73 Smith-Thomas, L. *et al.* (1994) An inhibitor of neurite outgrowth produced by astrocytes. *J. Cell Sci.* 107, 1687–1695
74 Smith-Thomas L. *et al.* (1995) Increased axon regeneration in astrocytes grown in the presence of proteoglycan synthesis inhibitors. *J. Cell Sci.* 108, 1307–1315
75 Moon, L.D.F. *et al.* (1999) Extensive axon regeneration following in vivo treatment of rat brain the chondroitinase ABC. *Soc. Neurosci. Abstr.* 25, p. 751
76 Bradbury, E.J. *et al.* (2000) Chrondroitinase ABC delivered to the site of a spinal cord injury upregulates GAP-43 expression in dorsal root ganglion neurons. *Soc. Neurosci. Abstr.* 26, p. 860
77 Asher, R.A. *et al.* (2000) Neurocan is upregulated in injured brain and in cytokine-treated astrocytes. *J. Neurosci.* 20, 2427–2438
78 Asher, R.A. *et al.* (1999) Versican is up-regulated in CNS injury and is a product of O-2A lineage cells. *Soc. Neurosci. Abstr.* 25, p. 750
79 Jaworski, D.M. *et al.* (1999) Intracranial injury acutely induces the expression of the secreted isoform of the CNS-specific hyaluronan-binding protein BEHAB brevican. *Exp. Neurol.* 157, 327–337
80 Dou, C.L. and Levine, J.M. (1994) Inhibition of neurite growth by the NG2 chondroitin sulfate proteoglycan. *J. Neurosci.* 14, 7616–7628
81 Fawcett, J.W. and Asher, R.A. (1999) The glial scar and CNS repair. *Brain Res. Bull.* 49, 377–391
82 Niederost, B.P. *et al.* (1999) Bovine CNS myelin contains neurite growth-inhibitory activity associated with chondroitin sulfate proteoglycans. *J. Neurosci.* 19, 8979–8989
83 McTigue, D.M. *et al.* (1998) Neurotrophin-3 and brain-derived neurotrophic factor induce oligodendrocyte proliferation and myelination of regenerating axons in the contused adult rat spinal cord. *J. Neurosci.* 18, 5354–5365

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.

Devin K. Binder*
Dept of Neurological Surgery, University of California, San Francisco, CA 94110, USA.
*e-mail: dbinder@itsa.ucsf.edu

Susan D. Croll
Regeneron Pharmaceuticals, 777 Old Saw Mill River Road, Tarrytown, NY 10591, USA.

Christine M. Gall
Dept of Anatomy and Neurobiology, University of California, Irvine, California 92697-4292, USA.

Helen E. Scharfman
CNRRR, Helen Hayes Hospital, West Haverstraw, NY 10993-1195 and the Depts of Pharmacology and Neurology, Columbia University, College of Physicians and Surgeons, NewYork, NY 10032, USA.

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)[1], 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[2] led to the idea that seizure-induced expression of neurotrophic factors might contribute to the lasting structural and functional changes underlying epileptogenesis[3]. Similarly, increases in neurotrophin (NT) expression that follow other insults (for example, ischemia and traumatic brain injury) could also contribute to epileptogenesis[4]. 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[5]. Signaling by RTKs is known to involve ligand-induced receptor dimerization and consequent trans-autophosphorylation[6]. Receptor autophosphorylation on multiple tyrosine residues creates specific binding sites for intracellular target proteins that bind to the activated receptor via SH2 domains[6]. 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 Ca^{2+}/cAMP-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[4].

http://tins.trends.com 0166-2236/01/$ – see front matter PII: S0166-2236(00)01682-9

