Signaling Mechanism III: Neurotrophic Factors

NS201B Lecture

UCSF Neuroscience Graduate Program

Wednesday, Nov 30th, 2016

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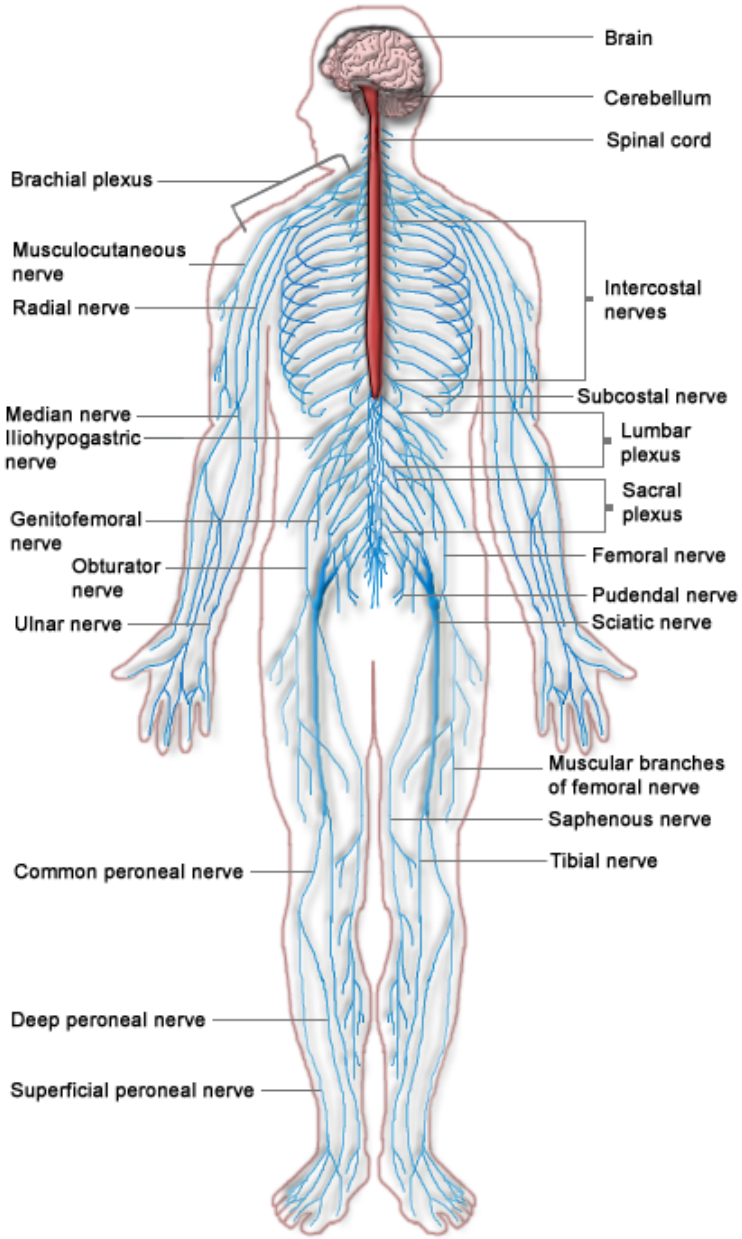
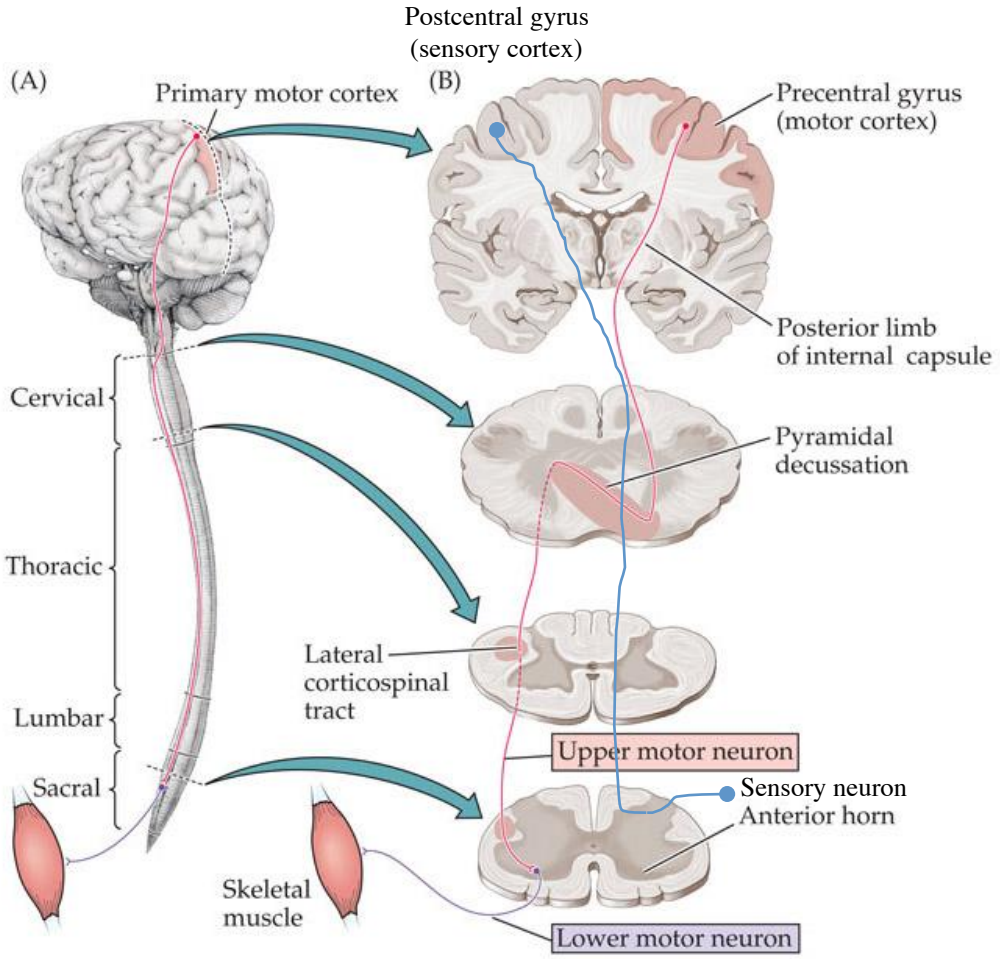
The challenges of building a functional nervous system





Topics for discussion

I. **Programmed cell death**
   - Mechanisms of PCD
   - Why PCD?

II. **Where & how do neurons obtain pro-survival cues?**
   - The discovery of NGF
   - Roles of neurotrophic factors in neural development

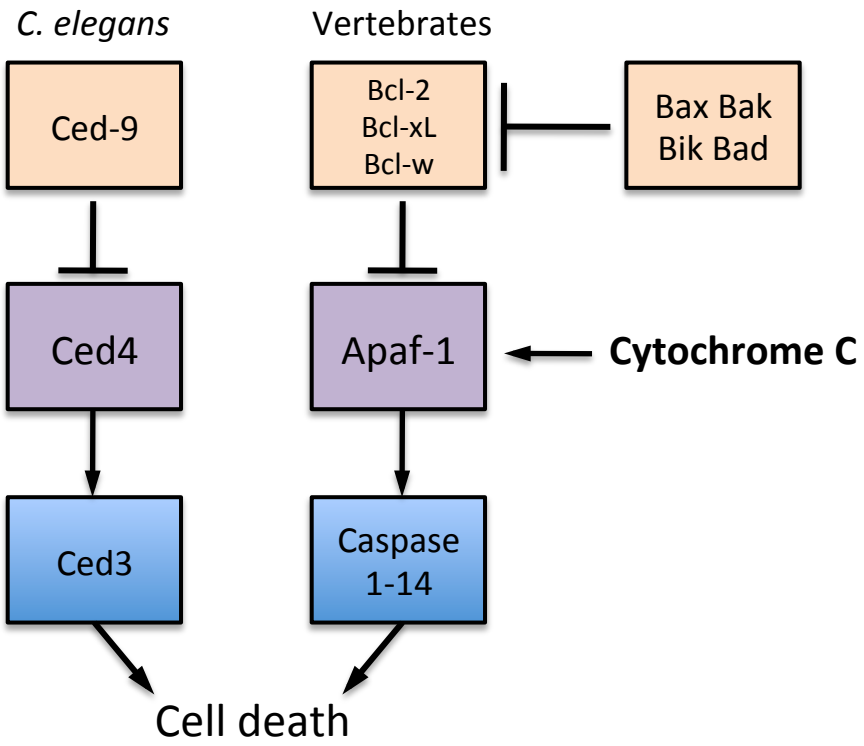
III. **Signaling mechanisms of neurotrophins**
   - Neurotrophins in axonal growth & maintenance
   - Signaling endosomes

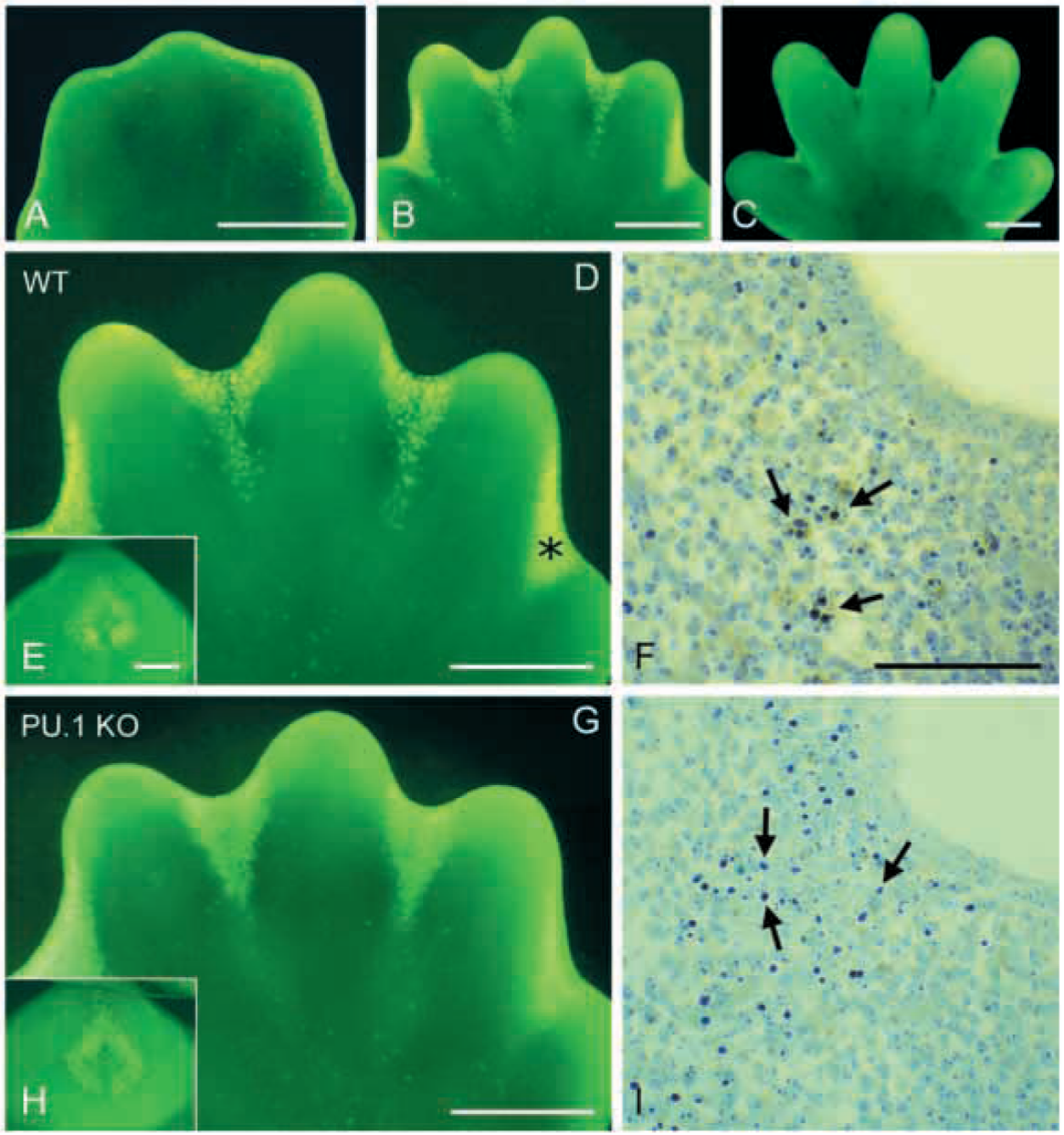
IV. **Neurotrophins and synaptic functions**
   - BDNF-TrkB signaling in synaptic functions

V. **Implications in neuropsychiatric disorders**

Programmed cell death in the nervous system

- Genetic analysis of PCD in *C. elegans*: evidence that intrinsic factors control the decision
- "Social controls" of cell death and proliferation regulate the timing and a fine balance of these events
- Intrinsic signals and extrinsic signals from neighboring cells
- Critical roles of neurotrophins in programmed cell death
- Caspases involved in apoptosis
  - Initiators: caspases 2, 8, 9, 10
  - Executioner: caspases 3, 6, 7
- Caspases involved in inflammation

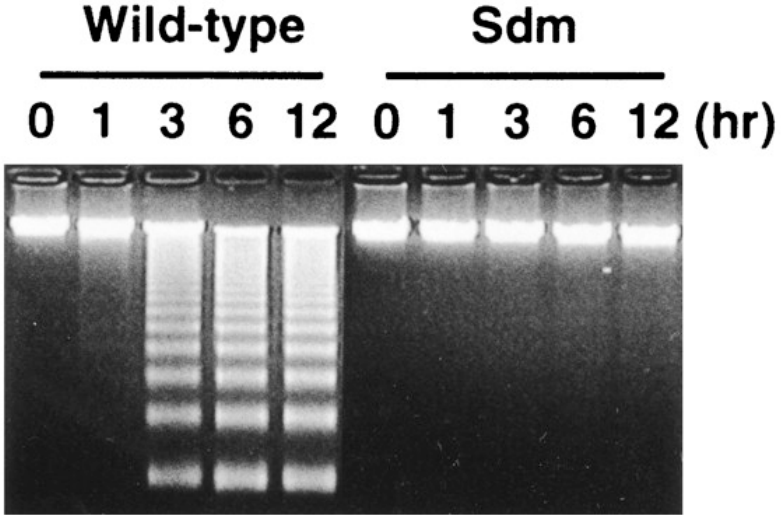


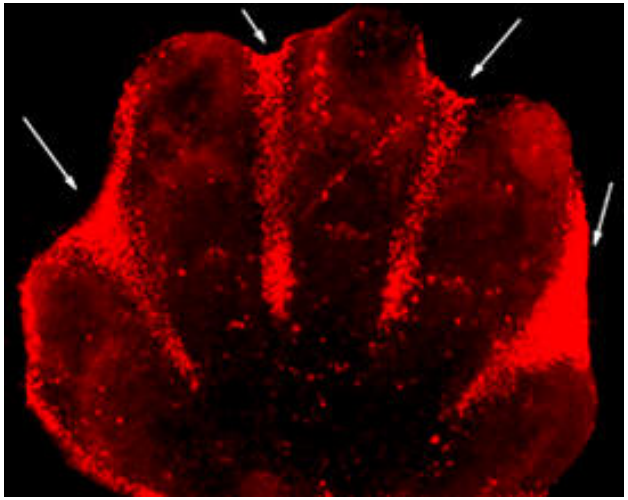


(Wood et al., Development 127: 5245-52, 2000)

Visualizing Cell Death

- DNA ladder in gel electrophoresis
- TUNEL: TdT-mediated dUTP nick end labeling
- Annexin V: anticoagulant protein specifically binds to phosphatidyl-serine ("eat me" signal)
- Activated caspase 3 Ab
- Histology





(McIlroy et al., Genes & Dev 14: 549-558, 2000)
(http://grupos.unican.es/apoptosis/pagina3.htm)

Morphological features of necrotic cell death and PCD

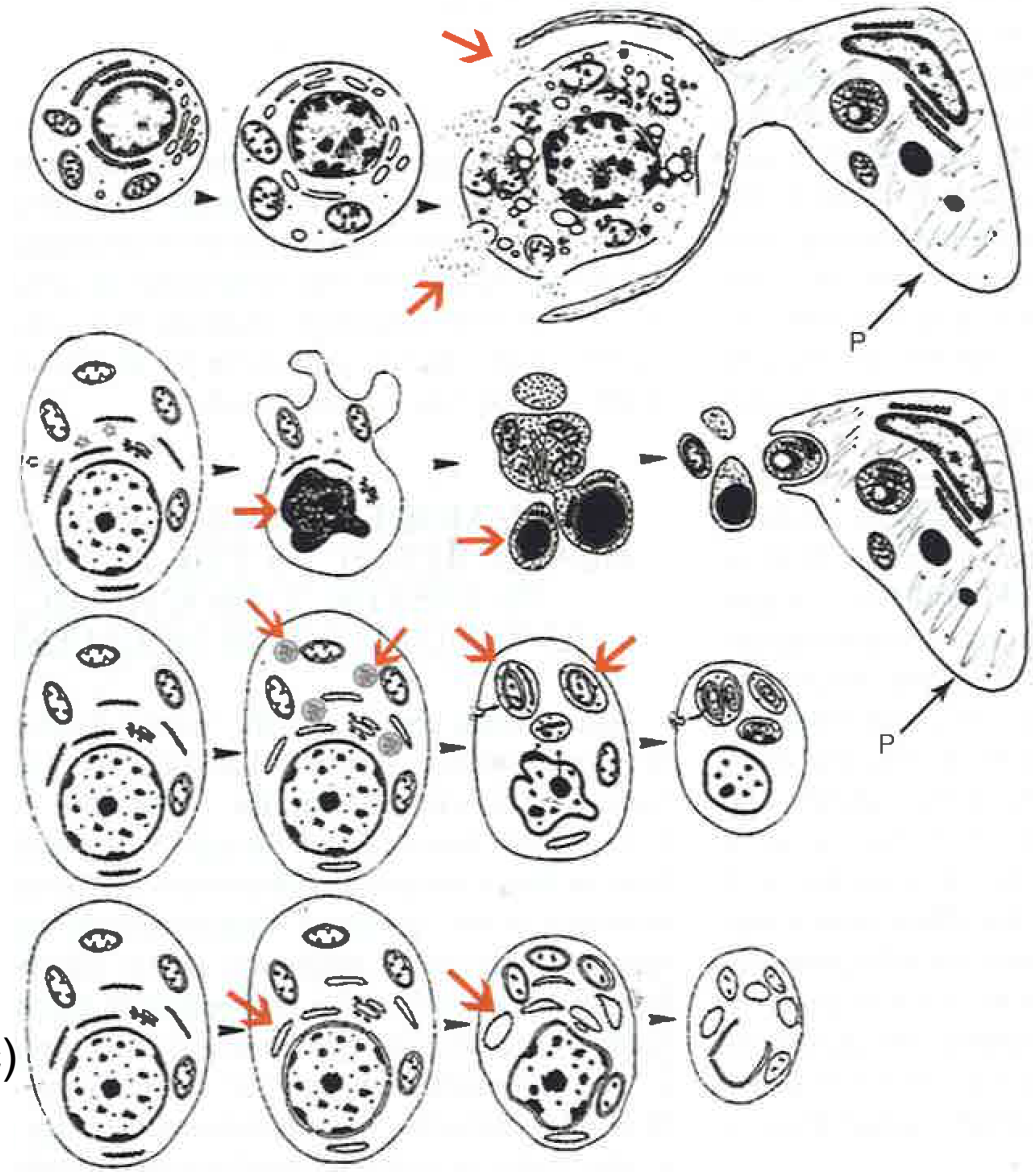
Necrosis

PCD

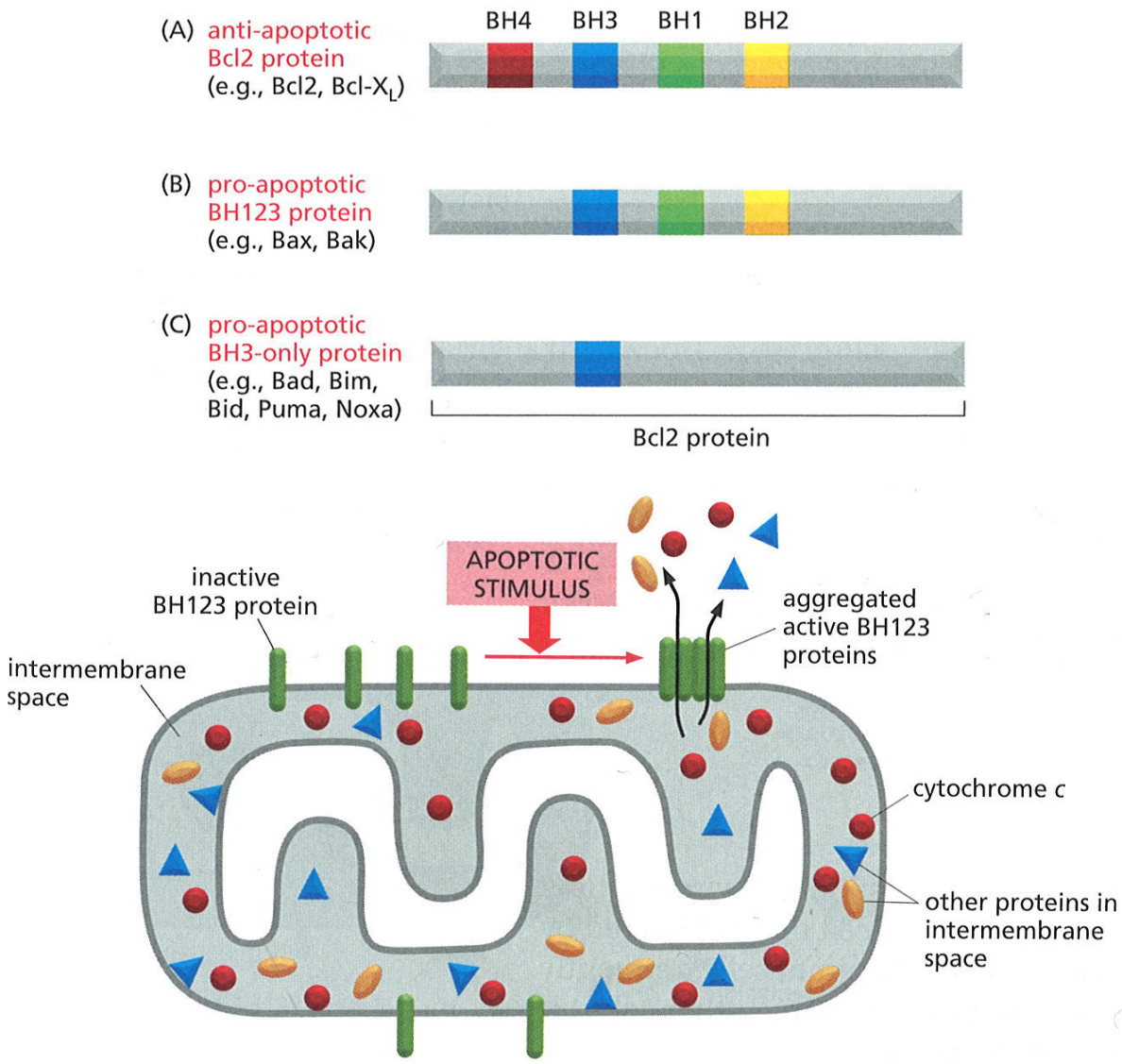
Type I
(apoptosis
or “nuclear”)

