Neurotrophins

Presented by:
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Lecturer of Medical Physiology
introduction

- Neurotrophins are a family of closely related proteins
- They were identified initially as survival factors for sensory and sympathetic neurons.
- They have been shown to control many aspects of survival, development and function of neurons in peripheral and central nervous systems.
Four neurotrophins are expressed in mammals: (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 & neurotrophin-4.

Each of them has been shown to activate one or more of the three members of the tropomyosin-related kinase (Trk) receptors.

In addition, they all activate p75NTR.
The Neurotrophic Hypothesis:

Targets of innervation secrete limiting amounts of survival factors to generate a balance between the size of the target organ and the number of innervating neurons.
1934: Victor Hamburger discovered that removal of a limb bud resulted in reduced numbers of sensory and motor neurons in the spinal cord.
1939: Victor Hamburger showed that transplantation of a supernumerary limb resulted in increased numbers of sensory and motor neurons in the spinal cord.
Based on his limb-bud experiments, V. Hamburger hypothesized that the targets of innervating neurons provide signals that recruit undifferentiated cells to develop into sensory or motor neurons.

(he was wrong)

In 1942, Levi-Montalcini and Levi proposed that target derived signals maintain survival of differentiating neurons. In 1949, Hamburger and Levi-Montalcini repeated the limb bud experiments and found that their results supported the neurotrophic hypothesis.

1954: neurite outgrowth assay

1960: NGF purified

1969: NGF purified to homogeneity

1986: Levi-Montalcini and Cohen split the Nobel prize for Physiology or Medicine “for their discovery of growth factors”
Neurotrophins in the CNS

1. In the CNS, neurotrophins have important roles in neuron and glial survival, as well as differentiation and growth (as they do in the PNS).

2. In fact, the functions stretch beyond the time of peak synapse formation (both before and after); e.g., BDNF mRNA increases to maximal levels in postnatal animals.
2. The many possible sources of trophic support are illustrated in the CNS – many types of neurotrophic factors with compensatory/synergistic effects exist. * this may be why transgenics missing one of them often have no blatant developmental abnormalities – or at least, can survive.

Note: autocrine, paracrine, afferants (anterograde transport).
3. BDNF, NT3, NT4, and their receptors are most widespread in the brain (NGF less so - mostly periphery), particularly in the cortex and hippocampus.
Regulation of Neurotrophin Synthesis by Physiological Activity

- The transcription of genes for CNS neurotrophins is regulated by various forms of neuronal activity.
Regulation of Neurotrophin Synthesis by Physiological Activity

- It has been observed that levels of BDNF mRNA in hippocampus, cortex, and cerebellum can be changed by:
  - depolarization and Ca$^{2+}$ influx
  - excitatory neurotransmission (glu, kainate increase; GABA decrease)
- seizure activity (generalized activation)
- stimulation of LTP
- normal physiological stimuli, such as light \(\rightarrow\) visual cortex; general physical activity, sensory stimulation, enriched environments.
Regulation of Synaptic Transmission by Neurotrophins

1. One important postnatal function of neurotrophins (after synaptogenesis and normal cell death):
   - from the anterograde transport (afferent sources)
   - including transient modulation of synaptic transmission (e.g., increased efficacy of inputs to CA1 pyramidal neurons (Schaffer collaterals)).
Regulation of Synaptic Transmission by Neurotrophins

2. Maintenance of LTP.
3. Alterations in morphology of synaptic elements.
4. Endocrine control of cell survival.
5. Maintenance of neuron size and arbourization.
6. Facilitation of activity-dependent enhancements (i.e., complexity of dendritic arbours or spine formation and remodeling).
Some Other Growth Factor Families: Cytokines

- Several other families of signaling molecules with actions both inside and outside the nervous system exist:
- Like neurotrophins, these diffusable factors regulated growth and maintenance: Cytokines = “cell movement factors”.
- So named because they were first known to regulated chemotaxis and migration.
• Include:
  - Interleukins (central changes in immune system).
  - TNF – proinflammatory.
  - Interferons – inhibit viral replication and growth.
• Several cytokines have activities in the developing and adult nervous system.
• The following are several families:
1. **Neuropoietic Cytokines:**

* e.g., ciliary neurotrophic factor – promote survival of developing motor neurons, hippocampus, sensory neurons, parasympathetic ciliary ganglionic neurons.

- induction of neural cell precursors to differentiate \( \rightarrow \) astrocytes.

* e.g., leukemia inhibitory factor – induces changes in gene expression that occurs in neurons after injury.
2. **TGF Superfamily:**

- recall role in early development and induction processes.
- may have distinct functions later in development.
- TGF and a close relative, GDNF (glial-derived neurotrophic factor) protect the survival and function of dopaminergic neurons (note the enhanced survival in animal models of Parkinson’s Disease).
- Also, survival of motor neurons.
  - peripheral sensory autonomic neurons.
- other systems (kidneys, enteric, nervous).
FGF:

- Mitogenesis during early embryonic development (stim proliferation of many embryonic tissues).
- In brain, FGF1 and FGF2 expression remain high in nervous system throughout life.
- Signal through tyr kinase receptors that are similar to the trks for neurotrophins.
- Have important roles promoting survival after injury and can also signal differentiation.
Transport of NGF
Structure of neurotrophins
Structure of neurotrophin receptors
Tyrosine kinase Receptor activation:
Tyrosine kinase Receptor activation:
Distribution of brain neurotrophins

- trkB
- BDNF
- trkC
- NT3
NGF: sympathetic neurons and some sensory neurons

BDNF: NGF-related factor purified in 1982 from pig brain (shares ~50% homolog with NGF)

NT-3 and NT-4/5: were obtained by PCR cloning

*All these factors are synthesized as ~250 aa precursors that are processed into 120 aa proteins*
The Trk Family of Receptor Tyrosine Kinases for the Neurotrophins

Trk: tropomyosin-related kinase, originally known as orphan receptors

p75_{NTR}: purified and cloned 1st, homology to TNFR
Alternative splicing generates many Trk receptor isoforms

