CHAPTER 1

General Introduction

TrkB/BDNF Signaling and Synaptic Plasticity

The development and the maturation of the central nervous system are brought about by the complex interplay of cellular signaling cascades. Many of the signaling molecules involved are temporally and spatially regulated during specific stages of development and each molecule may have distinct roles at different developmental time points. Therefore, it is quite fascinating that one protein, brain derived neurotrophic factor (BDNF), possesses such diverse and important roles not only during embryonic development, but also in the differentiation, survival and functioning of neural cells in the brain.

BDNF is a member of the neurotrophin (NT) family that includes nerve growth factor (NGF), NT3 and NT4/5 (Barde et al., 1982; Barde, 1990). Neurotrophins were traditionally thought to support survival and facilitate growth of neural cells, but more recent studies have shown that they also modulate synaptic plasticity. These effects are mediated by specific interaction of neurotrophins with their respective receptors (Klein et al., 1991a; Klein et al., 1991b; Klein et al., 1992; Lamballe et al., 1991). BDNF and its receptor tropomyosin related kinase B (TrkB) have proven to be the most important regulators of synaptic plasticity, and the work in this thesis was aimed at studying the cell autonomous roles of TrkB signaling in synaptic morphology and efficacy in the visual cortex.

In the central nervous system, BDNF protein and transcripts are expressed abundantly, with predominant expression in the hippocampus, followed by the cerebral cortex (Dugich-Djordjevic et al., 1992; Dugich-Djordjevic et al., 1995; Hofer et al., 1990). In the cerebral cortex, the expression of

BDNF increases with development and coincides with the developmental period when cortical circuitry undergoes structural and functional maturation (Castren et al., 1992; Lush et al., 2005).

BDNF, but also NT4, mediates its effects through its high affinity receptor, TrkB which belongs to the group of transmembrane proteins called receptor tyrosine kinases (Klein et al., 1989). Other members of the family include, TrkA (has a higher affinity for NGF) and TrkC (binds more specifically to NT3). Another NT receptor, $p75^{NTR}$, can be activated by all NTs non-specifically and is known to mediate programmed cell death (Kaplan and Miller, 2000) (Fig 1). Many of the neurotrophin receptors are expressed together in the same cells and interaction between subtypes has also been reported (Bibel et al., 1999).

TrkB consists of an IgG-like extracellular domain that is essential for ligand binding. There are 6 kinase domains in the intracellular part of the receptor (Klein et al., 1990a). Binding of BDNF or NT4 to TrkB homodimers results in the autophosphorylation of their kinase domains and thus starts a signaling cascade (Marsh et al., 1993).

TrkB is expressed in all neural tissues during embryonic development but shows restricted expression in specific regions and neuron types of the adult brain (Klein et al., 1990b). It is expressed in both the pre- and post-synaptic compartments and is evenly distributed in primary hippocampal and cortical neurons (Kryl et al., 1999). TrkB is enriched in postsynaptic densities (Wu et al., 1996) and colocalizes with NMDARs in the dendrites and synaptic vesicles in the axons of cortical neurons (Gomes et al., 2006). Surface TrkB is found in dendritic filopodia and axonal growth cones, structures that take part in synapse formation.

