UNIVERSITY OF TEXAS AT EL PASO

The Era of Neurodegenerative Metastasis

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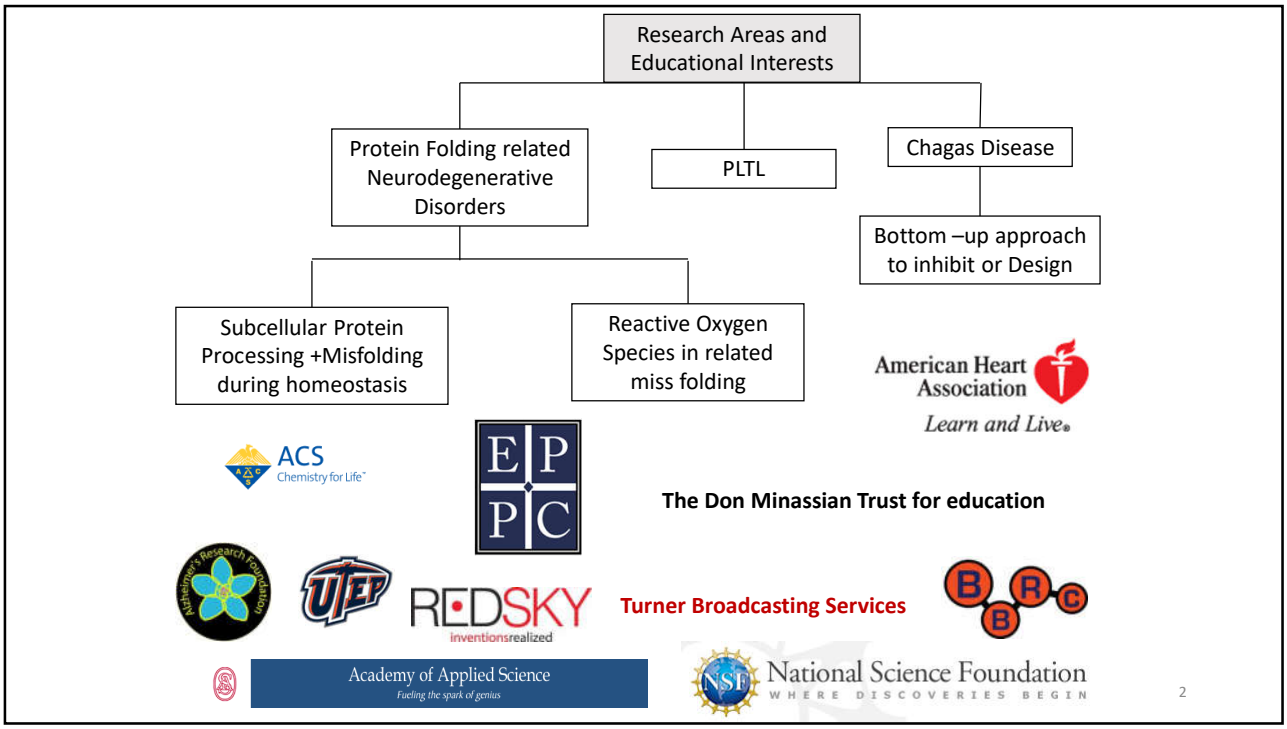

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Alzheimer's and Amyloid Beta (Aβ)

Alzheimer's disease is characterized by loss of cholinergic neurons; memory loss; abnormal behavior; shrinkage of the patient's brain

It is a protein misfolding disease

Its origins are within the cerebral cortex

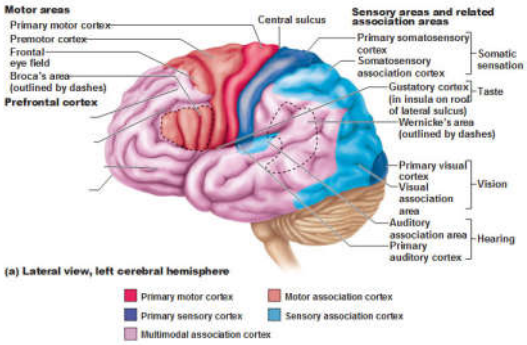
Key Biomarker: Amyloid β (1-42 aa; or 1-40 aa.) and Tau

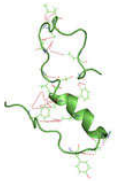
Peptides derive from the amyloid precursor protein (APP) which is cleaved by beta secretase and gamma secretase to yield Aβ.

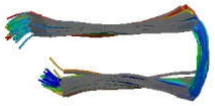
Aβ molecules can aggregate to form flexible soluble oligomers (which may exist in several forms.)

Implicated in: activation of kinase enzymes, protection against oxidative stress, regulation of cholesterol transport, a transcription, and anti-microbial activity

Functional Areas of the Cerebral Cortex







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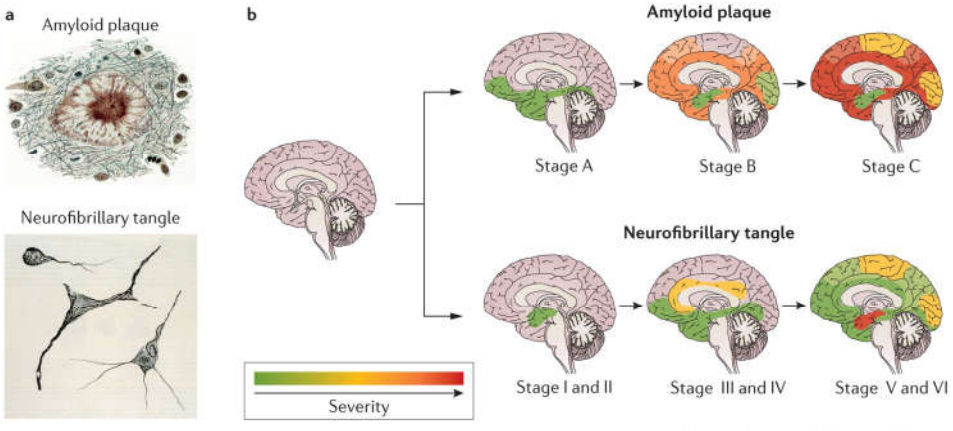
The spread of Alzheimer's



Amyloid plaques and neurofibrillary tangles **spread through the brain** as the disease progresses. In typical cases of Alzheimer's disease, amyloid-β (Aβ) deposition precedes neurofibrillary and neuritic changes with an apparent origin in the frontal and temporal lobes, hippocampus and limbic system (top row). Less commonly, the disease seems to emerge from other regions of the cerebral neocortex (parietal and occipital lobes) with relative sparing of the hippocampus. The neurofibrillary tangles and neuritic degeneration start in the medial temporal lobes and hippocampus, and progressively spread to other areas of the neocortex (bottom row).

