

A novel evolutionarily conserved neurotrophic factor, CDFN, in an experimental model of Parkinson's disease

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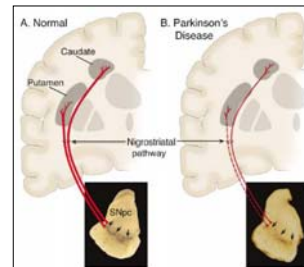
Overview

- Parkinson's disease
- Neurotrophic factors
- Glial cell-line derived neurotrophic factor, GDNF
- Neurturin, NTRN
- MANF protein family
- Conserved dopamine neurotrophic factor, CDFN
 - CDFN mRNA and protein expression
 - CDFN *in vivo* studies



Parkinson's disease

- Estimated to affect ~1% of the population (older than 65 years)
- Progressive neurodegenerative movement disorder
- Characterized by loss of dopaminergic neurons in the nigrostriatal pathway and a subsequent loss of dopamine in the striatum



Dauer and Przedborski 2003



Parkinson's disease

- Clinically most patient present with major symptoms of slowness of movement, resting tremor, rigidity and postural instability.
- Typically symptoms do not appear until there's
 - ~60 % loss of dopamine (DA) neurons in SN
 - ~80 % reduction in striatal DA
- Late onset of symptoms → high demands for the treatment for Parkinson's disease



Current therapy for Parkinson's disease (PD)

- Current available therapy (L-dopa or dopamine agonists) is symptomatic and does not arrest or attenuate the progression of the disease
- Interventions that slow or reverse the progression of the neuronal degeneration could be of significant benefit
- Slow and protracted degenerative process in PD creates opportunities for disease intervention:
 - prevent the degeneration
 - increase the functional activity of the remaining DAergic neurons



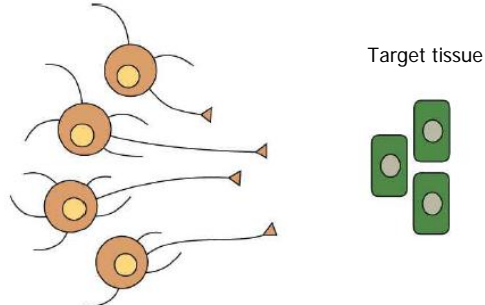
Neurotrophic factors

- Promote survival, differentiation and maintenance of neurons in developing and adult vertebrate nervous system
 - They bind to their cognate receptors and activate intracellular signaling that leads to the change in the receptor expressing cells
- Capable of making damaged neurons regrow their processes *in vitro* and *in vivo*
- New therapeutic agents for the treatment of PD ?



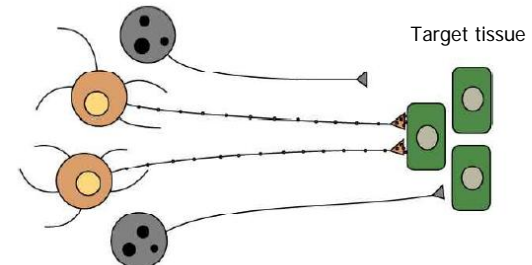
Target-field hypothesis for neurotrophic factors during development

- neurons are overproduced in development



Target-field hypothesis for neurotrophic factors during development

- Target tissue produce trophic factors in limited amount
 - survival of developing neurons depends on neuronal competition for the available factor





Glial cell-line derived neurotrophic factor (GDNF)

- Most potent and specific neurotrophic factor for dopaminergic neurons described so far is GDNF
- Promotes the survival and neuritogenesis of cultured embryonic nigrostriatal midbrain dopaminergic neurons *in vitro* (Lin *et al.* 1993)
- Protects nigral dopamine neurons against 6-OHDA *in vivo* (Kearns and Gash 1995, Kirik *et al.* 2000)
- Stimulates substantial axonal sprouting and reinnervation of dopaminergic afferents (Rosenblad *et al.* 1998, Aoi *et al.* 2000)

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9.6.2008 9



Clinical trials with GDNF

- GDNF delivery into the putamen of Parkinsonian patients has resulted in significant clinical improvement (Gill *et al.* 2003, Patel *et al.* 2005).
 - GDNF related adverse effects overbalanced the benefits → clinical trials stopped (Amgen study 2004, Lang *et al.* 2006)
 - Neurotrophic factors that could without adverse effects
 - slow or reverse the progression of neuronal degeneration
 - enhance recovery from neural injury
- are potentially very important therapeutic molecules.
→ warrants the search for novel neurotrophic factors.

