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REGULATION AND FUNCTION OF P75^{NTR}:

CYTOKINE MEDIATED P75^{NTR} REGULATION AND ITS FUNCTIONAL ROLE

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ABSTRACT OF THE DISSERTATION

Regulation and Function of $p75^{NTR}$:

Cytokine mediated $p75^{NTR}$ regulation and its functional role

by Soyoung Choi

Thesis Director: Dr. Wilma J. Friedman

The p75 neurotrophin receptor ($p75^{NTR}$) has been known to play multiple roles in cell survival and apoptosis (Friedman, 2000; Maggirwar et al., 1998; Rabizadeh et al., 1993), axonal growth (Bentley and Lee, 2000), and cell differentiation (Hosomi et al., 2003). This multifunctional receptor is more widely expressed during development than in adults (Friedman, 2000; Yan and Johnson, 1988). However, it is reexpressed in the brain after injury or in disease (Casha et al., 2001; Kokaia et al., 1998; Ramos et al., 2007; Roux et al., 1999; Yaar et al., 1997). Although previous studies demonstrated that $p75^{NTR}$ is induced by seizure in neurons, where it causes apoptosis, (Roux et al., 1999; Volosin et al., 2008), the mechanisms involved in regulating $p75^{NTR}$ expression after brain injury are unknown. The immediate response that occurs in many pathological conditions is the production of proinflammatory cytokines. Thus, I hypothesized that those essential proinflammatory cytokines may be responsible for the reexpression of $p75^{NTR}$ seen following brain injury or in disease. Using both *in vitro* and *in vivo* methods, this thesis evaluated the mechanisms that regulate $p75^{NTR}$ after cytokine treatment in distinct cellular contexts and the functional consequences of $p75^{NTR}$ expression on neuronal death. The results demonstrated that IL-1β and TNFα induce $p75^{NTR}$ expression in neurons and astrocytes by distinct signaling mechanisms. Moreover, IL-1β exacerbates proNGF-mediated neuronal death by

recruiting sortilin receptor and $p75^{NTR}$ to the cell surface. IL-1β preferentially induces the monomeric forms of $p75^{NTR}$, which associates with sortilin and is sufficient to activate proNGF mediated apoptotic signaling. IL-1β increases $p75^{NTR}$ expression and increases NGF in vivo without the context of injury, but does not induce cell death. Overall, these results suggest that IL-1β release following injury or in disease may facilitate proNGF-mediated cell death by regulating surface expression of the $p75^{NTR}$-sortilin complex to exacerbate neuronal death after injury that can be activated by proneurotrophins.

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Table of Contents

III. List of Illustrations

IV. List of abbreviations

BDNF	brain-derived neurotrophic factor
Cc3	cleaved caspase 3
CRD	cysteine rich domains
CSF	cerebrospinal fluid
Cys	cysteine
DISC	death-inducing signaling complex
DD	death domain
Erk	extracellular signal regulated kinase
ECD	extracellular domain
FADD	Fas-associated death domain
FRET	fluorescence resonance energy transfer
GFAP	glial fibrillary acidic protein
ICD	intracellular domain
IKK	ikappaB kinase
IL-1β	interleukin-1 beta
IP	intraperitoneal
IRAK	interleukin-1 receptor-associated kinase
JNK	c-Jun-N-terminal kinase
MAG	myelin associated glycoprotein
MAPK	mitogen-activated protein kinase
MEKK	MAP kinase kinase kinase
MEM	minimum essential medium
NGF	nerve growth factor

NT-3	neurotrophin 3
NT4/5	neurotrophin 4/5
NTR	neurotrophin receptor
NgR	nogo receptor
NADE	$p75^{NTR}$ -associated death executor
NFkB	nuclear factor kappa B
NRAGE	neurotrophin receptor-interacting MAGE homolog
NRIF	neurotrophin receptor interacting factor
LINGO-1	LRR and Ig domain containing, Nogo receptor-interacting protein
OMgp	oligodendrocyte myelin glycoprotein
p-p38	phospho-p38 MAPK
$p75^{NTR}$	p75 neurotrophin receptor
PAI-1	plasminogen activator inhibitor-1
PDE	phosphodiesterase
PKA	protein kinase A
PKC	protein kinase C
PI3K	phosphoinositide 3 kinase
PLCγ	phospholipase c gamma
RIP	receptor intramembrane proteolysis
SFM	serum free media
TACE	tumor necrosis factor-α-converting enzyme
TAK1	transforming growth factor β–activated kinase 1
TNFα	tumor necrosis factor alpha
TNFR	tumor necrosis factor receptor

tPA	tissue plasminogen activator
TRADD	TNFR type 1-associated death domain
TRAF	TNFR-associated factors
Trk	tropomyosin receptor kinase
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling

V. Introduction

The p75 neurotrophin receptor ($p75^{NTR}$) is known to play multiple roles in regulating neuronal survival, death, and axonal growth (Frade et al., 1996; Friedman, 2000; Greene and Rukenstein, 1981; Maggirwar et al., 1998; Rabizadeh et al., 1993). $p75^{NTR}$ is more widely expressed during development than in adults (Friedman, 2000; Yan and Johnson, 1988). However, $p75^{NTR}$ is reexpressed in several pathological conditions such as traumatic brain injury, seizure, ischemia, oxidative stress, axonal injury, and Alzheimer's disease (Casha et al., 2001; Kokaia et al., 1998; Ramos et al., 2007; Roux et al., 1999; Yaar et al., 1997). The role of $p75^{NTR}$ in those pathological conditions has been proposed to be involved in neurodegeneration and axonal regeneration. For example, pilocarpine-induced seizures induce $p75^{NTR}$ upregulation in the hippocampus (Roux et al., 1999) and mediates neuronal apoptosis by activating the intrinsic caspase-9,6,3 cascade (Troy et al., 2002). These studies suggest that there is a tight correlation between $p75^{NTR}$ expression after injury and neurodegeneration. Although there are many studies demonstrating that $p75^{NTR}$ is upregulated in several pathological conditions, the mechanisms regulating $p75^{NTR}$ expression after injury are not well defined. Thus, elucidating the mechanisms of $p75^{NTR}$ regulation is an important step toward identifying therapeutic targets for injury-induced cell death or neurodegenerative diseases.

Interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) are essential proinflammatory cytokines released from several cell types,

including astrocytes and microglia, after brain injury (Giulian et al., 1986). These cytokines are also highly expressed in Alzheimer's disease (Fillit et al., 1991; Griffin et al., 1989; Wang et al., 1997), Parkinson's disease (Mogi et al., 1996; Mogi et al., 1994), and spinal cord injury (Wang et al., 1997; Xu et al., 1998), conditions in which $p75^{NTR}$ is induced. IL-1β and TNFα are upregulated within 2 hours after these types of injury, before $p75^{NTR}$ induction and significant cell death (Buttini et al., 1994; Casha et al., 2001; Eriksson et al., 2000; Fan et al., 1996; Minami et al., 1992; Roux et al., 1999; Taupin et al., 1993). Therefore, cytokine induction precedes $p75^{NTR}$ induction after damage, and may play a role in regulation of $p75^{NTR}$ expression. Indeed, data from microarray analysis on astrocytes after IL-1β treatment showed increased $p75^{NTR}$ mRNA (John et al., 2005).

The studies in this thesis examined the mechanisms of $p75^{NTR}$ induction, as well as the functional consequences of elevated $p75^{NTR}$ on neuronal survival.

1. The p75 neurotrophin receptor

1.1 p75 structure and function

The p75 neurotrophin receptor ($p75^{NTR}$) is a member of the tumor necrosis factor receptor (TNFR) superfamily. It consists of an extracellular domain (ECD), a single transmembrane domain and an intracellular domain (ICD)

(Figure 1). The ECD contains four cysteine-rich domains (CRDs). Structural modeling and genetic studies suggest that the third CRD is responsible for binding neurotrophins (Baldwin et al., 1992; Chapman and Kuntz, 1995; Dechant and Barde, 2002; Shamovsky et al., 1999; Welcher et al., 1991; Yan and Chao, 1991).

Unlike the classical tyrosine kinase receptors, which are activated through ligand-mediated receptor dimerization, which consequently stabilizes the kinase domain (Hubbard, 1999; Schlessinger, 2002), TNFR superfamily members are self-associated to form trimers in the cell membrane and undergo a conformational change upon ligand binding, leading to a favorable conformational arrangement for interaction with intracellular adaptors (Chan, 2000). In TNF receptor family members such as Fas, TNFR1, and CD41, ligand-independent receptor trimerization occurs through CRD1 (Chan et al., 2000; Siegel et al., 2000). However, it is not yet been established whether CRD1 has the same function in $p75^{NTR}$, nor is it clear whether $p75^{NTR}$ exists as a preassociated form, like most TNF receptor superfamily members. Furthermore, there has been debate about the stoichiometry of $p75^{NTR}$-neurotrophin binding (Gong et al., 2008; He and Garcia, 2004). Recently, Vilar et al. has proposed a model suggesting that $p75^{NTR}$ exists as a dimer, and that nerve growth factor (NGF)-mediated activation occurs through a conformational rearrangement of these receptor dimers (Vilar et al., 2009). In their model, $p75^{NTR}$ forms disulfide-linked dimers through the Cys257 in the transmembrane domain, and a mutation of this cysteine residue to an alanine residue abolished NGF-mediated $p75^{NTR}$

activation. However, this mutation did not affect the myelin associated glycoprotein (MAG)-mediated $p75^{NTR}$ downstream signaling, implying that the mechanisms of $p75^{NTR}$ activation are unique to distinct ligands. Thus, it is still difficult to draw a general model for the mechanisms of $p75^{NTR}$ activation.

The ECD has a single asparagine-linked carbohydrate (N-glycosylation) in CRD1 and several O-glycosylation sites in the juxtamembrane stalk domain for modification of the protein (Large et al., 1989). Glycosylation of proteins has multiple roles such as mediating protein-protein interaction, proper folding or cellular localization. However, N-glycosylation of $p75^{NTR}$ does not seem to be involved in either trafficking to the plasma membrane or binding to NGF, as mutating the N-glycosylation site of $p75^{NTR}$ does not affect the cell surface expression of the mutant or its ability to bind to NGF (Baldwin and Shooter, 1995). Whereas O-glycosylation of $p75^{NTR}$ has been shown to direct $p75^{NTR}$ to the apical domain in polarized epithelial cells (Monlauzeur et al., 1998; Yeaman et al., 1997), a $p75^{NTR}$ mutant lacking the stalk domain where O-glycosylation sites are present is still expressed in the basolateral plasma membrane of the epithelial cells (Yeaman et al., 1997), or in the cell surface of COS 7 cells (Baldwin and Shooter, 1995). These studies suggest that the two glycosylation modifications on $p75^{NTR}$ may have different roles other than involving in trafficking to the plasma membrane.

$p75^{NTR}$ ICD contains a prominent feature called the death domain, which is structurally similar to the death domain found in TNFR1, Fas, and FADD

(Eberstadt et al., 1998; Huang et al., 1996; Liepinsh et al., 1997; Sukits et al., 2001). However, $p75^{NTR}$ and other TNFR superfamily members seem to have different activation mechanisms and signaling pathways. For example, although TNFR1 and Fas require ligand-mediated oligomerization of their death domains in order to interact with intracellular adaptors (Huang et al., 1996), $p75^{NTR}$ does not display any aggregation of its death domain upon activation (Liepinsh et al., 1997). Although $p75^{NTR}$ is also categorized as a death receptor due to the ability to induce cell death as other TNF receptors, the underlying signaling pathways are distinct from other death receptors. While TNF receptors mediate cell death through activating the extrinsic apoptotic signaling pathway, in which caspase-8 is involved to activate a cascade of sequential caspases (Locksley et al., 2001), the intrinsic apoptotic cascade, caspase-9, 6, and 3, is activated in $p75^{NTR}$ induced cell death (Troy et al., 2002).

The ICD has two different forms of post-translational modifications: palmitoylation at cysteine (Barker et al., 1994) and phosphorylation on serine and threonine residues (Grob et al., 1985; Taniuchi et al., 1986). Palmitoylation of $p75^{NTR}$ is required for γ-secretase-mediated receptor cleavage, generating a C-terminal fragment and consequently activating death signaling (Underwood et al., 2008) (Figure 1). A functional role for $p75^{NTR}$ phosphorylation has not been well established since most studies have shown that a ligand does not change the phosphorylation level of $p75^{NTR}$ (Grob et al., 1985; Taniuchi et al., 1986). However, it has been demonstrated that NGF increases PKA (protein kinase A) mediated $p75^{NTR}$ phosphorylation, leading to its localization to lipid rafts in cells

expressing $p75^{NTR}$ but not TrkA (Higuchi et al., 2003). Interestingly, phosphorylation or glycosylation of $p75^{NTR}$ is found only in monomeric $p75^{NTR}$ not oligomers (Gong et al., 2008; Grob et al., 1985), suggesting that these two posttranslational modifications may be involved in receptor oligomerization.

1.2 $p75^{NTR}$ ligands

$p75^{NTR}$ activation results in various cellular responses depending upon the binding of different ligands, combinations of coreceptors, and intracellular adaptors. There are four different neurotrophins, nerve growth factor (NGF), brain derived neurotrophin factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4) that bind $p75^{NTR}$ to regulate neuronal survival, differentiation, and axonal growth. These four neurotrophins also bind to tropomyosin-related kinase (Trk) receptors, which belong to the receptor tyrosine kinase family. The Trk family is comprised of 3 members, TrkA, TrkB, and TrkC, which have different specificities for each neurotrophin. The neurotrophins are synthesized as unprocessed pro-forms, and are proteolytically cleaved to their mature forms. While the mature neurotrophins bind to both Trk receptors and $p75^{NTR}$ with similar affinity (K_d=1 nM), the unprocessed forms bind to $p75^{NTR}$ with a much higher affinity (K_d=0.2nM) than to Trk receptors (K_d > 5nM) (Chao and Bothwell, 2002; Lee et al., 2001).

Other than neurotrophins, $p75^{NTR}$ has been shown to have various

ligands, such as prion protein fragment PrP (Della-Bianca et al., 2001), the Aβ peptide of the amyloid precursor protein (APP) (Kuner et al., 1998; Perini et al., 2002; Yaar et al., 2002; Yaar et al., 1997), which has been shown to result in neuronal cell death upon binding (Sotthibundhu et al., 2008). In addition, the rabies virus glycoprotein (RGV) has been reported to bind to $p75^{NTR}$, serving for the virus penetration into the nervous system (Tuffereau et al., 1998).

1.3 $p75^{NTR}$ coreceptors and their distinctive functions

$p75^{NTR}$ can function as a complex with various membrane proteins, including all three Trk receptors. Initially, most $p75^{NTR}$ -related studies had focused on neurotrophin-mediated Trk receptor signaling as a coreceptor because $p75^{NTR}$ lacks intrinsic catalytic activity. $p75^{NTR}$ in fact increases the activation of Trk receptors by enhancing the binding affinity of neurotrophins to Trk receptors (Hantzopoulos et al., 1994; Mahadeo et al., 1994; Verdi et al., 1994). However, recent focus has shifted toward identifying a role for $p75^{NTR}$ independent of Trk receptors, since it was demonstrated that $p75^{NTR}$ induces sphingomyelin hydrolysis and ceramide production (Dobrowsky et al., 1994). One of the outstanding findings is that $p75^{NTR}$ is involved in cell death independent of Trk receptors (Kaplan and Miller, 2000).

In the process of neuronal pruning during development, Trk receptor signaling mediated by target derived neurotrophins is a key factor for proper development. Neurons compete for the limited amount of adequate

neurotrophins and those neurons that failed to get supported by appropriate neurotrophins die due to the lack of survival signaling mediated by Trk receptor (Deppmann et al., 2008; Hamburger and Levi-Montalcini, 1949; Levi-Montalcini, 1987). As an alternative mechanism, $p75^{NTR}$ has been also shown to mediate apoptosis during the period of naturally occurring cell death, providing a molecular mechanism for active apoptosis in neurons that have weak Trk signaling (Bamji et al., 1998). In the $p75^{NTR}$ knockout mice, there are more cholinergic neurons and cholinergic hyperinnervation of the hippocampus (Van der Zee et al., 1996; Yeo et al., 1997). Furthermore, overexpression of $p75^{NTR}$ intracellular domain mediates neuronal apoptosis in many brain areas (Majdan et al., 1997), suggesting that $p75^{NTR}$ may contribute to programmed cell death in development.

Sortilin, which is a member of Vps10p-domain receptor family of sorting receptors, has been identified as a co-receptor for $p75^{NTR}$ (Nykjaer et al., 2004). Sortilin acts as a molecular switch to apoptosis in neurons coexpressing $p75^{NTR}$ and Trk receptors by increasing the binding affinity of proneurotrophins to the 75^{NTR}-sortilin complex. This tripartite complex of $p75^{NTR}$ with sortilin and pro-neurotrophin triggers cell death (Nykjaer et al., 2004). Indeed, age-dependent neurodegeneration and cell death following corticospinal lesion is prevented in the sortilin knockout mice (Jansen et al., 2007), suggesting sortilin has a distinct role as a $p75^{NTR}$ coreceptor in proneurotrophin-mediated apoptosis. Sortilin is exclusively expressed in cell bodies and dendrites throughout the CNS and PNS (Sarret et al., 2003),

and the majority of sortilin is located on intracellular membranes (Mazella, 2001; Nielsen et al., 2001; Petersen et al., 1997). It has been reported that NRH2, a mammalian $p75^{NTR}$ homologue, redistributes sortilin to the cell surface, rendering cells more susceptible to proneurotrophin- induced death (Kim and Hempstead, 2009).

In addition to trks and sortilin, $p75^{NTR}$ interacts with Nogo receptor (NgR), mediating the inhibition of neurite outgrowth by modulating RhoA activity (Yamashita and Tohyama, 2003). $p75^{NTR}$ forms a tripartite complex with Nogo receptor and the LINGO-1 (LRR and Ig domain containing, Nogo receptor-interacting protein) (Mi et al., 2004) and this receptor complex can be activated by three myelin associated inhibitory factors; oligodendrocyte myelin glycoprotein (OMgp), myelin associated glycoprotein (MAG) ,and Nogo (Wang et al., 2002).

1.4 $p75^{NTR}$ intracellular adaptors and downstream signaling pathways

TNFR superfamily members do not have an intrinsic kinase domain. They signal through an association between oligomerized intracellular domains and cytoplasmic adaptor proteins. TNFR- associated factors (TRAF) bind to TNFR superfamily members and convey the receptor signal to downstream effectors such as nuclear factor kappa B (NFκB), JNK, and Src (Bradley and Pober, 2001). Since it lacks a kinase domain, $p75^{NTR}$ is assumed to initiate signaling by recruiting an intracellular adaptor protein as

most TNFR superfamily members do. Several interacting molecules have been reported (Roux and Barker, 2002). Among the TRAF family, TRAF2, TRAF4, and TRAF6 have been shown to interact with $p75^{NTR}$ in HEK 293 cells, and it is suggested that TRAF2 preferentially binds to monomeric $p75^{NTR}$ (Ye et al., 1999). Whereas TRAF6 was found to cause apoptosis in Schwann cells and sympathetic neurons (Khursigara et al., 1999; Yeiser et al., 2004), we have shown that hippocampal neurons lack TRAF6 (Srinivasan et al., 2004), but express TRAF2 (Volosin et al., 2008).

Neurotrophin receptor interacting factor (NRIF) is another adaptor protein that interacts with the $p75^{NTR}$ ICD. In studies with sympathetic neurons and Schwann cells, NRIF was reported to transduce cell death signals through nuclear translocation with the help of TRAF6-mediated polyubiquitination, acting as a transcription factor (Geetha et al., 2005; Linggi et al., 2005). Interestingly, we have recently found that NRIF induces cell death through the same mechanisms with ubiquitination and nuclear translocation even in the absence of TRAF6, probably through TRAF2 (Volosin et al., 2008). Therefore, $p75^{NTR}$ may have various functions and mechanisms in different cellular contexts. Other adaptor proteins, NRAGE and NADE have also been shown to interact with $p75^{NTR}$ to mediate neurotrophin-induced cell death (Linggi et al., 2005; Park et al., 2000; Salehi et al., 2000). Another $p75^{NTR}$ adaptor protein, SC-1, has been found to regulate the cell cycle by forming a complex with histone deacetylases (HDACs) (Chittka et al., 2004).

$p75^{NTR}$ mediated death signaling through these adaptor proteins has been revealed to converge on the activation of Jun N-terminal kinase (JNK), p53, and caspases (Roux and Barker, 2002). Furthermore, $p75^{NTR}$ has been known to mediate intracellular signaling through regulated intramembrane proteolysis (RIP), which involves two sequential cleavages; the extracellular part of $p75^{NTR}$ is shed by α-secretase and the following intramembrane region is cleaved by γ-secretase (Figure 1). The released intracellular domain (ICD) travels to the nucleus and regulates transcription (Parkhurst et al., 2010). Recently, it has been shown that NRIF nuclear translocation is required for hippocampal neuronal cell death along with the $p75^{NTR}$ cleavage (Kenchappa et al., 2006; Volosin et al., 2008).

As noted earlier, $p75^{NTR}$ associates with Trk receptors to refine specificity for its ligands. Additionally, neurotrophins may simultaneously activate Trk and $p75^{NTR}$, resulting in different outcomes depending on spatial and temporal expression of the receptors. Activation of the Trk mediated phosphatidylinositol 3-kinase (PI3K) pathway is a dominant survival signaling over $p75^{NTR}$ promoted death signaling in sympathetic neurons. NGF treatment inhibited $p75^{NTR}$ mediated cell death in sympathetic neurons, which express TrkA and $p75^{NTR}$ (Bamji et al., 1998). In contrast, NGF induces $p75^{NTR}$ mediated apoptosis in a subpopulation of retinal ganglion cells which have decreased expression of TrkB or have failed to be supported by BDNF (Frade et al., 1999; Frade et al., 1996). In different types of cells such as basal forebrain neurons, Trk-mediated PI3K signaling

was not enough to overcome $p75^{NTR}$ -mediated apoptotic signaling (Song et al., 2010). Thus, neurotrophins may induce $p75^{NTR}$-mediated apoptosis depending upon distinct cellular context. Furthermore, $p75^{NTR}$ expression is limited in certain brain areas after development and its expression is increased in many pathological conditions, facilitating neurodegeneration. Therefore, the goal of this work is to identify mechanisms of $p75^{NTR}$ regulation following injury.

2. Proinflammatory cytokines

Interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) are essential proinflammatory cytokines released from several cell types, including astrocytes and microglia, in response to diverse brain insults such as infection, injury, and acute and chronic neurodegenerative diseases. IL-1β and TNFα have several physiological functions in neuroinflammation.

IL-1β is known to induce production of other cytokines and growth factors (Benveniste, 1992; Merrill and Benveniste, 1996), change blood flow (Maher et al., 2003; Monroy et al., 2001), and affect neuroendocrine responses and the activity of the HPA axis (Berkenbosch et al., 1987; Sapolsky et al., 1987). In addition, both IL-1β and TNFα influence neurodegeneration (Thornton et al., 2006; Zhao et al., 2001), and inhibition of endogenous IL-1β and TNFα protects against neuronal injury that occurs after cerebral ischemia (Meistrell et al., 1997; Relton and Rothwell, 1992).

In addition to effects on neurodegeneration, IL-1β also affects remyelination by oligodendrocytes after injury (Mason et al., 2001). These cytokines are highly expressed in Alzheimer's disease (Fillit et al., 1991; Griffin et al., 1989; Wang et al., 1997), Parkinson's disease (Mogi et al., 1996; Mogi et al., 1994), and spinal cord injury (Wang et al., 1997; Xu et al., 1998), conditions in which $p75^{NTR}$ is also induced.

2.1 IL-1 signaling

The interleukin-1 (IL-1) family includes two agonists, IL-1α and IL-1β, and a naturally occurring IL-1 receptor antagonist (IL-1ra). IL-1α and IL-1β are produced as inactive precursor forms. Most pro-IL-1α resides in the cell cytosol or is transported to the cell membrane, where it may remain as a precursor or get cleaved to its active form by membrane bound proteases (Endres et al., 1989; Lonnemann et al., 1989). In contrast to IL-1α, the processed form of IL-1β by IL- 1β converting enzyme (ICE, also known as caspase-1) is released into the extracellular space (Dinarello, 1996). When introduced exogenously, both mature IL-1α and IL-1β have similar functional outcomes (Dinarello, 1998; Dinarello and Thompson, 1991). They bind two different plasma membrane proteins, IL-1 receptor type I and II (Colotta et al., 1993; Loddick et al., 1998; Sims et al., 1993). Upon binding its ligand, IL-1 receptor type I associates with IL-1 receptor accessory protein (IL-1RAcP) (Sims and Dower, 1994; Wesche et al., 1997b). This heterodimeric receptor complex serves as a scaffold for the association of MyD88 and

Tollip, consequently recruiting other adaptor proteins, IRAK1 and IRAK4 (IL-1 receptor associated serine/threonine-specific protein kinase), triggering IRAK4 autophosphorylation and the phosphorylation of IRAK1(Burns et al., 2000; Cao et al., 1996a; Wesche et al., 1997a). Next, TRAF6, which is an E3 ubiquitin ligase, is recruited to the complex and synthesizes a polyubiquitin chain to itself with the help of E2 conjugating complexes (Ubc13/Uev1a) (Deng et al., 2000; Lamothe et al., 2007; Wang et al., 2001). This activates TAK1 (transforming growth factor β-activativated kinase 1), which subsequently activates several kinases in different downstream signaling pathways. Numerous signaling pathways activated by IL-1 have been reported so far, including activation of: NFκB, MAP-kinase family, phosphatidylinositol-3 kinase (PI3-K), sphingomyelinase, and protein kinase C (PKC) (Martin and Wesche, 2002). Previously, we have reported that IL-1β activates NFκB in astrocytes and the p38 MAPK pathway in neurons (Srinivasan et al., 2004), suggesting that cytokine-mediated signaling pathways may vary depending upon cellular context.

The NFκB pathway can be activated by TAK1, PKC, or MEKK3-dependent pathways upon IL-1 stimulation. TAK1 or PKC activates IKKβ, which subsequently phosphorylates IκBα, followed by poly-ubiquitination for degradation. Thereby, NFkB is freed from the NFkB-IkB complex, and translocates to the nucleus for gene activation, which is referred to as the classical NFkB pathway (Baeuerle and Baltimore, 1996; Thanos and Maniatis, 1995; Verma et al., 1995). Unlike TAK1 or PKC dependent NFkB

activation, MEKK3 mediated IKKβ activation phosphorylates IκBα in a way that avoids proteasome-dependent degradation but still releases NFκB (Yao et al., 2007).

Two MAPK pathways, including p38 and JNK, are activated by TAK1 (Sato et al., 2005) and other MAPKKKs, such as MEKK3, activate JNK but not p38 in mammalian cells (Blank et al., 1996).

IL-1RII, which lacks the intracellular domain, cannot signal, and has been known as a decoy receptor because it has lower affinity for IL-1α, thereby trapping IL-1β and preventing accessibility to IL-1RI (Colotta et al., 1993). The third member of the IL-1 family, IL-1ra, competes with IL-1α or IL-1β for binding to IL-1RI (Dinarello, 1998; Dripps et al., 1991). All members of the IL-1 and IL-1 receptor families are expressed throughout the brain at low or undetectable concentrations (Vitkovic et al., 2000). Therefore, most functional roles of IL-1 have been analyzed in disease or injury settings. In fact, IL-1α, IL-1β, and IL-1RI knockout mice show no physiological or developmental defects (Glaccum et al., 1997; Horai et al., 1998). Although the role of IL-1 in immune reactions following injury or in disease states have been more extensively accepted, several studies have been suggested the involvement of IL-1 in long-term potentiation (LTP) (Ross et al., 2003; Schneider et al., 1998) or in the regulation of sleep patterns (Krueger et al., 2001) in healthy conditions.

Various factors such as inflammatory stimuli (Hetier et al., 1988),

other cytokines (Lieberman et al., 1989), and cellular injury (Davies et al., 1999; Depino et al., 2005) induce IL-1 release in numerous cell types, including microglia (Giulian et al., 1986; Hetier et al., 1988; Yao et al., 1992), astrocytes (Knerlich et al., 1999; Lieberman et al., 1989; Zhang et al., 2000), oligodendrocytes (Blasi et al., 1999), and neurons (Lechan et al., 1990; Takao et al., 1990; Watt and Hobbs, 2000).

2.2 TNF signaling

TNFα is a member of the TNF superfamily of ligands and is produced as a monomeric type-2 transmembrane protein (tmTNF), which is cleaved to soluble TNF by the extracellular protease, TNFα convertase (TACE) (Idriss and Naismith, 2000). Both tmTNF and soluble TNF bind two TNFR superfamily members, TNFR1 and TNFR2. While TNFR1 is expressed in most cell types and has a preference for soluble TNF, TNFR2 expression is restricted to cells of the immune system with a preference for tmTNF. TNFR preexists as a trimer on the membrane prior to ligand binding and changes conformation upon binding to facilitate interaction with intracellular adaptor proteins. Cellular responses to TNF can be diverse depending on the activating intracellular signaling pathways, including nuclear factor kappa-B (NFκB), p38, c-jun N-terminal kinase (JNK), and the ceramide/sphingomyelinase signaling pathway (MacEwan, 2002). The most well characterized function of TNF is the induction of apoptosis. TNF binding to TNFRI recruits adaptor protein TRADD (TNF receptor-associated DD protein), RIP (receptor-interacting protein), TRAF2, and FADD (fas-associated DD protein).