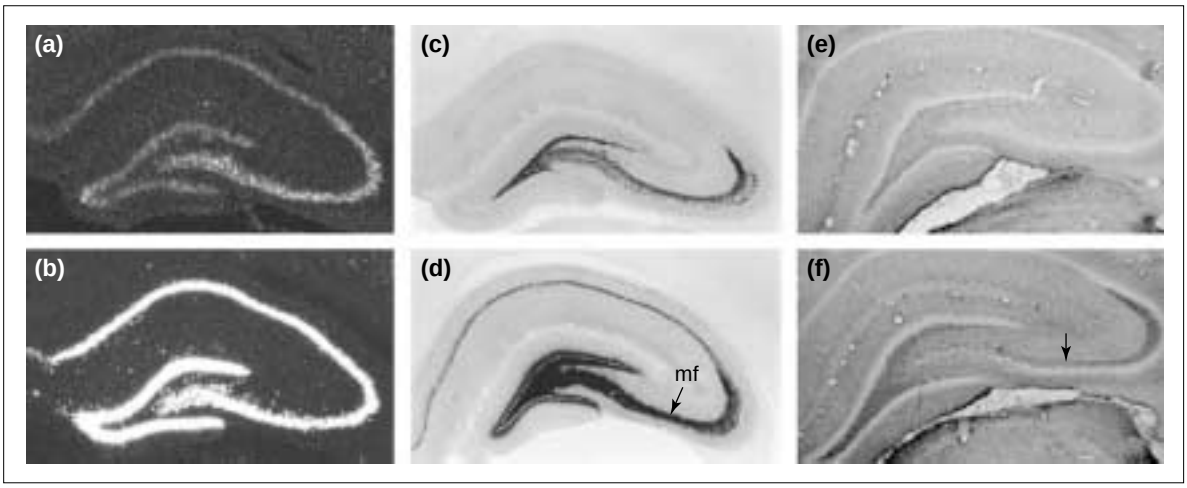

Fig. 1. Localization and regulation of brain-derived neurotrophic factor mRNA and protein and phospho-trk immunoreactivity. (a) and (b) Darkfield photomicrographs showing *in situ* hybridization for brain-derived neurotrophic factor (BDNF) mRNA in hippocampus in basal state (a) or 8 h after recurrent limbic seizures induced by contralateral hilus lesion (b). Note the basal distribution in the dentate gyrus and CA1–CA3 pyramidal cell layers and the dramatic increases in these areas following seizures. (c) and (d) Coronal sections showing BDNF immunoreactivity in hippocampus in basal state (c) or 12 h after hilus lesion-induced limbic seizures (d). Note the basal BDNF immunoreactivity in the mossy fiber pathway and the increase in the dentate gyrus granule cells, mossy fiber pathway (mf) and CA1–CA3 pyramidal cells following seizures. (e) and (f) Coronal sections showing phospho-trk immunoreactivity in hippocampus in sham-stimulated animal (e) or from animal 24 h after limbic seizures induced by hippocampal kindling stimulation (f). Note the low level of basal phospho-trk immunoreactivity and the increase, 24 h after seizures, in a pattern that corresponding to the mossy fiber pathway (arrow). For more detail, see Refs 3,37,46,57.

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 in the hippocampus following BDNF exposure *in vitro*[17]. The mechanism underlying synaptic potentiation by BDNF is currently unclear, but could involve the facilitation of transmitter release[18], phosphorylation of specific NMDA receptor subunits[19] and direct effects on ion channels and/or conductances[20,21]. Enhanced excitatory transmission might also arise indirectly, because BDNF is known to affect the structure and function of inhibitory (specifically, GABAergic) neurons[22].

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[4] (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[31,78].

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[3,24,37–39]. 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).