NGF $K_D$ for TrkA = $10^{-11}$M

NGF $K_D=10^{-9}$M  
(all neurotrophins can bind p75$^{NTR}$) 

Models for Trk and $p75^{NTR}$ interaction

Chao and Bothwell (2002) Neuron 33:9-12
p75^{NTR} is required for developmental myelination

## TABLE 21.1 The Neurotrophin Family and Its Receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Full-length kinase-containing isoforms&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Nonkinase forms&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Example of responsive neurons&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGF</td>
<td>trkA (trkA&lt;sub&gt;Ei&lt;/sub&gt;)</td>
<td>p75&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Cholinergic forebrain neurons</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sympathetic ganglia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DRG nociceptive</td>
</tr>
<tr>
<td>BDNF</td>
<td>trkB</td>
<td>p75&lt;sup&gt;LNTR&lt;/sup&gt;</td>
<td>Many CNS populations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trkB&lt;sub&gt;T₁&lt;/sub&gt;</td>
<td>Many CNS populations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trkB&lt;sub&gt;T₂&lt;/sub&gt;</td>
<td>Many CNS populations</td>
</tr>
<tr>
<td>NT-3</td>
<td>trkC (trkC&lt;sub&gt;TK+14&lt;/sub&gt;)</td>
<td>p75&lt;sup&gt;LNTR&lt;/sup&gt;</td>
<td>Many CNS populations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trkC&lt;sub&gt;TK-158&lt;/sub&gt;</td>
<td>Many CNS populations</td>
</tr>
<tr>
<td></td>
<td>(trkC&lt;sub&gt;TK+25&lt;/sub&gt; TrkC&lt;sub&gt;TK+39&lt;/sub&gt;)</td>
<td>trkC&lt;sub&gt;TK-143&lt;/sub&gt;</td>
<td>Choclear ganglia</td>
</tr>
<tr>
<td></td>
<td>trkB and trkA nonpreferred</td>
<td>trkC&lt;sub&gt;TK-113&lt;/sub&gt;</td>
<td>DRG proprioceptive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trkC&lt;sub&gt;TK-108&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>NT-4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>trkB</td>
<td>p75&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Many CNS populations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trkB&lt;sub&gt;T₁&lt;/sub&gt;</td>
<td>Nodose ganglia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trkB&lt;sub&gt;T₂&lt;/sub&gt;</td>
<td>Petrosal ganglia</td>
</tr>
<tr>
<td>NT-6&lt;sup&gt;f&lt;/sup&gt;</td>
<td>trkA</td>
<td>p75&lt;sup&gt;d&lt;/sup&gt;</td>
<td>(Found only in fish)</td>
</tr>
</tbody>
</table>
The effect of NT/NTR knockouts on neurons in the DRG

PERCENTAGES OF NEURONS AND SENSORY MODALITIES LOST IN DORSAL ROOT GANGLIA OF MUTANTS OF THE NEUROTROPHIN FAMILY

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Neuronal Loss (%)</th>
<th>Sensory Modality</th>
</tr>
</thead>
<tbody>
<tr>
<td>trkA</td>
<td>80</td>
<td>Nociceptors, thermoreceptors, low-threshold mechanoreceptors</td>
</tr>
<tr>
<td>NGF</td>
<td>80</td>
<td>Nociceptors, thermoreceptors, low-threshold mechanoreceptors</td>
</tr>
<tr>
<td>TrkB</td>
<td>30</td>
<td>Mechanoreceptors (Meissner)</td>
</tr>
<tr>
<td>BDNF</td>
<td>30</td>
<td>Mechanoreceptors (Meissner)</td>
</tr>
<tr>
<td>NT-4/5</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>TrkC</td>
<td>20–30</td>
<td>Proprioceptors</td>
</tr>
<tr>
<td>NT-3</td>
<td>60</td>
<td>Proprioceptors, D-hair, and mechanoreceptors</td>
</tr>
</tbody>
</table>
Trk receptor signaling

When a neurotrophin binds to a trk receptor, the kinase domain is activated resulting in autophosphorylation.

Autophosphorylation results in further activation of the kinase domain, leading to activation of three potential signaling cascades:

- MAPK
- PI3K
- PLC-γ
Our axons can be >1 m in length---how does the neurotrophin/receptor complex signal to the neuronal cell body?

Activated Trk can signal locally and retrogradely using different signalling pathways.

Differential control of TrkA trafficking and signaling may also be the basis for the different functions of NGF and NT-3.
*In vitro* assays have shown that neurotrophins enhance both axonal and dendritic growth

*In vivo*, the situation is more difficult to study.

**Why?** In standard knockouts, it is difficult to separate the survival effects of neurotrophins from their effects on the morphology of neurons.

This problem has begun to be addressed by using conditional knockouts, or by crossing neurotrophin knockouts with mouse mutants lacking pro-apoptotic genes.

Recent evidence from these kinds of experiments suggests that long distance peripheral sensory axon growth in vivo is neurotrophin-dependent.
Neurotrophins’ roles in neuronal development and function

NT’s are expressed in regions of the developing embryo that are traversed by sensory axons en route to their targets.

NT’s affect the proliferation and differentiation of CNS neuroepithelial progenitors, neural crest cells, and progenitors of enteric neurons \textit{in vitro} (and in some cases also confirmed \textit{in vivo}).

In the CNS, BDNF/TrkB signaling is implicated in the development and maintenance of cortical circuits.
Neurotrophins in the CNS

The highest levels of neurotrophins are found in the hippocampus.

