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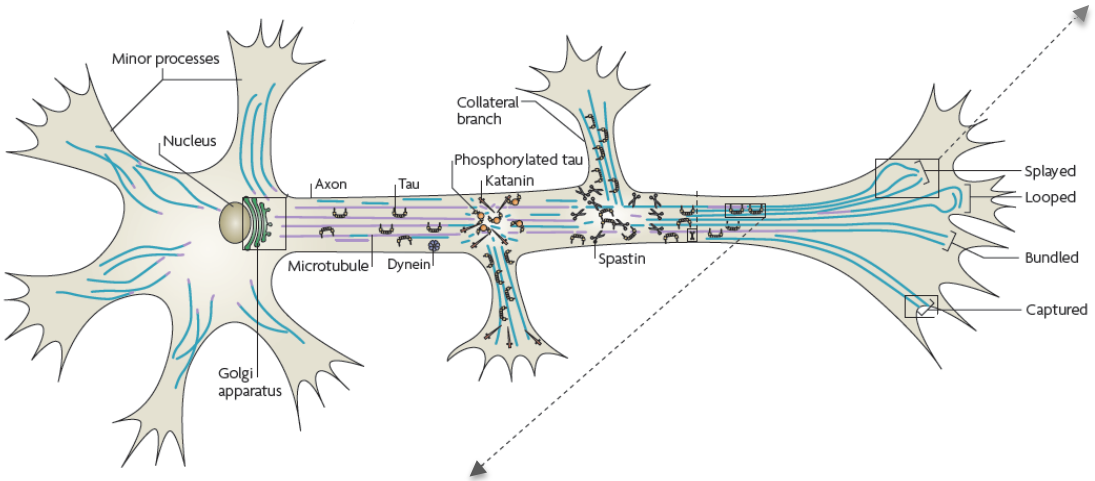
Applied Translational Medicine

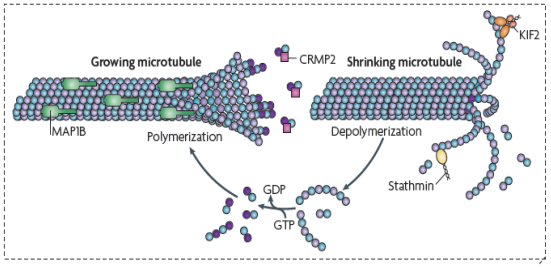
Neuroprotection in preclinical models of Parkinson disease by the NAPVSIPQ peptide

Bruce H. Morimoto, Ph.D.
Executive Director, Applied Translational Medicine

Microtubules

- Microtubules essential for neuronal structure & function
- Destabilization occurs in many neurodegenerative diseases

