. The increase in cytosolic Ca^{2+} was shown by direct Ca^{2+} imaging with the fluorescence dye Fluo-3 in separate cultures, through use of methods previously described [Y. Dan and M.-m. Poo, *Nature* **359**, 733 (1992)].
18. The transection was performed in normal culture medium to facilitate resealing of the plasma membrane. The medium was replaced with Ca^{2+}-free solution [115 mM NaCl, 2 mM $MgCl_2$, 2.5 mM KCl, 10 mM Hepes, 3 mM EGTA, and 0.1% BSA (pH 7.3)] 20 min after the neurite transection.
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24. Addition of actinomycin D at 2.5 μg/ml to *Xenopus* embryonic cell cultures was shown to inhibit >95% of [^{3}H]uridine incorporation into the trichloroacetic acid–precipitable cell fraction from these cultures [D. K. O'Dowd, *Nature* **303**, 619 (1983)]. For blocking of RNA synthesis in *Xenopus* embryos, actinomycin D (25 μg/ml) has been used [G. Grafi and G. Galili, *FEBS Lett.* **336**, 403 (1993)]. Incubation with cycloheximide at 0.6 μM (or 0.17 μg/ml) for 2 hours was found to achieve about 90% inhibition of [^{3}H]leucine incorporation into the acid-precipitable fraction of these *Xenopus* cultures [L. A. C. Blair, *J. Neurosci.* **3**, 1430 (1983)]. Higher concentrations of these drugs were used in the present study to ensure more complete inhibition.
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26. We thank Regeneron Inc. for providing CNTF and BDNF, L.-q. Liu for technical assistance, and B. Lu, R. Girod, and J. Alder for helpful comments on the manuscript. Supported by grants from the NSF (IBN 22106) and the Office of Naval Research (N00014-90-J1865).

16 August 1994; accepted 15 November 1994

In Vivo Evidence of Structural Brain Asymmetry in Musicians

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Certain human talents, such as musical ability, have been associated with left-right differences in brain structure and function. In vivo magnetic resonance morphometry of the brain in musicians was used to measure the anatomical asymmetry of the planum temporale, a brain area containing auditory association cortex and previously shown to be a marker of structural and functional asymmetry. Musicians with perfect pitch revealed stronger leftward planum temporale asymmetry than nonmusicians or musicians without perfect pitch. The results indicate that outstanding musical ability is associated with increased leftward asymmetry of cortex subserving music-related functions.

A number of studies have demonstrated that the left hemisphere of the brain is dominant in the production and comprehension of language in the vast majority of persons (*1*). Similar attempts to localize musical functions have yielded conflicting data, mainly because studies of amusia—that is, impairment of musical skills as a result of cerebral lesions—have failed to reveal structural-functional maps similar to those of language organization (*2*). This situation has now changed with the introduction of positron emission tomography (PET) to measure regional cerebral blood flow and metabolism during the processing of verbal and nonverbal stimuli. Whereas left hemispheric activation sites are seen during phonological, lexical, or semantic language task performance (*3*), right hemispheric preponderances are found for melodic and pitch perception, at least in musically naïve subjects (*4*). However, processing strategies may differ among individuals depending on prior musical experience (or giftedness), as suggested by PET experiments (*5*) and by behavioral (*6*) and neurophysiological (*7*) studies.

These proposed functional differences have only been related to anecdotal postmortem descriptions of gross anatomical differences in the brains of eminent musicians compared to nonmusicians as well as pronounced interhemispheric asymmetry mainly of temporal lobe structures (*8*). In an unselected postmortem sample that established an anatomical marker for cerebral asymmetry, the size of a well-defined portion of the posterior superior temporal gyrus, termed the planum temporale (PT), was larger on the left side in the majority of brains (*9*). Asymmetry of the PT has been increasingly accepted as a substrate of left hemisphere dominance for language-related auditory processing because (i) asymmetry of the PT first appears in higher primates, suggesting a relation with the evolution of language (*10*); (ii) the left PT coincides with the center of Wernicke's speech area as identified by lesion studies (*11*); (iii) macroscopic asymmetry of the PT correlates with cytoarchitectonic asymmetry of association cortices thought to play a role in higher order auditory processing (*12*); and (iv) asymmetry of the PT is correlated with handedness, with left-handers being anatomically more symmetrical (*13*).

Rightward deviation from the usual pattern of cerebral asymmetry may be associated with increased giftedness for talents for which the right hemisphere is assumed to be important (*14*). This proposed relation has been partially substantiated by connections between nonrighthandedness, atypical visuospatial lateralization, spatial giftedness, and musical talent (*15*). We have used high-resolution in vivo magnetic resonance morphometry of the PT as an index of laterality in 30 healthy, right-handed professional musicians and compared the results with those from nonmusicians matched for age, sex, and handedness (*16–18*).

Table 1. Means (±SD) for age, degree of anatomical planum temporale asymmetry (δPT), and size of left and right PT determined with in vivo magnetic resonance morphometry in healthy, right-handed musicians and nonmusicians.

Subjects	Age	δPT†	PT size (mm^2)	
			Left	Right
Musicians (n = 30)	26 (4)	−0.36 (0.25)*	1063 (189)	750 (187)
Perfect pitch (n = 11)	27 (5)	−0.57 (0.21)**	1097 (202)	611 (105)
No perfect pitch (n = 19)	26 (4)	−0.23 (0.17)	1043 (183)	830 (178)
Nonmusicians (n = 30)	26 (3)	−0.23 (0.24)	896 (236)	736 (263)

†Negative values indicate leftward asymmetry of the PT (*16*). *P = 0.028 compared to nonmusicians. **P < 0.001 compared to musicians without perfect pitch (*21*).

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