Type II
(autophagic)

Type III
(cytoplasmic)



Intrinsic Pathway of Apoptosis Depends on Mitochondria



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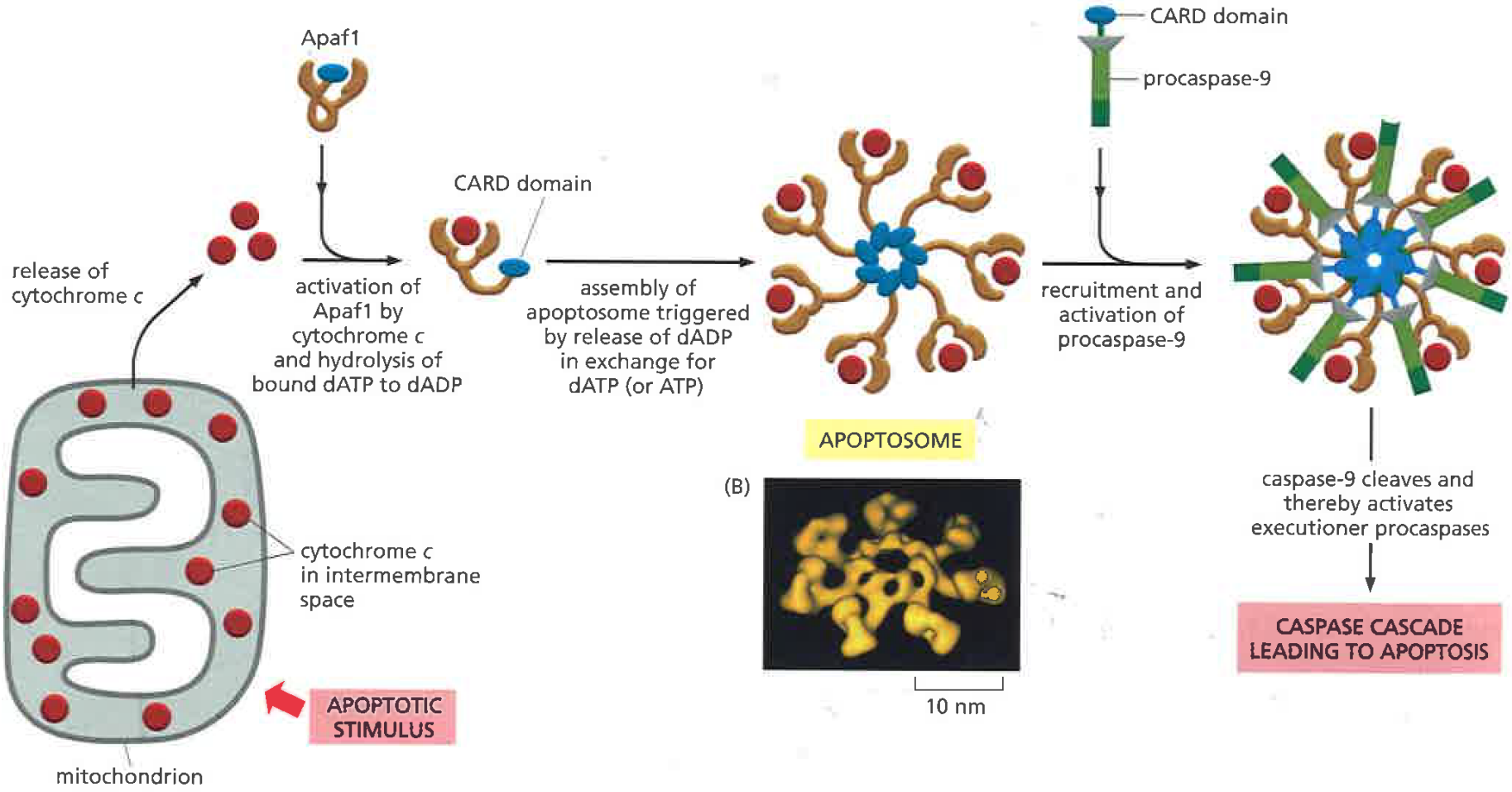
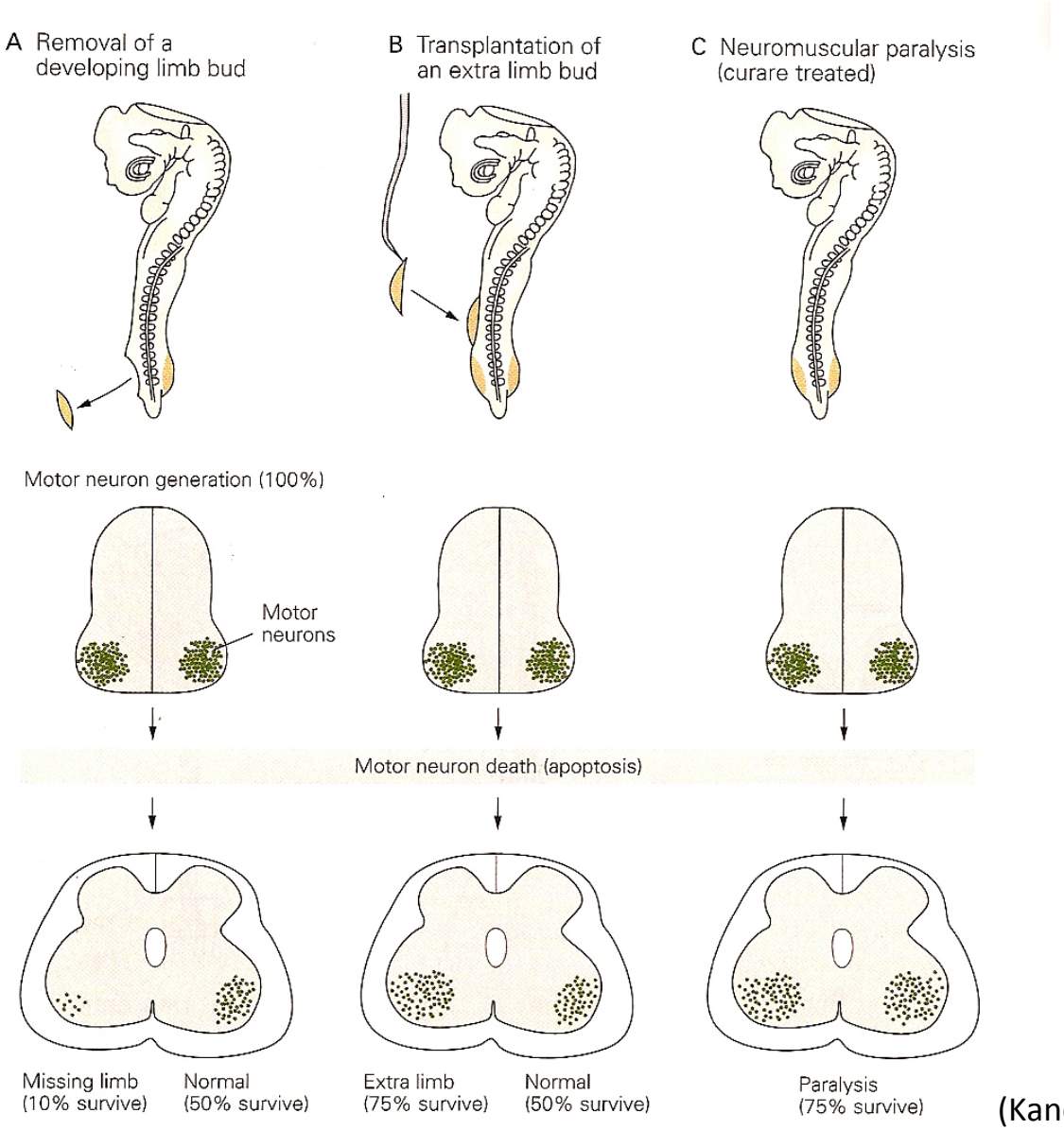


What does PCD do in the nervous system?

- Regulating the size of progenitor cell population
- Production of excess neurons may serve as “buffer”
- Pattern formation and morphogenesis
- Differential remove of neurons in males and females
  - Sexually dimorphic nucleus in spinal cord and brain
- “Get rid of” progeny of a specific sublineage no longer needed
- Negative selection of an inappropriate phenotype
- Removal of cells that serve transient functions
- System-matching by optimizing innervation between neurons
- “Error correction” of misrouted, mis-positioned or mis-targeted neurons
- Experience-dependent plasticity
- Removal of harmful or damaged neurons

Classical chick limb ablation & transplantation experiments



(Kandel et al., Figure 53-11)

The Discovery of NGF

Viktor Hamburger

- Relationship between the developing nervous system and the limbs
- Wing bud extirpation and limb transplantation experiments (1934) – focus on motor columns in spinal cord and sensory ganglia (minor)
- Possible mechanisms for “Hypoplasia in LMC”:
  - (a) effects on the proliferation of motor progenitors
  - (b) failure of young neurons to migrate into LMC
  - (c) failure to induce differentiation of other motor neurons
  - [never recognized “cell death” as a potential mechanism]

The Discovery of NGF

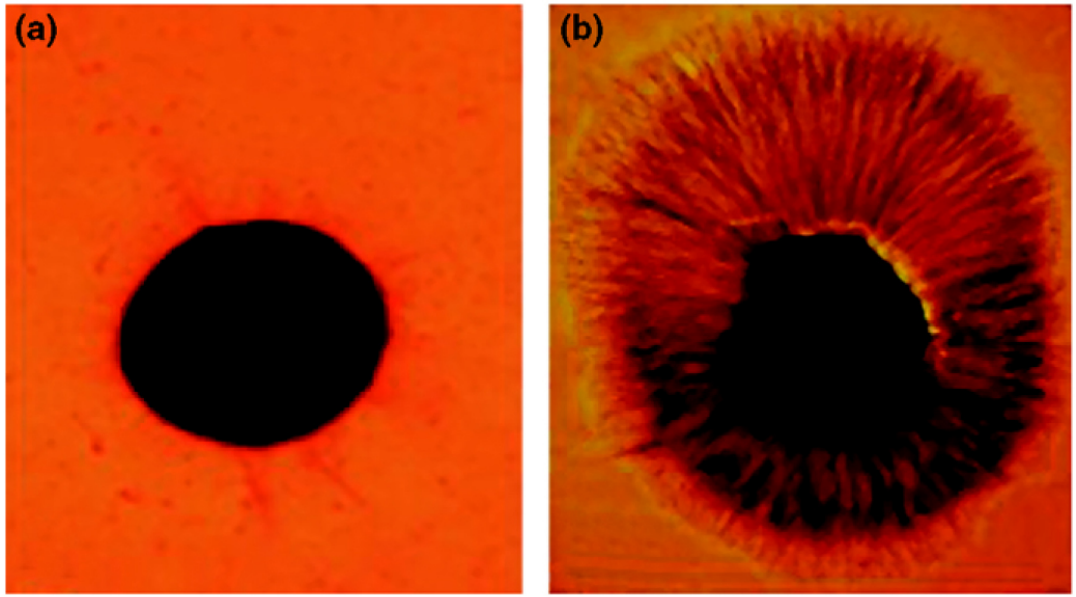
Rita Levi-Montalcini

- Read Viktor Hamburger's 1934 paper on the effects of wing bud extirpation on the development of CNS in chick embryos (June 1940)
- Repeated Hamburger's experiments and extended it in 3 significant ways: (1) analyzed the effects of hind limb bud removal, focusing on spinal sensory ganglia and motor columns of spinal cord, (2) examined embryos over a wider range of survival periods, (3) Nissl and modified silver method (De Castro's modification of Cajal)
- Major conclusions: (1) "Hypoplasia" is the direct consequence of the death of previously differentiated motor neurons, (2) clear degeneration of cells in spinal ganglia (thanks to the silver stain)
- So, "not proliferation or differentiation, but rather survival"

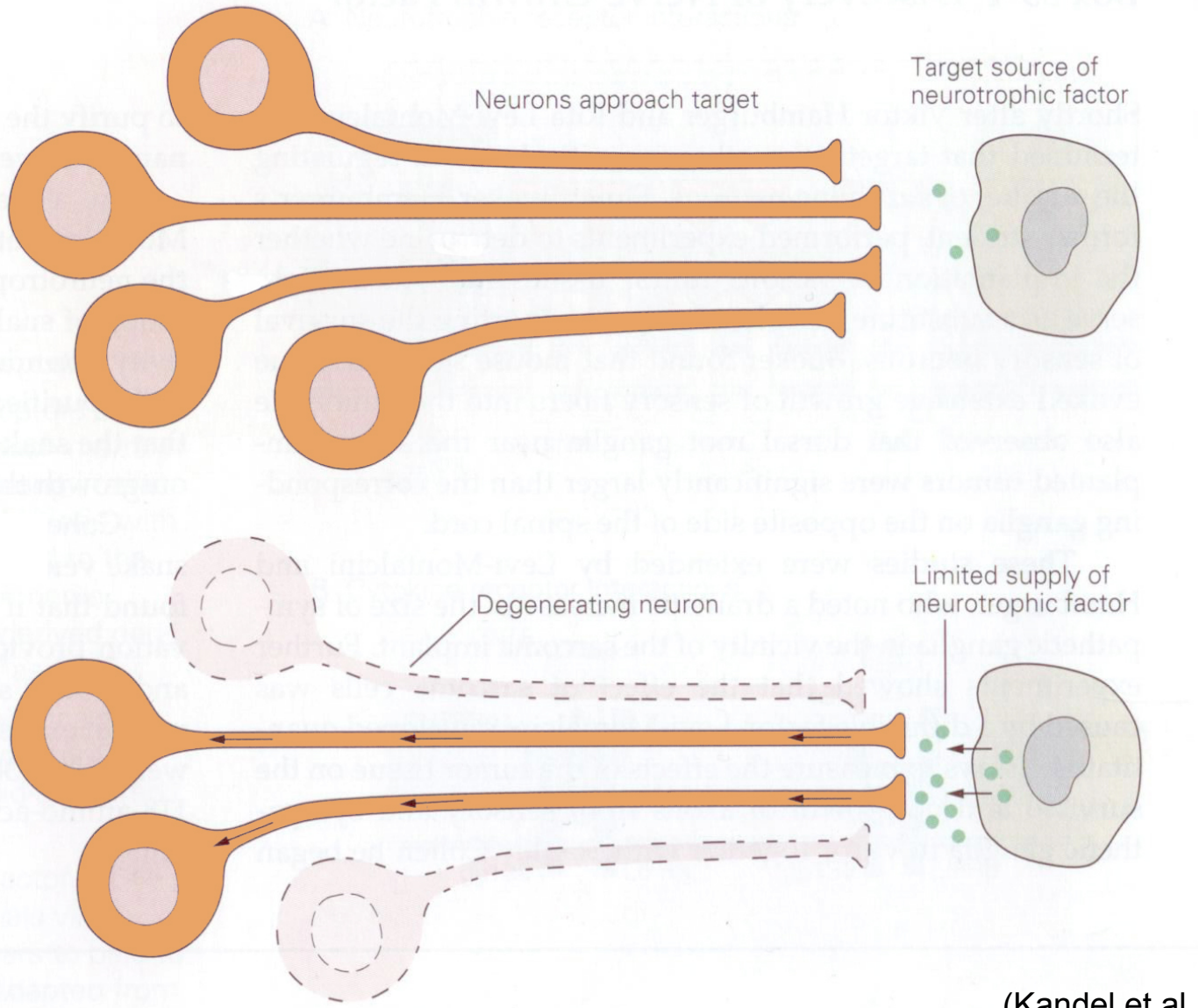
The Discovery of NGF

- from limb bud extirpation, to sarcoma, and to golden halo

- Evidence that the neural growth-promoting effects of sarcomas are due to a diffusible factor
- Nerve fibers have no direct connection with the tumors
- A bioassay for the nerve growth-promoting factor and the discovery of the “Golden Halo”
- Stanley Cohen and the isolation of NGF



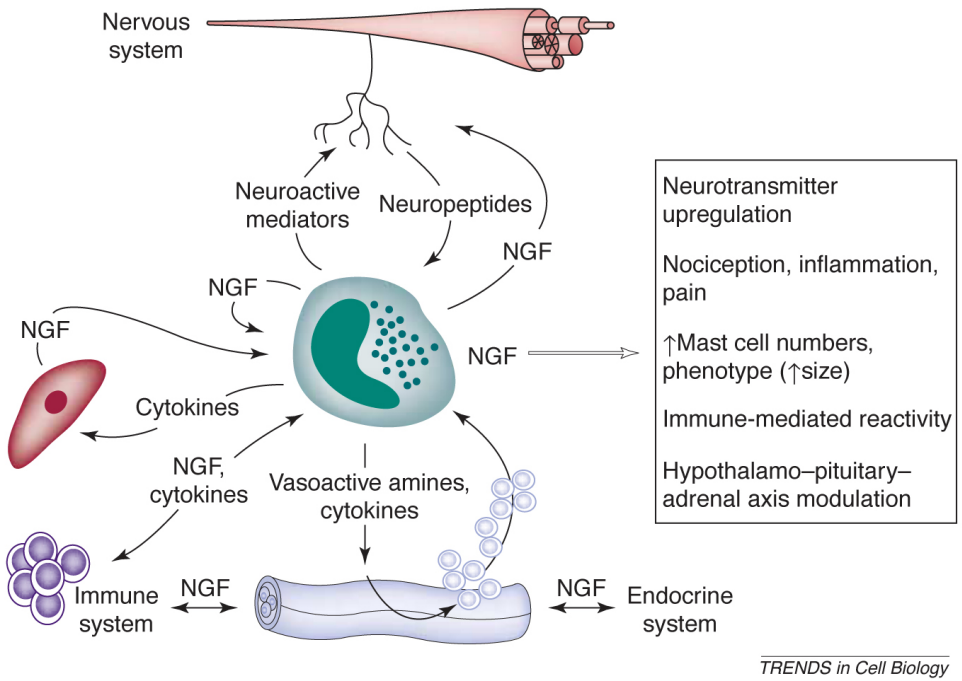
The neurotrophic factor hypothesis



(Kandel et al., Figure 53-12)

