BDNF and epilepsy: too much of a good thing?

Devin K. Binder, Susan D. Croll, Christine M. Gall and Helen E. Scharfman

Various studies have shown that brain-derived neurotrophic factor (BDNF) increases neuronal excitability and is localized and upregulated in areas implicated in epileptogenesis. Seizure activity increases the expression of BDNF mRNA and protein, and recent studies have shown that interfering with BDNF signal transduction inhibits the development of the epileptic state in vivo. These results suggest that BDNF contributes to epileptogenesis. Further analysis of the cellular and molecular mechanisms by which BDNF influences excitability and connectivity in adult brain could provide novel concepts and targets for anticonvulsant or anti-epileptogenic therapy.

Epilepsy is a disorder of the brain characterized by the periodic and unpredictable occurrence of seizures. Although complex partial epilepsy is the most common form in adults (40% of all cases), control of seizures can be achieved only in a minority of these cases by optimal current anticonvulsant treatment. Consequently, complex partial epilepsy remains a major public health problem affecting approximately one million people in the USA.

Elucidating the cellular and molecular mechanisms of epileptogenesis could provide novel therapeutic approaches aimed at the prevention or management of the disease. The discovery that limbic seizures increase nerve growth factor (NGF) mRNA levels led to the idea that seizure-induced expression of neurotrophic factors might contribute to the lasting structural and functional changes underlying epileptogenesis. Similarly, increases in neurotrophin (NT) expression that follow other insults (for example, ischemia and traumatic brain injury) could also contribute to epileptogenesis. In the past several years, there has been an exciting confluence of in vitro and in vivo findings that strongly implicate the NT brain-derived neurotrophic factor (BDNF) in particular limbic circuits in the cascade of electrophysiological and behavioral changes underlying the epileptic state. The purpose of this review is to critically examine the evidence for a role of BDNF in epileptogenesis.

**BDNF signal transduction**

BDNF is a member of the neurotrophin family of neurotrophic factors, which also includes NGF, NT-3, NT-4/5 and NT-6. NTs bind with high affinity, but differing specificity, to NT receptors (trkA, trkB and trkC) and each NT binds with low affinity to the p75 receptor. Trk proteins are transmembrane receptor tyrosine kinases (RTKs) homologous to other RTKs, such as the epidermal growth factor (EGF) receptor and insulin receptor family. Signaling by RTKs is known to involve ligand-induced receptor dimerization and consequent trans-autophosphorylation. Receptor autophosphorylation on multiple tyrosine residues creates specific binding sites for intracellular target proteins that bind to the activated receptor via SH2 domains. Activation of target proteins can result in activation of a variety of intracellular signaling cascades, including the Ras–mitogen-activated protein kinase (MAPK) cascade and phosphorylation of Ca2+/AMP-response-element-binding proteins (CREB; Ref. 7).

**BDNF effects in vitro**

The classical view of NT function includes effects on the growth and survival of neurons during development. BDNF, in particular, appears to regulate neuronal morphology and synaptogenesis and have neuroprotective effects in diverse areas of the CNS (Ref. 8). Whereas the functions of NTs in the adult brain are less clear, they might include neuroprotective and morphological effects following pathologic upregulation in response to seizures.
However, little direct evidence exists to support such actions. By contrast, more recently described effects of NTs in the adult brain suggest that they have striking influences on neuronal excitability\(^9\). For example, application of NTs (including BDNF) has been shown to potentiate synaptic transmission in vitro\(^10\) and in vivo\(^11\). BDNF enhances excitatory synaptic transmission\(^10,12\) and reduces inhibitory synaptic transmission\(^13\). In the hippocampus, a critical level of BDNF/trkB activation appears to be vital for modulating synaptic efficacy. Pretreatment of adult hippocampal slices with the BDNF scavenger, trkB-Fc, reduces LTP (Ref. 14) and hippocampal slices from BDNF knockout animals exhibit impaired LTP induction that can be restored by reintroducing BDNF (Refs 15,16). In addition, trk receptor antagonists such as K252a block hyperexcitability with the BDNF scavenger, trkB-Fc, reduces LTP (Ref. 14) and hippocampal slices from BDNF knockout animals exhibit impaired LTP induction that can be restored by reintroducing BDNF (Ref.s 15,16). \(</3\>\)

