



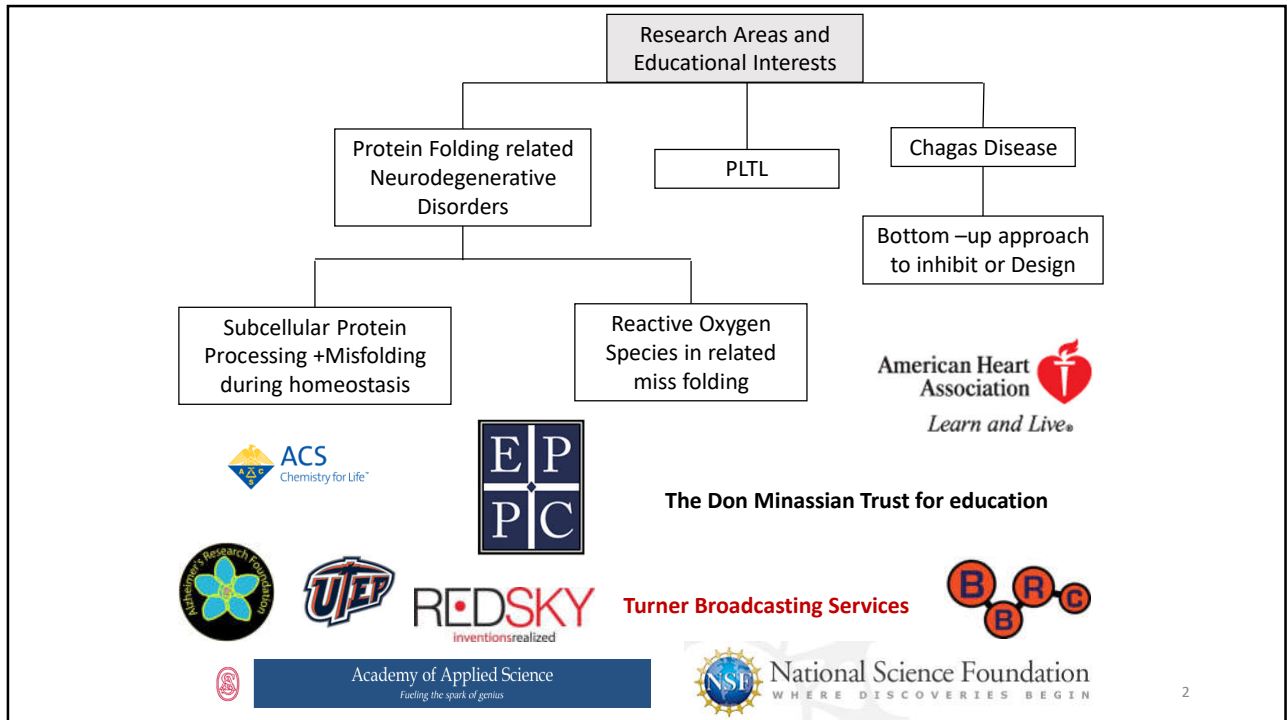
UNIVERSITY OF TEXAS AT EL PASO

The Era of Neurodegenerative Metastasis

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Yogyakarta, Indonesia

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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

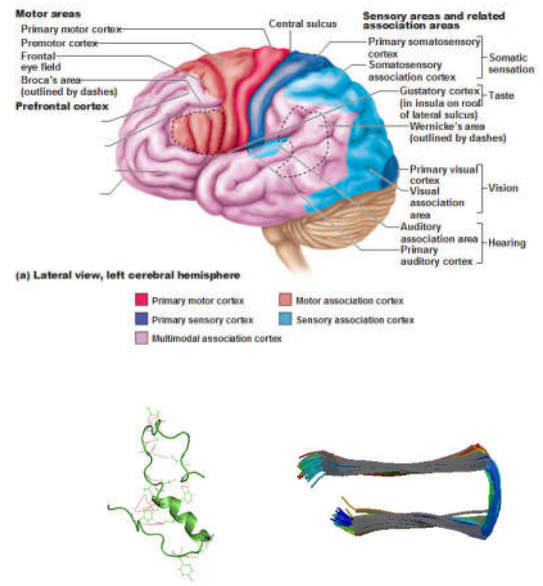
Key Biomarker: Amyloid β (1-42 aa; or 1-40 aa.) and Tau

