Impact of Myosin 5a Mutation In Neurodegenerative Disorders. Rat Model

EX MORTE VENIT VITA

Externus timor maximum concordiae vinculum. Livy

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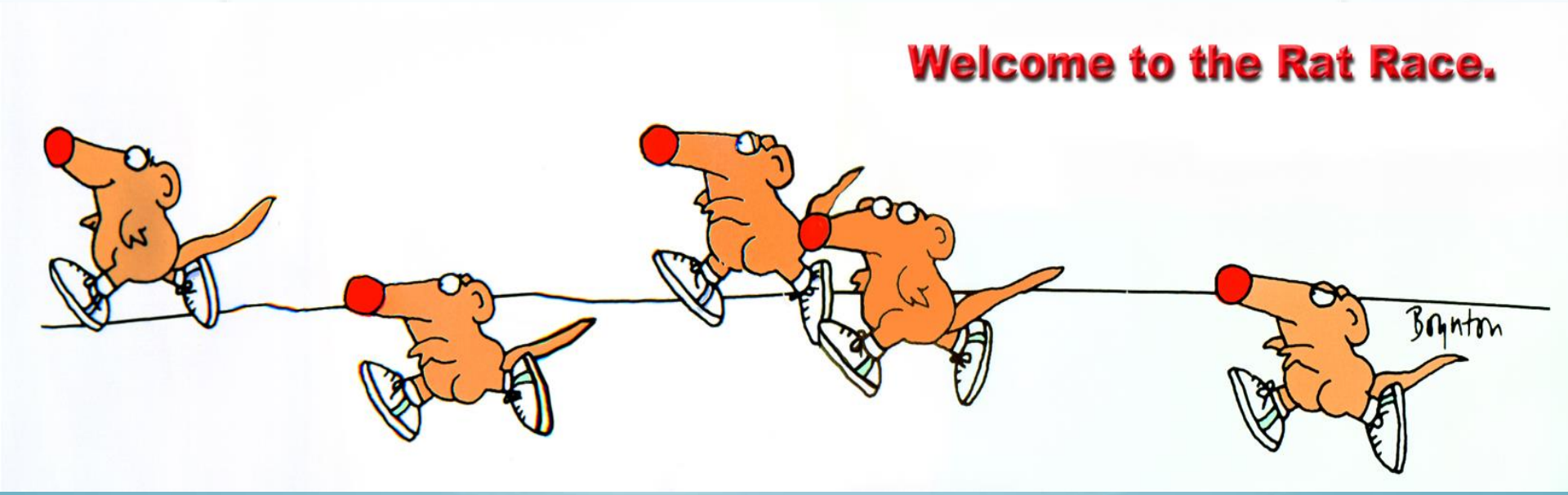
Berlin-Druckrey (BD-IV) rat model for PD/AD
Potential role of Myo5A in neurodegeneration



Presentation Outline

- Class V Myosins
- Myo5A human/animal diseases
- BD-IV rat genetic analysis
- Myo5A interaction with α-syn/tau
- Myo5A dopamine metabolism alteration
- Myo5A miRNA alteration in BD-IV rat
- Conclusions/ Future Directions

Class V Myosins

- Actin-dependent motor proteins
- Involved in intracellular transport of organelles

- Highly Expressed in CNS/PNS
- Three myosin V heavy chain genes (Myo5A,B,C)

Myo5a mutations cause pigmentation and neurological defects in humans and animals

- Mutations in human *MYO5A* cause Griscelli syndrome, type 1 in humans (Griscelli et al., 1978)
- Mutations in horse *MYO5A* cause Lavender Foal Syndrome (Brooks et al., 2010)
- *Myo5a* is mutated in *dilute* mice, (Mercer et al., 1991)
- *Myo5a* is mutated in *dilute opisthotonus* rats (Futaki et al.,2000)
- Myo 5a is mutated in *shaker* BD-IV rat. Stoica et al.,

Griscelli Syndrome type I

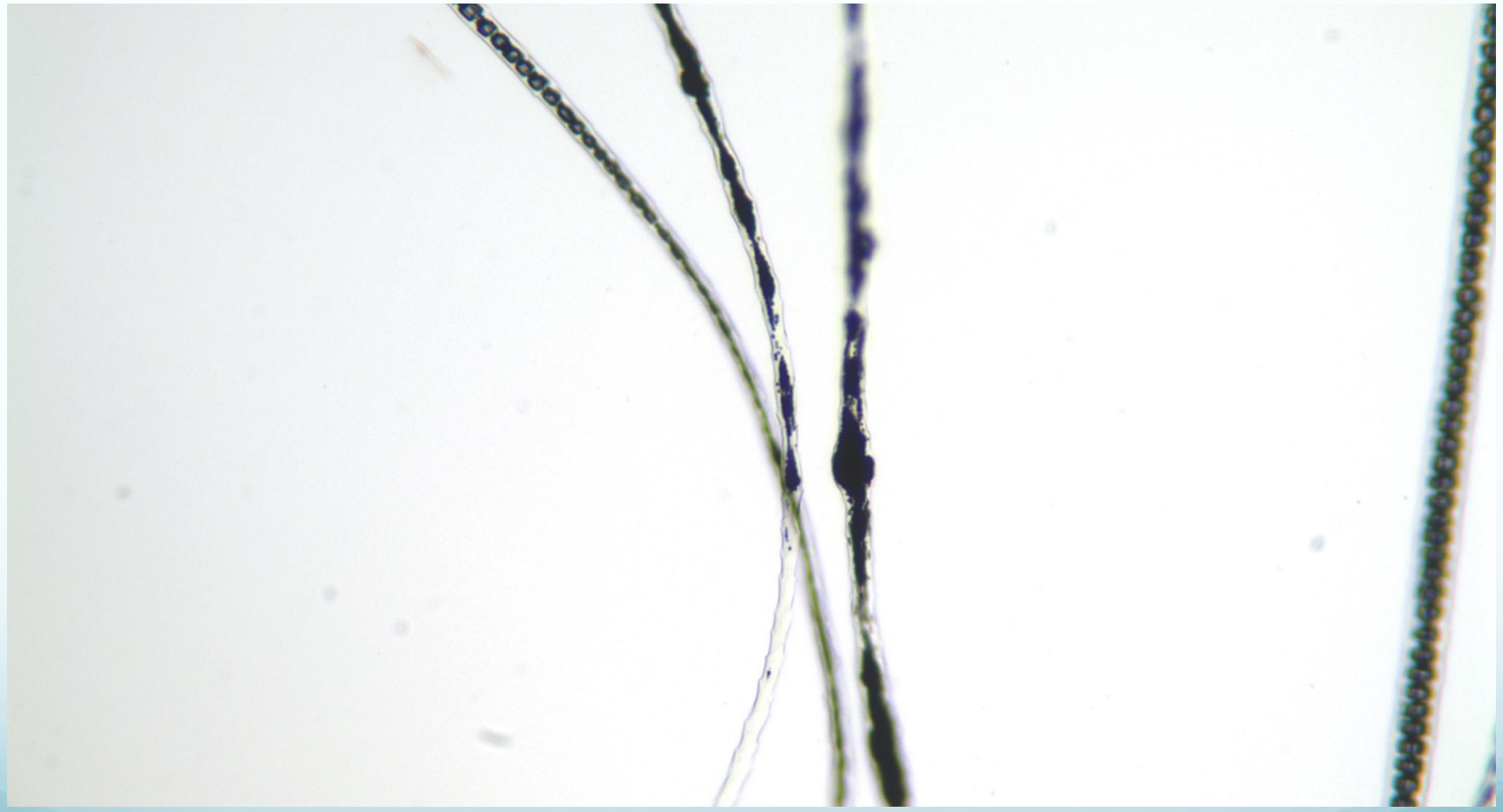
Lavender Foal Syndrome

Myo5a is mutated in mice: *dilute-lethal*

Normall

Mutant

Shaker's BD-IV hair



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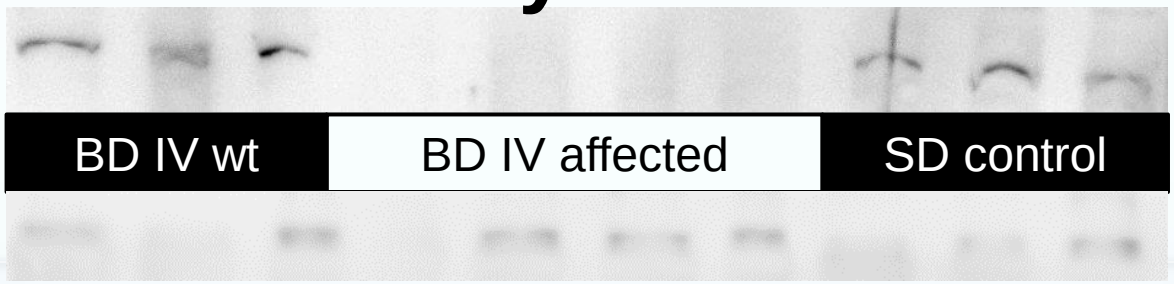
Myo5A

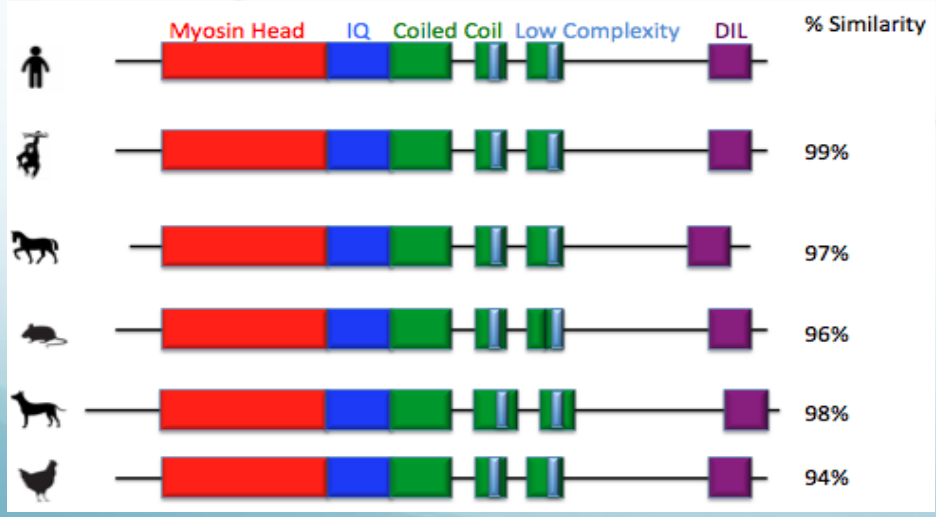


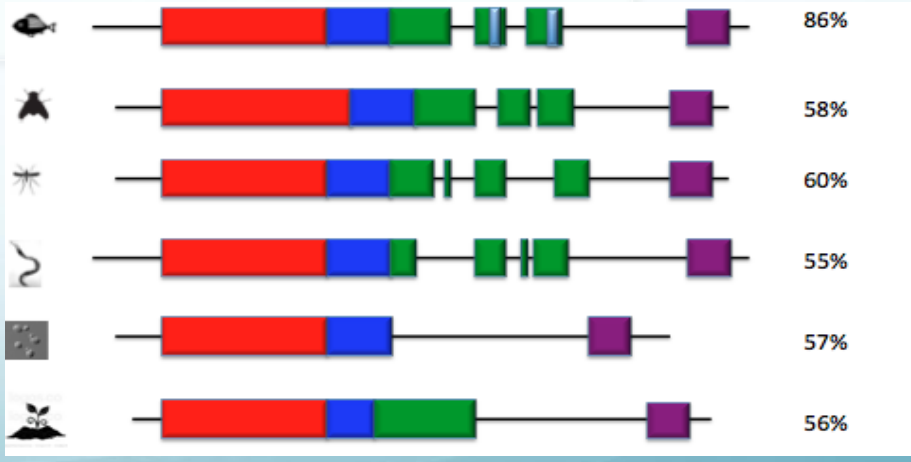
GAPDH

Myo5A

Mammalian genome contains three myosine V genes-Myo5A, B and C- that display differential expression patterns and tissue-specific alternative splice variants. *Myo5A* gene encodes the molecular motor protein Myo5A, found on:

- chromosome 1 in horses
- chromosome 15 in humans
- chromosome 9 in mice
- **chromosome 8 in rat**

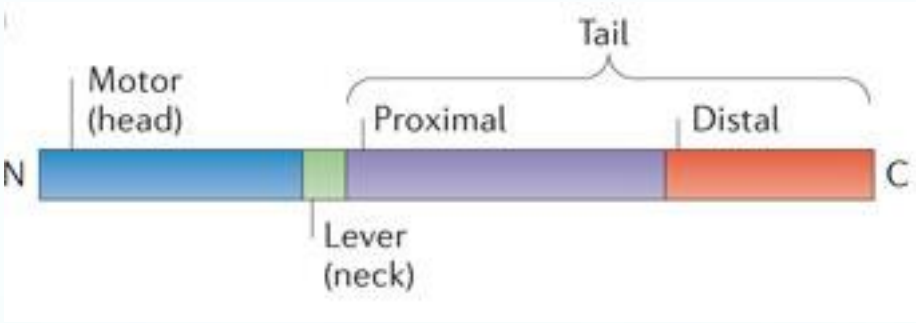


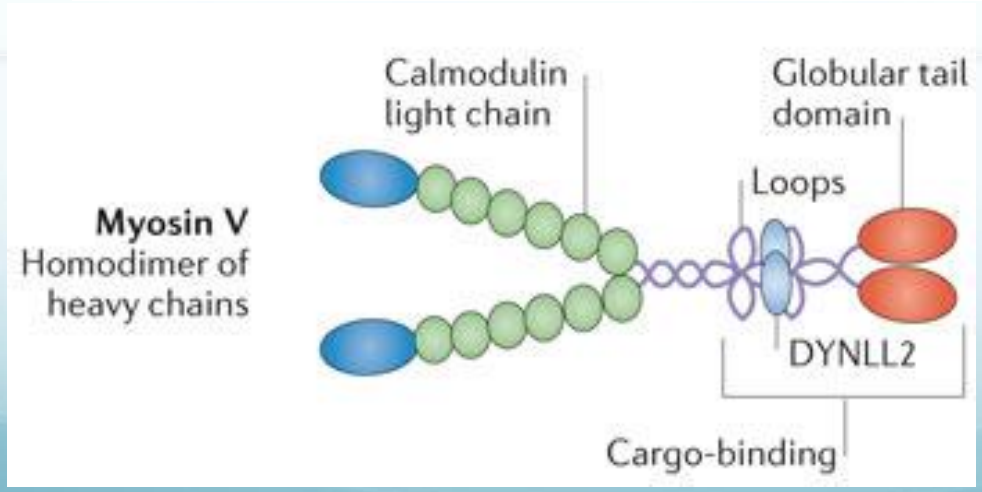


Myo5a is a highly conserved protein from plants to human

Myo5A

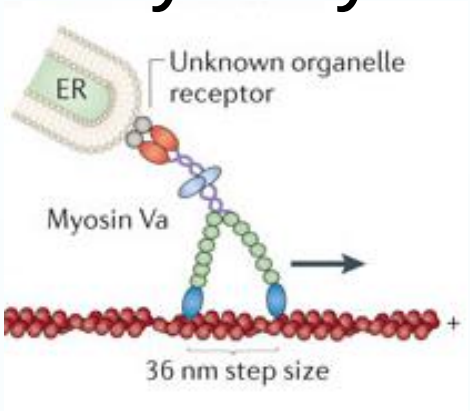
Myo 5A comprises a homodimer of heavy chains, each of which has six IQ-motifs that bind to calmodulin light chains. The Myo5A heavy chains dimerize via a coiled-coil region (purple) that is interrupted by loops. In neuronal Myo5A, the dimeric light chain dynein light chain 2 (DYNLL2; light blue) binds in the coiled-coil region. Both the coiled-coil region and the globular tail domain (red) are involved in cargo binding.





Rudolf *et al.*, 2011

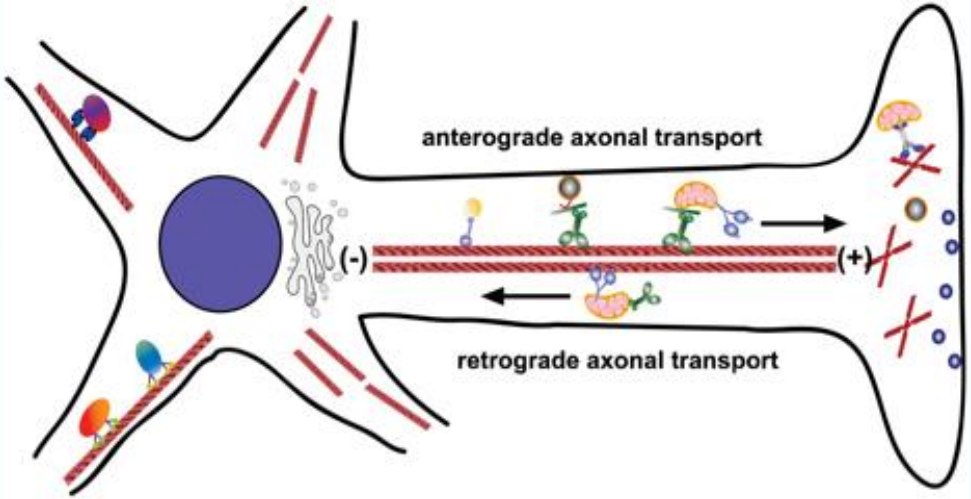
Myo5A-binds actin and produce mechanical force through ATP hydrolysis



- **Myo5A** regulates organelle transport in both melanocytes and neuronal cells (highly expressed in neurons)
- Is highly expressed in the central and peripheral nervous system
- **Myo5A** is a motor protein that is involved in local, actin-based organelle transport
- In Purkinje cells **Myo5A** appears to be involved in transport of smooth endoplasmic reticulum into the spines.

- Kneussel and Wagner, Nature Reviews, 2013

Myo5A is associated with mitochondria and secretory vesicles



Cai, Q.Davis, ML. Sheng, Z. (2011) Regulation of axonal mitochondria transport and its impact on synaptic transmission. *Neuroscience Research.* 70(1): 9---15.

BD- IV Rat Genetic analysis

- Whole genome sequencing

- Hugo Bellen, Professor&Head, Baylor College of Medicine, Houston
Chen Rui, Associate Professor, Baylor College of Medicine, Houston

Mutation found in the
affected rat (*Myo5A*) by whole genome sequencing

Berlin-Druckrey (BD-IV) rats

Control	Control (Carriers) heterozygous	Affected homozygous

Myo5A gene

Results of whole genome sequencing

- located on 8q24 and its size is 118kb (118,043 bp). 182kb (including all the regulatory regions)

- protein size approx. 190 KDa

Shaker rats are homozygous for a splice site mutation in *Myo5a*

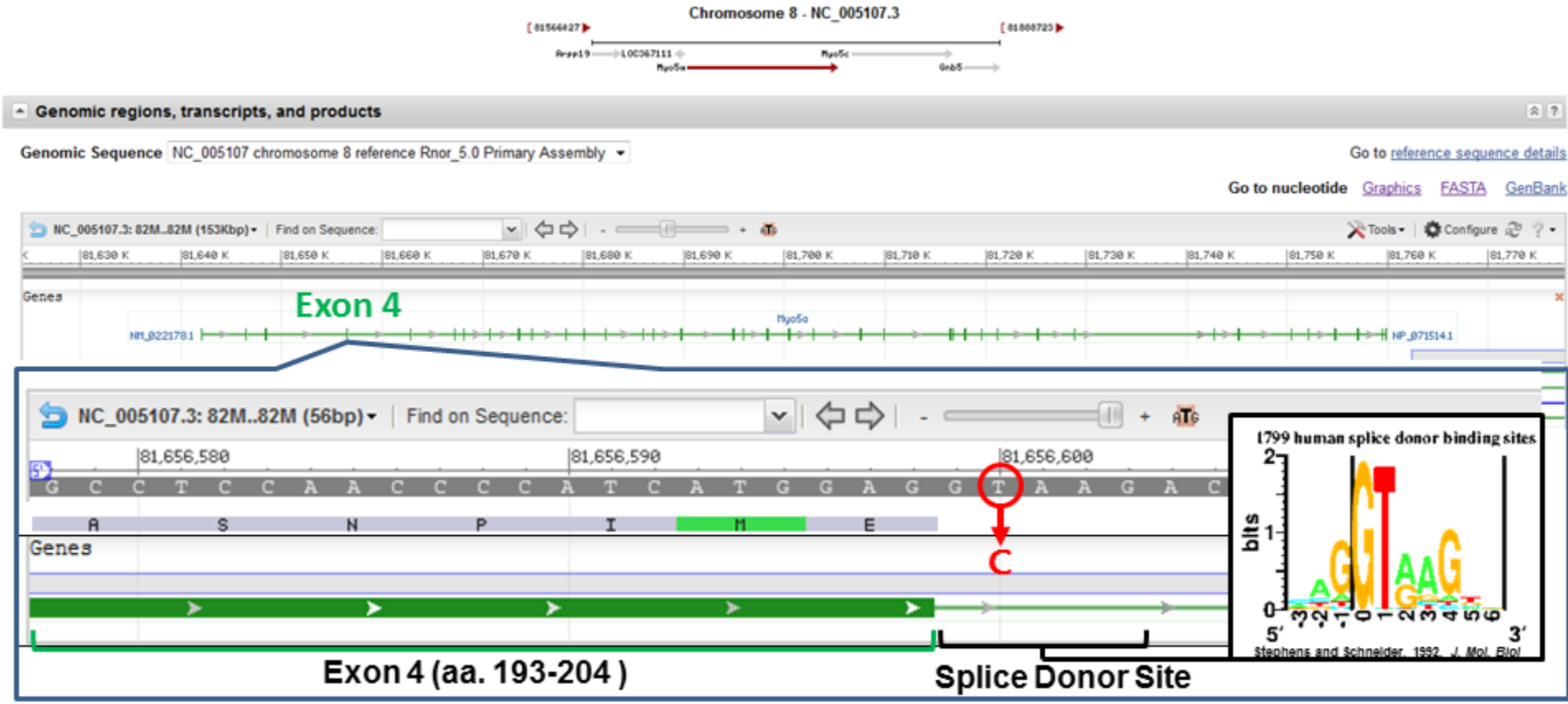
Myo5A Variant Found in *shaker* Rats: Splicing Mutation Variant (Information from Yumei and Rui (HGSC, BCM)