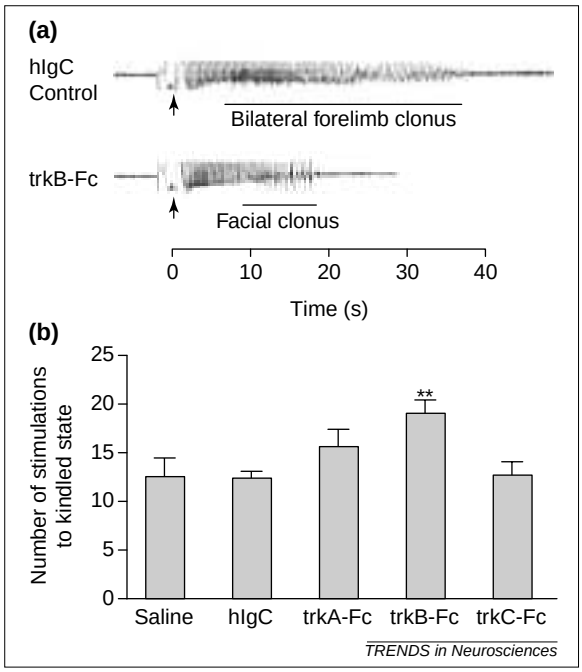

Fig. 2. Inhibition of kindling by trkB-Fc. (a) Representative electroencephalograms of seizures in animals treated either with human IgG (hIgG) or trkB-Fc during kindling stimulations. Arrows mark the stimulation artifact. Whereas the hIgG-treated (control) animal had a 36 s seizure discharge with a clonic motor component of 30 s, the trkB-Fc-treated animal had a 19 s seizure discharge with facial clonus. Shorter and behaviorially less-intense seizures were consistently observed in the trkB-Fc-treated animals compared with controls. (b) Number (mean ± SE) of stimulations to kindling criterion (three consecutive clonic motor seizures) by treatment group. All groups (except saline-treated) received intracerebroventricular doses of 50 μg/day. TrkB-Fc-treated animals required significantly (**$P < 0.01$) more stimulations to reach the kindled state. For more detail see Ref. 53.

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 activity[40,41] and increases in BDNF protein content have been described following hilar lesion-induced limbic seizures, kindling and kainate administration[42–45].

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[3]. 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 episode[42].

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 levels[46] (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 antisera. However, the lack of specificity of the antisera 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 seizures[50]. 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 *de novo*[51].