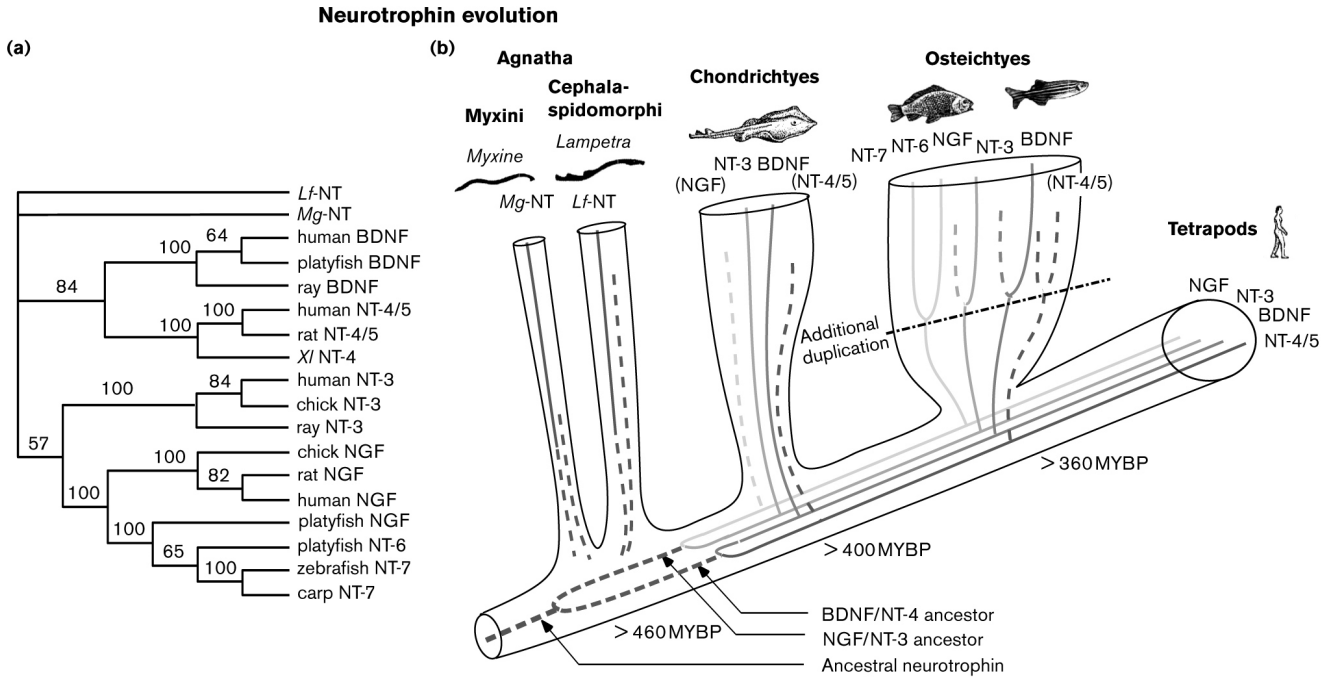
Diverse functions of NGF in the nervous system

- Required for survival of sensory and sympathetic neurons
- Expressed in the target tissue
- Captured at nerve terminals by endocytosis and transported retrogradely through axons to neuronal cell bodies and trans-synaptically on targets of the central afferents of these neurons
- Under injury conditions: NGF is synthesized and released by mast cells, Schwann cells and fibroblasts (paracrine mechanisms)



The family of neurotrophins

- NGF, BDNF, NT-3, NT-4/5: Evolutionarily conserved
- **Provide permissive signals that support survival of neurons**
- **Provide instructive cues to promote and guide axonal outgrowth**
- Transduce sustainable signals that regulate neuronal functions



(Hallböök, Curr Op Neurobio 1999)

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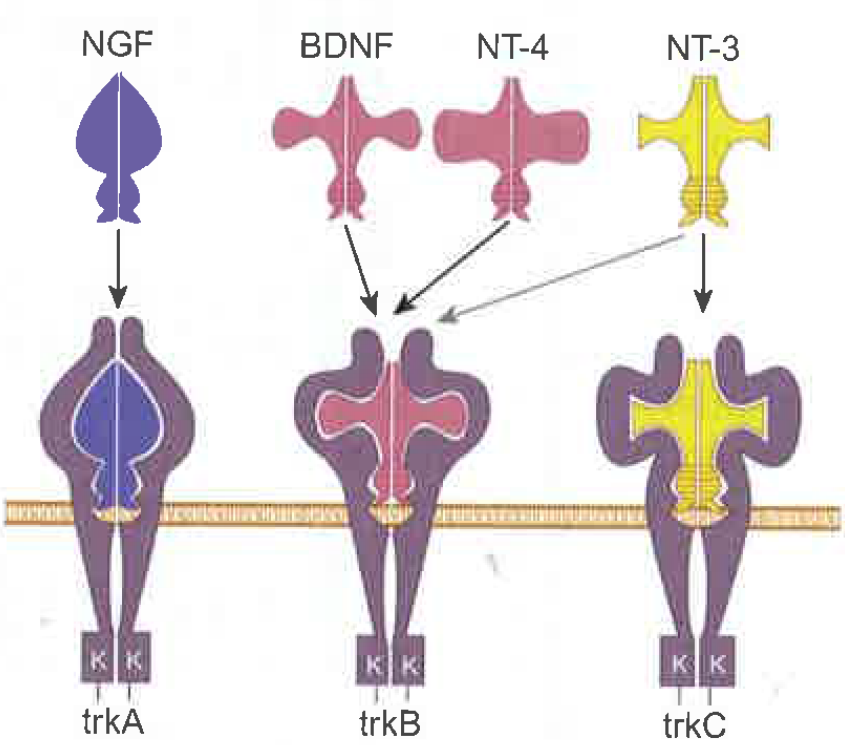
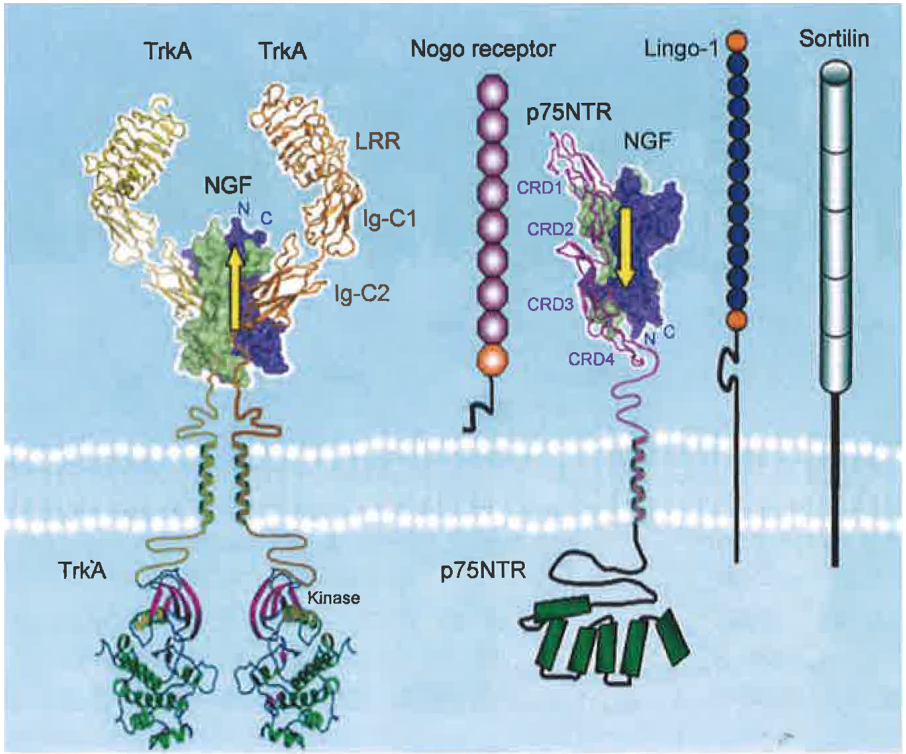
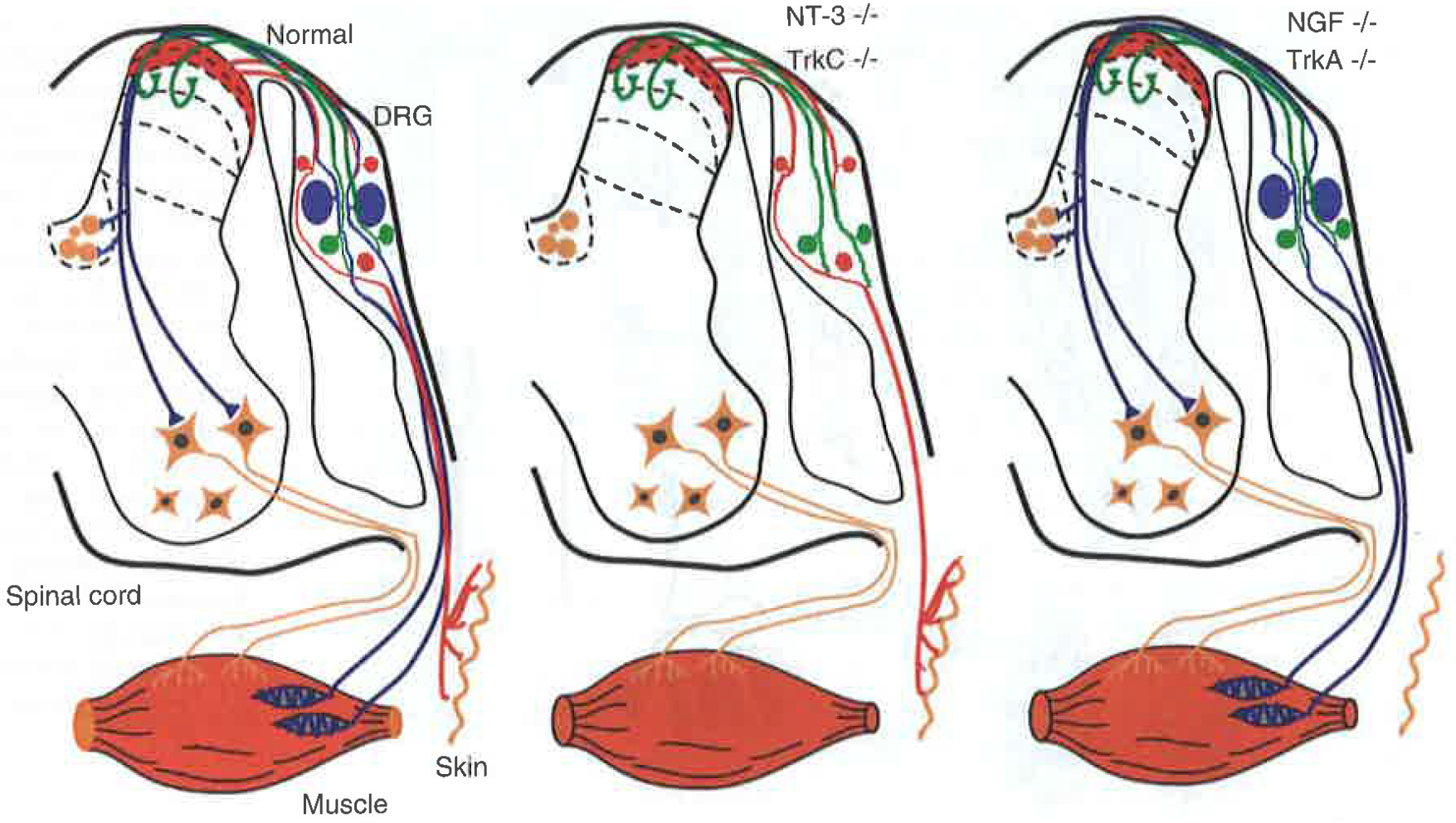
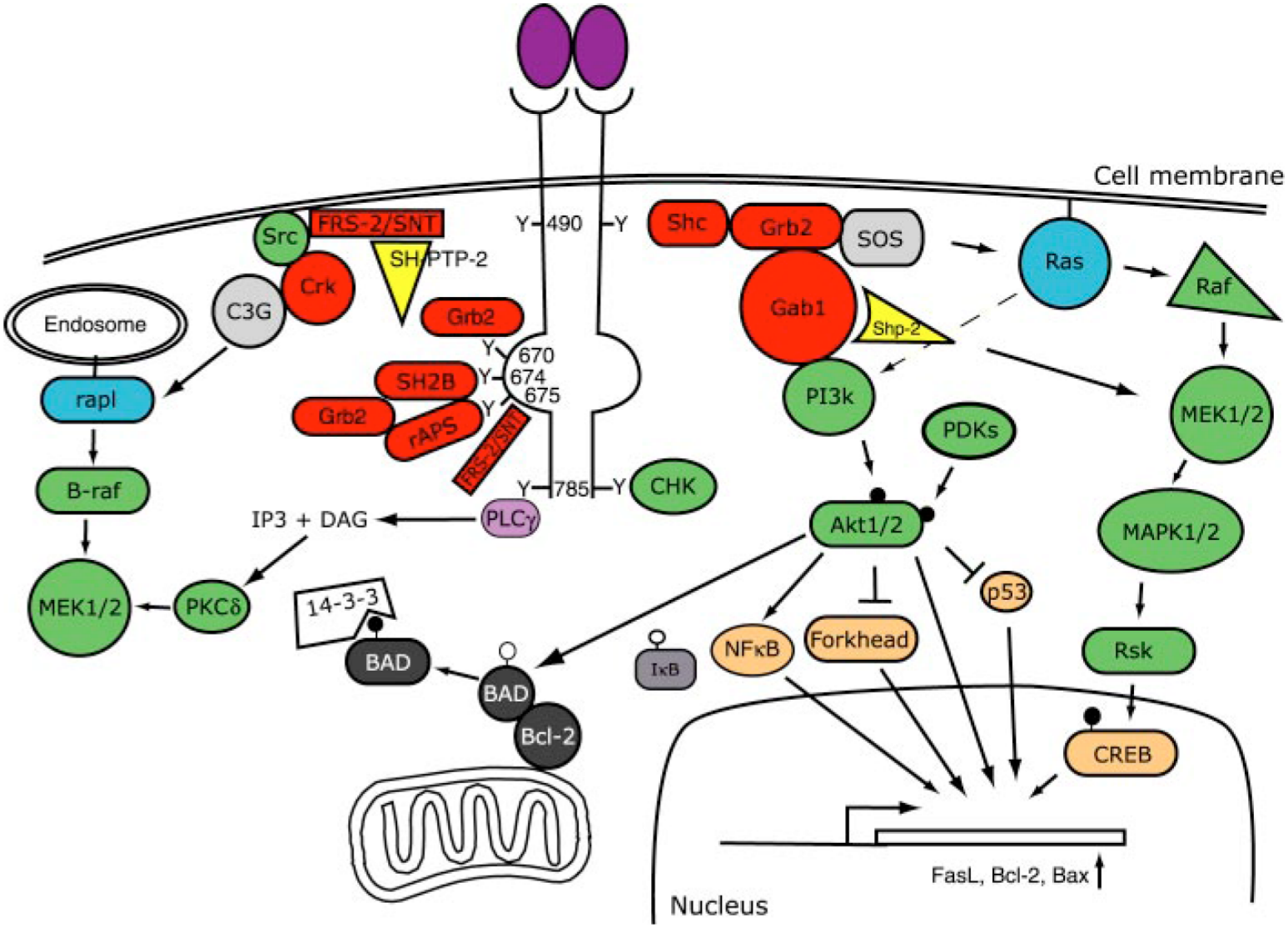




Phenotypic similarities between NGF/TrkA & NT-3/TrkC null mutants



Signaling mechanisms of neurotrophin receptors, Trk



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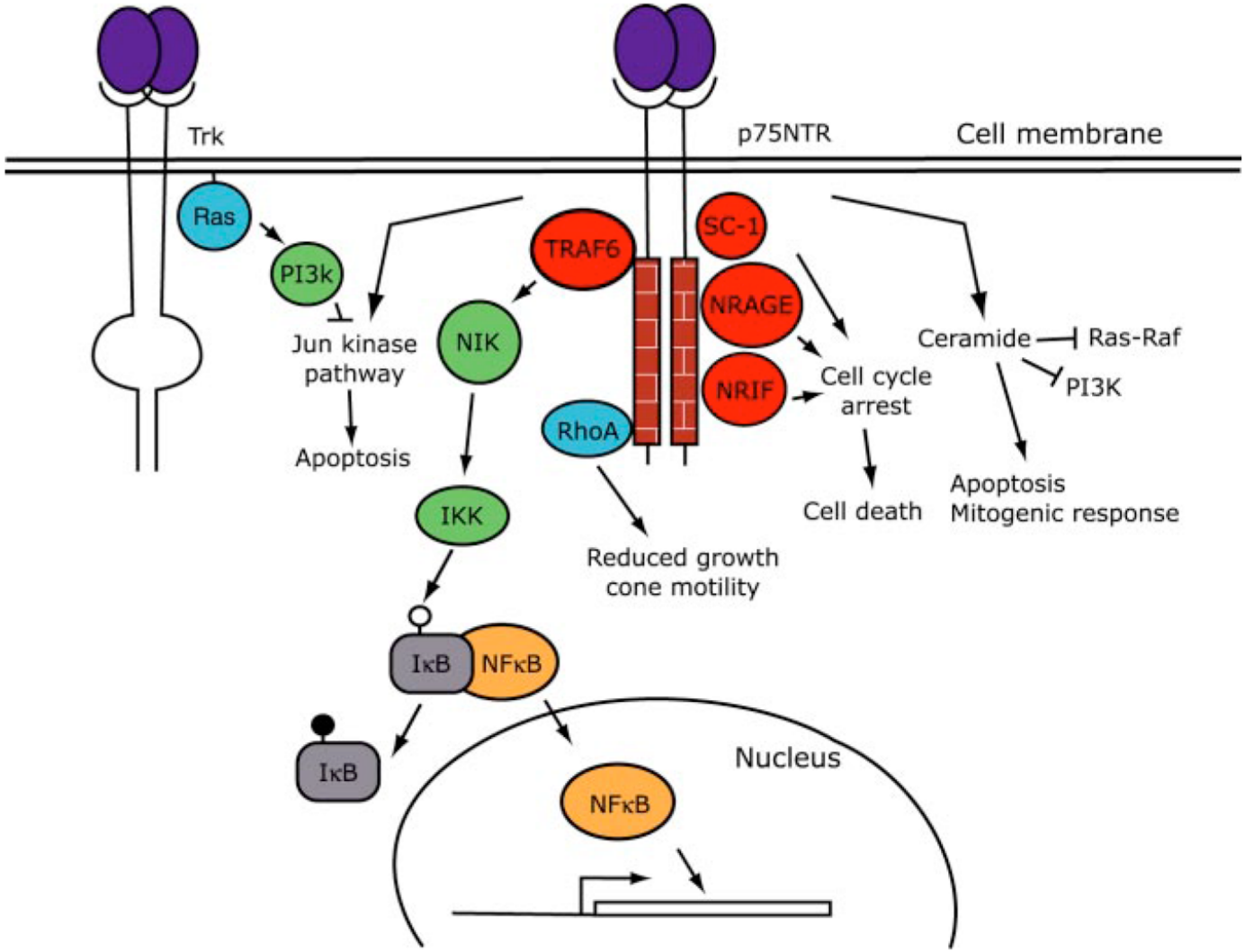
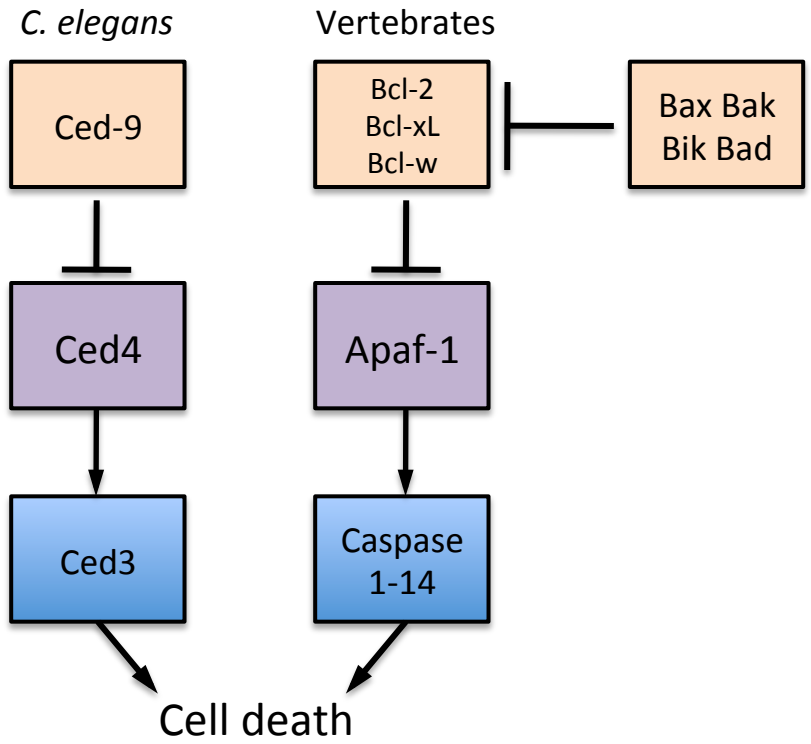


Signaling mechanisms of neurotrophin receptors, Trk

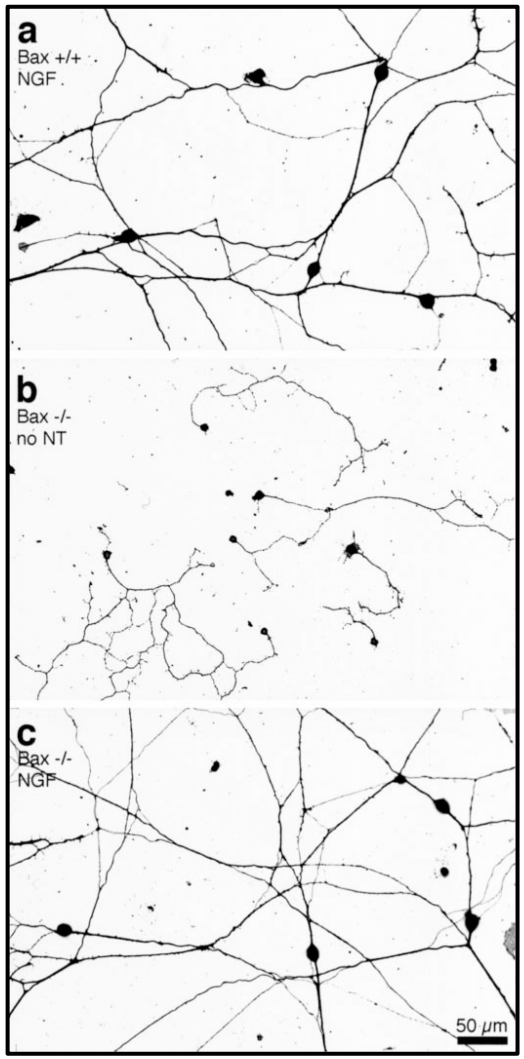
- What do we learn from the signaling pathways of neurotrophin?
  - Specificity
  - Compartmentalized signals
  - Positive and negative regulations
  - From cell membrane to nucleus