Localization, transport and release of BDNF
BDNF mRNA has a widespread distribution in the CNS (Ref. 23). Similarly, mRNA encoding the high affinity receptor for BDNF, trkB, is located throughout the brain\(^24,25\). Notably, high levels of BDNF and trkB mRNA expression are found in brain areas that have been associated with seizure susceptibility, such as hippocampus and entorhinal cortex (Fig. 1a). Within hippocampus, the granule cells, pyramidal cells and some hilar GABAergic neurons express mRNA encoding BDNF and trkB. BDNF protein immunoreactivity also appears to be widespread and preferentially localized in cell bodies and axons, compared to dendrites\(^23\). Similar to BDNF mRNA, constitutive BDNF protein expression is high in hippocampus, where the mossy fiber axons of dentate granule cells are intensely immunoreactive\(^23,26\) (Fig. 1c). By contrast to the classical target-derived trophic factor model, in which NTs are retrogradely transported, abundant evidence has shown that BDNF is anterogradely transported in brain\(^23,27,28\). In particular, within hippocampus, it appears that BDNF within the hilus and CA3 stratum lucidum is synthesized by granule cells, anterogradely transported and preferentially stored in mossy fiber terminal boutons\(^29\). Biochemical studies suggest that endogenous BDNF might be packaged in a releasable vesicular pool\(^30\) and recent evidence indicates that NTs are released acutely following depolarization\(^31\).

BDNF upregulation by seizure activity
Seizures have been shown to stimulate the expression of a variety of genes, including those that encode transcription factors\(^32,33\), neuropeptides\(^34\), growth associated proteins (GAP-43; Ref. 35), proteases\(^36\) and also NTs and trk receptors. In particular, BDNF, NGF and trkB mRNA concentrations are increased in kindling and other seizure models, whereas NT-3 mRNA concentrations are decreased\(^37\). The magnitude of increase for BDNF mRNA is greatest in the hippocampus, being markedly upregulated in the dentate gyrus and CA1–CA3 pyramidal cell layers (Fig. 1b).

http://tins.trends.com
This upregulation has also been shown at the protein level. Extracts and in vivo microdialysates from animals given chemical convulsions show marked increases in neurotrophic activity40,41 and increases in BDNF protein content have been described following hilar lesion-induced limbic seizures, kindling and kainate administration42-45.

Seizure-induced increases in BDNF mRNA levels are transient, whereas the increase in BDNF protein content persists longer. For example, following lesion-induced recurrent limbic seizures, BDNF mRNA concentrations peak 6 h after the seizure onset and return to control levels ~12 h after seizures have ended. By contrast, initial increases in BDNF protein content lag behind mRNA changes by 4 h, but remain well elevated for four days after the seizure episode42.

It appears that, following seizures, newly expressed BDNF is anterogradely transported. Using hippocampal microdissection and quantification of BDNF by two-site ELISA, Elmer et al.43 showed that after seizures BDNF protein levels were maximal at 12 h in the dentate gyrus, but were maximal after 24 h in CA3. This is consistent with (but does not prove) anterograde transport of seizure-induced BDNF protein. More recent evidence regarding the anatomical distribution and time course of BDNF immunoreactivity following seizures has shown that there is increased BDNF immunoreactivity in dentate granule cells by 4 h after seizure induction, followed by large increases in hilus and CA3 stratum lucidum 12–24 h later. During the latter time period, BDNF immunoreactivity within the granule cell bodies had returned to control levels46 (Fig. 1d).

Effects of inhibition of BDNF/trkB in seizure models

Evidence that BDNF is upregulated by seizures and positively modulates neuronal excitability within hippocampus suggests that BDNF and possibly other NTs play a role in epileptogenesis. This view is supported by recent studies using the kindling model. In this model, repeated, focal application of initially subconvulsive electrical stimuli eventually results in intense focal and tonic–clonic seizures. Once established, this enhanced sensitivity to electrical stimulation persists throughout the life of the animal. The kindling model has been an important tool, because it enables experimental control over seizures and precise quantitation of the effects of in vivo experimental manipulation of epileptogenesis.