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Gene therapy of PD - Neurturin

- Neurturin belongs to GDNF family
 - Only two neurotrophic factors, GDNF and neurturin, are known to possess neurorestorative effects when given after intrastriatal 6-OHDA injection in the rat (Hoffer *et al.* 1994, Rosenblad *et al.* 1999.)
- Delivery of Neurturin by AAV2 (CERE-120) :
 - Neuroprotection and neurorestoration in a rat and a primate model of Parkinson's Disease (Kordower *et al.* 2006, Herzog 2007, Gasmí 2007)
- 12 patients have received NTN-gene using AAV vector to putamen (Marks *et al.* 2008)
 - Phase 2 continuing
 - So far no complications

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MANF = Mesencephalic astrocyte-derived neurotrophic factor

- 20kD, secreted protein, was discovered in 2003
- Initially discovered from a rat mesencephalic type-1 astrocyte cell line
- rhMANF selectively protects *in vitro* nigral dopaminergic neurons, versus GABAergic or serotonergic neurons (Petrova *et al.* 2003)

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9.6.2008 12



CDNF = Conserved Dopamine Neurotrophic Factor

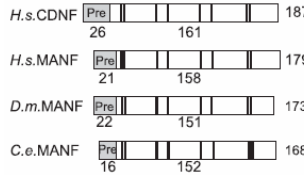
- Novel neurotrophic factor which was found using bioinformatic tools
→ comparison of amino acid sequences in databases
- CDNF and MANF form a novel MANF family of conserved secreted factors with eight cysteine residues

H.s.CDNF 187

H.s.MANF 179


D.m.MANF 173

C.e.MANF 168



Schematic illustration of MANF & CDNF molecules

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CDNF = Conserved Dopamine Neurotrophic Factor

- CDNF and MANF are evolutionarily conserved homologous proteins found in mammals and invertebrates (*Drosophila melanogaster*, *C.elegans*)
- Human CDNF shows 59 % amino acid identity with hMANF
49 % amino acid identity with *D.melanogaster*
46 % amino acid identity with *C.elegans*

Amino acid identity (%)* of CDNF and MANF proteins between selected organisms.

	H.s.CDNF	H.s.MANF	M.m.CDNF	M.m.MANF	D.m.MANF
H.s.MANF	59				
M.m.CDNF	80	58			
M.m.MANF	59	98	58		
D.m.MANF	49	53	47	53	
C.e.MANF	46	50	46	50	50

*Signal sequences omitted. H.s., *Homo sapiens*; M.m., *Mus musculus*; D.m., *Drosophila melanogaster*; C.e., *Caenorhabditis elegans*.

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Novel neurotrophic factor CDNF protects and rescues midbrain dopamine neurons *in vivo*

Päivi Lindholm¹, Merja H. Voutilainen², Juha Laurén^{1,†}, Johan Peränen¹, Veli-Matti Leppänen¹, Jaan-Olle Andressoo¹, Maria Lindahl¹, Sanna Janhunen^{2,†}, Nisse Kalkkinen¹, Tõnis Timmusk^{1,3}, Raimo K. Tuominen² & Mart Saarma¹