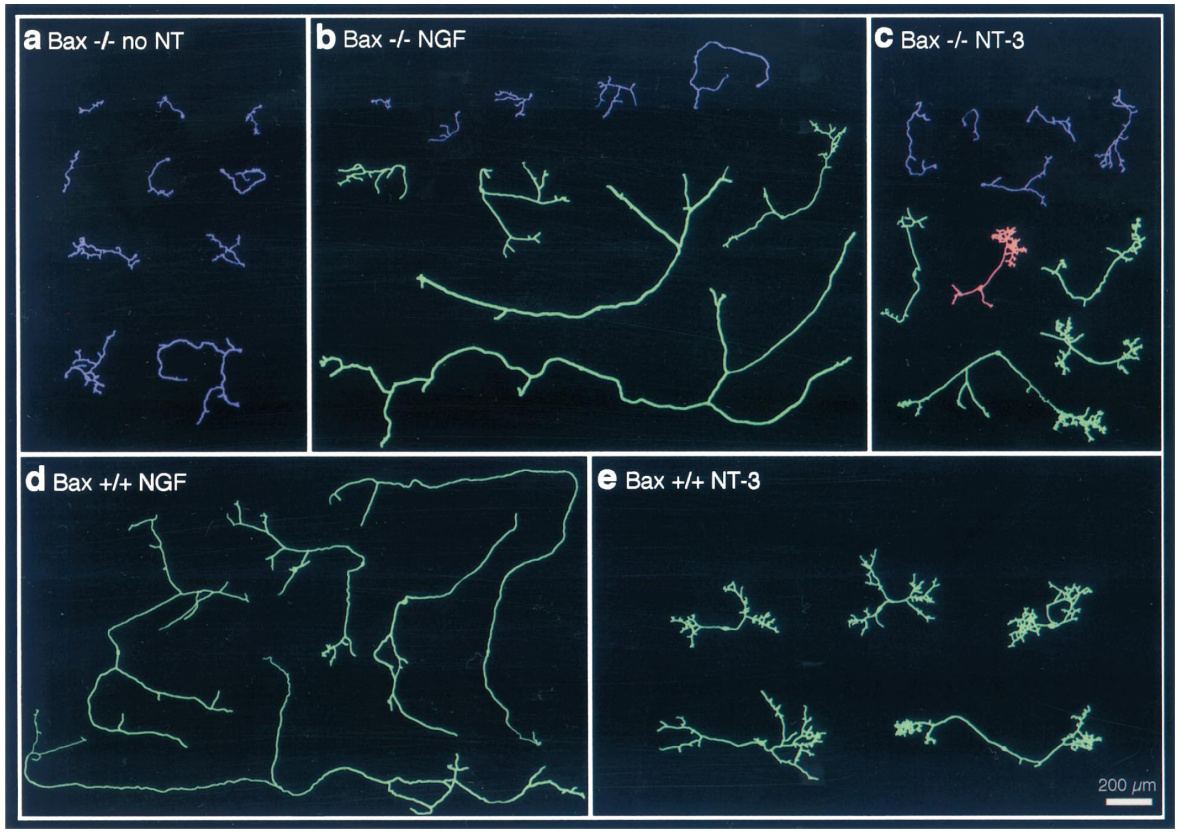
Signaling mechanisms of neurotrophin in axonal growth & maintenance

- Can neurotrophins provide instructive cues to promote axonal outgrowth, extension, branching and increase in caliber?
- How to dissociate the survival function of neurotrophins from other important roles, e.g. axonal growth and maintenance?



Signaling mechanisms of neurotrophin in axonal growth & maintenance

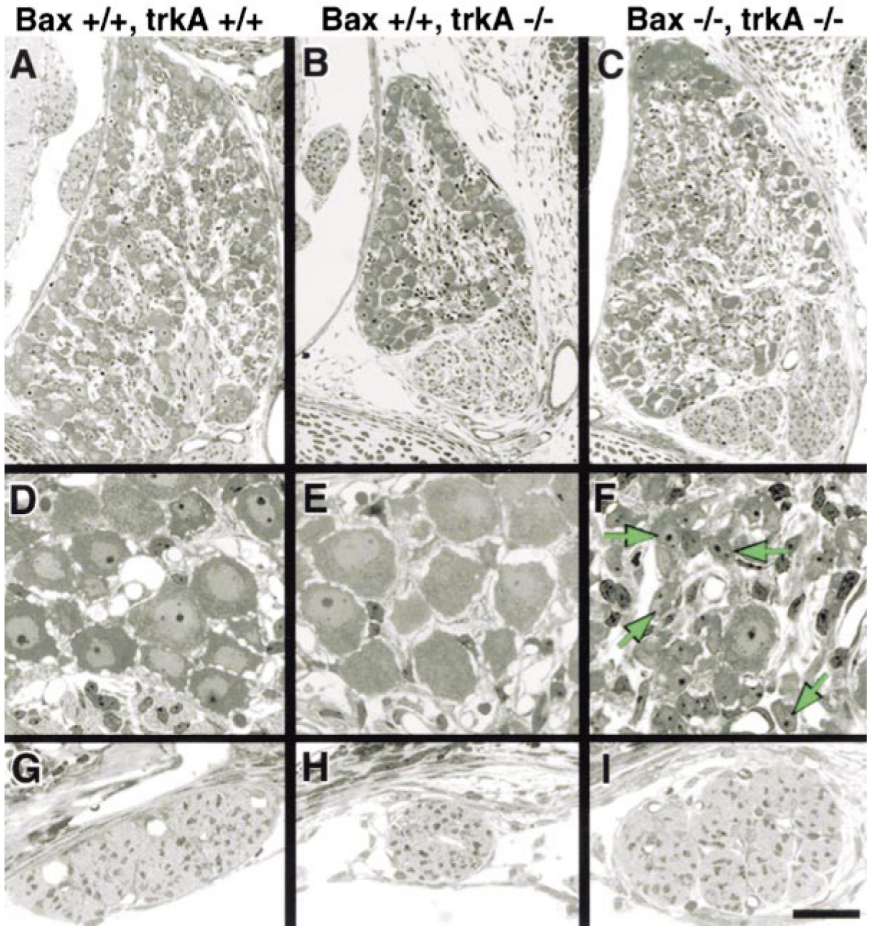


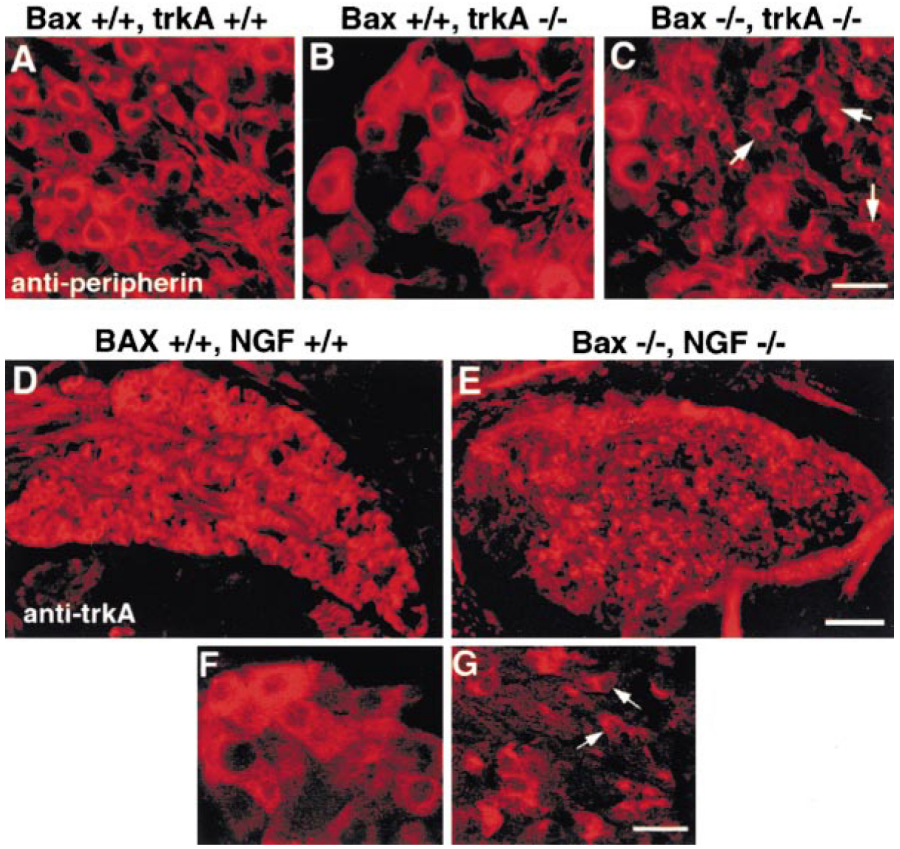


(Lentz, Knudsen, Korsmeyer and Snider, J Neurosci 1999)

Signaling mechanisms of neurotrophin in axonal growth & maintenance

- Loss of Bax protects sensory neurons in *Ngf* KO mice from cell death



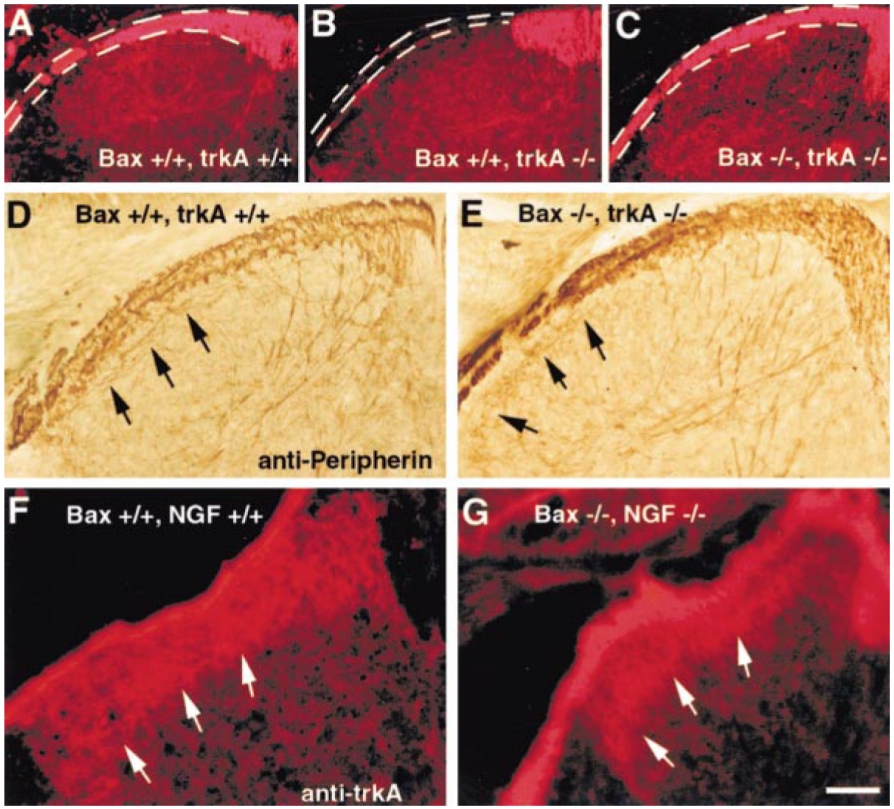


(Patel et al., Neuron 2000)

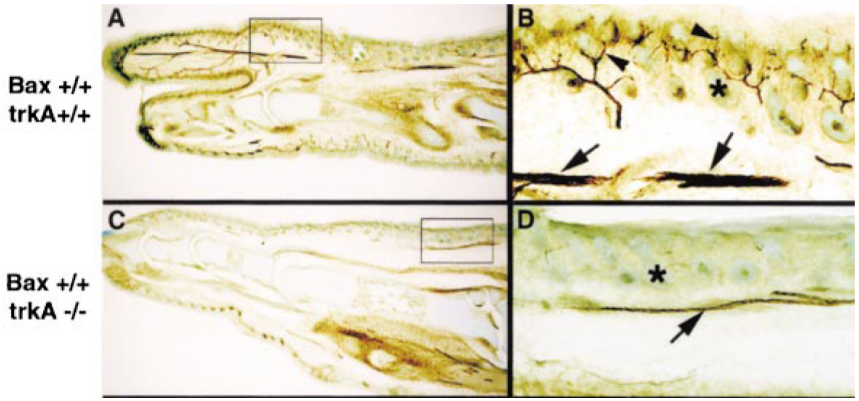
Signaling mechanisms of neurotrophin in axonal growth & maintenance

- Essential role of NGF in peripheral, but not in central, axon innervation in sensory neurons

Central projection

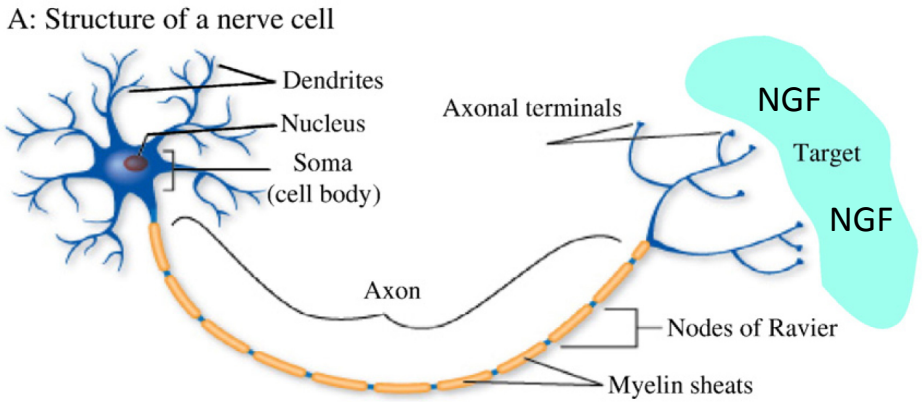


Peripheral projection



(Patel et al., Neuron 2000)

How to propagate signals from nerve terminals to the cell body?

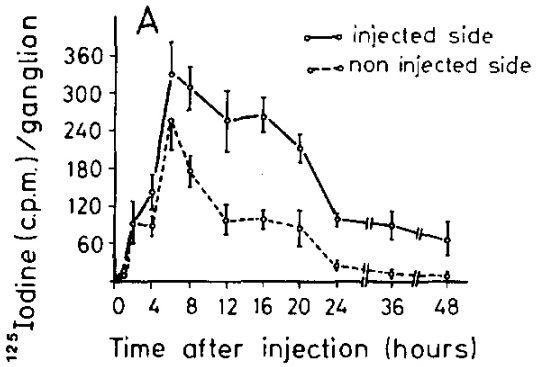


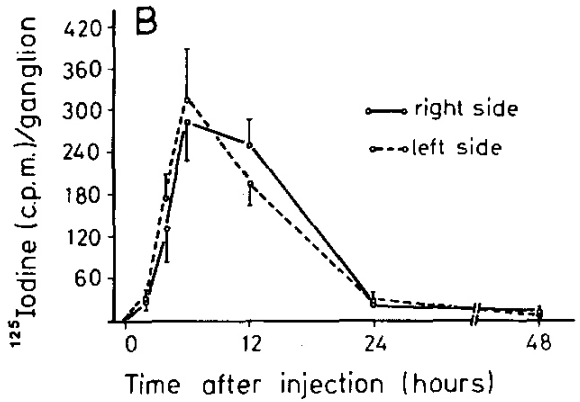
- The wave model
- The signaling effector model
- The signaling endosome hypothesis
    - specific for NGF & receptors
    - highly organized & compartmentalized
    - sustainable
- NGF-independent retrograde signaling

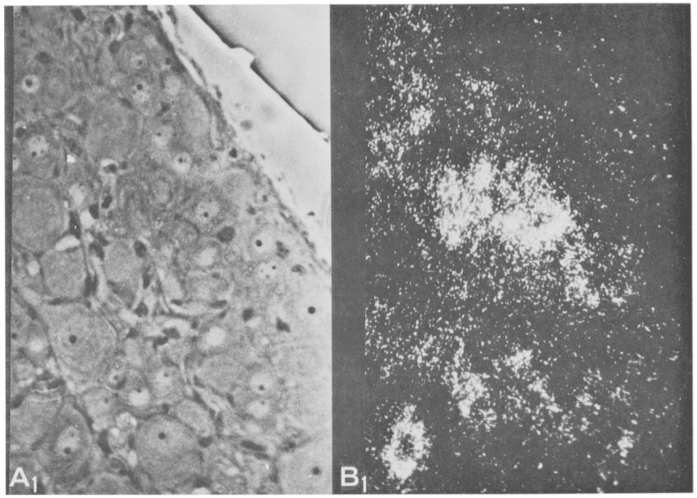
Evidence for retrograde transport of neurotrophins

- NGF injection in peripheral tissues leads to uptake in sensory ganglia
- Cell biology and biochemical evidence in PC12 cells and sensory neurons identifying NGF-TrkA complex in vesicular structures, including clathrin-coated vesicles (CCV), endosomes and multivesicular bodies (MVB)
- NGF-TrkA+ vesicles contain activated Ras-MAPK pathway
- Compartmentalized chamber cultures
- Quantum dot technology: real time imaging the dynamics of NGF-TrkA containing vesicles

Evidence for retrograde transport of neurotrophins







Treatment	*Time before ^{125}I-NGF injection (h)*	*Difference in ganglionic ^{125}I (counts/min)*
Albumin in saline	2	217 ± 22
Transection of postganglionic nerve fibres	0	10 ± 34***
Colchicine 2 µl (20 mg/ml)	0	229 ± 42*
	2	70 ± 31**
	12	25 ± 37***

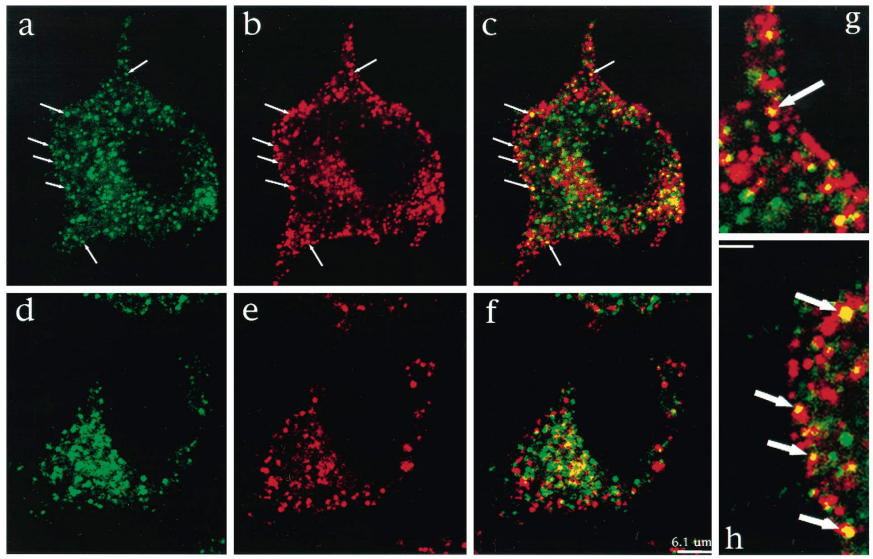
* Differs from 0, $P < 0.005$, but not from control animals, $P > 0.05$.
** Differs from 0 and control animals, $P < 0.05$.
*** Differs from control animals, $P < 0.01$, but not from 0, $P > 0.05$.