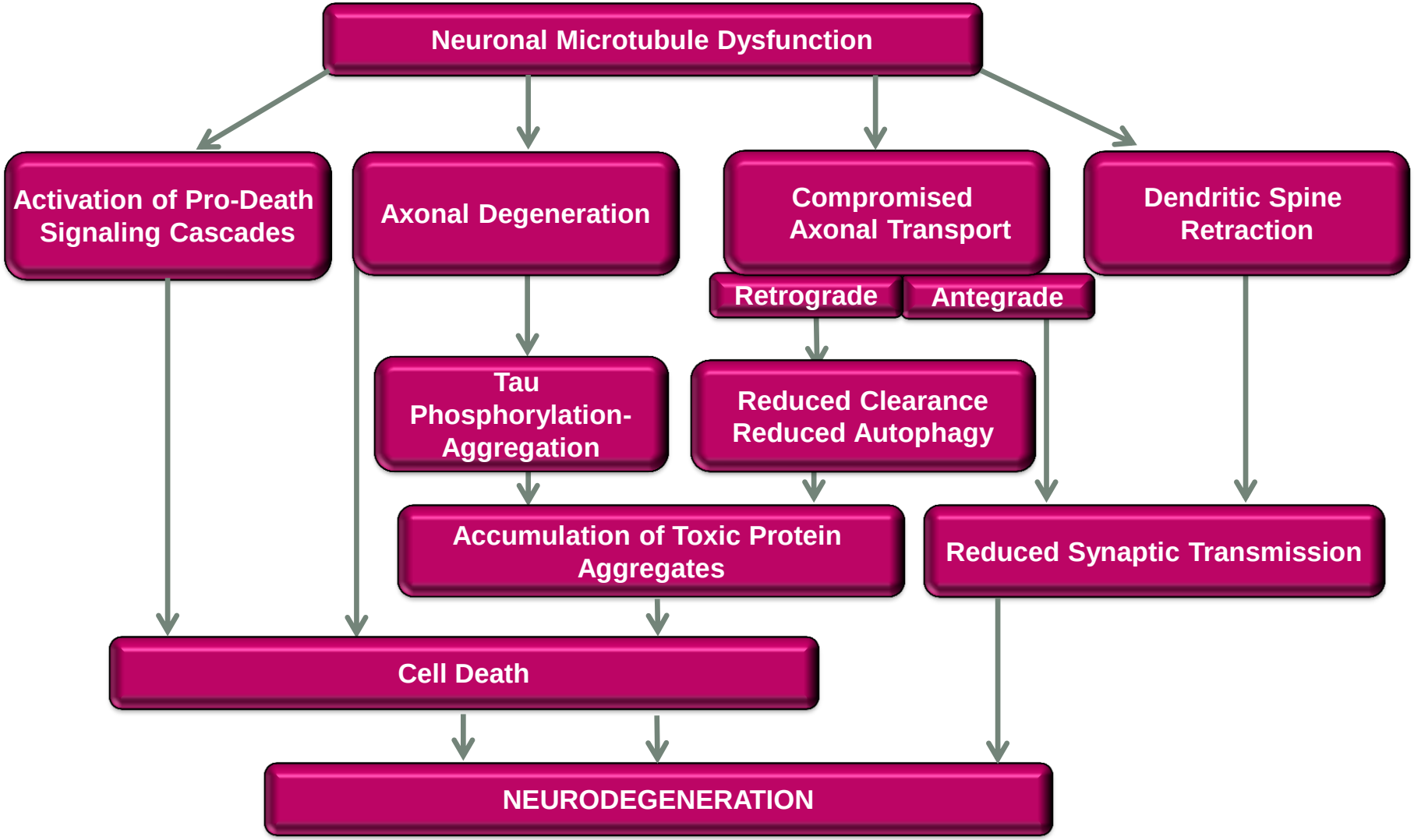




Conde & Caceres, *Nat Rev Neurosci* 2009: 10; 319-332

Perlson, et al. *TINS* 2010: 33; 335-344

Microtubules and Neurodegeneration



NAPVSIPQ Discovery

- **NAPVSIPQ** is the smallest active fragment of ADNP which provides neuroprotection

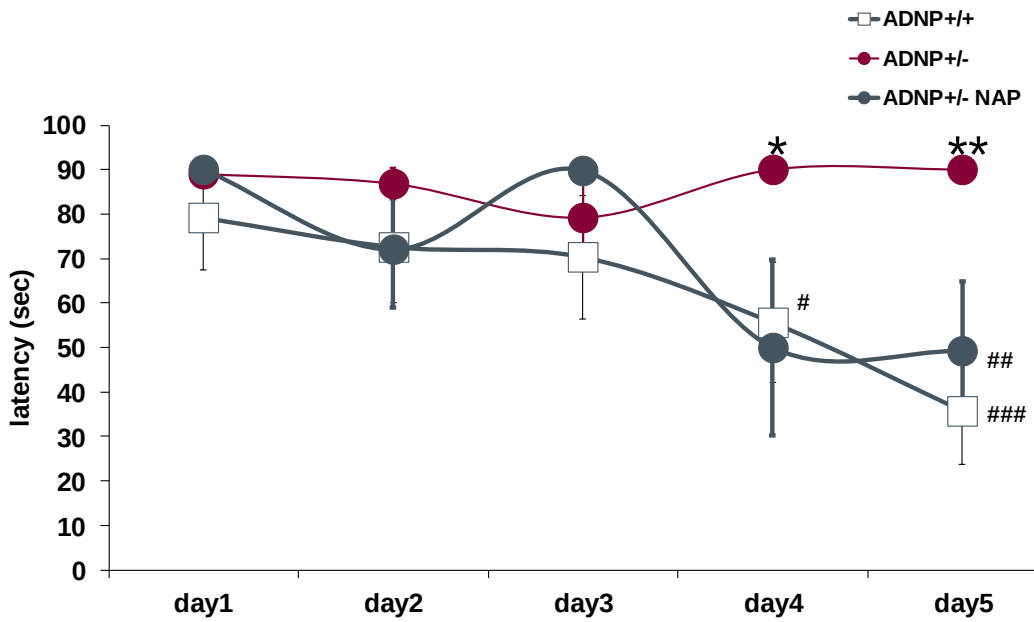
J. Neurochem. 1999; 72, 1283-1293
Developmental Brain Res. 2003;144: 83-90
J Mol. Neurosci. 2004; 24(2):181-187
Neuroscience Letters 2005; 373(1):73-78
Current Alzheimer's Res. 2005; 2(2):149-153
Peptides. 2005; 26(8):1520-1527
Peptides. 2005; 26(8):1520-1527
CNS Drug Rev. 2005;11(4):353-68
Mol Cell Endocrinol. 2006;252:148-53.
Pharmacol Ther. 2007;114(2):146-154
Developmental Biology, 2007; 303(2): 814-824

ADNP Summary

- ADNP knockout (-/-) mice
  - Embryonic lethal
  - Failure of neural tube closure
- ADNP heterozygous (+/-) mice
  - Express 50% less ADNP
  - Reduced cognitive function
  - Tau hyperphosphorylation
  - Tauopathy-like phenotype
- ADNP—1100 amino acid protein

J Pharmacol Exp Ther. 2007 Nov;323(2):438-49. Epub 2007 Aug 24.