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

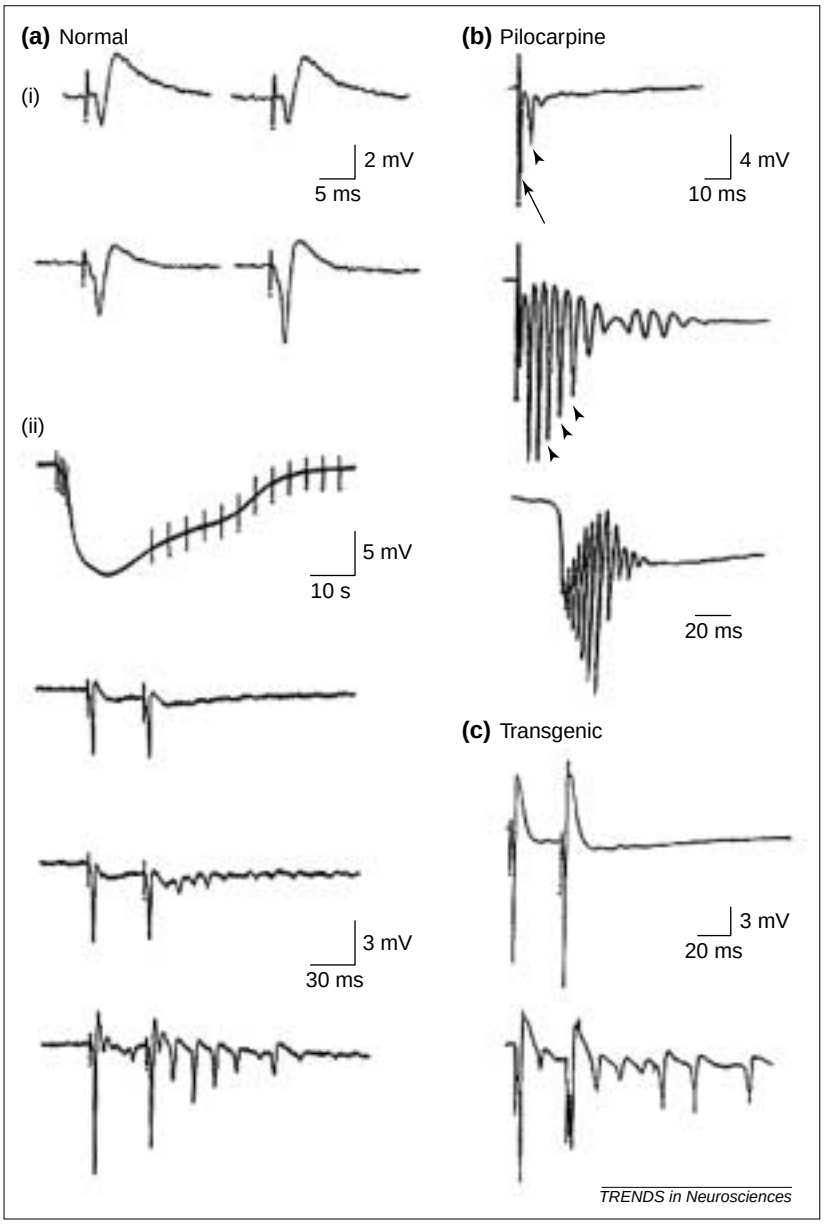

Fig. 3. Brain-derived neurotrophic factor-induced hyperexcitability of the mossy fiber pathway. (a) Responses of CA3 pyramidal cells, recorded extracellularly from the pyramidal cell layer, to single and repetitive afferent stimulation in hippocampal slices, before and after brain-derived neurotrophic 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 absence 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[59]. (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 cell layer, recorded extracellularly, in the absence of BDNF. The bottom trace shows that after several pairs of stimuli, multiple population spikes were evoked. For more detail see Refs 17,50,59.

suggested that, under some circumstances, trkB-Fc can act as a carrier for BDNF, enhancing both its distribution and activity[53]. However, for carrier effects, molar ratios of trkB-Fc to BDNF were not tested past a 2:1 excess and *in vitro* data indicate total BDNF inhibition occurs at ratios of 10:1 or greater. The 55 μg/day doses used in the kindling study[52] should have represented a vast excess of trkB-Fc relative to BDNF and therefore should have blocked the effects of BDNF.

By contrast, chronic intrahippocampal infusion of BDNF inhibits the development of hippocampal kindling and reduces the duration of electrographic seizure[54]. However, prolonged exposure to increased concentrations of BDNF suppresses trkB receptor responsiveness and reduces trkB mRNA and protein levels *in vitro*[55,56]. Similarly, a six-day infusion of BDNF into the adult hippocampus *in vivo* decreases trkB receptor levels by 80% (Ref. 55). Thus, it is possible that chronic BDNF infusion in these kindling studies led to trkB downregulation and reduced responsiveness. In such a case, the retarded kindling development observed is consistent with the findings of the trkB-Fc infusion studies and those of BDNF heterozygotes[49] in implicating trkB receptor activation in kindling development. Alternatively, BDNF infusion could have upregulated the inhibitory molecule neuropeptide Y (NPY) in these studies (see below).

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[5], 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[57]. 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.

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[57] suggesting that the phospho-trk

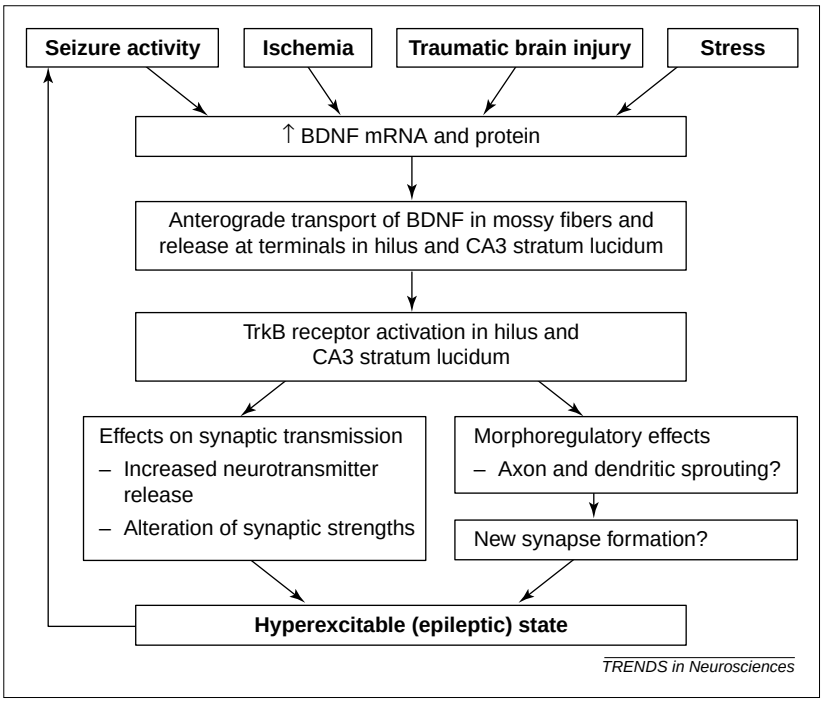

Fig. 4. Brain-derived neurotrophic factor (BDNF) mechanisms of epileptogenesis. Various stimuli (e.g. seizure, ischemia, traumatic brain injury, stress) could lead to upregulation of *BDNF* mRNA and protein in particular anatomic networks in the hippocampus, leading to long-lasting effects on synaptic structure and function and ultimately to hyperexcitability.