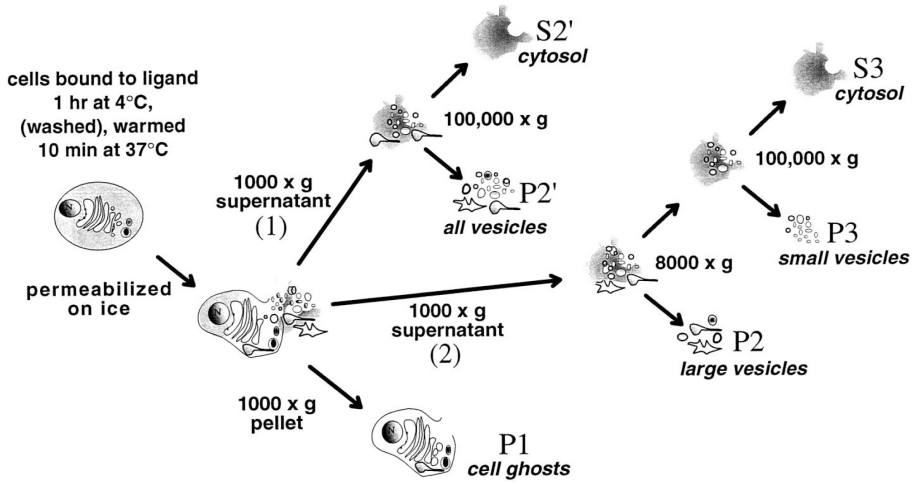
• Retrograde axonal transport of NGF is estimated at a rate of about 2.5 mm/h & depends on a colchicine-sensitive mechanism

(Hendry et al., Brain Res 1974)

Evidence for endocytosis of NGF-TrkA complex after NGF treatment

TrkA/Clathrin heavy chain





(Grimes et al., J Neurosci 1996)

- PC12 cells: permeabilized, homogenized and fractionate by centrifugation
- All fractions confirmed by EM
- **P1:** cell ghosts
- **P2:** mitochondria, dense bodies, ribosomes & clear uncoated vesicles
- **P3:** more homogeneous population of organelles
    1. Small, uncoated with a dense core
    2. Clear, coated vesicles
    3. Clear, uncoated with variable sizes, mitochondria and dense bodies

Evidence for endocytosis of NGF-TrkA complex after NGF treatment

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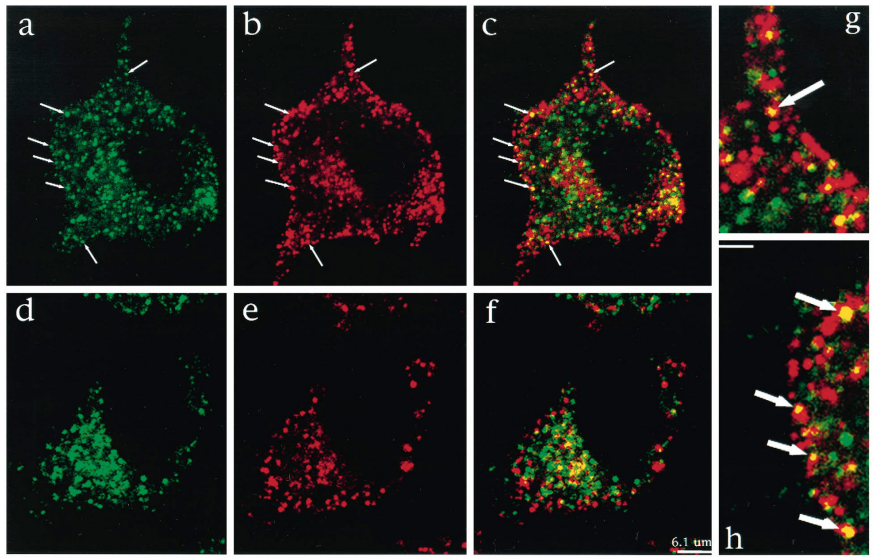
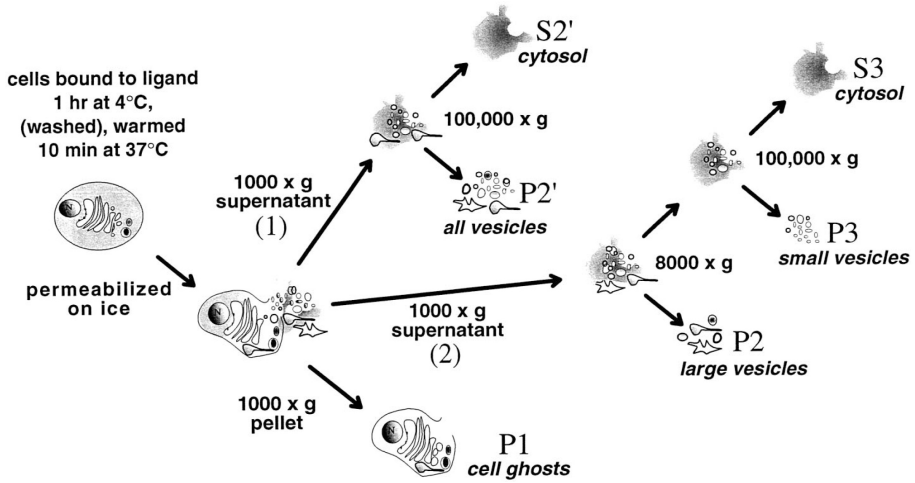
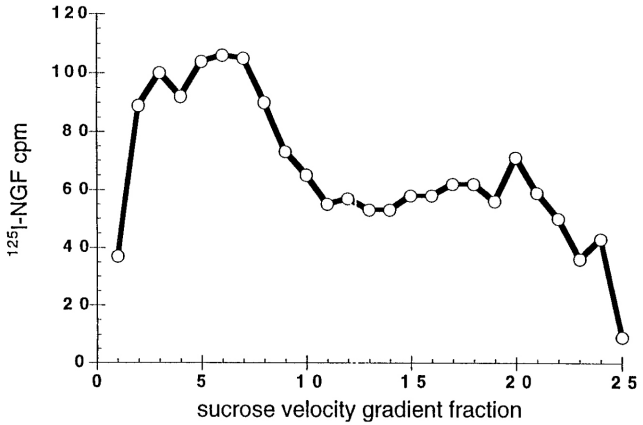
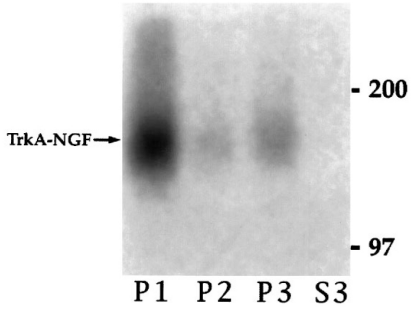




(Grimes et al., J Neurosci 1996)

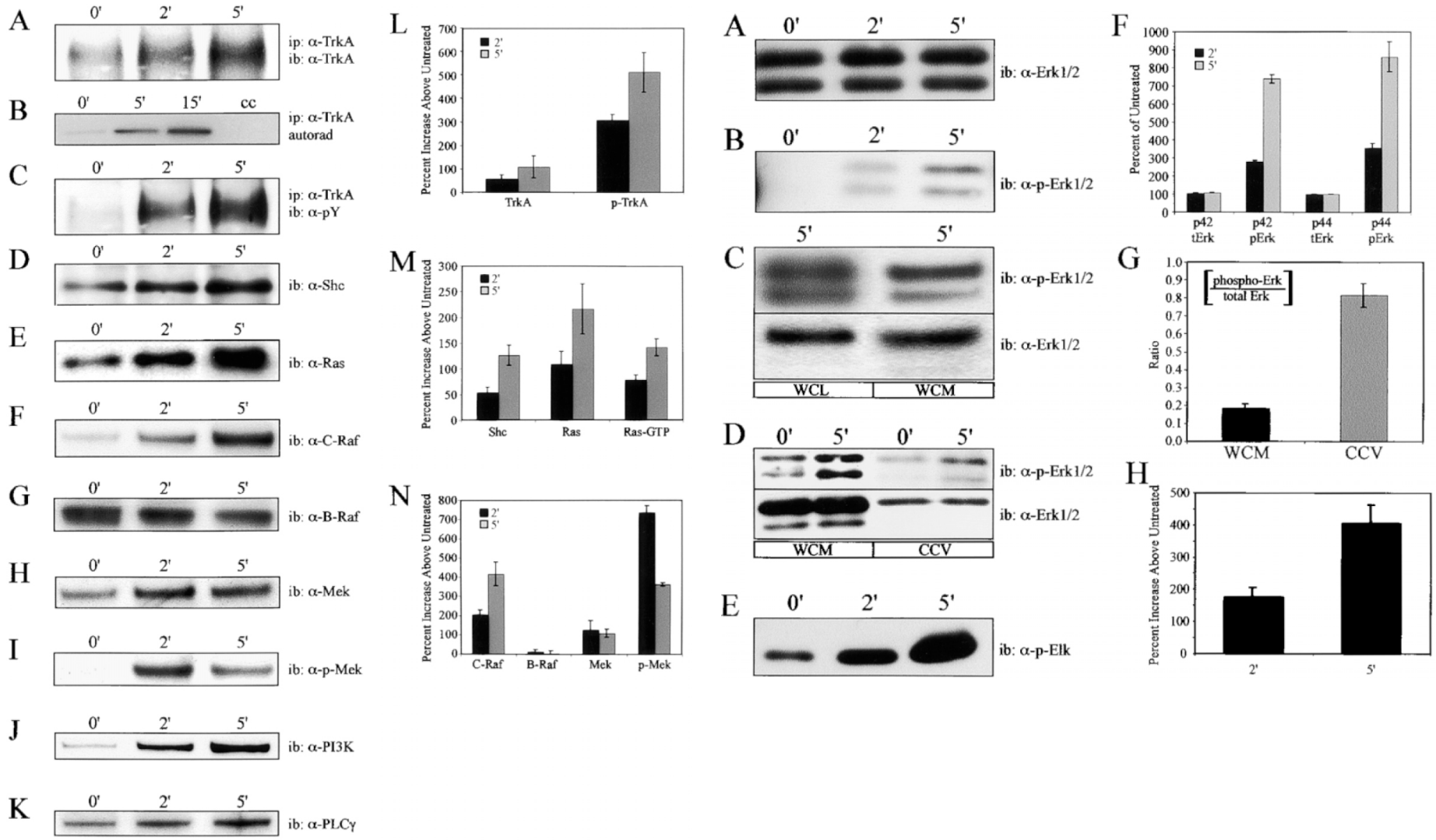




125I-NGF cross-linked to TrkA

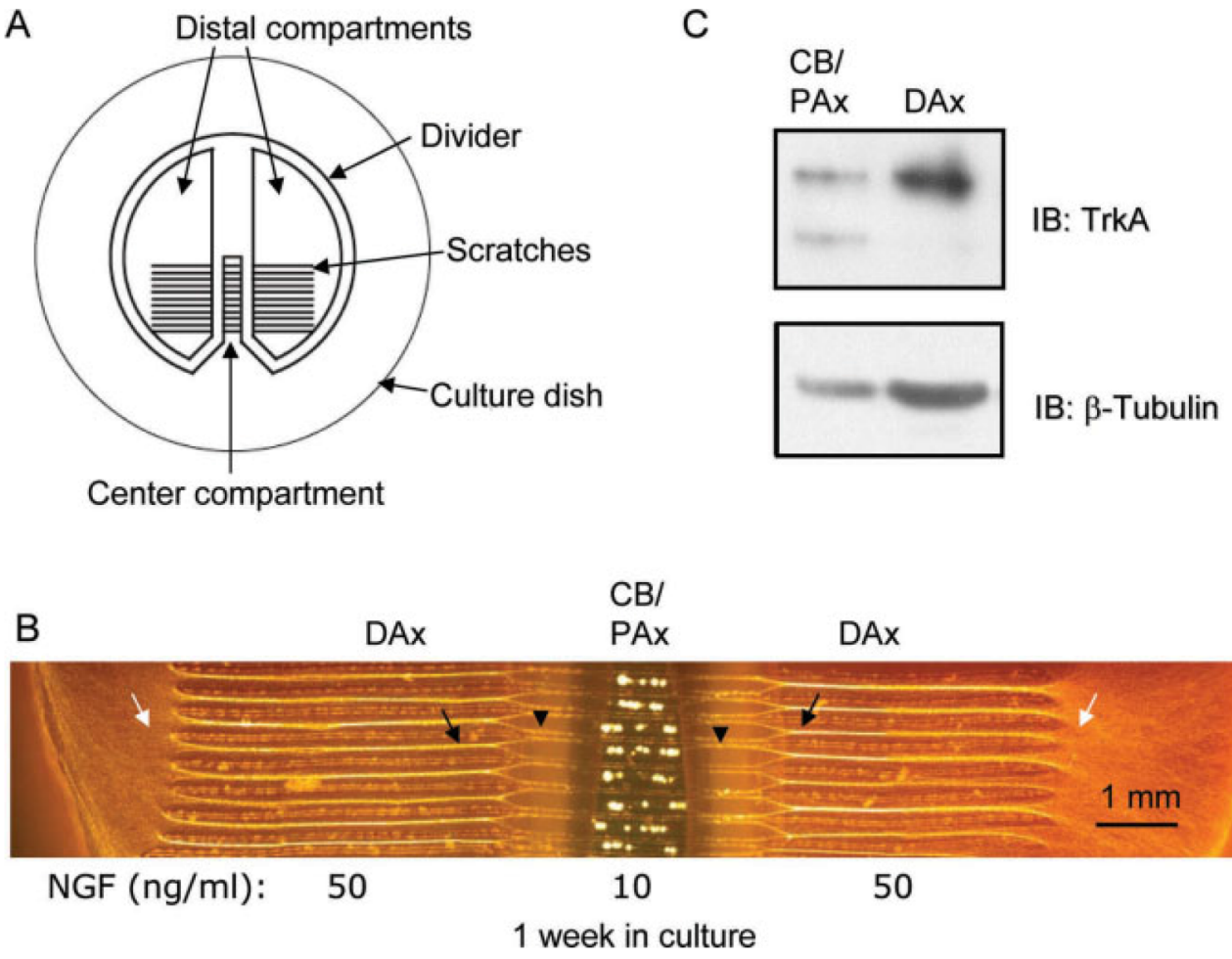
- Following NGF treatment, NGF is bound to TrkA in endocytic vesicles
- TrkA is tyr-phosphorylated and associated with PLCγ
- Signaling endosomes contain active receptors

Clathrin-coated vesicles contained NGF bound to TrkA and activated signaling proteins of the Ras-MAP kinase pathway



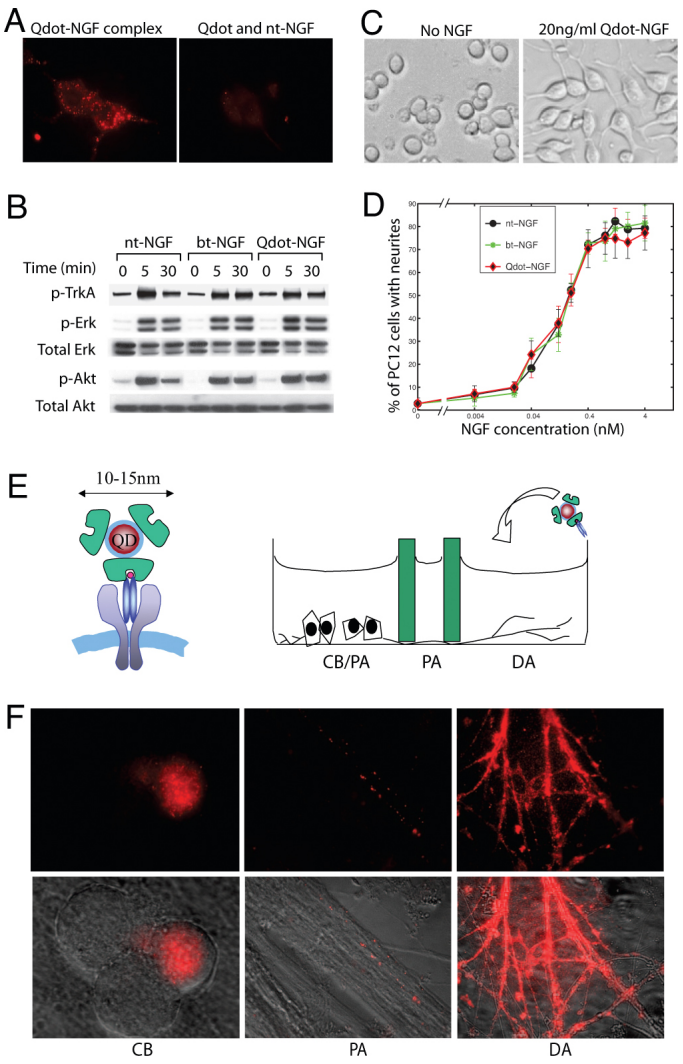
(Howe et al., Neuron 2001)

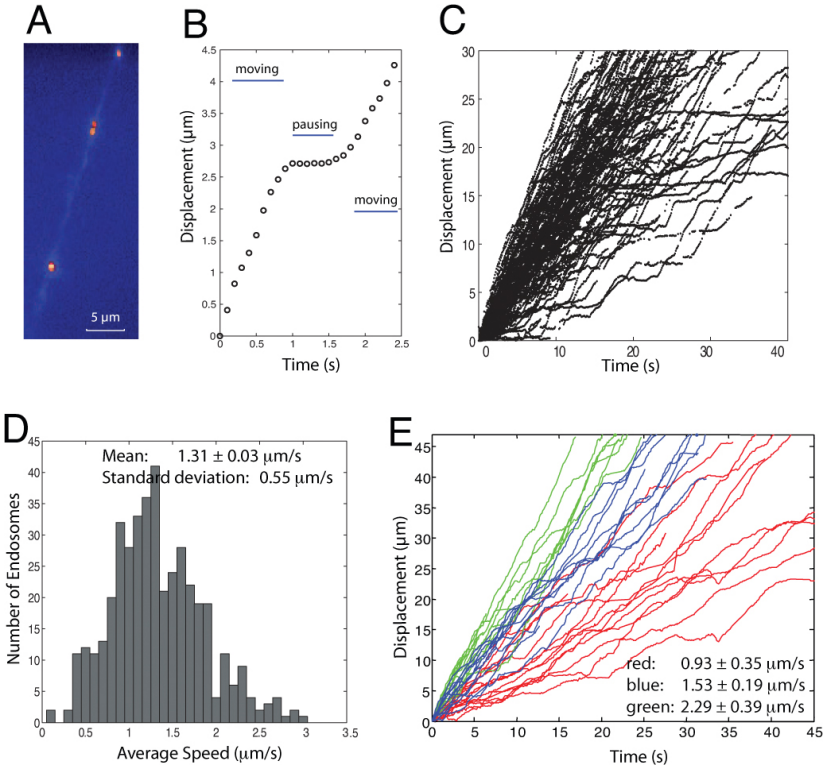
Evidence for retrograde transport of neurotrophins



(Campenot & MacInnis., J Neurobio 2003
& Ye et al., Neuron 2003)

Retrograde transport of NGF revealed by quantum dot technology





- Endosomes containing QD-NGF exhibit stop-and-go motion
- Speed and the duration of pauses varied greatly from one endosome to another
- Average speed of endosomal movement seems to vary considerably between axons

(Cui et al., PNAS 2007)

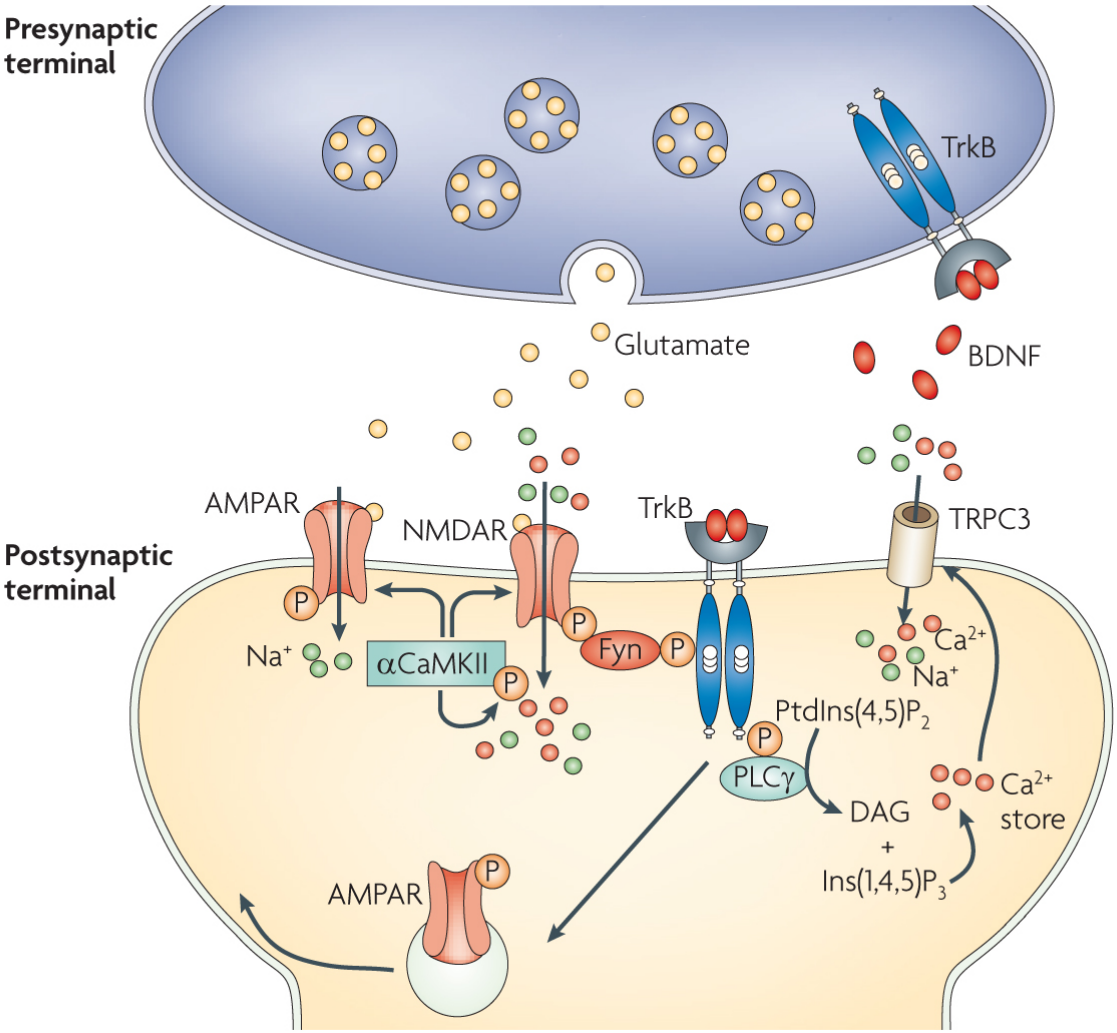
Summary I

- Neurotrophins and receptors: highly evolutionarily conserved & important for neuronal survival, axonal growth, target innervation, synaptic plasticity
- Neurotrophin signaling mechanisms are interconnected and show significant redundancy, but it is possible to “dissociate” the prosurvival function of neurotrophins from axonal growth
- Long range transmission of neurotrophin signals: models and the “signaling endosome hypothesis”