ADNP (+/-) Cognitive Deficiency

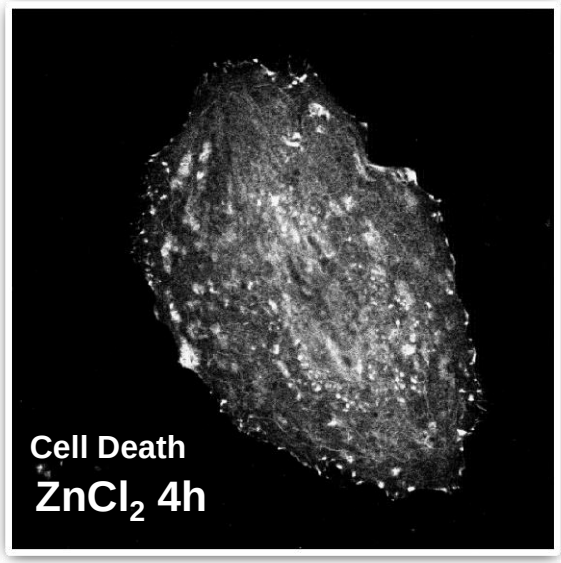


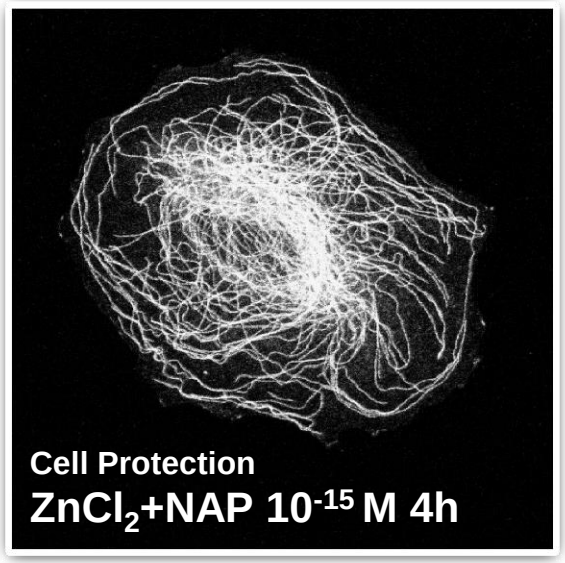
- ADNP heterozygous mice display impaired learning and memory in the Morris water maze
- Impairment is significantly reversed by treatment with NAP

J Pharmacol Exp Ther. 2007 Nov;323(2):438-49. Epub 2007 Aug 24.

Cytoskeletal Protection

- NAPVSIPQ protects astrocytes through interaction with microtubules promoting proper organization of the cellular skeleton





J Biol Chem. 2004 Jul 2;279(27):28531-8.

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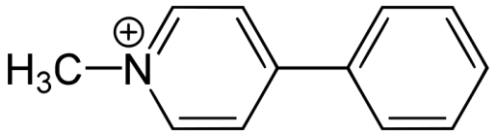
Overview: Mechanism of Action

- NAPVSIPQ and microtubules
  - Co-localizes with microtubules
  - Interacts with brain-specific tubulin
  - Transiently stabilizes structure & function
  - Protects microtubule disassembly
  - Reverses nocodazole-induced depolymerization
- Modulation of microtubule dynamics
  - Increased microtubule integrity
  - Preservation of microtubule function (axonal transport)
  - Cell survival

Can NAPVSPIQ protect dopaminergic neurons?

- Cell-based model
  - Rat mesencephalic neurons (cultured for 5 days) express tyrosine hydroxylase (TH)
  - Neurotoxin: MPP+ (1-methyl-4-phenylpyridinium) blocks mitochondrial oxidative phosphorylation



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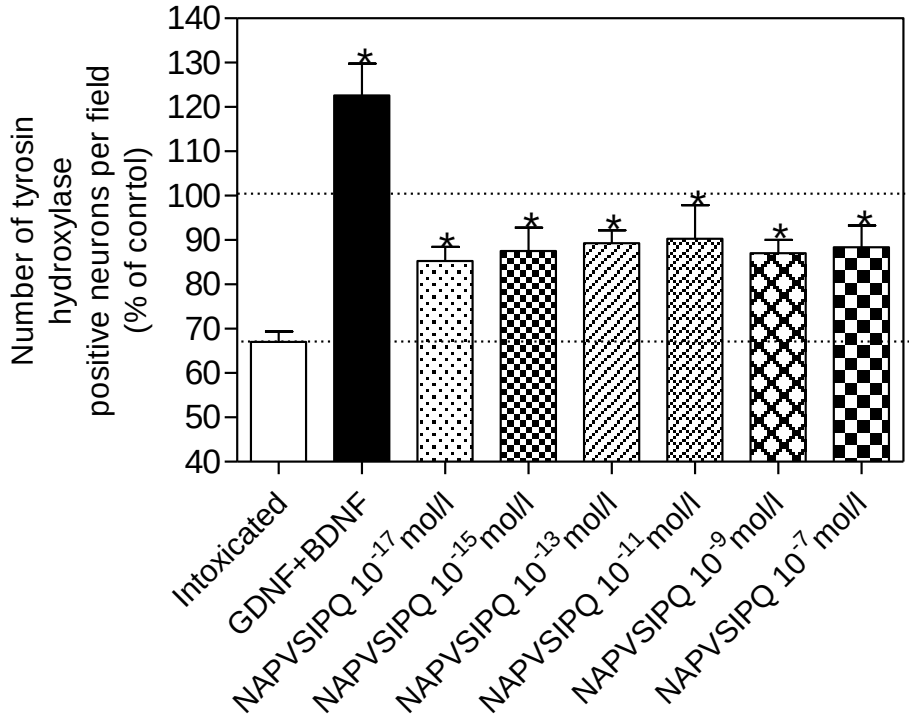
Protection mode