Activation of the different signaling pathways leading to NFκB activation or apoptosis upon TNF binding has been shown to be dependent on different complex formation. Rapid and transient TNFRI-TRADD-RIP-TRAF2 complex formation at the plasma membrane triggers NFκB activation. If this fails and the complex is dissociated from TNFRI, then FADD can be associated to the complex, recruiting pro-caspase-8 to form a DISC (death–inducing signaling complex) that can activate the extrinsic apoptosis pathway (Micheau and Tschopp, 2003). Although both cytokines have specific receptors, it has been known that they share intracellular signaling effectors such as NFκB and MAPK.

3. The role of proinflammatory cytokines and $p75^{NTR}$ in CNS injury or disease

In the CNS, proinflammatory cytokines such as IL-1β and TNFα have immediate actions under inflammatory conditions following acute CNS disorders such as trauma, stroke, or epilepsy and chronic neurodegenerative diseases such as Alzheimer's and Parkinson's disease or multiple sclerosis (Allan et al., 2005). Proinflammatory cytokines, particularly IL-1β, are initially produced by microglia within minutes to hours after insult/injury, and subsequently their expression is observed in astrocytes (Nieto-Sampedro and Berman, 1987). Many studies have shown that IL-1β is involved in promoting harmful effects following insult/injury by inducing other proinflammatory cytokines such as TNFα, IL-6 (Chung and Benveniste, 1990; Sparacio et al., 1992), chemokines (Peterson et al., 1997; Rivieccio et al.,

2005), adhesion molecules (Kyrkanides et al., 1999), matrix metalloproteinases (Thornton et al., 2008), cyclooxygenases-2 (Molina-Holgado et al., 2000), and neurotoxic factors such as nitric oxide (NO) (Chao et al., 1997). Although IL-1β alone is not neurotoxic in the absence of insult or injury (Loddick and Rothwell, 1996; Relton and Rothwell, 1992; Shaftel et al., 2007; Yamasaki et al., 1995), IL-1β has a synergistic effect on neurotoxicity when administered with other cytokines (Chao et al., 1995; Hu et al., 1997) and exacerbates neurodegeneration following ischemia or epilepsy (Lawrence et al., 1998; Yamasaki et al., 1995), possibly with the release of these neurotoxins.

However, not only does IL-1β produce neurotoxic factors, but many studies have shown that IL-1β also induces trophic factors such as ciliary neurotrophic factor (CNTF) (Herx et al., 2000), fibroblast growth factor (FGF) (Ho and Blum, 1997), and NGF (Carman-Krzan et al., 1991; Friedman et al., 1996; Spranger et al., 1990). For example, IL-1β mediated NGF release from astrocytes enhances neuronal survival after injury in specific brain areas where TrkA is present, such as basal forebrain cholinergic neurons (Kromer, 1987; Williams et al., 1986). The contradictory aspects of IL-1β effects following injury implies that it may have influence on a variety of cell types in the CNS, thereby the balance between neuroprotective and neurotoxic factors released from various cells might be a contributing element for IL-1β mediated cellular outcome in injury/disease onset.

$p75^{NTR}$ is induced following many types of brain injury and in several

diseases, and this expression has been tightly correlated with cell death occurring in pathological conditions (Armstrong et al., 1991; Casha et al., 2001; Ernfors et al., 1989; Kokaia et al., 1998; Martinez-Murillo et al., 1998; Roux et al., 1999; Syroid et al., 2000). For example, $p75^{NTR}$ expression is increased in entorhinal, piriform cortex, and hippocampus where neuronal apoptosis is induced following pilocarpine mediated seizure (Roux et al., 1999). More direct evidence has been shown in $p75^{NTR}$ knockout mice, in which the amount of cell death was significantly attenuated in the knockout mice compared to wild type animals following pilocarpine induced seizure (Troy et al., 2002). These studies indicated a role for $p75^{NTR}$ in injury-mediated cell death. Furthermore, it has been suggested that $p75^{NTR}$ plays a role in cholinergic neuronal degeneration which is an early and key feature of Alzheimer's disease (Wu et al., 2005; Yan and Feng, 2004). Increased levels of proNGF have been detected in AD (Fahnestock et al., 2001) and proNGF isolated from AD patients has been revealed to cause $p75^{NTR}$ mediated neuronal cell death (Pedraza et al., 2005). These studies indicate a role for $p75^{NTR}$ in cell death following injury or in diseases, in addition to developmental programed cell death.

Although $p75^{NTR}$ has been implicated in developmental apoptosis as well as neurodegeneration in several pathological situations, there is a possibility of different $p75^{NTR}$-mediated cell death mechanisms being used in development versus pathological conditions. Indeed, sortilin, which is a necessary $p75^{NTR}$ coreceptor for cell death signaling, appeared to be involved in $p75^{NTR}$-mediated cell death after injury or in age-dependent neurodegeneration but not during

development (Jansen et al., 2007). Additionally, many studies have demonstrated that high levels of proNGF are detected as a result of altering the activity of proNGF processing enzymes such as tissue plasminogen activator (tPA) or matrix metalloproteinase-7 (MMP7) (Le and Friedman, 2012), activating $p75^{NTR}$ mediated cell death following brain injury/insults (Lee et al., 2001). Interestingly, increased level of proNGF has been reported in Alzheimer's disease (Fahnestock et al., 2001) and proNGF purified from Alzheimer's patients appears to be more potent in inducing apoptosis than proNGF from healthy brain (Podlesniy et al., 2006). As the disease progresses, a decrease in the TrkA to p75 ratio has been observed (Mufson et al., 2010), thus favoring apoptotic proNGF/p75 signaling in Alzheimer's disease. These studies may suggest that proNGF binding to $p75^{NTR}$-sortilin complex might be a major mechanism in cell death occurring specifically after injury or in Alzheimer's disease.

It is interesting to note that common changes have been observed in various pathological conditions including pilocarpine mediated seizure and Alzheimer's disease. proNGF and $p75^{NTR}$ are common proteins upregulated in those situations and these consequences throughout the variety of pathological situations imply that there might be a common mechanism that is responsible for proNGF and $p75^{NTR}$ regulation.

4. Significance

The $p75^{NTR}$ has various functions throughout the nervous system. Often referred to as a promiscuous receptor, $p75^{NTR}$ has several binding partners and

ligands, eliciting distinct consequences depending upon the cellular context. Thus, this receptor does not likely have “canonical” activation mechanisms, which is supported by recent findings demonstrating that different ligands require distinct $p75^{NTR}$ activation mechanisms (Vilar et al., 2009). What makes this receptor more complicated is that p75 can initiate signaling even in the absence of ligands through γ-secretase mediated receptor proteolysis (Le Moan et al., 2011). $p75^{NTR}$ not only mediates cell death by neurotrophins, proneurotrophins, and Aβ or other factors, possibly activating RIP, but also inhibits axonal growth and regeneration following injury. All the complexity of $p75^{NTR}$ demands more intensive and specific studies on identifying its function and mechanism of action in distinct cellular contexts. Therefore, finding biological factors leading to $p75^{NTR}$ regulation in different cell types especially after injury or disease may contribute to understanding the specific function of $p75^{NTR}$ following brain injury and in neurodegenerative disease.

Proinflammatory cytokines are induced early after brain insults, which precedes by several hours the $p75^{NTR}$ induction in the same tissue. The increase in brain IL-1 has also been reported in several neurodegenerative disorders including Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis (ALS). A study demonstrating that IL-1 increases $p75^{NTR}$ mRNA in astrocytes (John et al., 2005) may suggest IL-1 as a possible player for p75 regulation in neurons as well after brain injury/insults. IL-1 acts on a variety of cells in the CNS, resulting in numerous functions, sometimes contradictory outcomes in conjunction with the variety of cellular contexts. It therefore is

critically meaningful to investigate the distinct mechanisms of $p75^{NTR}$ regulation by IL-1 in the absence of additional environmental changes occurring after injury/insults to tease out possible different mechanisms of $p75^{NTR}$ regulation in specific cell types. Furthermore, neurotrophins have been potential therapeutic targets for the treatment of various neurodegenerative diseases such as Alzheimer's disease (Lad et al., 2003), indicating the importance of understanding the $p75^{NTR}$ activation mechanisms under different situations. Therefore, it will be crucial to understand the influences of $p75^{NTR}$ upregulation following injury/insults.

VI. Research Aims

1. Specific Aim 1: Determine whether cytokines regulate $p75^{NTR}$ expression in the absence of injury *in vivo* and *in vitro*.

Although there have been numerous studies demonstrating increased levels of $p75^{NTR}$ expression in the brain after injury, the specific signals have been unknown. We hypothesized that proinflammatory cytokines which are produced during the initial response to injury may be responsible for $p75^{NTR}$ regulation. To examine the specific effect of IL-1β on $p75^{NTR}$ regulation, cannulated rats were utilized in the absence of injury that might give numerous secondary influences. Since IL-1β affects glia as well as neurons, the experiments in this aim were done in hippocampal neurons and astrocytes to determine whether both IL-1β and TNFα regulate $p75^{NTR}$ in both cell types.

2. Specific Aim 2: Identify the mechanism of $p75^{NTR}$ regulation after cytokine treatment in different cellular contexts *in vitro*.

Our previous study suggested that the signaling pathway activated by IL-1β is cell type-specific. IL-1β activates NFkB in hippocampal astroyctes, but not in neurons (Srinivasan et al., 2004). Therefore, we studied the signaling mechanisms leading to $p75^{NTR}$ expression both in neurons and astrocytes. Two major signaling pathways, NFkB and p38 MAPK pathways that are known to be activated by cytokines, were examined using the different cell types *in vitro*. The experiments in this aim defined the mechanism of $p75^{NTR}$ regulation by IL-1β and TNFα in neurons and astrocytes.

3. Specific Aim 3: Examine functional roles of $p75^{NTR}$ elevated after cytokine treatment.

Increased expression of $p75^{NTR}$ in neurons after injury has been shown to lead to cell death via the intrinsic caspase cascade. Thus, the experiments in this

aim investigated cell death following cytokine mediated $p75^{NTR}$ upregulation. *In vitro*, cell death inducing ligands such as high dose of NGF or low dose of proNGF were applied to IL-1β primed neurons to examine whether IL-1β exacerbates NGF or proNGF mediated neuronal cell death. The experiments in this aim defined the distinct $p75^{NTR}$ activation mechanisms by specific ligands following IL-1β priming.

4. Specific Aim 4: Elucidate the consequences of $p75^{NTR}$ induction following IL-1 treatment in vivo.

IL-1β was infused into the cannulated rat hippocampus to determine whether IL-1β itself has any effect on hippocampal neuronal cell death *in vivo*. In vivo, many cell types are present in the hippocampus including microglia, astrocytes, and neurons that are known to be sources of neurotrophins. $p75^{NTR}$ expression, NGF/proNGF level, and cell death were tested following IL-1β infusion. The experiments in this aim examined consequences of $p75^{NTR}$ expression following cytokine treatment.

VII. Materials and Methods

Materials. IL-1β was generously provided by Dr. Ron Hart, (Rutgers University, Piscataway, NJ) and NGF was provided by Genentech. TNFα was purchased

from R&D systems, Inc. (Minneapolis, MN). SB203580 and SN-50 were obtained from Alexis Biochemicals (San Diego, CA). Eagle's MEM, Ham's F12, and penicillin-streptomycin were purchased from Invitrogen (Carlsbad, CA). All other materials were obtained from Sigma (St.Louis, MO).

1. Primary hippocampal neuronal cultures

Neuronal cultures were prepared as described previously (Farinelli et al., 1998; Friedman, 2000). Rat hippocampi were dissected from embryonic day 18, dissociated, plated on poly-D-lysine (0.1mg/ml)-coated dishes, and maintained in a serum-free environment. The medium consisted of a 1:1 mixture of Eagle's MEM and Ham's F12 supplemented with glucose (6mg/ml), insulin (25μg/ml), putrescine (60μM), progesterone (20nM), transferrin (100μg/ml), selenium (30nM), penicillin (0.5U/ml), and streptomycin (0.5μ/ml). Cultures were maintained in 5% CO_2 at 37°C for 5 days and subjected to different treatments.

2. Primary hippocampal astrocyte cultures

Astrocyte cultures were prepared as described previously (McCarthy and de Vellis, 1980; Srinivasan et al., 2004). Rat hippocampi were dissected from embryonic day 21, dissociated, plated on poly-D-lysine (0.1mg/ml)-coated flasks, and maintained in Eagle's MEM with Earle's salts, 2mM L-glutamine, 15% heat-inactivated fetal bovine serum, 6mg/ml glucose, penicillin (0.5U/ml), and streptomycin (0.5μ/ml). After 7-9 days incubation in 5% CO_2 at 37°C, cells were

shaken at 450 rpm for 10 min to remove microglia and neurons, followed by fresh medium change and returned to the incubator. Cells were shaken at 225 rpm overnight to eliminate additional non-astrocytic cells. Cells were replenished with fresh medium containing 0.1mM cytosine arabinoside, maintained for 3 days. Cells were trypsinized and replated at subconfluent density and were kept in 5% CO_2 at 37°C for 4 days before treatment.

3. Western blot analysis and Immunoprecipitation.

Cells were lysed in RIPA buffer (50mM Tris-HCl, pH7.5 150mM NaCl, 5mM EDTA, 1% Nonidet P-40, 0.5% deoxycholic acid, 0.5% SDS) supplemented with a protease inhibitor mixture (Roche Products, Welwyn Garden City, UK), 1mM sodium vanadate, and 5mM sodium fluoride. Proteins were quantified by Bradford assay (Bio-Rad, Hercules, CA) and equal amounts of proteins were run on 10% polyacrylamide gel, transferred onto a nitrocellulose membrane. Membranes were blocked in 5% non-fat milk in TBST and then probed with antibodies to $p75^{NTR}$ (Upstate Biotechnology, Inc., Lake Placid, NY), actin (Sigma, St. Louis, MO), phospho-p38 (P-p38), phospho-IκB (P-IκB), or phospho-CREB (P-CREB) (Cell Signaling Technology, Beverly, MA). P-p38, P-IκB, P-CREB blots were stripped and reprobed with anti-p38, anti-IκB, anti-CREB (Cell Signaling Technology), respectively. Bands were visualized by x-ray film exposure using enhanced chemical luminescence (Pierce, Rockford, IL). For IκB ubiquitination studies, cells were treated with IL-1β or TNFα for the indicated periods, then lysed in buffer (50mM Tris-HCl, pH7.5, 150mM NaCl, 1mM EDTA, 5mM NaF,

1mM Na_3VO_4, 1% Triton X-100, and protease inhibitors), and centrifuged. Supernatants were incubated with anti-IκB overnight at 4°C and then incubated with protein A agarose at 4°C for 2h. Immunopricipitates were washed three times with lysis buffer, and analyzed by Western blot for ubiquitin (Santa Cruz Biotechnology, Santa Cruz, CA).

4. Biotinylation of cell surface proteins

Cells were treated with IL-1β for 6 h and washed with pre-chilled PBS once and with PBS++ (PBS containing 1mM MgCl2 and 2.5 mM CaCl2) twice. Cell surface proteins were biotinylated with sulfo-NHS-SS-Biotin (Pierce) at 4°C for 1 h, quenched with glycine, and washed with PBS++ twice. Biotinylated cells were lysed in buffer containing 50mM Tris, 150mM NaCl, 1mM EDTA, 1% Nonidet P40, 0.5% deoxycholate, protease inhibiotor mixture, 1mM sodium vanadate and 5mM sodium fluoride, and lysates were incubated with streptavidin-agarose (Pierce) overnight at 4°C. After centrifugation (4500g for 3min at 4°C), supernatants were saved and pellets were washed with lysis buffer three times. Pellets and supernatants were analyzed by Western blot for $p75^{NTR}$ (upstate), sortilin (BD), and transferrin receptor (Invitrogen).

5. Immunostaining

To visualize nuclear translocation of NFκB, cells were plated on plastic Lab-Tek slide wells and treated with IL-1β or TNFα with or without preincubation

with SN-50 for 30 min. Cells were fixed in 4% paraformaldehyde, washed with PBS, permeabilized with PBS plus 0.3% Triton X-100, blocked in PBS/5% goat serum/0.3% Triton X-100, and then incubated with anti-p65 (Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. Slides were washed three times with PBS, incubated with secondary antibodies coupled to the Alexa 555 fluophore (Molecular Probes Inc., Eugene, OR) for 1h at room temperature, then washed with PBS three times. Hoechst 33342 (1μg/ml, Sigma) was used to visualize the nuclei.

6. Quantitative real-time reverse transcription PCR

Primary hippocampal neurons or astrocytes were treated with IL-1β or TNFα for 2, 4 or 8 h, and mRNA was isolated using TRIzol reagent (Invitrogen). cDNA was generated using SuperScriptTM II RT with random hexamers (Invitrogen), and SYBR-green-based quantitative real-time PCR was performed using primers specific for

p75NTR (rat, forward 5'-CTGATGCTGAATGCGAAGAG-3' and reverse 5'-TCACCATATCCGCCACTGTA-3') or

actin (forward 5'-TCATGAAGTGTGACGTTGACATCCGT-3' and reverse 5'-CTTAGAAGCATTTGCGGTGCACGATG-3') with the comparative CT method (ΔΔCT)(ABI).

7. Survival assay.

To assess cell viability following ligand treatment, primary hippocampal neurons were lysed, and only healthy nuclei were counted using a hemocytometer as described previously (Farinelli et al., 1998; Maroney et al., 1999; Friedman, 2000). Nuclei of dead cells were discriminated from nuclei of healthy cells by pyknotic and irregular shapes, typical representation of apoptotic cells. Cell counts were performed in triplicate wells.

8. Cell transfection, immunostaining, and co-immunoprecipitation

Cells were transfected with $p75^{NTR}$ shRNAs alone or cotransfected with wild-type or mutant $p75^{NTR}$ by electroporation with recommended program by manufacturer on an Amaxa Nucleofactor device. For neurons, cells were kept in serum (DMEM+10% HS) for 2-3 h, then medium was changed into serum-free medium and cells were used for Western blot assay on the third day after transfection. For immunostaining, neurons were plated on poly-D-lysine (0.1mg/ml)-coated cover glass in SFM, transfected with lipofectamine2000 (Invitrogen) after 3 DIV and, were used on the second day after transfection. Cells were fixed in 4% paraformaldehyde, washed with PBS, permeabilized with PBS plus 0.3% Triton X-100, blocked in 5% goat serum with permeabilizing buffer, and then incubated with anti-cleaved caspase 3 (Cell Signaling) and anti-HA (covance) to identify $p75^{NTR}$ constructs overnight at 4°C. Glass cover slips were washed three times with PBS, incubated with secondary antibodies coupled to the Alexa 594 and 488 fluorophores (Molecular Probes Inc., Eugene, OR) for 1h at room temperature, then washed with PBS three times. Hoechst 33342

(1μg/ml, Sigma) was applied to visualize the nuclei. For co-immunoprecipitation, transfected cells were treated with proNGF for 30 min, lysed in modified TNE buffer (50mM Tris, 137mM NaCl, 2mM CaCl) containing 0.2% triton, 60mM octylglucoside and protease inhibitors (Roche). 400μg of protein were immunoprecipitated with anti-p75 (Chemicon) or anti-HA (Covance) overnight at 4°C and then incubated with Protein G-sepharose beads for 4 h at 4°C. Beads were washed three times with lysis buffer and analyzed by Western blot for sortilin (BD) and p75 (Upstate).

9. Confocal microscopy and acceptor photobleaching

A Zeiss LSM 510 confocal laser scanning microscope (Carl Zeiss AG, Jena, Germany) was used to perform the acceptor photobleaching. Neurons were treated with proNGF or NGF for 30 min, then fixed in 4% paraformaldehyde, washed with PBS, blocked in 5% goat serum in PBS, and then incubated with anti-sortilin (Alpha-Diagnostic) and anti-HA (Covance) overnight at 4°C. The secondary antibodies for FRET pairs were goat-anti-mouse 488 and goat-anti-rabbit 555 (Alexa). Ten fluorescence images were taken, four before and six after illuminating at 545 nm (bleaching beam). Regions of interest were analyzed by comparing the mean fluorescence intensity, which was adjusted by subtraction of the background fluorescence, before and after photobleaching of the acceptor. FRET was considered to have occurred when the donor intensity was at least 10% higher after acceptor bleaching.

10. Animals and Stereotaxic cannulation of the hippocampus

Male Sprague Dawley rats (250-275g) were anaesthetized with ketamine (50mg/kg)/xylazine (10mg/kg) and placed in stereotaxic frame for bilateral implantation of cannula into dorsal hippocampus. The following coordinates were used: anterior-posterior = -3.1mm from bregma, lateral = ±2 mm from midline, dorsoventral = -3mm from skull (Paxinos et al., 1985). Skull holes were made with a dental drill and the guide cannula and support screw were fixed with dental cement. After 7 days, 10μg (in 0.5μl) of IL-1β was infused unilaterally into the hippocampus via the guide cannula at a rate of 0.5μl/min. Animals found to have an incorrectly placed cannula were excluded.

All animal studies were conducted using the NIH (National Institutes of Health) guidelines for the ethical treatment of animals with approval of the Rutgers Institutional Animal Care and Facilities Committee.

11. Brain preparation for Immunohistochemistry

Two days after IL-1β infusion, rats were anesthetized with ketamine/xylazine and perfused transcardially with saline followed by 4% paraformaldehyde. The brains were removed and postfixed in 4% paraformaldehyde before being cryoprotected in 30% sucrose for 2 d. Brains were then sectioned on a cryostat (Leica), mounted onto charged slides for immunostaining. Frozen brain sections (12 μm) were warmed at 37°C for 1 min,

washed with PBS , blocked in 10% goat serum with PBS plus 0.3% Triton X-100, and then incubated with anti- $p75^{NTR}$ (192 IgG; Chemicon; 1:500) and anti-GFAP (generously provided by Dr. James Goldman, Columbia; 1:500), or anti- $p75^{NTR}$ (Upstate; 1:500) and anti-NeuN (Chemicon; 1:500) overnight at 4°C. The sections were then washed three times with PBS for 15 min, followed by incubation with the secondary antibodies, donkey anti-rabbit, goat anti-mouse, texas red conjugated anti-mouse (Jackson; 1:500) in the dark for 1 h, washed three times with PBS for 15 min, and coverslipped. Hoechst 33342 dye (1 μg/ml; Sigma) was added into PBS during the last wash to label nuclei. Sections were mounted with anti-fading medium (ProLong Gold; Invitrogen) and were examined by fluorescence microscopy (Nikon). Cell death was examined by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) staining following manufacturer's manual (Roche, Mannheim, Germany).

12. Brain preparation for Quantitative real-time reverse transcription PCR

Dorsal hippocampi were freshly dissected and mRNA and proteins were isolated using TRIZOL reagent (Invitrogen, Carlsbad, CA). cDNA was generated using SuperScriptTM II Reverse Transcriptase with random hexamers (Invitrogen), and SYBR-green based quantitative real-time PCR was performed using primers specific for

$p75^{NTR}$ (rat, forward: 5¢- CTGATGCTGAATGCGAAGAG-3¢, reverse: 5¢-TCACCATATCCGCCACTGTA-3¢),

NGF (rat, forward: 5¢-CAAGGACGCAGCTTTCTATCCTG-3¢, reverse: 5¢-

CTTCAGGGACAGAGTCTCCCTCT-3¢), or

actin (forward:5¢-TCATGAAGTGTGACGTTGACATCCGT-3¢,reverse :5¢-CTTAGAAGCATTTGCGGTG CACGATG-3¢) with the comparative CT method (ΔΔCT)(ABI).

13. Analysis of Cerebrospinal fluid (CSF)

Rats were anesthetized with ketamine/xylazine and placed in a stereotaxic frame for collecting CSF from cisterna magna using a 25 gauge needle. Only CSF samples that did not contain blood contamination were mixed with protease inhibitors, flash frozen, and stored at -80°C until analysis.

14. In Situ Zymography

One day following IL-1β infusion, rats were anesthetized with ketamine/xylazine, and their brains were flash frozen, and sectioned on a cryostat (Leica), mounted onto charged slides for in situ zymography. Fresh frozen sections (12μm) were covered with a buffer containing 1% low-melting point agarose (BioRad, Hercules, CA), 0.1 M Tris, pH 7.5, 2.5% milk and 20 μg/mL plasminogen. Areas of tPA acivity were detected in black spots on dark field of views, where the endogenous enzyme degraded the substrate.

VIII. Results

Overview

Previous work has demonstrated that increased $p75^{NTR}$ plays a role in neurodegeneration following brain insults and in neurodegenerative diseases. However, the mechanisms of $p75^{NTR}$ regulation in these pathological conditions have not been identified. Using both in vivo and in vitro approaches to investigate the effect of IL-1β as a potential player in $p75^{NTR}$ regulation without the context of additional injury, IL-1β was found to have direct effect on $p75^{NTR}$ regulation both in hippocampal neurons and astrocytes in vitro. TNFα, another type of proinflammatory cytokine, also increased $p75^{NTR}$ expression both in neurons and astrocytes (Chapter 1). Because $p75^{NTR}$ may act differently depending upon cellular context, identifying the cell specific mechanisms of $p75^{NTR}$ regulation is critical. Investigating the roles of the NFkB and p38 MAPK pathways leading to $p75^{NTR}$ expression showed the mechanisms governing this regulation are cytokine- and cell-type specific (Chapter 2). The functional role of $p75^{NTR}$ expressed following IL-1β treatment was examined with regard to its capability to induce cell death upon ligand binding. IL-1β was found to exacerbate only proNGF, not NGF, mediated cell death by recruiting sortilin and $p75^{NTR}$, which are required for proNGF-mediated apoptosis, to the cell surface. IL-1β also increased the ratio of monomeric to dimeric $p75^{NTR}$. We used a $p75^{NTR}$ monomeric mutant, which lacks the ability to form a disulfide bond at Cys257 located in the transmembrane domain, thereby failing to form a dimer. proNGF was found to preferentially activate monomeric $p75^{NTR}$, resulting in cell death. In accordance with a previous study (Vilar et al., 2009), NGF was not able to respond to monomeric $p75^{NTR}$, indicating that IL-1β mediated monomeric $p75^{NTR}$ induction is favorable to respond to proNGF rather than NGF (Chapter 3). Given

previous studies demonstrating that IL-1β itself is not neurotoxic to healthy neurons, the effect of IL-1β infusion into healthy brain was assessed since IL-1β is a well-known factor to increase NGF mRNA in astrocytes (Friedman et al., 1996). Although the exogenous IL-1β increased NGF and $p75^{NTR}$ expression in the hippocampus (Chapter 1), no cell death was detected. Furthermore, IL-1β induced NGF release rather than proNGF, possibly by increasing tPA activity (Chapter 4). These findings are consistent with the idea that IL-1β itself is not neurotoxic, probably due in part to the lack of death-inducing ligands such as proNGF in the healthy brain, which is altered in pathological conditions where high levels of proNGF have been reported. Altogether, proinflammatory cytokines induced in numerous pathophysiological situations, particularly IL-1β, may play a key role in the induction of $p75^{NTR}$, leading to increased vulnerability to cell death occurring particularly after injury or in disease.

Chapter 1. $p75^{NTR}$ regulation following proinflammatory cytokine treatment in the hippocampus in vivo and in vitro

1.1 $p75^{NTR}$ expression following IL-1β infusion in vivo

To investigate whether IL-1β induces $p75^{NTR}$ expression in vivo, IL-1β (10ng) was directly infused into one hippocampal hemisphere through a cannula. As a control, saline was infused into the other side of hippocampus. To examine $p75^{NTR}$ mRNA level, dorsal hippocampus was dissected and analyzed by quantitative PCR. $p75^{NTR}$ mRNA level was increased in the tissue sample that had been infused with IL-1β for 4h as compared to the contralateral side with saline infusion (Figure 2A). Western blot analysis of tissue samples taken from dorsal hippocampus 2 days after IL-1β infusion shows that $p75^{NTR}$ protein level was also elevated by IL-1β (Figure 2B). Since IL-1β can affect multiple cell types, sections were double labeled with anti- $p75^{NTR}$ and a neuronal marker, NeuN, or a glial marker, GFAP to examine where $p75^{NTR}$ was regulated following IL-1β infusion. Increased $p75^{NTR}$ was colocalized with NeuN staining (Figure 2C) and GFAP staining (Figure 2D), suggesting that IL-1β mediates $p75^{NTR}$ expression in hippocampal neurons and also in non-neuronal cells *in vivo*, as expected.

1.2 IL-1β and TNFα increases $p75^{NTR}$ expression in primary hippocampal neurons and astrocytes in vitro

To further investigate whether proinflammatory cytokines such as IL-1β and TNFα directly induce $p75^{NTR}$ in different cell types, cultured hippocampal

neurons and astrocytes were treated with IL-1β or TNFα for 1, 4, 8, 12, or 24 hours and the levels of $p75^{NTR}$ were evaluated by Western blot analysis. The expression of $p75^{NTR}$ was increased by IL-1β and TNFα both in neurons (Figure 3A-D) and astrocytes (Figure 3E-H) relative to actin. Elevated $p75^{NTR}$ expression in astrocytes was detected within 1 hour and showed a peak after 8 hour treatment with cytokines (Figure 3E-H), while in neurons increases were detected at 4 hr and peaked at 8-12hr (Figure 3A-D). The increased $p75^{NTR}$ expression induced by IL-1β was transient in both cell types, returning to baseline by 24 hour (Figure 3A, E), while treatment with TNFα induced a more sustained $p75^{NTR}$ increase in both neurons and astrocytes, remaining elevated at 24 hour (Figure 3B, F). $p75^{NTR}$ mRNA was determined by quantitative PCR and mRNA expression peaked after 4 hour of treatment of both cytokines in both neurons (Figure 3I) and astrocytes (Figure 3J). These results suggest that both cytokines have direct effect on $p75^{NTR}$ induction in neurons and astrocytes.