Funabashi et al.47 and Van der Zee et al.48 found that kindling development could be delayed by intraventricular infusion of anti-NGF antiserum. However, the lack of specificity of the antiserum limited interpretation of these experiments. Kokaia et al.49 reported a greater than twofold reduction in the rate of kindling development in BDNF heterozygous (+/−) mice, in which one BDNF allele had been inactivated by gene targeting. Both the basal and seizure-induced concentrations of BDNF mRNA were lower in the BDNF−/− compared to wild-type mice, consistent with the idea that reduced trkB receptor activation in the BDNF−/− mice contributed to the inhibition of kindling development. The twofold reduction in kindling rate in the heterozygotes is striking, given that presumably there was some reduction (but not elimination) of trkB receptor signaling. Conversely, transgenic mice that overexpress BDNF have more severe seizures in response to kainic acid and some display spontaneous seizures50. Obviously, results from both the BDNF+/− knockouts and the BDNF transgenic mice must be interpreted with caution given potential developmental effects of altered BDNF levels. The availability of conditional knockouts for trkB will enable analysis of the importance of trkB signaling in adult animals denovo51.

A recent approach involved selective blockade of trkB receptors during kindling development using trk-specific ‘receptor bodies’52. These compounds are divalent homodimers that contain the ligand-binding domain of a given trk receptor and thus act as false receptors or ‘receptor bodies’ that putatively sequester endogenous NT. Intracerebroventricular (ICV) infusion of trkB receptor body, trkB-Fc, inhibited the development of kindling in comparison to animals treated with saline, human IgG, trkA-Fc or trkC-Fc (Ref. 52; Fig. 2). Furthermore, the degree of immunohistochemical penetration of trkB-Fc into hippocampus, but not striatum, septum or other structures, correlated with the magnitude of inhibition of kindling development. These results suggest that activation of trkB receptors contributes to the development of kindling and that the hippocampus might be a primary site of trkB action.

Although the finding that ICV infusion of trkB-Fc interferes with kindling strongly suggests that BDNF is involved in the development of kindled seizures, an alternative explanation exists. Recent data have
Orthodromic population spike (arrowhead), presumably the direct granule cell axon stimulation, before BDNF application. This elicited an antidromic population spike (arrow) and a second, pilocarpine-treated rat with mossy fiber sprouting. The top trace shows the response to a stimulus absence of BDNF. (b) Responses of dentate gyrus granule cells to mossy fiber stimulation in a which never occurred in response to stimulation of other inputs, or stimulation of mossy fibers in the absence of BDNF. (c) Responses to the same stimuli, applied after bath application of BDNF, showing a greater response to mossy fiber but not fimbria stimulation.

Fig. 3. Brain-derived neurotrophic factor-induced hyperexcitability of the mossy fiber pathway. (a) Responses of CA3 pyramidal cells, recorded extracellularly from the pyramidal cell layer, to single and repetitive afferent stimulation in hippocampal slices, before and after brain-derived neurotrophic factor (BDNF) exposure. (i) Traces on left show responses to single stimuli applied to the fimbria (top) or mossy fibers (bottom) at submaximal intensity, before BDNF exposure. Traces on right show responses to the same stimuli, applied after bath application of BDNF, showing a greater response to mossy fiber but not fimbria stimulation. (ii) The top trace shows extracellularly recorded responses to repetitive stimulation of the mossy fibers in the presence of BDNF leading to spreading depression in the CA3 pyramidal cell layer. Traces below show responses to the first three pairs of stimuli that led to the spreading depression episode shown above, illustrating the addition of multiple population spikes as stimulation was initiated. Only four pairs of stimuli at 1 Hz evoked spreading depression, which never occurred in response to stimulation of other inputs, or stimulation of mossy fibers in the presence of BDNF. (b) Responses of dentate gyrus granule cells to mossy fiber stimulation in a pilocarpine-treated rat with mossy fiber sprouting. The top trace shows the response to a stimulus before BDNF application. This elicited an antidromic population spike (arrow) and a second, orthodromic population spike (arrowhead), presumably the direct granule cell axon stimulation, followed by excitation of recurrent mossy fibers innervating granule cells. The middle trace shows the response to the same stimulus, but after BDNF exposure, evoking a burst of population spikes. The bottom trace shows that, in addition to evoked bursts after BDNF application, spontaneous bursts were recorded. This activity was not recorded in the CA1 or CA3 regions, and thus was probably generated from granule cells, an hypothesis consistent with intracellular recordings. (c) Responses of CA3 pyramidal cells to mossy fiber stimulation in transgenic mice overexpressing BDNF. The top trace shows the response to a pair of stimuli to the hilus, which evoked large population spikes in the CA3 stratum lucidum of the hippocampus. (Fig. 1f). This distribution coincides with the mossy fiber pathway arising from the dentate gyrus granule cells. Interestingly, the anatomical distribution, time course and threshold for seizure-induced phospho-trk immunoreactivity correspond with the pattern of BDNF upregulation following seizures (Fig. 1d). That is, both phospho-trk and BDNF immunoreactivity are most prominently increased in hippocampal CA3 stratum lucidum and are maximal 24 h after seizure onset, suggesting that the phospho-trk