Location: chr8:79923871 (Using reference rn4)
chr8: 81656600 (Using reference rn5)

Reference Allele: T , Variant Allele: C

Primer used for validation:

RAT_MYO5A_101F	CTCGTGTAAAACGACGGCCAGTcttctgctttgccttcacactc	chr8:79923590+79923987 (rn4)
RAT_MYO5A_101R	CTGCTCAGGAAACAGCTATGACtggctggaaaccctagactttc	chr8:79923590+79923987 (rn4)



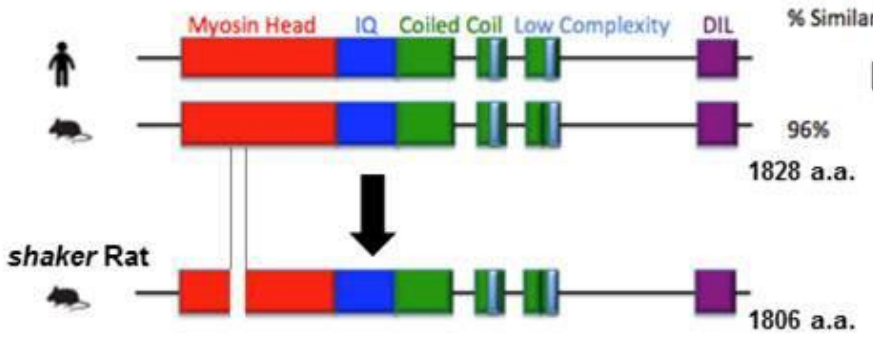
http://www.ncbi.nlm.nih.gov/gene/25017

2 possibilities, both of which are likely to lead to strong loss of function alleles, if not null

Myosin 5A mutation in BD-IV rat

Scenario 1: internal deletion

Internal deletion generated by the splice donor mutation is likely to be deleterious to the motor domain of Myo5a

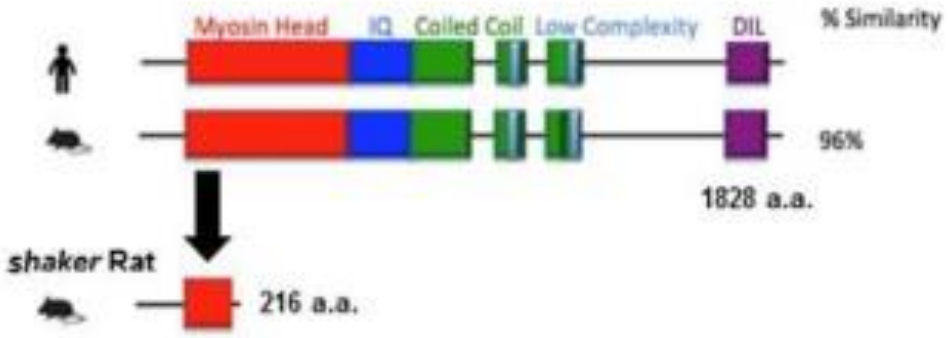


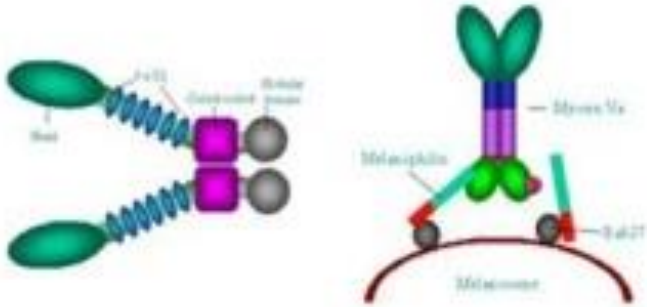
- If this is the case, RT-PCR of mRNA would allow one to detect a band that is 66bp smaller in the mutant (and even heterozygous) animal compared to homozygous wild-type animals

http://blackburngen677s13.weebly.com/domains.html

Scenario 2: read-through and early termination

The alternative scenario is that the intronic sequence will be transcribed and translated. This adds 12 random amino acids after E204 and terminate, leading to an early truncation of Myo5a. The protein will most likely be non-functional.





Myo5a is most known for its role in melanosome trafficking. Deletion of 2/3 of the motor domain together with rest of the protein will most likely kill the function of the protein

- If this is the case, western blotting or immunohistochemistry using Myo5a will fail to detect a signal, or very detection of a 20-30 kDa. protein if the truncated protein is stable.

Myo5a and alpha-synuclein

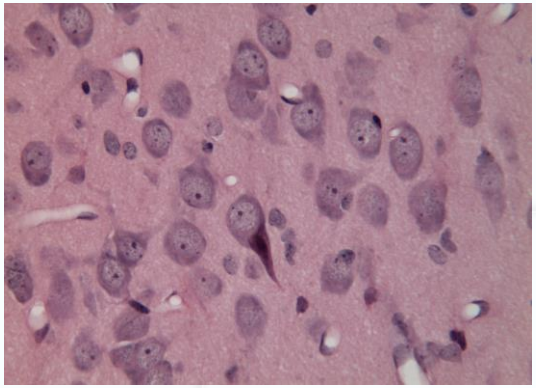
Myo5A interacts with alpha-synuclein at the presynaptic terminals?

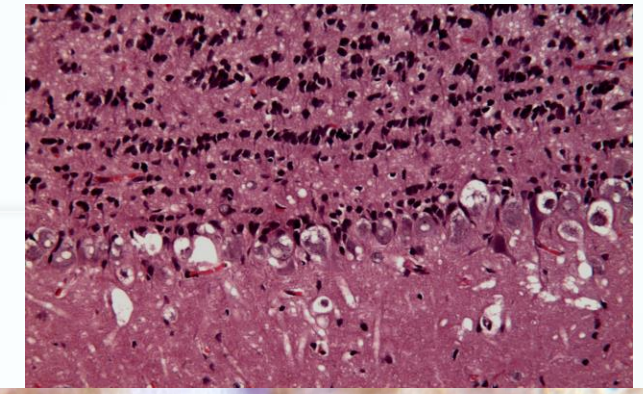
Myo5A influences accumulation and/or aggregation of alpha-synuclein at the presynaptic terminals?

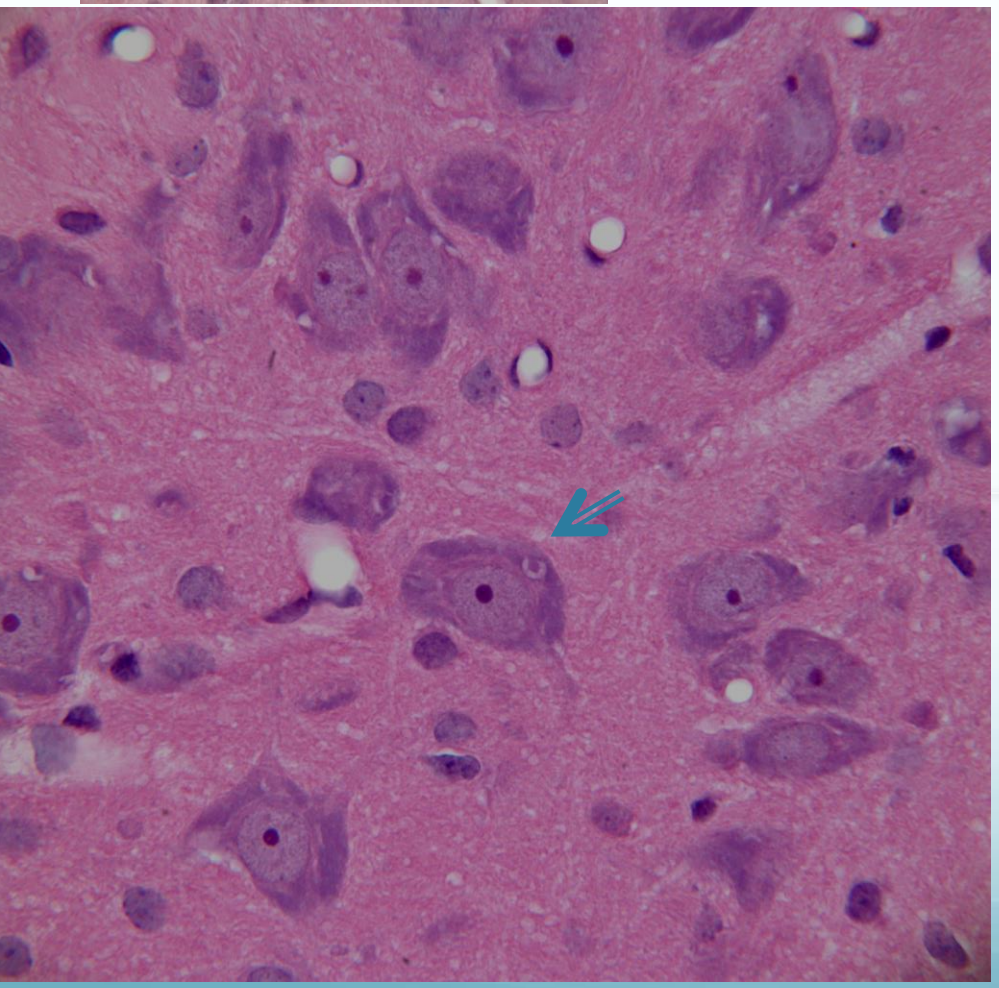
Myo5A plays a role in PD/AD?!