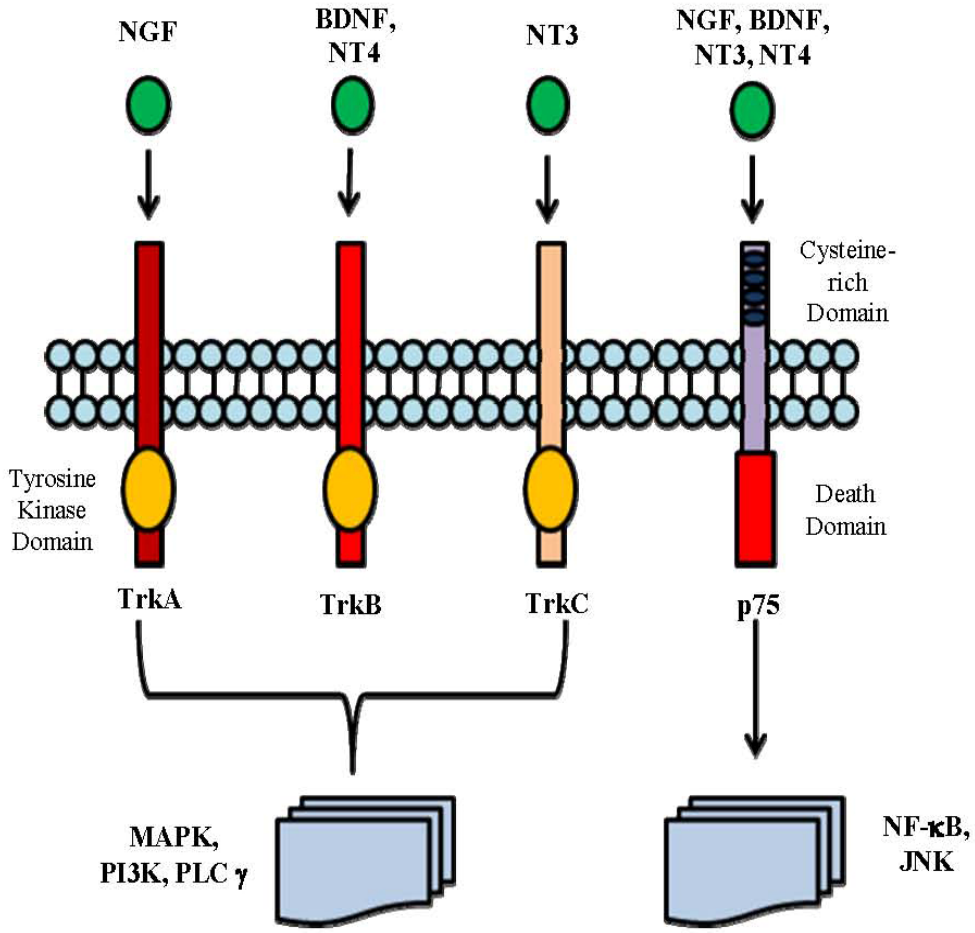

Fig 1. Interaction of Neurotrophins and their Receptors. Neurotrophins (NT) bind selectively to their respective receptors – NGF to TrkA; BDNF and NT-4 to TrkB; NT3 to TrkC. All the NTs bind non-specifically to p75 receptor. NTs bind to the extracellular immunoglobulin G (IgG) domains of Trk receptors to initiate receptor dimerization. This activates the intracellular catalytic tyrosine kinase domain to initiate diverse signal transduction pathways. There are four cysteine-rich repeats in the extracellular domain of p75 and a death domain intracellularly. Heterodimeric interactions between Trk and p75 receptors leads to changes in the affinity of NT binding.

In addition to the full length TrkB receptor, two C-terminally truncated splice variants of TrkB have been identified - TrkB.T1 (Klein et al., 1990a) and TrkB.T2 (Barbacid, 1994). TrkB, TrkB.T1 and TrkB.T2 share the same extracellular and transmembrane domains and the first 12 amino acid sequences of the intracellular domain. The truncated isoforms lack the kinase domain of the TrkB and instead have isoform specific C-terminal sequences (11AA for TrkB.T1 and 9AA for TrkB.T2).

Although TrkB.T1 receptors form homodimers upon ligand binding, downstream signaling does not occur due to the lack of functional kinase domains. Along with TrkB, TrkB.T1 is expressed in dendrites and axons of cortical and hippocampal neurons, with moderate enrichment in postsynaptic densities (Kryl et al., 1999). It can act as a dominant negative form of TrkB by forming a heterodimer with the latter and inhibiting BDNF signaling (Fig. 2).

TrkB.T1 has been shown to impede phosphorylation of TrkB (Knusel et al., 1994) and TrkB-dependent calcium efflux (Eide et al., 1996). Interestingly, TrkB.T1 does not abolish BDNF induced gene expression (Offenhauser et al., 2002). A recent study showed that TrkB.T1 can activate the release of calcium from intracellular stores in glial cells but not in hippocampal neurons (Rose et al., 2003). It remains unclear whether endogenous TrkB.T1 elicits any signaling in neurons.

TrkB is expressed in abundance when compared to TrkB.T1 during the early course of postnatal development, but TrkB.T1 forms the predominant isoform in the adult brain (Allendoerfer et al., 1994; Escandon et al., 1994). Lesions to the adult hippocampus result in a decrease of BDNF and TrkB

mRNA, whereas TrkB.T1 expression increases in non-neuronal cells (Beck et al., 1993). This suggests a role for TrkB.T1 as a ligand-presenter during axonal regeneration.