Trophic support for peripheral and central nervous system neurons

Other growth factors:
- CNTF
- TGFs
- LIF
- ILs
- IGFs
- EGF
- FGFs
- EGF
- PDGFs

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BDNF can be secreted by the presynaptic neuron in an activity-dependent fashion

**Indirect Evidence:**
- BDNF is found in synaptic vesicle preparations
- TrkB receptors are found in dendrites
- Axotomy of axons from BDNF-expressing neurons results in a depletion of BDNF in their target area

**Direct Evidence:**
Selection of CNS synapses via BDNF

- Strong presynaptic activity results in release of more BDNF. The postsynaptic site responds by elevating the amount of AMPA receptors and nNOS. This mechanism could contribute to selective facilitation (e.g. maintenance of LTP).
Cytokines and Growth Factors in the Nervous System

**CNTF, LIF** (oncostatin M, cardiotrophin-1): neuropoietic cytokines. These factors may be important in neuronal response to injury.
Cytokines and Growth Factors in the Nervous System

**GDNF, neurturin, artemin, persephin:** exhibit distant homology with the TGF-β family. They signal through a receptor complex composed of the Ret tyrosine kinase and a GPI-linked binding subunit (GFRα family; GFRα1, α2, α3, and α4). These factors are potent axon-promoting growth factors *in vivo* for developing sympathetic and parasympathetic neurons.
MAP kinase cascade:
Growth factors in the CNS
Depolarization and Ca influx enhances growth factor expression
Neurotrophins can protect striatal neurons from excitotoxic injury


In the striatum, TrkB receptors are the most abundant, followed by TrkC
NEUROTROPHINS AND LEARNING
**Critical Period**

An extreme form of Sensitive Period. Appropriate expression is essential for the normal development of a pathway or set of connections (and after this period, it cannot be repaired).

*e.g.*, There was a critical period for the formation of ocular dominance columns, based on neuronal activity/input from both spontaneous firing and visual inputs from the eyes.
If appropriate information is not received during the critical period (from experience), this pathway never attains the ability to process information in a normal fashion, and as a result, perception or behavior can be permanently impaired.

E.g., development of appropriate social and emotional responses to others.

E.g., development of language skills in humans.
What happens to neurons in the absence of neurotrophic factors?
Morphological types of cell death

**Apoptosis**: originally defined according to a set of characteristic ultrastructural features that include nuclear and cytoplasmic condensation, cell fragmentation and phagocytosis.

**Necrosis**: cell death as the result of injury, disease, or pathological state (usually involves large numbers of cells and is associated with inflammation). Chromatin condenses in multiple small clumps and at later stages, cell membranes and organelles disintegrate.

**Autophagy**: (from the Greek, self-eating) Cytoplasm is destroyed by lysosomal enzymes before any nuclear changes become visible. A characteristic feature is the appearance of large autophagic vacuoles in the cytoplasm. At later stages, chromatin condenses, DNA laddering is evident and phagocytosis occurs.
Morphology of cell death

Necrosis

PCD

apoptotic

autophagic

Morphology of cell death
Ultrastructure of cell death
Stages during neuronal development where PCD occurs
PCD also occurs in the glial lineage

A

Glial precursor

→ Growth factor

Newly formed glia

→ Surviving glial cell

If no axon is present:

Glial precursor

→ Dead glial cell

B

<table>
<thead>
<tr>
<th>Dying Schwann cells</th>
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<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>150</td>
</tr>
<tr>
<td>125</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>75</td>
</tr>
<tr>
<td>50</td>
</tr>
<tr>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cont (+) axon</th>
<th>Exp (-) axon</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>150</td>
</tr>
</tbody>
</table>
Genes involved in PCD are highly conserved

C. elegans

Mammalian homologs of the *C. elegans* PCD genes

Ced-3 = caspases (cysteine aspartate proteases)

Ced-4 = Apaf-1

Ced-9 = Bcl-2 family

Pro-death: Bax, Bak [Bim, Brd, Dp5/Hrk, Bad]

Pro-life: Bcl-2, Bcl-X<sub>L</sub>, IAPs
Death signaling pathways

Extrinsic

FasL

\[ \text{FasR} \]

FADD

\[ \text{pro-casp8} \rightarrow \text{casp8} \rightarrow \text{casp9} \rightarrow \text{pro-casp3} \rightarrow \text{casp} \rightarrow \text{APOPTOSIS} \]

Intrinsic

Bim

\[ \text{Bcl-2} \]

Bax

\[ \text{PTP} \]

Cyt C

\[ \text{APAF} \]

"Apoptosome"

Bid

APOPTOSIS
NERVE GROWTH FACTOR
Structure of NGF

• NGF was the first discovered neurotrophin

• The mature form of NGF consists of two $\alpha$, two $\beta$ (resemble that of insulin), & two $\gamma$ subunits.

• $\alpha$ subunits have trypsin-like activity.
• $\beta$ subunits have nerve growth-promoting activity
• $\gamma$ subunits are serine proteases of unknown function.
NGF receptors

NGF interacts with two entirely distinct classes of receptors.

a) P75NTR:

• It was the first receptor to be discovered and was identified as a low-affinity receptor for NGF, but was subsequently shown to bind each of the neurotrophins with a similar affinity.

• It is a member of the tumour necrosis receptor superfamily.
• It has an extracellular domain that includes four cysteine-rich motifs, a single transmembrane domain, and a cytoplasmic domain that includes a ‘death’ domain similar to those present in other members of this family.

• P75 NTR transmits signals important for regulating neuronal survival and differentiation as well as synaptic plasticity.
b) TrkA:

- TrkA is a single-pass transmembrane protein.

- It serves as a receptor tyrosine kinase (RTK) for NGF.

- NGF signaling through TrkA elicits many of the classical neurotrophic actions described to NGF.

- These include precursor proliferation and commitment, cell survival, axon and dendrite growth, membrane trafficking, synapse formation and function, as well as glial differentiation and interactions with neurons.
The two receptor classes of the neurotrophins

Bibel M., Barde Y. Genes Dev. 2000; 14: 2919-2937
p75NTR impact TrkA signal transduction.

It enhance binding of NGF to TrkA, by increasing the specificity of TrkA for NGF binding.

Moreover, TrkA signaling also impacts signaling through p75NTR, through the direct association of TrkA and p75NTR.
P75(NTR) can form a heterodimer with trkA monomer and that dimer has increased affinity and specificity for NGF.