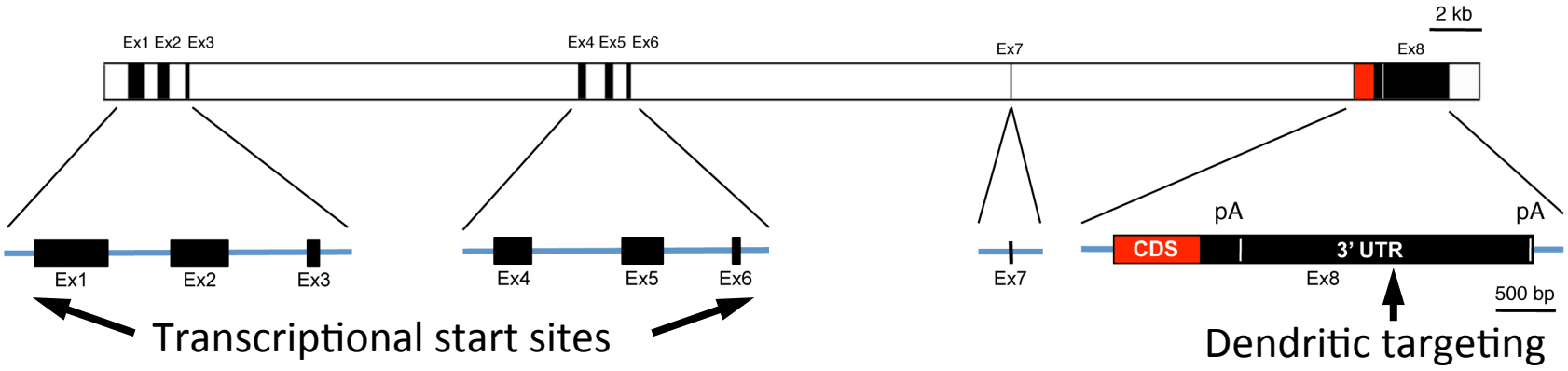
Non-survival functions of neurotrophic factors

- Cell fate decision (stem cells/precursors)
- Axon guidance
- Dendrite growth and branching
- Myelination
- Synapse development, stabilization and plasticity
- LTP, learning, memory
- Pain physiology and pathophysiology
- Neurotransmitter phenotype
- Ion channel regulation/function
- Neurological disease and neurodegeneration
- Psychiatric disease

BDNF-TrkB Signaling in LTP and Learning

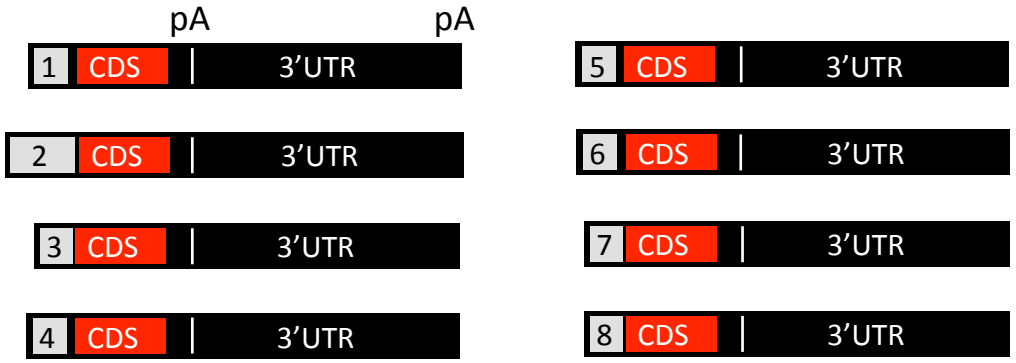


Extensive Splicing of 5' Alternative Non-coding Exons in mouse *Bdnf* Gene

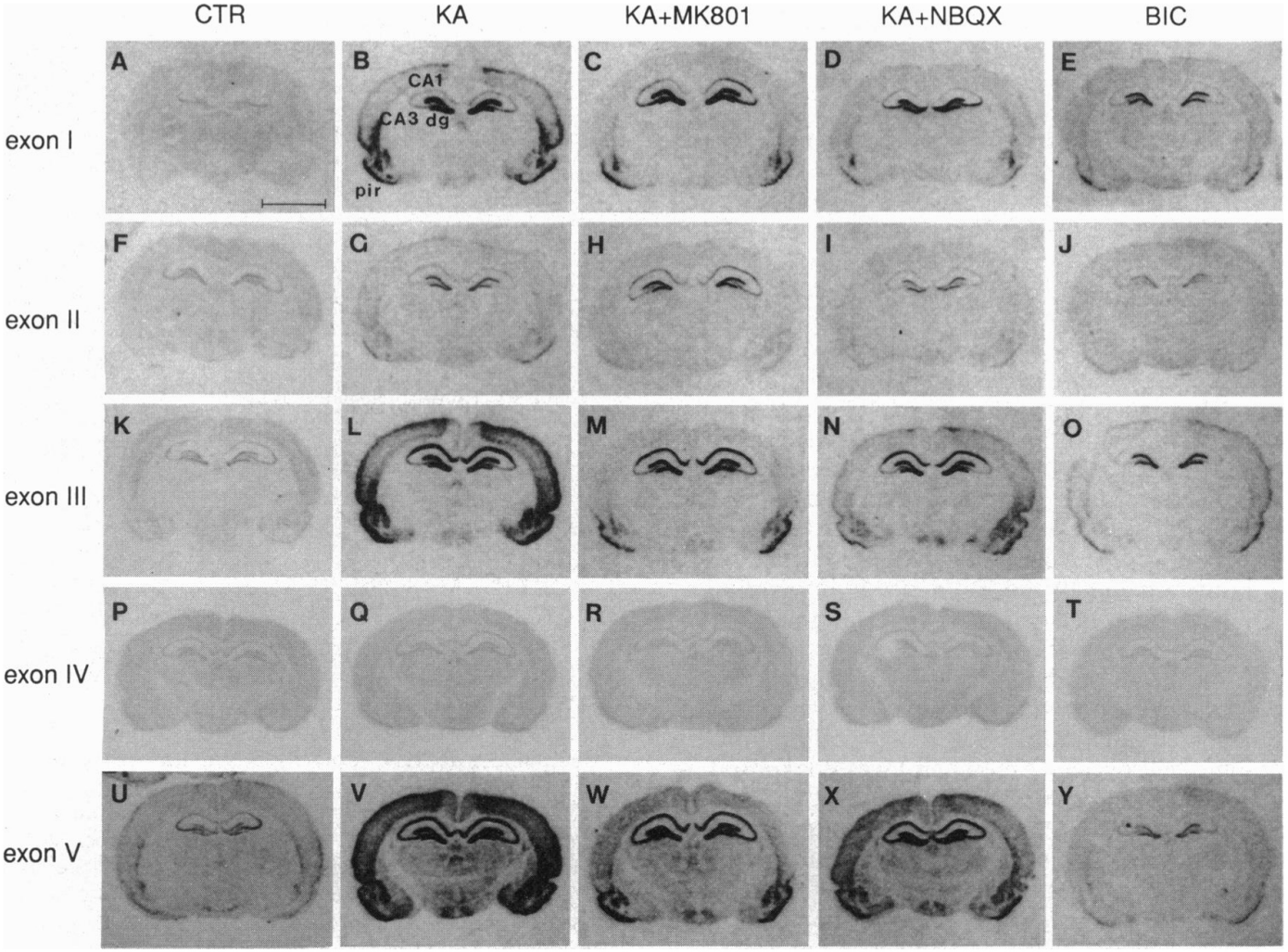


Extensive splicing of 5' alternative exons

Diverse *Bdnf* mRNA transcripts:



Activity dependent transcription of *Bdnf* mRNA

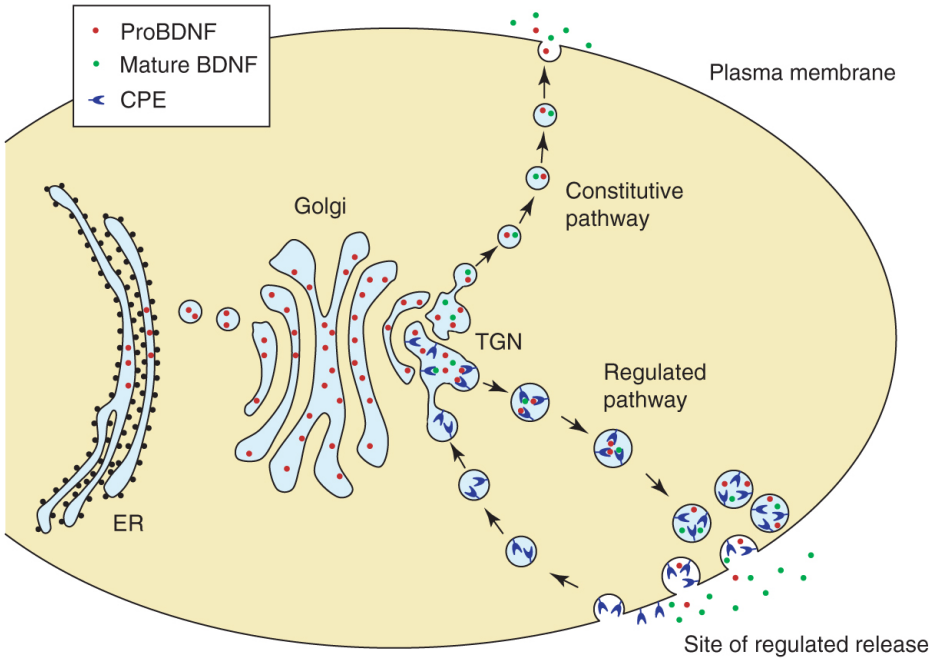


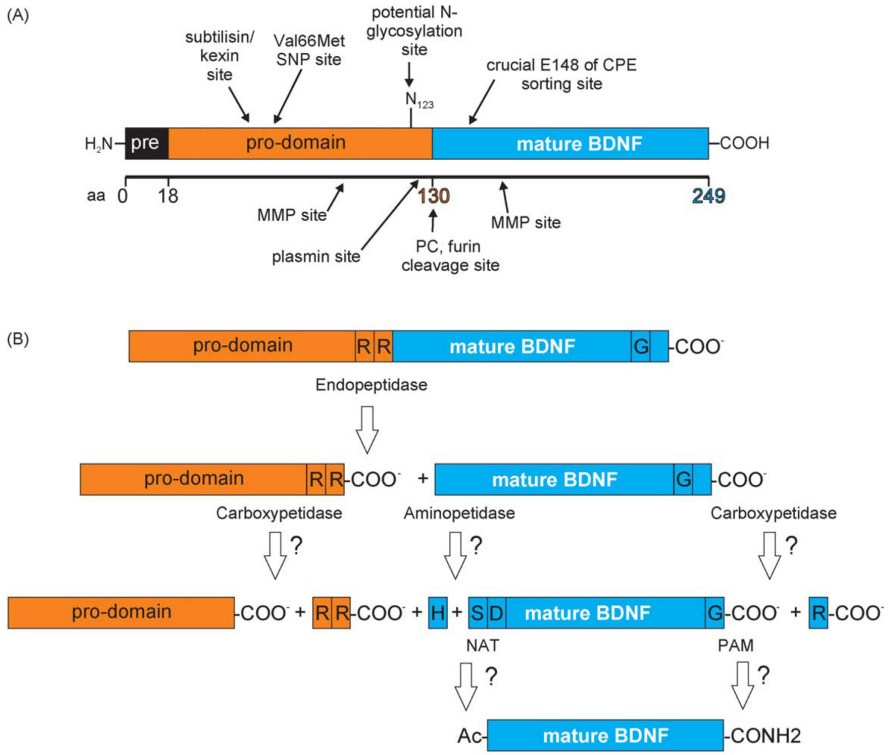
MK801: NMDA receptor antagonist
NBQX: AMPA receptor antagonist
Bicuculine: $GABA_A$ antagonist

(Timmusk et al., Neuron 1993;
Metsis et al., PNAS 1993)

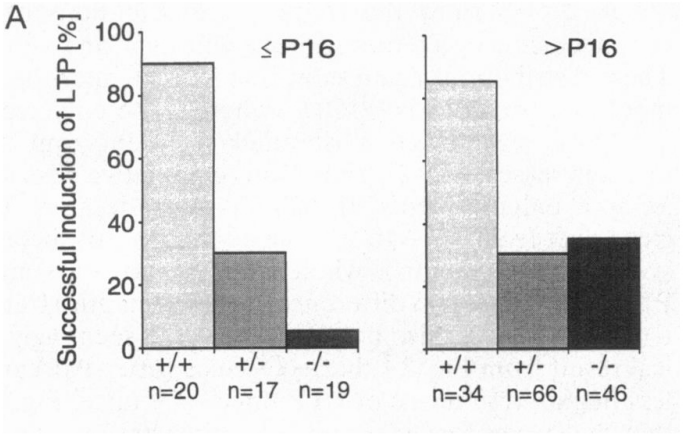
Processing and release of BDNF

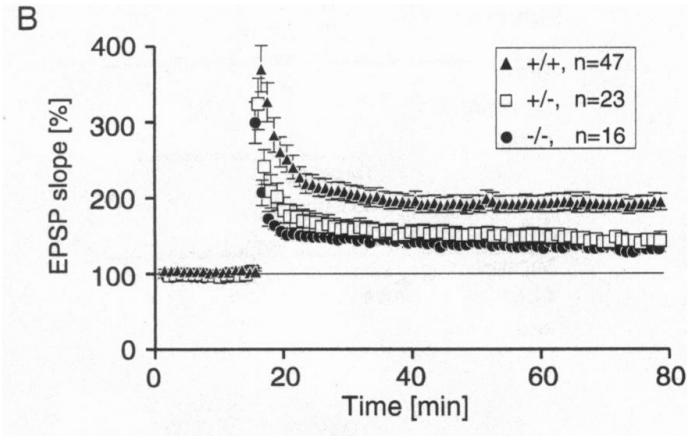
- Synthesized in ER as a 32 KDa precursor protein (proBDNF)
- Processed in Golgi and TGN to become mature BDNF
- Released via constitutive and regulated secretory pathways

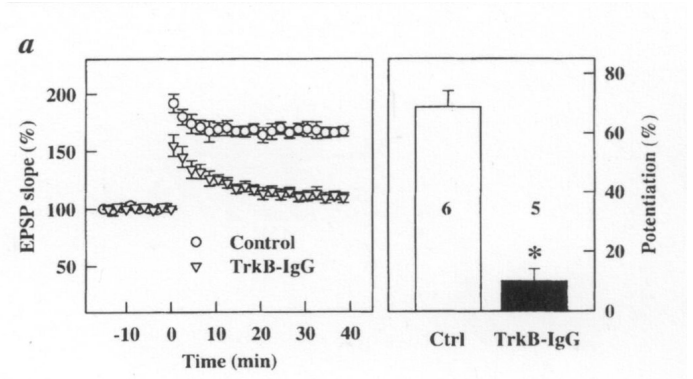




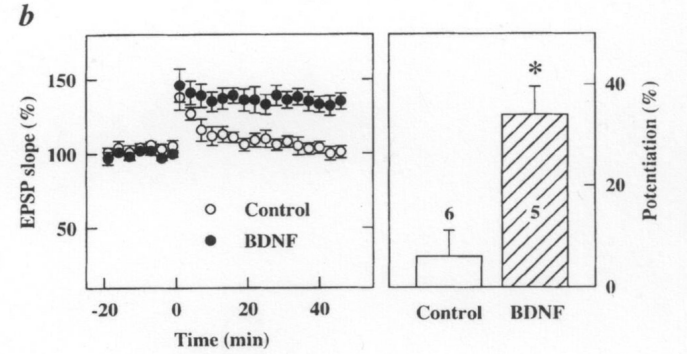
Role of BDNF in LTP

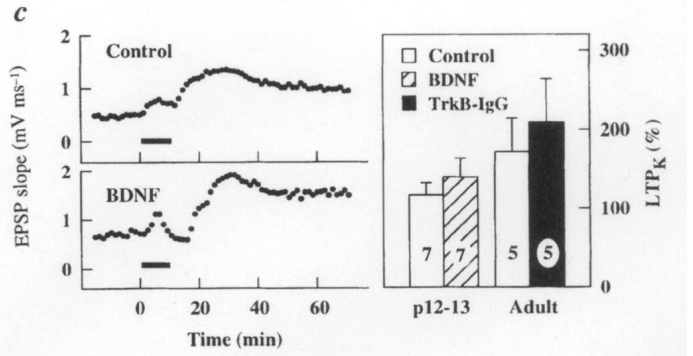






LTP_T



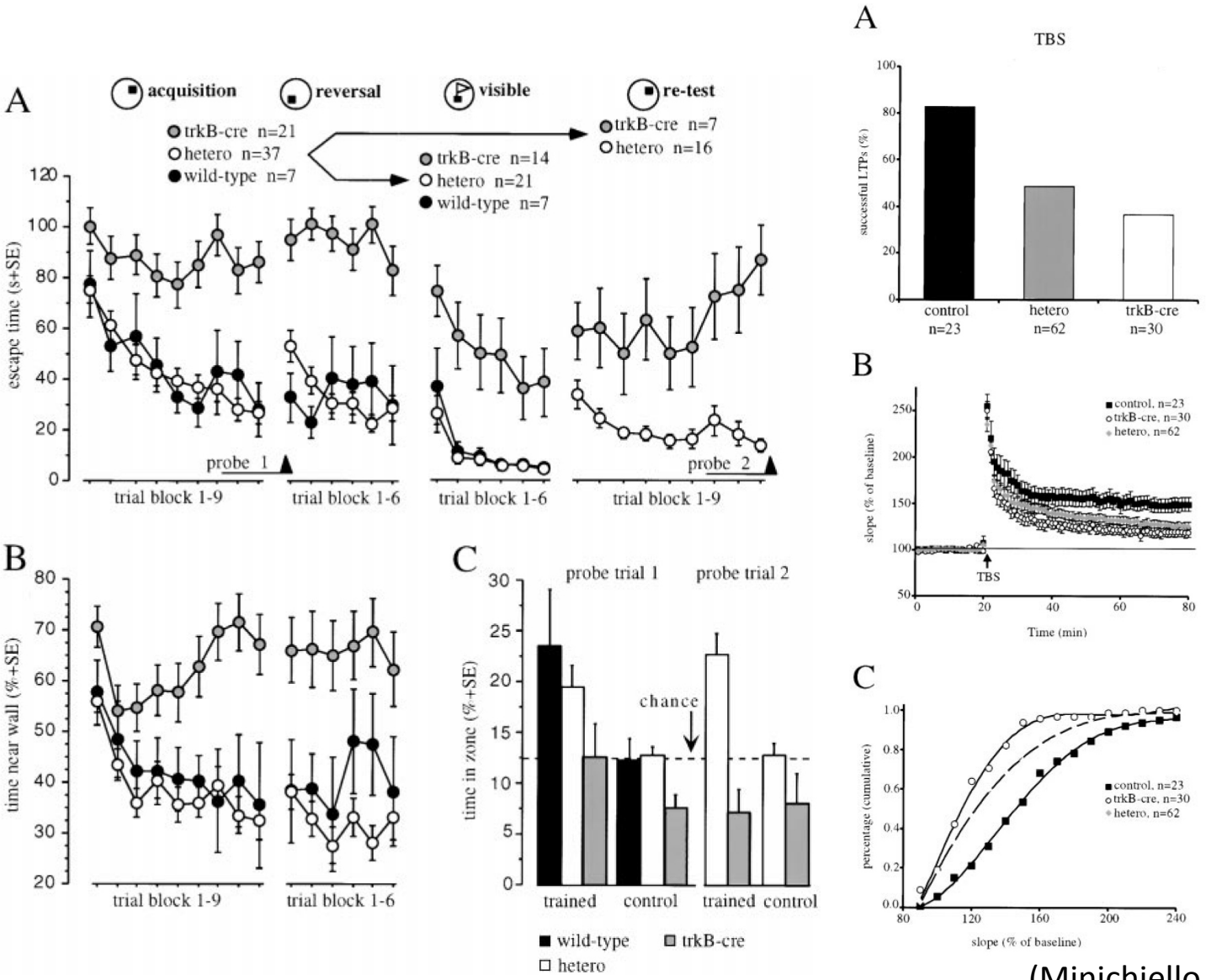


LTP_K

(Korte et al., PNAS 1995 & 1996;
Figurov et al., Nature 1996)