Nature Reviews Disease Primers **volume 1**, Article number: 15056 (2015)

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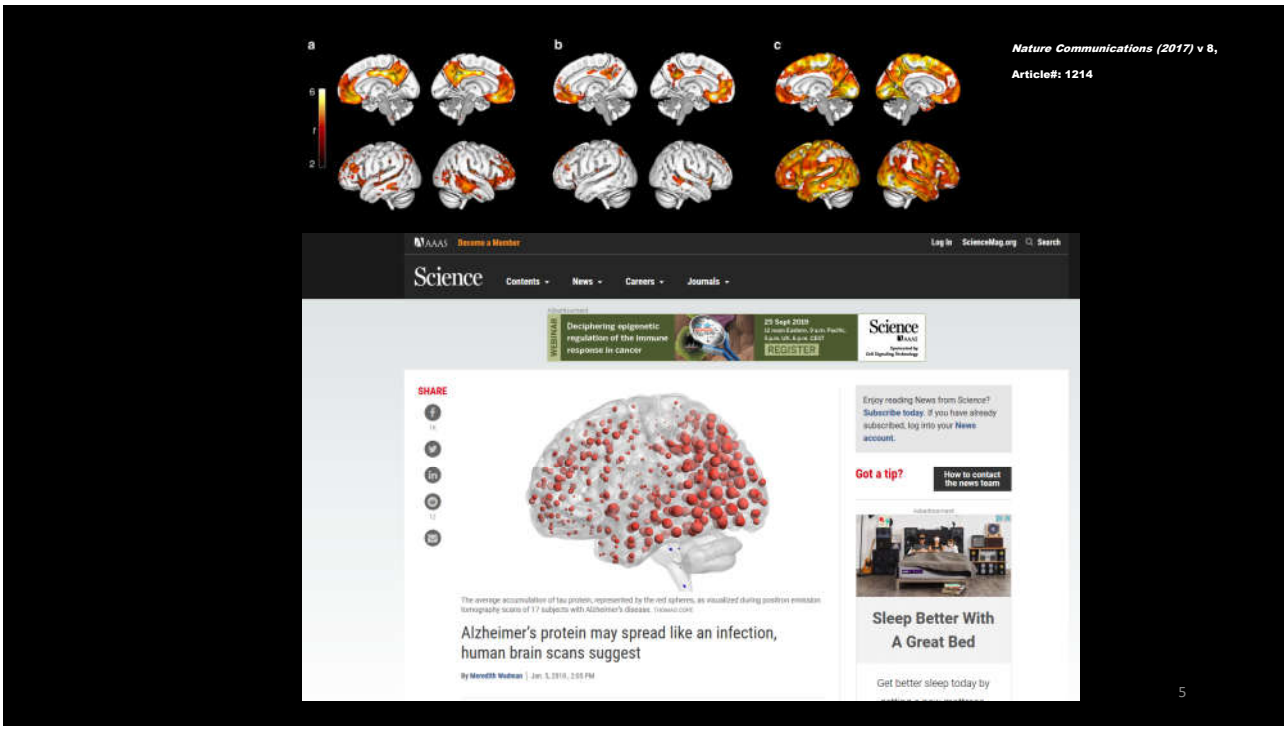

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Parkinson's and its biomarkers

Degenerative disorder of the central nervous system that mainly affects the motor system

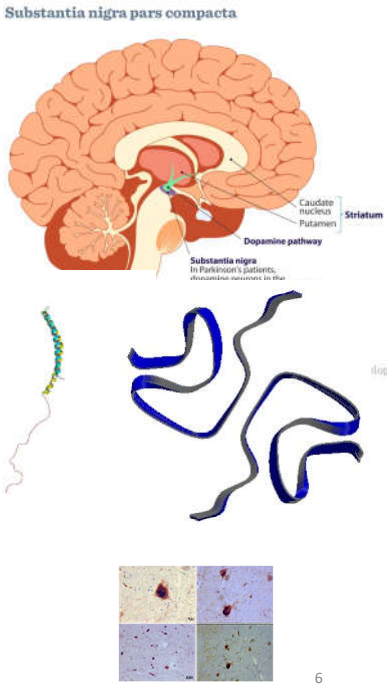
Tremor, slowness of movement (bradykinesia), rigidity, and postural instability.

Location – *Substantia Nigra pars compacta* (SNpc)

Key Biomarkers: α-synuclein and synphilin-1 (co-localize to form Lewy bodies)

α-synuclein: Regulation of membrane stability and/or turnover (?)

synphilin-1: may affect dopamine release



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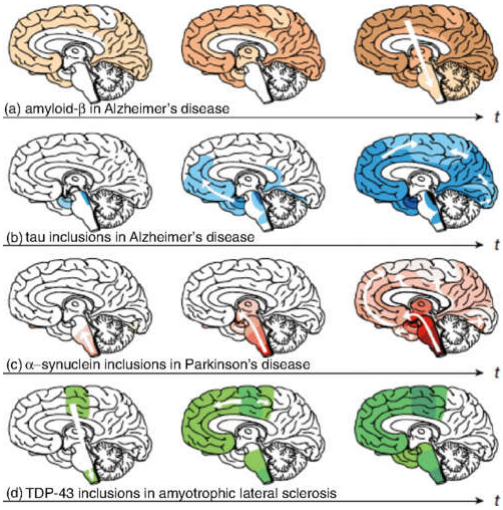

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Based on their physiological signatures and the presence of distinct biomarkers, AD and PD have historically been categorized as two different disease entities

AD –identified by Aβ pathology, originates in the memory hub

PD-identified by Lewy body pathology in the SNpc- the locomotor seat

HOWEVER

- **Clinically, ~30%- 40% of PD patients present with dementia during their disease course**
- **~30% of patients with AD develop Parkinsonism,**
- **A relatively higher percentage develop PD when AD occurs in conjunction with Lewy bodies.**
- **Both AD and PD patients experience similar symptoms, including: severe depression, hallucination, and psychosis in advanced stages of the disease**
- ***APOE* status, a strong risk factor and modifier of onset age for AD, has been associated with PD as well**
- **Tau polymorphisms have also been found to potentiate risk of PD.**