However, p75NTR receptors can form homodimers that in the absence of trk receptors cause apoptosis.
Nerve Growth Factor Receptor Signaling
Production of NGF and its receptors expression

• Both NGF and its receptors are produced during development, adult life, and aging by many cell types in the CNS and PNS, immune and inflammatory system.
# A-Peripheral Nervous System and Peripheral Tissues:

<table>
<thead>
<tr>
<th>NGF producing cells</th>
<th>TrkA expressing cells</th>
<th>P75 NTR expressing cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non neuronal target cells of sympathetic and sensory neurons throughout the body <em>(during development)</em></td>
<td>Sympathetic neurons, peripheral sensory neurons that mediate nociception <em>(during development and in adult life)</em></td>
<td>Sympathetic neurons, peripheral sensory neurons that mediate nociception, <em>(during development and in adult life)</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most α-motor neurons <em>(transiently, during the phase of axon elongation)</em>, Schwann cells in peripheral nerves <em>(during development)</em></td>
</tr>
</tbody>
</table>

**NB:** in normal adults, expression is down regulated to undetectable levels, but returns after injury or inflammation.
**C-Immune and Inflammatory System**

<table>
<thead>
<tr>
<th>NGF producing cells</th>
<th>TrkA expressing cells</th>
<th>P75 NTR expressing cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>mast cells, monocytes, macrophages, T lymphocytes (CD3C and CD4C T cells), and B lymphocytes.</td>
<td>Several types of bone marrow-derived leukocytes (eg: mast cells, CD4C T lymphocytes, B lymphocytes, monocytes, and macrophages.)</td>
<td>Follicular dendritic cells and B lymphocytes</td>
</tr>
</tbody>
</table>
FUNCTION & SIGNALLING
NGF mediates several types of intercellular communication and has been shown to act as:

- A retrograde transported, target-derived factor that influences afferent neurons.
- A locally released paracrine factor that affects both neurons and non-neuronal cells.
- An autocrine factor acting on the same cells that produce and release it.
- An endocrine factor that acts after transport through the blood stream.
I- NGF functions in the nervous system

1. NGF controls survival of neurons.
2. Differentiation & growth of neuronal processes.
3. Role in synaptic transmission and plasticity.
7. Role of Neurotrophins in behaviour
i- Neurotrophins control the survival of neurons

• **Neurotrophins prevent programmed cell death**
  
  Neurotrophins control the development of specific subpopulations of sensory neurons.

  NGF is necessary for the survival of essentially all small neurons with unmyelinated or thinly myelinated axons.
Neurotrophins cause programmed cell death

- Overexpression of p75 in transgenic mice causes death of much of neurons in the developing CNS.

- Ligand-mediated cell death was first shown in the developing avian retina.

- Experiments using antibodies blocking the biological activity of NGF or of p75 indicated reduction of programmed cell death.
• Similar results were obtained in the developing mouse neural tube.

• Cell death in both structures correlates with the development of axonal tracts, resulting in the formation of the optic nerve and the commissural pathway, and cell elimination seems to be accelerated by a NGF/p75-dependent mechanism.
• The dual receptor system allows the transduction of different signals following ligand binding, as signaling cell death through p75 or cell survival through the Trk receptors.

• These receptors directly interact, allowing fine tuning and cross talk.
Signaling pathway linking p75 with cell death.

- Caspase activation, as well as bax/bad, bcl-2, and bcl-xl, are involved in apoptosis.

- Jun kinase-signalling cascade which results in activation of p53 and apoptosis through targeting the pro-apoptotic gene, bax.

- In addition, the Jun kinase cascade induces expression of Fas ligand promoting apoptosis through activation of the Fas receptor.
• A further recently identified interactor of p75, designated NRAGE homolog (neurotrophin receptor-interacting MAGE).

• MAGE (a protein family expressed in cancer tissues) mediates NGF-dependent apoptosis in sympathetic neuron precursor cells.

• It causes cell cycle arrest when overexpressed in transfected cells.

• Moreover, NRAGE disrupts the interaction between p75 and TrKA.

**NB.:** Neurotrophin binding to *p75NTR* also promotes activation of *NF-κB*, promoting NF-κB-dependent neuronal survival.
Signaling pathway linking TrkA with cell survival.

- Upon binding of NGF to TrkA, the receptor is subjected to a series of events that characterize RTK signaling, that finally leads to the generation of a cascade of receptor-independent signaling pathways.

- One of the main signalling cascade involves IRS (insulin receptor substrates IRS-1 and IRS-2) and Gab-1 (Grb-associated binder-1)

- These are adapter proteins that mediate the association and activation of PI3K, as PI3K does not directly interact with the Trk receptors.
TrkA and P75NTR coactivation

- There is evidence for NGF-mediated cell death through p75, especially in structures where p75 is expressed in development before TrkA.

- In general, it seems that neurotrophins can only induce cell death through p75 in cells not expressing their specific trk receptors.
• Coactivation of the appropriate trk receptors inhibits p75-mediated apoptosis.

• Trk activation can block p75-mediated cell death by several mechanisms.

• For example, the interaction of both receptors may disrupt the interaction of p75 with a death-transducing protein.
• Interactions between p75NTR and Trk receptor signalling are facilitated by assembly of multiprotein complexes that include both receptors as constituents.

• A protein, named ARMS interacts with both receptors and is phosphorylated following Trk activation, resulting in prolonged activation of signalling pathways for neuronal differentiation.
NGF homodimer (red/yellow)

TrkA binding sites

p75 binding induces conformational change on opposing NGF binding site, preventing second p75 interaction
Neurotrophins regulate neuronal growth and differentiation

*Peripheral nervous system*

- In vivo work with sympathetic ganglia revealed that NGF levels regulate dendritic growth.

- The increased number of dendrites induced by NGF is accompanied by a very large increase in the number of preganglionic axons innervating NGF-responsive ganglia.
This figure shows a clump of sympathetic neurone cell bodies in a culture dish in the absence of NGF (upper figure) compared with a clump in the presence of NGF (lower figure).
b-Central nervous system

- Neurotrophins regulate axonal branching during the development of CNS neurons.

- NGF has been reported to affect apical dendrites.

- It appears that neither p75 nor TrkA are expressed by the responsive neurons !!!