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

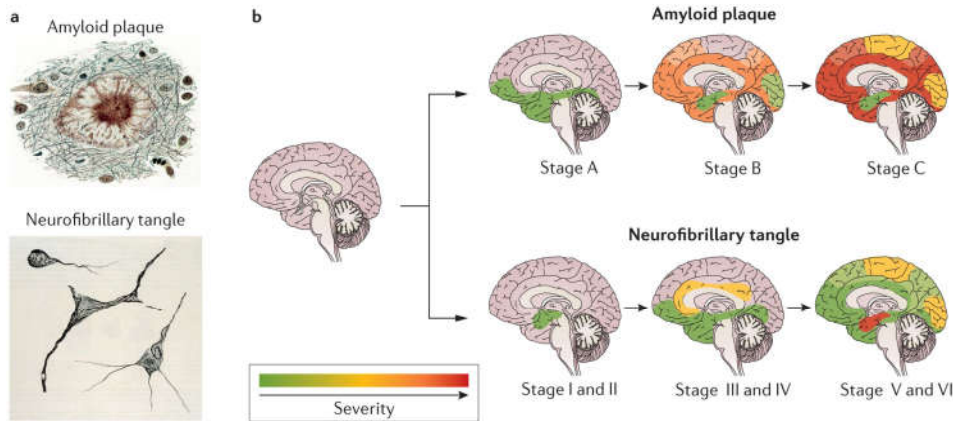
Functional Areas of the Cerebral Cortex



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The spread of Alzheimer's



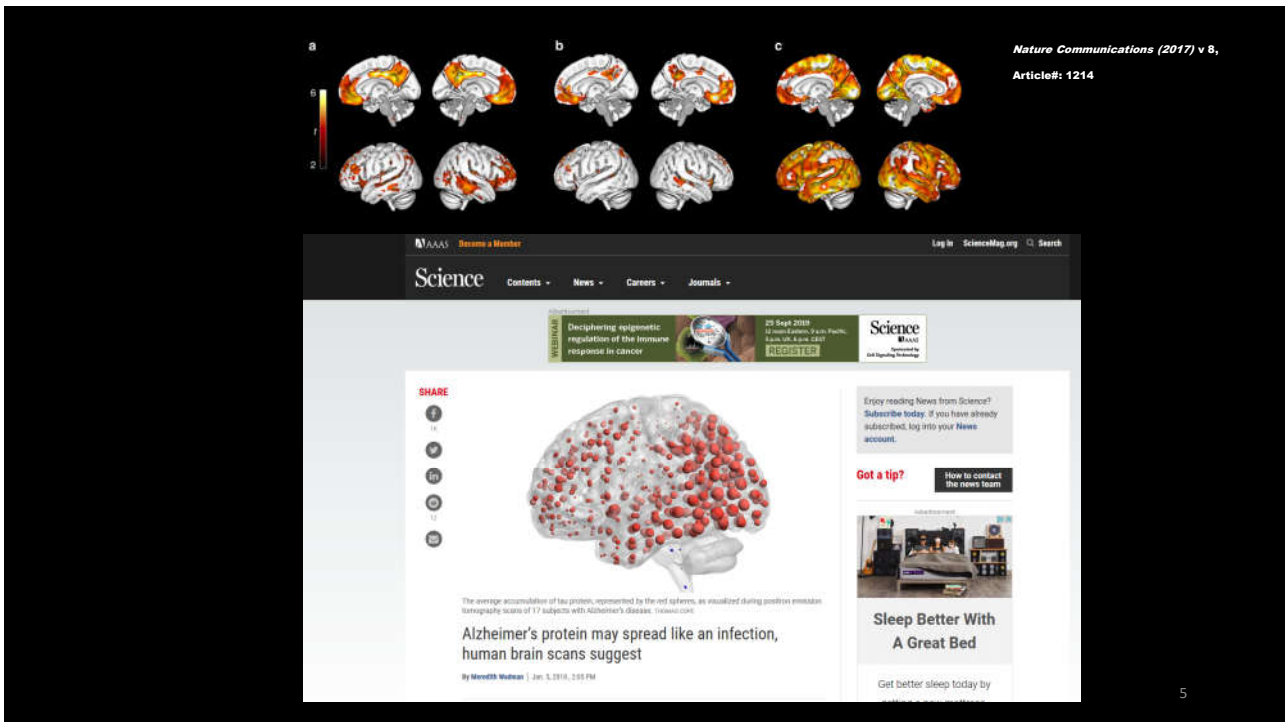
Nature Reviews | Disease Primers

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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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)