1.3 Surface expression of $p75^{NTR}$ following cytokine treatment

$p75^{NTR}$ is a cell surface receptor that can bind a variety of different ligands to mediate distinct cellular functions. To assess whether the elevated $p75^{NTR}$ was localized to the cell surface, biotinylation assays were performed on cytokine treated neurons and astrocytes. Nearly all the detectable $p75^{NTR}$ was present at the cell surface after 8 hr of treatment with IL-1β or TNFα in neurons and little $p75^{NTR}$ was detected in the intracellular fraction (Figure 4A). Similar to neurons, IL-1β and TNFα treated

astrocytes had elevated p75 ^{NTR} at the cell surface, but not in cytosolic compartment (Figure 4B), suggesting that p75^{NTR} induced by cytokines is present at the cell surface in both neurons and astrocytes. To assess specificity of the biotin pull-down, cells were incubated with or without biotin, and lysates were pulled down with the streptavidin beads, and then subjected to Western blot analysis. Only samples labeled with biotin showed p75^{NTR} band (Figure 4C).

Chapter 2. Mechanisms of p75^{NTR} regulation after cytokine treatment in different cellular contexts in vitro

2.1 Signaling pathways required for IL-1β induction of p75^{NTR} in hippocampal neurons and astrocytes

In our previous studies, we have demonstrated that IL-1β activates distinct signaling pathways in neurons and astrocytes. IL-1β activates the classical NFκB pathway in astrocytes but not in neurons, whereas p38 MAPK (but not JNK (c-Jun N-terminal kinase) or ERK (extracellular-signal-regulated kinase) MAPK) is activated by IL-1β in hippocampal neurons (Srinivasan et al., 2004). Therefore, the underlying signaling mechanisms governing IL-1β regulation of p75^{NTR} in both cell types was investigated.

To confirm the activation of specific signaling pathways in the different cell types, cultured cells treated with IL-1β were lysed and analyzed by Western blot for phopho-p38 and phopho-IκB. IL-1β induced

robust transient phosphorylation of p38 MAPK in hippocampal neurons (Figure 5A). Activation of NFκB signaling was monitored by phosphorylation and degradation of IκB, which is necessary for nuclear translocation of NFκB. In neurons, no IκB phoshorylation was detected (results not shown), consistent with our previous study showing that IL-1β does not activate NFκB in hippocampal neurons (Srinivasan et al., 2004). However, in astrocytes there was robust increase in phospho-IκB 5 min after IL-1β treatment, with a subsequent loss of IκB protein (Figure 5B). Interestingly, IL-1β also activated p38 MAPK signaling in astrocytes, phospho-p38 MAPK increased within 5min after IL-1β treatment and peaked at 10 min in astrocytes (Figure 5C). Thus, IL-1β activates both NFκB and p38 MAPK signaling in astrocytes, but only p38 MAPK in hippocampal neurons.

To examine which signaling pathway was responsible for the IL-1β mediated $p75^{NTR}$ upregulation in the different cell types, neurons or astrocytes were pretreated either with a p38 MAPK inhibitor SB203580 (10 μM) or an NFκB inhibitor SN-50 (10 μM) for 30 min. IL-1β was then applied to the cells for 8 hr, and $p75^{NTR}$ levels were analyzed by Western blot. In hippocampal neurons, the p38 MAPK inhibitor reduced the IL-1β mediated $p75^{NTR}$ upregulation (Figure 6A, C). As expected, the NFκB inhibitor, SN-50, did not prevent the $p75^{NTR}$ expression in neurons. In astrocytes, both the p38 MAPK inhibitor and the NFκB inhibitor completely prevented the IL-1β induction of $p75^{NTR}$ expression (Figure 6B, D), indicating that $p75^{NTR}$ induction in astrocytes requires both the p38 MAPK and NFκB pathways. To confirm that p38 MAPK inhibitor blocked the p38

MAPK activation, astrocytes were pretreated with the inhibitor prior to IL-1β treatment and p-p38 levels were analyzed by Western blot. Both IL-1β and TNFα increased p-p38 level and SB203580 completely blocked the p-p38 level increased by IL-1β (Figure 7A) and TNFα (Figure 7B). Confirmation that the NFκB inhibitor blocked the NFκB signaling pathway is shown by staining cells with p65. SN-50 treated cells showed the absence of nuclear p65 staining (Figure 7C), indicating that SN-50 blocked the NFκB nuclear translocation.

2.2 TNFα induces $p75^{NTR}$ via NFκB in both neurons and astrocytes

Both IL-1β and TNFα elicited increased $p75^{NTR}$ expression in hippocampal neurons; however, the signaling mechanisms activated by these cytokines were distinct. In contrast to the effects of IL-1β, TNFα elicited a modest transient phosphorylation of IκB (Figure 8A, C), but failed to induce activation of p38 MAPK (Figure 8B, D). Moreover, p65 staining in nuclei was visible with TNFα treated neurons, but not with IL-1β treated neurons (Figure 8E), confirming activation of the NFκB pathway by TNFα in neurons.

In astrocytes, TNFα treatment induced phosphorylation of IκB, which peaked at 10 min (Figure 8F) and decreased afterwards. IκB protein was degraded after phosphorylation (Figure 8F). TNFα also activated the p38 MAPK pathway in astrocytes, inducing robust phosphorylation that peaked at 10-20 min after treatment (Figure 8G). Thus, TNFα, like IL-1β, induced

activation of both the NFκB and p38 MAPK pathways in hippocampal astrocytes.

Interestingly, although TNFα induced phosphorylation of IκB in hippocampal neurons and astrocytes, loss of IκB protein was only observed in astrocytes (Figure 8F), not in neurons (Figure 8A). Once IκB is phosphorylated, it goes through polyubiquitination and proteasome-dependent degradation (Karin and Ben-Neriah, 2000). To investigate whether the ubiquitination of IκB occurred in neurons, astrocytes and neurons were treated with TNFα for the indicated times and the cell lysates were immunoprecipitated with anti-IκB antibody and analyzed by Western blot for detecting ubiquitination. There was increased ubiquitinated IκB in the TNFα treated astrocytes; however, no ubiquitination was detected in neurons treated with TNFα (Figure 8H), consistent with the lack of IκB degradation.

TNFα induced phosphorylation of IκB and nuclear translocation of NFκB in hippocampal neurons, suggesting that the increase in $p75^{NTR}$ expression induced by this cytokine might be mediated by NFκB. Treatment of the neurons with the NFκB inhibitor SN50, but not the p38 MAPK inhibitor SB203580, prevented the TNFα-evoked increase in $p75^{NTR}$ (Figure 9A, C), demonstrating that TNFα regulates $p75^{NTR}$ expression via NFκB signaling, whereas IL-1β regulates $p75^{NTR}$ via p38 MAPK signaling in hippocampal neurons (Figure 6).

Similar to the effects in neurons, the p38 MAPK inhibitor did not prevent the TNFα mediated $p75^{NTR}$ upregulation in astrocytes (Figure 9B, D), whereas NFκB inhibition blocked the TNFα mediated $p75^{NTR}$ expression (Figure 9B, D). Confirmation that the inhibitors blocked their respective signaling pathways is

shown in figure 7. Thus, in astrocytes, IL-1β requires both the p38 MAPK and NFκB pathways to regulate $p75^{NTR}$ expression; however, TNFαaregulates $p75^{NTR}$ only through the NFκB pathway. Moreover, TNFα signaled via the NFκB pathway to regulate $p75^{NTR}$ in both hippocampal neurons and astrocytes, whereas IL-1β signaled via the p38 MAPK pathway in both cell types and via NFκB only in astrocytes (Figure 10).

Chapter 3. Functional roles of $p75^{NTR}$ after IL-1β treatment

3.1 IL-1β primed neurons are more vulnerable to proNGF than NGF

Previous studies established that high dose of NGF or low dose of proNGF induces $p75^{NTR}$ mediated cell death in hippocampal neurons which do not express TrkA (Friedman, 2000; Volosin et al., 2008). To investigate the functional consequences of IL-1β mediated $p75^{NTR}$ upregulation, hippocampal neurons were pretreated with IL-1β for 4-6 hours to allow $p75^{NTR}$ induction, and then subjected to either NGF or proNGF treatment overnight. Either NGF (100ng/mL) or proNGF (1-10ng/mL) caused hippocampal neuronal death (Figure 11), consistent with previous studies. Interestingly, whereas IL-1β did not affect NGF mediated cell death in IL-1β primed neurons (Figure 11A), proNGF induced more cell death (Figure 11B), suggesting that IL-1β may make neurons more vulnerable to proNGF.

3.2 Recruitment of p75^{NTR} and sortilin to the cell surface and generation of monomeric p75^{NTR} following IL-1β treatment

Since sortilin is a required coreceptor for p75^{NTR} in proNGF mediated neuronal cell death (Nykjaer et al., 2004), the level of sortilin at the cell surface was examined. Biotinylation assays showed increased sortilin on the cell surface in IL-1β primed neurons (Figure 12). Increased surface level of p75^{NTR} was also detected as shown earlier (Figure 4). Transferrin receptor level wasn't changed by IL-1β treatment.

It has been recently shown that disulfide linked p75^{NTR} dimerization through Cys257 is necessary for NGF mediated p75^{NTR} signaling (Vilar et al., 2009). Mutation of cysteine to alanine at 257 (p75^{NTR}-C257A) results in monomeric form of p75^{NTR}, which does not respond to NGF. To evaluate which form of p75^{NTR} was found in IL-1β primed neurons, cultured hippocampal neurons were treated with IL-1β for 4, 8, 12, or 24h and analyzed on non-reducing gels. The levels of p75^{NTR} monomers gradually increased, while dimers were unchanged over time (Figure 13A, C). The gradual increase of p75^{NTR} monomers were also detected with the increasing doses of IL-1β treatment (Figure 13B, D), suggesting that p75^{NTR} induced by IL-1β may be monomeric rather than dimeric.

3.3 Monomeric p75^{NTR} is responsive to proNGF

To investigate whether monomeric $p75^{NTR}$ associated with sortilin following proNGF treatment, the monomeric $p75^{NTR}$ mutant ($p75^{NTR}$-C257A) was compared to wt-$p75^{NTR}$. Either wt-$p75^{NTR}$ or $p75^{NTR}$-C257A was introduced into hippocampal neurons together with $p75^{NTR}$ shRNA to knock down endogenous $p75^{NTR}$. To validate the $p75^{NTR}$ mutants, cell lysates were collected and subjected gel electrophoresis under non-reducing conditions 2 days after transfection. Both dimers and monomers were observed in wt-$p75^{NTR}$ and only monomers were detected with mutation of Cys257 to alanine ($p75^{NTR}$-C257A) (Figure 14).

To examine whether monomeric mutant ($p75^{NTR}$-C257A) associated with sortilin following proNGF treatment, transfected neurons were treated with 10ng/mL proNGF for 30 mins and then cell lysates were subjected to co-immunoprecipitation analysis. Whereas non-transfected neurons showed increased level of association between $p75^{NTR}$ and sortilin upon proNGF treatment (Figure 15A, B), the association between wt-$p75^{NTR}$ and sortilin was not changed by the ligand (Figure 15A, B). However, proNGF significantly increased the association between $p75^{NTR}$-C257A and sortilin (Figure 15A,B). To further confirm the molecular association between $p75^{NTR}$ and sortilin, acceptor photobleaching FRET was performed. Following acceptor photobleaching of sortilin (Alexa-555), donor fluorescence of HA-tagged wt- $p75^{NTR}$ (Figure 16A) or $p75^{NTR}$-C257A (Figure 16B) was increased upon proNGF treatment, indicating that the distance between $p75^{NTR}$ and sortilin was within the close proximity FRET could detect. The average of FRET efficiency showed that proNGF increased more sortilin

interaction with $p75^{NTR}$-C257A than with wt-$p75^{NTR}$ (Figure 16C). These data shows that monomeric $p75^{NTR}$ has a capability to interact with sortilin in the presence of proNGF.

To assess whether the proNGF was able to induce cell death through monomeric $p75^{NTR}$, wt-$p75^{NTR}$ or monomeric $p75^{NTR}$ mutant ($p75^{NTR}$-C257A) along with $p75^{NTR}$ shRNA to knock down the endogenous $p75^{NTR}$ were introduced into hippocampal neurons. These transfected neurons were treated with 10ng/mL proNGF for 8 hours and then analyzed the cleaved caspase 3 level using Western blot analysis (Figure 17A, C) and immunohistochemistry (Figure 17B, D). Caspase-3 mediated cell death is a well-established biological outcome after $p75^{NTR}$ activation by neurotrophins (Troy et al., 2002). Mock transfection showed a slight increase of cleaved caspase 3 level with proNGF and transfection with p75 shRNA showed p75 knock-down, which is correlated with the reduced amount of cleaved caspase 3 level even in the presence of proNGF (Figure 17A). Whereas wt-$p75^{NTR}$ transfected neurons was not affected with proNGF on the level of cleaved caspase-3, proNGF significantly increased the level of cleaved caspase-3 with $p75^{NTR}$-C257A (Figure 17A, B, C, and D), which is correlated with the previous co-immunoprecipitation analysis showing the increased association of sortilin only with $p75^{NTR}$-C257A (Figure 15A,B). To further confirm the level of cleaved caspase-3 following proNGF treatment, transfected neurons were treated with 10ng/mL proNGF for 6hrs, and then cells were fixed and stained for cleaved caspase-3 and HA, which allows to discriminate the transfected neurons since both wt-$p75^{NTR}$ and mutated

$p75^{NTR}$ have HA tag. Consistent with Western blot analysis, proNGF showed the significant increase of cleaved caspase-3 level only in $p75^{NTR}$-C257A transfected neurons (Figure 17B, D), indicating that monomeric $p75^{NTR}$ responds to proNGF to activate the underlying signaling pathway as well as to enhance the interaction with sortilin.

To ascertain whether the monomeric $p75^{NTR}$ mediated the enhanced vulnerability to proNGF after IL-1β treatment, we introduced wt-$p75^{NTR}$ and monomeric $p75^{NTR}$ mutant ($p75^{NTR}$-C257A) into neurons and analyzed NGF induced apoptosis by analyzing activation of caspase-3. Consistent with the study done by Vilar et al, the C257A mutant was not responsive to NGF in our system (Figure 18A, B), supporting the concept that dimers are necessary for NGF mediated $p75^{NTR}$ activation. These results suggest that monomeric $p75^{NTR}$ can be activated by proNGF in a complex with sortilin as opposed to the NGF requirement for disulfide-linked $p75^{NTR}$ dimers.

Chapter 4. Effects of IL-1β on $p75^{NTR}$ expression and cell death in vivo

4.1 No effect of IL-1β infusion on cell death in vivo

In light of previous work showing cell death mediated by $p75^{NTR}$, I examined whether IL-1β induced cell death as a result of $p75^{NTR}$ induction *in vivo*. Neither IL-1β nor saline infusion induced TUNEL positive cells (Figure 19A), which is consistent with many studies demonstrating that IL-1β itself doesn't induce cell death (Lawrence et al., 1998). To validate the

TUNEL assay, the consecutive section was treated with DNase-I to generate DNA fragments and then performed the TUNEL assay, showing positive staining (Figure 19B). These findings confirm that IL-1β alone is not sufficient to mediate cell death even with the $p75^{NTR}$ induction.

4.2 IL-1β releases NGF into the CSF

IL-1β is well known to regulate NGF expression and secretion in the brain (Spranger et al., 1990; Yasuda et al., 2007). To confirm the NGF regulation by IL-1β, tissue samples were taken from dorsal hippocampus 4 hour after IL-1β or saline infusion. NGF mRNA levels were increased by IL-1β compared to saline infusion (Figure 20A). Since we demonstrated that IL-1β also increased $p75^{NTR}$ and proNGF has been suggested to be involved in cell death in concert with $p75^{NTR}$ following injury (Beattie et al., 2002), we investigated which forms of NGF were secreted following infusion of IL-1β. The cerebrospinal fluid (CSF) was collected from the rats and analyzed by Western blot. The CSF taken from the IL-1β infused rat showed increased mature NGF compared to the CSF samples from control rat (Figure 20B). ProNGF was not detected in either of the samples (Figure 20B). These results suggest that IL-1β regulates NGF gene expression, with the subsequent secretion of mature NGF.

Our lab has recently demonstrated that enzymes such as MMP7 and tPA that process proNGF cleavage are reduced following seizures, leading to increased extracellular proNGF. To investigate whether IL-1β may alter proNGF processing enzyme activity to result in increased NGF rather than

proNGF, in situ zymography for MMP7 and tPA was performed. IL-1β infused side showed no change in MMP7 activity, but increases of tPA activity was detected compared to saline infused side (Figure 21), suggesting that IL-1β increases NGF possibly by increasing the tPA mediated cleavage of proNGF to NGF. Taken together, these data suggest that IL-1β induces $p75^{NTR}$ and NGF expression, but does not stabilize proNGF *in vivo* and therefore is not sufficient to induce cell death.

IX. Discussion

The p75 neurotrophin receptor is induced in CNS neurons after many types of injury and in neurodegenerative disease (Kokaia et al., 1998; Roux et al., 1999; Casha et al., 2001; Ramos et al., 2007; Yaar et al., 1997), and can mediate many different cellular responses, including neuronal apoptosis or survival, and regulation of axonal growth (Greene and Rukenstein, 1981; Rabizadeh et al., 1993; Frade et al., 1996; Maggirwar et al., 1998; Friedman, 2000). However, the signals that regulate $p75^{NTR}$ induction are not well characterized. Since many different types of pathophysiological conditions induce expression of $p75^{NTR}$, this study investigated whether specific proinflammatory cytokines, which are highly expressed in the brain following injury or in neurodegenerative disease (Minami et al., 1992; Taupin et al., 1993; Buttini et al., 1994; Fan et al., 1996; Eriksson et al., 2000), might play a role in $p75^{NTR}$ regulation.

Mechanism of $p75^{NTR}$ regulation is cytokine and cell type specific

In addition to the seizure-induced regulation of $p75^{NTR}$ previously

observed in brain neurons (Troy et al., 2002; Roux et al., 1999), our lab has recently demonstrated that this receptor is induced astrocytes as well, following pilocarpine-induced seizure (Volosin et al., 2008). In astrocytes, we have determined that stimulation of $p75^{NTR}$ with NGF negatively regulates cell proliferation (Cragnolini et al., 2009). Thus, this thesis investigated the mechanisms that regulate $p75^{NTR}$ induction in both primary hippocampal neurons and astrocytes. This study demonstrated that the proinflammatory cytokines IL-1β and TNFα induce $p75^{NTR}$ expression in both hippocampal neurons and astrocytes. However, the underlying signaling pathways leading to $p75^{NTR}$ induction was cytokine- and cell type-specific. Whereas IL-1β induced $p75^{NTR}$ via p38 MAPK in neurons, and via both p38 MAPK and NFκB in astrocytes, TNFα induced $p75^{NTR}$ via NFκB both in neurons and astrocytes. Thus, inflammatory cytokines play a key role in regulating $p75^{NTR}$ expression, and the cellular specificity may provide a possible therapeutic target for the CNS diseases.

Mechanisms of IL-1β regulation of $p75^{NTR}$

The release of IL-1β after injury elicits diverse effects including the production of other cytokines and growth factors, thereby promoting inflammatory activity. This study has shown that IL-1β induced $p75^{NTR}$ expression in primary hippocampal neurons and astrocytes. The increased $p75^{NTR}$ expression was transient and returned to baseline by 24 hr, which may be due to the actions of IL-1Ra, the naturally occurring IL-1β receptor antagonist (Hannum et al., 1990), providing negative feedback. Many signaling pathways can be activated by IL-1β

in different cell types, including the classic NFκB pathway, as well as the JNK, ERK, and p38 MAP kinase pathways (Dunne and O'Neill, 2003; O'Neill, 2002). Our lab has previously established that hippocampal neurons utilize a different signaling pathway compared to astrocytes (Srinivasan et al., 2004). While IL-1β activated NFκB in astrocytes, it failed to do so in hippocampal neurons, activating p38 MAPK signaling instead (Srinivasan et al., 2004). It has been reported that TRAF6 is necessary for IL-1β mediated NFκB activation (Cao et al., 1996b) and our lab has previously shown that TRAF6 is detected in astrocytes, but not in neurons (Srinivasan et al., 2004). Thus, IL-1β failed to activate NFκB in neurons, probably in part due to the absence of TRAF6 in hippocampal neurons.

It is important to note that only pathological concentrations (nM range) of IL-1β induced the p75 expression, which is correlated with the concentrations of IL-1β that activate p38 MAPK in neurons. Moreover, IL-1β activation of p38 MAPK was necessary for $p75^{NTR}$ induction in the hippocampal neurons. p38 MAPK is one of mitogen-activated protein kinase (MAPK) and has been known to be activated by various stress related signals (Chen et al., 2001; Kyriakis and Avruch, 2001), brain inflammation (Kaminska et al., 2009), HIV-associated neurocognitive disorders (Medders and Kaul, 2011), and ischemic injury (Barone et al., 2001). Specifically, p38 MAPK inhibitor, SB239063 has been shown to facilitate neuroprotection following a severe stroke (Barone et al., 2001), possibly through inhibiting $p75^{NTR}$ expression in part. Thus, the amount of IL-1β released in pathological conditions could be a critical factor to determine its functions in traditional inflammation and neurodegeneration versus other functions such as sleep regulation or synaptic plasticity in the brain. Indeed, our lab has recently

reported that IL-1β plays a role in synaptic plasticity through src activation only with physiological concentrations (pM range) (Huang et al., 2011). Interestingly, pathological concentrations activate only p38 MAPK, which is suggested to inhibit src activation, and the IL-1β mediated src activation is a neuron-specific effect through a splice variant of the IL-1β receptor accessory protein (AcPb) which is found in neurons, but not in astrocytes (Huang et al., 2011). This suggests that IL-1β may have distinct effect in different cellular contexts due to the presence of diverse intracellular protein compositions.

In contrast to neurons, IL-1β activated both the p38 MAPK pathway and the NFκB pathway in astrocytes. Pharmacological inhibitors were used to determine which of these pathways regulated the induction of $p75^{NTR}$ expression in astrocytes. Interestingly, inhibition of either pathway prevented the increase in $p75^{NTR}$ expression, indicating that both p38 MAPK signaling and the NFκB pathways are required for $p75^{NTR}$ induction in astrocytes.

Mechanisms of TNFα regulation of $p75^{NTR}$

TNFα is another major proinflammatory cytokine that is produced in the brain after injury (Taupin et al., 1993; Fan et al., 1996), and frequently acts synergistically with IL-1β. In contrast to the actions of IL-1β, TNFα activated the NFκB pathway in hippocampal neurons, indicated by phosphorylation of IκB, demonstrating that these two inflammatory cytokines signal via distinct pathways. This is probably due to the fact that different signaling pathways converging to NFκB activation are being engaged. TRAF6 is a necessary adaptor protein in IL-1β-mediated NFκB activation. As mentioned earlier, TRAF2, which is detected in

neurons instead of TRAF6, is a key adaptor protein in TNFα-mediated NFκB activation, possibly rendering cytokine specific effects on neurons. Inhibition of NFκB nuclear translocation with SN50 prevented TNFα-mediated $p75^{NTR}$ induction in the hippocampal neurons, confirming that the distinct pathways activated by the two different cytokines both lead to induction of $p75^{NTR}$.

Interestingly, although TNFα treatment of hippocampal neurons lead to IκB phosphorylation, there was no subsequent ubiquitination and degradation of the protein as normally seen in astrocytes and other cell types (Verstrepen et al., 2008). An alternative pathway for activation of NFκB has been demonstrated as a MEKK3 (MAPK/ERK kinase kinase 3)-dependent pathway in which IκB is phosphorylated and dissociated from NFkB, but is not degraded (Yao et al., 2007). Since that is what we observed in response to TNFα treatment of neurons, this pathway is likely to be the one involved.

In astrocytes, TNFα activated both the NFκB and p38 MAPK pathways, similar to the effects of IL-1β. However, blocking NFκB nuclear translocation with SN50 prevented TNFα-mediated induction of $p75^{NTR}$, while the p38 MAPK inhibitor had no effect, indicating that the NFκB pathway mediates $p75^{NTR}$ induction by TNFα in both neurons and astrocytes. This is in contrast to the regulation of $p75^{NTR}$ by IL-1β in astrocytes, which required signaling via both the NFκB and p38 MAPK pathways. Thus, these two key inflammatory cytokines have a common target in regulation of the $p75^{NTR}$, but the mechanisms by which they regulate expression of this receptor are distinct both in hippocampal neurons and astrocytes. To date, only two transcription factors, Sp1 and Egr-1 have been known to regulate $p75^{NTR}$ gene expression. Hypo-osmotic stress

regulates $p75^{NTR}$ by increasing cellular levels of Sp1 in primary cortical neuron (Ramos et al., 2007). Given the fact that activation of p38 MAPK signaling pathway is the major stress response, it is highly plausible that p38 MAPK might be a key intermediate molecule to activate Sp1 to regulate $p75^{NTR}$ expression in neurons. Furthermore, Egr-1 has been suggested to upregulate $p75^{NTR}$ in Schwann cells following injury (Nikam et al., 1995). Thus, different signaling pathways may turn on distinct transcription factors to modulate $p75^{NTR}$ expression in various cell types.

Since $p75^{NTR}$ is a cell surface receptor that binds a variety of ligands to mediate different cellular effects, it was important to determine whether the inflammatory cytokines not only increased expression of this receptor in the neurons and astrocytes, but whether the elevated levels of receptor expression were on the cell surface where it may be activated by ligand. Biotinylation experiments determined that the increased $p75^{NTR}$ expression was nearly completely present on the cell surface, confirming that the receptor would be accessible for ligand binding.

Functional roles of elevated $p75^{NTR}$ after cytokine treatment

The $p75^{NTR}$ plays an important role in neuronal apoptosis, serving as a receptor for proneurotrophins that are released in the hippocampus following seizures or in neurodegenerative disease (Volosin et al., 2008; Yaar et al., 1997). Thus, understanding the mechanisms that regulate expression of this receptor may serve as a potential therapeutic target for neuroprotection following injury or in disease. There has been contradictory

data on the effect of direct administration of IL-1β to the rat brain. Whereas one group has shown that IL-1β and TNFα administration caused neuronal loss in the substantia nigra (Carvey et al., 2005), most studies have shown that IL-1β alone did not directly affect healthy neurons either in vitro or in vivo (Chao et al., 1995; Lawrence et al., 1998). However, IL-1β is known to exacerbate neuronal degeneration, and the naturally occurring IL-1Ra has been shown to be neuroprotective in models of ischaemia and excitotoxicity (Simi et al., 2007). The neuronal induction of $p75^{NTR}$ by inflammatory cytokines may contribute to neurodegeneration in these pathological conditions.

Since IL-1β can exacerbate neuronal death after injury, this study investigated whether the increases in $p75^{NTR}$ mediated enhanced cell death in IL-1β primed neurons. Interestingly, results showed that $p75^{NTR}$ elevated by IL-1β increased vulnerability to proNGF, but not NGF, in hippocampal neurons.

In addition, in order for proNGF to activate $p75^{NTR}$ mediated cell death, sortilin is required as a coreceptor for $p75^{NTR}$. This study showed that IL-1β recruits sortilin to the cell surface as well as $p75^{NTR}$. Sortilin is mostly present in intracellular membranes and IL-1β-mediated sortilin recruiting to plasma membrane may be a crucial factor to cause the $p75^{NTR}$ mediated cell death observed following injury or in disease. Mechanisms of how IL-1β recruits sortilin to the cell surface are undefined. However, recent a study has demonstrated that NRH2, a mammalian homologue of $p75^{NTR}$, may regulate sortilin trafficking to the cell surface (Kim and Hempstead,

2009). Furthermore, coexpression of NRH2 and $p75^{NTR}$ has been reported in subpopulations of cells where proNGF mediated cell death was elicited (Kanning et al., 2003; Murray et al., 2004). Thus, it is highly plausible that IL-1β may regulate NRH2 as well as $p75^{NTR}$, to mediate sortilin trafficking.

Mechanisms of proNGF mediated $p75^{NTR}$ activation

The mechanisms of $p75^{NTR}$ activation and the stoichiometry of $p75^{NTR}$, its coreceptor, and ligands are not fully understood. Recently, the concept of a ligand specific $p75^{NTR}$ activation mechanism was proposed in which NGF requires disulfide-linked dimers through the Cys^{257} in the transmembrane domain for $p75^{NTR}$ activation (Vilar et al., 2009).

This study found that hippocampal neurons contained both monomeric and dimeric $p75^{NTR}$, and that monomeric $p75^{NTR}$ was specifically elevated by IL-1β. The IL-1β primed neurons responded only to proNGF, reflecting the concept of a ligand specific $p75^{NTR}$ activation mechanism. Indeed, replacing endogenous $p75^{NTR}$ with a monomeric $p75^{NTR}$ mutant induced proNGF mediated neuronal apoptosis. Furthermore, monomeric $p75^{NTR}$ mutant was able to associate with sortilin upon proNGF treatment. Therefore, the current study demonstrated for the first time that monomeric $p75^{NTR}$ had the capability of association with sortilin upon proNGF treatment, leading to neuronal cell death. This is distinct from the mechanisms of NGF mediated $p75^{NTR}$ activation, where dimeric $p75^{NTR}$ is required for receptor conformational changes upon NGF binding. Although proNGF increased the association between monomeric $p75^{NTR}$ and sortilin, the association between

wt-p75^{NTR} and sortilin was not increased by proNGF, probably due to the presence of high level of dimers rather than monomers in wt-p75^{NTR} transfected neurons.