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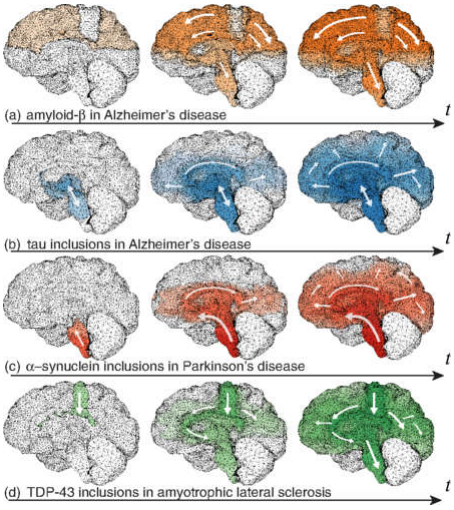

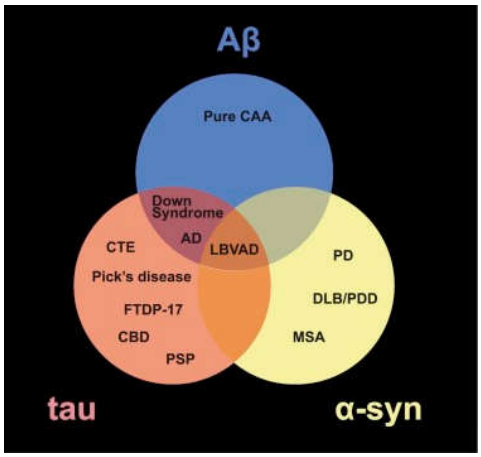

The era of neurodegenerative metastasis

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Hypothesis: amyloidogenic seeds can provoke cross-pathology

Test: To determine the impact of an amyloidogenic seed on heterotypic neurons

Heterotypic = different in form, arrangement, or type

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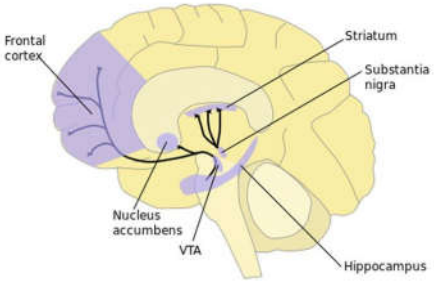
Impact of an amyloidogenic seed in a heterotypic environment?

Prion-like seeding observed in Aβ, α-synuclein, tau, and polyQ mutant Huntingtin (mHTT).

Able to seed their associated pathologies (homotoxic) at locations distant from origin

Seed spread via endocytosis, neuronal tunneling, etc.



Are amyloidogenic seeds hetertoxic?

Are seeds capable of provoking cross-pathology (clinically measurable outcomes)?

What is the neurometabolic trajectory to cross-reactivity?

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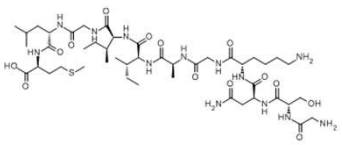
Or Clinically: Is amyloid beta a risk factor for PD?



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In vitro assays

Hypothesis: amyloidogenic seeds can provoke cross-pathology.

To test this *in vitro*, we examine the impact of Aβ in a heterotypic cellular vehicle (i.e. in a cell line wherein it is not normally expressed)

To complete this objective, SH-SY5Y cell lines were insulted with Aβ 25-35. As a function of this insult, we examined outputs associated with the Parkinsonian phenotype

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In Vitro Model

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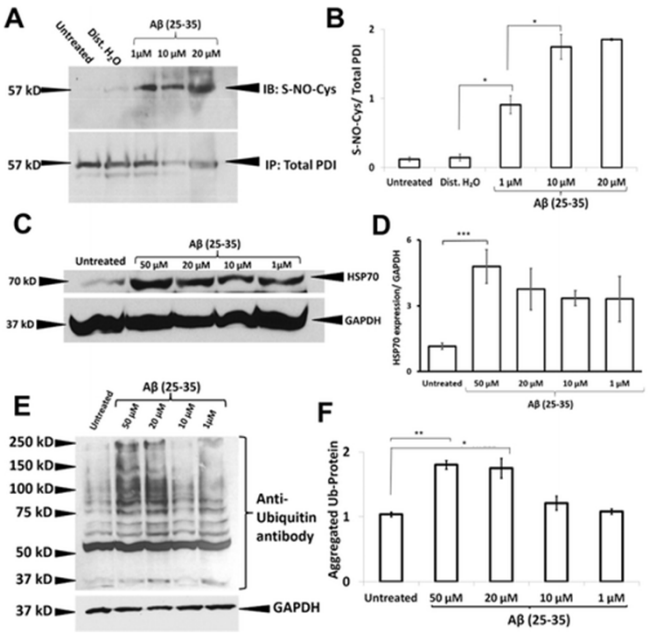
Cell Health

Aβ induces

- S-Nitrosylation of Protein Disulfide Isomerase (a Parkinson Disease target)
- Upregulation of HSP-70
- Ubiquitinated debris



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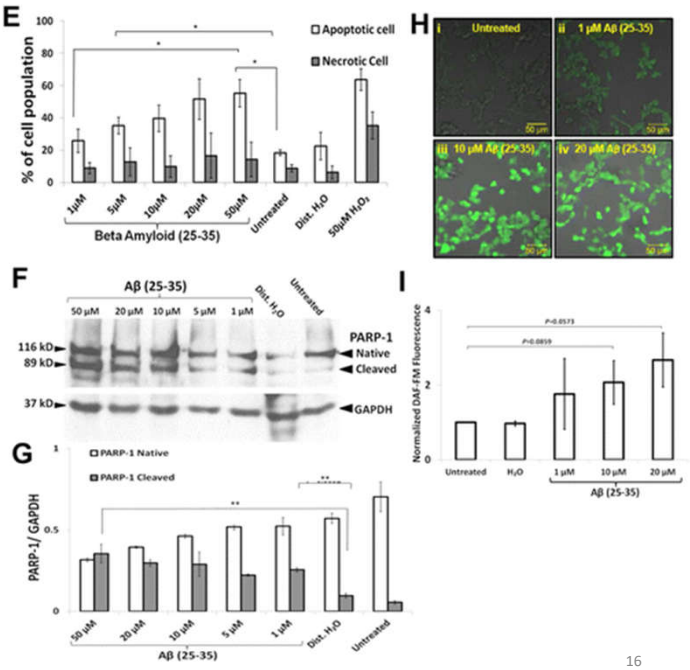
RNS and Apoptosis