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[17] (Fig. 3a). Acute application of exogenous BDNF to hippocampal slices appears to preferentially enhance the efficacy of excitatory mossy fiber synapses onto CA3 pyramidal cells[17] (Fig. 3a).

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 cell dendrites[58]. In hippocampal slices isolated from pilocarpine-treated rats, BDNF enhanced responses to stimulation of the mossy fiber collaterals that were recorded in the inner molecular layer[59] (Fig. 3b). These effects were blocked by K252a, an inhibitor of trk receptor activation, confirming a preferential enhancement of mossy fiber synaptic transmission by BDNF. BDNF immunoreactivity was intense in sprouted mossy fibers, similar to the intensity of normal mossy fibers[59]. In addition, BDNF exposure in these epileptic animals led to seizure-like events, suggesting that BDNF might be more potent after seizures compared to normal tissue[59]. Consistent with this interpretation are observations of heightened seizure susceptibility, spontaneous seizures and hyperexcitability of hippocampal field CA3 in transgenic mice that overexpress BDNF (Ref. 50; Fig. 3c).

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 (Fig. 4). *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[60,61]. TrkB receptor activation could lead to acute depolarization[21], enhanced glutamate-mediated synaptic transmission[12,18] or reduced inhibitory synaptic transmission[13]. 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 (Fig. 4).

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 hippocampi of patients with temporal lobe epilepsy[62]. Second, evidence for the modulation of ionotropic receptors during epilepsy comes from studies showing altered electrophysiology of dentate granule cells in kindling[63,64] and other animal models[65–67] and also in human epileptic tissue[68]. Third, increased excitability of CA3 pyramidal cells is observed in kindled animals, as indicated by increased epileptiform bursting induced by elevated K^+ or lowered Mg^{2+} in isolated hippocampal slices[69,70]. CA3 excitability is also present in other animal models[71]. Fourth, tetanic stimulation of the mossy fiber pathway in hippocampal slices (such as might occur during a seizure) induces synaptic potentiation onto CA3 pyramidal cells while inducing depression onto stratum lucidum interneurons[72].

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 trk receptor activation in CA3 stratum lucidum[57] and the fact that constitutive and seizure-induced BDNF immunoreactivity within the hippocampus is most intense in the mossy fiber pathway[23,26], 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[73]. Interestingly, mossy fiber sprouting was greater in $BDNF^{+/-}$ compared to wild-type mice, in spite of kindling development being inhibited in the mutants[49]. In addition, bath-applied trkB-Fc failed to inhibit kainate-induced mossy fiber sprouting in hippocampal explant cultures[74]. Therefore, there is little evidence to date to suggest that BDNF upregulation is responsible for synaptic reorganization in the adult brain during epileptogenesis.

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[75]. NPY is thought to inhibit seizure generation, because NPY knockout animals are more susceptible to seizure[76]. Interestingly, both kindling and kainate-induced seizures increase NPY immunoreactivity in the mossy fibers[45,77] in a distribution that is strikingly similar to phospho-trk immunoreactivity. This suggests that BDNF-induced trk activation could lead to NPY upregulation in an overlapping anatomical distribution, which might subsequently limit excitability[54].

Concluding remarks

Just over 10 years ago, Gall and Isackson[2] 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 trk 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 anticonvulsant and anti-epileptogenic therapies, provide fascinating and fundamental questions for future study.

Acknowledgements
We acknowledge the contributions of many investigators to this rapidly growing field that we were unable to cite owing to space constraints.This work was supported by the NIH.