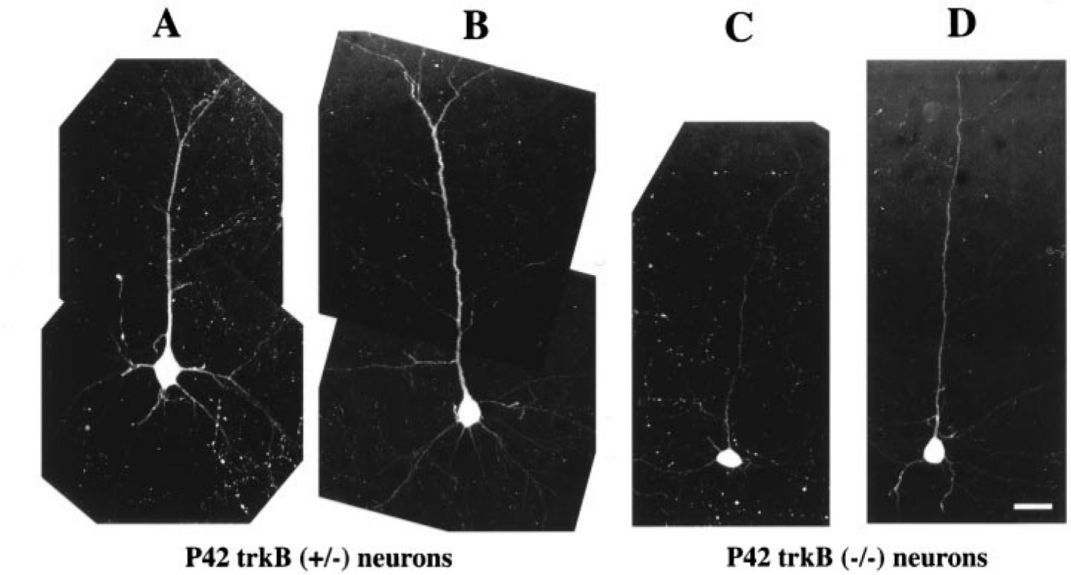
- LTP_T: theta burst stimulation (TBS)-induced LTP
- LTPK: LTP elicited by potassium blocker

CaMKII-Cre-TrkB mutant mice show hippocampus-based learning/memory defects



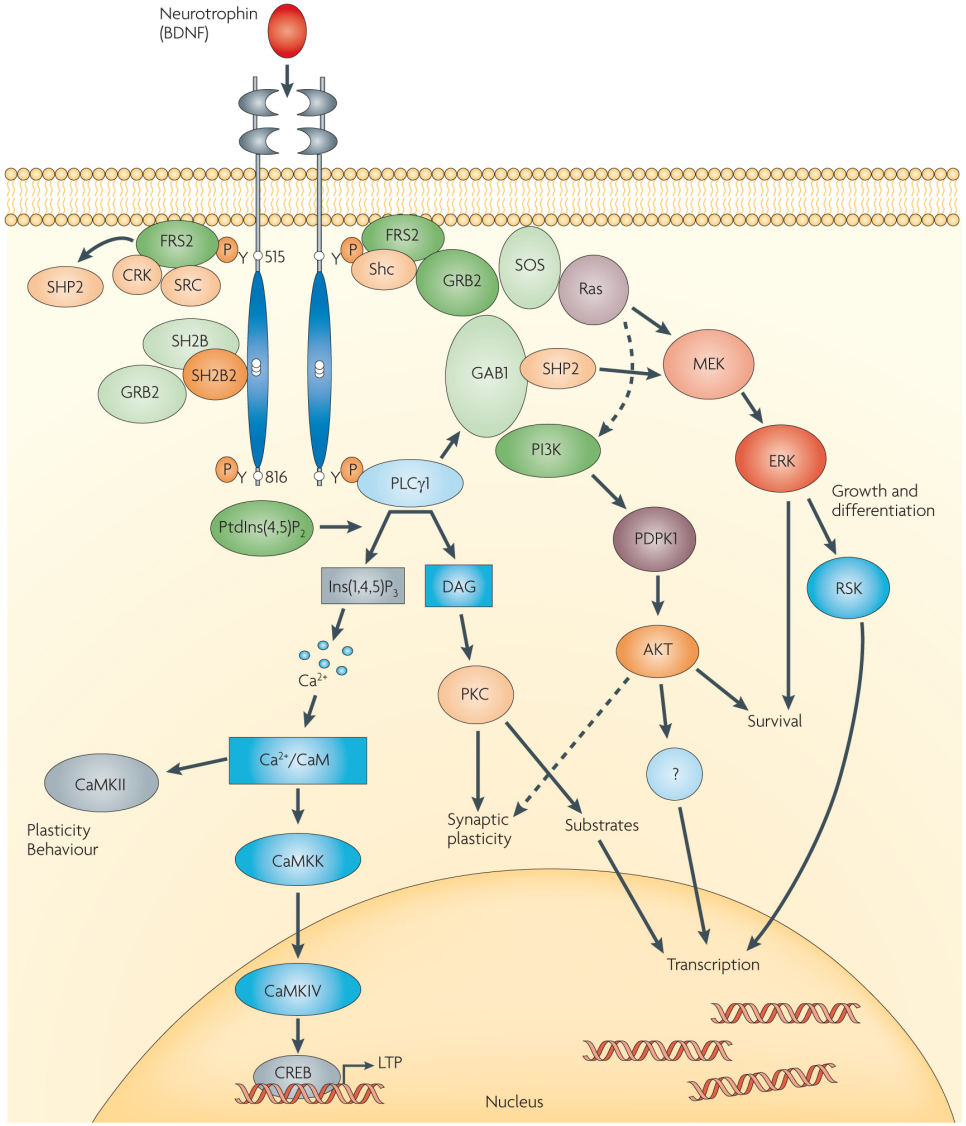
(Minichiello et al., 1999)

Loss of BDNF-TrkB signaling leads to dendritic arboriziation defects in cortical neurons



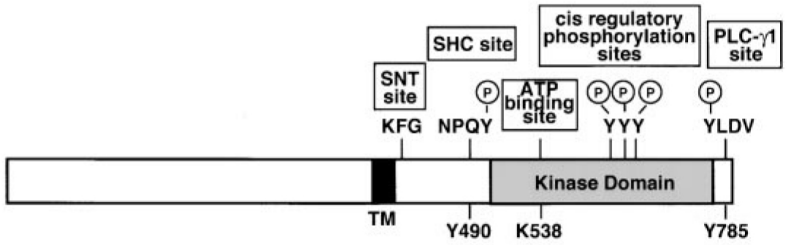
(Xu et al., Neuron 2000)

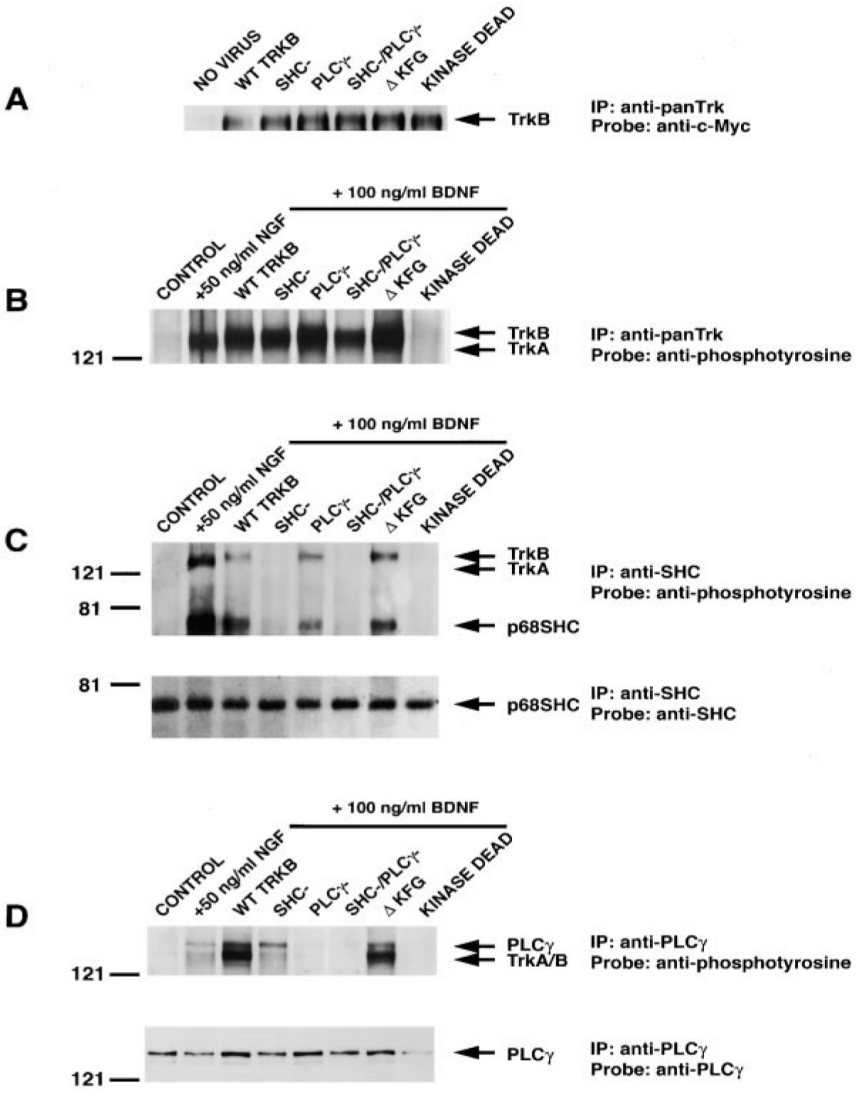
BDNF-TrkB Signaling in neuronal functions



- Signal tranduction downstream of Trk receptors is mediated thru modular pathways
- Phosphorylation on tyrosine residues is critical to transduce and propagate signals
- How to test this hypothesis?

Biochemical approaches to characterize the modular signaling transduction downstream of TrkB

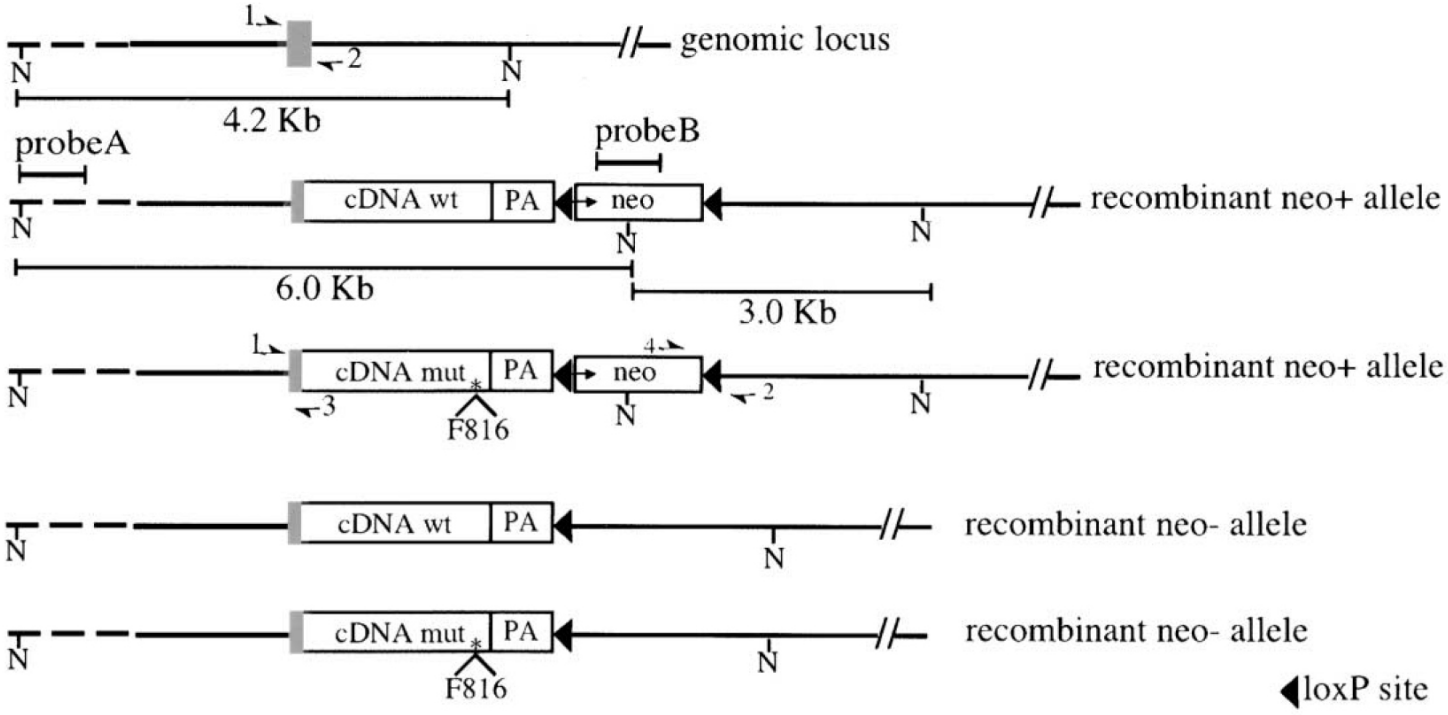




How to study the *in vivo* functions of TrkB cytoplasmic tyrosine residues in neuronal functions?

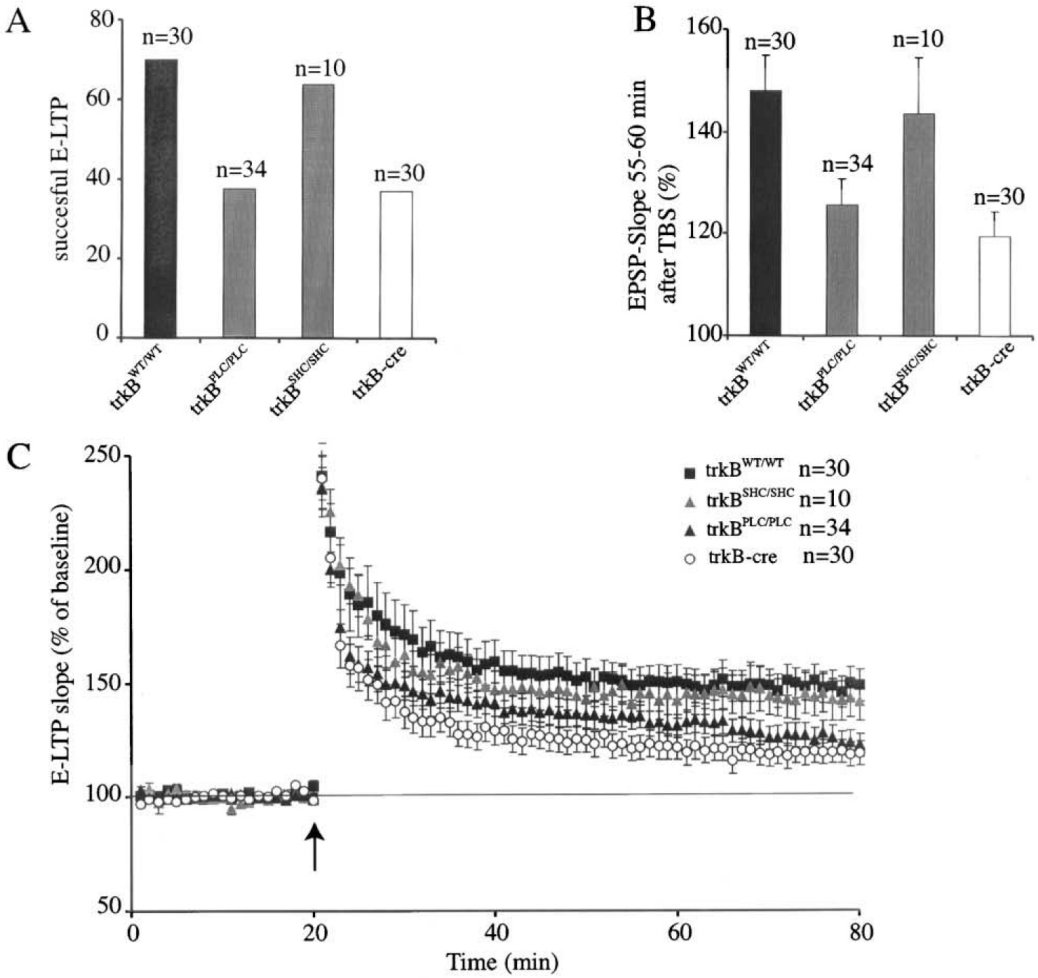
(Atwal et al., Neuron 2000)

Genetic approach to dissection TrkB downstream signaling mechanism



(Minichiello et al., Neuron 2002)

Impaired Hippocampal Synaptic Plasticity in *trkB$^{PLC/PLC}$* Mutants



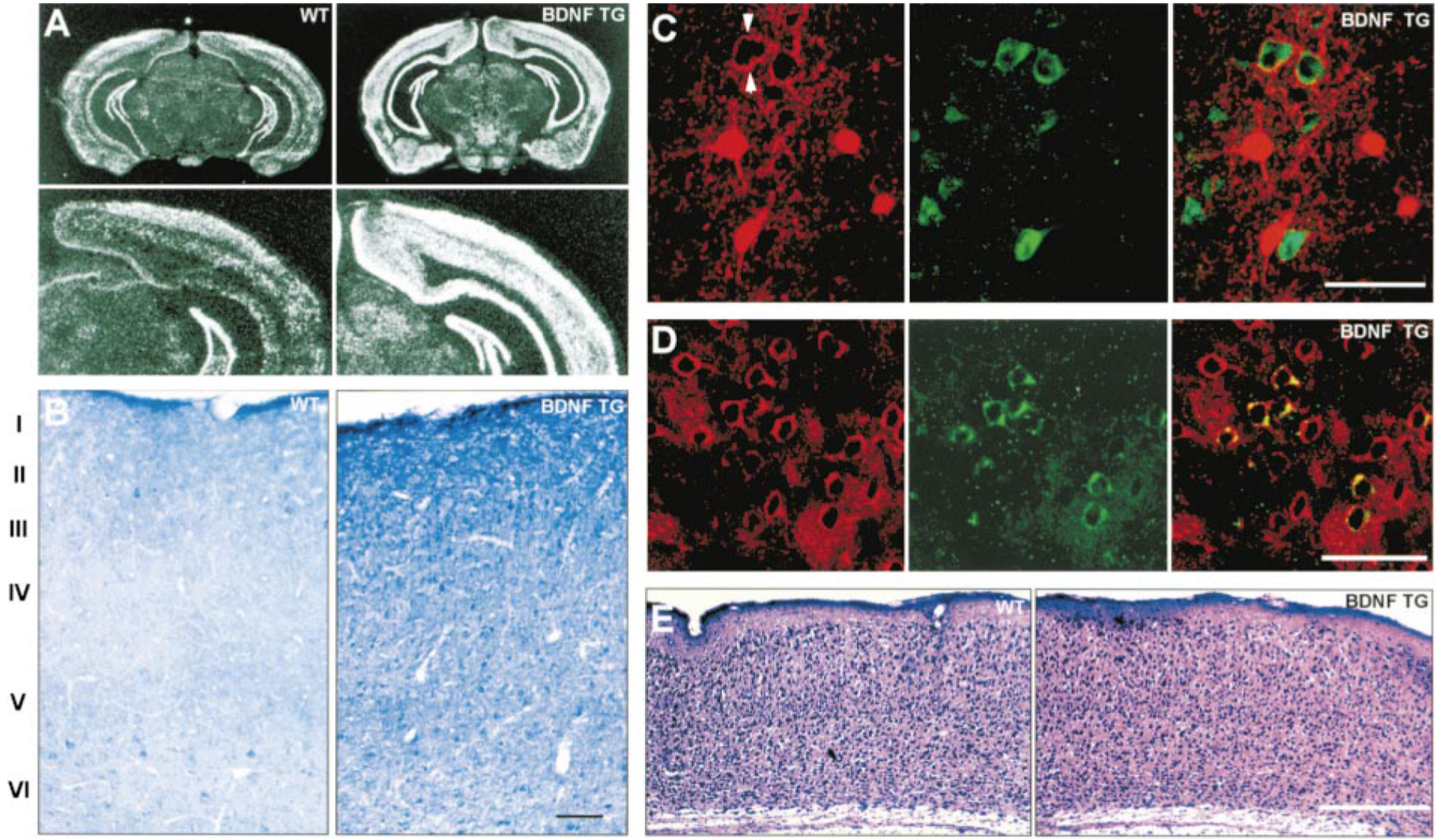
- Basic synaptic transmission thru NMDA receptor is intact, but synaptic strengthening is impaired in trkB$^{PLC/PLC}$ mutants (comparable to trkB conditional mutants)
- trkB$^{PLC/PLC}$ mutants show defects in both early and late phase LTP
- Impaired CREB and CAMKIV phosphorylation in trkB$^{PLC/PLC}$ mutants

(Minichiello et al., Neuron 2002)