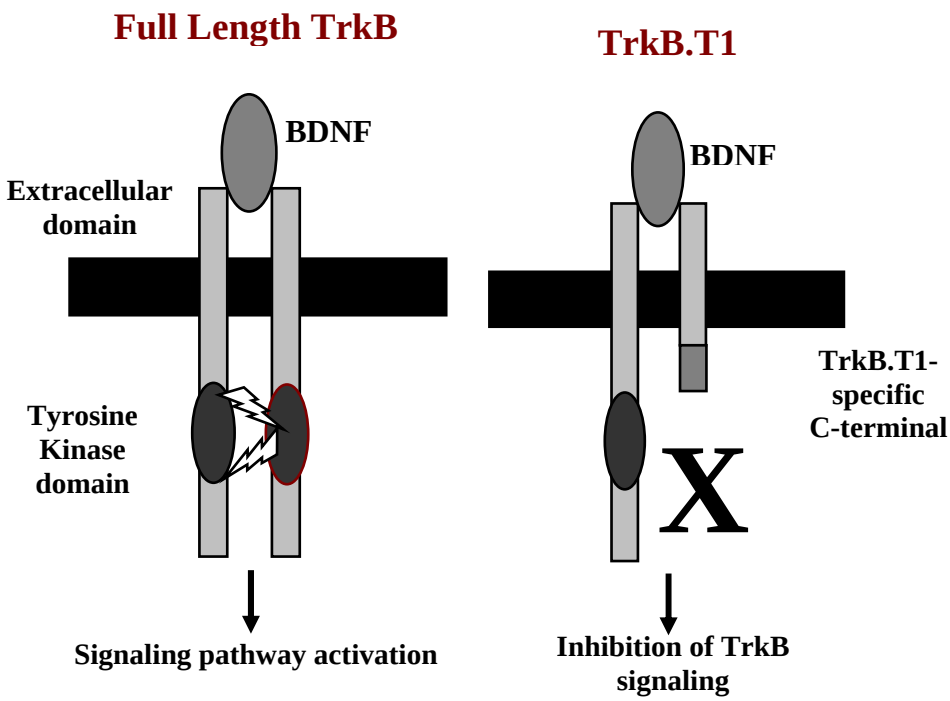

Fig 2. TrkB.T1 is a dominant negative isoform of TrkB. BDNF binding to the full length TrkB receptor results in homo-dimerization, leading to autophosphorylation of the intracellular tyrosine kinase domains. Activation of TrkB homodimers results in initiation of downstream signaling pathways. When BDNF binds to a heterodimer formed between the full length TrkB and the dominant negative TrkB.T1 isoform, TrkB mediated signaling is disrupted as TrkB.T1 does not have a functional kinase domain.

Activity-dependent expression, synthesis and release of BDNF

Classically, neurotrophins are thought to be expressed and released as "target-derived" factors, i.e., a presynaptic neuron activates the target cell, which in turn releases BDNF. The released BDNF is then thought to bind to TrkB receptors on the presynaptic neuron and initiate signaling. This would require an activity-dependent release of BDNF.

BDNF is expressed abundantly in the central nervous system (Hofer et al., 1990). In contrast to NGF and its receptors TrkA and $p75^{NTR}$, TrkB is expressed in both BDNF-synthesizing and –releasing cells as well as in cells that do not express BDNF (Klein et al., 1990b). Unlike the classical neurotrophin, BDNF is released from both presynaptic- and postsynaptic-terminals (Haubensak et al., 1998; Kojima et al., 2001) and can influence neuronal activity in both cells. These properties allow BDNF to have both autocrine and paracrine roles. Moreover, BDNF can be taken up and released again by neurons not expressing the BDNF gene themselves, causing BDNF/TrkB signaling to be even more flexible and complex.