- Rat mesencephalic neurons (cultured for 5 days)
- 2 µM MPP+, co-culture for 3 days

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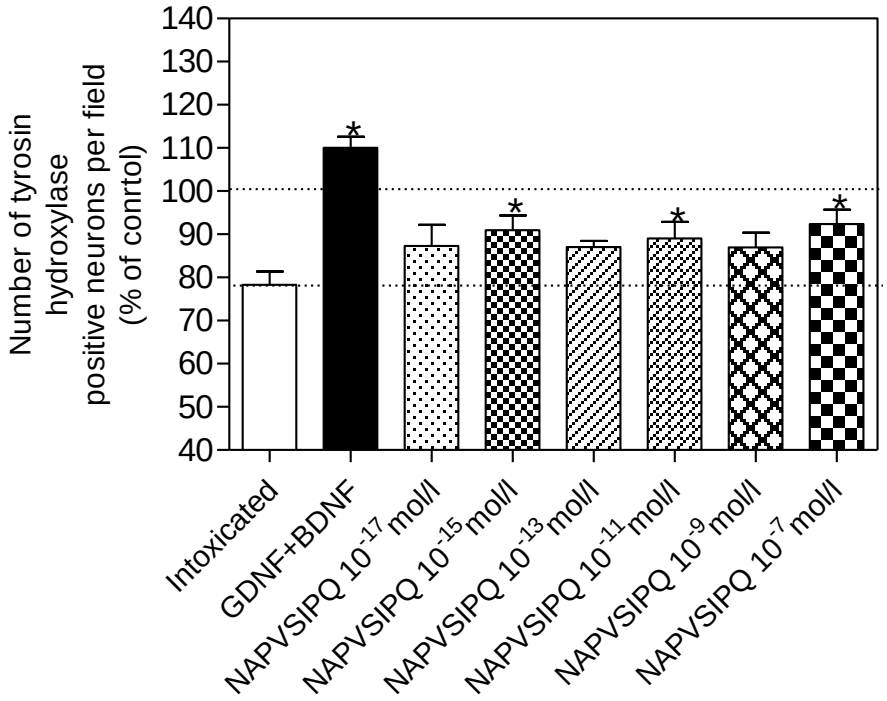
Reversal mode



- Rat mesencephalic neurons (cultured 5 days)
- 2 µM MPP+ for 1 day
- MPP+ washed out
- Culture with test articles for 2 days

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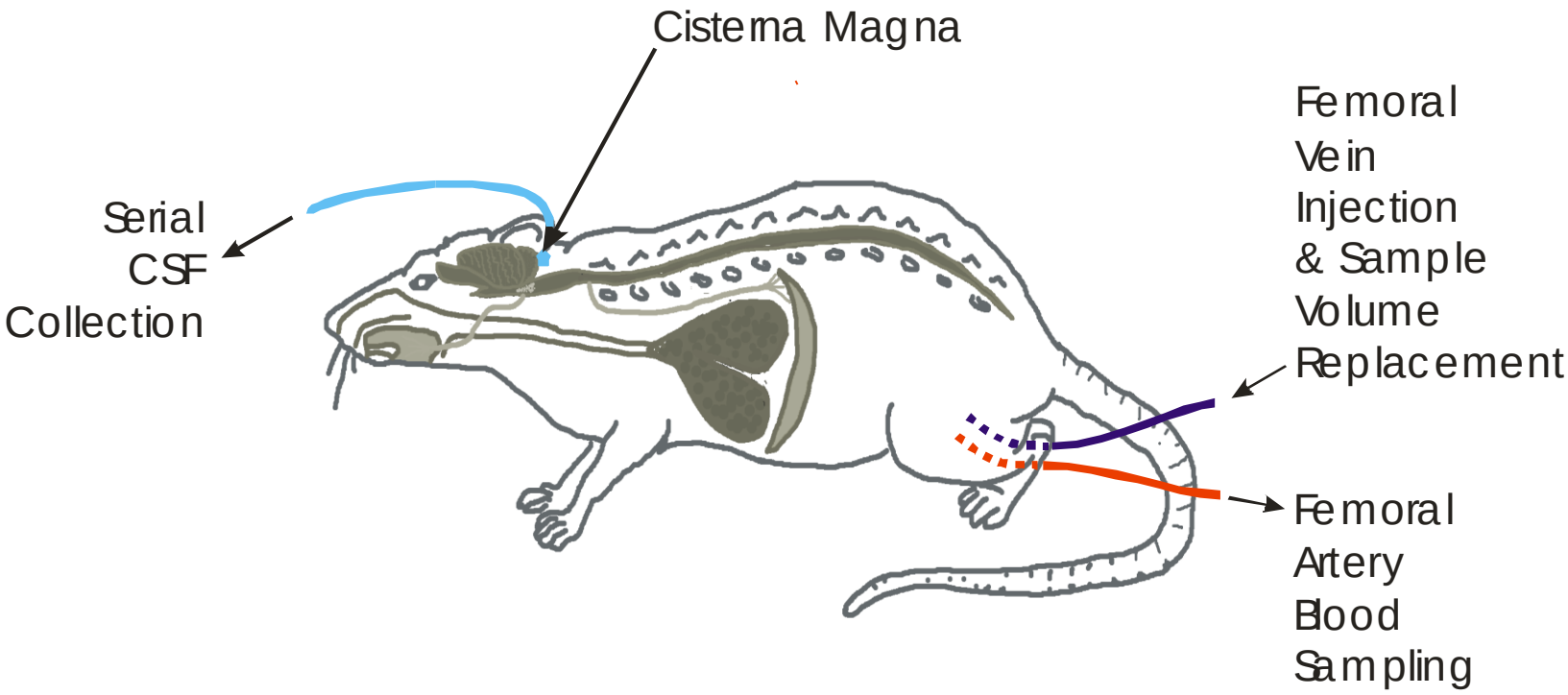
Pharmacokinetics