Activation of trk receptors after seizures

The work described above suggests that limiting activation of the trkB receptor inhibits epileptogenesis, but whether and where NT receptor activation occurs during epileptogenesis remained unclear. Because ligand-induced receptor tyrosine phosphorylation is essential for NT-induced cellular responses, receptor tyrosine phosphorylation seems a logical index of the level of biological NT activity. Using antibodies that selectively recognize the phosphorylated form of trk receptors (phospho-trk), investigators found that, in contrast to the low level of phospho-trk immunoreactivity that is constitutively expressed in the hippocampus of adult rats (Fig. 1e), phospho-trk immunoreactivity was strikingly increased following partial kindling or kainate-induced seizures. Furthermore, following seizures, phospho-trk immunoreactivity was selectively increased in the dentate hilus and CA3 stratum lucidum of the hippocampus (Fig. 1f). This distribution coincides with the mossy fiber pathway arising from the dentate gyrus granule cells.
immunoreactivity might be caused by seizure-induced increases in BDNF expression and release.

**BDNF-induced hyperexcitability of the mossy fiber-CA3 synapse**

Based on data from normal mature animals described above, one might speculate that BDNF upregulation in the adult brain could predispose certain areas to seizures or even cause seizures. Indeed, in adult rat hippocampal slices, exposure to BDNF can produce multiple discharges and spreading depression in area CA3 and the entorhinal cortex upon afferent stimulation. Acute application of exogenous BDNF to hippocampal slices appears to preferentially enhance the efficacy of excitatory mossy fiber synapses onto CA3 pyramidal cells. Actions of BDNF have also been examined after pilocarpine-induced status epilepticus and chronic seizures, in which sprouting of mossy fiber collaterals occurs. The new collaterals innervate processes in the inner molecular layer, including granule cells and increased anterograde transport of BDNF in mossy fibers and release at terminals in hilus and CA3 stratum lucidum. These effects were blocked by K252a, an inhibitor of trk receptor activation, confirming a preferential enhancement of mossy fiber synaptic transmission by BDNF.

**Cellular model of BDNF-trkB interaction**

The studies summarized above suggest that: (1) upregulation of BDNF mRNA, protein and receptor activation occurs during epileptogenesis; (2) this upregulation is functionally relevant to increased excitability; and (3) the hippocampus and closely associated limbic structures might be particularly important in the pro-epileptogenic effects of BDNF. These observations suggest the following cellular and molecular model of the actions of BDNF in promoting excitability in the hippocampus. BDNF mRNA upregulation by seizure or perhaps by other stimuli, such as ischemia or traumatic brain injury, leads to increased BDNF production by the dentate granule cells and increased anterograde transport and release of BDNF from mossy fiber axons resulting in activation of trkB receptors in the hilus and CA3 stratum lucidum. The locus of activation of trkB receptors by released BDNF could be either pre- or postsynaptic. TrkB receptor activation could lead to acute depolarization, enhanced glutamate-mediated synaptic transmission or reduced inhibitory synaptic transmission. These alterations in synaptic transmission, either alone or in combination with other changes (see below) could be sufficiently long-lived to underlie a permanent hyperexcitability of the hippocampal network, that is, an epileptic state.

Evidence implicating BDNF in the modulation of synaptic transmission underlying epileptogenesis is crucially dependent on whether such modulation occurs in epileptic tissue. Several lines of evidence suggest this is the case. First, BDNF expression is increased in hippocampal patients with temporal lobe epilepsy. Second, evidence for the modulation of ionotropic receptors during epilepsy comes from studies showing altered electrophysiology of dentate granule cells in kindling and other animal models and also in human epileptic tissue. Third, increased excitability of CA3 pyramidal cells is observed in kindled animals, as indicated by increased epileptiform burst activity induced by elevated K+ or lowered Mg2+ in isolated hippocampal slices. CA3 excitability is also present in other animal models. Fourth, tetanic stimulation of the mossy fiber pathway in hippocampal slices (such as might occur during a seizure) induces synaptic potentiation on CA3 pyramidal cells while inducing depression onto stratum lucidum interneurons.