Consequences of p75^{NTR} induction following IL-1β treatment

The most immediate response to injury is the induction of proinflammatory cytokines which are responsible for various cellular functions such as inflammation, the production of other cytokines, growth factors, and neurotrophins. Most cell types such as microglia, astrocytes, neurons, oligodendrocytes, endothelial cells, and invading leukocytes in the brain have been reported to express IL-1β as well as IL-1RI even under normal conditions. Although mice lacking IL-1α, IL-1β, and IL-1RI have shown no physiological or developmental defects, abundant IL-1RI expression in the hippocampus indicates a possible physiological role of IL-1β in this area. In accordance with this receptor expression pattern, IL-1β has been shown to be highly involved in synaptic function by modulating NMDA receptor in the hippocampus. Furthermore, it is highly possible that such a high expression level of IL-1RI in the hippocampus may contribute the vulnerability of this area following brain injury or in Alzheimer's patients.

IL-1β is the first proinflammatory cytokine that is released by microglia initially in response to brain injury. The mechanism of how IL-1β is released from cells has been suggested to involve in cleavage of pro-IL-1β by ICE-1 (caspase-1) and activation of purinergic P2X7 receptor by extracellular ATP released from damaged cells (Di Virgilio, 1995; Ferrari et al., 1997). Initial release of IL-1β has

been known to activate other cell types such as astrocytes in a paracrine manner, resulting in inducing the release of IL-1 family, such as IL-1β and IL-1ra, other growth factors such as NGF, and nitric oxide. Thus, balance between neuroprotective and detrimental effectors released by IL-1β may influence secondary cellular effects accordingly.

Extensive evidence has suggested that $p75^{NTR}$ plays an important role in neuronal death occurring after injury or in neurodegenerative diseases. Specifically, our lab has established that $p75^{NTR}$ is involved in neuronal death following seizure, acting as a receptor for proNGF, which is released in response to injury. In this thesis, increased levels of $p75^{NTR}$ were observed following direct infusion of IL-1β into the hippocampus without injury, suggesting increased levels of $p75^{NTR}$ in response to seizure or in disease may be due to the effect of proinflammatory cytokines that are elevated. Consistent with previous studies, direct infusion of IL-1β into the hippocampus of the rat brain increased NGF mRNA levels. Although many studies have shown the IL-1β mediated NGF release from various types of cells, those studies did not clarify the forms of NGF that were produced. Here, IL-1β caused the release of mature NGF, not proNGF which is observed after seizure treatment and in disease. Interestingly, IL-1β increased tPA activity, which activates plasmin, in turn enhancing proNGF processing. This is contrary to what we found previously with seizure-induced injury, where reduced activity of the proNGF processing enzymes, tPA and MMP7, was observed following seizure. Furthermore, IL-1β itself was not sufficient to mediate cell death *in vivo* even with the elevated $p75^{NTR}$ expression. This is consistent with *in vitro* data in which NGF did not activate monomeric $p75^{NTR}$

generated by IL-1β. Compared to injury or in disease, it is likely the presence of NGF rather than proNGF, that fails to elicit $p75^{NTR}$ mediated cell death, such as occurs in the hippocampus after injury or in Alzheimer's disease (Table 1).

Injury to the brain is accompanied by neuroinflammation, which is characterized by activation of microglia, astrocytes, and endothelial cells, and release of cytokines. Microglia have been believed to be the first to be activated and to migrate to damaged tissue. IL-1β is initially released from microglia due in part to the slight changes in ionic balance such as extracellular potassium level following brain insults (Gehrmann et al., 1995) and this initial production of IL-1β may have diverse actions on microglia and other cell types such as astrocytes through an autocrine and paracrine manner. Not only is IL-1β produced as an early event after microglia activation, other types of inflammatory mediators, which can lead to leakage of the blood brain barrier and neuronal hyperexcitability, are released, resulting changes in brain parenchyma. Chronic neuroinflammation has been suggested to be a causative factor for several neurodegenerative diseases. In those diseases, sustained NFκB activation has been reported in astrocytes. Furthermore, this thesis has found sustained $p75^{NTR}$ expression specifically in astrocytes, implying that astrocytes may act as replenishing source for IL-1β throughout the disease development.

Whereas the majority of studies have demonstrated that IL-1β alone did not initiate damage in healthy cells, IL-1β is known to exacerbate

neuronal death with other cytokines or after injury. However, the mechanisms by which IL-1β contributes to neuronal degeneration have been unknown. Here, this study demonstrated that IL-1β may sensitize neurons and make them more vulnerable to proNGF mediated cell death by modifying the $p75^{NTR}$/sortilin complex. Specifically, generation of monomeric $p75^{NTR}$ and recruiting sortilin to cell surface favors proNGF mediated $p75^{NTR}$ activation. Interestingly, this correlates with the environmental changes in Alzheimer's disease where the increased level of IL-1β and proNGF has been reported. A previous study has indicated that the relative proportion of disulfide-linked $p75^{NTR}$ dimers is variable depending on different cell types (Grob et al., 1983; Grob et al., 1985; Vilar et al., 2009). Thus, IL-1β may serve as a key switch altering the ratio of monomeric to dimeric $p75^{NTR}$ following injury or in disease, possibly through receptor modifications; thereby supporting proNGF mediated signaling rather than NGF. Overall, this study suggests that the cellular environment modified following injury or in disease may determine the different ratio of $p75^{NTR}$ species (monomers, dimers, or oligomers), changing various cellular outcomes depending on the presence of distinct ligands with the various combinations of $p75^{NTR}$ coreceptor.

X. Conclusion and Future Directions

The work in this thesis aimed to identify the mechanisms of $p75^{NTR}$ upregulation that were observed under various pathological conditions. $p75^{NTR}$ mediated neuronal death is well established both in development and pathological conditions where extensive $p75^{NTR}$ upregulation is observed.

The data presented in this thesis suggest that IL-1β may exacerbate neuronal death by creating a cellular environment more vulnerable to proNGF mediated neuronal death following injury or in neurodegenerative disease. Neurotrophins have been therapeutic targets in numerous disorders. Specifically,

NGF has been suggested to treat the Alzheimer's disease because cholinergic basal forebrain neurons, which are severely damaged in this disease, are dependent on NGF for their survival. It is therefore crucial to understand how NGF treatment may overcome the monomeric p75 if it is truly found in disease.

Although studies have been pursued to understand the $p75^{NTR}$ activation mechanisms, its complex character with the existence of several ligands and coreceptors may impede clarification of how this receptor is activated. It is clear that $p75^{NTR}$ may have distinct activation mechanisms depending upon different cellular environments as a recent study has demonstrated that NGF requires a conformational change upon binding to dimeric $p75^{NTR}$ in order to signal. However, the work in this thesis showed that proNGF may not necessarily require dimeric $p75^{NTR}$ to induce signaling, since monomeric $p75^{NTR}$, which is not activated by NGF, was able to respond to proNGF. When considering the stoichiometry of proNGF and the $p75^{NTR}$-sortilin complex, in which the mature domain of proNGF binds $p75^{NTR}$ and the pro-region interacts with sortilin, this is more likely the scenario for proNGF mediated $p75^{NTR}$ activation mechanisms. Nonetheless, the possibility of dimeric $p75^{NTR}$ activation by proNGF remains to be further investigated.

As mentioned previously, different cells contain various ratios of $p75^{NTR}$ forms (monomers, dimers, or oligomers). This work suggested that IL-1β may alter the functional outcome of $p75^{NTR}$ by increasing monomeric $p75^{NTR}$, hence favoring proNGF mediated cell death signaling. However, the mechanisms of

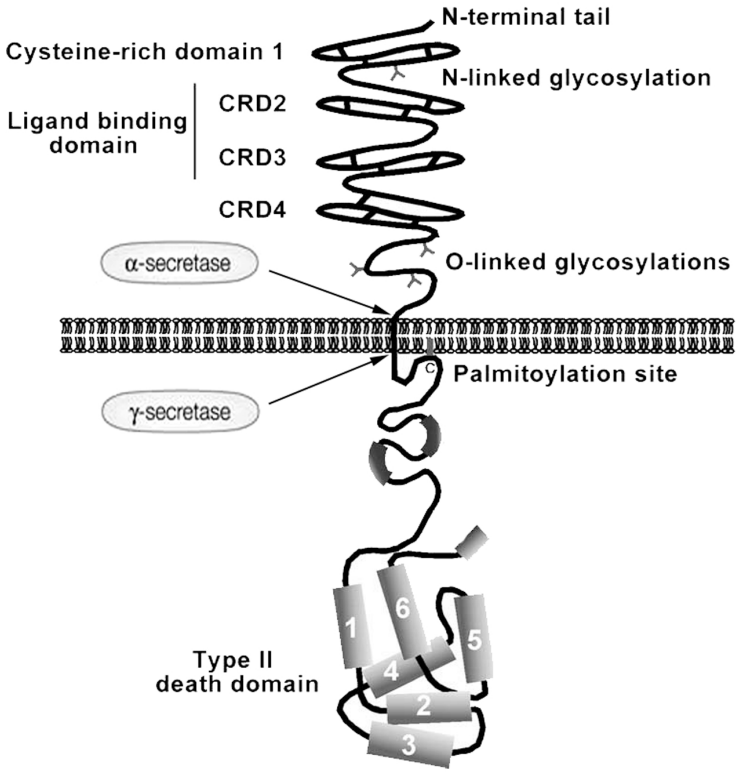
generating monomeric $p75^{NTR}$ remain unknown. Possible mechanisms may include $p75^{NTR}$ modifications such as glycosylation, phosphorylation, or s-nitrosylation at cysteine 257 where the disulfide bond is linked.

Although this study demonstrated that IL-1β may be involved in $p75^{NTR}$ regulation following injury or in disease, the elevated $p75^{NTR}$ by IL-1β was not sufficient to induce cell death in the absence of proNGF, from the results showing that IL-1β infusion directly into the hippocampus *in vivo* induced $p75^{NTR}$ expression but failed to mediate cell death, probably due to the increased activity of tPA, resulting in the increase of NGF, which is in contrast to the injury situation (Figure 22A). It indicates that additional mechanisms may be involved in regulating proNGF processing enzymes, or some novel signals to stabilize proNGF may be engaged following injury or in disease (Figure 22B). It has been demonstrated that phosphodiesterase PDE4A4/5 interaction with $p75^{NTR}$ increases PAI-1 (plasminogen activator inhibitor-1) and decreases tPA following injury (Sachs et al., 2007), suggesting a possible mechanism for the reduced tPA activity found after injury or in disease. Additionally, it has been reported that proNGF isolated from Alzheimer's patients is highly glycosylated and more stable than proNGF from normal individuals, suggesting possible additional modification mechanisms for proNGF.

Our recent studies have demonstrated that $p75^{NTR}$ is also induced in astrocytes and serves to attenuate proliferation of these cells (Cragnolini et al, 2009). Since excessive glial proliferation can lead to scarring and inhibition of the

potential for regeneration, the induction of $p75^{NTR}$ on astrocytes may be beneficial. Thus, it may be therapeutically beneficial to prevent $p75^{NTR}$ induction in hippocampal neurons, to prevent neuronal loss, yet permit $p75^{NTR}$ induction in astrocytes to attenuate glial proliferation. The distinct mechanisms of cytokine-mediated $p75^{NTR}$ regulation that was identified in this study may facilitate the cell type-specific regulation of this receptor, and the functional consequences. Thus, further investigation is required to find the functional role of $p75^{NTR}$ in astrocytes or other cell types in pathological conditions.

Figure 1



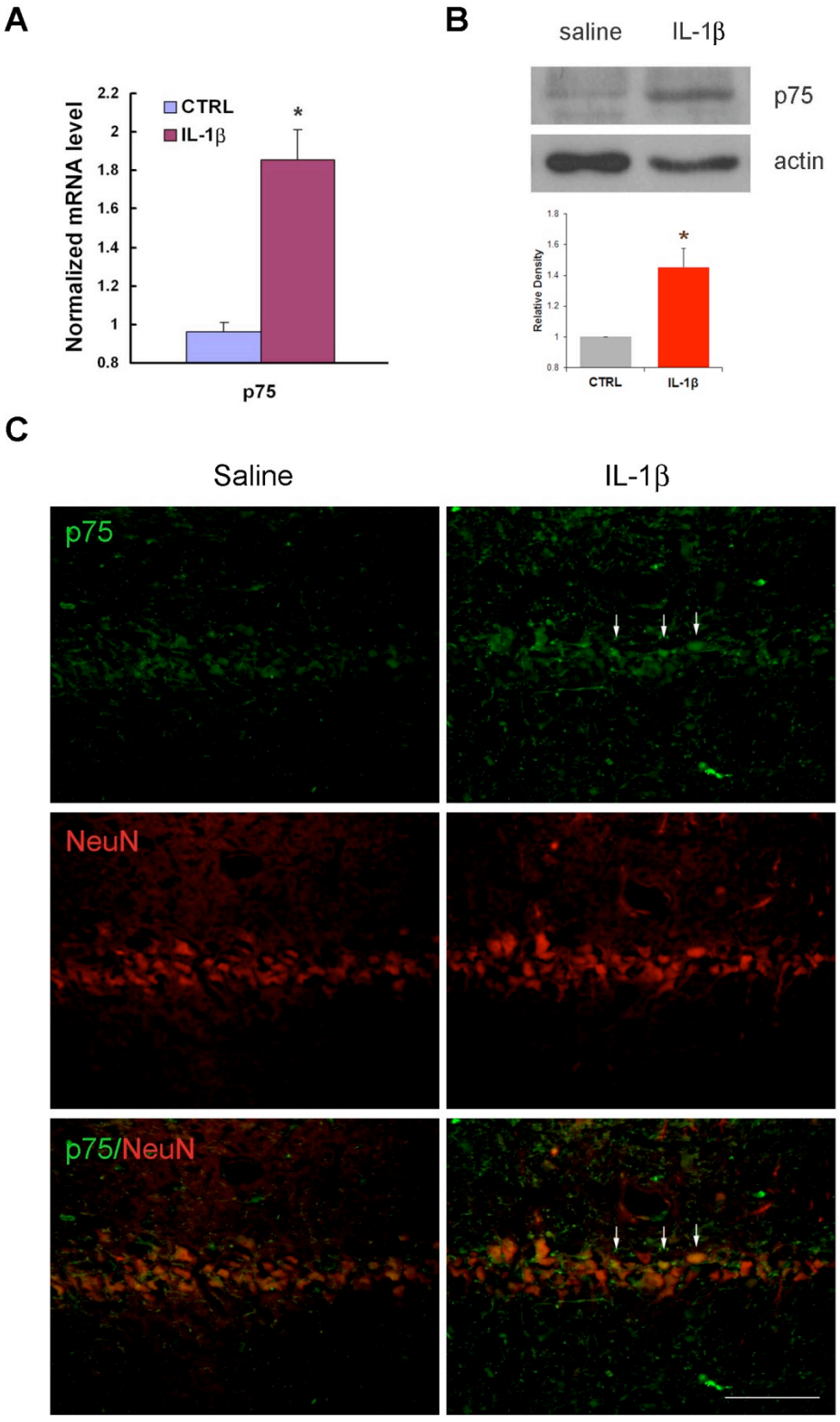
Roux and Barker, 2002

Figure 1. Schematic illustration of the structure of $p75^{NTR}$.

$p75^{NTR}$ consists of an extracellular domain (ECD), a single transmembrane domain and an intracellular domain (ICD). The ECD contains four cysteine rich domains (CRDs), and the ICD has the death domain. There are several modifications in the ECD and ICD of $p75^{NTR}$. Note two cleavage sites by α-secretase and γ-secretase consecutively, generating a signaling C-terminal fragment (CTF).

Adapted from Roux and Barker, 2002

Figure 2


A
Normalized mRNA level
2.2
2
1.8
1.6
1.4
1.2
1
0.8
CTRL
IL-1β
*
p75
B
saline
IL-1β
p75
actin
Relative Density
1.8
1.6
1.4
1.2
1
0.8
*
CTRL
IL-1β
C
Saline
IL-1β
p75
NeuN
p75/NeuN

D

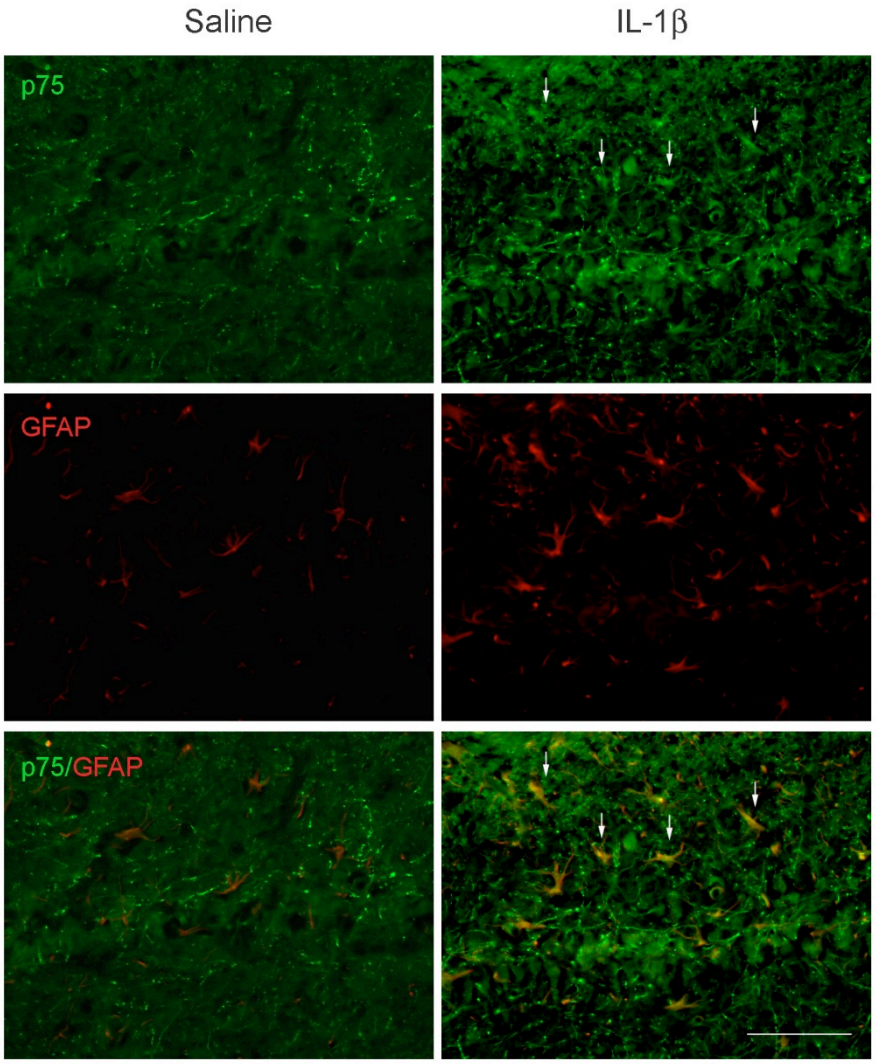


Figure 2. Unilateral IL-1β infusion increases $p75^{NTR}$ expression *in vivo*

Rats were cannulated 7 days before infusion with IL-1β (10ng). A. $p75^{NTR}$ mRNA is induced by IL-1β. Tissue was taken after 4 hr treatment with IL-1β (mean±SEM, n = 3). Asterisk denotes difference from saline ($p<0.05$). B. 2 days after the infusion, each hippocampus was taken for Western blot assay and $p75^{NTR}$ expression was increased with IL-1β infusion. Quantification of blots from three experiments and densitometric values were normalized to actin and are expressed relative to the CTRL. Error bars represent SEM. * $p<0.05$ relative to CTRL, two-tailed t test. C-D. 2 days after the IL-1β infusion, brains were perfused, sectioned through the hippocampus, immunostained with $p75^{NTR}$ (green) antibody and NeuN (red) (C) or GFAP (D). IL-1β infusion increased $p75^{NTR}$ expression (arrows) both in neurons and astrocytes in the CA1 of Hippocampus (right column) compared to saline infusion (left column). Scale bars = 50μm. Images are representative of two independent experiments.

Figure 3

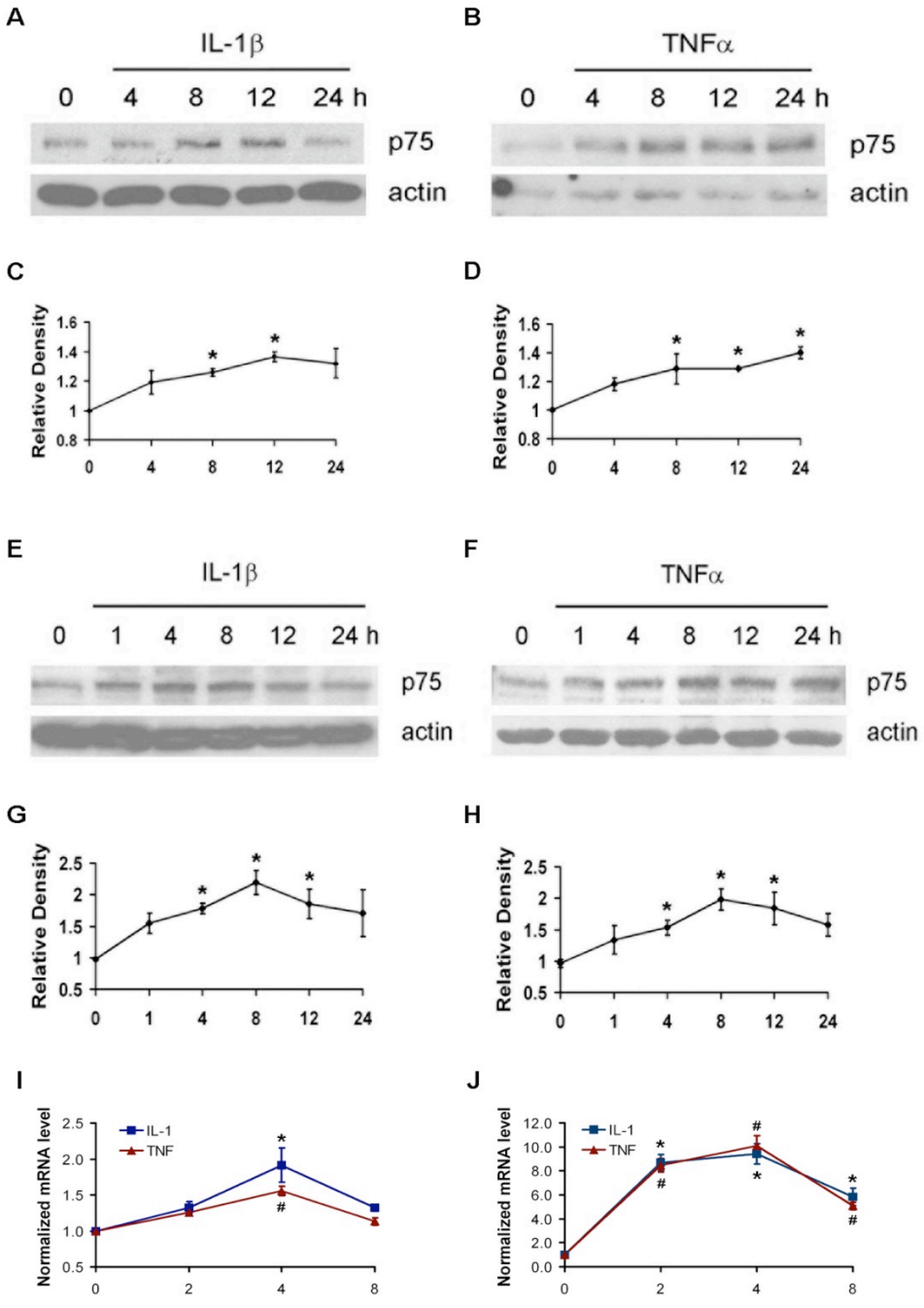

A
IL-1β
0 4 8 12 24 h
p75
actin
B
TNFα
0 4 8 12 24 h
p75
actin
C
Relative Density
D
Relative Density
E
IL-1β
0 1 4 8 12 24 h
p75
actin
F
TNFα
0 1 4 8 12 24 h
p75
actin
G
Relative Density
H
Relative Density
I
Normalized mRNA level
IL-1
TNF
J
Normalized mRNA level
IL-1
TNF

Figure 3. IL-1β and TNFα induce $p75^{NTR}$ in neurons and astrocytes

(A–D) Cultured hippocampal neurons were treated with either (A) IL-1β (10 ng/ml) or (B) TNFα (10 ng/ml) for 4, 8, 12 or 24 h, and were then lysed and analysed by Western blot for $p75^{NTR}$ and actin. (C) and (D) Quantification of blots from three experiments as in (A) and (B) respectively. Densitometric values were normalized to actin and are expressed relative to the untreated cells (time 0).

(E–H) Cultured hippocampal astrocytes were treated with either (E) IL-1β (10 ng/ml) or (F) TNFα (10 ng/ml) for 1, 4, 8, 12 or 24 h, and were then lysed and analysed by Western blot for $p75^{NTR}$ and actin. (G) and (H) Quantification of blots from three experiments as in (E) and (F) respectively. Densitometric values were normalized to actin and are expressed relative to the untreated cells (time 0).

(I and J) Quantitative real-time PCR analysis of $p75^{NTR}$ mRNA in hippocampal neurons (I) or astrocytes (J) treated with IL-1β or TNFα expressed relative to untreated control cultures. The significance was determined by ANOVA with Tukey's post-hoc analysis. Asterisk indicates values significantly different from control at $P < 0.05$.

Figure 4

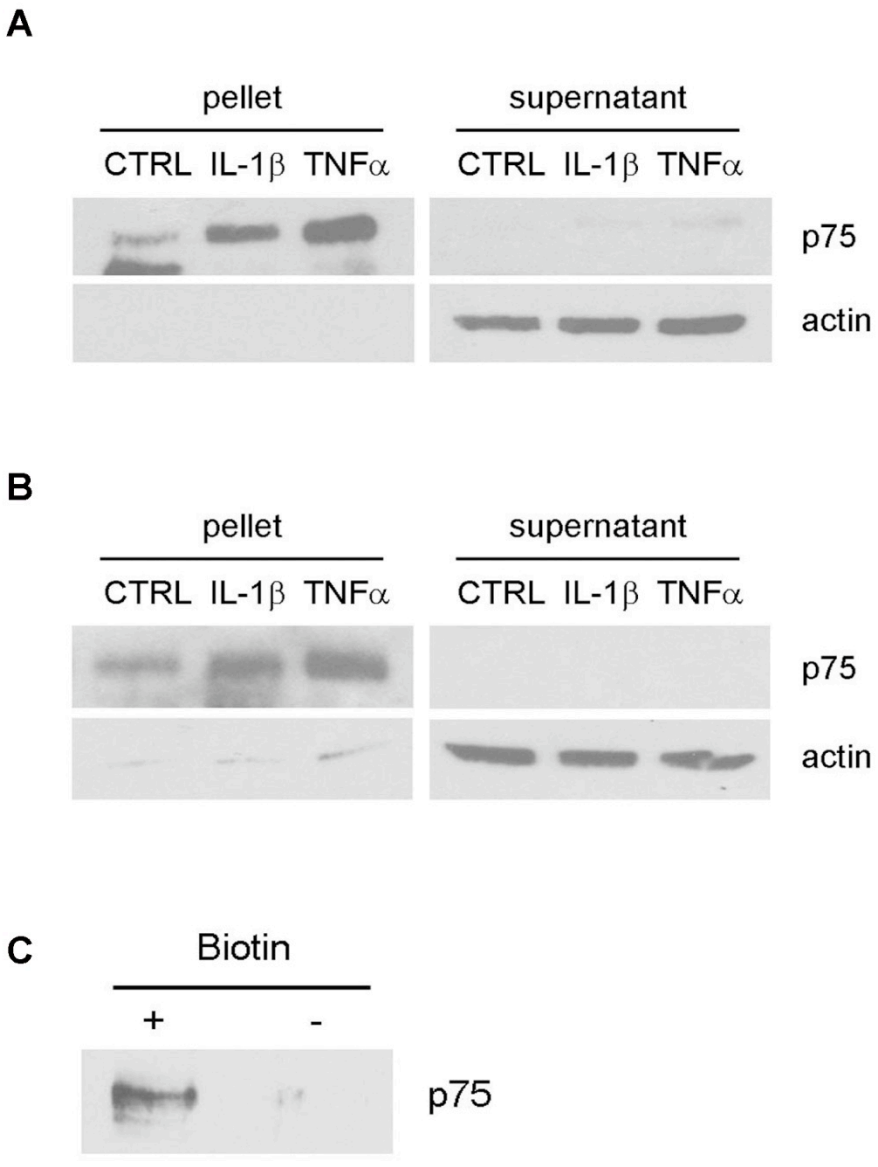

A
pellet
supernatant
CTRL IL-1β TNFα
CTRL IL-1β TNFα
p75
actin
B
pellet
supernatant
CTRL IL-1β TNFα
CTRL IL-1β TNFα
p75
actin
C
Biotin
+
-
p75

Figure 4. Increased $p75^{NTR}$ expression induced by IL-1β and TNFα is present on the cell surface in both neurons and astrocytes.

Cultured hippocampal neurons (A) or astrocytes (B) were treated with IL-1β or TNFα for 8 h, and incubated with biotin for 1 h. Cell lysates were precipitated with streptavidin. Biotinylated cell surface protein and non-biotinylated intracellular proteins were analyzed by Western blotting for $p75^{NTR}$. Blots were stripped and reprobed for actin, which was only present in the intracellular fraction. CTRL, control. Blots are representative of three independent experiments. C. Hippocampal neurons were treated with or without biotin. In the absence of biotin, no $p75^{NTR}$ was detected.