- Next step…move to animal studies…
  - Understand drug concentrations versus time
  - Does NAPVSIPQ get to the brain?
    - Need to verify the peptide gets to the pharmacodynamic compartment
    - For CNS drugs, this would be the brain or CSF as a surrogate
  - How much NAPVSIPQ gets into the body and brain after intranasal administration?
    - Intravenous administration (assumed as 100%)
    - Compare exposure after IV administration with intranasal

Serial Plasma-CSF Pharmacokinetic Model



Experimental Model

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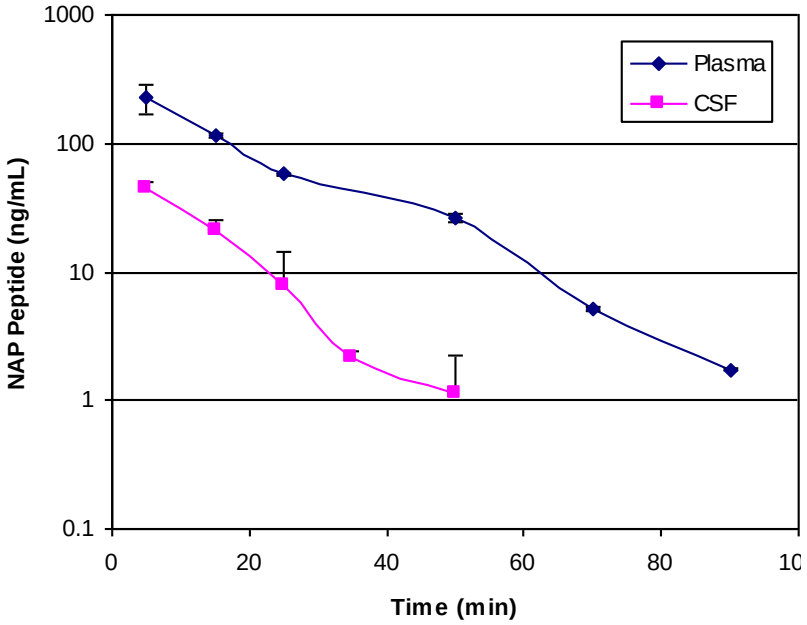
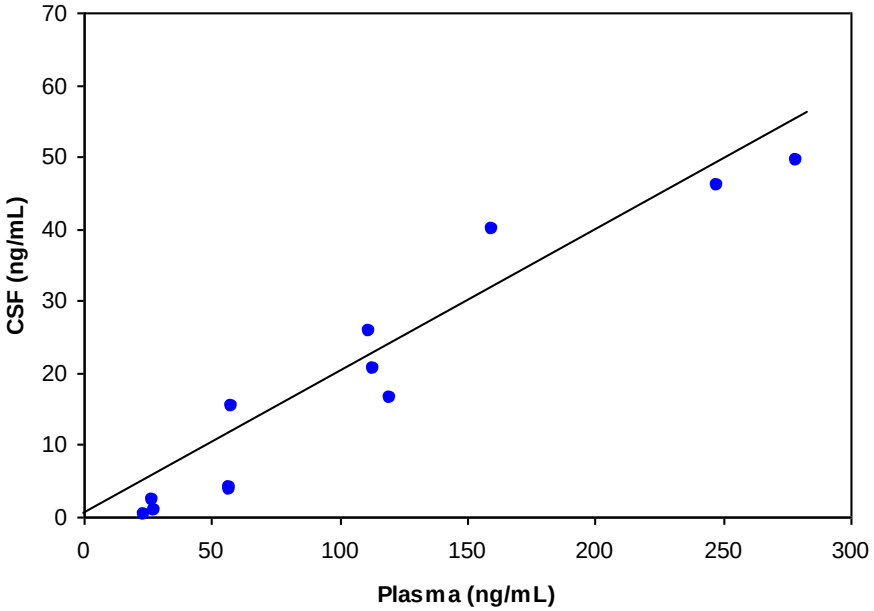
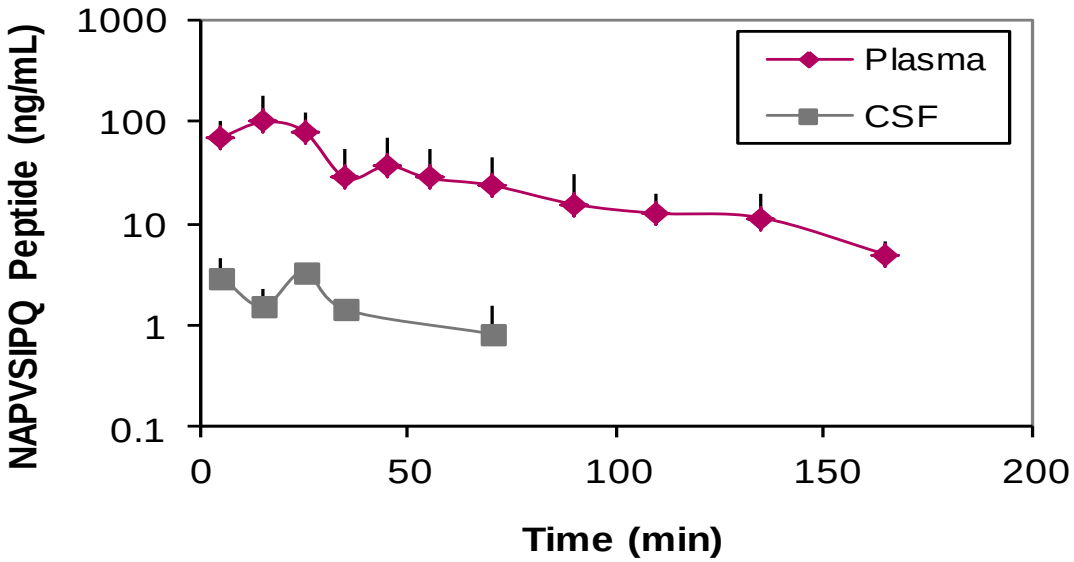
IV: Plasma & CSF Pharmacokinetic Profile (Rat)