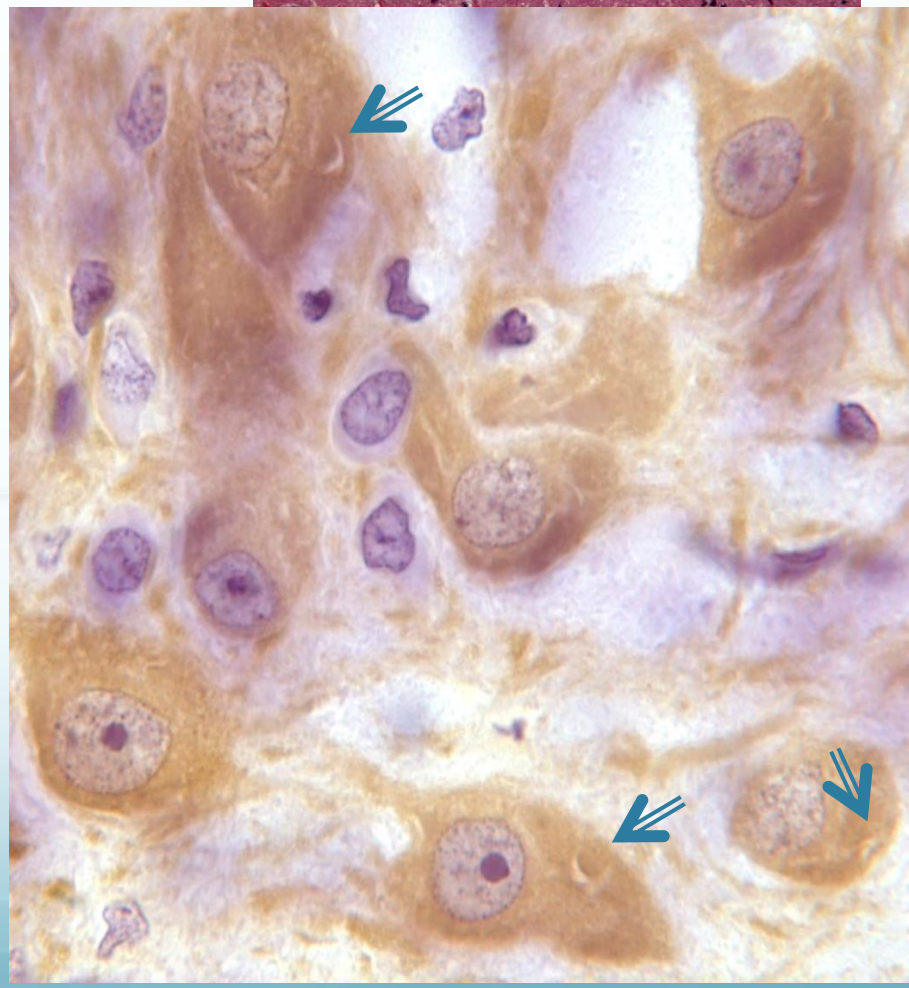
- **α-syn is the most enriched mRNA associated with Myo5a**
- **RNA localization and local protein synthesis may be involve in neurodegenerative disorders such as PD (**Calliari et al., Developmental Neurobiology, 2013)