- Aβ activates Apoptosis
- Induces PARP-cleavage



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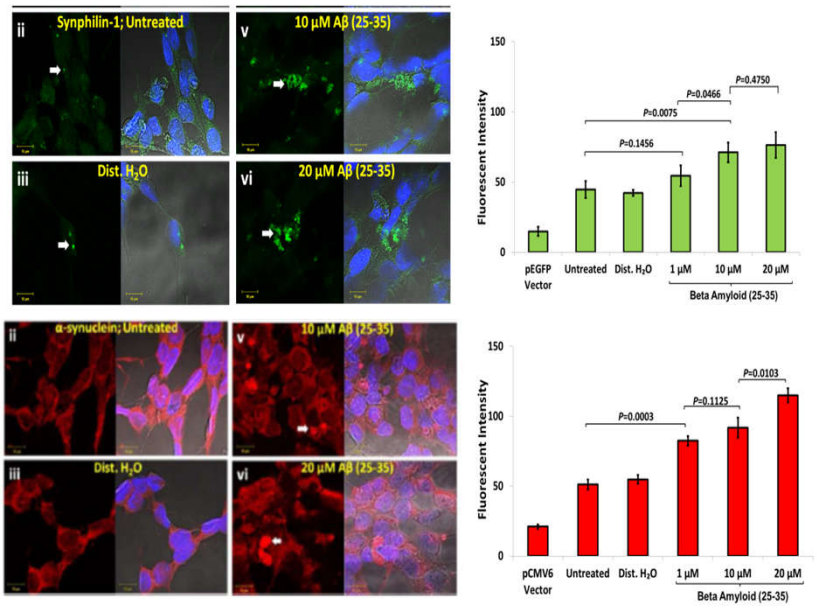
SH-SY5Y cells after Aβ insult

Immunofluorescence images of SH-SY5Y cells. We observed, in a dose dependent manner, the presence of:

- **cytoplasmic aggregates of synphilin-1 (top panel)**
- **Cytoplasmic aggregates of alpha-synuclein (bottom panel).**



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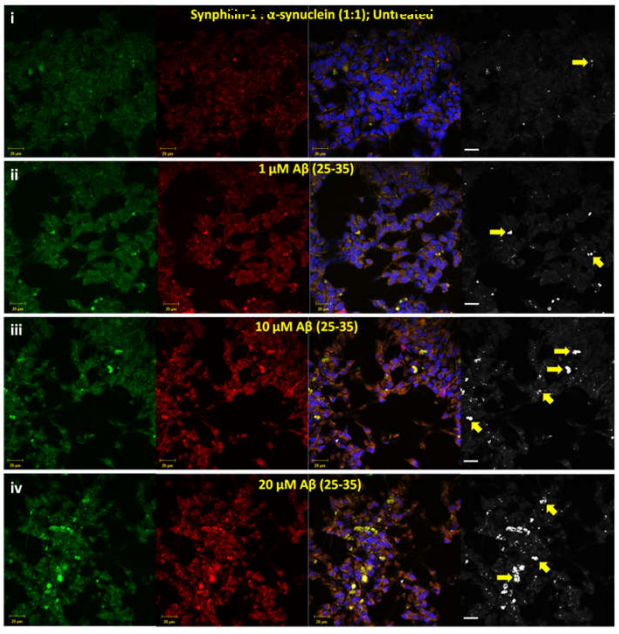
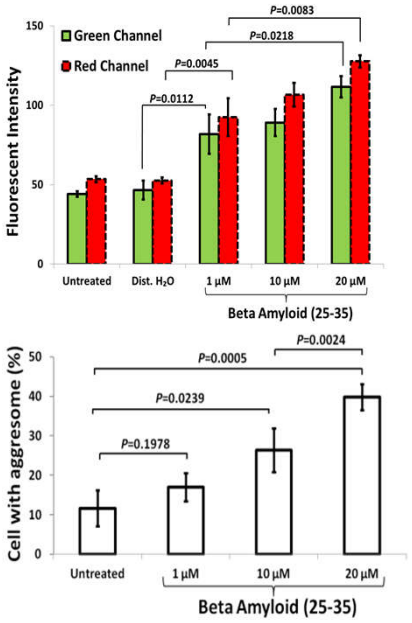
Lewy-body like Aggregates





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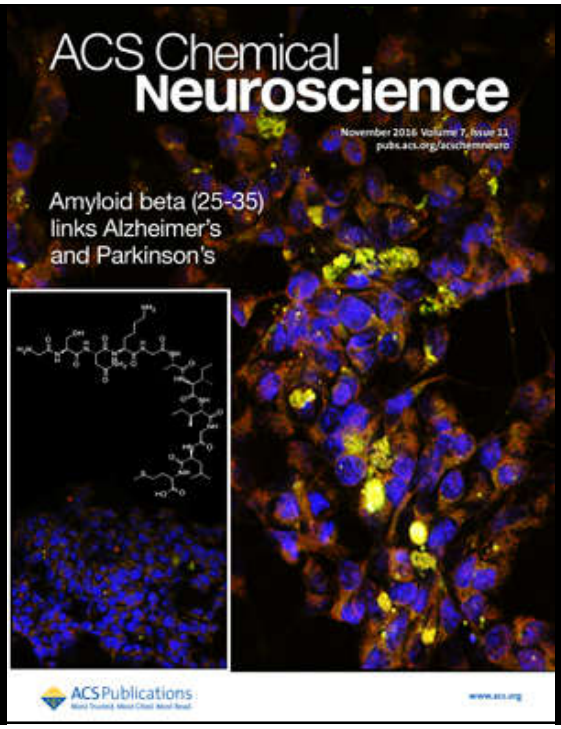

November 16, 2016
Volume 7, Issue 11
Pages 1469-1619
About the Cover: Overlaid confocal image of dopaminergic (SH-SY5Y) cells transfected with α-synuclein (red) and synphilin-1 (green) upon incubation (24 h) with amyloid β (25−35) peptide resulting in Parkinsonian Lewy-body-like aggregates (colocalization of α-synuclein and synphilin-1; yellow). Nuclei were counterstained with DAPI (blue). Left bottom inset: Dopaminergic (SH-SY5Y) cells transfected as above but prior to amyloid β (25−35) peptide incubation are displayed along with the sequence of the amyloidogenic 11-mer. Microscopy data was gathered at the BBRC (UTEP). Art Designers: Jose E. Marin, Parijat Kabiraj, Armando Varela-Ramirez, and Mahesh Narayan.