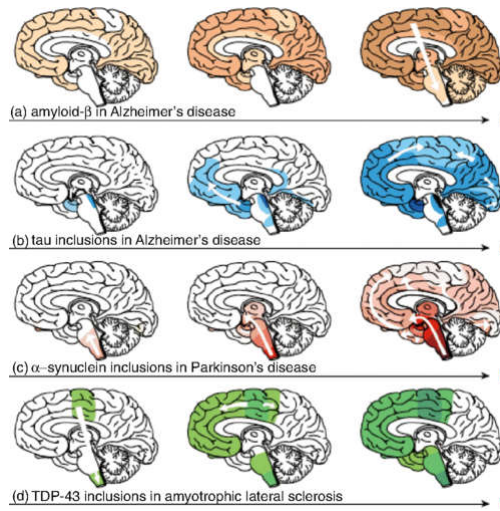
Key Biomarkers: α -synuclein and synphilin-1 (co-localize to form Lewy bodies)

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

synphilin-1: may affect dopamine release

Substantia nigra pars compacta

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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

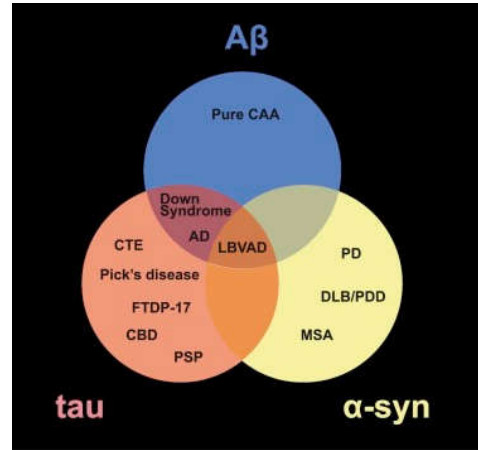
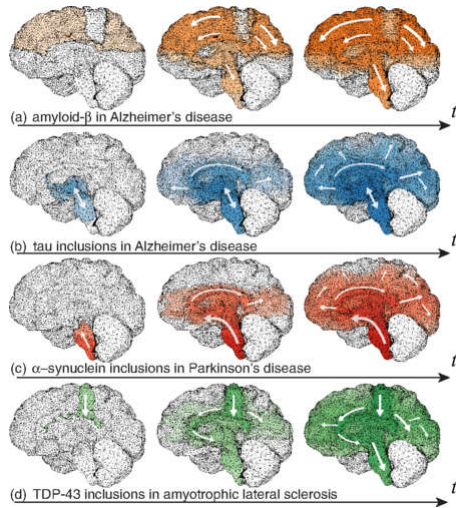
AD –identified by A β pathology,
originates in the memory hub

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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In vitro assays

Hypothesis: amyloidogenic seeds can provoke cross-pathology.

To test this *in vitro*, we examine the impact of A β 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 A β 25-35. As a function of this insult, we examined outputs associated with the Parkinsonian phenotype