In Parkinson's disease, brain dopamine neurons degenerate most prominently in the substantia nigra¹. Neurotrophic factors promote survival, differentiation and maintenance of neurons in developing and adult vertebrate nervous system^{2,3}. The most potent neurotrophic factor for dopamine neurons described so far is the glial-cell-line-derived neurotrophic factor (GDNF)⁴. Here we have identified a conserved dopamine neurotrophic factor (CDNF) as a trophic factor for dopamine neurons. CDNF, MANF, but not genes homologous to MANF or CDNF in other species, including mouse, contains an amino-terminal arginine-rich sequence. In human MANF the arginine-rich region is considered as a non-translated sequence⁵. CDNF and MANF proteins form a novel MANF family of conserved secreted factors with eight cysteine residues of similar spacing (Fig. 1a, b), suggesting a unique protein fold. Human and mouse CDNF messenger RNAs encode 187 amino acid proteins with pre-

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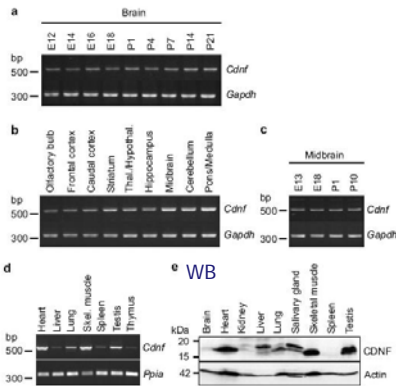
CDNF mRNA and protein expression

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In mice, CDNF mRNA and protein are expressed in brain and in peripheral tissue

mRNA

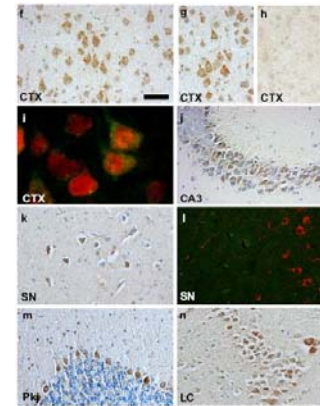


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9.6.2008 17



Mouse CDNF protein expression



g) CDNF antibodies pre-incubated with MANF protein detect CDNF
h) No CDNF signal after antibodies incubated with CDNF

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CDNF *In vivo* studies

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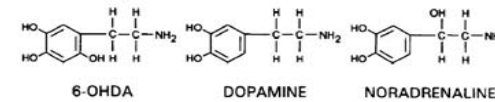
9.6.2008 19



Unilateral 6-OHDA-lesion model of PD

6-OHDA (6-hydroxydopamine):

- Selectively destroys the dopaminergic nigrostriatal pathway
- Cannot penetrate blood brain barrier → direct injection to brain
- The membrane uptake systems of catecholaminergic neurotransmitters transport 6-OHDA into the cell as a "false transmitter" (due to its similar structure)



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Intrastriatal injection of 6-OHDA toxin and trophic factors

Striatum

CP GP SN NSP

Intact

Modified: Kirik et al., Nature Neuroscience 2004

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Degenerative changes seen in the nigral DA neurons following axon terminal damage induced by intrastriatal 6-OHDA

- The 6-OHDA lesion causes acute destruction of axon terminals in the striatum surrounding the injection site
- This leads to retrograde degeneration of the axons → followed by delayed death of the DA neurons in the SN
- The cellular degeneration in nigra is progressive → takes place within 3-4 weeks

6-OHDA lesion (day 0)

Phase 1 (0-7 days) Atrophy No cell death

Phase 2 (1-4 wks) Rapid cell death Long-term atrophy TH downregulation

Phase 3 (4 wks+) Delayed cell death Persistent atrophy

Björklund et al. 1997, Neurobiology of Disease 9.6.2008 22

Unilateral 6-OHDA-lesion model of PD: Ungerstedt turning model

- 6-OHDA selectively destroys the dopaminergic nigrostriatal pathway (including presynaptic DA-neurons in the striatum)
- Causes supersensitivity of the postsynaptic dopamine receptors in the striatum
- An imbalance in DA activity between two striata causes rotational asymmetry
- Rats turn away from the bigger DA content

Indirect DA-agonist (e.g. amphetamine) → ipsilateral turns

Direct DA-agonist (e.g. apomorphine) → contralateral turns

6-OHDA

Striatum Nigrostriatal tract Substantia nigra

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Experimental plan for neuroprotection studies