Morphological changes



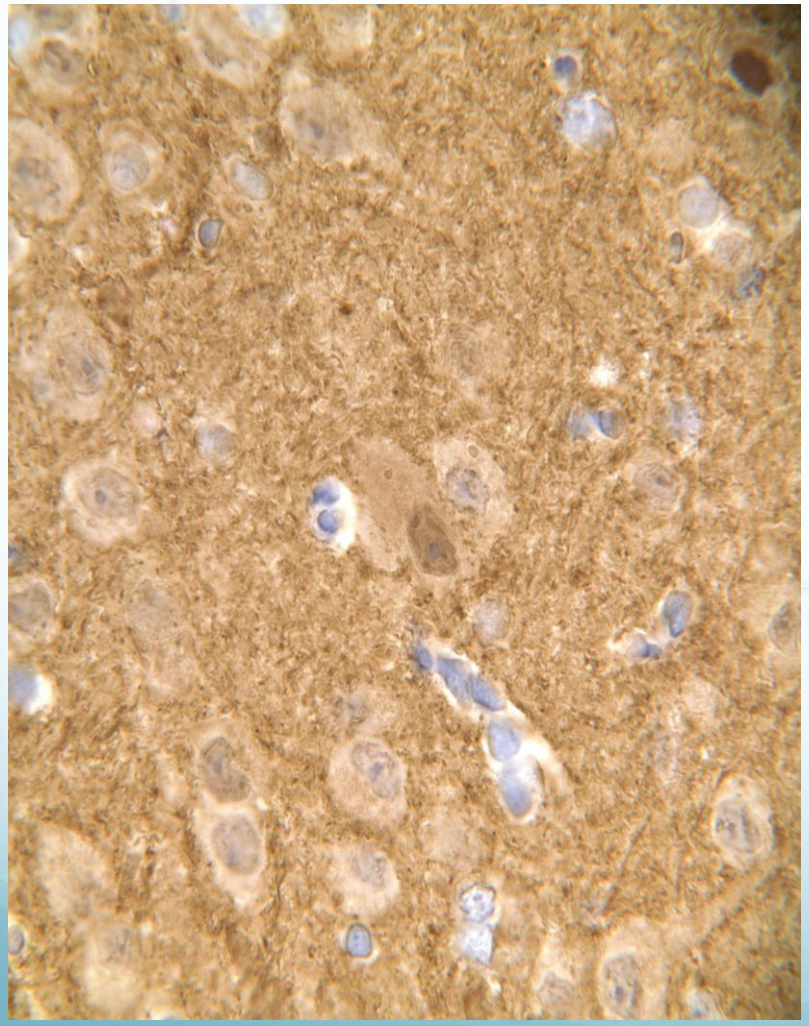




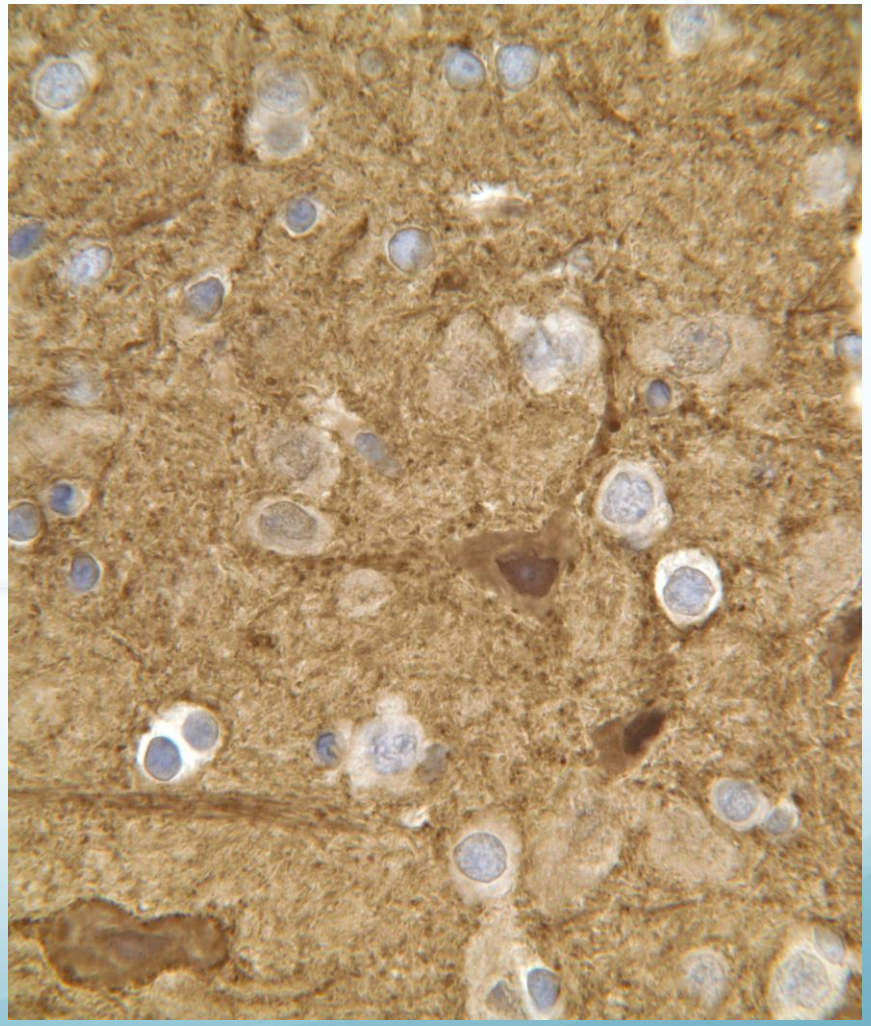


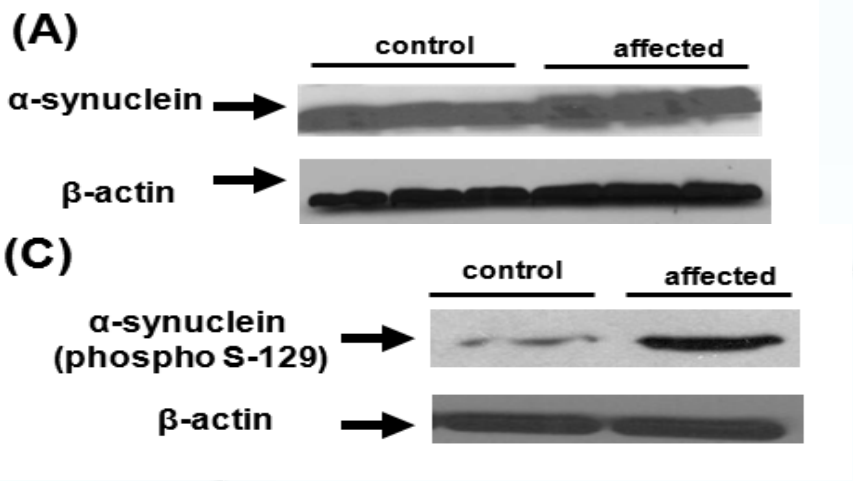
α-synuclein LB

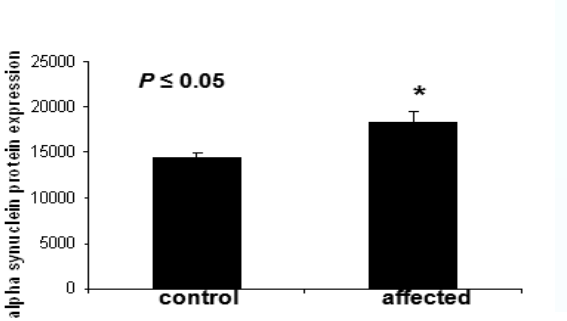
Striatum

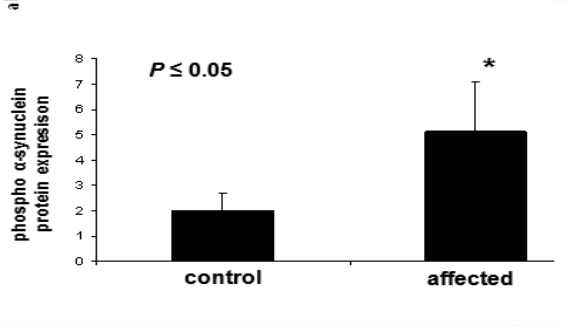


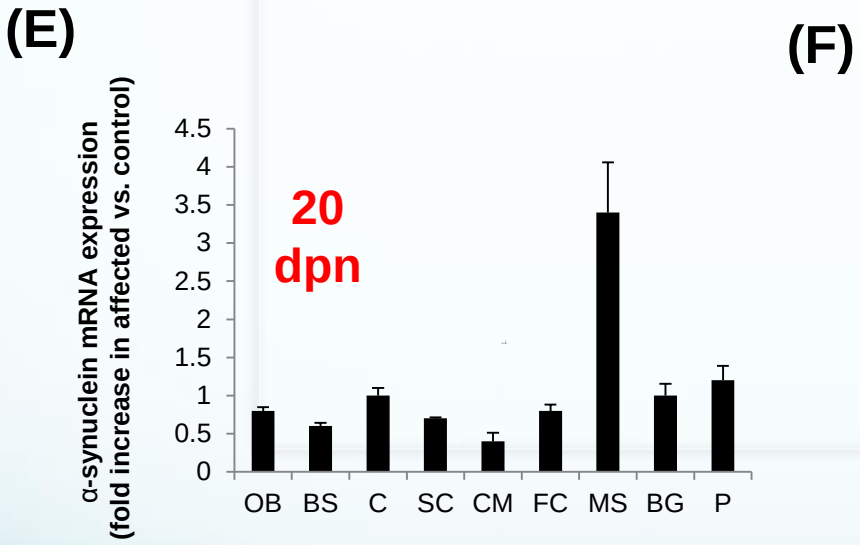
SN

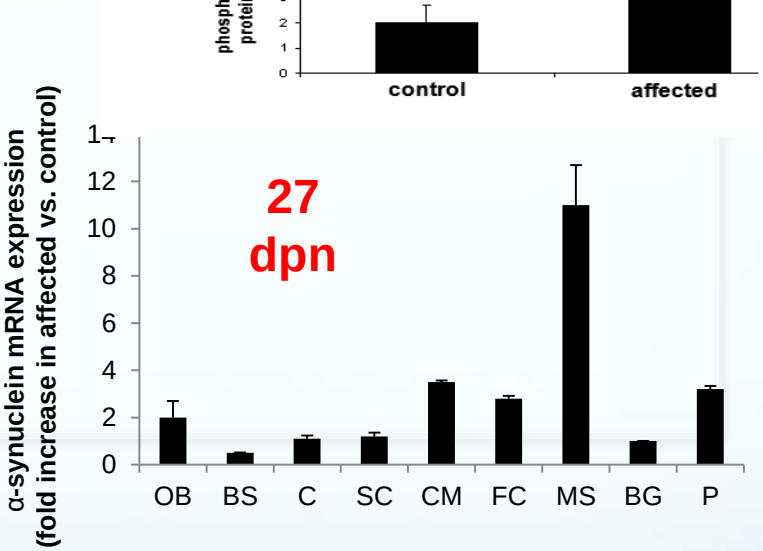




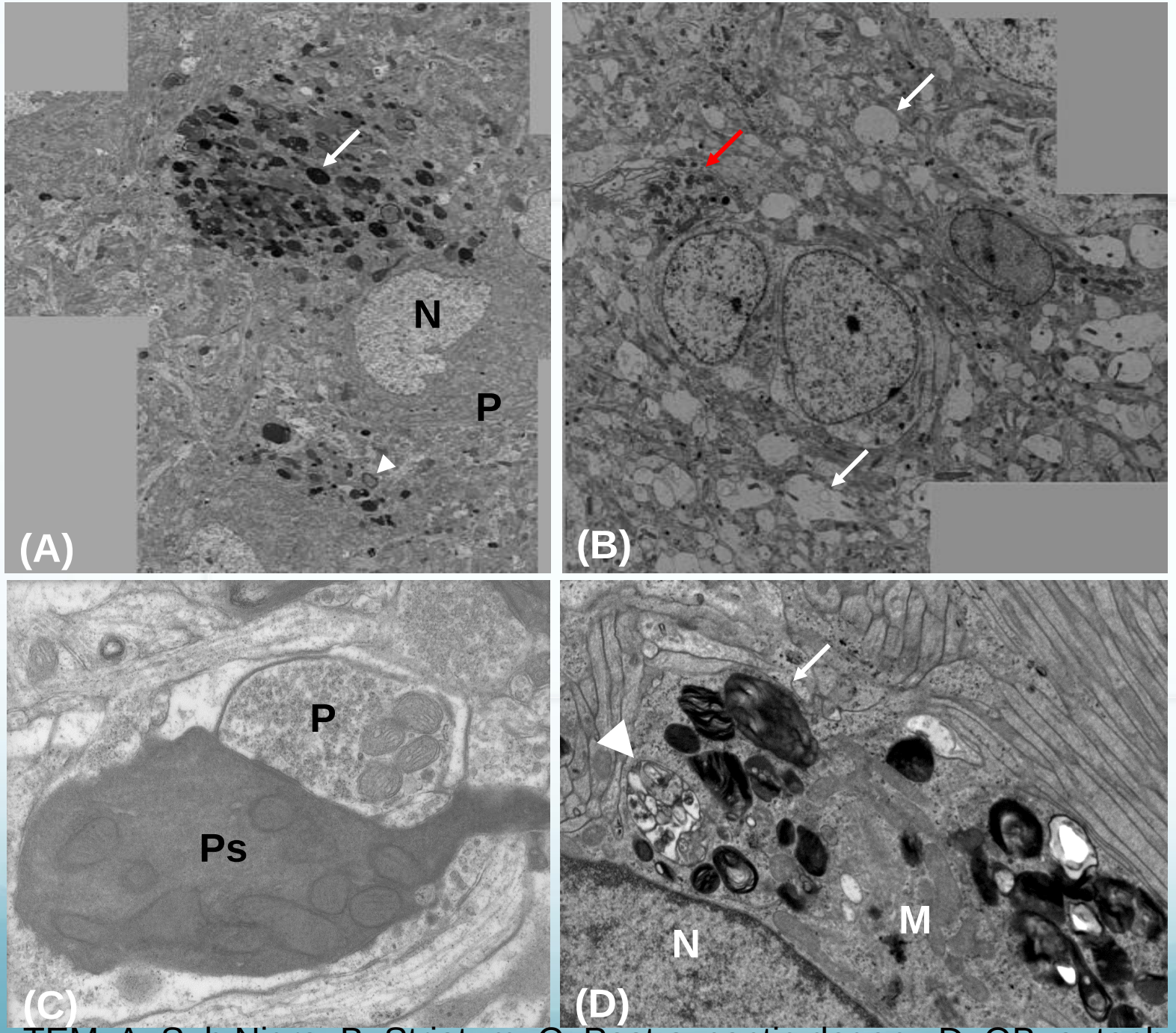






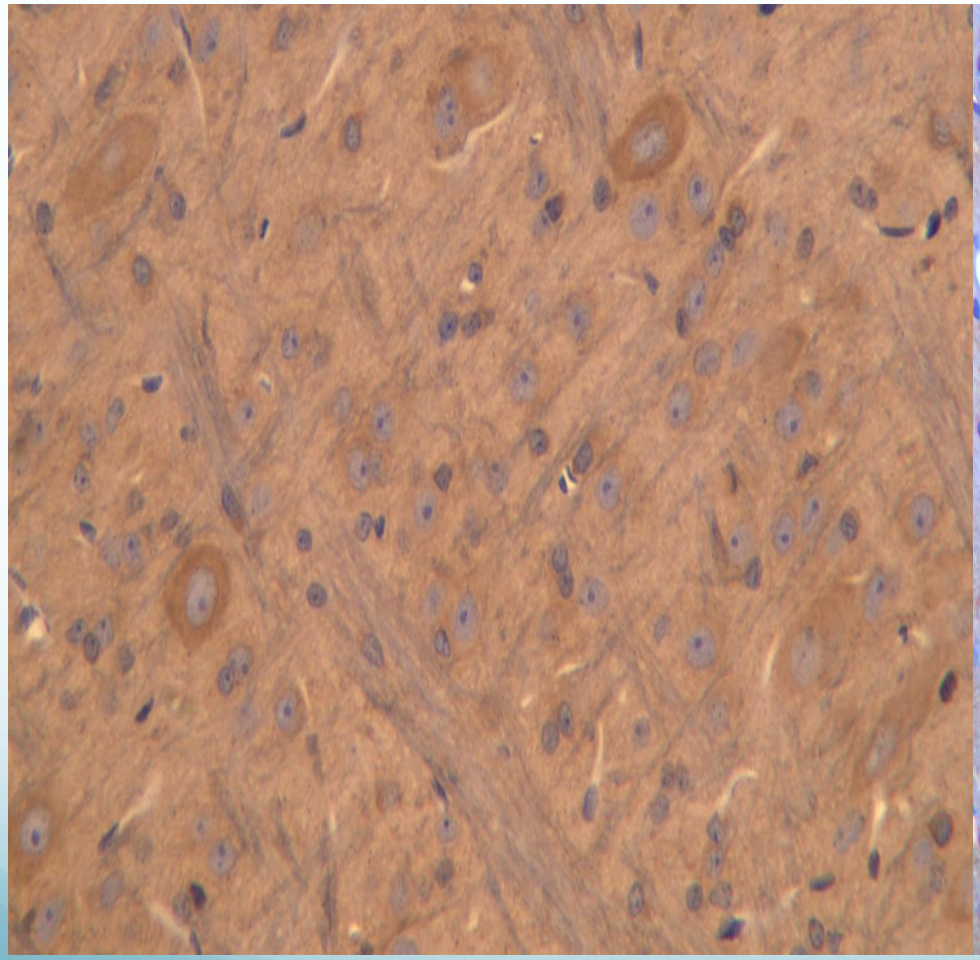