The expression of BDNF transcripts as well as protein is known to be regulated by neural activity. BDNF transcripts are elevated by depolarization, and kainic acid administration on hippocampal cultures in vitro (Zafra et al., 1992) and in developing rat hippocampi (Dugich-Djordjevic et al., 1992). At the subcellular level, BDNF and TrkB transcripts are found in the dendrites and cytoplasm of cortical and hippocampal neurons (Tongiorgi et al., 1996). When hippocampal cells are subjected to depolarizing conditions, there is a two-fold increase in the levels of both BDNF and TrkB transcripts in the dendrites (Tongiorgi et al., 1997). This increase is mediated by activation of glutamate receptors and

requires Ca^{2+} influx through voltage gated calcium channels (VGCC). There is also substantial accumulation of BDNF and TrkB proteins in the distal parts of the dendrites, close to their sites of synaptic activity. Similar increases in protein levels of BDNF and TrkB are also observed upon electrical stimulation of hippocampal neurons in the same study.

In the visual cortex, expression of BDNF transcripts and protein – but not of NT4 (Pollock and Frost, 2003) - is regulated by light both during development and in adulthood (Castren et al., 1992; Tropea et al., 2001). Dark rearing of adult rats results in lower BDNF transcript levels both in the cell body and in dendrites. This can however be rescued with brief exposure to light (Capsoni et al., 1999). Diurnal changes also show regulation with increased BDNF protein levels in the retina, primary visual cortex and superior colliculus during the light phase (Pollock et al., 2001). TrkB mRNA levels are reduced in dark-reared rats (Castren et al., 1992). Interestingly, the amount of TrkB receptors remain the same between light-reared and dark-reared rats (Tropea and Domenici, 2001). However, dark rearing reduces the phosphorylation of TrkB receptors, which can be rescued by brief exposure to either light or BDNF (Viegi et al., 2002).

Similar regulation of BDNF is observed in the somatosensory cortex. Unilateral stimulation of whiskers by mechanical deflection results in an increase of BDNF transcripts in the excitatory neurons of the contralateral barrel cortex of adult mice (Rocamora et al., 1996). Cauterization of whiskers in neonatal mice results in a decrease of BDNF and TrkB transcripts in the corresponding barrels of the somatosensory cortex (Singh et al., 1997).

Regulation of BDNF release is also known to be effected by neural activity. Depolarization of hippocampal neurons results in a five-fold increase in BDNF release (Goodman et al., 1996). Similarly, hippocampal neurons subjected to high frequency stimulation show increased BDNF release by activating ionotropic glutamate receptors, accompanied by an increase in postsynaptic influx of Ca^{2+} (Hartmann et al., 2001). Interestingly, short high frequency stimulation that induces hippocampal LTP also facilitates secretion of BDNF but by release of Ca^{2+} from internal stores through the activation of Inositol-tri-phosphate (Balkowiec and Katz, 2002; Gartner and Staiger, 2002). However, low-frequency stimulation, that initiates long term depression, does not result in BDNF release. Thus, expression in the adult brain and regulation of BDNF release in response to neuronal activity suggest a role for BDNF in neuronal plasticity.

TrkB/BDNF mediated potentiation of synaptic activity

Expression and release of BDNF, as stated earlier, are regulated by neuronal activity. Does BDNF itself, in turn, influence changes in neuronal activity? BDNF selectively increases integrated synaptic current in hippocampal neurons by activating TrkB receptors and increasing activity of NMDA receptors (Black, 1999). Local application of low amounts of BDNF is sufficient to induce synaptic transmission in a TrkB-dependent manner (Kafitz et al., 1999). This neurotrophin-induced depolarization is mediated by a tetrodotoxin-insensitive sodium channel, $Na_V1.9$ (Blum et al., 2002). BDNF also increases both the frequency and amplitude of miniature excitatory postsynaptic currents in cortical neurons. This is accompanied by

an increase in intracellular Ca^{2+} levels that requires activation of voltage gated calcium channels (He et al., 2005).

BDNF also regulates the release of neurotransmitter from presynaptic terminals. It augments quantal neurotransmitter release and increases the number of docked vesicles at the active zones of hippocampal excitatory synapses (Tyler and Pozzo-Miller, 2001). In addition it increases the probability of vesicle release and the size of the readily releasable pool of vesicles in these cells (Tyler et al., 2006). Low levels of BDNF, in conjugation with brief presynaptic depolarization, activate TrkB receptors to potentiate neurotransmitter release from presynaptic neurons at the developing neuromuscular junction (Boulanger and Poo, 1999).