Intrastriatal CDNF (10µg/rat) or GDNF (10µg/rat)

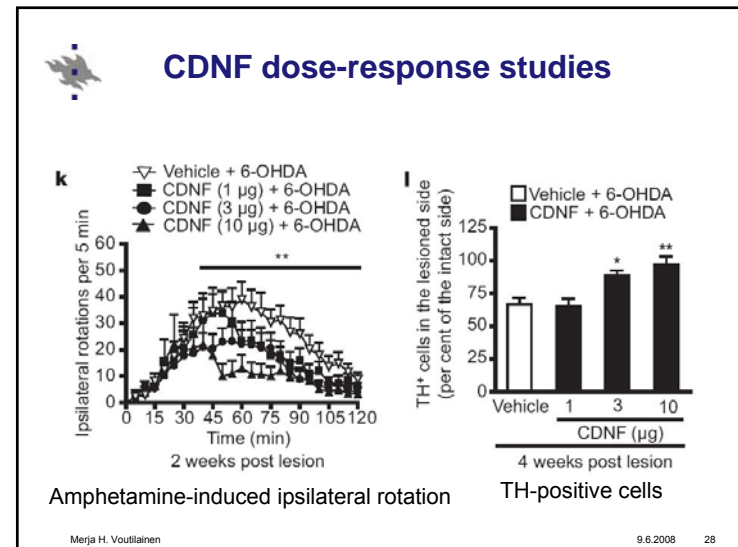
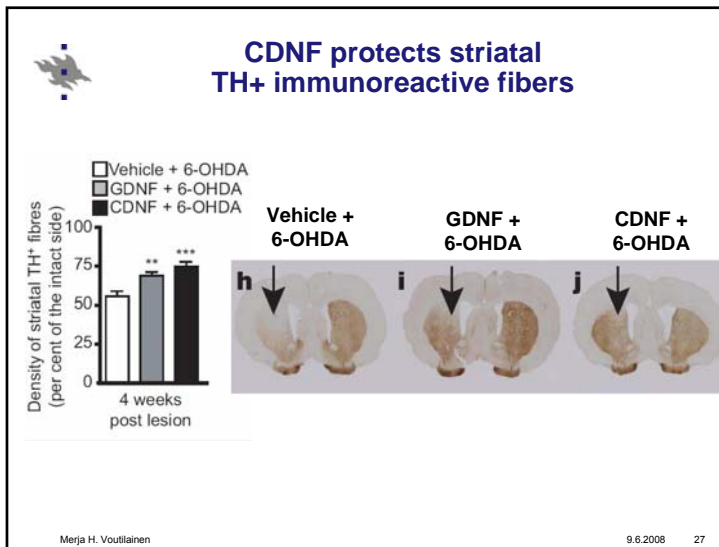
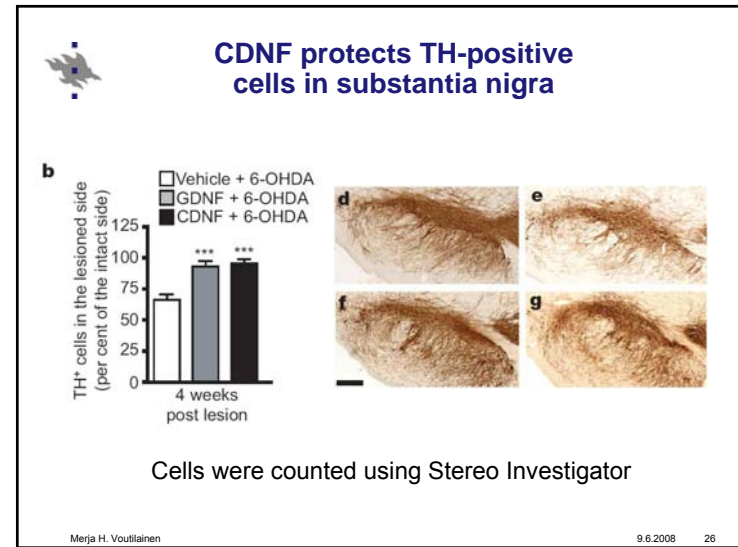
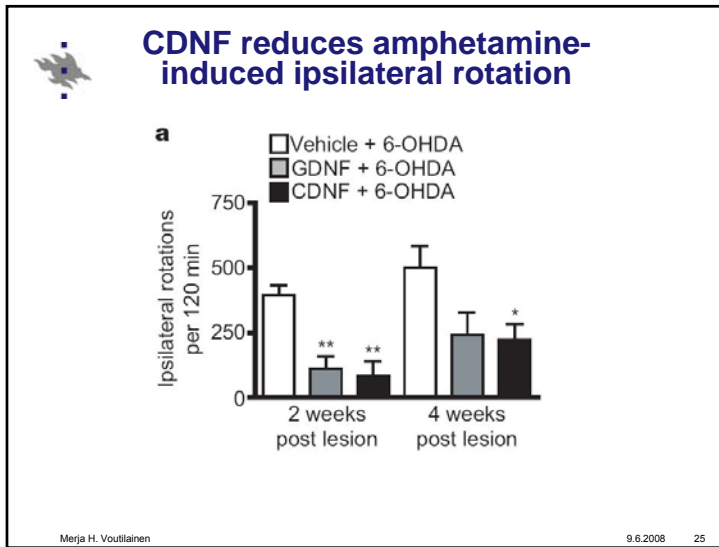
Intrastriatal 6-OHDA (8µg/rat)