Figure 5

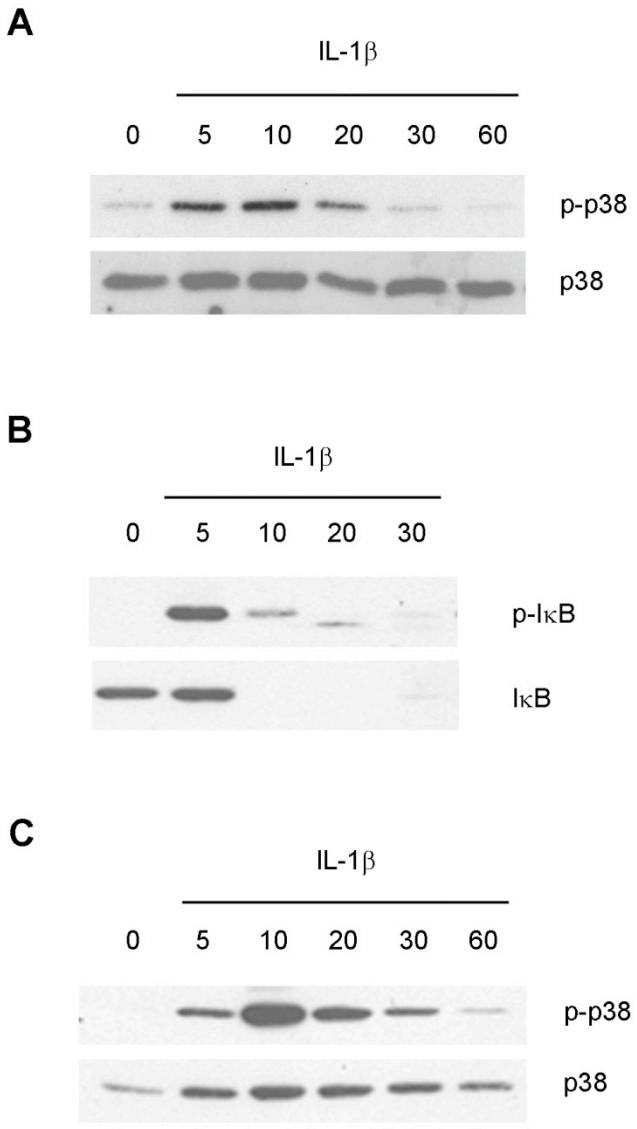

A
IL-1β
0
5
10
20
30
60
p-p38
p38
B
IL-1β
0
5
10
20
30
p-IκB
IκB
C
IL-1β
0
5
10
20
30
60
p-p38
p38

Figure 5. IL-1β activates p38 MAPK in both neurons and astrocytes, and NFκB only in astrocytes.

(A) Western blot analysis of phospho-p38 MAPK activation by IL-1β in neurons. Hippocampal neurons were cultured for 5 days, and treated with 10ng/mL IL-1β for 5, 10, 20, 30 and 60 min and probed for p-p38 MAPK. Blot was stripped and reprobed for total p38 MAPK (bottom). (B) Western blot analysis of phospho-IκB activation by IL-1β in astrocytes. Cultured astrocytes were treated with 10ng/mL IL-1β for 5, 10, 20, and 30 min and probed for phospho-IκB. Blot was stripped and re-probed for total IκB, which was degraded after phosphorylation (bottom). (C) Hippocampal astrocytes were treated with 10ng/mL IL-1β for 5, 10, 20, 30 and 60 min and probed for p-p38 MAPK. Blot was stripped and reprobed for total p38 MAPK (bottom).

Figure 6

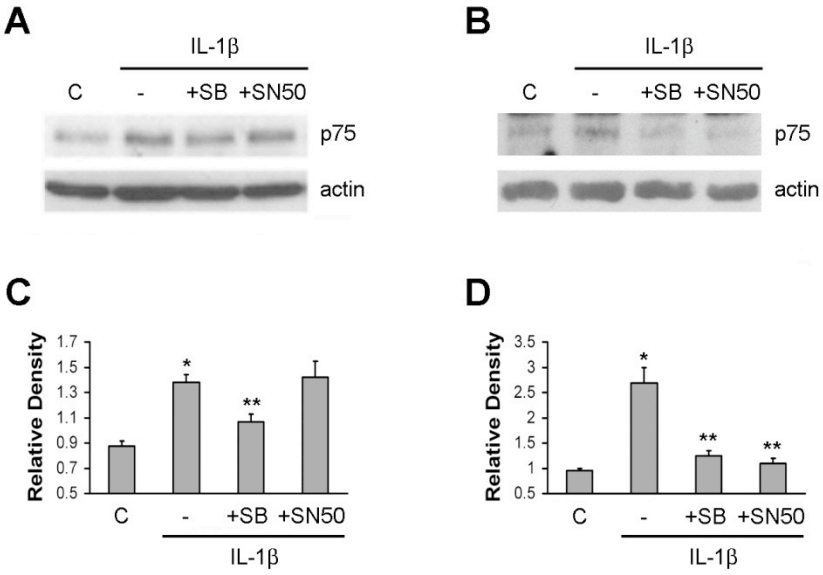

A
IL-1β
C
-
+SB
+SN50
p75
actin
B
IL-1β
C
-
+SB
+SN50
p75
actin
C
Relative Density
1.7
1.5
1.3
1.1
0.9
0.7
0.5
*
**
C
-
+SB
+SN50
IL-1β
D
Relative Density
3.5
3
2.5
2
1.5
1
0.5
*
**
**
C
-
+SB
+SN50
IL-1β

Figure 6. Signaling pathways required for IL-1β induction of $p75^{NTR}$ in hippocampal neurons and astrocytes.

(A) Hippocampal neurons were pretreated with SB203580 (10μM) or SN-50 (10μM) for 30 min and then 10ng/mL IL-1β was added to the culture for 8 hr. Western blot analysis was used to assess $p75^{NTR}$ and actin. (C) Quantification of 3 blots as shown in A. * indicates IL-1β treatment is significantly different from control, ** indicates IL-1β+SB is significantly different from IL-1β alone. (B) Hippocampal astrocytes were pretreated for 30 min with the p38 MAPK inhibitor SB203580 (10μM) or the NFκB inhibitor SN-50 (10μM) prior to IL-1β treatment for 8 hours. Western blot analysis was used to detect the $p75^{NTR}$ and actin. IL-1β induces $p75^{NTR}$ receptor through both the NFκB and p38 MAPK pathways. (D) Quantification of 3 blots as shown in B. * indicates IL-1β treatment is significantly different from control, ** indicates IL-1β+SB is significantly different from IL-1β alone.

Figure 7

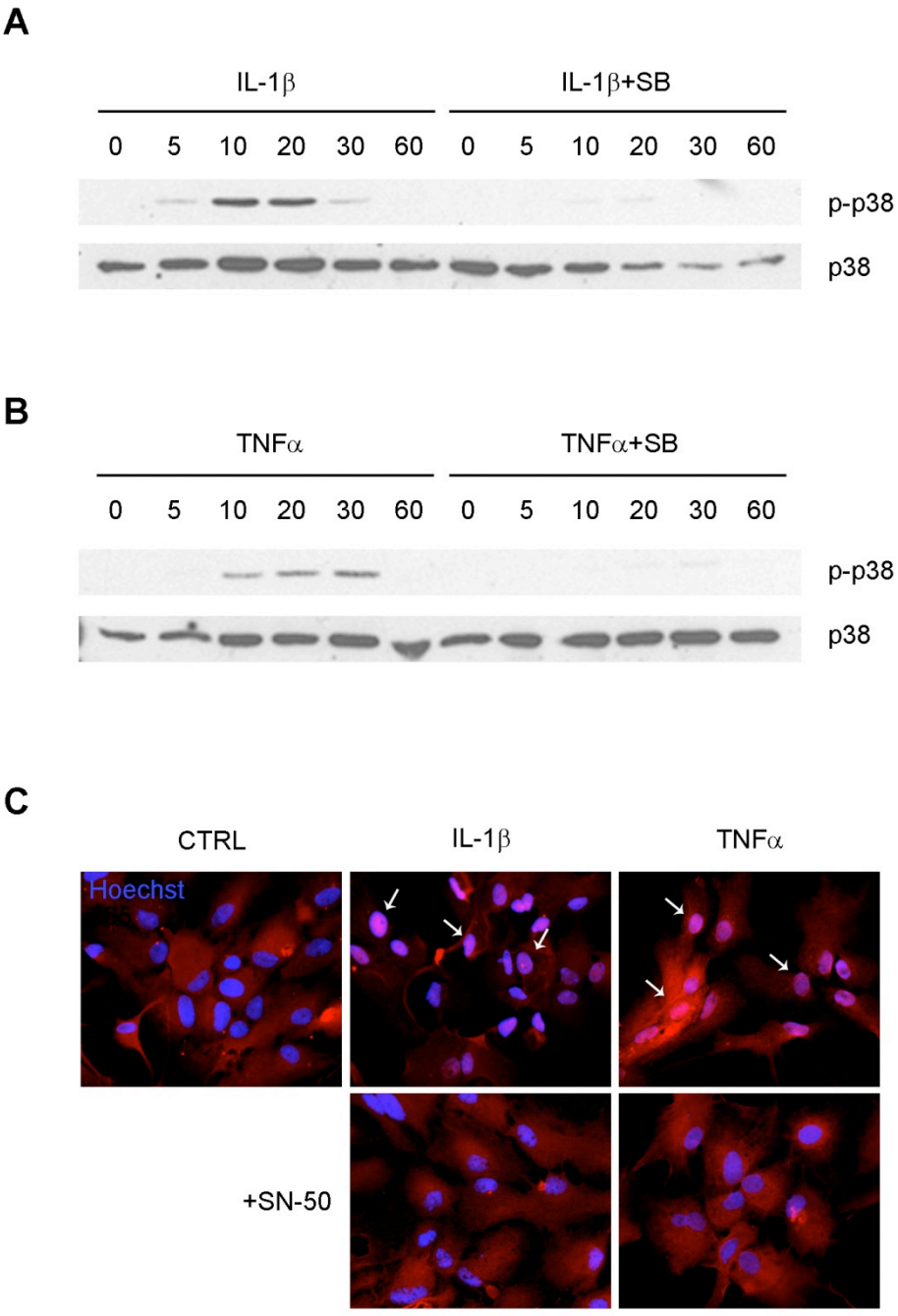

A
IL-1β
IL-1β+SB
0 5 10 20 30 60 0 5 10 20 30 60
p-p38
p38
B
TNFα
TNFα+SB
0 5 10 20 30 60 0 5 10 20 30 60
p-p38
p38
C
CTRL
IL-1β
TNFα
Hoechst
+SN-50

Figure 7. Confirmation that inhibitors of p38 MAPK and NFκB inhibit their respective pathways.

Treatment of hippocampal astrocytes with (A) IL-1β or (B) TNFα in the absence or presence of the p38 MAPK inhibitor showed that SB203580 inhibited p38 MAPK phosphorylation induced by either ligand. (C) To confirm the inhibitory effect of SN-50 on NFκB nuclear translocation, astrocytes were pretreated with vehicle or 10 μM SN-50 for 30 min and then treated with IL-1β or TNFα for 30 min. Cells were fixed and stained for p65, and nuclei were labeled with Hoechst. Nuclear staining of p65 was observed in IL-1β or TNFα treated astrocytes without SN50 (arrows, note pink color of the nuclei) and nuclear translocation of p65 was blocked by SN-50 (bottom).

Figure 8

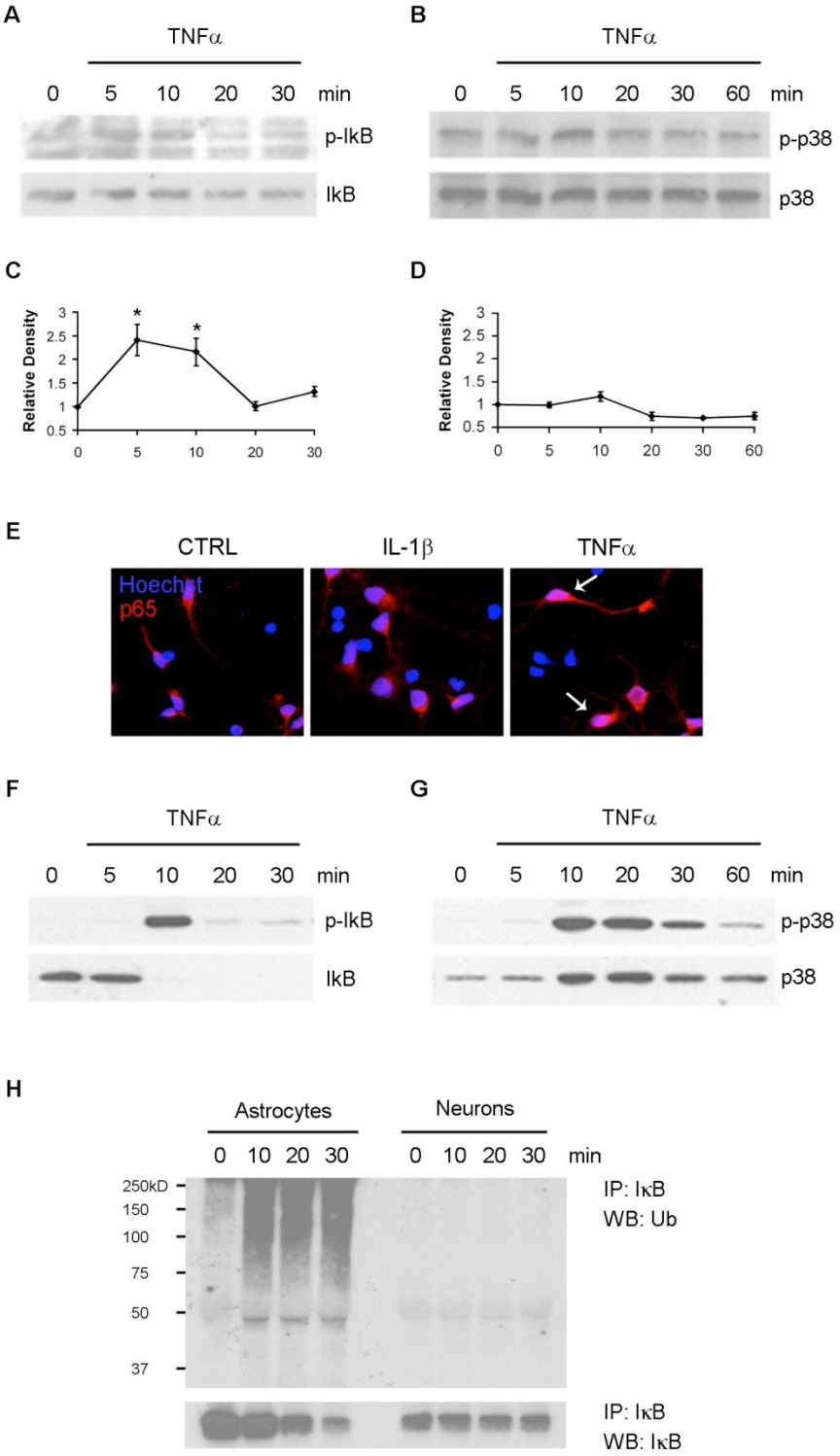

A
TNFα
0 5 10 20 30 min
p-IkB
IkB
B
TNFα
0 5 10 20 30 60 min
p-p38
p38
C
Relative Density
3
2.5
2
1.5
1
0.5
0 5 10 20 30
*
*
D
Relative Density
3
2.5
2
1.5
1
0.5
0 5 10 20 30 60
E
CTRL
IL-1β
TNFα
Hoechst
p65
F
TNFα
0 5 10 20 30 min
p-IkB
IkB
G
TNFα
0 5 10 20 30 60 min
p-p38
p38
H
Astrocytes
Neurons
0 10 20 30 0 10 20 30 min
250kD
150
100
75
50
37
IP: IκB
WB: Ub
IP: IκB
WB: IκB

Figure 8. TNFα phosphorylates IκB in neurons, and both IκB and p38 MAPK in astrocytes

(A) Hippocampal neurons were cultured for 5 days and treated with TNFα for the indicated times. Lysates were probed for P-IκB, stripped and re-probed for total IκB. Note the lack of degradation of IκB. (B) Neuronal lysates were probed for P-p38 MAPK, stripped and re-probed for total p38 MAPK. (C) Quantification of 3 blots as shown in (A). Densitometric values were normalized to IκB and are expressed relative to the untreated cells (time 0). TNFα treatment significantly induced P-IκB at 5 and 10 min, $p<0.05$. (D) Quantification of 3 blots as shown in (B). Densitometric values were normalized to p38 and are expressed relative to the untreated cells (time 0). TNFα treatment did not induce phosphorylation of p38 MAPK in hippocampal neurons. (E) Neurons were treated with IL-1β or TNFα for 30 min, fixed and immunostained for p65. Nuclei were identified with Hoechst labeling. Note the presence of nuclear p65 in TNFα but not IL-1β treated neurons. (F) Cultured astrocytes were treated with TNFα for the indicated times. Astrocyte lysates were probed for P-IκB, stripped and re-probed for total IκB. (G) Astrocyte lysates were probed for P-p38 MAPK, stripped and re-probed for total p38 MAPK. (H) Analysis of ubiquitinated IκB in astrocytes and neurons. Cells were treated with TNFα as indicated. Cell lysates were immunoprecipitated with anti-IκB and probed for ubiquitin. Blots were stripped and re-probed for IκB. Note that levels of total IκB decreased over time in the astrocytes, but not in the neurons.

Figure 9

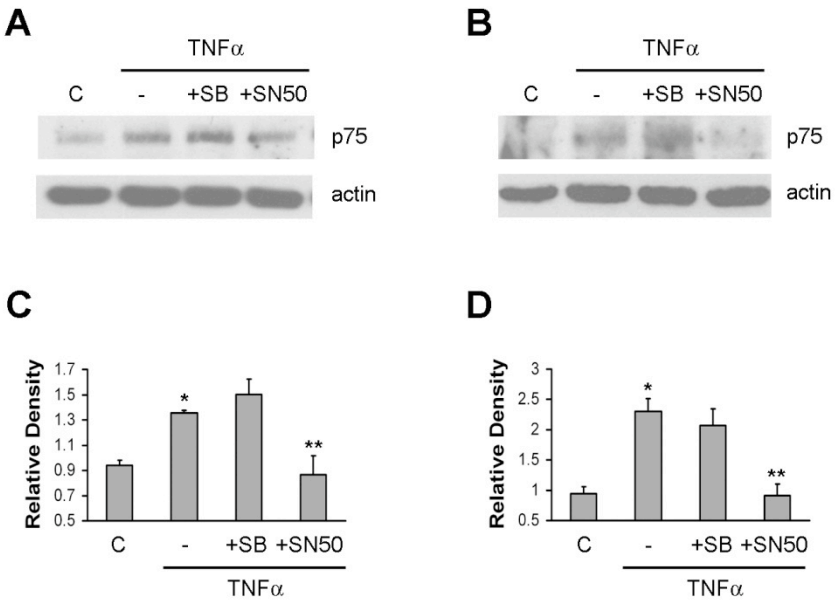

A
TNFα
C
-
+SB
+SN50
p75
actin
B
TNFα
C
-
+SB
+SN50
p75
actin
C
Relative Density
1.7
1.5
1.3
1.1
0.9
0.7
0.5
*
**
C
-
+SB
+SN50
TNFα
D
Relative Density
3
2.5
2
1.5
1
0.5
*
**
C
-
+SB
+SN50
TNFα

Figure 9. TNFα induces $p75^{NTR}$ via NFkB in both neurons and astrocytes.

(A) Hippocampal neurons were pretreated with SB203580 (10 μM) or SN-50 (10 μM) for 30 min and then 10 ng/ml TNFα was provided for 8 h. Western blot analysis was used to assess $p75^{NTR}$ and actin levels. (C) Quantification of three blots as shown in (A). * indicates levels are significantly different from control, $P<0.05$; ** indicates TNFα+SN-50 is significantly different from TNFα alone, $P<0.05$. (B) Hippocampal astrocytes were pretreated with SB203580 (10 μM) or SN-50 (10 μM) for 30 min and then 10 ng/ml TNFα was added to the culture for 8 h. Western blot analysis was used to assess $p75^{NTR}$ and actin. (D) Quantification of three blots as shown in (B). * indicates levels are significantly different from control, $P<0.05$; ** indicates TNFα+SN-50 is significantly different from TNFα alone, $P<0.05$. SB, SB203580. C, control.

Figure 10

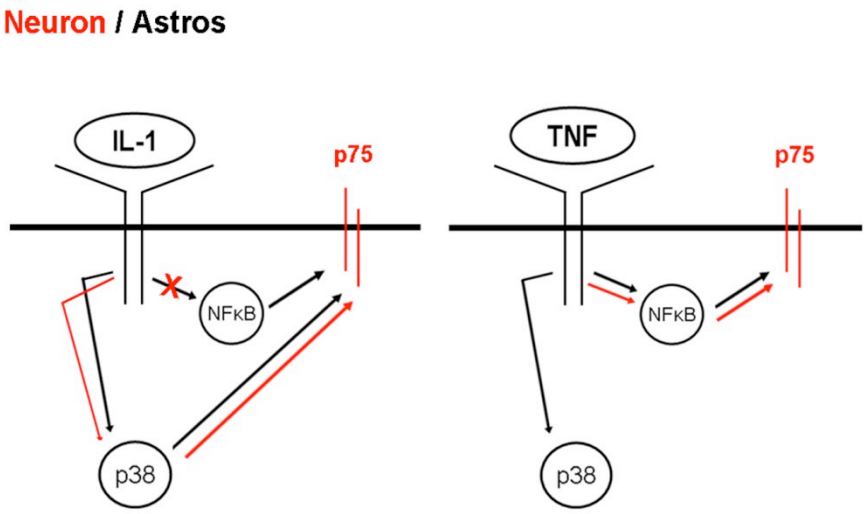
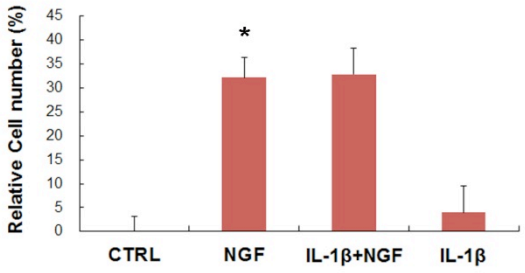


Figure 10. Schematic diagram showing the signaling pathways activated by IL-1β and TNFα in neurons (red) and astrocytes (black) leading to induction of $p75^{NTR}$.

Figure 11

A


Relative Cell number (%)
45
40
35
30
25
20
15
10
5
0
*
CTRL
NGF
IL-1β+NGF
IL-1β

B

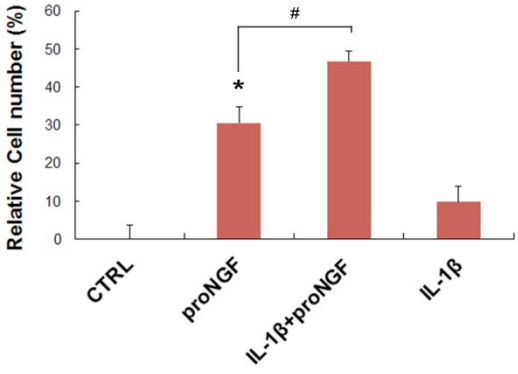

Relative Cell number (%)
60
50
40
30
20
10
0
#
*
CTRL
proNGF
IL-1β+proNGF
IL-1β

Figure 11. IL-1β primed neurons are more vulnerable to proNGF than NGF

A. Bars show percent cell death (mean±SEM, n = 4) in hippocampal neuronal cultures. Cells were treated for 4-6 h with IL-1β (10ng/mL), followed by overnight treatment with NGF (100ng/mL). IL-1β didn't exacerbate NGF-mediated neuronal death. Asterisks denote difference from untreated control ($p < 0.05$). B. Bars indicate percent cell death (mean±SEM, n = 4) in hippocampal neuronal cultures treated with IL-1β (10 ng/mL) for 4-6 hrs, followed by proNGF (1-10ng/mL). ProNGF elicited more cell death in IL-1β primed neurons. Asterisk denotes difference from no treatment and a dagger indicates difference from proNGF ($p<0.05$; one-way ANOVA and Tukey's post hoc analysis).

Figure 12

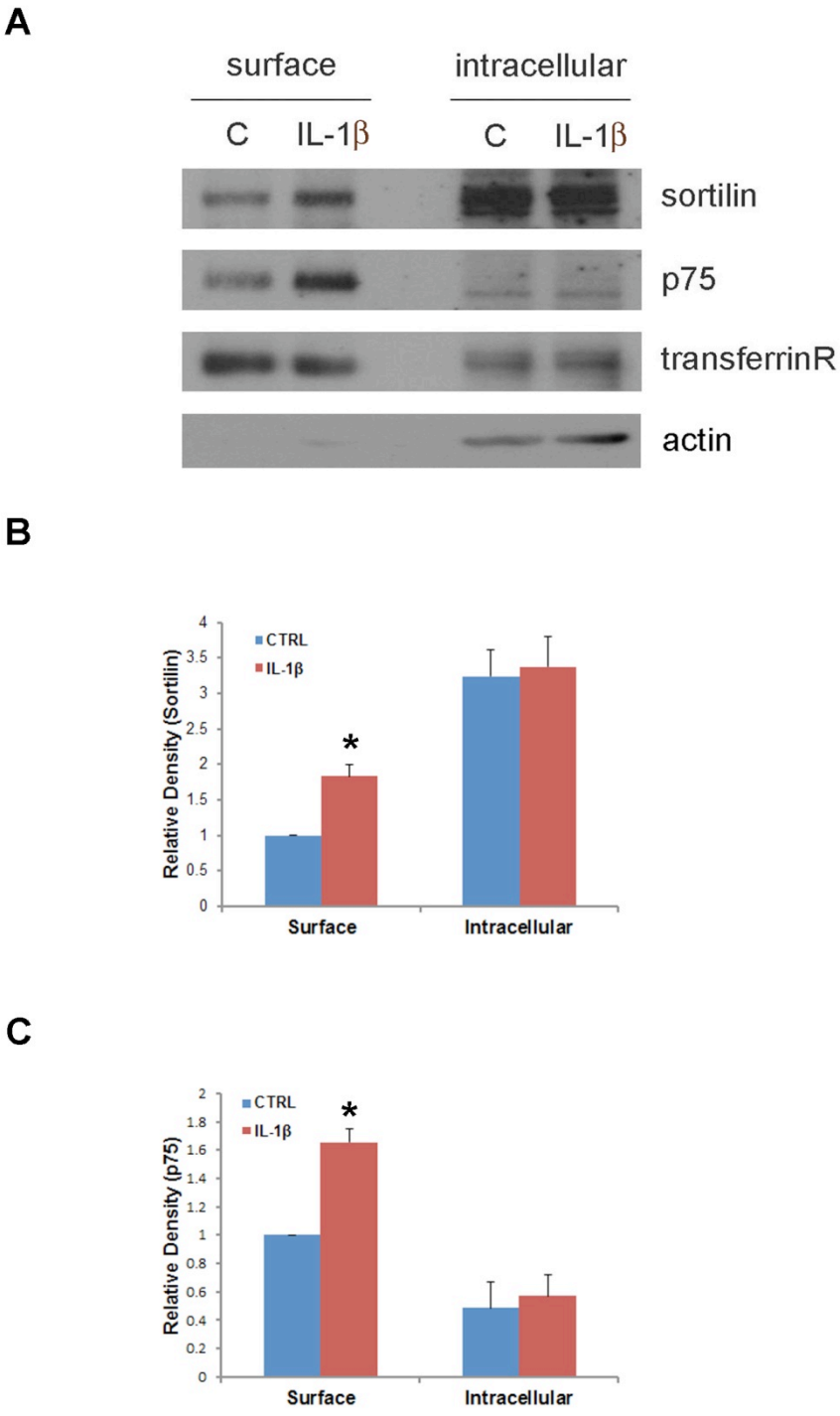

A
surface
intracellular
C
IL-1β
C
IL-1β
sortilin
p75
transferrinR
actin
B
CTRL
IL-1β
Relative Density (Sortilin)
*
Surface
Intracellular
C
CTRL
IL-1β
Relative Density (p75)
*
Surface
Intracellular

Figure 12. IL-1β recruits sortilin receptors to the plasma membrane as well as $p75^{NTR}$.

A. Cultured hippocampal neurons were treated with IL-1β for 8h, and incubated with biotin for 1h. Cell lysates were precipitated with streptoavidin. Biotinylated cell surface protein and nonbiotinylated intracellular proteins were analyzed by Western blotting for sortilin, $p75^{NTR}$ and transferrin receptor. Blots were stripped and re-probed for actin, which was only present in the intracellular fraction. B (sortilin) and C ($p75^{NTR}$) show quantification from three blots. Asterisk denotes difference from no treatment ($p<0.05$; one-way ANOVA and Tukey's post hoc analysis).