- The possibilities exist, that unidentified NGF receptors may mediate its action OR that the effects are indirect and necessitate the presence of other cells.
IN GENERAL:

- NGF regulates the cell body size, terminal sprouting, dendritic arborization, and gene expression in the PNS (sympathetic neurons and small nociceptive sensory neurons), and in the CNS (basal forebrain and striatal cholinergic neurons).
signaling cascades involved in neuronal differentiation and growth

- the activation of the Ras/Raf/MEK/MAPK pathway results from the formation of a variety of complexes of adapter molecules.

- This complexity of activation allows a sustained activation of the MAPK pathway in response to neurotrophins, as well as a fine-tuning of the responses.
iii-NGF role in synaptic transmission and plasticity

- Neurotrophins modulate the number of synapses and the efficacy of synaptic transmission.

- Manipulations of NGF levels were shown early on to regulate both the strength and the number of presynaptic inputs.
Plasticity of NGF-Responsive Neurons

- Neurotrophins are implicated as molecular mediators of specific forms of both structural and functional plasticity.

- NGF has reported effects on stimulus dependant activity in adult somatosensory cortex that appear to be mediated by TrkA and facilitated by p75NTR.

- The mechanistic basis for these effects is unclear but may involve NGF-dependent modulation of cholinergic function and subsequent modification of cortical plasticity.
Signaling cascades involved in synaptic plasticity and effects on synaptic transmission

- the association of PLC-γ with Trk regulates intracellular Ca2+ levels and protein kinase C activity via cleavage of the substrate PIP2 to DAG and IP3.

- This pathway seems to play an important role in neurotrophin-mediated neurotrophin release and in synaptic plasticity.
Signaling through the Trk receptors — the main pathways

Differentiation Neurite Growth

Survival

Activity-Dependent Plasticity

MAPK

MEK

Raf

Ras

SOS

F RS 2

SHP-2

Grb2

Gab-1

PI3K

PKB AKT
iv-NGF And Neuroprotection

- NGF acts to protect neurons from endogenous toxic events generated during the response to tissue injury and this signaling facilitates regrowth & repair.

- The signaling mechanisms engaged in neuroprotection have not been defined.
**Protection from Axotomy:**

- Among NGF-responsive neurons, sensory neurons and cholinergic neurons in the basal nucleus survive axotomy but exhibit moderate to severe atrophy.

- In contrast, about 50% (but not all) of axotomized sympathetic neurons and septal cholinergic neurons rapidly die.

- Neither the mechanism of axotomy-induced cell death, nor the reasons that some neurons die while others survive but atrophy, are understood.
• In the adult septum, axotomy, induced cell death can be largely prevented in both rodents and primates by NGF infusions at the time of the axotomy.

• This effect appears to be mediated via TrkA signaling, and NGF need only be given transiently for a few weeks after the axotomy and can then be discontinued without subsequent loss of neurons.

• In addition, NGF is able to prevent axotomy-induced atrophy in cells that are not killed by axotomy, as well as to reverse atrophy that has already occurred.
**Protection from Glutamate Excitotoxicity (in stroke):**

- NGF has protective effect against glutamate receptor–mediated excitotoxicity, such that NGF infusions reduced the overall size of excitotoxic lesions in the striatum and prevent the death of cholinergic, NGF receptor–expressing, striatal neurons.

- NGF has also been reported to protect PC12 cells from anoxia and glucose deprivation, or from nitric oxide cytotoxicity.
NGF Protection of CNS Neurons that do not Appear to Express NGF Receptors

• NGF is reported to protect a broad spectrum of neurons from ischemia, glutamate receptor–mediated excitotoxicity, and metabolic insults such as glucose deprivation and oxidative stress.

• Protective effects of NGF on neurons not known to express NGF receptors have been described and confirmed by numerous research groups using many different in vivo and in vitro models.
Potential Mechanisms of NGF-Mediated Neuroprotection of Non-NGF Receptor-Expressing Neurons

• The signaling mechanisms underlying NGF-mediated protection of neurons that do not express NGF receptors are not understood.

• In some cases, protection has been shown to involve stabilization of intracellular Ca2+ levels and prevention of the surge in cytoplasmic Ca2+ associated with cell death, as triggered by excess glutamate signaling or oxidative stress.
• In a recent study, protection of cortical neurons from glutamate excitotoxicity in vitro by both NGF and estrogen were found to require activation of the MAPK pathway.

• The means by which NGF signaling leads to widespread neuroprotective effects is not known, and there are several different options to consider.
The first is simply that TrkA and p75NTR expression is more widespread among neurons than is currently appreciated, and very low levels of TrkA or p75NTR might be able to mediate the protective signaling.

The second option is that a novel NGF receptor exists.

A third option is that these neuroprotective effects are mediated via NGF signaling through NGF-responsive nonneuronal cells as glia and inflammatory cells.
v-NGF and neuronal Repair

a-PNS

-Glia and Inflammatory Cells

• After a peripheral nerve injury that causes axotomy, both myelinating and nonmyelinating Schwann cells distal to the injury differentiate and reenter the cell cycle.

• Proliferating and reactive Schwann cells produce growth factors, cytokines, and growth-associated proteins, which are likely to play key roles in axon regeneration and nerve repair.
Changes in gene expression by reactive Schwann cells include a marked upregulation of both NGF and p75NTR.

Peripheral nerve injury also leads to substantial infiltration of inflammatory cells, and among these, macrophages, mast cells, and subsets of T cells have the capacity to express NGF.

The precise roles and functions of NGF signaling for different cell types during the response to peripheral nerve injury are not certain.
-Neurons

- After axotomy, TrkA and p75NTR expression by sensory neurons decreases, whereas motor neurons begin to reexpress detectable levels of p75NTR.

- Neither the reasons for, nor the consequences of, the decline in TrkA expression by axotomized sensory neurons are understood.