- Anesthetized rats (n=3), 30 mg/kg IV
- Serial collection of plasma and CSF
- Linear correlation between plasma and CSF
- 14-20% CSF exposure

IN: Plasma & CSF Pharmacokinetic Profile (Rat)



- Anesthetized rats (n=5), 10 mg per animal IN (~30 mg/kg)
- Serial collection of plasma and CSF

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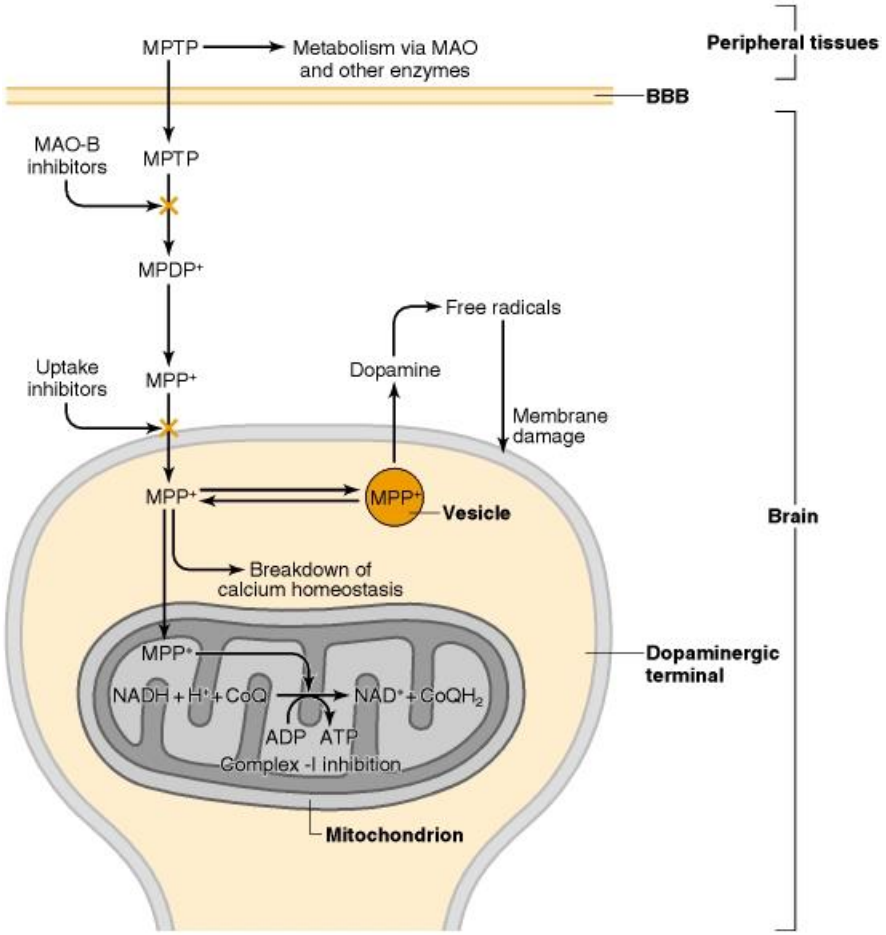
Summary: Pharmacokinetics