Figure 13

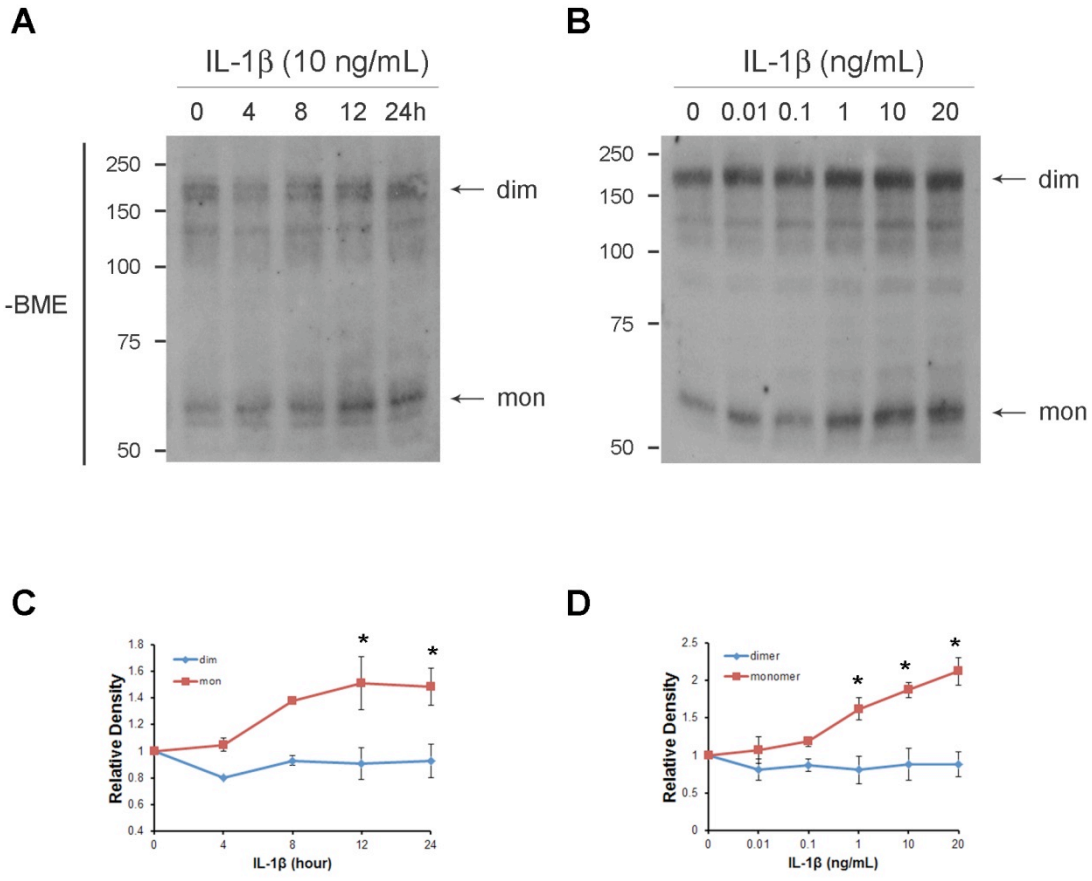

A
IL-1β (10 ng/mL)
0 4 8 12 24h
-BME
250
150
100
75
50
dim
mon
B
IL-1β (ng/mL)
0 0.01 0.1 1 10 20
250
150
100
75
50
dim
mon
C
Relative Density
dim
mon
IL-1β (hour)
D
Relative Density
dimer
monomer
IL-1β (ng/mL)

Figure 13. IL-1β increases monomeric $p75^{NTR}$

A. Cultured hippocampal neurons were treated with IL-1β (10ng/mL) for 4, 8, 12, 24 hr as indicated, then lysed and analyzed for $p75^{NTR}$ in a non-reducing gel. $p75^{NTR}$ dimers and monomers were detected. Levels of monomer were increased while dimers were remained the same following IL-1β treatment. C. Quantification of blots from three experiments (A). Densitometric values were expressed relative to the untreated cells (time 0). The significance was determined by ANOVA with Tukey's post-hoc analysis. * indicates values significantly different from time 0 at $P<0.05$. B. Cultured hippocampal neurons were treated with IL-1β for 12 hr with different doses of IL-1β, then lysed and analyzed for $p75^{NTR}$ in non-reducing gel. Levels of monomer were gradually increased from 1 ng/mL of IL-1β. D. Quantification of blots from three experiments. Densitometric values were expressed relative to the untreated cells. The significance was determined by ANOVA with Tukey's post-hoc analysis. * indicates values significantly different from control at $P<0.05$.

Figure 14

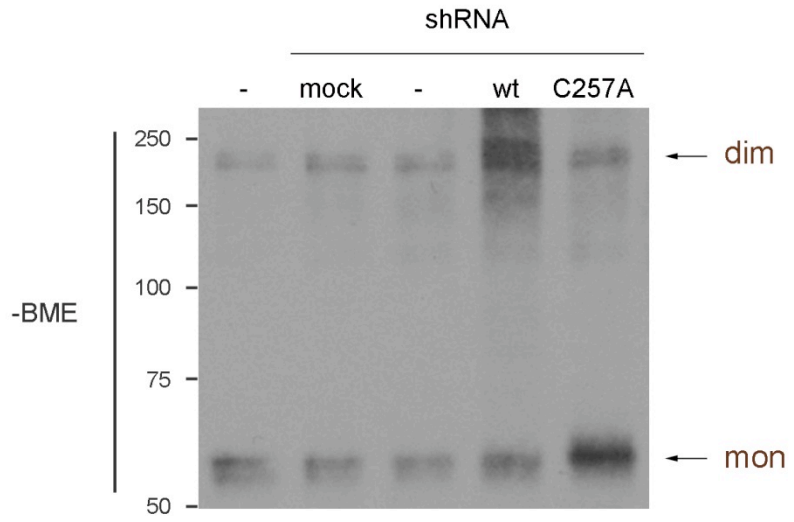


Figure 14. Characterization of $p75^{NTR}$ mutants in hippocampal neurons.

Western blot from a non-reducing gel shows distinct $p75^{NTR}$ constructs transfected in hippocampal neurons. Both dimeric and monomeric forms were observed with wt-$p75^{NTR}$. Mutation of cysteine 257 (C257A) formed only monomers, confirming loss of disulfide bond.

Figure 15

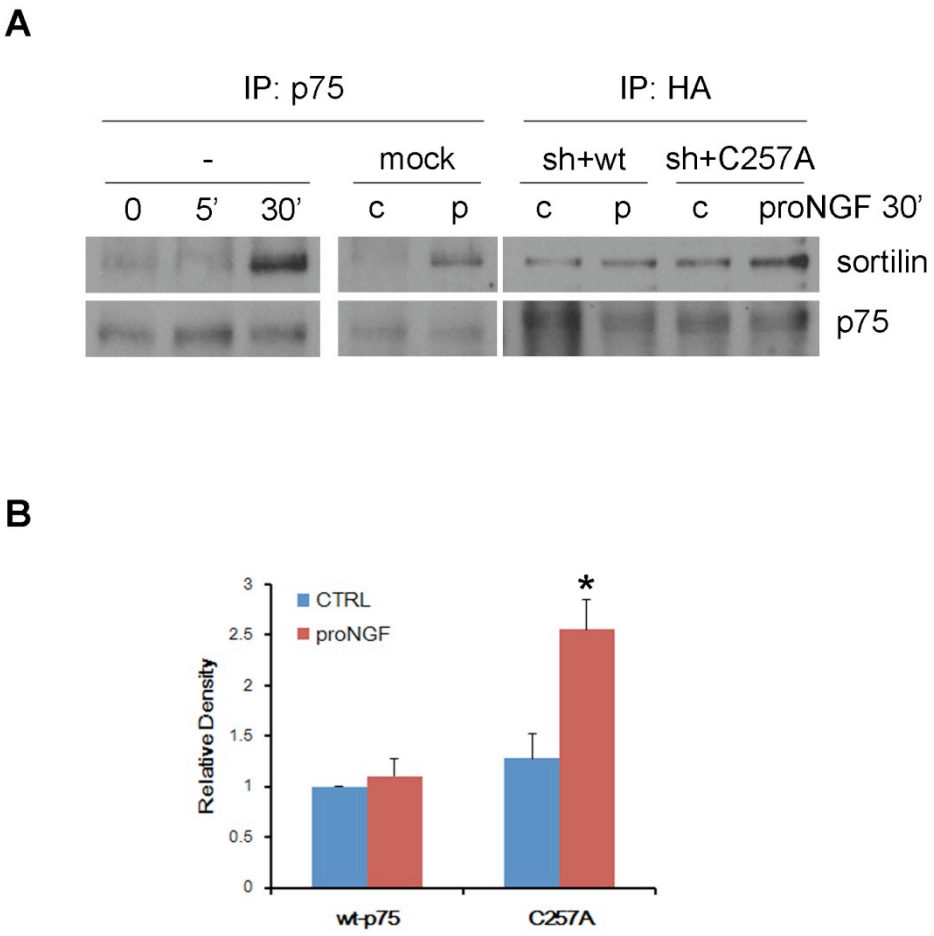

A
IP: p75
IP: HA
-
mock
sh+wt
sh+C257A
0
5'
30'
c
p
c
p
c
proNGF 30'
sortilin
p75
B
CTRL
proNGF
Relative Density
3
2.5
2
1.5
1
0.5
0
*
wt-p75
C257A

Figure 15. proNGF increases the association between sortilin receptor and C257A $p75^{NTR}$ mutant.

A. Transfected cells were treated with proNGF for 30 mins. Protein lysates were immunoprecipitated with either anti-$p75^{NTR}$ or anti-HA and immunoblotted for sortilin and $p75^{NTR}$. Elevated association between $p75^{NTR}$-C257A and sortilin were observed. B. Densitometric values were expressed relative to the untreated cells, showing quantification from three blots. The significance was determined by ANOVA with Tukey's post-hoc analysis. * indicates values significantly different from control at $P<0.05$.

Figure 16

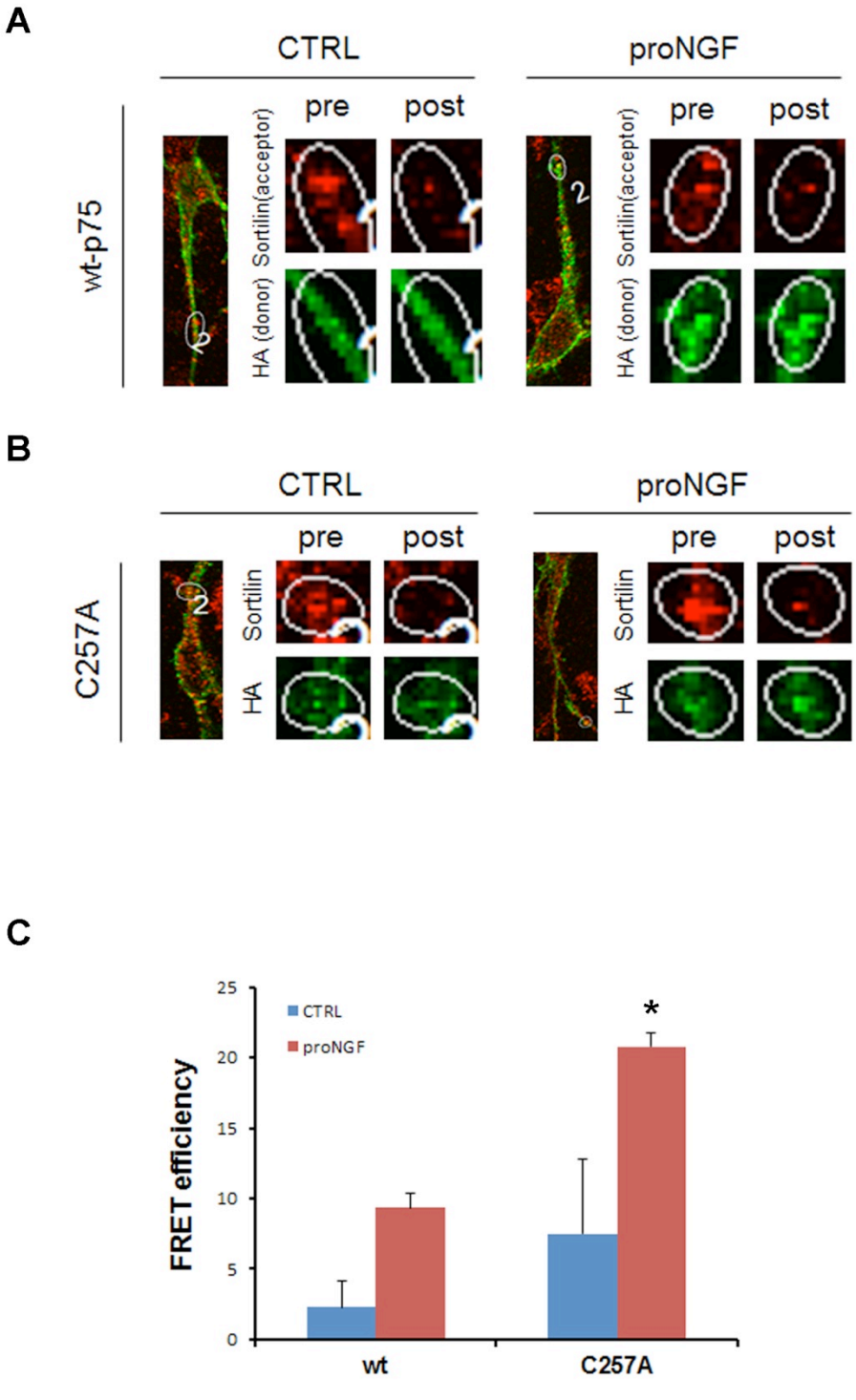

A
CTRL
proNGF
pre
post
wt-p75
Sortilin(acceptor)
HA (donor)
B
C257A
Sortilin
HA
C
FRET efficiency
CTRL
proNGF
wt
C257A
*

Figure 16. FRET analysis of the association between sortilin receptor and $p75^{NTR}$ mutant upon proNGF treatment.

Representative example of sortilin-p75NTR association in hippocampal neurons transfected with (A) wt-p75, and (B) C257A-p75NTR; images were acquired before and after bleaching the acceptor within ROI (white circle). C. Bars show quantification of number of cells that showed positive FRET events between HA tagged $p75^{NTR}$ constructs and sortilin using acceptor photobleaching FRET assay (mean±SEM, n = 2). Transfected cells were treated with pro-NGF for 30 min, fixed, and stained with anti-sortilin and anti-HA. proNGF increased the association between $p75^{NTR}$-C257A and sortilin. Asterisk indicates difference from sh +wt-p75 CTRL.

Figure 17

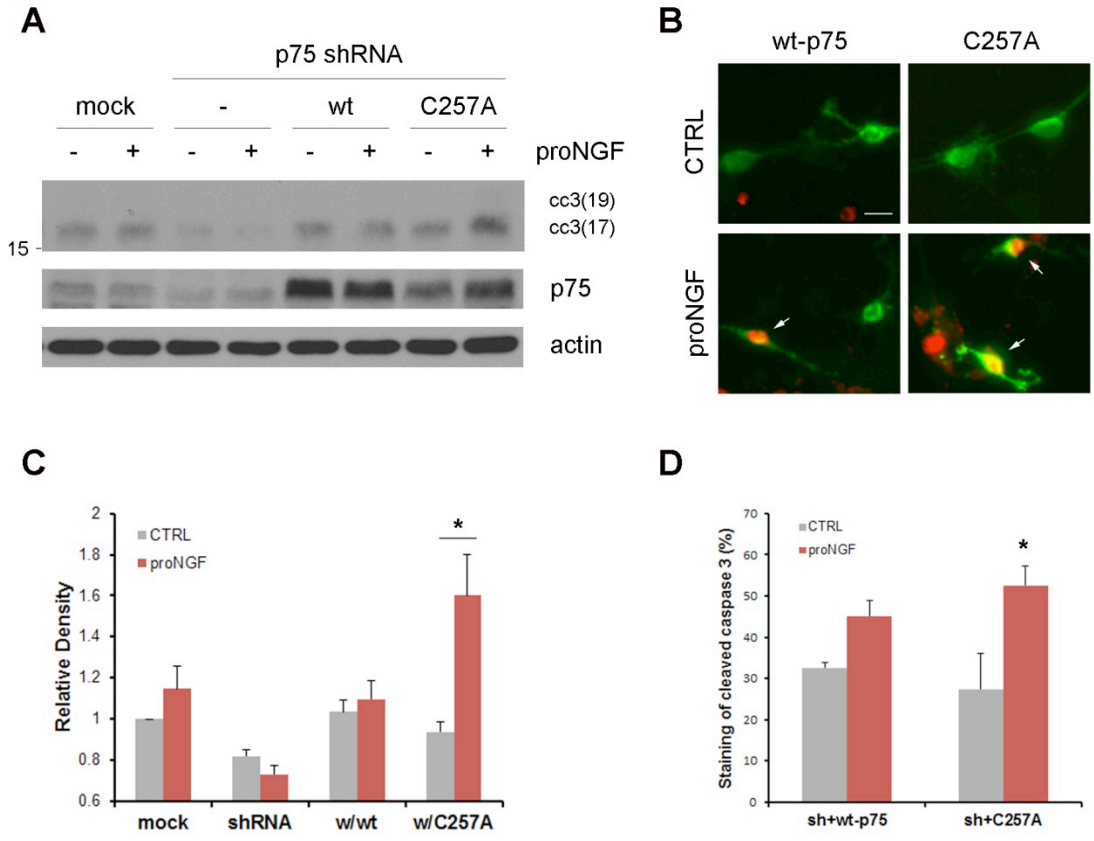

A
p75 shRNA
mock
-
wt
C257A
- + - + - + - +
proNGF
cc3(19)
cc3(17)
15
p75
actin
B
wt-p75
C257A
CTRL
proNGF
C
CTRL
proNGF
Relative Density
2
1.8
1.6
1.4
1.2
1
0.8
0.6
*
mock
shRNA
w/wt
w/C257A
D
CTRL
proNGF
Staining of cleaved caspase 3 (%)
70
60
50
40
30
20
10
0
*
sh+wt-p75
sh+C257A

Figure 17. proNGF activates C257A $p75^{NTR}$ mutant

A. Western blots for cleaved caspase-3, $p75^{NTR}$, and actin of samples prepared from transfected cells with wt or C257A $p75^{NTR}$ constructs. More cleaved caspase-3 was detected in C257A-$p75^{NTR}$ after proNGF treatment. C. Quantification of blots from three experiments. Densitometric values were expressed relative to the untreated cells. The significance was determined by ANOVA with Tukey's post-hoc analysis. * indicates values significantly different from control at $P<0.05$. B. Hippocampal neurons were transfected with HA tagged wt-$p75^{NTR}$ or C257A. Cells were assessed for anti-cc3 staining (red) to see cell death and for anti-HA staining (green) to visualize the transfected $p75^{NTR}$ constructs 48 hour after transfection. $p75^{NTR}$-shRNA was cotransfected with all the constructs. Arrows indicate double labeled cells. Scale bars = 10μm. D. Quantification of positive cleaved caspase-3 staining of transfected cells from B (mean±SEM, n = 4). Asterisk indicates difference from wt-$p75^{NTR}$ CTRL ($p<0.05$; one-way ANOVA and Tukey's post hoc analysis).

Figure 18

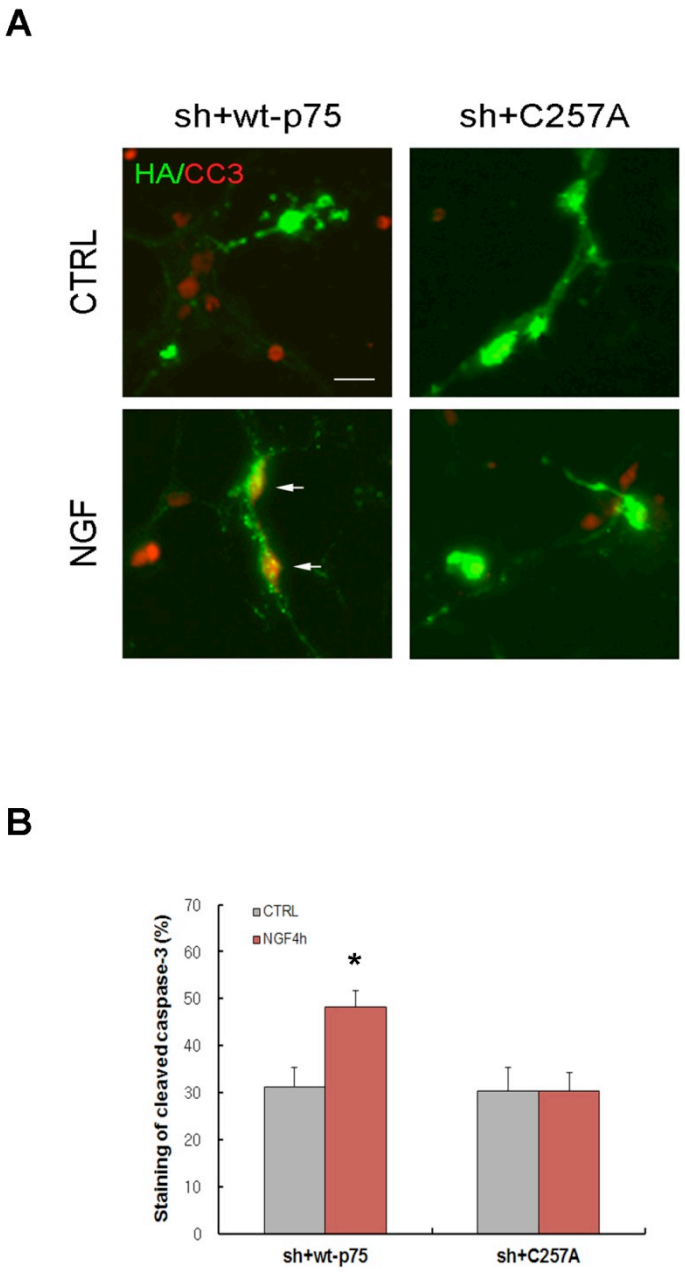

A
sh+wt-p75
sh+C257A
HA/CC3
CTRL
NGF
B
Staining of cleaved caspase-3 (%)
CTRL
NGF4h
*
sh+wt-p75
sh+C257A

Figure 18. C257A mutant $p75^{NTR}$ is not responsive to NGF

Cultured hippocampal neurons were transfected with HA tagged wt-$p75^{NTR}$ or C257A. Cells were assessed for anti-cleaved caspase-3 (cc3) staining (red) to see cell death and for anti-HA staining (green) to visualize the transfected $p75^{NTR}$ constructs 48 hour after transfection. $p75^{NTR}$-shRNA was cotransfected with all the constructs to knockdown endogenous $p75^{NTR}$. Arrows indicate double labeled cells. Scale bars = 10μm. B. Quantification of CC3-positive transfected cells from B (mean±SEM, n = 4). Note no responsive effect of NGF in C257A. Asterisk indicates difference from CTRL in wt-$p75^{NTR}$ ($p<0.05$; one-way ANOVA and Tukey's post hoc analysis).

Figure 19

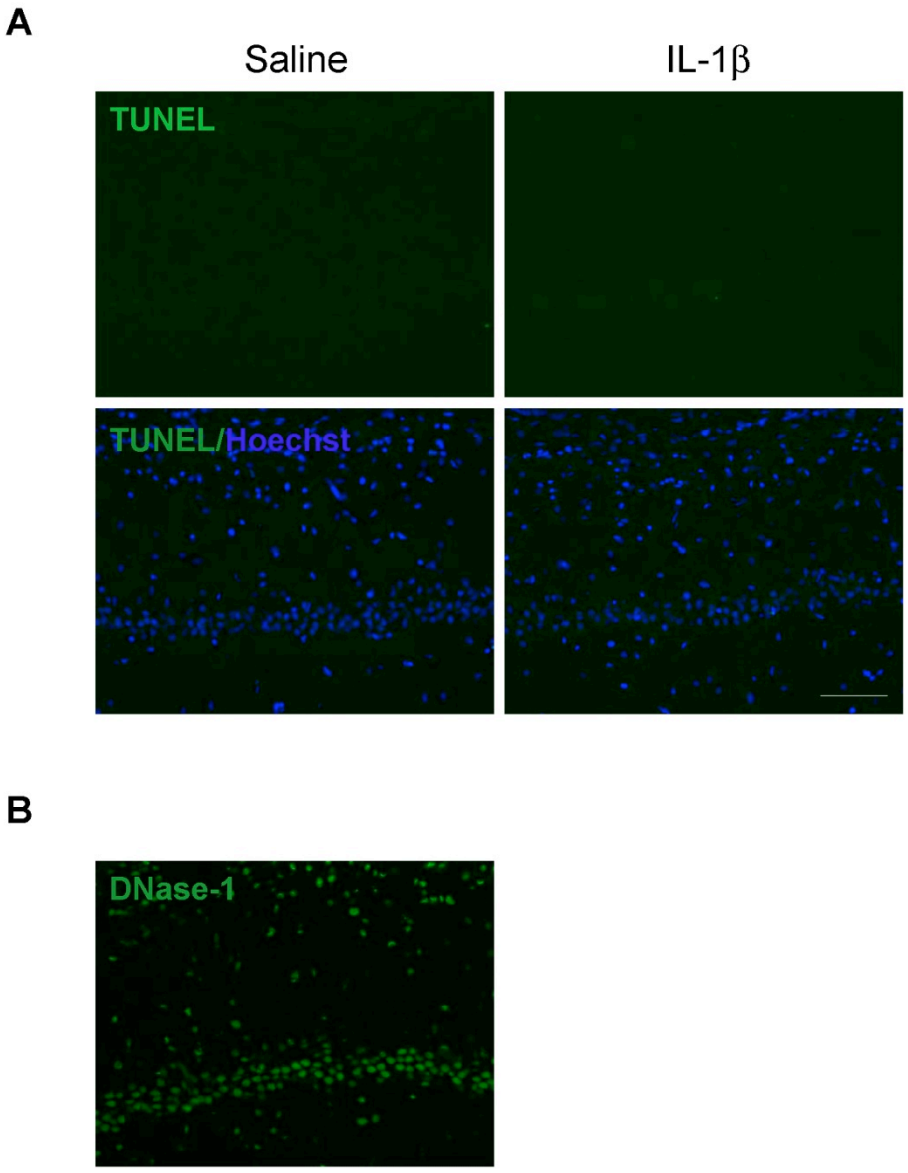


Figure 19. IL-1β is not sufficient to mediate cell death *in vivo*

A. TUNEL assay showed negative staining with both saline (Left column) and IL-1β (Right column) infusion, suggesting that IL-1β itself does not directly elicit cell death. Double staining for TUNEL (green) and Hoechst (blue). Images are representative of two independent experiments. B. Positive control TUNEL assay with DNase-I. Scale bars = 50μm.

Figure 20

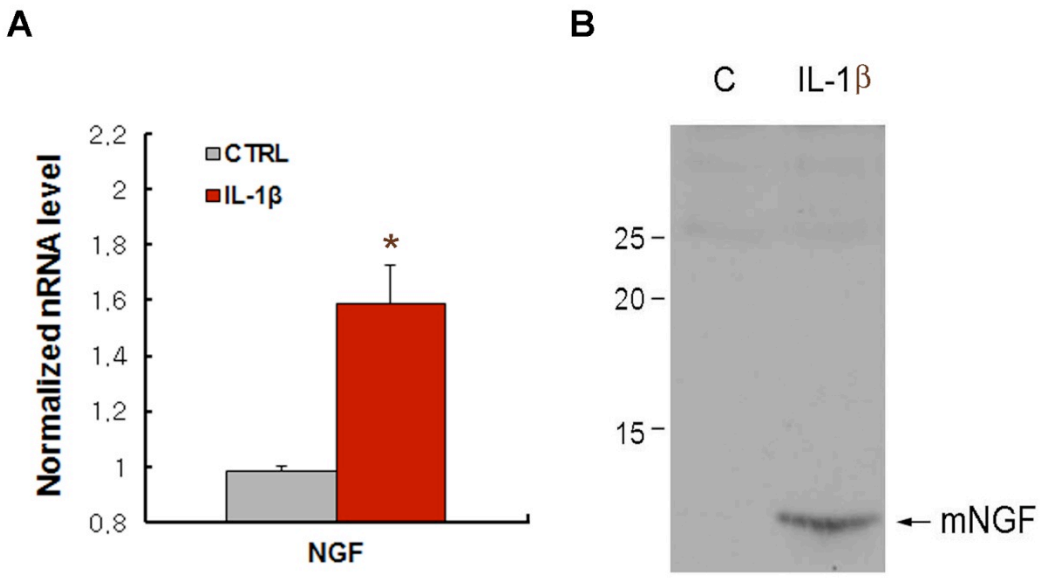

A
B
C
IL-1β
Normalized nRNA level
2,2
2
1,8
1,6
1,4
1,2
1
0,8
CTRL
IL-1β
*
NGF
25
20
15
mNGF

Figure 20. IL-1β release NGF into the CSF

A. Bars indicates NGF mRNA level (mean±SEM, n = 3) by qPCR after 4 h treatment with IL-1β. Asterisk denotes difference from saline ($p<0.05$). B. Western blots show that mature NGF was detected in the Cerebrospinal fluid (CSF) collected from IL-1β infused rats. Blot is representative of three independent experiments.