BDNF differentially regulates excitation and inhibition depending on the cell type. While it increases the amplitude of excitatory postsynaptic currents (EPSCs) and reduces the amplitude of inhibitory postsynaptic currents (IPSCs) in excitatory neurons of hippocampal cultures (Wardle and Poo, 2003), it increases IPSC amplitude in GABAergic neurons.

Activity-dependent synaptic changes are thought to be governed by a Hebbian mechanism, wherein the active synapses, that fire together, are strengthened. Long term potentiation (LTP) is the most-studied *in vitro* model for synaptic plasticity (Bliss and Lomo, 1973; Lomo, 2003). Even though the correlation between LTP and behavioral learning paradigms is not clear, various signaling molecules have been implicated in LTP modulation.

BDNF application to mature hippocampal slices results in persistent enhancement of synaptic strength at the Schaffer collateral to CA1 synapses. This is mediated by the phosphorylation of postsynaptic TrkB receptors

(Levine et al., 1995) as it can be inhibited by the tyrosine kinase blocker – K252a (Kang and Schuman, 1995) and is brought about by the activation of NMDARs (Levine et al., 1998). In an in vivo demonstration of LTP induction by BDNF, intracortical injection of BDNF by acute microinfusion induced potentiation of synaptic transmission between the basolateral amygdala-insular cortex pathway (Escobar et al., 2003). Similarly, acute intrahippocampal infusion of BDNF resulted in induction of late form of LTP in the medial perforant pathway-granule cell synapses (Messaoudi et al., 2002). The role of BDNF signaling in facilitating potentiation of excitatory synapses is further strengthened by a study that showed inhibition of LTD induction by BDNF in visual cortical slices of young rats (Akaneya et al., 1996).

In mice that are homozygously or heterozygously deficient for BDNF, both early and late forms of hippocampal LTP are significantly impaired (Korte et al., 1995; Korte et al., 1998). It can be restored by addition of BDNF exogenously (Patterson et al., 1996) or by viral-mediated injection of BDNF (Korte et al., 1996). BDNF heterozygous mice showed selective impairment in LTP induction from layer IV-III of the visual cortex, while LTP from white matter-layer III LTP occurred normally (Bartoletti et al., 2002).

Mice that are homozygously deficient for TrkB show drastic reduction in potentiation when subjected to conditions that induce LTP (Minichiello et al., 1999) with heterozygous mice showing a milder phenotype. Transgenic mice with point mutations to the Shc and PLCγ docking sites of the TrkB receptor showed that TrkB-mediated hippocampal plasticity is effected by initial recruitment of PLCγ followed by phosphorylation of CREB and CaMKIV (Minichiello et al., 2002).

Mice that lack TrkB presynaptically in the hippocampus failed to induce LTP in the Schaffer Collateral-CA1 pathway. When TrkB was eliminated from postsynaptic neurons, it did not affect LTP induction suggesting a predominantly presynaptic role for BDNF signaling to induce LTP in this pathway (Xu et al., 2000a). However, in the hippocampal dentate gyrus, BDNF was found to induce LTP by activating postsynaptic Ca^{2+} channels and NMDA receptors (Kovalchuk et al., 2002). This indicates that the mode and site of LTP induction through BDNF signaling is pathway specific. Experiments involving NT4 deficient animals suggest that most of the changes in hippocampal LTP caused by TrkB deficiency are caused by BDNF signaling. In the absence of NT4, intermediate LTP is not affected at all. NT4 is involved in the induction of long term LTP, however, affecting both its strength and its persistence (Xie et al., 2000).

TrkB/BDNF dependent regulation of neuronal morphology

Synaptic plasticity, which effects long-lasting alterations to the network, can be mediated by either changing the efficacy of existing synapses or by rewiring neuronal connections. This can involve the growth or retraction of neurites and the loss and formation of synapses. It has been known for long that plasticity in the visual cortex is accompanied by growth and retraction of thalamocortical and intracortical axons (Antonini et al., 1999; Shatz and Stryker, 1978). Live imaging studies in recent years have added important information about the relationships between functional plasticity and structural changes, especially at the level of dendritic spine- loss and – formation (Grutzendler et al., 2002; Holtmaat et al., 2008; Majewska et al., 2006; Trachtenberg et al., 2002)**.** Experiments in barrel cortex have shown