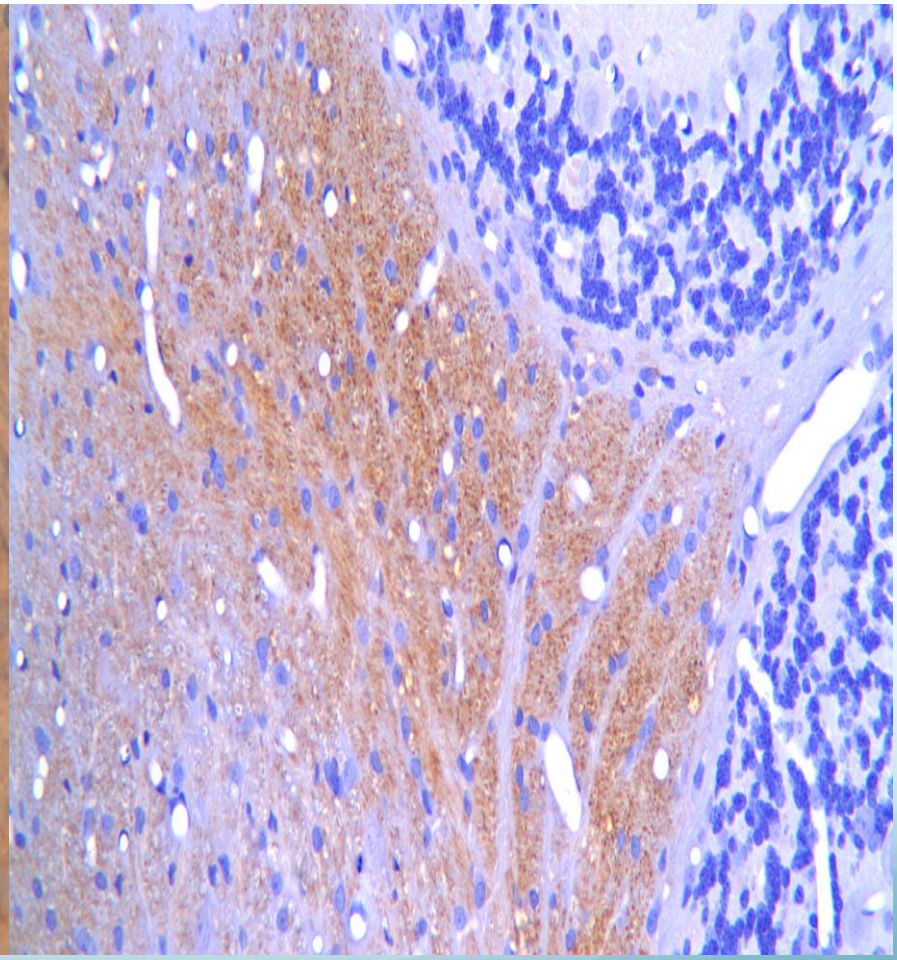
Stoica et al., 2012



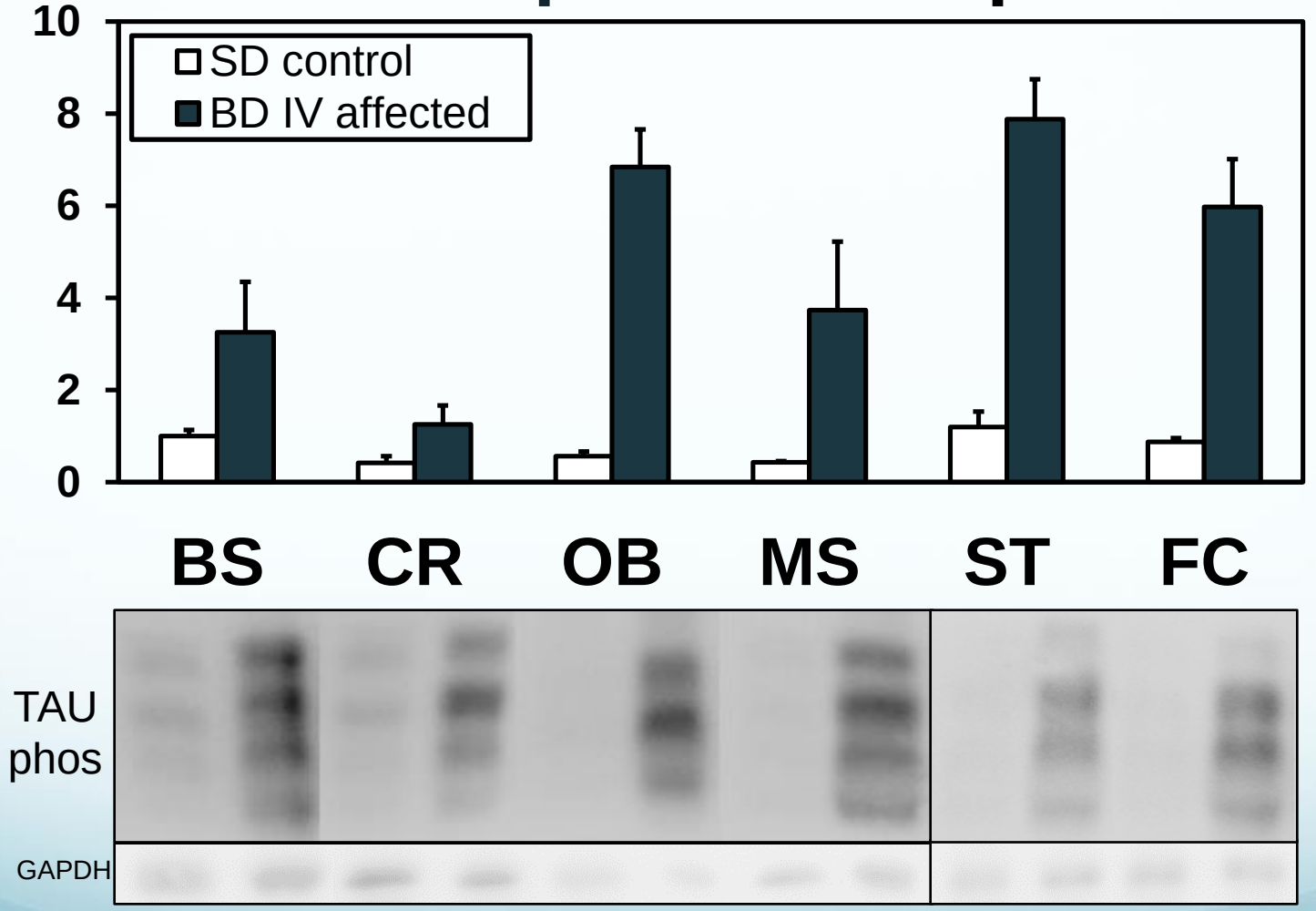
TEM: A. Sub.Nigra, B. Striatum, C. Post-synaptic degen., D. OB, autophagy

BD-IV shaker rat: Brain stem and cerebellum nuclei Ph. Tau

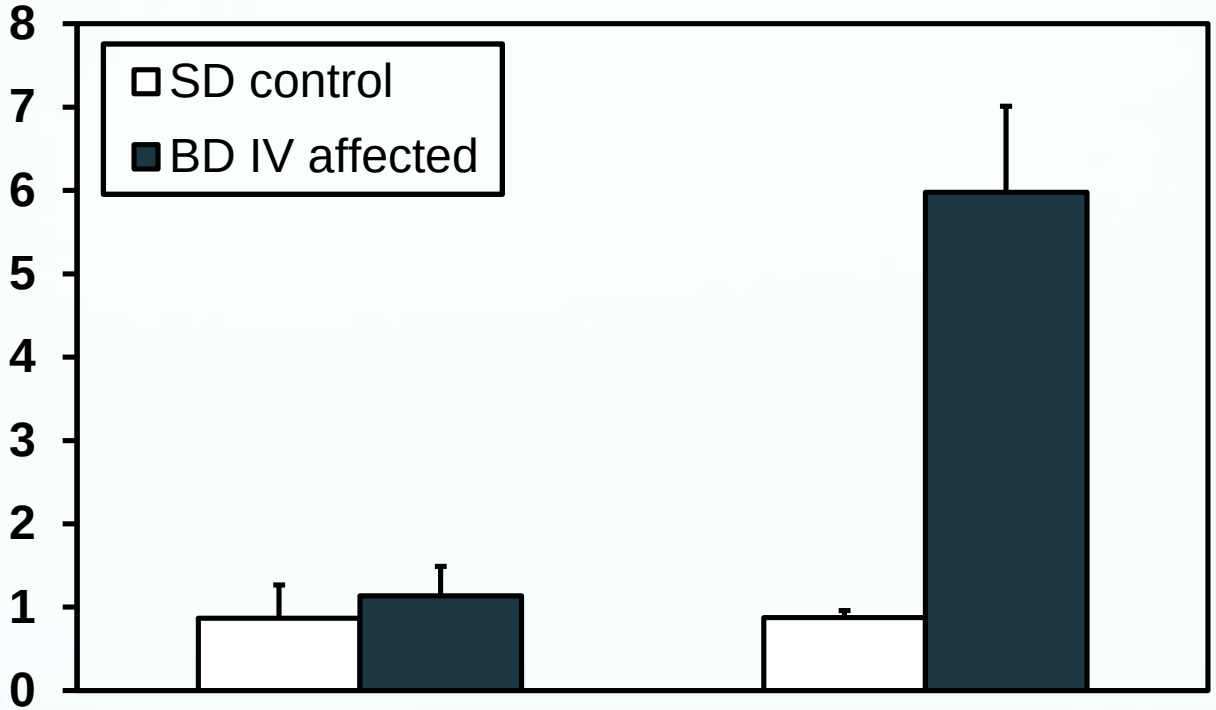


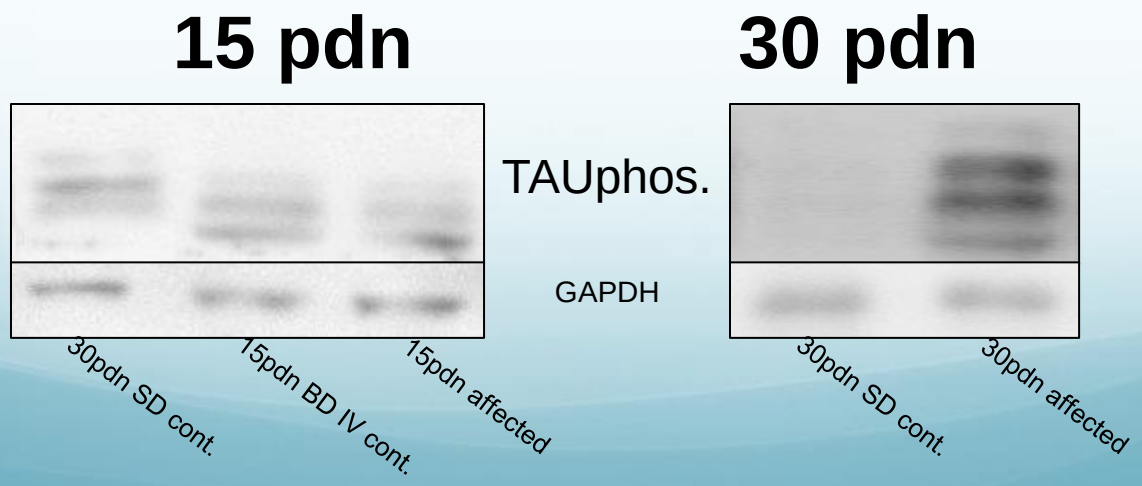


TAU phos. in 30dpn rats



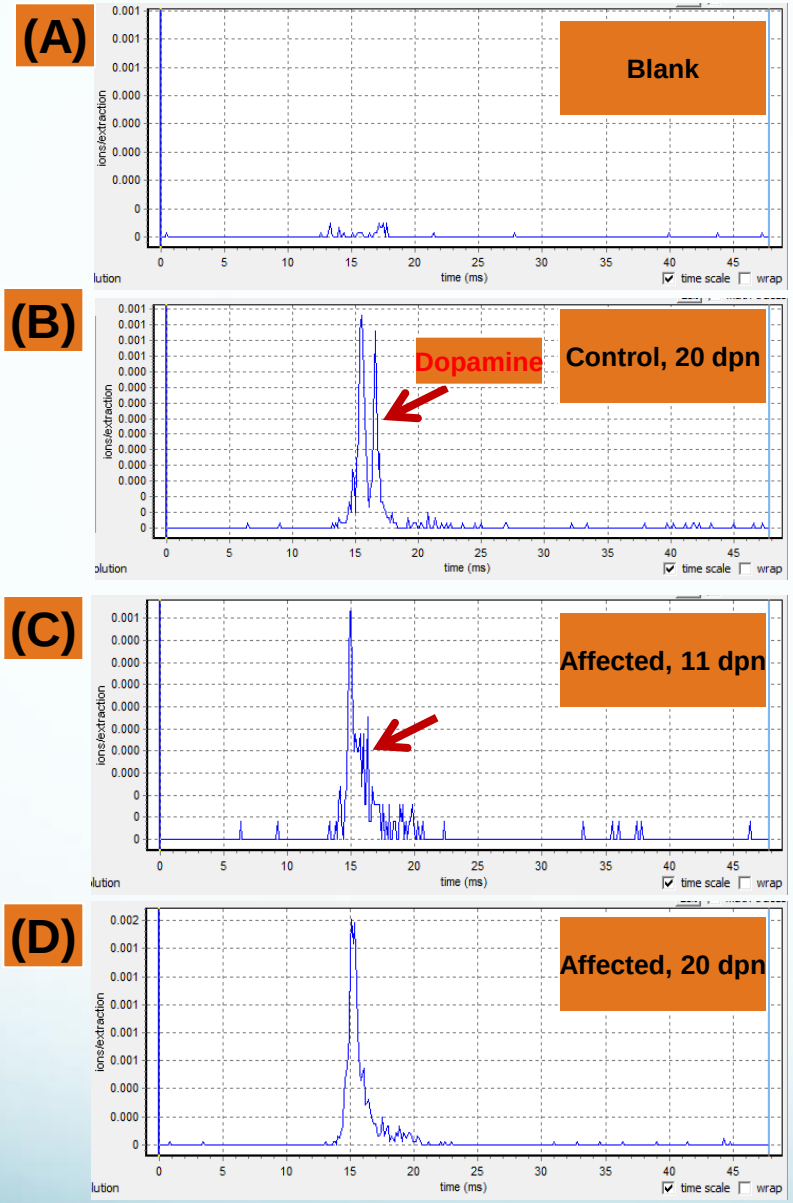
anti-TAU (ph S396)
in the Frontal Cortex