- Recent findings show that p75NTR signaling through Rho and ceramide pathways may be involved in promoting axon elongation.
The process of brain regeneration is a product of stem cells and of nerve growth factors, and it requires physical and mental activity to work.
• In the CNS, as in the PNS, injury or insults such as trauma, ischemia, or degenerative disease trigger rapid and substantial upregulation in the expression of NGF and NGF receptors by cell types involved in the repair process, including:

(a) Local astrocytes and microglia.
(b) Invading inflammatory cells, including macrophages, mast cells, and subsets of T cells.
(c) Certain neurons.
• Several lines of evidence suggest that NGF signaling may in some of these instances facilitate the repair or reorganization of neural connections.

• NGF induces neurite outgrowth in developing hippocampal pyramidal neurons in vitro, via a p75NTR-ceramide–mediated signaling pathway, and in retinal neurons, p75NTR signaling activates Rho pathways, which have been associated with axon growth.
vi-Nociception

- Small nociceptive sensory neurons express both types of NGF receptor throughout life.

- NGF has a variety of effects on these cells, including upregulation of TrkA and p75NTR, CGRP (calcitonin gene related peptide), and tachykinin expression, as well as modulation of cell size, activity, and neuropeptide release.

- NGF signaling in these cells leads to hypersensitivity to nociceptive stimuli in the form of allodynia and hyperalgesia in both animals and patients given NGF in clinical trials for peripheral neuropathies.
NGF is expressed and released in many tissues in response to injury.

Blockade of NGF signaling using function blocking antibodies in experimental animals with skin injury and inflammation prevents the development of hyperalgesia.

It may accomplish this effect in part by inducing mast cell degranulation and the consequent release of serotonin, histamine, and NGF itself.
• In terms of mechanical hyperalgesia, NGF may act centrally by upregulating CGRP, substance P, and BDNF.

• Recent findings suggest that PKC may mediate the actions of NGF on peripheral nociception.

• NGF may also be an important mediator in pain due to visceral inflammation and in neuropathic pain syndromes induced by peripheral nerve irritation.
The selective postnatal ablation of TrkB revealed a substantial reduction of LTP in the hippocampus. These animals became impaired over time with regard to their spatial learning behavior. Extensive behavioral tests of these mice indicate that they behave as if they had a hippocampal lesion.
Animals with low levels of BDNF show learning deficits and, enhanced aggressiveness and hyperphagia, accompanied with weight gain.

These findings are reminiscent of dysfunctions of the serotoninergic system. (BDNF has trophic effects on serotoninergic neurons).

With regard to cognitive functions, it had been noted that the administration of NGF to age-impaired rats significantly ameliorates their cognitive performance, presumably as a result of the action of NGF on the cholinergic forebrain neurons.
• Neurotrophic factors, may be as a co-therapeutic agent in neurodegenerative disease.

• However, in practice, their clinical use is limited because of difficulties in protein delivery and pharmacokinetics in the central nervous system.

• Research is underway on neurotrophic factors and their receptors, To overcome these disadvantages and to facilitate the development of drugs with improved pharmacotherapeutic profiles, together with the development of new technologies for their delivery into the brain.
In Alzheimer’s disease

NTF are dysregulated and, unevenly distributed because of impaired retrograde axonal transport.

- This leads to an accumulation of NGF where it is synthesized (hippocampus and neocortex) and to a loss of NGF in the basal forebrain.

- NGF accumulation in the target regions may lead to increased signaling through p75NTR, which is increasingly expressed in the aged brain, and thus mediates cell death and degeneration of BFCN.
Figure 1. Diagram showing retrograde transport of NGF (in red). NGF is released from the hippocampal neuron and binds to its receptor on the basal forebrain cholinergic neuron (BFCN). NGF and its receptor are enclosed in a signaling endosome. The signaling endosome travels to the cell body and nucleus of the BFCN, where NGF triggers a complex cascade of events that ensures proper cell maintenance and function.
The most important concern regarding a future therapy with NTF is the mode of delivery.

An ongoing gene therapy focusing on NGF-grafted autologous fibroblasts that are implanted into the basal forebrain of AD patients predicts a slower progression of the dementia, some cognitive improvement and sprouting of axons on the site of injection (NTF don’t cross BBB).
II- Extra-neural Roles of NGF

i. Immune and inflammatory system.
ii. Atopic conditions.
iii. Diabetes mellitus and its complications.
iv. CVS.
v. Liver injury and fibrosis.
vi. Non neuronal cancers.
vii. Occular diseases.
viii. Skin diseases.
ix. Sensory-neural hearing loss.
**Immune and Inflammatory System**

- NGF has numerous effects on immune and inflammatory cells that are generally directed at inducing their state of activation and effector functions.

- NGF increases mast cell number, induces mast cell degranulation and increases mast cell expression of cyclooxygenase and interleukin-6.
• NGF activates monocytes, macrophages, and CNS microglia by increasing their phagocytic activity and by inducing their expression of interleukin-1 and lysosomal proteases of the cathepsin family.

• NGF is chemotactic for neutrophils and influences T and B lymphocyte proliferation.

• It is an autocrine survival factor for B lymphocytes, and stimulates immunoglobulin production.
• NGF exerts different actions depending on which type of inflammatory reaction and in which type of tissue it is released.

• There is increasing evidence that during the allergic reaction NGF plays an important role in promoting the inflammatory cascade and tissue repair.

• Serum levels of NGF were increased in several chronic inflammatory or fibrotic disorders such as SLE, psoriasis.
The left side mainly represents the innate and adaptive nerve growth factor (NGF) mediating response. The right side shows NGF effects on cells mainly involved in the T-helper cell (Th) network. All these cells express at least the tyrosine kinase receptor trkA. The straight arrows indicate the NGF effects on both immune and structural cells. Curved arrows indicate well-established autocrine NGF effects. The diagram in the box depicts NGF’s position in the stromal-epithelial interaction.

Innate and adaptive response

Recruitment, proliferation, differentiation, survival, release of mediators/cytokines

Th2 inflammation
• In general, NGF is proinflammatory substance.

• This makes it a double edged weapon.

• It is beneficial for processes of defence against external invaders, and for tissue repair.

• At the same time, it’s needed to antagonize NGF or its receptors in some autoimmune diseases, and in atopic conditions.
NGF and atopic conditions

**Allergic rhinitis and atopic dermatitis:**

- Neurotrophins have been shown to be increased in allergic rhinitis (AR) and atopic dermatitis (AD).