Modulation of multiple synaptic stations in the limbic system probably contributes to
hyperexcitability following seizures. However, the pivotal role of the CA3 pyramidal cells in promoting epileptiform activity in the hippocampus; the role of BDNF in hippocampal synaptic transmission; the localization of seizure-induced trkB receptor activation in CA3 stratum lucidum and the fact that constitutive and seizure-induced BDNF immunoreactivity within the hippocampus is most intense in the mossy fiber pathway suggest that strengthening of the excitatory mossy fiber input onto CA3 pyramidal cells might be a primary mechanism by which BDNF promotes epileptogenesis.

**Other effects of BDNF**

Based on the known effects of BDNF, it is possible that trkB receptor activation could contribute to epileptogenesis not only via synaptic effects on excitability, but also by inducing changes in dendritic or axonal sprouting, synaptic morphology and synapse formation on a slower time scale. The most prominent synaptic reorganization known to occur in the epileptic brain is sprouting of the dentate granule cell mossy fibers. Interestingly, mossy fiber sprouting was greater in BDNF−/− compared to wild-type mice, in spite of kindling development being inhibited in the mutants. In addition, bath-applied trkB-Fc failed to inhibit kainate-induced mossy fiber sprouting in hippocampal explant cultures. Therefore, there is little evidence to date to suggest that BDNF upregulation is responsible for synaptic reorganization in the adult brain during epileptogenesis.

**BDNF**

BDNF is known to modulate the expression of neurotransmitters and neuropeptides, many of which potentially play a role in seizures. Perhaps the best characterized of these is NPY. BDNF (but not NGF) is known to increase NPY concentrations. NPY is thought to inhibit seizure generation, because NPY knockout animals are more susceptible to seizures. Interestingly, both kindling and kainate-induced seizures increase NPY immunoreactivity in the mossy fibers in a distribution that is strikingly similar to phospho-trkB immunoreactivity. This suggests that BDNF-induced trkB activation could lead to NPY upregulation in an overlapping anatomical distribution, which might subsequently limit excitability.

**Concluding remarks**

Just over 10 years ago, Gall and Isackson discovered that limbic seizures upregulate the mRNA encoding NGF. In the past decade, much has been learned about the importance of NTs to epileptogenesis. Although the expression of many growth-related genes is altered by seizure activity, the upregulation of BDNF and activation of trkB receptors appear to play a key role in the development of hyperexcitability in vitro and in vivo, in particular in the hippocampus via modulation of mossy fiber–CA3 synapses. The precise downstream effectors of BDNF and trkB responsible for epileptogenesis, together with the potential for novel anti-convulsant and anti-epileptic therapies, provide fascinating and fundamental questions for future study.

**References**


Isakovson, P. J. et al. (1991) BDNF mRNA expression is increased in adult rat forebrain after limbic seizures: temporal patterns of induction distinct from NGF. Neuron 6, 937–948

Audo, G. et al. (1996) Change in neurotrophins and their receptor mRNAs in the rat forebrain after status epilepticus induced by pilocarpine. Epilepsia 37, 198–207


Rudge, J. S. et al. (1998) Endogenous BDNF protein is increased in adult rat hippocampus after a kaicic acid induced excitotoxic insult but exogenous BDNF is not neuroprotective. Exp. Neurol. 149, 398–410

Vezzani, A. et al. (1999) Brain-derived neurotrophic factor immunoreactivity in the limbic system of rats after acute seizures and during spontaneous convulsions: temporal evolution of changes as compared to neuropeptide Y. Neuroscience 90, 1445–1461


Croll, S. D. et al. (1999) Brain-derived neurotrophic factor transgenic mice exhibit passive avoidance deficits, increased seizure severity and in vitro hyperexcitability in the hippocampus and entorhinal cortex. Neuroscience 93, 1491–1506


Wu, K. et al. (1996) Functional trkB neurotrophin receptors are intrinsic components of the adult brain postsynaptic density. Mol. Brain Res. 43, 260–269


Kohr, G. et al. (1993) Properties of NMDA receptor channels in neurons acutely isolated from epileptic (kindled) rats. J. Neurosci. 13, 3612–3627


Croll, S. D. et al. (1994) Regulation of neuropeptides in adult rat forebrain by the neurotrophins BDNF and NGF. Eur. J. Neurosci. 6, 1343–1353