- Rapid distribution in plasma and CSF
- Linear correlation between plasma and CSF levels
- Bioavailability proportional to residence time in the nasal cavity. Bioavailability in plasma: ~100%
- Bioavailability in CSF: ~30%

Animal model of PD

- MPTP crosses the BBB
- Selectively destroys dopamine neurons
- Causes “Parkinson-like” phenotype



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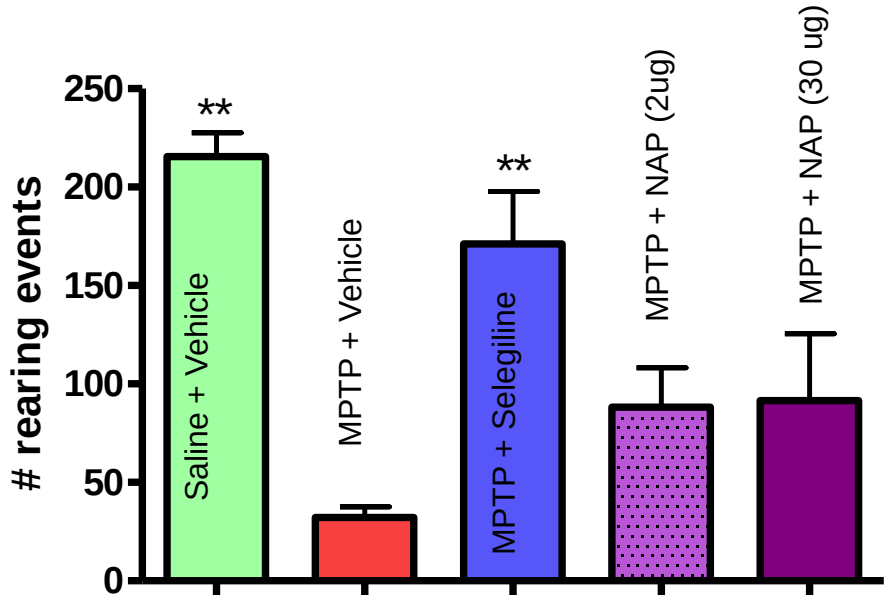
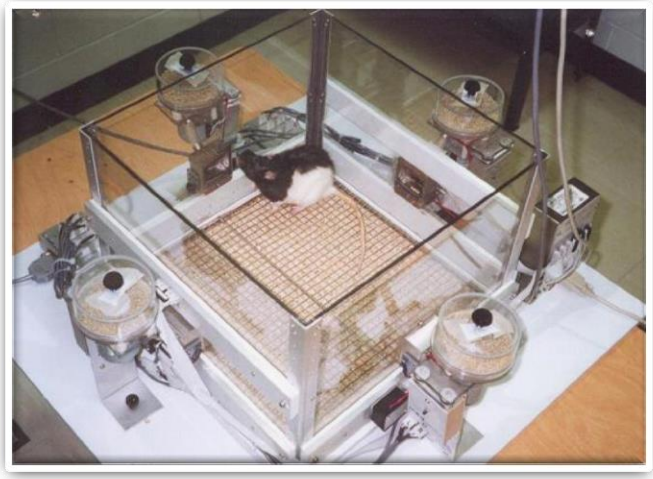
MPTP mouse model: behavior





- 40 mg/kg sc MPTP on Day 1 & 2 (n=10 animals per group)
- NAP peptide administered IN daily for 6 days (start: Day 0)
- Automated open field test
- No change in horizontal locomotor activity

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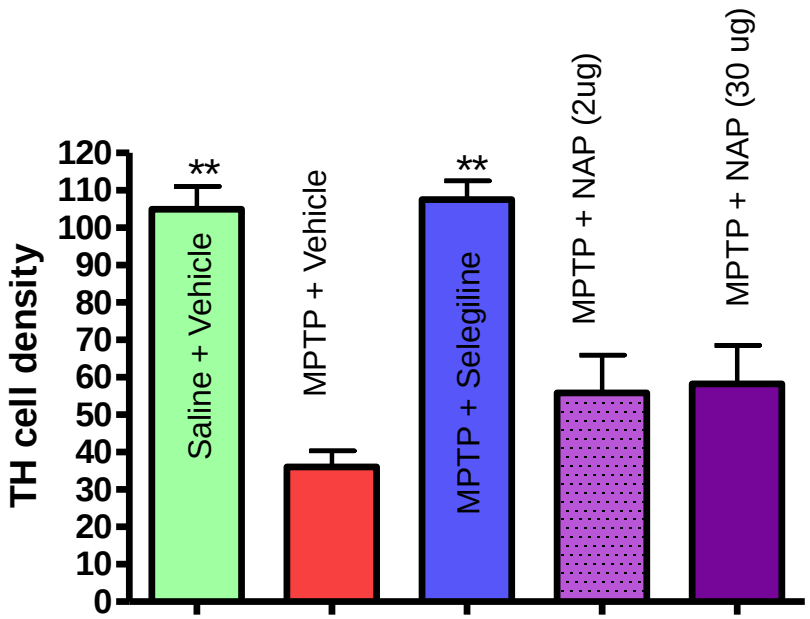
MPTP mouse model: pathology



