REVIEW ARTICLES

# Neurotrophic regulation of synapse development and plasticity*

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**Abstract** Neurotrophic factors are traditionally thought to be secretory proteins that regulate long-term survival and differentiation of neurons. Recent studies have revealed a previously unexpected role for these factors in synaptic development and plasticity in diverse neuronal populations. Here we review experiments carried out in our own laboratory in the last few years. We have made two important discoveries. First, we were among the first to report that brain-derived neurotrophic factor (BDNF) facilitates hippocampal long-term potentiation (LTP), a form of synaptic plasticity believed to be involved in learning and memory. BDNF modulates LTP at CA1 synapses by enhancing synaptic responses to high frequency, tetanic stimulation. This is achieved primarily by facilitating synaptic vesicle docking, possibly due to an increase in the levels of the vesicle protein synaptobrevin and synaptophysin in the nerve terminals. Gene knockout study demonstrates that the effects of BDNF are primarily mediated through presynaptic mechanisms. Second, we demonstrated a form of long-term, neurotrophin-mediated synaptic regulation. We showed that long-term treatment of the neuromuscular synapses with neurotrophin-3 (NT3) resulted in an enhancement of both spontaneous and evoked synaptic currents, as well as profound changes in the number of synaptic varicosities and synaptic vesicle proteins in motoneurons, all of which are indicative of more mature synapses. Our current work addresses the following issues: (i) activity-dependent trafficking of neurotrophin receptors, and its role in synapse-specific modulation; (ii) signal transduction mechanisms mediating the acute enhancement of synaptic transmission by neurotrophins; (iii) acute and long-term synaptic actions of the GDNF family; (iv) role of BDNF in late-phase LTP and in the development of hippocampal circuit.

**Keywords:** **neurotrophic, synaptic plasticity, LTP, activity-dependent, hippocampus, neuromuscular junction.**

Neurotrophic factors are traditionally viewed as secretory proteins that regulate neuronal survival and differentiation. However, a series of recent studies have revealed an unexpected role for these factors in synaptic development and plasticity[1,2]. Brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) have been shown to acutely potentiate synaptic transmission at the neuromuscular junction and in the brain. These factors also promote long-term maturation of the neuromuscular synapses. In the visual system, neurotrophins are involved in the formation of eye-specific synaptic connections and activity-dependent synaptic competition. Gene knockout and physiological experiments demonstrated that the neurotrophin BDNF plays an important role in hippocampal long-term potentiation (LTP), a long-lasting enhancement in synaptic efficacy often used as a cellular model for learning and memory. These findings have brought together two hotly pursued areas of neuroscience, namely the function of neurotrophic factors and the mechanisms for synaptic plasticity. Continuous studies in this emerging field will help understand how synapses develop and function in the brain, and may have general implications in treating neurological disorders in both children and adults. Our laboratory was among the first to demonstrate the novel, synaptic function of neurotrophic factors. Currently, we are focusing on the mechanisms by which neurotrophic factors regulate synapses, using the neuromuscular junction and hippocampus as model systems. In this review, we summarize the

work done in our laboratory. The readers are encouraged to read a number of reviews published in recent years[1~4].

Synapse formation is a highly organized, multi-stage process. After initial contact is made between pre- and postsynaptic elements, these components undergo a series of activity-dependent events, leading a mature synapse. Retrograde messengers have long been thought to mediate synapse development. However, so far there is no definitive proof of any molecules as the retrograde messengers. In a series of studies using Xenopus nerve-muscle co-cultures, we have demonstrated that the neurotrophin NT3 may be involved in the reciprocal interaction between the motoneurons and muscle cells. The NT3 receptor TrkC has been found in the presynaptic motoneurons. We have shown that NT3 gene is expressed in the postsynaptic muscle cells, and its expression is activity-dependent[5]. Innervation and consequent membrane depolarization leads to a rapid but specific increase in NT3 mRNA in developing muscle cells. Long-term treatment of the nerve-muscle co-culture with NT3 induces a series of presynaptic changes indicative of synaptic maturation[6]. These include an increase in the frequency and amplitude of spontaneous synaptic currents; characteristic change in the amplitude distribution of spontaneous synaptic currents, and an increase in the amplitude and a decrease in the variability of evoked synaptic currents. NT3 treatment also lead to a significant increase in synaptic varicosities as well as an enhancement of the expression of various synaptic vesicle proteins in the motoneurons. These results provide direct evidence that muscle-derived NT3 may serve as a retrograde messenger for activity-dependent synaptic strengthening at the developing neuromuscular junction. We are currently investigating the molecular mechanisms underlying the NT3 effect, and its physiological relevance in synaptic competition and elimination.