that in young animals in which plasticity can be induced readily, spine turnover is high (Zuo et al., 2005a; Zuo et al., 2005b)**.** It has also become clear that strong correlations exist between the morphology of dendritic spines (Table 1) and their stability. Especially thin spines lacking a clear spine head and long spines carrying a small head are short lived. In contrast, large mushroom shaped spines are mostly long lived and can persist for months or longer (Grutzendler et al., 2002; Holtmaat et al., 2005; Trachtenberg et al., 2002; Zuo et al., 2005a). New spines are formed through the formation of filopodia, long finger shaped protrusions that do not yet carry a synapse (Portera-Cailliau et al., 2003; Ziv and Smith, 1996). Little is known about the stability or maturity of stubby spines, which do not have a discernable spine neck. This is mostly due to the difficulty of visualizing them in live preparations. Altered sensory input, for example the trimming of whiskers or the closure of one eye, results in an increased loss and formation of synaptic protrusions (Hofer et al., 1990; Holtmaat et al., 2006; Zuo et al., 2005b) leaving total synapse numbers almost unchanged, but probably causing changes in the functionality of the neuronal network. Moreover, it was found that the size of dendritic spines is strongly correlated with the efficacy of the synapse it carries. These observations have allowed a much better interpretation of the functional meaning of structural changes in spines and accentuated the importance of studying the involvement of signaling cascades that regulate neuronal morphology in neuronal plasticity.

Shape	Protrusion Type	Distinct Head	Length (in μm)
	Mushroom	Yes	< 2.0
	Long	Yes	> 2.0
	Stubby	-	< 1.0
	Thin	-	< 2.0
	Filopodia	-	> 2.0

Table 1. Spine Classification. Dendritic protrusions were classified as spines and filopodia. Spines were further categorized to mushroom, long, stubby and thin depending on the presence of a distinct head and spine length. These, in turn, were further classified into mushrooms with larger and smaller heads. The thin spines and filopodia were distinguished based on their length, i.e., headless protrusions that were lesser than 1 μm were classified as thin spines whereas those longer than 1 μm were classified as filopodia.

As mentioned in previous sections, BDNF signaling regulates synaptic plasticity by modulating physiological properties of synapses. There is enough evidence that BDNF also regulates synapse formation, dendrite and axonal morphology. BDNF induces outgrowth of primary dendrites in cortical neurons by activating phosphatidylinositol 3-kinase (PI3-K) and MAP kinase pathways in a protein synthesis-independent manner (Dijkhuizen and Ghosh, 2005). BDNF also causes an increase in total length and number of axonal branches of GABAergic neurons in hippocampal cultures (Vicario-Abejon et al., 1998) and dendritic arborization of basket cells and stellate cells in cerebellar cultures (Mertz et al., 2000). Cerebellar cultures grown in the presence of BDNF for two weeks show an increase in the density of dendritic spines, without showing effects on the dendritic arborization of Purkinje cells (Shimada et al., 1998).

Overexpression of BDNF in ferret slice cultures results in increased dendritic branching through an autocrine mechanism by activating TrkB receptors (Horch et al., 1999). Apart from inducing dendritic sprouting, BDNF also destabilizes cortical dendrites and spines. This destabilization could be a way to reconfigure dendrites and synapses to adapt to potentially better and new inputs. However, these drastic changes brought about by autocrine effects of BDNF to modify entire dendrite arbors are in contrast with its paracrine effects. BDNF released from neighboring cells has to be in close proximity to bring about any change in the dendritic arbors of the recipient neuron (Horch and Katz, 2002). This indicates that synaptic modifications initiated by released BDNF are very local and highly synapse-specific.

Many studies indicate that the effects of BDNF signaling on synaptic morphology are complementary to neuronal activity. BDNF differentially regulates synaptic structure and shape of CA1 neurons depending on whether action potentials or vesicular release is disrupted (Tyler and Pozzo-Miller, 2003). In rat olfactory bulb slice cultures, acute treatment of BDNF causes a reduction in spine size without affecting the length and density of spines in granule cells (Matsutani and Yamamoto, 2004). These rapid effects are brought about by enhanced glutamatergic signaling by activation of NMDARs. Expression of BDNF in layer 4 neurons in slice cultures of the developing ferret visual cortex results in increased branching, number and length of basal dendrites and spine number (McAllister et al., 1995). Inhibiting either spontaneous activity, glutamatergic synaptic transmission or L-type calcium channels prevents these BDNF induced structural modifications, suggesting that these effects of BDNF are dependent on neuronal activity (McAllister et al., 1996).