- 40 mg/kg sc MPTP on Day 1 & 2 (n=10 animals per group)
- NAP peptide administered IN daily for 6 days (start: Day 0)
- Animals sacrificed on Day 5, brains fixed in neutral formalin
- Immunohistochemistry, blind read, cells counted in 3 sections of substantia nigra for all brains

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α-Synuclein (Thy-1) mouse model

- Progressive nigrostriatal motor dysfunction and non-motor olfactory & GI impairment
- Daily IN administration of 2 µg NAPVSIPQ for 8 weeks
- Males (n=12 per group), 1 month of age at start of study
- Behavioral Tests
  - Challenging beam (motor performance & coordination)
  - Pole test (motor coordination)
  - Cylinder test (spontaneous activity)
  - Buried pellet test (olfaction)
- Brain histology
- Collaboration with Sheila Fleming & Marie-Francoise Chesselet (UCLA)

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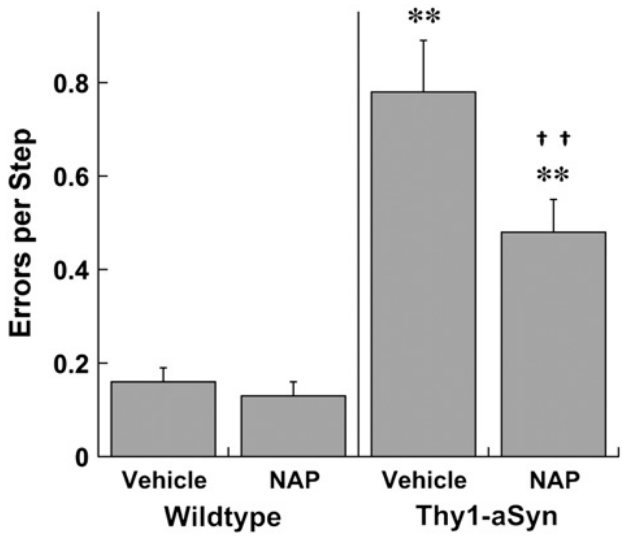
Neuroprotection in Alpha-Synuclein Mouse Model

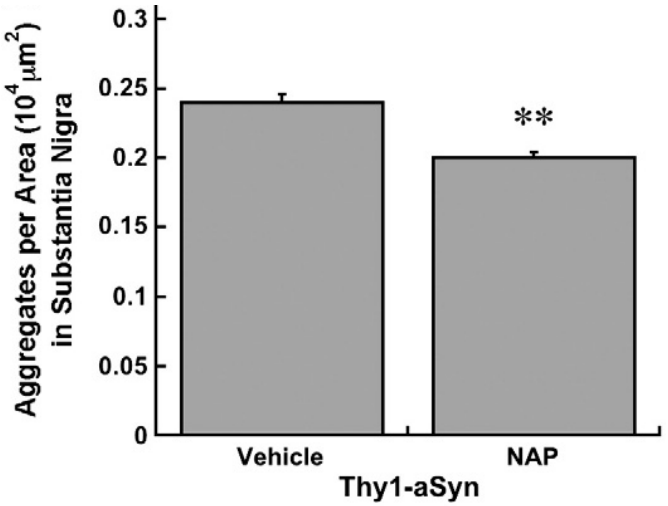
Th1-aSyn Tg mouse model – Motor function on Challenging beam



- Statistically significant improvement in motor function
  - Errors per step on the challenging beam in WT (Veh=15; NAP=16) and Thy1-aSyn (Veh=12; NAP=13) mice treated with intranasal Vehicle or AL-108
  - NAPVSIPQ given at 2ug per day for 2 months, starting at age 1 month of age
  - Challenging beam carried out at 3 months of age

Reduction in Synuclein Pathology in Mouse Model

Th1-aSyn Tg mouse model – Synuclein aggregates



**represent p<0.01 compared to vehicle treated Thy1-aSyn mice.

- Statistically significant reduction in synuclein pathology
  - Average proteinase K-resistant alpha synuclein aggregates from two adjacent sections within the substantia nigra in Thy1-aSyn mice treated with vehicle (n=6) or NAP (n=7)
  - NAPVSIPQ given at 2ug per day for 2 months, starting at age 1 month of age
  - Histology carried out at 4 months of age

Summary: NAPVSIPQ in Parkinson Disease

Brain penetration (intranasal)

MPP+ Toxicity (in vitro)

MPTP Toxicity (in vivo)

Alpha-synuclein Tg (in vivo)

- Plasma-CSF pharmacokinetics
  - NoAb (Missisaagua, Canada)
- Protection and reversal of damage to cultured neurons
  - Neurofit SAS (Illkirch, France)
- Improved motor activity and protection of dopaminergic neurons
  - MDS Pharma Services (Bothell, WA)
- Improved motor performance and reduction in synuclein aggregates in Th1-SynA mice
  - S. Flemming and M-F.Chesselet (UCLA)

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Questions?

bruce.morimoto@celerion.com