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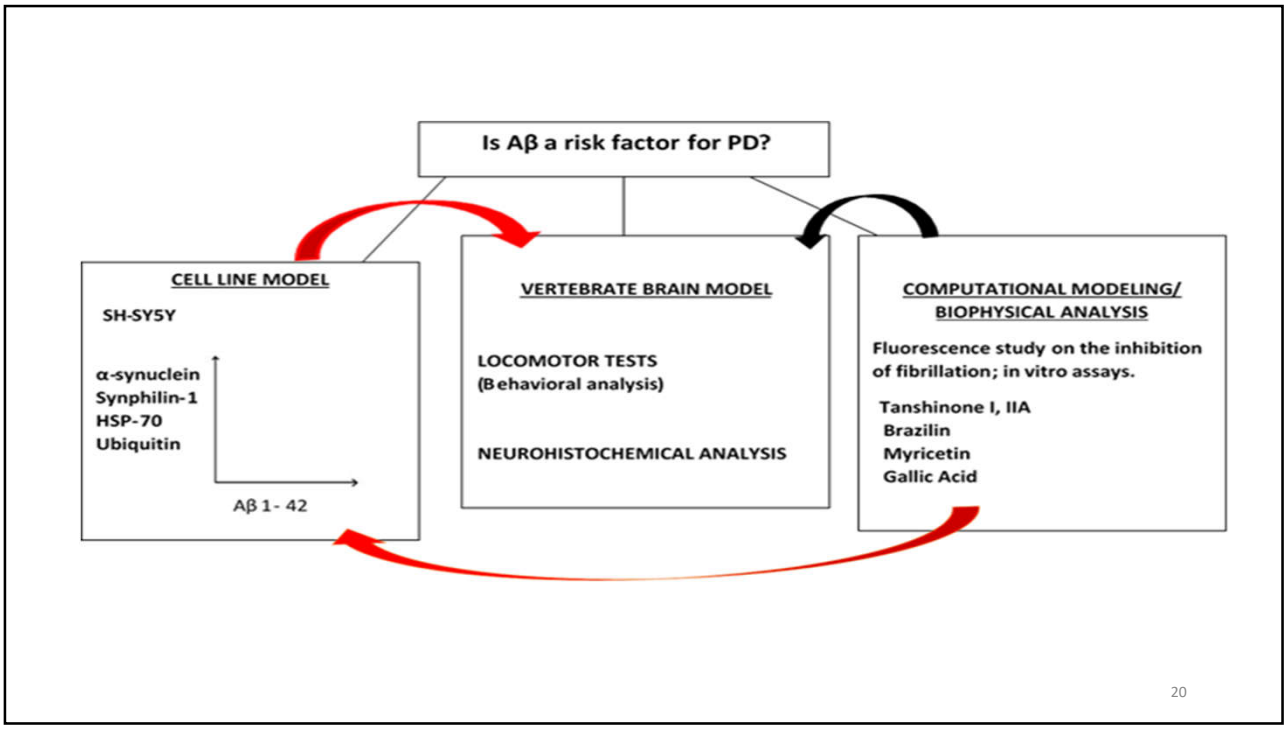

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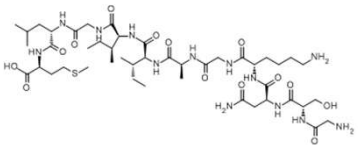
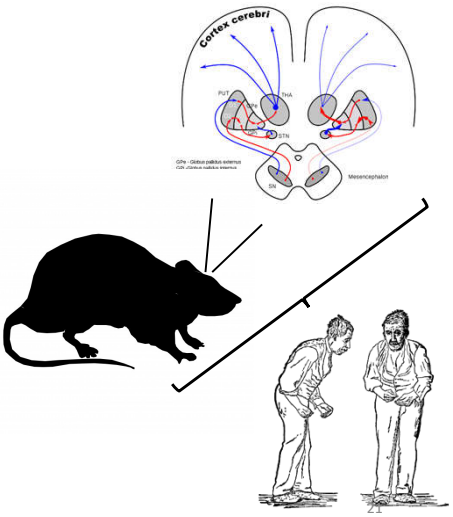
In vitro assays

Hypothesis: **amyloidogenic seeds can provoke cross-pathology.**





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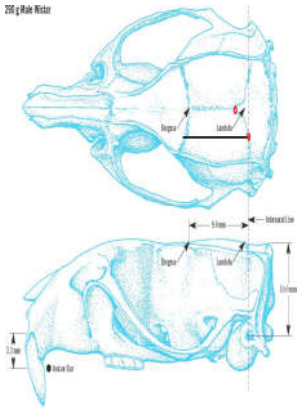
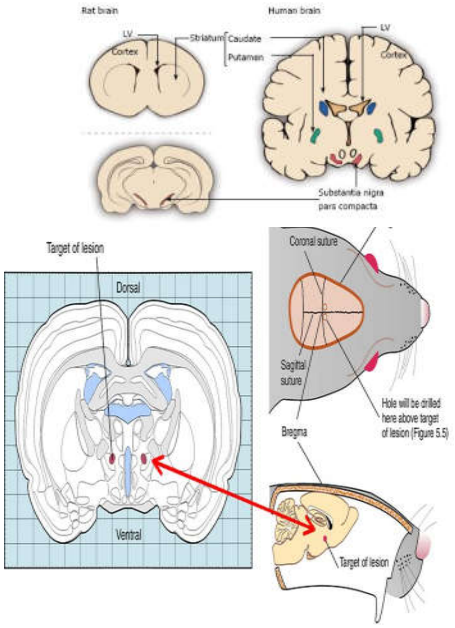
In Vivo Stereotactic Infusion