Mice that lack both alleles of BDNF show reduced maturation of glutamatergic synapses (Itami et al., 2000). Addition of BDNF results in the conversion of the predominantly N-methyl-D-aspartate receptor (NMDAR) expressing “silent synapses” into more mature AMPA receptor containing synapses in these mice (Itami et al., 2003). In adult animals, stimulation of whiskers results in synapse formation in the somatosensory cortex, which is compromised in mice heterozygous for BDNF (Genoud et al., 2004). However, perhaps as a compensatory mechanism, there is an increase in spine volume when compared to controls, indicating that BDNF mediates synaptic modifications of the adult cortical circuitry in response to altered sensory input.

BDNF also regulates axonal arborization during development when neurons are still innervating their target cells and new synapses are being formed. When BDNF is injected to optic tectum of Xenopus leavis tadpoles, there is a rapid increase in the branching and complexity of axonal arbors of retinal ganglion cells (Cohen-Cory and Fraser, 1995) followed by an increase in the number of synapses on tectal neurons (Alsina et al., 2001; Sanchez et al., 2006). Both BDNF and TrkB have been implicated in maintenance of dendritic arbors in the neocortex. Mice that lack BDNF in excitatory neurons of the forebrain develop normally but have thinner cortices at 5 weeks and show a reduced neuropil (Gorski et al., 2003). Although initial development of neurons seems unaffected, neurons in layers 2/3 start retracting their dendrites after three weeks, suggesting that BDNF is essential for the maintenance of structure and function of cortical neurons. Similarly, in mice where TrkB is deleted in excitatory, CaMKII-expressing cells, cortical layers 2/3 and 5 are compressed and pyramidal neurons have shorter and thinner dendrites. The thinning of the cortex is partly due to the reduction in the size of the neuropil and reduced synaptic connectivity (Xu et al., 2000b).

Expressing TrkB.T1 in hippocampal neurons results in outgrowth of filopodia, independent of BDNF and TrkB signals (Hartmann et al., 2004). However, coexpression of p75 receptor that lacks the intracellular domain inhibits this effect, suggesting that TrkB.T1 induces filopodia outgrowth in distinct cell-type specific pathways. Expression of TrkB and TrkB.T1 in ferret visual cortical slices results in differential regulation of cortical dendrite development (Yacoubian and Lo, 2000). While TrkB causes an increase in proximal dendrite branching, TrkB.T1 regulates the elongation

of distal dendrites. Overexpression of TrkB.T1 in adult muscles results in the loss of acetylcholine receptors in the neuromuscular junctions (Gonzalez et al., 1999) demonstrating that TrkB-signaling is necessary for maintenance of these synapses.

Role of TrkB and BDNF in learning and memory

Activity-dependent synaptic modifications, both electrical as well as structural, are thought to be the basis for learning and memory. Since BDNF affects various forms of synaptic plasticity both *in vitro* and *in vivo*, many studies have looked at the possibility of BDNF modulating learning and memory.

Rats trained in a radial arm maze test show an increase in BDNF mRNA in the hippocampus (Mizuno et al., 2000). Treating rats with antisense oligonucleotides against BDNF results in compromised spatial learning, accompanied by a reduction of BDNF transcript and protein levels and reduced phosphorylation of TrkB in a PI3-K-dependent manner (Mizuno et al., 2003b). Moreover, injection of antisense BDNF oligonucleotides into the hippocampi of extensively trained rats results in impairment of reference and working memory, suggesting that BDNF is required both for acquisition and consolidation of spatial memory. Interaction between NMDARs and BDNF-TrkB signals is crucial for spatial learning and memory (Mizuno et al., 2003a). TrkB-deficient mice show impaired spatial learning in the Morris water maze and eight-arm maze tests (Minichiello et al., 1999). Similarly, mice overexpressing TrkB.T1 also show impaired spatial learning (Saarelainen et al., 2000). Again, NT4 seems to contribute less significantly to TrkB dependent learning paradigms, as in NT4 deficient animals

contextual fear condition is unaffected. However, retention is diminished in the absence of NT4, indicating that BDNF and NT4 may have their specific roles in learning and memory (Xie et al., 2000).