Amphetamine 2.5 mg/kg, i.p., induced rotation

Perfusion & Immunohistochemistry

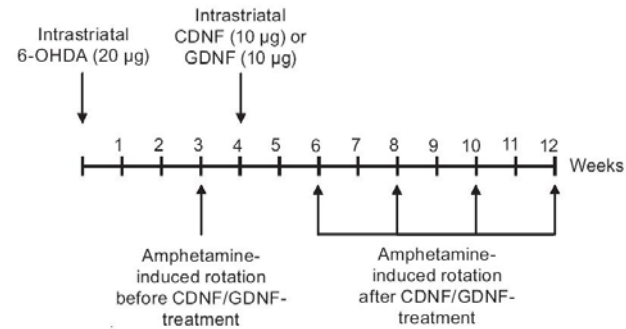
6 hours 2 weeks 4 weeks

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Time course of neurorestoration studies

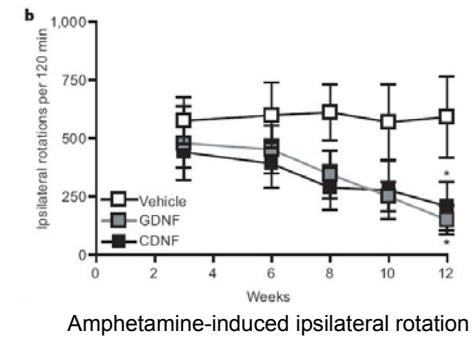


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9.6.2008 29



CDNF restores motor function

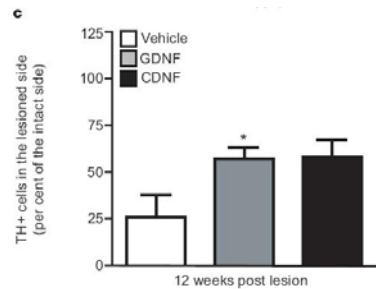


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CDNF induces a partial recovery of TH-positive cells



Stereological counting of cells using Stereo Investigator

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Conclusions

- CDNF is a member of the highly conserved MANF protein family
- The expression of CDNF mRNA and protein was detected in postnatal and adult mouse striatum and substantia nigra
 - striatal expression suggests that CDNF supports the survival and function of DAergic axon terminals
 - In midbrain CDNF might provide a local trophic support for DAergic neurons with paracrine mechanism
- CDNF had both neuroprotective and neurorestorative properties *in vivo*
 - CDNF might have potential as a therapeutic protein or as a basis for the development of drugs for the treatment of Parkinson's disease

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9.6.2008 32



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Thank you for your attention!