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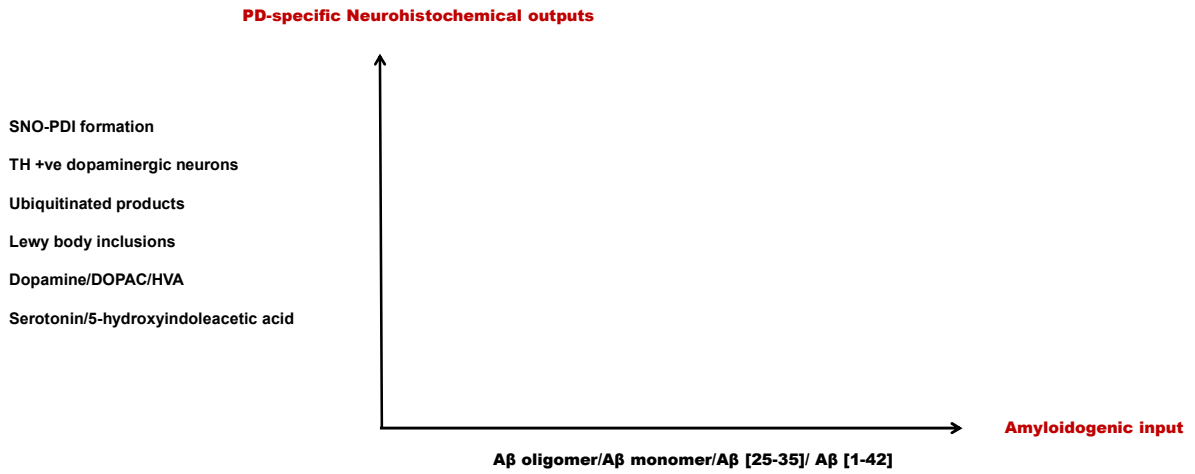
Neurohistochemistry of nigral neurons



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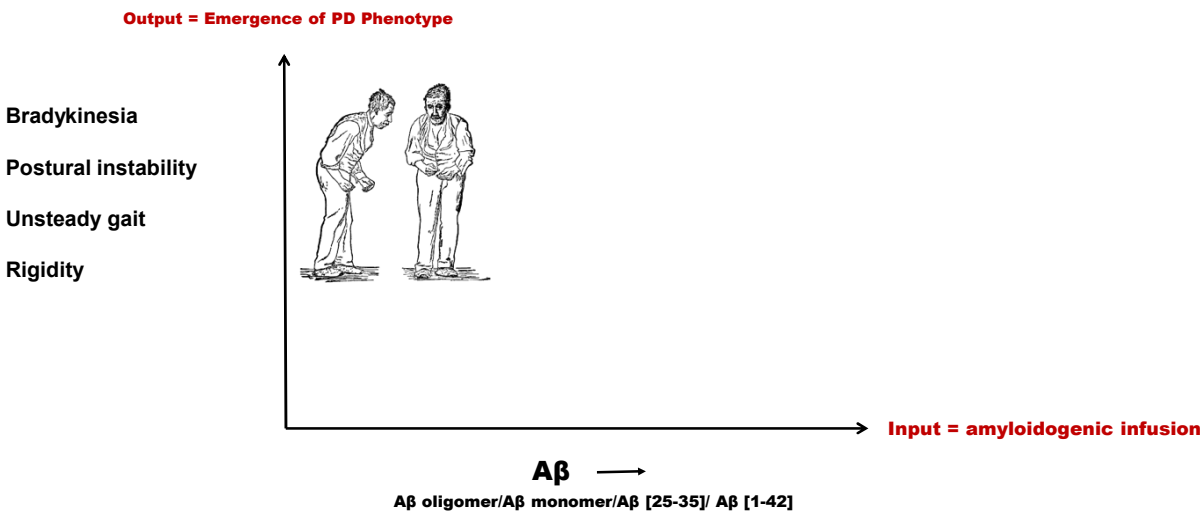
Qualitative Behavioral Aberrations



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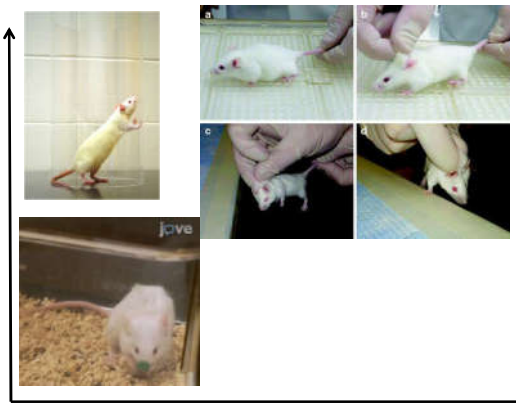
Quantitative Locomotor Deficits

Output = Emergence of PD Phenotype

Single Akinesia Test
(Cylinder Test)

Forelimb Placement

Adhesive Removal Test
(Sticky Dot Test)



Input = amyloidogenic infusion

Aβ ⟶

Aβ oligomer/Aβ monomer/Aβ [25-35]/ Aβ [1-42]

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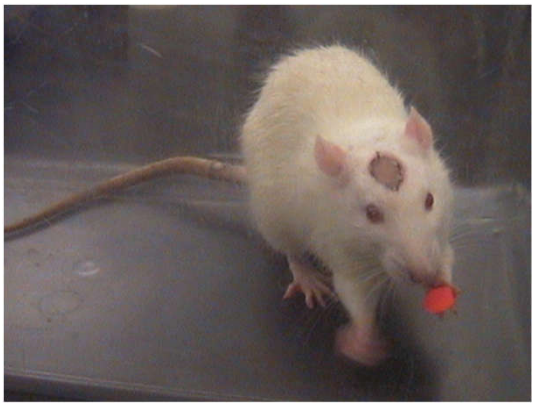
Sticky Dot Test

This test assesses both the sensory capability (time to contact) of the rats as well as their motor capabilities (time to remove stimulus).