- Expression of trkA-C and p75NTR was significantly higher in peripheral blood eosinophils of AD and AR.

- Apoptosis was significantly inhibited by BDNF, NGF, NT-3/-4 in AD, and AR eosinophils.

- With this study, new pathophysiologic insights are provided into atopic diseases.
**Bronchial asthma:**

- Airway inflammation and hyper-responsiveness are interconnected by bidirectional signalling between immune and neural cells, rather than being independent processes.

- Recent studies have demonstrated that nerve growth factor (NGF) acts as an important mediator in neuro-immune mechanisms of asthma by regulating neuronal plasticity and connecting neuro-immune mechanisms.

- It induces airway inflammation by causing neurogenic inflammation & amplifying the effects of immune cells, via trkA.
• Alveolar macrophages (AM) express NGF and TrkA.

• They are extensively distributed over the bronchial and alveolar surfaces.

• AM release large quantities of inflammatory mediators and pro-inflammatory cytokines such as IL-1β, IL-8, IL-6 and TNF-α playing a role in the initiation and progression of airway inflammation.

• They appear to have a versatile role in the pathogenesis of asthma & this seems to be mediated by NGF/TrkA interaction.
NGF and DM.

- Pacreatic B-cells secrete NGF and express both of its receptors, TrkA and P75 (autocrine action).

- NGF was described to be able to induce pancreatic B-cells (represented by 2 insululinaoma cell lines), to undergo specific changes in gene expression.

- This induces proliferative differentiation of islet cells.

- NGF is supposed to have a role in islet cell development.

- It’s also suggested to be the basis of new therapeutic lines for DM.
• A recent study showed that Na current density in cells treated with NGF increases 97% compared with control cells.

• NGF-induced increase in Na current density is dependent on protein synthesis and mRNA transcription.

• The rise in insulin secretion could be partially explained by the NGF-increased Na current because it can lead to stronger depolarizations that increase calcium entry and exocytosis.

• Blocking endogenous NGF markedly decreases glucose induced insulin secretion.
NGF and Diabetic neuropathy

There are several mechanisms playing a key role leading to neuronal loss in diabetes, including:

- Metabolic disruption resulting from chronic hyperglycemia,
- Altered vascular function leading to defective nutritive support for peripheral nerve fibers;
- \textit{Altered neurotrophic/growth factor availability}
- Autoimmune processes
- \textit{Oxidative stress causing neuronal loss and dysfunction.}
• In normal skin, NGF is produced by basal keratinocytes, and acts via its (trk A) on nociceptor nerve fibers to increase their sensitivity, particularly in inflammation.

• In vitro studies show that keratinocytes express both NGF and trk A, and that NGF may increase keratinocyte proliferation and its own expression via an autocrine loop.

• NGF is reduced in epidermal keratinocytes in human diabetic skin and this decrease has been related to dysfunction of cutaneous sensory fibers.

• This suggests that abnormal availability of target-derived NGF may be responsible for early small-fiber neuropathy
• Low levels of NGF could be due to either decreased production or transport of NGF in diabetes or both, possibly as a result of glucose-induced oxidative stress.

• Another possibility is that structural and biochemical similarities between NGF and the insulin family of peptides, makes it possible that antibodies to insulin may cross-react with NGF and contribute to an effective reduction in NGF available to nerves, thereby contributing to the development of neuropathy.
• It has now been shown that NGF treatment ameliorates diabetic sensory neuropathy in animals.

• Reduction in pain sensation was prevented.

• Treatment of rats with NGF normalized the levels of CGRP and SP peptides in lumbar DRGs.
Recent studies indicate that apoptosis may be a mechanism whereby diabetes induces nerve damage and that diabetes opposes the antiapoptotic mechanisms that normally protect nerves.

Since NGF can rescue neuronal cells from apoptosis, thus, with its reduced levels seen in diabetes, and the concomitant increase in peroxynitrites in local tissues, lead to cleavage of caspases and activation of the apoptotic cascade.
• Hyperglycemia induces an increase in reactive oxygen species (ROS) of cells of diabetic rats in diabetic dorsal root ganglion neurons (DRG) and Schwann cells (SC).

• This is associated with swelling and disruption of the mitochondrial cristae with mitochondrial hyperpolarization, then depolarization.

• This is coupled with cleavage of caspases, and programmed cell death (PCD).

• NGF can block induction of ROS and/or stabilize the mitochondrial membrane, and this inhibits PCD.
Recent studies also showed elevated FAS levels in serum of diabetic patients, and in vitro, this serum could induce apoptosis in neuronal cells.

This indicates that FAS mediated apoptosis may have a role in aetiopathogenesis of diabetic neuropathy.

This apoptosis can be blocked or inhibited by anti-FAS antibody.
• Although systemic administration of NGF, and neurotrophins in general, may prove disappointing, gene therapy might provide an effective mode of delivery for these powerful agents.
NGF and diabetic retinopathy:

- Recent studies reported that NGF exerts a protective action on cells of the visual system, including retinal cells.

- Neurotrophins, are not only important regulators of retinal development, but also play a key role in regeneration of neural circuits in the visual system in retinal degenerative diseases.

- Diabetes increases oxidative stress and tyrosine nitration in the retina.
Increase in \textit{peroxynitrite}, (by tyrosine nitration) correlates with accelerated retinal endothelial cell death, breakdown of the \textit{brain-retinal barrier}, and accelerated neuronal cell death in models of experimental diabetes and neurotoxicity.

It’s suggested that peroxynitrite impairs NGF neuronal survival by \textit{nitrating TrkA receptor} and \textit{enhancing p75NTR expression}.

Neutralizing peroxynitrite restores NGF prosurvival signal in RGCs.
Cardiovascular Actions of Neurotrophins

- During cardiovascular development, neurotrophins and their receptors are essential in the formation of the heart and regulation of vascular development.