BDNF regulates excitation-inhibition synapse homeostasis

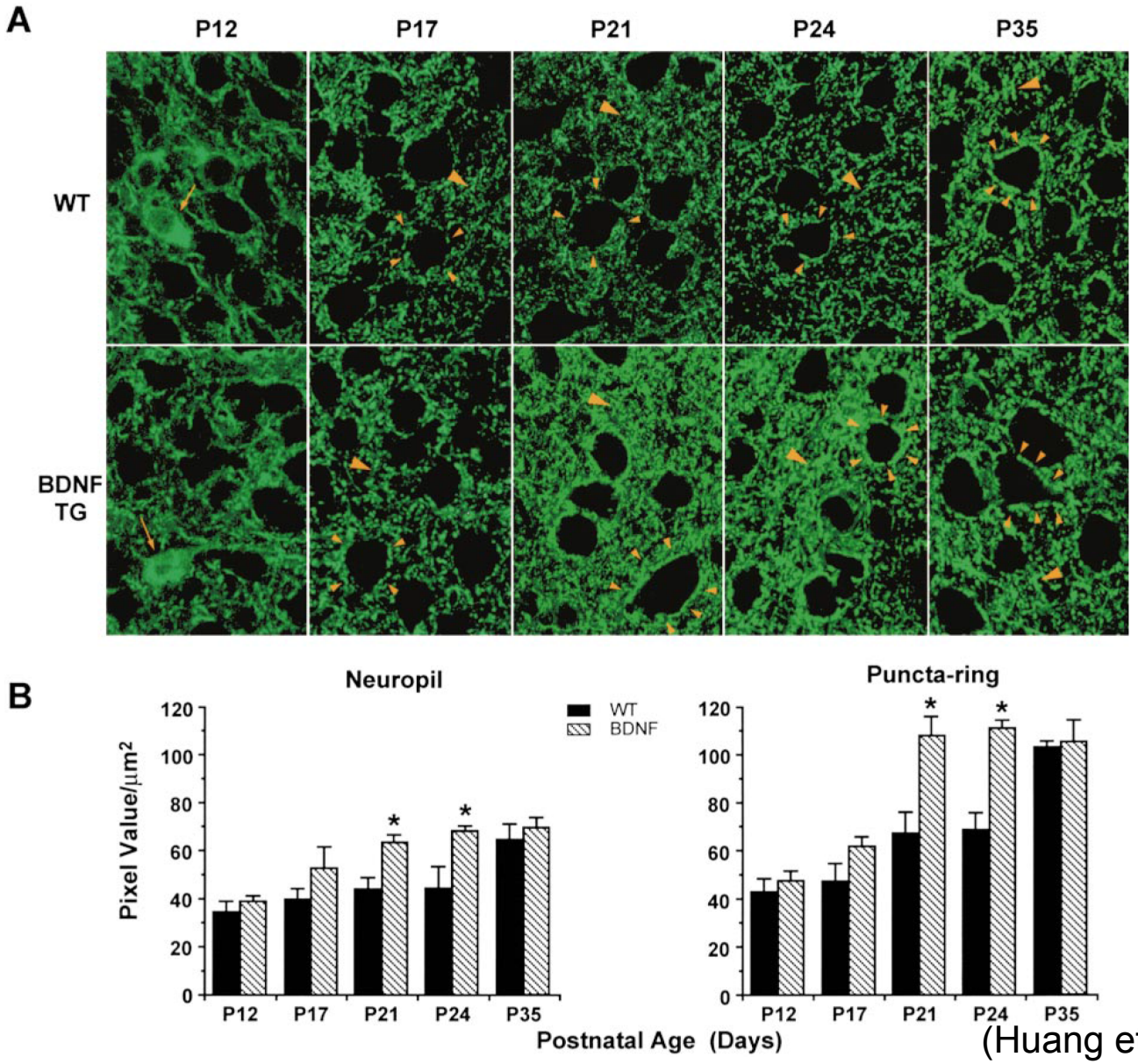
- **Cortical neuron cultures:**
- BDNF promotes interneuron neurite growth
- BDNF stimulates expression of GABA and other calcium-binding proteins
- BDNF regulates the strength of synaptic inhibition
- **Intracortical injection:**
- Ocular dominance in visual cortex as a model system
- trkB-Ig fusion protein alters the formation and plasticity of ocular dominance in visual cortex
- But, other mechanisms of BDNF can provide alternative interpretations

Transgenic expression of *Bdnf* in excitatory neurons



(Huang et al., Cell 1999)

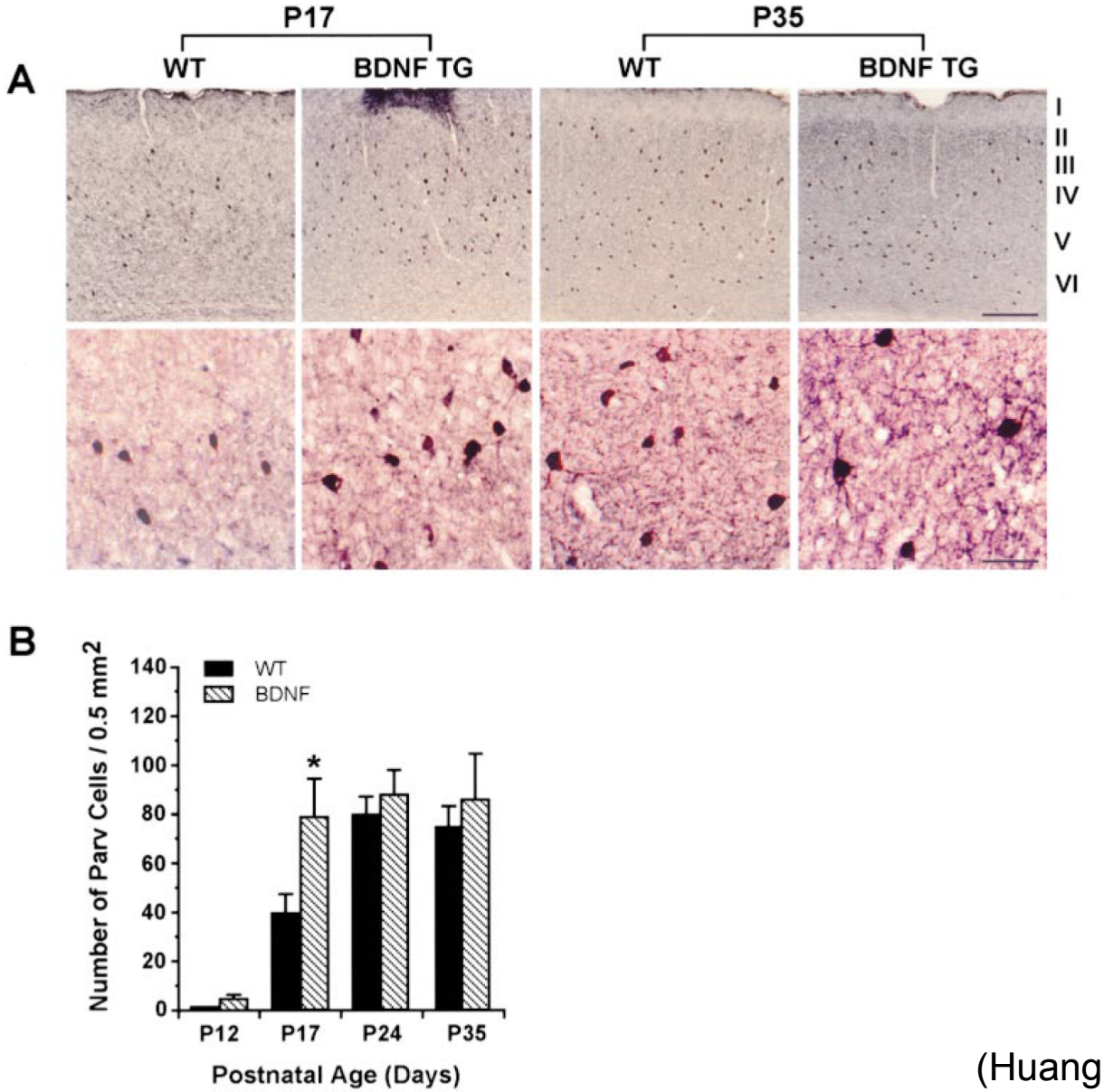
Expression of *Bdnf* in excitatory neurons accelerates maturation of GABA synapse



GAD65 (IF)

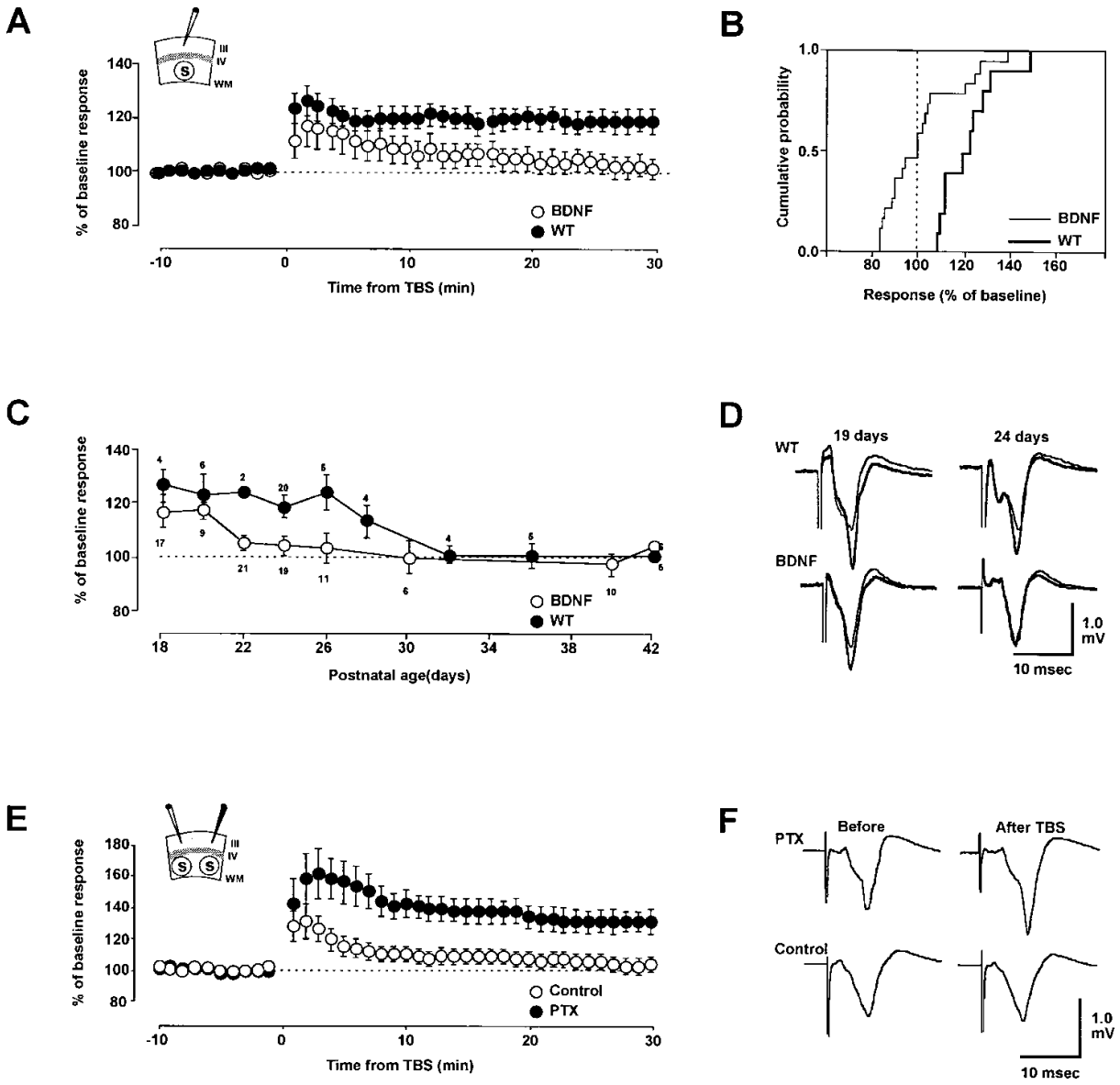
(Huang et al., Cell 1999)

Expression of *Bdnf* in excitatory neurons accelerates maturation of GABA synapse



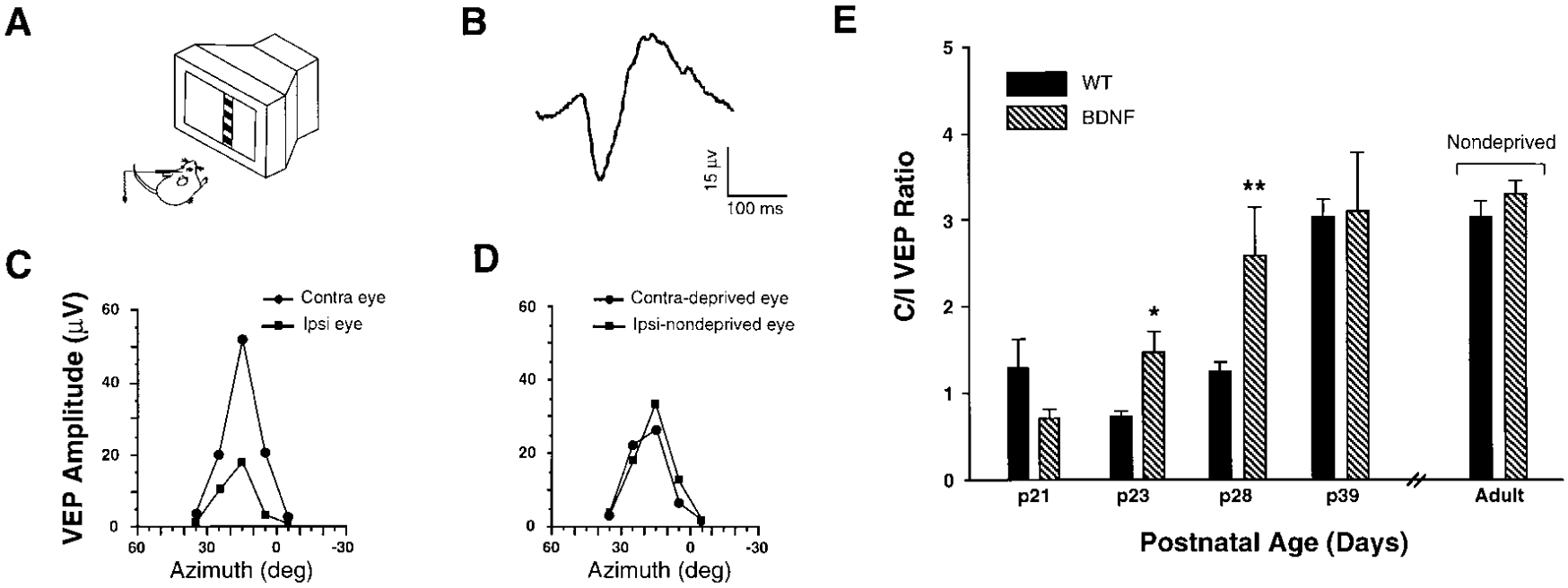
(Huang et al., Cell 1999)

Bdnf Tg visual cortex shows accelerated decline of WM➔III LTP



Bdnf Tg mice show precocious termination of the critical period of ocular dominance

VEPs (Visual Evoked Potentials): (1) recorded from the binocular region of the primary visual cortex, (2) represent the integrated response of a population of neurons to patterned visual stimuli, (3) used to measure visual acuity, contrast sensitivity, cortical retinopy and binocularity

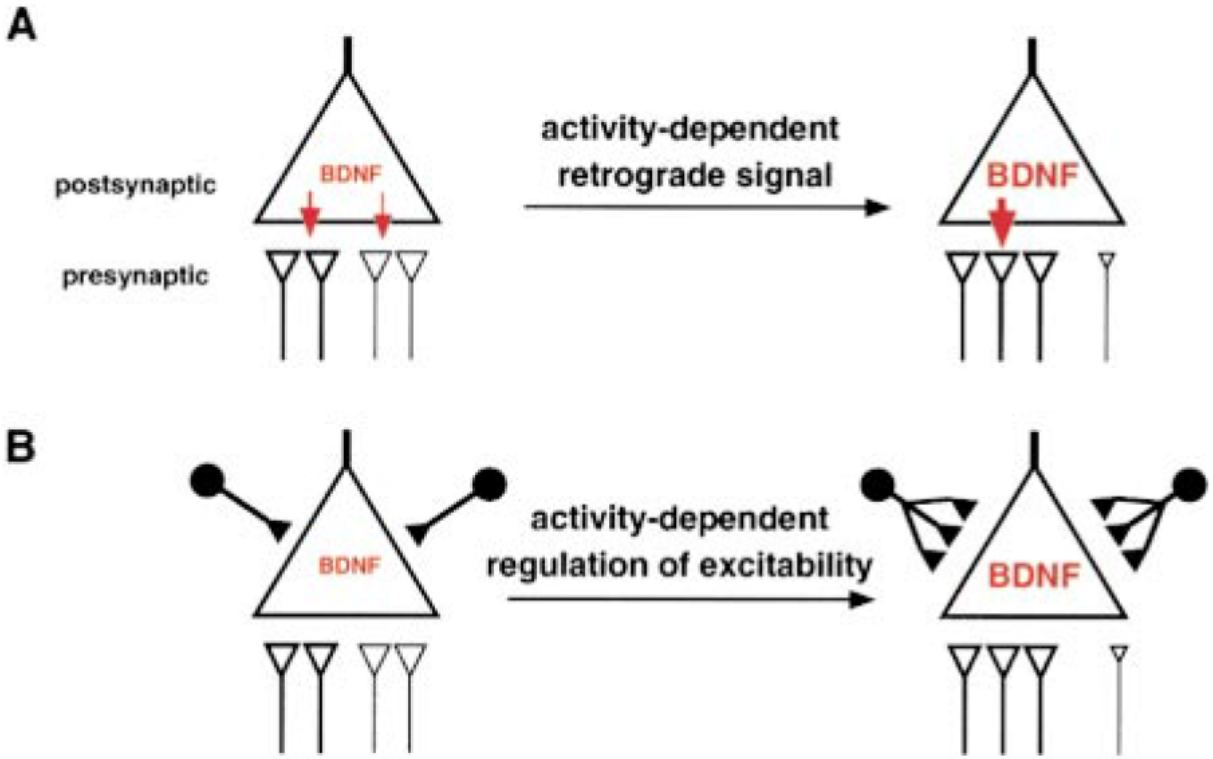


(non-deprived WT adult)

(4 days of MD in WT adult)

(Huang et al., Cell 1999)

Models of BDNF function in synaptic plasticity



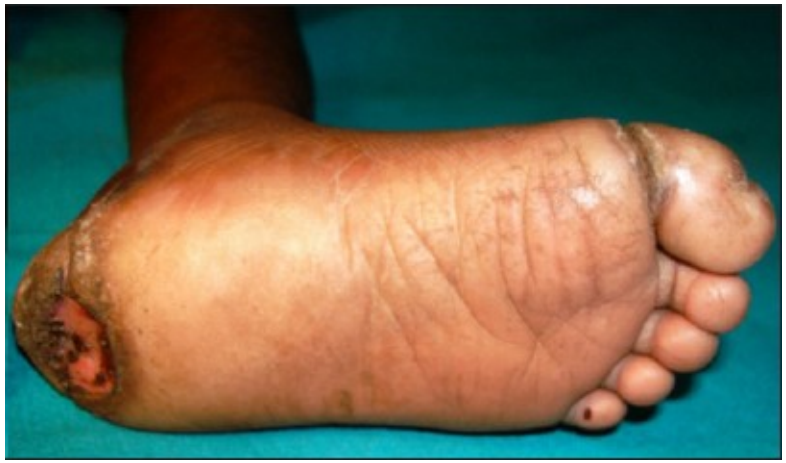
(Huang et al., Cell 1999)

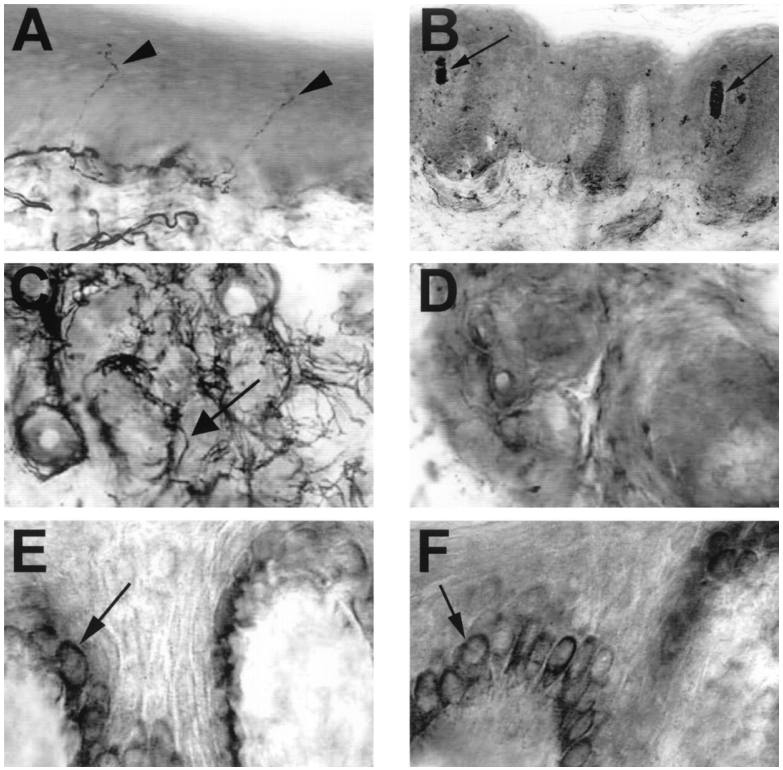
Summary II

- Transcription of *Bdnf* mRNA can be regulated by activity-dependent mechanisms
- Processing and release of proBDNF and mature BDNF involve proteolytic cleavage, and have been implicated in certain neuropsychiatric diseases
- BDNF-TrkB signaling is critical for LTP, learning/memory and homeostasis of energy balance and obesity
- Biochemical and genetic approaches reveal signal transduction through PLCγ-$Ca2^{+}$ downstream of TrkB is required for LTP
- BDNF regulates excitation-inhibition synapse homeostasis in visual cortex

Congenital Insensitivity to Pain with Anhidrosis (CIPA)

- Aka Hereditary sensory and autonomic neuropathy type IV (HSANIV), autosomal recessive (AR)
- 2 characteristic features: (1) inability to feel pain and temperature, and (2) decreased or absent sweating (anhidrosis)
- Repeated trauma can lead to chronic bone infections (osteomyelitis) or a condition called Charcot joints
- Mental retardation





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