Figure 21

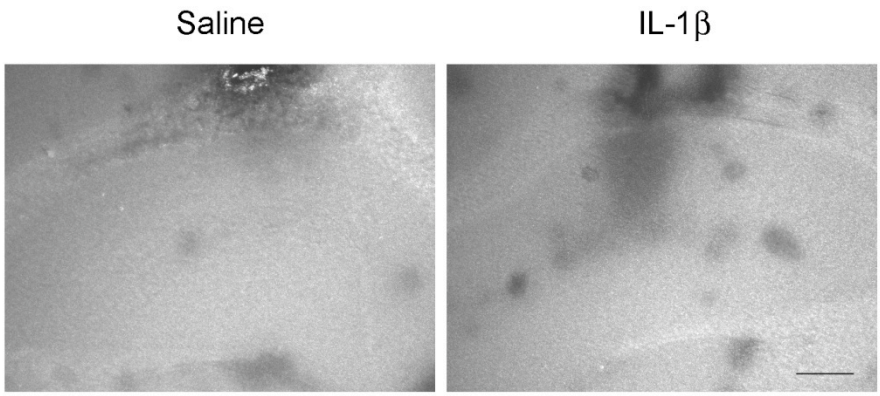


Figure 21. IL-1β increases tPA activity

In situ zymogram showed increased tPA activity with IL-1β infusion. tPA activity was increased in response to IL-1β infusion. Fresh frozen sections were covered with an in situ zymogram assay buffer containing 20 μg/mL plasminogen. Areas of tPA activity was detected in block spots on dark field of views. Images are representative of two independent experiments. Scale bars = 250μm

Figure 22

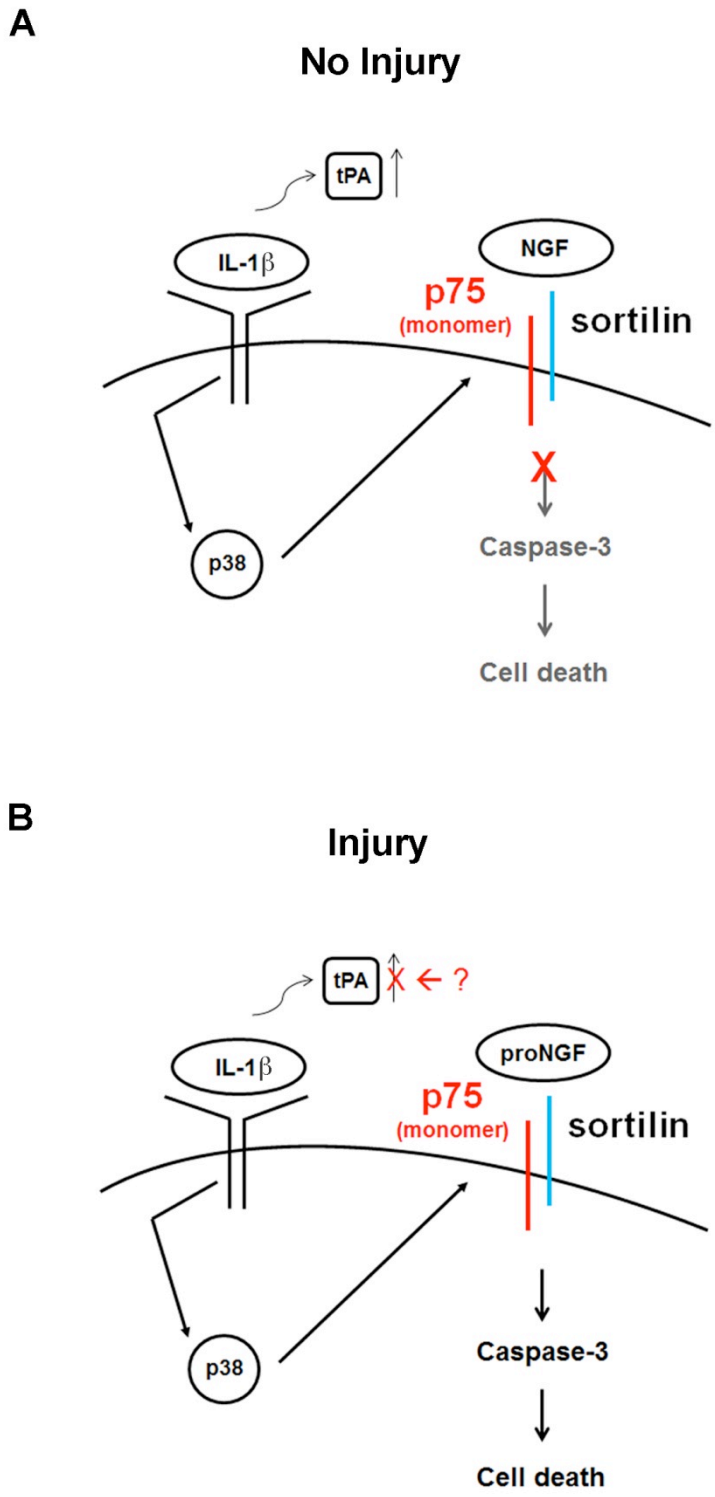

A
No Injury
tPA
IL-1β
NGF
p75
(monomer)
sortilin
X
p38
Caspase-3
Cell death
B
Injury
tPA
← ?
IL-1β
proNGF
p75
(monomer)
sortilin
p38
Caspase-3
Cell death

Figure 22. Proposed model of IL-1β mediated cellular changes in non-injury vs. injury situations

IL-1β may increase monomeric $p75^{NTR}$ through p38 MAPK pathway in neurons. A. In the absence of additional brain injury, IL-1β may increase the secretion of NGF due to the increased activity of tPA, hence NGF may not respond the monomeric p75 induced by IL-1β. B. In injury onset, however, unknown mechanism may decrease the tPA activity, resulting in increased level of extracellular proNGF, which is now able to activate monomeric $p75^{NTR}$ to mediate cell death mechanism.

Table 1

	Injury	AD	**IL-1β**
p75	↑	↑	↑
NGF mRNA	↑	↑	↑
(proNGF↔NGF)	(proNGF)	(proNGF)	**(NGF)**
tPA/plasmin	↓	↓	↑
Neuronal death	↑	↑	–

Table 1. Comparison of IL-1β effects with injury and disease

XII. References

Allan, S.M., P.J. Tyrrell, and N.J. Rothwell. 2005. Interleukin-1 and neuronal injury. *Nat Rev Immunol*. 5:629-640.

Armstrong, D.M., R. Brady, L.B. Hersh, R.C. Hayes, and R.G. Wiley. 1991. Expression of choline acetyltransferase and nerve growth factor receptor within hypoglossal motoneurons following nerve injury. *The Journal of comparative neurology*. 304:596-607.

Baeuerle, P.A., and D. Baltimore. 1996. NF-kappa B: ten years after. *Cell*. 87:13-20.

Baldwin, A.N., C.M. Bitler, A.A. Welcher, and E.M. Shooter. 1992. Studies on the structure and binding properties of the cysteine-rich domain of rat low affinity nerve growth factor receptor (p75NGFR). *J Biol Chem*. 267:8352-8359.

Baldwin, A.N., and E.M. Shooter. 1995. Zone mapping of the binding domain of the rat low affinity nerve growth factor receptor by the introduction of novel N-glycosylation sites. *J Biol Chem*. 270:4594-4602.

Bamji, S.X., M. Majdan, C.D. Pozniak, D.J. Belliveau, R. Aloyz, J. Kohn, C.G. Causing, and F.D. Miller. 1998. The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. *The Journal of cell biology*. 140:911-923.

Barker, P.A., G. Barbee, T.P. Misko, and E.M. Shooter. 1994. The low affinity neurotrophin receptor, p75LNTR, is palmitoylated by thioester formation through cysteine 279. *J Biol Chem*. 269:30645-30650.

Barone, F.C., E.A. Irving, A.M. Ray, J.C. Lee, S. Kassis, S. Kumar, A.M. Badger, J.J. Legos, J.A. Erhardt, E.H. Ohlstein, A.J. Hunter, D.C. Harrison, K. Philpott, B.R. Smith, J.L. Adams, and A.A. Parsons. 2001. Inhibition of p38 mitogen-activated protein kinase provides neuroprotection in cerebral focal ischemia. *Med Res Rev*. 21:129-145.

Beattie, M.S., A.W. Harrington, R. Lee, J.Y. Kim, S.L. Boyce, F.M. Longo, J.C. Bresnahan, B.L. Hempstead, and S.O. Yoon. 2002. ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. *Neuron*. 36:375-386.

Bentley, C.A., and K.F. Lee. 2000. p75 is important for axon growth and schwann cell migration during development. *J Neurosci*. 20:7706-7715.

Benveniste, E.N. 1992. Cytokines: influence on glial cell gene expression and

function. *Chem Immunol*. 52:106-153.

Berkenbosch, F., J. van Oers, A. del Rey, F. Tilders, and H. Besedovsky. 1987. Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. *Science*. 238:524-526.

Blank, J.L., P. Gerwins, E.M. Elliott, S. Sather, and G.L. Johnson. 1996. Molecular cloning of mitogen-activated protein/ERK kinase kinases (MEKK) 2 and 3. Regulation of sequential phosphorylation pathways involving mitogen-activated protein kinase and c-Jun kinase. *The Journal of biological chemistry*. 271:5361-5368.

Blasi, F., M. Riccio, A. Brogi, M. Strazza, M.L. Taddei, S. Romagnoli, A. Luddi, R. D'Angelo, S. Santi, E. Costantino-Ceccarini, and M. Melli. 1999. Constitutive expression of interleukin-1beta (IL-1beta) in rat oligodendrocytes. *Biol Chem*. 380:259-264.

Bradley, J.R., and J.S. Pober. 2001. Tumor necrosis factor receptor-associated factors (TRAFs). *Oncogene*. 20:6482-6491.

Burns, K., J. Clatworthy, L. Martin, F. Martinon, C. Plumpton, B. Maschera, A. Lewis, K. Ray, J. Tschopp, and F. Volpe. 2000. Tollip, a new component of the IL-1RI pathway, links IRAK to the IL-1 receptor. *Nature cell biology*. 2:346-351.

Buttini, M., A. Sauter, and H.W. Boddeke. 1994. Induction of interleukin-1 beta mRNA after focal cerebral ischaemia in the rat. *Brain Res Mol Brain Res*. 23:126-134.

Cao, Z., W.J. Henzel, and X. Gao. 1996a. IRAK: a kinase associated with the interleukin-1 receptor. *Science*. 271:1128-1131.

Cao, Z., J. Xiong, M. Takeuchi, T. Kurama, and D.V. Goeddel. 1996b. TRAF6 is a signal transducer for interleukin-1. *Nature*. 383:443-446.

Carman-Krzan, M., X. Vige, and B.C. Wise. 1991. Regulation by interleukin-1 of nerve growth factor secretion and nerve growth factor mRNA expression in rat primary astroglial cultures. *Journal of neurochemistry*. 56:636-643.

Carvey, P.M., E.Y. Chen, J.W. Lipton, C.W. Tong, Q.A. Chang, and Z.D. Ling. 2005. Intra-parenchymal injection of tumor necrosis factor-alpha and interleukin 1-beta produces dopamine neuron loss in the rat. *J Neural Transm*. 112:601-612.

Casha, S., W.R. Yu, and M.G. Fehlings. 2001. Oligodendroglial apoptosis occurs along degenerating axons and is associated with FAS and p75 expression

following spinal cord injury in the rat. *Neuroscience*. 103:203-218.

Chan, F.K. 2000. The pre-ligand binding assembly domain: a potential target of inhibition of tumour necrosis factor receptor function. *Annals of the rheumatic diseases*. 59 Suppl 1:i50-53.

Chan, F.K., H.J. Chun, L. Zheng, R.M. Siegel, K.L. Bui, and M.J. Lenardo. 2000. A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. *Science*. 288:2351-2354.

Chao, C.C., S. Hu, L. Ehrlich, and P.K. Peterson. 1995. Interleukin-1 and tumor necrosis factor-alpha synergistically mediate neurotoxicity: involvement of nitric oxide and of N-methyl-D-aspartate receptors. *Brain Behav Immun*. 9:355-365.

Chao, C.C., J.R. Lokensgard, W.S. Sheng, S. Hu, and P.K. Peterson. 1997. IL-1-induced iNOS expression in human astrocytes via NF-kappa B. *Neuroreport*. 8:3163-3166.

Chao, M.V., and M. Bothwell. 2002. Neurotrophins: to cleave or not to cleave. *Neuron*. 33:9-12.

Chapman, B.S., and I.D. Kuntz. 1995. Modeled structure of the 75-kDa neurotrophin receptor. *Protein Sci*. 4:1696-1707.

Chen, Z., T.B. Gibson, F. Robinson, L. Silvestro, G. Pearson, B. Xu, A. Wright, C. Vanderbilt, and M.H. Cobb. 2001. MAP kinases. *Chem Rev*. 101:2449-2476.

Chittka, A., J.C. Arevalo, M. Rodriguez-Guzman, P. Perez, M.V. Chao, and M. Sendtner. 2004. The p75NTR-interacting protein SC1 inhibits cell cycle progression by transcriptional repression of cyclin E. *J Cell Biol*. 164:985-996.

Chung, I.Y., and E.N. Benveniste. 1990. Tumor necrosis factor-alpha production by astrocytes. Induction by lipopolysaccharide, IFN-gamma, and IL-1 beta. *Journal of immunology*. 144:2999-3007.

Colotta, F., F. Re, M. Muzio, R. Bertini, N. Polentarutti, M. Sironi, J.G. Giri, S.K. Dower, J.E. Sims, and A. Mantovani. 1993. Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science*. 261:472-475.

Cragnolini, A.B., Y. Huang, P. Gokina, and W.J. Friedman. 2009. Nerve growth factor attenuates proliferation of astrocytes via the p75 neurotrophin receptor. *Glia*. 57:1386-1392.

Davies, C.A., S.A. Loddick, S. Toulmond, R.P. Stroemer, J. Hunt, and N.J. Rothwell. 1999. The progression and topographic distribution of interleukin-1beta expression after permanent middle cerebral artery occlusion in the rat. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 19:87-98.

Dechant, G., and Y.A. Barde. 2002. The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system. *Nat Neurosci*. 5:1131-1136.

Della-Bianca, V., F. Rossi, U. Armato, I. Dal-Pra, C. Costantini, G. Perini, V. Politi, and G. Della Valle. 2001. Neurotrophin p75 receptor is involved in neuronal damage by prion peptide-(106-126). *The Journal of biological chemistry*. 276:38929-38933.

Deng, L., C. Wang, E. Spencer, L. Yang, A. Braun, J. You, C. Slaughter, C. Pickart, and Z.J. Chen. 2000. Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell*. 103:351-361.

Depino, A., C. Ferrari, M.C. Pott Godoy, R. Tarelli, and F.J. Pitossi. 2005. Differential effects of interleukin-1beta on neurotoxicity, cytokine induction and glial reaction in specific brain regions. *Journal of neuroimmunology*. 168:96-110.

Deppmann, C.D., S. Mihalas, N. Sharma, B.E. Lonze, E. Niebur, and D.D. Ginty. 2008. A model for neuronal competition during development. *Science*. 320:369-373.

Di Virgilio, F. 1995. The P2Z purinoceptor: an intriguing role in immunity, inflammation and cell death. *Immunol Today*. 16:524-528.

Dinarello, C.A. 1996. Biologic basis for interleukin-1 in disease. *Blood*. 87:2095-2147.

Dinarello, C.A. 1998. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. *Int Rev Immunol*. 16:457-499.

Dinarello, C.A., and R.C. Thompson. 1991. Blocking IL-1: interleukin 1 receptor antagonist in vivo and in vitro. *Immunol Today*. 12:404-410.

Dobrowsky, R.T., M.H. Werner, A.M. Castellino, M.V. Chao, and Y.A. Hannun. 1994. Activation of the sphingomyelin cycle through the low-affinity neurotrophin receptor. *Science*. 265:1596-1599.

Dripps, D.J., B.J. Brandhuber, R.C. Thompson, and S.P. Eisenberg. 1991. Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. *The Journal of biological chemistry*. 266:10331-10336.

Dunne, A., and L.A. O'Neill. 2003. The interleukin-1 receptor/Toll-like receptor superfamily: signal transduction during inflammation and host defense. *Sci STKE*. 2003:re3.

Eberstadt, M., B. Huang, Z. Chen, R.P. Meadows, S.C. Ng, L. Zheng, M.J. Lenardo, and S.W. Fesik. 1998. NMR structure and mutagenesis of the FADD (Mort1) death-effector domain. *Nature*. 392:941-945.

Endres, S., J.G. Cannon, R. Ghorbani, R.A. Dempsey, S.D. Sisson, G. Lonnemann, J.W. Van der Meer, S.M. Wolff, and C.A. Dinarello. 1989. In vitro production of IL 1 beta, IL 1 alpha, TNF and IL2 in healthy subjects: distribution, effect of cyclooxygenase inhibition and evidence of independent gene regulation. *European journal of immunology*. 19:2327-2333.

Eriksson, C., R. Tehranian, K. Iverfeldt, B. Winblad, and M. Schultzberg. 2000. Increased expression of mRNA encoding interleukin-1beta and caspase-1, and the secreted isoform of interleukin-1 receptor antagonist in the rat brain following systemic kainic acid administration. *J Neurosci Res*. 60:266-279.

Ernfors, P., A. Henschen, L. Olson, and H. Persson. 1989. Expression of nerve growth factor receptor mRNA is developmentally regulated and increased after axotomy in rat spinal cord motoneurons. *Neuron*. 2:1605-1613.

Fahnestock, M., B. Michalski, B. Xu, and M.D. Coughlin. 2001. The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease. *Molecular and cellular neurosciences*. 18:210-220.

Fan, L., P.R. Young, F.C. Barone, G.Z. Feuerstein, D.H. Smith, and T.K. McIntosh. 1996. Experimental brain injury induces differential expression of tumor necrosis factor-alpha mRNA in the CNS. *Brain Res Mol Brain Res*. 36:287-291.

Ferrari, D., P. Chiozzi, S. Falzoni, M. Dal Susino, L. Melchiorri, O.R. Baricordi, and F. Di Virgilio. 1997. Extracellular ATP triggers IL-1 beta release by activating the purinergic P2Z receptor of human macrophages. *Journal of immunology*. 159:1451-1458.

Fillit, H., W.H. Ding, L. Buee, J. Kalman, L. Altstiel, B. Lawlor, and G. Wolf-Klein.

1991. Elevated circulating tumor necrosis factor levels in Alzheimer's disease. *Neurosci Lett*. 129:318-320.

Frade, J.M., P. Bovolenta, and A. Rodriguez-Tebar. 1999. Neurotrophins and other growth factors in the generation of retinal neurons. *Microscopy research and technique*. 45:243-251.

Frade, J.M., A. Rodriguez-Tebar, and Y.A. Barde. 1996. Induction of cell death by endogenous nerve growth factor through its p75 receptor. *Nature*. 383:166-168.

Friedman, W.J. 2000. Neurotrophins induce death of hippocampal neurons via the p75 receptor. *J Neurosci*. 20:6340-6346.

Friedman, W.J., S. Thakur, L. Seidman, and A.B. Rabson. 1996. Regulation of nerve growth factor mRNA by interleukin-1 in rat hippocampal astrocytes is mediated by NFkappaB. *The Journal of biological chemistry*. 271:31115-31120.

Geetha, T., R.S. Kenchappa, M.W. Wooten, and B.D. Carter. 2005. TRAF6-mediated ubiquitination regulates nuclear translocation of NRIF, the p75 receptor interactor. *EMBO J*. 24:3859-3868.

Gehrmann, J., Y. Matsumoto, and G.W. Kreutzberg. 1995. Microglia: intrinsic immuneffector cell of the brain. *Brain Res Brain Res Rev*. 20:269-287.

Giulian, D., T.J. Baker, L.C. Shih, and L.B. Lachman. 1986. Interleukin 1 of the central nervous system is produced by ameboid microglia. *The Journal of experimental medicine*. 164:594-604.

Glaccum, M.B., K.L. Stocking, K. Charrier, J.L. Smith, C.R. Willis, C. Maliszewski, D.J. Livingston, J.J. Peschon, and P.J. Morrissey. 1997. Phenotypic and functional characterization of mice that lack the type I receptor for IL-1. *Journal of immunology*. 159:3364-3371.

Gong, Y., P. Cao, H.J. Yu, and T. Jiang. 2008. Crystal structure of the neurotrophin-3 and p75NTR symmetrical complex. *Nature*. 454:789-793.

Greene, L.A., and A. Rukenstein. 1981. Regulation of acetylcholinesterase activity by nerve growth factor. Role of transcription and dissociation from effects on proliferation and neurite outgrowth. *J Biol Chem*. 256:6363-6367.

Griffin, W.S., L.C. Stanley, C. Ling, L. White, V. MacLeod, L.J. Perrot, C.L. White, 3rd, and C. Araoz. 1989. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc Natl Acad*

Sci U S A. 86:7611-7615.

Grob, P.M., C.H. Berlot, and M.A. Bothwell. 1983. Affinity labeling and partial purification of nerve growth factor receptors from rat pheochromocytoma and human melanoma cells. *Proceedings of the National Academy of Sciences of the United States of America*. 80:6819-6823.

Grob, P.M., A.H. Ross, H. Koprowski, and M. Bothwell. 1985. Characterization of the human melanoma nerve growth factor receptor. *J Biol Chem*. 260:8044-8049.

Hamburger, V., and R. Levi-Montalcini. 1949. Proliferation, differentiation and degeneration in the spinal ganglia of the chick embryo under normal and experimental conditions. *J Exp Zool*. 111:457-501.

Hannum, C.H., C.J. Wilcox, W.P. Arend, F.G. Joslin, D.J. Dripps, P.L. Heimdal, L.G. Armes, A. Sommer, S.P. Eisenberg, and R.C. Thompson. 1990. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature*. 343:336-340.

Hantzopoulos, P.A., C. Suri, D.J. Glass, M.P. Goldfarb, and G.D. Yancopoulos. 1994. The low affinity NGF receptor, p75, can collaborate with each of the Trks to potentiate functional responses to the neurotrophins. *Neuron*. 13:187-201.

He, X.L., and K.C. Garcia. 2004. Structure of nerve growth factor complexed with the shared neurotrophin receptor p75. *Science*. 304:870-875.

Herx, L.M., S. Rivest, and V.W. Yong. 2000. Central nervous system-initiated inflammation and neurotrophism in trauma: IL-1 beta is required for the production of ciliary neurotrophic factor. *Journal of immunology*. 165:2232-2239.

Hetier, E., J. Ayala, P. Denefle, A. Bousseau, P. Rouget, M. Mallat, and A. Prochiantz. 1988. Brain macrophages synthesize interleukin-1 and interleukin-1 mRNAs in vitro. *Journal of neuroscience research*. 21:391-397.

Higuchi, H., T. Yamashita, H. Yoshikawa, and M. Tohyama. 2003. PKA phosphorylates the p75 receptor and regulates its localization to lipid rafts. *The EMBO journal*. 22:1790-1800.

Ho, A., and M. Blum. 1997. Regulation of astroglial-derived dopaminergic neurotrophic factors by interleukin-1 beta in the striatum of young and middle-aged mice. *Experimental neurology*. 148:348-359.

Horai, R., M. Asano, K. Sudo, H. Kanuka, M. Suzuki, M. Nishihara, M. Takahashi, and Y. Iwakura. 1998. Production of mice deficient in genes for interleukin (IL)-1alpha, IL-1beta, IL-1alpha/beta, and IL-1 receptor antagonist shows that IL-1beta is crucial in turpentine-induced fever development and glucocorticoid secretion. *The Journal of experimental medicine*. 187:1463-1475.

Hosomi, S., T. Yamashita, M. Aoki, and M. Tohyama. 2003. The p75 receptor is required for BDNF-induced differentiation of neural precursor cells. *Biochem Biophys Res Commun*. 301:1011-1015.

Hu, S., P.K. Peterson, and C.C. Chao. 1997. Cytokine-mediated neuronal apoptosis. *Neurochemistry international*. 30:427-431.

Huang, B., M. Eberstadt, E.T. Olejniczak, R.P. Meadows, and S.W. Fesik. 1996. NMR structure and mutagenesis of the Fas (APO-1/CD95) death domain. *Nature*. 384:638-641.

Huang, Y., D.E. Smith, O. Ibanez-Sandoval, J.E. Sims, and W.J. Friedman. 2011. Neuron-specific effects of interleukin-1beta are mediated by a novel isoform of the IL-1 receptor accessory protein. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 31:18048-18059.

Hubbard, S.R. 1999. Structural analysis of receptor tyrosine kinases. *Prog Biophys Mol Biol*. 71:343-358.

Idriss, H.T., and J.H. Naismith. 2000. TNF alpha and the TNF receptor superfamily: structure-function relationship(s). *Microscopy research and technique*. 50:184-195.

Jansen, P., K. Giehl, J.R. Nyengaard, K. Teng, O. Lioubinski, S.S. Sjoegaard, T. Breiderhoff, M. Gotthardt, F. Lin, A. Eilers, C.M. Petersen, G.R. Lewin, B.L. Hempstead, T.E. Willnow, and A. Nykjaer. 2007. Roles for the pro-neurotrophin receptor sortilin in neuronal development, aging and brain injury. *Nature neuroscience*. 10:1449-1457.

John, G.R., S.C. Lee, X. Song, M. Rivieccio, and C.F. Brosnan. 2005. IL-1-regulated responses in astrocytes: relevance to injury and recovery. *Glia*. 49:161-176.

Kaminska, B., A. Gozdz, M. Zawadzka, A. Ellert-Miklaszewska, and M. Lipko. 2009. MAPK signal transduction underlying brain inflammation and gliosis as therapeutic target. *Anat Rec (Hoboken)*. 292:1902-1913.

Kanning, K.C., M. Hudson, P.S. Amieux, J.C. Wiley, M. Bothwell, and L.C.

Schecterson. 2003. Proteolytic processing of the p75 neurotrophin receptor and two homologs generates C-terminal fragments with signaling capability. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 23:5425-5436.

Kaplan, D.R., and F.D. Miller. 2000. Neurotrophin signal transduction in the nervous system. *Curr Opin Neurobiol*. 10:381-391.

Karin, M., and Y. Ben-Neriah. 2000. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu Rev Immunol*. 18:621-663.

Kenchappa, R.S., N. Zampieri, M.V. Chao, P.A. Barker, H.K. Teng, B.L. Hempstead, and B.D. Carter. 2006. Ligand-dependent cleavage of the P75 neurotrophin receptor is necessary for NRIF nuclear translocation and apoptosis in sympathetic neurons. *Neuron*. 50:219-232.

Khursigara, G., J.R. Orlinick, and M.V. Chao. 1999. Association of the p75 neurotrophin receptor with TRAF6. *J Biol Chem*. 274:2597-2600.

Kim, T., and B.L. Hempstead. 2009. NRH2 is a trafficking switch to regulate sortilin localization and permit proneurotrophin-induced cell death. *The EMBO journal*. 28:1612-1623.

Knerlich, F., L. Schilling, C. Gorlach, M. Wahl, H. Ehrenreich, and A.L. Siren. 1999. Temporal profile of expression and cellular localization of inducible nitric oxide synthase, interleukin-1beta and interleukin converting enzyme after cryogenic lesion of the rat parietal cortex. *Brain research. Molecular brain research*. 68:73-87.

Kokaia, Z., G. Andsberg, A. Martinez-Serrano, and O. Lindvall. 1998. Focal cerebral ischemia in rats induces expression of P75 neurotrophin receptor in resistant striatal cholinergic neurons. *Neuroscience*. 84:1113-1125.

Kromer, L.F. 1987. Nerve growth factor treatment after brain injury prevents neuronal death. *Science*. 235:214-216.

Krueger, J.M., F.J. Obal, J. Fang, T. Kubota, and P. Taishi. 2001. The role of cytokines in physiological sleep regulation. *Annals of the New York Academy of Sciences*. 933:211-221.

Kuner, P., R. Schubenel, and C. Hertel. 1998. Beta-amyloid binds to p57NTR and activates NFkappaB in human neuroblastoma cells. *Journal of neuroscience research*. 54:798-804.

Kyriakis, J.M., and J. Avruch. 2001. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation.

Physiol Rev. 81:807-869.

Kyrkanides, S., J.A. Olschowka, J.P. Williams, J.T. Hansen, and M.K. O'Banion. 1999. TNF alpha and IL-1beta mediate intercellular adhesion molecule-1 induction via microglia-astrocyte interaction in CNS radiation injury. *Journal of neuroimmunology*. 95:95-106.

Lad, S.P., K.E. Neet, and E.J. Mufson. 2003. Nerve growth factor: structure, function and therapeutic implications for Alzheimer's disease. *Curr Drug Targets CNS Neurol Disord*. 2:315-334.

Lamothe, B., A. Besse, A.D. Campos, W.K. Webster, H. Wu, and B.G. Darnay. 2007. Site-specific Lys-63-linked tumor necrosis factor receptor-associated factor 6 auto-ubiquitination is a critical determinant of I kappa B kinase activation. *The Journal of biological chemistry*. 282:4102-4112.

Large, T.H., G. Weskamp, J.C. Helder, M.J. Radeke, T.P. Misko, E.M. Shooter, and L.F. Reichardt. 1989. Structure and developmental expression of the nerve growth factor receptor in the chicken central nervous system. *Neuron*. 2:1123-1134.

Lawrence, C.B., S.M. Allan, and N.J. Rothwell. 1998. Interleukin-1beta and the interleukin-1 receptor antagonist act in the striatum to modify excitotoxic brain damage in the rat. *Eur J Neurosci*. 10:1188-1195.

Le, A.P., and W.J. Friedman. 2012. Matrix metalloproteinase-7 regulates cleavage of pro-nerve growth factor and is neuroprotective following kainic acid-induced seizures. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 32:703-712.

Le Moan, N., D.M. Houslay, F. Christian, M.D. Houslay, and K. Akassoglou. 2011. Oxygen-dependent cleavage of the p75 neurotrophin receptor triggers stabilization of HIF-1alpha. *Molecular cell*. 44:476-490.

Lechan, R.M., R. Toni, B.D. Clark, J.G. Cannon, A.R. Shaw, C.A. Dinarello, and S. Reichlin. 1990. Immunoreactive interleukin-1 beta localization in the rat forebrain. *Brain research*. 514:135-140.

Lee, R., P. Kermani, K.K. Teng, and B.L. Hempstead. 2001. Regulation of cell survival by secreted proneurotrophins. *Science*. 294:1945-1948.

Levi-Montalcini, R. 1987. The nerve growth factor 35 years later. *Science*. 237:1154-1162.

Lieberman, A.P., P.M. Pitha, H.S. Shin, and M.L. Shin. 1989. Production of tumor necrosis factor and other cytokines by astrocytes stimulated with

lipopolysaccharide or a neurotropic virus. *Proceedings of the National Academy of Sciences of the United States of America*. 86:6348-6352.

Liepinsh, E., L.L. Ilag, G. Otting, and C.F. Ibanez. 1997. NMR structure of the death domain of the p75 neurotrophin receptor. *EMBO J*. 16:4999-5005.