In an attempt to identify new neurotrophic factors that regulate different stages of synaptogenesis, we have recently discovered a novel mechanism for long-term modulation of synaptic transmission by glial cell line-derived neurotrophic factor (GDNF)[1)]. Long-term application of GDNF potentiates both spontaneous and evoked transmitter releases at the neuromuscular synapses, in ways reminiscent of presynaptic over-expressing frequenin, a neural specific $Ca^{2+}$-binding protein we cloned a few years ago[7]. The frequency of spontaneous and the amplitude of evoked synaptic currents are increased. Moreover, synaptic delay is decreased, and paired-pulse facilitation (PPF) is reduced by GDNF treatment, suggesting an enhancement of transmitter release. GDNF enhances the expression of frequenin in the nervous system. Transfection of frequenin antisense oligonucleotides or loading of anti-frequenin antibody into presynaptic motoneurons prevents the synaptic action of GDNF. $Ca^{2+}$ imaging experiments indicate that GDNF treatment enhances $Ca^{2+}$ influx during evoked synaptic transmission. Moreover, both GDNF and frequenin potentiate $Ca^{2+}$ channels in the spinal neurons, and the effect of GDNF is also blocked by frequenin antisense oligonucleotides. Thus, the long-term facilitation of synaptic transmission by GDNF is mediated through up-regulation of frequenin, leading to potentiation of $Ca^{2+}$ channel activity in the nerve terminals. This study has identified, for the first time, a molecular target that mediates the long-term, synaptic action of a neurotrophic factor.

In addition to the long-term effects, neurotrophic factors may also acutely modulate synaptic

1) Wang, C. et al. Unpublished paper.

transmission and synaptic plasticity. In 1993, Mu-ming Poo and his colleagues reported for the first time an acute potentiation of neurotransmitter release at the neuromuscular synapses by the neurotrophins BDNF and NT3. Since this pioneering work, studies of the acute neurotrophic effects on synapses have become a very active field in neuroscience. Our laboratory has studied the signaling mechanisms of the acute synaptic potentiation by neurotrophins. We found that unlike BDNF which requires $Ca^{2+}$ influx for its acute effect, NT3 rapidly enhances spontaneous transmitter release at the developing neuromuscular synapses even when $Ca^{2+}$ influx is completely blocked, suggesting that the NT3 effect is independent of extracellular $Ca^{2+}$ [8]. Depletion of intracellular $Ca^{2+}$ stores, or blockade of IP3 inositol 1,4,5-triphosphate or ryanodine receptors prevents the NT3-induced synaptic potentiation. Blockade of IP3 receptors cannot prevent BDNF-induced potentiation, suggesting that BDNF and NT3 use different mechanisms to potentiate transmitter release. Inhibition of $Ca^{2+}$/Calmodulin-dependent kinase II (CaMKII) completely blocks the acute effect of NT3. Further, the NT3-induced potentiation requires a continuous activation of CaMKII, because application of the CaMKII inhibitor KN62 reverses the previously established NT3 effect. Thus, NT3 potentiates neurotransmitter secretion by stimulating $Ca^{2+}$ release from intracellular stores through IP3 and/or ryanodine receptors, leading to an activation of CaMKII. These results have revealed an unusual mechanism for synaptic regulation by neurotrophic factors.

Based on studies primarily using the PC12 cells, we know that the signal transduction of neurotrophins could be mediated through three major pathways. The phospholipase C-$\gamma$(PLC-$\gamma$) pathway which generates IP3 and diacylglycerol, leading to the release of $Ca^{2+}$ from internal stores and the activation of protein kinase C, respectively. The phosphoinositide 3-kinase (PI3 kinase) phosphorylates the D3 position of phosphatidylinositol lipids to produce PtdIns(3, 4)P2 and PtdIns(3,4,5)P3. The third pathway involves SHC/Grb2/Sos interaction, Ras activation, and a series of phosphorylation reactions that include Raf, MEK and mitogen associated protein kinase (MAP kinase). Signal transduction mechanisms for neurotrophins in the central nervous system are difficult to study[9]. To gain further insights into how a specific neurotrophic function is achieved under a particular physiological condition, we systematically examined the signaling events necessary and/or sufficient to mediate the acute modulation of synaptic transmission at the neuromuscular synapses by NT3[1]. NT3-induced synaptic potentiation was blocked either by inhibition PI3 kinase, PLC-$\gamma$ or its downstream IP3 receptors, but not by MAP kinase. However, presynaptic expression of a constitutively active form of PI3 kinase (PI3K*) was not sufficient to elevate basal synaptic transmission, and application of NT3 on top of that still elicited a marked enhancement of synaptic activity. Stimulation of $Ca^{2+}$ release from intracellular stores in presynaptic neurons by IP3 also had no effect. Remarkably, photo-uncaging of IP3 in neurons loaded with PI3K* mimicked the NT3 effect. These results reveal a novel function of PI3 kinase in synaptic transmission, and suggest that simultaneous activation of PI3 kinase and IP3 pathways is necessary and sufficient to mediate the NT3-induced synaptic potentiation.