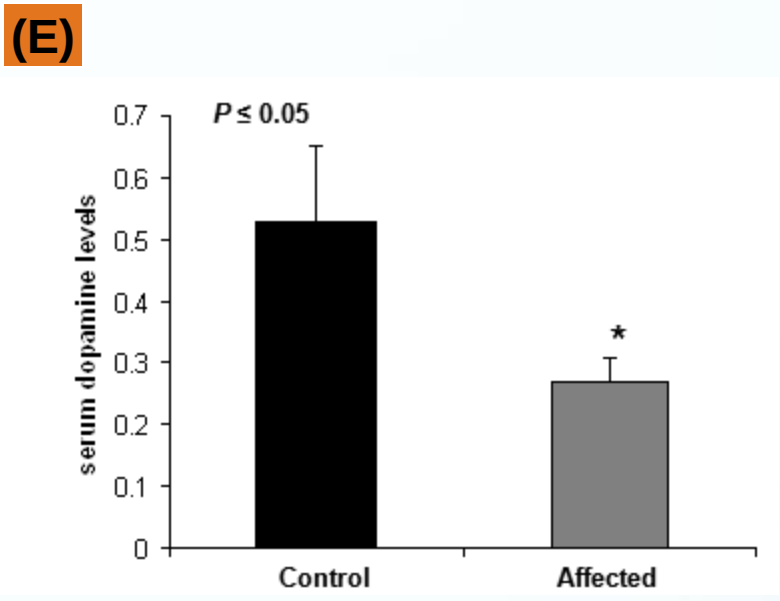


Alteration of Dopamine metabolism

Catecholaldehyde hypothesis



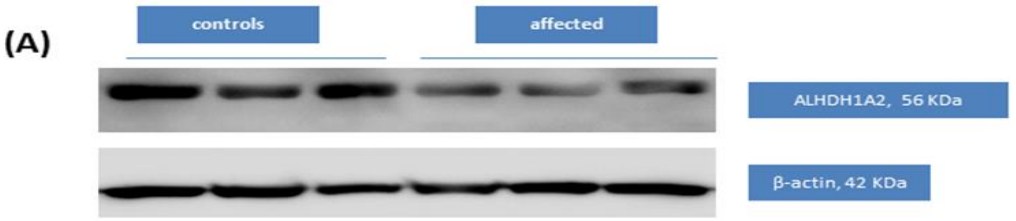
Zhang *et al*., 2014. Metabolic analysis of striatum tissues from Parkinson's disease-like rats by electrospray ion mobility mass spectrometry.

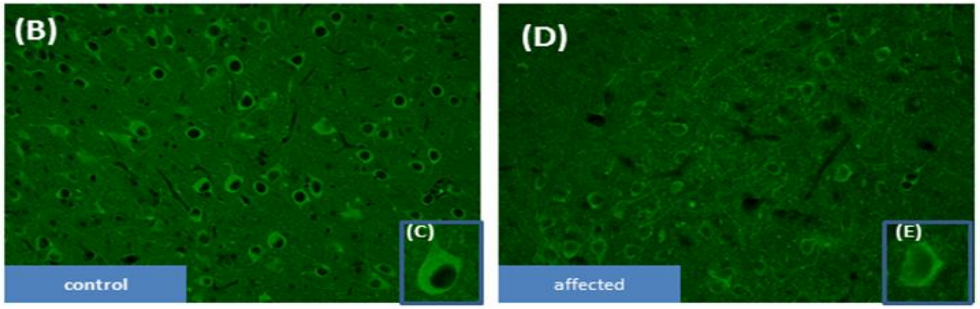


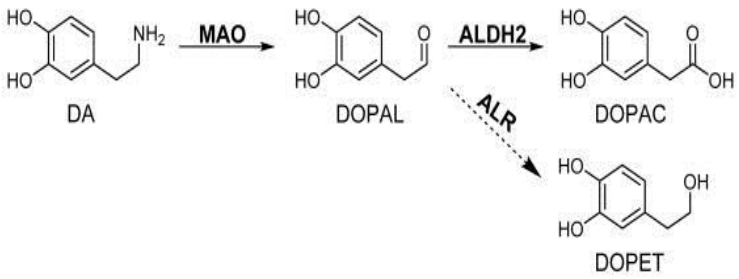
Serum dopamine levels from control and affected BD-IV rats sacrificed at 24 dpn.

Ion mobility mass spectrum for blanks **(A)** as well as normal (B) and affected rat striatal tissues at 11 (C) and 20 dpn (D). The labeled peak was selected for at the reduced mobility of dopamine and at m/z = 154, the mass of the dopamine ion. The striatal level of dopamine in affected rats was reduced by > 90%, relative to control rats.

ALDH1A2



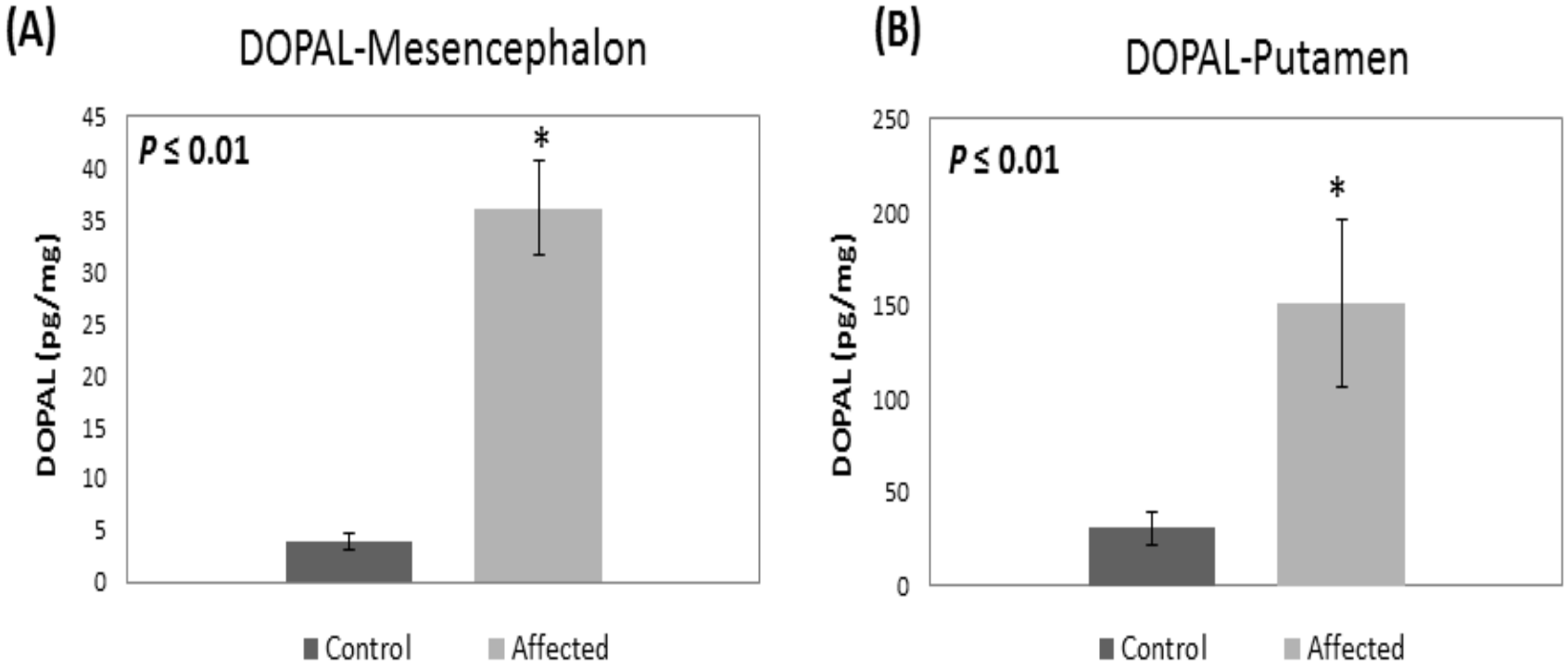




Aldehyde dehydrogenase participates in the metabolism of catecholamines including dopamine (DA) and converts 3,4- dihydroxyphenylacetaldehyde (**DOPAL**), a potentially toxic aldehyde, to 3,4-dihydroxyphenylacetic acid (**DOPAC)**, a non-toxic metabolite.

The decreased levels of aldehyde dehydrogenases were associated with loss of neurons in SN and decline in motor function, supporting the hypothesis that impaired detoxification of biogenic aldehydes are important in the pathophysiology of PD.

HPLC analysis of DOPAL



DOPAL neurotoxicity and its role in PD was demonstrated both *in vivo* and *in vitro* and supports of “catecholaldehyde hypothesis” as an important link in the pathogenesis of PD.

DOPAL causes α-syn aggregation

- Goldstein *et al*., 2012 showed that DOPAL potently oligomerizes α-syn and appears to aggregate mutant form of the protein.

- Burke *et al*., 2008 showed that DOPAL injection into the SN of Sprague-Dawley rats resulted in DA neuron loss and the accumulation of high molecular weight oligomers of α-syn detected by Western blot. These findings support the hypothesis that DA metabolism via DOPAL can cause both DA neuron loss and α-syn aggregation observed in PD.