- **Small, circular adhesive stickers will be placed on their forelimb palms.**
- **The test was repeated 3 times and the three trials were averaged.**



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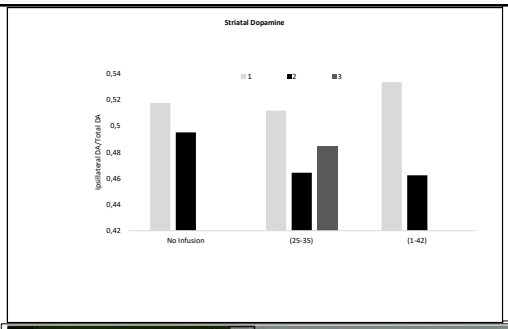

Figure 1*:* **The image shows the quantified levels of dopamine via HPLC-ECD from striatal tissue (n=1). The data shown reflects the amount in the lesioned hemisphere over the total amount of striatal dopamine.**

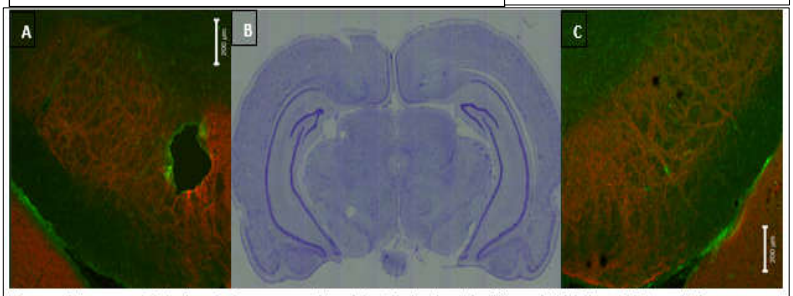

Figure 2: Immunohistochemistry was used to detect whether the β-amyloid (Alexa488) peptide co-localized with α-synuclein (Texas Red). Panel A shows the hemisphere contralateral to the infusion, Panel C shows the hemisphere ipsilateral to the infusion and Panel B shows the representative Nissl.

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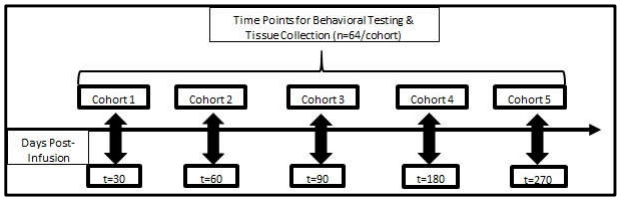

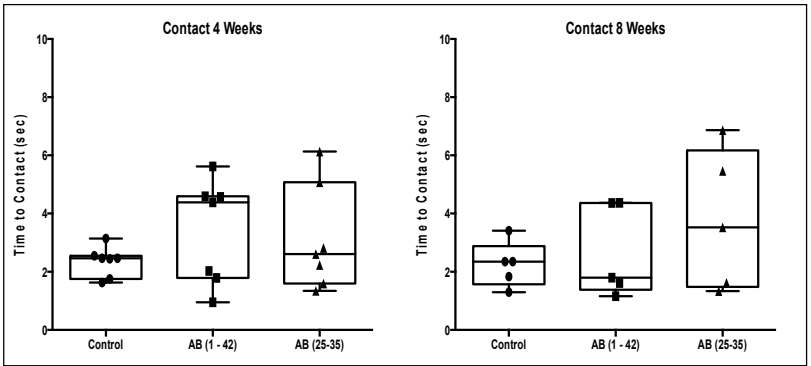

Figure 6: Preliminary Data from the Sticky Dot Test, measures shown above are contact time recorded at 4 weeks post infusion (n=7 rats/group) and at 8 weeks post-infusion (n=5 rats/group).

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Findings….

In Vitro

Our findings suggest that Aβ [25-35] initiates a cascade of physico-chemical events *in vitro* that provokes the Parkinsonian phenotype:

1) initiation of cell death *via* apoptosis
2) elevated levels of RNS, but not ROS
3) cleavage of poly(ADP-ribose) polymerase-1 (PARP-1)
4) aggregation of i) α-syn, ii) synphilin-1, and iii) their co-localization to form Lewy-like bodies
5) Aβ dependent co-localization of α-syn with PDI (perinuclear)
6) co-localization of Aβ (25-35) with α-syn
7) chemical mutation of PDI to SNO-PDI
8) elevated levels of HSP- 70
9) accumulation of ubiquitinated proteins.

In Vivo

Preliminary data indicate but locomotory deficits and neurohistochemical aberrations associated with Aβ-associated infiltration of the vertebrate nigral mass.

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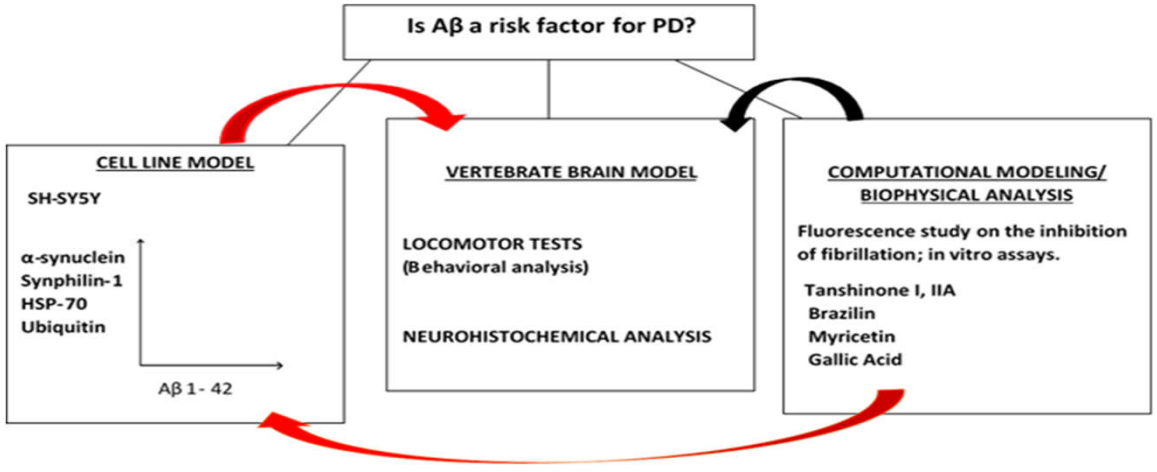