- Postnatally, neurotrophins control the survival of endothelial cells, vascular smooth muscle cells, and cardiomyocytes and regulate angiogenesis and vasculogenesis, by autocrine and paracrine mechanisms.
Recent studies suggest the capacity of neurotrophins, via their trk receptors, to promote therapeutic neovascularization in animal models of hind limb ischemia.

Finally, nerve growth factor looks promising in treating microvascular complications of diabetes or reducing cardiomyocyte apoptosis in the infarcted heart.
NGF and Liver fibrosis

• In liver fibrosis, there’s an excessive formation of myofibroblasts.

• Loss of these myofibroblasts is essential for recovery.

• During the early phase of recovery, level of NGF increases.

• This facilitates apoptosis of myofibroblasts as they express P75NTR.

• In the absence of p75NTR signaling, fibrosis develops and resolution is retarded.
NGF and non-neuronal cancers

• In recent years, many findings have indicated that (NGF) is involved in aspects of tumor biology such as growth, invasion and metastasis.

• Many kinds of carcinomas, including HCC, breast, prostate, pancreas, medulloblastomas, and primitive neuroectodermal tumors have been shown to express NGF or its receptors.

• Furthermore, NGF was detected also in stromal fibroblasts in esophageal, prostate and breast carcinomas suggesting the presence of paracrine mechanism.

• Roles for NGF in invasion and migration were suggested for prostatic and pancreatic cancer cells, and its role in metastasis and progression were suggested for malignant melanoma, prolactinoma and small cell lung cancer cells.
**HCC:**

- NGF and its receptor trkA are not detected in the liver of healthy people.

- They are both expressed in the liver of the patients with liver cirrhosis and/or hepatocellular carcinoma (HCC).

- NGf was found to be secreted by HCC cells.

- TrkA was demonstrable in the walls of hepatic arteries, which may contribute to the development of tumor-associated arteries.
Furthermore, the stromal tissues adjacent to HCC contained abundant nerve fibers, which may be also caused by NGF.

Therefore, NGF produced by HCC cells was considered to have a paracrine action toward non-tumor cell components in the HCC tissues.

These important discoveries indicate that NGF is playing a critical role in the development of liver cirrhosis and its progression towards HCC.

Based on this discovery, inactivation of the NGF or blockage of its specific receptor trkA in diseased liver may suppress or prevent the development of liver cirrhosis and HCC.
Prostate cancer:

- It has been demonstrated that the simultaneous high expression of TrkA might confer a proliferative advantage to PCa cells.
- NGF induced TrkA activation and stimulated cell proliferation of PCa cells.
- This event was only partially inhibited by the pan Trk inhibitor.
**Malignant melanoma:**

- Primary and metastatic melanoma cells secrete all NTs.

- They also express (p75NTR) and (Trk) receptors.

- The inhibition of Trk receptors prevents proliferation, indicating that autocrine NTs are responsible for this effect.

- NT-3, NT-4, and nerve growth factor (NGF) induce cell migration, with a stronger effect on metastatic cell lines.

- Transfection with p75NTR small interfering RNA inhibits NT-induced melanoma cell migration.

- These results indicate that NTs play a critical role in the progression of melanoma.
**Breast cancer:**

- Analysis of a series of biopsies revealed widespread expression of NGF in the majority of human breast tumors, with anti-NGF immunoreactivity concentrated in the epithelial cancer cells.

- Immunodeficient mice xenografted with human breast cancer cells and treated with anti-NGF antibodies displayed inhibited tumor growth and metastasis.

- Such treatments directed against NGF, induced a decrease in cell proliferation with a concomitant increase in apoptosis of breast cancer cells and an inhibition of tumor angiogenesis.

- Together, these data indicate that targeting NGF in breast cancer may have therapeutic ramifications.
NGF and ocular diseases

• Recent studies proved that topical ocular adminstration of NGF, caused marked clinical and cytological improvement in different ocular conditions including:
  
  dry eye, superficial keratitis, and corneal ulcer.

• This report demonstrated that NGF exerts an antibacterial and anti inflammatory activity.

• Results suggest a potential therapeutic use of NGF in different ocular inflammatory surface.
• Corneal neurotrophic ulcers associated with impairment of sensory innervation of the cornea may lead to loss of vision, and there is no effective treatment for these ulcers.

• With topical application of NGF, healing began 2 to 14 days after the initiation of treatment and all patients had complete healing after 10 days to 6 weeks of treatment, and has all restored their corneal integrity.

• There were no systemic or local side effects of treatment.
NTs in pathological skin conditions

- NTs are involved in the pathogenesis of inflammatory skin diseases characterized either by cell loss (stress-induced alopecia) or hyperproliferation (inflammation, wound healing, psoriasis, atopic dermatitis) via neurogenic inflammatory processes or by inducing a misbalance in cytokine production and autoimmune responses.
Systemic administration of NGF for the treatment of peripheral neuropathies has to cope with the problem of pain induction.

To develop adequate pharmacological tools for targeting only the clinically desired elements of NT signalling in the skin and to be able to dissociate the antiapoptotic and proliferation-stimulatory effects of NGF on epidermal keratinocyte from its proinflammatory ones is an important challenge.
• Progress in these areas is a prerequisite for the development of satisfactory new treatment modalities for several major pathological skin conditions, such as alopecia, psoriasis, atopic dermatitis, and tumor growth, based on the well-targeted modulation of selected aspects of NT signalling.
Neurotrophic factors as therapeutic agents for SGN rescue

- The application of neurotrophic factors to the cochlea has proven highly successful in animal studies in terms of protecting SGNs from degeneration, and results from studies combining neurotrophins and cochlear prostheses are particularly promising.

- In terms of clinical application, however, the side effects and risks associated with neurotrophin administration must always be considered, especially in view of the free communication between the cochlea and the CSF.
• Cell-based therapies, perhaps in conjunction with gene transfer, are likely to provide a safe and efficient means of delivering neurotrophic factors to the cochlea at physiologically relevant levels, and over long periods of time.

• Neurotrophic factors therefore retain high potential as therapeutic agents for the rescue of SGNs in deafness, for application both in combination with cochlear implants, as well as for innervating regenerated hair cells.
Nerve growth factor and its role in rheumatoid arthritic pain