References

1 Hauser, W.A. and Kurland, L.T. (1975) The epidemiology of epilepsy in Rochester, Minnesota, 1935 through 1967. *Epilepsia* 16, 1–66
2 Gall, C.M. and Isackson, P.J. (1989) Limbic seizures increase neuronal production of messenger RNA for nerve growth factor. *Science* 245, 758–761
3 Gall, C.M. (1993) Seizure-induced changes in neurotrophin expression: implications for epilepsy. *Exp. Neurol.* 124, 150–166
4 Lindvall, O. *et al.* (1994) Neurotrophins and brain insults. *Trends Neurosci.* 17, 490–496
5 Barbacid, M. (1994) The trk family of neurotrophin receptors. *J. Neurobiol.* 25, 1386–1403
6 Schlessinger, J. and Ulrich, A. (1992) Growth factor signaling by receptor tyrosine kinases. *Neuron* 9, 381–391
7 Segal, R.A. and Greenberg, M.E. (1996) Intracellular signaling pathways activated by neurotrophic factors. *Annu. Rev. Neurosci.* 19, 463–489
8 Barde, Y.A. (1989) Trophic factors and neuronal survival. *Neuron* 2, 1525–1534
9 Thoenen, H. (1995) Neurotrophins and neuronal plasticity. *Science* 270, 593–598
10 Lohof, A.M. *et al.* (1993) Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. *Nature* 363, 350–353
11 Messaoudi, E. *et al.* (1998) Acute intrahippocampal infusion of BDNF induces lasting potentiation of synaptic transmission in the rat dentate gyrus. *J. Neurophysiol.* 79, 496–499
12 Kang, H. and Schuman, E.M. (1995) Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. *Science* 267, 1658–1662
13 Tanaka, T. *et al.* (1997) Inhibition of $GABA_A$ synaptic responses by brain-derived neurotrophic factor (BDNF) in rat hippocampus. *J. Neurosci.* 17, 2959–2966
14 Figurov, A. *et al.* (1996) Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature* 381, 706–709
15 Korte, M. *et al.* (1996) Virus-mediated gene transfer into hippocampal CA1 region restores long-term potentiation in brain-derived neurotrophic factor mutant mice. *Proc. Natl. Acad. Sci. U. S. A.* 93, 12547–12552
16 Patterson, S.L. *et al.* (1996) Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron* 16, 1137–1145
17 Scharfman, H.E. (1997) Hyperexcitability in combined entorhinal/hippocampal slices of adult rat after exposure to brain-derived neurotrophic factor. *J. Neurophysiol.* 78, 1082–1095
18 Takei, N. *et al.* (1997) Brain-derived neurotrophic factor increases the stimulation-evoked release of glutamate and the levels of exocytosis-associated proteins in cultured cortical neurons from embryonic rats. *J. Neurochem.* 68, 370–375
19 Suen, P-C. *et al.* (1997) Brain-derived neurotrophic factor rapidly enhances phosphorylation of the postsynaptic N-methyl-D-aspartate receptor subunit 1. *Proc. Natl. Acad. Sci. U. S. A.* 94, 8191–8195
20 Levine, E.S. *et al.* (1995) Differential effects of NGF and BDNF on voltage-gated calcium currents in embryonic basal forebrain neurons. *J. Neurosci.* 15, 3084–3091
21 Kafitz, K.W. *et al.* (1999) Neurotrophin-evoked rapid excitation through trkB receptors. *Nature* 401, 918–921
22 Marty, S. *et al.* (1997) Neurotrophins and activity-dependent plasticity of cortical interneurons. *Trends Neurosci.* 20, 198–202
23 Conner, J.M. *et al.* (1997) Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. *J. Neurosci.* 17, 2295–2313
24 Bengzon, J. *et al.* (1993) Regulation of neurotrophin and trkA, trkB and trkC tyrosine kinase receptor messenger RNA expression in kindling. *Neuroscience* 53, 433–446
25 Fryer, R.H. *et al.* (1996) Developmental and mature expression of full-length and truncated trkB receptors in the rat forebrain. *J. Comp. Neurol.* 374, 21–40

26 Yan, Q. *et al.* (1997) Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. *Neuroscience* 78, 431–448

27 Zhou, X.-F. and Rush, R.A. (1996) Endogenous brain-derived neurotrophic factor is anterogradely transported in primary sensory neurons. *Neuroscience* 74, 945–951

28 Fawcett, J.P. *et al.* (1998) Functional evidence that BDNF is an anterograde neuronal trophic factor in the CNS. *J. Neurosci.* 18, 2808–2821