In the central nervous system, hippocampus is an area in the brain important for learning and memory. Tetanic stimulation induces LTP, which is an electrophysiological manifestation of learning

[1] Yang, F. He, X. Mizuno, K. et al. PI3 kinase pathway mediates the NT3 – induced potentiation of neurotransmitter release at the developing neuromuscular synapse. revision in Nature Neurosci.

and memory. The expression of BDNF gene is enhanced by tetanic stimulation. We have discovered that exogenous application of BDNF promotes LTP in neonatal hippocampus where endogenous BDNF level is low, while application of the BDNF antagonist TrkB-IgG inhibits LTP in adult hippocampus where endogenous BDNF level is high[10]. The BDNF effect on LTP is restricted to tetanized synapse (input specific)[11]. This effect is achieved by an attenuation of the synaptic fatigue induced by high frequency, tetanic stimulation. We have also shown that BDNF preferentially enhances synaptic transmission at high frequency, through a presynaptic mechanism[11]. These results provide the basis for a role of BDNF as a retrograde messenger in the Hebbian model, which predicts that more active synapses are favorable during synaptic competition. Using BDNF knockout mice, we have investigated the mechanisms by which BDNF regulates high frequency synaptic transmission[12]. We found a severe impairment in hippocampal LTP in the heterozygous mice, primarily due to deficits in presynaptic properties. These mice showed a pronounced synaptic fatigue induced by tetanus. Synaptic fatigue is known to be due to a depletion of synaptic vesicles during high frequency stimulation. Electron microscopic study showed that there are less synaptic vesicles docked at presynaptic active zone in the mutant mice. Biochemical experiments indicated that proteins involved in synaptic vesicle docking are markedly reduced in these mice. Treatment of the mutant slices with BDNF reversed the electrophysiological and biochemical deficits in the hippocampal synapses. Using a conditional knockout mouse with specific deletion of the BDNF receptor TrkB in the CA1 region, we showed that BDNF modulates LTP and HFS response in the CA1 synapses through mechanisms independent of postsynaptic CA1 pyramidal neurons[13]. Taken together, these results suggest a novel role for BDNF in the mobilization and/or docking of synaptic vesicles to presynaptic active zones. Our studies may have general implications in understanding the mechanisms of learning and memory, and in treatment of learning disorders in both children and adults. We are currently using biochemical and physiological approaches to determine the molecular targets as well as signal transduction mechanism of the BDNF regulation.

As a diffusable molecule, how does BDNF distinguish active and inactive neurons or synapses, and restrict its action preferentially on active neurons/synapses? One possible mechanism is that cellular responsiveness of neurons to BDNF is enhanced by neuronal activity. We recently found that the number of the BDNF receptor TrkB on the surface of hippocampal neurons can be enhanced by high frequency neuronal activity and synaptic transmission, and this effect is mediated by $Ca^{2+}$ influx[14]. Using membrane protein biotinylation as well as receptor binding assays, we showed that field electric stimulation increased the number of TrkB on the surface of cultured hippocampal neurons. Immunofluorescence staining suggested that the electric stimulation facilitated the movement of TrkB from intracellular pool to the cell surface, particularly on neuronal processes. The number of surface TrkB was regulated only by high frequency, tetanic stimulation, but not by low frequency stimulation. The activity-dependent modulation appeared to require $Ca^{2+}$ influx, since treatment of the neurons with blockers of voltage-gated $Ca^{2+}$ channels or NMDA receptors, or removal of extracellular $Ca^{2+}$, severely attenuated the effect of electric stimulation. Moreover, inhibition of CaMKII significantly reduced the effectiveness of the tetanic stimulation. These findings may help understand the role of neuronal activity in neurotrophin function and the mechanism for receptor tyrosine kinase signaling.

In summary, results from our laboratory as well as many other laboratories have proved that neu-

rotrophic factors indeed play an important role in synaptic transmission, plasticity and development. Future studies should be directed towards the cellular and molecular mechanisms underlying specific neurotrophic regulations, and their physiological relevance to the development and function of the nervous system.

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