TrkB/BDNF signaling and effects on experience dependent plasticity in the visual cortex

During the development of the primary visual cortex (V1) in higher mammals, eye-specific thalamic afferents from the lateral geniculate nucleus segregate to form ocular dominance columns in V1 (LeVay et al., 1978). Although the rodent primary visual cortex lacks this columnar structure, most neurons in V1 receive binocular input, albeit dominated by the contralateral eye (Antonini et al., 1999; Gordon and Stryker, 1996). During a postnatal developmental period, the visual cortex is very susceptible to sensory experience in many different species (Fagiolini et al., 1994; Gordon and Stryker, 1996; Hubel et al., 1977; Shatz and Stryker, 1978). Visual deprivation to one of the eyes in this "critical period" results in a shift of responsiveness towards the open eye (Hubel et al., 1977). This Ocular Dominance (OD) plasticity during the critical period involves both functional and structural modifications of cells within the primary visual cortex (Fagiolini et al., 1994; Gordon and Stryker, 1996; Hubel et al., 1977; Majewska and Sur, 2003; Mataga et al., 2004; Matsubara et al., 1985; Oray et al., 2004; Trachtenberg and Stryker, 2001). OD plasticity is a competitive process, as deprivation of visual input to both eyes does not result in this shift, nor does the monocular visual cortex lose responsiveness to the deprived eye. Recent studies have shown that OD plasticity also occurs to a limited extent in the adult visual cortex (Hofer et al., 2006; Pham et al.,

2004; Sawtell et al., 2003). This limitation of plasticity in the adult visual cortex is thought to be brought about by the development of the extracellular matrix (Mataga et al., 2004; Oray et al., 2004; Pizzorusso et al., 2002) which may limit axonal growth and retraction.

The beginning of the critical period can be delayed by dark rearing (Mower, 1991) or accelerated by BDNF expression (Hanover et al., 1999). Overexpression of BDNF also brings about the maturation of inhibition (Huang et al., 1999) which is a crucial determinant of the onset of the critical period (Fagiolini and Hensch, 2000; Hensch et al., 1998), both modulated by BDNF and its receptor TrkB (Huang et al., 1999). Maybe through this mechanism, overexpression of BDNF in transgenic mice suppresses the effects of dark rearing and rescues the delay in the onset of the critical period. Moreover, it counteracts the reduction of visual acuity normally caused by dark rearing (Gianfranceschi et al., 2003). Although NT4 expression is not regulated by visual input (Pollock and Frost, 2003), down regulation of inhibitory and excitatory synaptic proteins caused by dark rearing can be rescued by infusion of NT4 (Cotrufo et al., 2003).

The expression of BDNF protein starts with eye-opening and increases to adulthood, thus making it a good candidate for mediating OD plasticity (Castren et al., 1992; Huang et al., 1999; Tropea et al., 2001). Indeed, the formation of ocular dominance can be disrupted by infusion of BDNF in the visual cortex of higher mammals (Cabelli et al., 1995). This has resulted in the hypothesis that BDNF acts as a competitive factor during OD plasticity: when depolarized by thalamic inputs, layer IV stellate neurons in the visual cortex would release limiting amounts of BDNF, which would in turn act retrogradely on the presynaptic thalamic neurons to stimulate synapse

formation. The restricted amount of BDNF is thought to be the substrate for activity-dependent competition between the thalamic neurons and to influence the OD shift. Since it is experimentally difficult to dissect out the pre- and post-synaptic effects of BDNF, this hypothesis has never been addressed convincingly. In fact, a recent study (Kaneko et al., 2008) has shown that TrkB signaling is not involved in the induction of an OD shift by monocular deprivation.

Questions posed

As is evident from the literature, BDNF has diverse roles in modulating neuronal properties. Most of the studies are carried out in vitro or in the developing brain where compounding conditions are not at play. It has been difficult to study the effects of BDNF in the intact adult brain as it is technically demanding to target its expression to a specific cell type without disturbing its development. In view of these shortcomings in our understanding of BDNF/TrkB signaling in the adult brain, this study aimed at addressing these broad but fundamental questions.

- What is the involvement of TrkB signaling in the regulation of neuronal structure and function in the adult visual cortex?
- Are the effects of TrkB signaling in the adult visual cortex based on competitive mechanisms?