29 Smith, M.A. *et al.* (1997) Anterograde transport of endogenous brain-derived neurotrophic factor in hippocampal mossy fibers. *NeuroReport* 8, 1829–1834

30 Fawcett, J.P. *et al.* (1997) Detection of brain-derived neurotrophic factor in a vesicular fraction of brain synaptosomes. *J. Biol. Chem.* 272, 8837–8840

31 Blöchl, A. and Thoenen, H. (1995) Characterization of nerve growth factor (NGF) release from hippocampal neurons: evidence for a constitutive and an unconventional sodium-dependent regulated pathway. *Eur. J. Neurosci.* 7, 1220–1228

32 Morgan, J.I. and Curran, T. (1991) Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes *fos* and *jun*. *Annu. Rev. Neurosci.* 14, 421–451

33 Kiessling, M. and Gass, P. (1993) Immediate early gene expression in experimental epilepsy. *Brain Pathol.* 3, 381–393

34 Gall, C. *et al.* (1991) Seizures and the regulation of neurotrophic factor and neuropeptide gene expression in brain. *Epilepsy Res. Suppl.* 4, 225–245

35 Meberg, P.J. *et al.* (1993) Induction of F1/GAP-43 gene expression in hippocampal granule cells after seizures. *Mol. Brain Res.* 17, 295–299

36 Qian, Z. *et al.* (1993) Tissue plasminogen activator is induced as an immediate-early gene during seizure, kindling, and long-term potentiation. *Nature* 361, 453–457

37 Isackson, 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

38 Mudo, 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

39 Gall, C.M. *et al.* (1997) Seizures and the regulation of neurotrophic factor expression: associations with structural plasticity in epilepsy. In *Advances in Neurology: Neuronal Regeneration, Reorganization and Repair* (Vol. 72) (Seil, R.J., ed.), pp. 9–24, Lippincott-Raven

40 Lowenstein, D.H. *et al.* (1993) Prolonged increases in neurotrophic activity associated with kainate-induced hippocampal synaptic reorganization. *Neuroscience* 56, 597–604

41 Humpel, C. *et al.* (1995) Monitoring release of neurotrophic activity in the brains of awake rats. *Science* 269, 552–554

42 Nawa, H. *et al.* (1995) BDNF protein measured by a novel enzyme immunoassay in normal brain and after seizure: partial disagreement with mRNA levels. *Eur. J. Neurosci.* 7, 1527–1535

43 Elmer, E. *et al.* (1998) Dynamic changes of brain-derived neurotrophic factor protein levels in the rat forebrain after single and recurring kindling-induced seizures. *Neuroscience* 83, 351–362

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

45 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

46 Gall, C.M. *et al.* (1996) Cellular localization of BDNF protein after recurrent seizures in rat: evidence for axonal transport of the newly synthesized factor. *Epilepsia* 37 (Suppl. 5), 47

47 Funabashi, T. *et al.* (1988) Intraventricular injection of antiserum to nerve growth factor delays the development of amygdaloid kindling. *Brain Res.* 458, 132–136

48 Van der Zee, C.E. *et al.* (1995) Intraventricular administration of antibodies to nerve growth factor retards kindling and blocks mossy fiber sprouting in adult rats. *J. Neurosci.* 15, 5316–5323

49 Kokaia, M. *et al.* (1995) Suppressed epileptogenesis in BDNF mutant mice. *Exp. Neurol.* 133, 215–224

50 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

51 Xu, B. *et al.* (2000) Cortical degeneration in the absence of neurotrophin signaling: dendritic retraction and neuronal loss after removal of the receptor trkB. *Neuron* 26, 233–245

52 Binder, D.K. *et al.* (1999) Selective inhibition of kindling development by intraventricular administration of trkB receptor body. *J. Neurosci.* 19, 1424–1436

53 Croll, S.D. *et al.* (1998) Co-infusion with a trkB-Fc receptor body carrier enhances BDNF distribution in the adult rat brain. *Exp. Neurol.* 152, 20–33

54 Larmet, Y. *et al.* (1995) Protective effects of brain-derived neurotrophic factor on the development of hippocampal kindling in the rat. *NeuroReport* 6, 1937–1941

55 Frank, L. *et al.* (1996) BDNF downregulates neurotrophin responsiveness, trkB protein and trkB mRNA levels in cultured rat hippocampal neurons. *Eur. J. Neurosci.* 8, 1220–1230