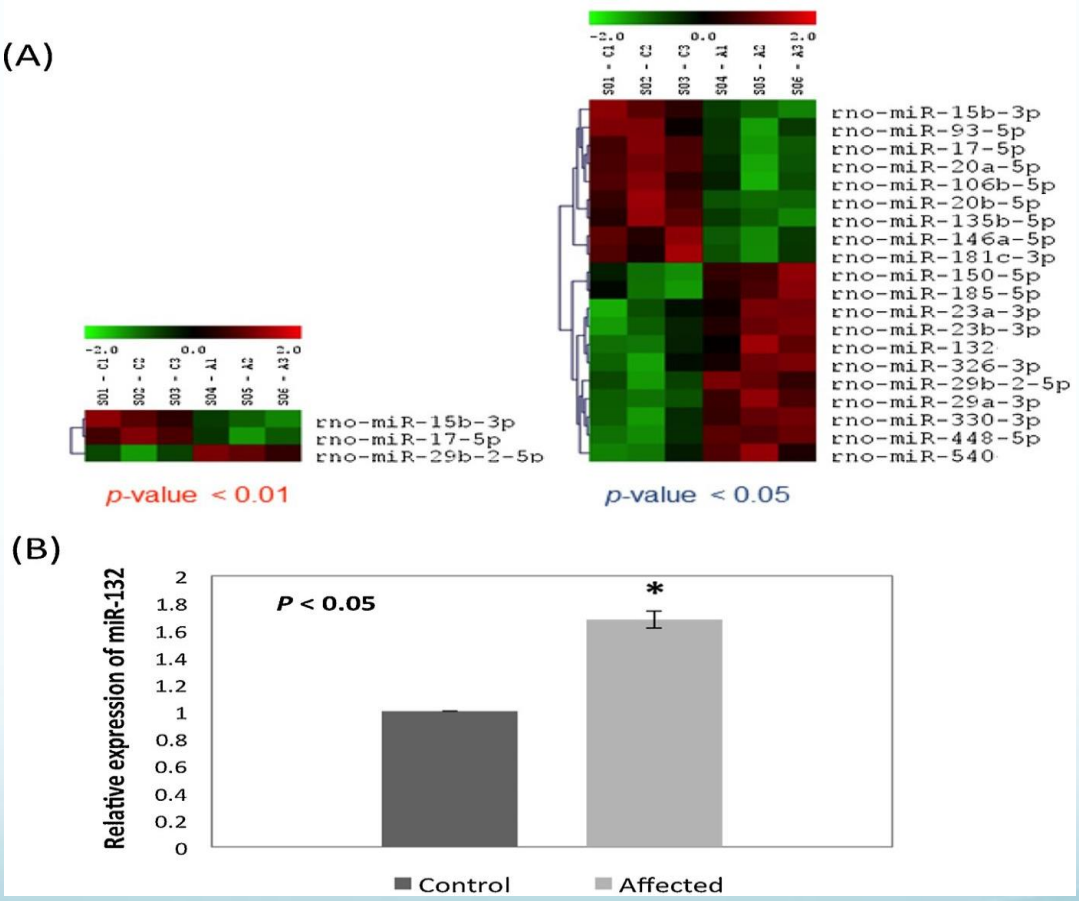
Conclusions

- Aldehyde dehydrogenase decreased
- DOPAL levels increased
- **Catechol aldehyde hypothesis**
  proof of concept in this model

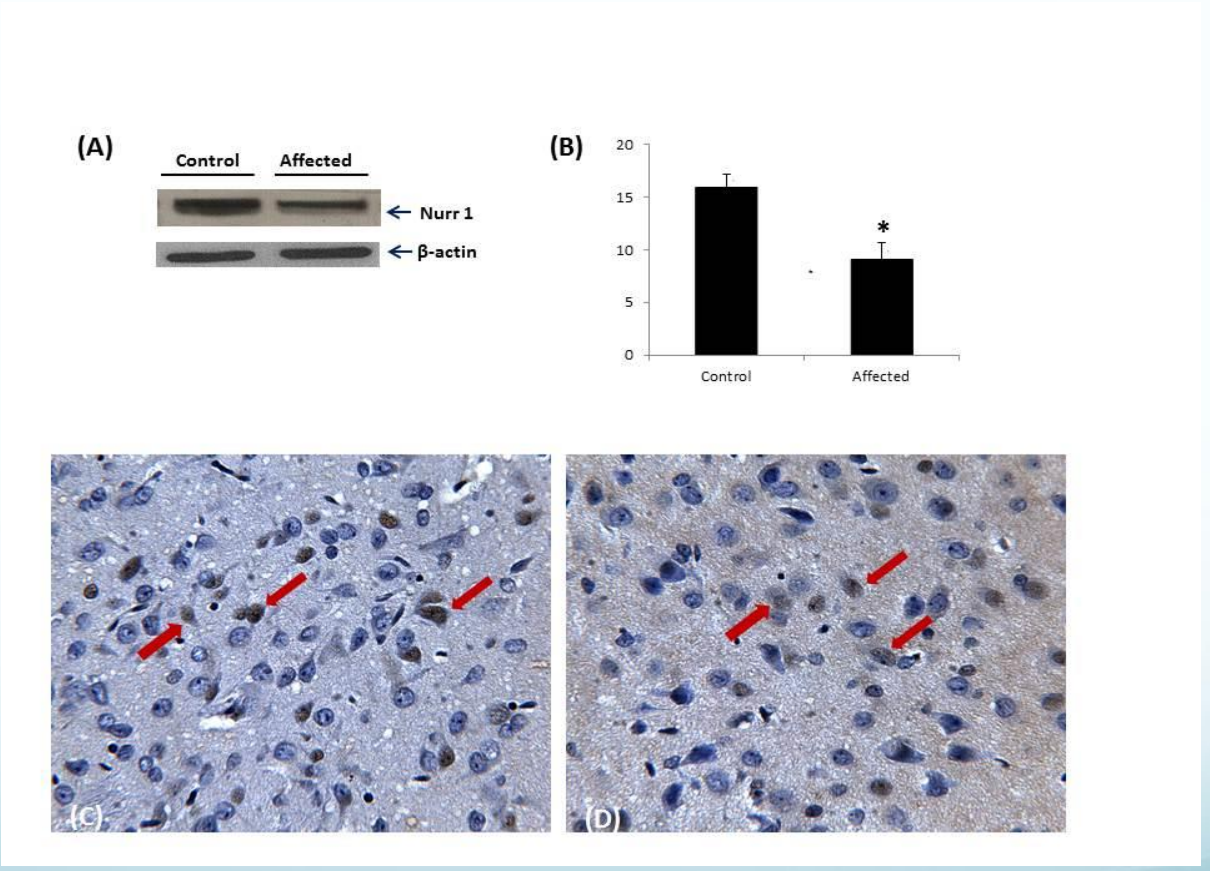
The severity of pathology is directly related to the overexpression of **α-syn/tau and parallel decrease in DA level in striatum and blood**

Stoica G. et al., JNC, 2012

Brain miRNA Expression Profile

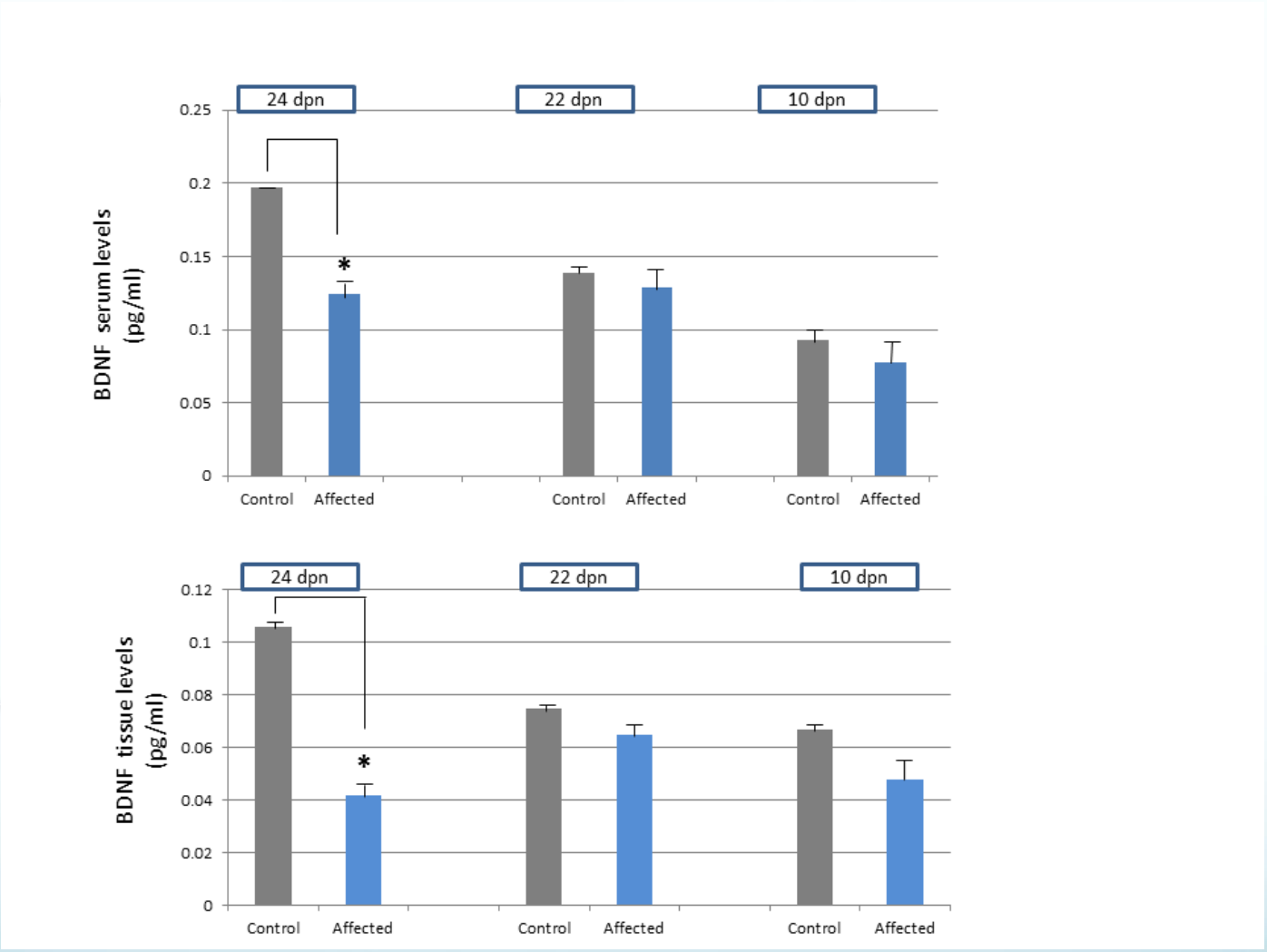


Brain Nurr-1 Protein Expression



Lungu *et al*., 2013

Significant reduction in BDNF, major regulator of neuronal survival in the serum and mesencephalon of affected rats



Lungu *et al*., 2013

Conclusions

- DOPAMINE level decreased
- Perikarya and neurites Lewy bodies
- Neuronal loss
- Gliosis and release of inflammatory cytokines
- Decreased nerve growth factors
- α -SYN level increased
- **α-syn is the most enriched mRNA associated with Myo5a**
- **RNA localization and local protein synthesis may be involve in neurodegenerative disorders such as PD (**Calliari et al., Developmental Neurobiology, 2013)

Future Directions

- Continue to explore genetic alterations responsible for disease
- Understanding the functions of myosins in neurons is significant for molecular mechanisms at synapses and their plasticity
- Identify proteins/myosins interactions for developing disease-modifying therapies
- Explore the potential involvement of Myo 5A genetic alteration in human neurodegenerative disorders such as: Parkinson's Disease, Alzheimer and others
- Understanding neuronal functions of myosins help explain how these motors contribute to brain function in health and neurological disorders

A surprise guest! Michael J. Fox addressed the audience to a rousing standing ovation! Fox encouraged the group to interact and do what they need to do to find the answers. He added that the answers don't just fall from the sky--you have to get up on your ladders and get them. (2012 NYAS)

Thank for the generous support from MJ Fox Foundation for Parkinson's Disease!