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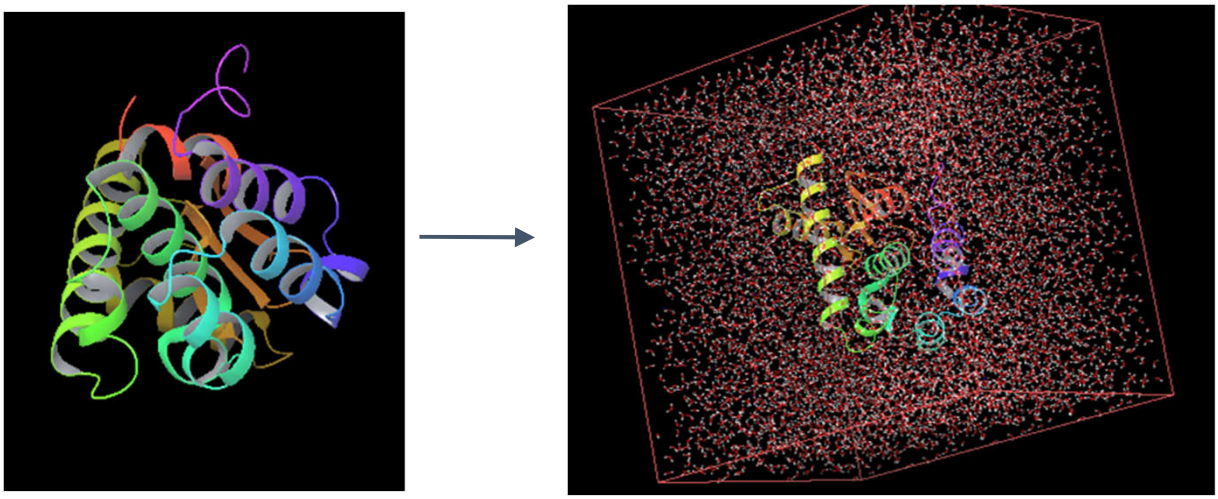

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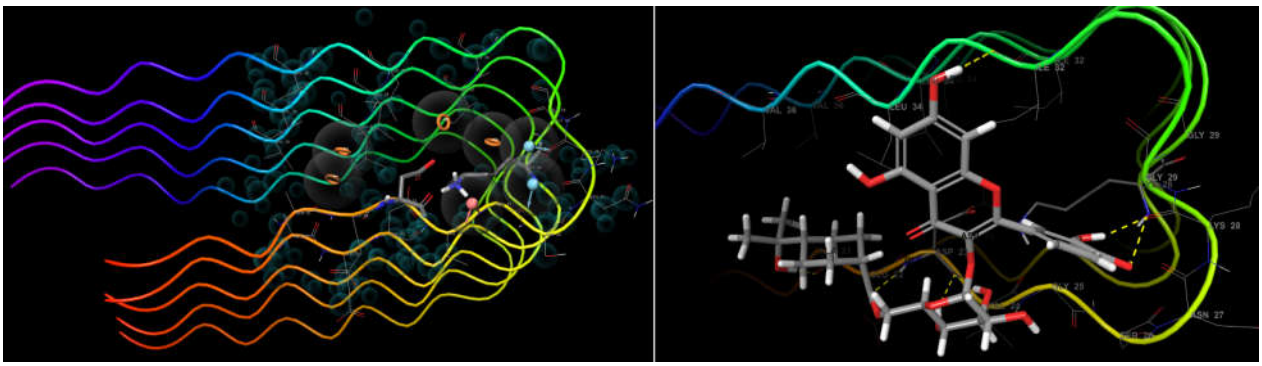

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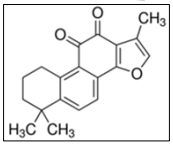
Phytochemicals

Salvia miltiorrhiza

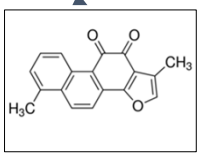
Curcuma Longa

Family:Rosaceae Rubus

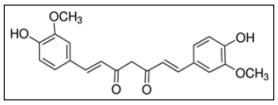
Caesalpinia sappan



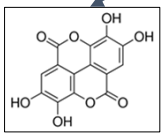
Tanshinone IIA



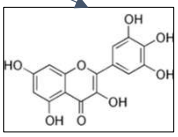
Tanshinone I



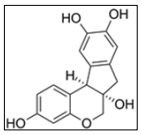
Curcumin



Ellagic acid



Myricetin



Brazilin

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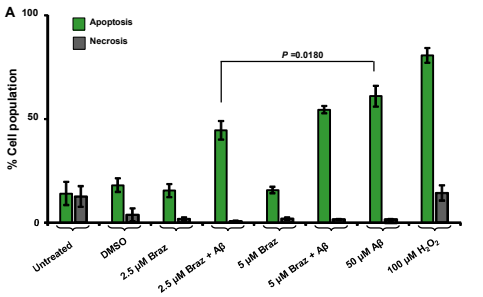

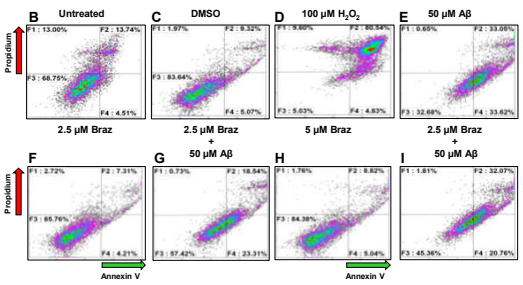

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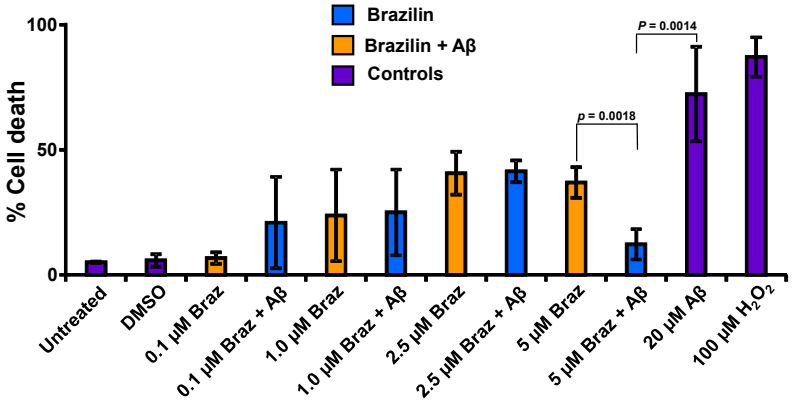

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ACS Chemical Neuroscience

Nanoscopic Portrait of an Amyloidogenic Pathway Visualized through Tip-Enhanced Raman Spectroscopy

Sreenivasan Sreeprasad* and Mahesh Narayan*

ACS Chemical Neuroscience

Learnings from Protein Folding Projected onto Amyloid Misfolding

Sreeprasad Sreenivasan and Mahesh Narayan*

ACS Chemical Neuroscience

The Era of Neurodegenerative Metastasis

Mahesh Narayan*

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Acknowledgements

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- Dr. Parijat Kabiraj
- Dr. Alok Kumar
- Emmanuel Zubia
- Gabriela Henriquez
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