Linggi, M.S., T.L. Burke, B.B. Williams, A. Harrington, R. Kraemer, B.L. Hempstead, S.O. Yoon, and B.D. Carter. 2005. Neurotrophin receptor interacting factor (NRIF) is an essential mediator of apoptotic signaling by the p75 neurotrophin receptor. *J Biol Chem*. 280:13801-13808.

Locksley, R.M., N. Killeen, and M.J. Lenardo. 2001. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell*. 104:487-501.

Loddick, S.A., C. Liu, T. Takao, K. Hashimoto, and E.B. De Souza. 1998. Interleukin-1 receptors: cloning studies and role in central nervous system disorders. *Brain Res Brain Res Rev*. 26:306-319.

Loddick, S.A., and N.J. Rothwell. 1996. Neuroprotective effects of human recombinant interleukin-1 receptor antagonist in focal cerebral ischaemia in the rat. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 16:932-940.

Lonnemann, G., S. Endres, J.W. Van der Meer, J.G. Cannon, K.M. Koch, and C.A. Dinarello. 1989. Differences in the synthesis and kinetics of release of interleukin 1 alpha, interleukin 1 beta and tumor necrosis factor from human mononuclear cells. *European journal of immunology*. 19:1531-1536.

MacEwan, D.J. 2002. TNF receptor subtype signalling: differences and cellular consequences. *Cellular signalling*. 14:477-492.

Maggirwar, S.B., P.D. Sarmiere, S. Dewhurst, and R.S. Freeman. 1998. Nerve growth factor-dependent activation of NF-kappaB contributes to survival of sympathetic neurons. *J Neurosci*. 18:10356-10365.

Mahadeo, D., L. Kaplan, M.V. Chao, and B.L. Hempstead. 1994. High affinity nerve growth factor binding displays a faster rate of association than p140trk binding. Implications for multi-subunit polypeptide receptors. *J Biol Chem*. 269:6884-6891.

Maher, C.O., R.E. Anderson, H.S. Martin, R.L. McClelland, and F.B. Meyer. 2003. Interleukin-1beta and adverse effects on cerebral blood flow during long-term global hypoperfusion. *J Neurosurg*. 99:907-912.

Majdan, M., C. Lachance, A. Gloster, R. Aloyz, C. Zeindler, S. Bamji, A. Bhakar, D. Belliveau, J. Fawcett, F.D. Miller, and P.A. Barker. 1997. Transgenic mice expressing the intracellular domain of the p75 neurotrophin receptor undergo neuronal apoptosis. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 17:6988-6998.

Martin, M.U., and H. Wesche. 2002. Summary and comparison of the signaling mechanisms of the Toll/interleukin-1 receptor family. *Biochimica et biophysica acta*. 1592:265-280.

Martinez-Murillo, R., A.P. Fernandez, M.L. Bentura, and J. Rodrigo. 1998. Subcellular localization of low-affinity nerve growth factor receptor-immunoreactive protein in adult rat purkinje cells following traumatic injury. *Exp Brain Res*. 119:47-57.

Mason, J.L., K. Suzuki, D.D. Chaplin, and G.K. Matsushima. 2001. Interleukin-1beta promotes repair of the CNS. *J Neurosci*. 21:7046-7052.

Mazella, J. 2001. Sortilin/neurotensin receptor-3: a new tool to investigate neurotensin signaling and cellular trafficking? *Cell Signal*. 13:1-6.

Medders, K.E., and M. Kaul. 2011. Mitogen-activated protein kinase p38 in HIV infection and associated brain injury. *J Neuroimmune Pharmacol*. 6:202-215.

Meistrell, M.E., 3rd, G.I. Botchkina, H. Wang, E. Di Santo, K.M. Cockroft, O. Bloom, J.M. Vishnubhakat, P. Ghezzi, and K.J. Tracey. 1997. Tumor necrosis factor is a brain damaging cytokine in cerebral ischemia. *Shock*. 8:341-348.

Merrill, J.E., and E.N. Benveniste. 1996. Cytokines in inflammatory brain lesions: helpful and harmful. *Trends Neurosci*. 19:331-338.

Mi, S., X. Lee, Z. Shao, G. Thill, B. Ji, J. Relton, M. Levesque, N. Allaire, S. Perrin, B. Sands, T. Crowell, R.L. Cate, J.M. McCoy, and R.B. Pepinsky. 2004. LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nature neuroscience*. 7:221-228.

Micheau, O., and J. Tschopp. 2003. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell*. 114:181-190.

Minami, M., Y. Kuraishi, K. Yabuuchi, A. Yamazaki, and M. Satoh. 1992. Induction of interleukin-1 beta mRNA in rat brain after transient forebrain ischemia. *J Neurochem*. 58:390-392.

Mogi, M., M. Harada, H. Narabayashi, H. Inagaki, M. Minami, and T. Nagatsu.

1996. Interleukin (IL)-1 beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. *Neurosci Lett*. 211:13-16.

Mogi, M., M. Harada, P. Riederer, H. Narabayashi, K. Fujita, and T. Nagatsu. 1994. Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. *Neurosci Lett*. 165:208-210.

Molina-Holgado, E., S. Ortiz, F. Molina-Holgado, and C. Guaza. 2000. Induction of COX-2 and PGE(2) biosynthesis by IL-1beta is mediated by PKC and mitogen-activated protein kinases in murine astrocytes. *British journal of pharmacology*. 131:152-159.

Monlauzeur, L., L. Breuza, and A. Le Bivic. 1998. Putative O-glycosylation sites and a membrane anchor are necessary for apical delivery of the human neurotrophin receptor in Caco-2 cells. *J Biol Chem*. 273:30263-30270.

Monroy, M., J.W. Kuluz, D. He, W.D. Dietrich, and C.L. Schleien. 2001. Role of nitric oxide in the cerebrovascular and thermoregulatory response to interleukin-1 beta. *Am J Physiol Heart Circ Physiol*. 280:H1448-1453.

Mufson, E.J., J. Wuu, S.E. Counts, and A. Nykjaer. 2010. Preservation of cortical sortilin protein levels in MCI and Alzheimer's disease. *Neuroscience letters*. 471:129-133.

Murray, S.S., P. Perez, R. Lee, B.L. Hempstead, and M.V. Chao. 2004. A novel p75 neurotrophin receptor-related protein, NRH2, regulates nerve growth factor binding to the TrkA receptor. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 24:2742-2749.

Nielsen, M.S., P. Madsen, E.I. Christensen, A. Nykjaer, J. Gliemann, D. Kasper, R. Pohlmann, and C.M. Petersen. 2001. The sortilin cytoplasmic tail conveys Golgi-endosome transport and binds the VHS domain of the GGA2 sorting protein. *EMBO J*. 20:2180-2190.

Nieto-Sampedro, M., and M.A. Berman. 1987. Interleukin-1-like activity in rat brain: sources, targets, and effect of injury. *Journal of neuroscience research*. 17:214-219.

Nikam, S.S., G.I. Tennekoon, B.A. Christy, J.E. Yoshino, and J.L. Rutkowski. 1995. The zinc finger transcription factor Zif268/Egr-1 is essential for Schwann cell expression of the p75 NGF receptor. *Molecular and cellular neurosciences*. 6:337-348.

Nykjaer, A., R. Lee, K.K. Teng, P. Jansen, P. Madsen, M.S. Nielsen, C. Jacobsen,

M. Kliemannel, E. Schwarz, T.E. Willnow, B.L. Hempstead, and C.M. Petersen. 2004. Sortilin is essential for proNGF-induced neuronal cell death. *Nature*. 427:843-848.

O'Neill, L.A. 2002. Signal transduction pathways activated by the IL-1 receptor/toll-like receptor superfamily. *Curr Top Microbiol Immunol*. 270:47-61.

Park, J.A., J.Y. Lee, T.A. Sato, and J.Y. Koh. 2000. Co-induction of p75NTR and p75NTR-associated death executor in neurons after zinc exposure in cortical culture or transient ischemia in the rat. *J Neurosci*. 20:9096-9103.

Parkhurst, C.N., N. Zampieri, and M.V. Chao. 2010. Nuclear localization of the p75 neurotrophin receptor intracellular domain. *The Journal of biological chemistry*. 285:5361-5368.

Pedraza, C.E., P. Podlesniy, N. Vidal, J.C. Arevalo, R. Lee, B. Hempstead, I. Ferrer, M. Iglesias, and C. Espinet. 2005. Pro-NGF isolated from the human brain affected by Alzheimer's disease induces neuronal apoptosis mediated by p75NTR. *The American journal of pathology*. 166:533-543.

Perini, G., V. Della-Bianca, V. Politi, G. Della Valle, I. Dal-Pra, F. Rossi, and U. Armato. 2002. Role of p75 neurotrophin receptor in the neurotoxicity by beta-amyloid peptides and synergistic effect of inflammatory cytokines. *The Journal of experimental medicine*. 195:907-918.

Petersen, C.M., M.S. Nielsen, A. Nykjaer, L. Jacobsen, N. Tommerup, H.H. Rasmussen, H. Roigaard, J. Gliemann, P. Madsen, and S.K. Moestrup. 1997. Molecular identification of a novel candidate sorting receptor purified from human brain by receptor-associated protein affinity chromatography. *J Biol Chem*. 272:3599-3605.

Peterson, P.K., S. Hu, J. Salak-Johnson, T.W. Molitor, and C.C. Chao. 1997. Differential production of and migratory response to beta chemokines by human microglia and astrocytes. *J Infect Dis*. 175:478-481.

Podlesniy, P., A. Kichev, C. Pedraza, J. Saurat, M. Encinas, B. Perez, I. Ferrer, and C. Espinet. 2006. Pro-NGF from Alzheimer's disease and normal human brain displays distinctive abilities to induce processing and nuclear translocation of intracellular domain of p75NTR and apoptosis. *The American journal of pathology*. 169:119-131.

Rabizadeh, S., J. Oh, L.T. Zhong, J. Yang, C.M. Bitler, L.L. Butcher, and D.E. Bredesen. 1993. Induction of apoptosis by the low-affinity NGF receptor. *Science*. 261:345-348.

Ramos, A., W.C. Ho, S. Forte, K. Dickson, J. Boutilier, K. Favell, and P.A. Barker. 2007. Hypo-osmolar stress induces p75NTR expression by activating Sp1-dependent transcription. *J Neurosci*. 27:1498-1506.

Relton, J.K., and N.J. Rothwell. 1992. Interleukin-1 receptor antagonist inhibits ischaemic and excitotoxic neuronal damage in the rat. *Brain research bulletin*. 29:243-246.

Rivieccio, M.A., G.R. John, X. Song, H.S. Suh, Y. Zhao, S.C. Lee, and C.F. Brosnan. 2005. The cytokine IL-1beta activates IFN response factor 3 in human fetal astrocytes in culture. *Journal of immunology*. 174:3719-3726.

Ross, F.M., S.M. Allan, N.J. Rothwell, and A. Verkhratsky. 2003. A dual role for interleukin-1 in LTP in mouse hippocampal slices. *Journal of neuroimmunology*. 144:61-67.

Roux, P.P., and P.A. Barker. 2002. Neurotrophin signaling through the p75 neurotrophin receptor. *Progress in neurobiology*. 67:203-233.

Roux, P.P., M.A. Colicos, P.A. Barker, and T.E. Kennedy. 1999. p75 neurotrophin receptor expression is induced in apoptotic neurons after seizure. *J Neurosci*. 19:6887-6896.

Sachs, B.D., G.S. Baillie, J.R. McCall, M.A. Passino, C. Schachtrup, D.A. Wallace, A.J. Dunlop, K.F. MacKenzie, E. Klussmann, M.J. Lynch, S.L. Sikorski, T. Nuriel, I. Tsigelny, J. Zhang, M.D. Houslay, M.V. Chao, and K. Akassoglou. 2007. p75 neurotrophin receptor regulates tissue fibrosis through inhibition of plasminogen activation via a PDE4/cAMP/PKA pathway. *The Journal of cell biology*. 177:1119-1132.

Salehi, A.H., P.P. Roux, C.J. Kubu, C. Zeindler, A. Bhakar, L.L. Tannis, J.M. Verdi, and P.A. Barker. 2000. NRAGE, a novel MAGE protein, interacts with the p75 neurotrophin receptor and facilitates nerve growth factor-dependent apoptosis. *Neuron*. 27:279-288.

Sapolsky, R., C. Rivier, G. Yamamoto, P. Plotsky, and W. Vale. 1987. Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science*. 238:522-524.

Sarret, P., P. Krzywkowski, L. Segal, M.S. Nielsen, C.M. Petersen, J. Mazella, T. Stroh, and A. Beaudet. 2003. Distribution of NTS3 receptor/sortilin mRNA and protein in the rat central nervous system. *J Comp Neurol*. 461:483-505.

Sato, S., H. Sanjo, K. Takeda, J. Ninomiya-Tsuji, M. Yamamoto, T. Kawai, K. Matsumoto, O. Takeuchi, and S. Akira. 2005. Essential function for the

kinase TAK1 in innate and adaptive immune responses. *Nature immunology*. 6:1087-1095.

Schlessinger, J. 2002. Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. *Cell*. 110:669-672.

Schneider, H., F. Pitossi, D. Balschun, A. Wagner, A. del Rey, and H.O. Besedovsky. 1998. A neuromodulatory role of interleukin-1beta in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*. 95:7778-7783.

Shaftel, S.S., T.J. Carlson, J.A. Olschowka, S. Kyrkanides, S.B. Matousek, and M.K. O'Banion. 2007. Chronic interleukin-1beta expression in mouse brain leads to leukocyte infiltration and neutrophil-independent blood brain barrier permeability without overt neurodegeneration. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 27:9301-9309.

Shamovsky, I.L., G.M. Ross, R.J. Riopelle, and D.F. Weaver. 1999. The interaction of neurotrophins with the p75NTR common neurotrophin receptor: a comprehensive molecular modeling study. *Protein Sci*. 8:2223-2233.

Siegel, R.M., J.K. Frederiksen, D.A. Zacharias, F.K. Chan, M. Johnson, D. Lynch, R.Y. Tsien, and M.J. Lenardo. 2000. Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. *Science*. 288:2354-2357.

Simi, A., N. Tsakiri, P. Wang, and N.J. Rothwell. 2007. Interleukin-1 and inflammatory neurodegeneration. *Biochem Soc Trans*. 35:1122-1126.

Sims, J.E., and S.K. Dower. 1994. Interleukin-1 receptors. *European cytokine network*. 5:539-546.

Sims, J.E., M.A. Gayle, J.L. Slack, M.R. Alderson, T.A. Bird, J.G. Giri, F. Colotta, F. Re, A. Mantovani, K. Shanebeck, and et al. 1993. Interleukin 1 signaling occurs exclusively via the type I receptor. *Proceedings of the National Academy of Sciences of the United States of America*. 90:6155-6159.

Song, W., M. Volosin, A.B. Cragnolini, B.L. Hempstead, and W.J. Friedman. 2010. ProNGF induces PTEN via p75NTR to suppress Trk-mediated survival signaling in brain neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 30:15608-15615.

Sotthibundhu, A., A.M. Sykes, B. Fox, C.K. Underwood, W. Thangnipon, and E.J. Coulson. 2008. Beta-amyloid(1-42) induces neuronal death through the

p75 neurotrophin receptor. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 28:3941-3946.

Sparacio, S.M., Y. Zhang, J. Vilcek, and E.N. Benveniste. 1992. Cytokine regulation of interleukin-6 gene expression in astrocytes involves activation of an NF-kappa B-like nuclear protein. *Journal of neuroimmunology*. 39:231-242.

Spranger, M., D. Lindholm, C. Bandtlow, R. Heumann, H. Gnahn, M. Naher-Noe, and H. Thoenen. 1990. Regulation of Nerve Growth Factor (NGF) Synthesis in the Rat Central Nervous System: Comparison between the Effects of Interleukin-1 and Various Growth Factors in Astrocyte Cultures and in vivo. *Eur J Neurosci*. 2:69-76.

Srinivasan, D., J.H. Yen, D.J. Joseph, and W. Friedman. 2004. Cell type-specific interleukin-1beta signaling in the CNS. *J Neurosci*. 24:6482-6488.

Sukits, S.F., L.L. Lin, S. Hsu, K. Malakian, R. Powers, and G.Y. Xu. 2001. Solution structure of the tumor necrosis factor receptor-1 death domain. *J Mol Biol*. 310:895-906.

Syroid, D.E., P.J. Maycox, M. Soilu-Hanninen, S. Petratos, T. Bucci, P. Burrola, S. Murray, S. Cheema, K.F. Lee, G. Lemke, and T.J. Kilpatrick. 2000. Induction of postnatal schwann cell death by the low-affinity neurotrophin receptor in vitro and after axotomy. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 20:5741-5747.

Takao, T., D.E. Tracey, W.M. Mitchell, and E.B. De Souza. 1990. Interleukin-1 receptors in mouse brain: characterization and neuronal localization. *Endocrinology*. 127:3070-3078.

Taniuchi, M., E.M. Johnson, Jr., P.J. Roach, and J.C. Lawrence, Jr. 1986. Phosphorylation of nerve growth factor receptor proteins in sympathetic neurons and PC12 cells. In vitro phosphorylation by the cAMP-independent protein kinase FA/GSK-3. *J Biol Chem*. 261:13342-13349.

Taupin, V., S. Toulmond, A. Serrano, J. Benavides, and F. Zavala. 1993. Increase in IL-6, IL-1 and TNF levels in rat brain following traumatic lesion. Influence of pre- and post-traumatic treatment with Ro5 4864, a peripheral-type (p site) benzodiazepine ligand. *J Neuroimmunol*. 42:177-185.

Thanos, D., and T. Maniatis. 1995. NF-kappa B: a lesson in family values. *Cell*. 80:529-532.

Thornton, P., E. Pinteaux, S.M. Allan, and N.J. Rothwell. 2008. Matrix

metalloproteinase-9 and urokinase plasminogen activator mediate interleukin-1-induced neurotoxicity. *Molecular and cellular neurosciences*. 37:135-142.

Thornton, P., E. Pinteaux, R.M. Gibson, S.M. Allan, and N.J. Rothwell. 2006. Interleukin-1-induced neurotoxicity is mediated by glia and requires caspase activation and free radical release. *J Neurochem*. 98:258-266.

Troy, C.M., J.E. Friedman, and W.J. Friedman. 2002. Mechanisms of p75-mediated death of hippocampal neurons. Role of caspases. *J Biol Chem*. 277:34295-34302.

Tuffereau, C., J. Benejean, D. Blondel, B. Kieffer, and A. Flamand. 1998. Low-affinity nerve-growth factor receptor (P75NTR) can serve as a receptor for rabies virus. *The EMBO journal*. 17:7250-7259.

Underwood, C.K., K. Reid, L.M. May, P.F. Bartlett, and E.J. Coulson. 2008. Palmitoylation of the C-terminal fragment of p75(NTR) regulates death signaling and is required for subsequent cleavage by gamma-secretase. *Mol Cell Neurosci*. 37:346-358.

Van der Zee, C.E., G.M. Ross, R.J. Riopelle, and T. Hagg. 1996. Survival of cholinergic forebrain neurons in developing p75NGFR-deficient mice. *Science*. 274:1729-1732.

Verdi, J.M., S.J. Birren, C.F. Ibanez, H. Persson, D.R. Kaplan, M. Benedetti, M.V. Chao, and D.J. Anderson. 1994. p75LNGFR regulates Trk signal transduction and NGF-induced neuronal differentiation in MAH cells. *Neuron*. 12:733-745.

Verma, I.M., J.K. Stevenson, E.M. Schwarz, D. Van Antwerp, and S. Miyamoto. 1995. Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation. *Genes & development*. 9:2723-2735.

Verstrepen, L., T. Bekaert, T.L. Chau, J. Tavernier, A. Chariot, and R. Beyaert. 2008. TLR-4, IL-1R and TNF-R signaling to NF-kappaB: variations on a common theme. *Cell Mol Life Sci*. 65:2964-2978.

Vilar, M., I. Charalampopoulos, R.S. Kenchappa, A. Simi, E. Karaca, A. Reversi, S. Choi, M. Bothwell, I. Mingarro, W.J. Friedman, G. Schiavo, P.I. Bastiaens, P.J. Verveer, B.D. Carter, and C.F. Ibanez. 2009. Activation of the p75 neurotrophin receptor through conformational rearrangement of disulphide-linked receptor dimers. *Neuron*. 62:72-83.

Vitkovic, L., J. Bockaert, and C. Jacque. 2000. "Inflammatory" cytokines: neuromodulators in normal brain? *Journal of neurochemistry*. 74:457-471.

Volosin, M., C. Trotter, A. Cragnolini, R.S. Kenchappa, M. Light, B.L. Hempstead, B.D. Carter, and W.J. Friedman. 2008. Induction of proneurotrophins and activation of p75NTR-mediated apoptosis via neurotrophin receptor-interacting factor in hippocampal neurons after seizures. *J Neurosci*. 28:9870-9879.

Wang, C., L. Deng, M. Hong, G.R. Akkaraju, J. Inoue, and Z.J. Chen. 2001. TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature*. 412:346-351.

Wang, C.X., J.A. Olschowka, and J.R. Wrathall. 1997. Increase of interleukin-1beta mRNA and protein in the spinal cord following experimental traumatic injury in the rat. *Brain Res*. 759:190-196.

Wang, K.C., J.A. Kim, R. Sivasankaran, R. Segal, and Z. He. 2002. P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature*. 420:74-78.

Watt, J.A., and N.K. Hobbs. 2000. Interleukin-1beta immunoreactivity in identified neurons of the rat magnocellular neurosecretory system: evidence for activity-dependent release. *Journal of neuroscience research*. 60:478-489.

Welcher, A.A., C.M. Bitler, M.J. Radeke, and E.M. Shooter. 1991. Nerve growth factor binding domain of the nerve growth factor receptor. *Proc Natl Acad Sci U S A*. 88:159-163.

Wesche, H., W.J. Henzel, W. Shillinglaw, S. Li, and Z. Cao. 1997a. MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. *Immunity*. 7:837-847.

Wesche, H., C. Korherr, M. Kracht, W. Falk, K. Resch, and M.U. Martin. 1997b. The interleukin-1 receptor accessory protein (IL-1RAcP) is essential for IL-1-induced activation of interleukin-1 receptor-associated kinase (IRAK) and stress-activated protein kinases (SAP kinases). *The Journal of biological chemistry*. 272:7727-7731.

Williams, L.R., S. Varon, G.M. Peterson, K. Wictorin, W. Fischer, A. Bjorklund, and F.H. Gage. 1986. Continuous infusion of nerve growth factor prevents basal forebrain neuronal death after fimbria fornix transection. *Proceedings of the National Academy of Sciences of the United States of America*. 83:9231-9235.

Wu, C.K., L. Thal, D. Pizzo, L. Hansen, E. Masliah, and C. Geula. 2005. Apoptotic signals within the basal forebrain cholinergic neurons in Alzheimer's disease. *Experimental neurology*. 195:484-496.

Xu, I.S., S. Grass, X.J. Xu, and Z. Wiesenfeld-Hallin. 1998. On the role of galanin

in mediating spinal flexor reflex excitability in inflammation. *Neuroscience*. 85:827-835.

Yaar, M., S. Zhai, R.E. Fine, P.B. Eisenhauer, B.L. Arble, K.B. Stewart, and B.A. Gilchrest. 2002. Amyloid beta binds trimers as well as monomers of the 75-kDa neurotrophin receptor and activates receptor signaling. *The Journal of biological chemistry*. 277:7720-7725.

Yaar, M., S. Zhai, P.F. Pilch, S.M. Doyle, P.B. Eisenhauer, R.E. Fine, and B.A. Gilchrest. 1997. Binding of beta-amyloid to the p75 neurotrophin receptor induces apoptosis. A possible mechanism for Alzheimer's disease. *The Journal of clinical investigation*. 100:2333-2340.

Yamasaki, Y., N. Matsuura, H. Shozuhara, H. Onodera, Y. Itoyama, and K. Kogure. 1995. Interleukin-1 as a pathogenetic mediator of ischemic brain damage in rats. *Stroke; a journal of cerebral circulation*. 26:676-680; discussion 681.

Yamashita, T., and M. Tohyama. 2003. The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. *Nature neuroscience*. 6:461-467.

Yan, H., and M.V. Chao. 1991. Disruption of cysteine-rich repeats of the p75 nerve growth factor receptor leads to loss of ligand binding. *J Biol Chem*. 266:12099-12104.

Yan, Q., and E.M. Johnson, Jr. 1988. An immunohistochemical study of the nerve growth factor receptor in developing rats. *J Neurosci*. 8:3481-3498.

Yan, Z., and J. Feng. 2004. Alzheimer's disease: interactions between cholinergic functions and beta-amyloid. *Curr Alzheimer Res*. 1:241-248.

Yao, J., J.E. Keri, R.E. Taffs, and C.A. Colton. 1992. Characterization of interleukin-1 production by microglia in culture. *Brain research*. 591:88-93.

Yao, J., T.W. Kim, J. Qin, Z. Jiang, Y. Qian, H. Xiao, Y. Lu, W. Qian, M.F. Gulen, N. Sizemore, J. DiDonato, S. Sato, S. Akira, B. Su, and X. Li. 2007. Interleukin-1 (IL-1)-induced TAK1-dependent Versus MEKK3-dependent NFkappaB activation pathways bifurcate at IL-1 receptor-associated kinase modification. *The Journal of biological chemistry*. 282:6075-6089.

Yasuda, K., L. Churchill, T. Yasuda, K. Blindheim, M. Falter, and J.M. Krueger. 2007. Unilateral cortical application of interleukin-1beta (IL1beta) induces asymmetry in fos, IL1beta and nerve growth factor immunoreactivity: implications for sleep regulation. *Brain Res*. 1131:44-59.

Ye, X., P. Mehlen, S. Rabizadeh, T. VanArsdale, H. Zhang, H. Shin, J.J. Wang, E.

Leo, J. Zapata, C.A. Hauser, J.C. Reed, and D.E. Bredesen. 1999. TRAF family proteins interact with the common neurotrophin receptor and modulate apoptosis induction. *J Biol Chem*. 274:30202-30208.

Yeaman, C., A.H. Le Gall, A.N. Baldwin, L. Monlauzeur, A. Le Bivic, and E. Rodriguez-Boulan. 1997. The O-glycosylated stalk domain is required for apical sorting of neurotrophin receptors in polarized MDCK cells. *J Cell Biol*. 139:929-940.

Yeiser, E.C., N.J. Rutkoski, A. Naito, J. Inoue, and B.D. Carter. 2004. Neurotrophin signaling through the p75 receptor is deficient in traf6-/- mice. *J Neurosci*. 24:10521-10529.

Yeo, T.T., J. Chua-Couzens, L.L. Butcher, D.E. Bredesen, J.D. Cooper, J.S. Valletta, W.C. Mobley, and F.M. Longo. 1997. Absence of p75NTR causes increased basal forebrain cholinergic neuron size, choline acetyltransferase activity, and target innervation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 17:7594-7605.

Zhang, W., C. Smith, C. Howlett, and D. Stanimirovic. 2000. Inflammatory activation of human brain endothelial cells by hypoxic astrocytes in vitro is mediated by IL-1beta. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 20:967-978.

Zhao, X., B. Bausano, B.R. Pike, J.K. Newcomb-Fernandez, K.K. Wang, E. Shohami, N.C. Ringger, S.M. DeFord, D.K. Anderson, and R.L. Hayes. 2001. TNF-alpha stimulates caspase-3 activation and apoptotic cell death in primary septo-hippocampal cultures. *J Neurosci Res*. 64:121-131.

XIII. Curriculum Vitae

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Major Publications

Choi S, Friedman WJ (2012) IL-1ß regulates p75NTR expression and increases vulnerability to proNGF. *In preparation*

Vilar M, Charalampopoulos I, Kenchappa RS, Reversi A, Klos-Applequist JM, Karaca E, Simi A, Spuch C, **Choi S**, Friedman WJ, Ericson J, Schiavo G, Carter BD, Ibáñez CF (2009) Ligand-independent signaling by disulfide-crosslinked dimers of the p75 neurotrophin receptor. *J Cell Sci*. 122(Pt 18):3351-7. Epub 2009 Aug 25.

Choi S, Friedman WJ (2009) Inflammatory cytokines IL-1beta and TNF-alpha regulate p75NTR expression in CNS neurons and astrocytes by distinct cell-type-specific signalling mechanisms. *ASN Neuro*. 2009 May 20;1(2). pii: e00010. doi: 10.1042/AN20090009.

Vilar M, Charalampopoulos I, Kenchappa RS, Simi A, Karaca E, Reversi A, **Choi S**, Bothwell M, Mingarro I, Friedman WJ, Schiavo G, Bastiaens PI, Verveer PJ, Carter BD, Ibáñez CF (2009) Activation of the p75 neurotrophin receptor through conformational rearrangement of disulphide-linked receptor dimers. *Neuron.* 62(1):72-83.

Jung-Jin Hwang, Mi-Ha Park, **So-Young Choi**, Jae-Young Koh (2005) Activation of the Trk Signaling Pathway by Extracellular Zinc: ROLE OF METALLOPROTEINASES. *J Biol Chem*. 280(12):11995-2001.

So-Young Choi, Jung-Jin Hwang and Jae-Young Koh (2003) NR2A induction and NMDA receptor dependent neuronal death by neurotrophin-4/5 in cortical cell culture. *J. Neurochem.* 88(3):708-16.

Jung-Jin Hwang, **So-Young Choi** and Jae-Young Koh (2002) The role of NADPH oxidase, neuronal nitric oxide synthase and poly (ADP ribose) polymerase in oxidative neuronal death induced in cortical cultures by brain-derived neurotrophic factor and neurotrophin-4/5. *J. Neurochem.* 82: 894-902.