56 Knusel, B. *et al.* (1997) Ligand-induced down-regulation of trk messenger RNA, protein and tyrosine phosphorylation in rat cortical neurons. *Neuroscience* 78, 851–862

57 Binder, D.K. *et al.* (1999) Immunohistochemical evidence of seizure-induced activation of trk receptors in the mossy fiber pathway of adult rat hippocampus. *J. Neurosci.* 19, 4616–4626

58 Okazaki, M.M. *et al.* (1995) Hippocampal mossy fiber sprouting and synapse formation after status epilepticus in rats: visualization after retrograde transport of biocytin. *J. Comp. Neurol.* 352, 515–534

59 Scharfman, H.E. *et al.* (1999) Actions of brain-derived neurotrophic factor in slices from rats with spontaneous seizures and mossy fiber sprouting in the dentate gyrus. *J. Neurosci.* 19, 5619–5631

60 Wu, K. *et al.* (1996) Functional trkB neurotrophin receptors are intrinsic components of the adult brain postsynaptic density. *Mol. Brain Res.* 43, 286–290

61 Drake, C.T. *et al.* (1999) Ultrastructural localization of full-length trkB immunoreactivity in rat hippocampus suggests multiple roles in modulating activity-dependent synaptic plasticity. *J. Neurosci.* 19, 8009–8026

62 Murray, K.D. *et al.* (2000) Altered mRNA expression for brain derived neurotrophic factor and type II calcium/calmodulin dependent protein kinase in the hippocampus of patients with intractable temporal lobe epilepsy. *J. Comp. Neurol.* 418, 411–422

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

64 McNamara, J.O. (1994) Cellular and molecular basis of epilepsy. *J. Neurosci.* 14, 3413–3425

65 Wuarin, J-P. and Dudek, F.E. (1996) Electrographic seizures and new recurrent excitatory circuits in the dentate gyrus of hippocampal slices from kainate-treated epileptic rats. *J. Neurosci.* 16, 4438–4448

66 Gibbs, J.W. *et al.* (1997) Differential epilepsy-associated alterations in postsynaptic GABA(A) receptor function in dentate granule and CA1 neurons. *J. Neurophysiol.* 77, 1924–1938

67 Okazaki, M.M. *et al.* (1999) Recurrent mossy fiber pathway in rat dentate gyrus: synaptic currrents evoked in presence and absence of seizure-induced growth. *J. Neurophysiol.* 81, 1645–1660

68 Williamson, A. *et al.* (1999) Decrease in inhibition in dentate granule cells from patients with medial temporal lobe epilepsy. *Ann. Neurol.* 45, 92–99

69 Behr, J. *et al.* (1998) Enhanced propagation of epileptiform activity through the kindled dentate gyrus. *J. Neurophysiol.* 79, 1726–1732

70 King, G.L. *et al.* (1985) Abnormal neuronal excitability in hippocampal slices from kindled rats. *J. Neurophysiol.* 54, 1295–1304

71 Buzsaki, G. *et al.* (1989) Neuronal activity in the subcortically denervated hippocampus: a chronic model for epilepsy. *Neuroscience* 28, 527–538

72 Maccaferri, G. *et al.* (1998) Target-specific expression of presynaptic mossy fiber plasticity. *Science* 279, 1368–1370

73 Sutula, T. *et al.* (1988) Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science* 239, 1147–1150

74 Routbort, M.J. *et al.* (1997) TrkB-IgG does not inhibit mossy fiber sprouting in an *in vitro* model. *Soc. Neurosci. Abstr.* 23, 888

75 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

76 Baraban, S.C. *et al.* (1997) Knock-out mice reveal a critical antiepileptic role for neuropeptide Y. *J. Neurosci.* 17, 8927–8936

77 Marksteiner, J. *et al.* (1990) Neuropeptide Y biosynthesis is markedly induced in mossy fibers during temporal lobe epilepsy of the rat. *Neurosci. Lett.* 112, 143–148

78 Balkoweic, A. and Katz, D.M. (2000) Activity-dependent release of endogenous brain-derived neurotropic factor from primary sensory neurons detected by ELISA *in situ*. *J. Neurosci.* 20, 7417–7423