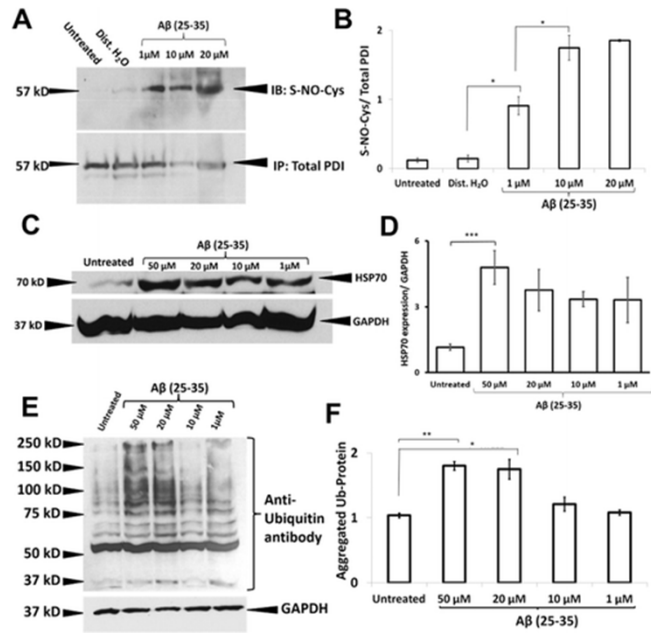
In Vitro Model



Cell Health

A β induces

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

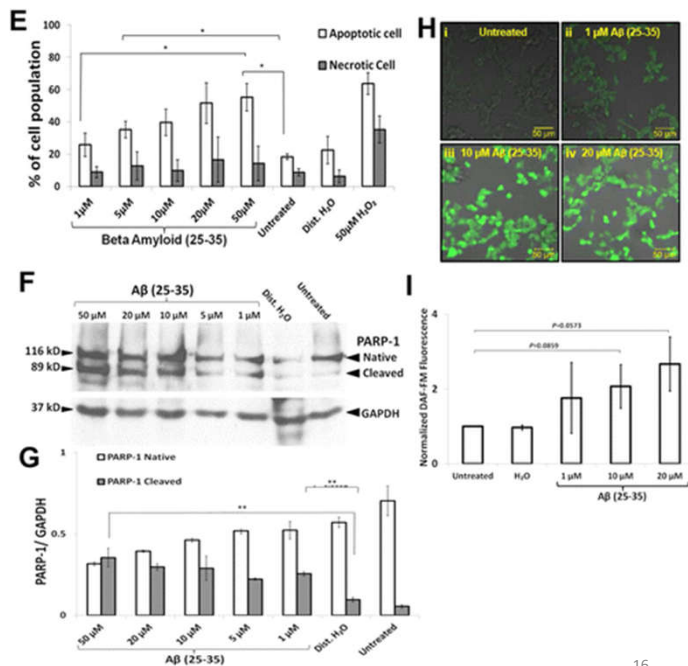


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RNS and Apoptosis

- A β activates Apoptosis
- Induces PARP-cleavage



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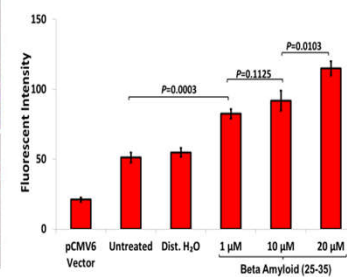
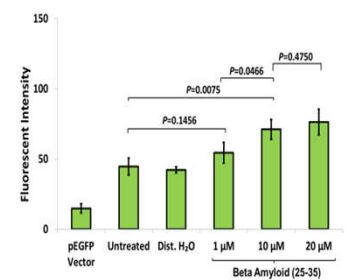
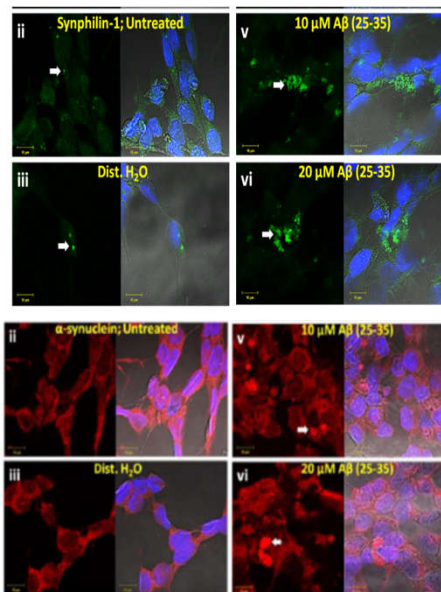
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SH-SY5Y cells after A β insult

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).

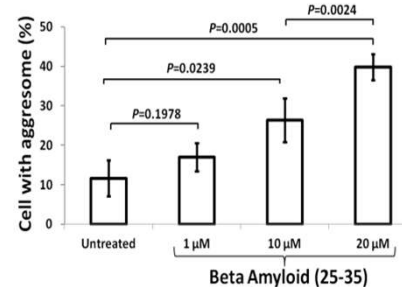
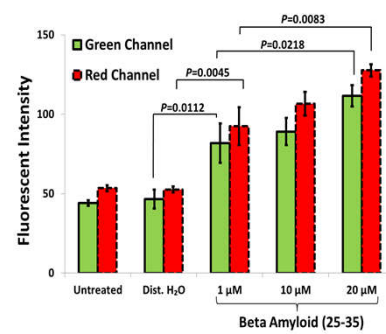
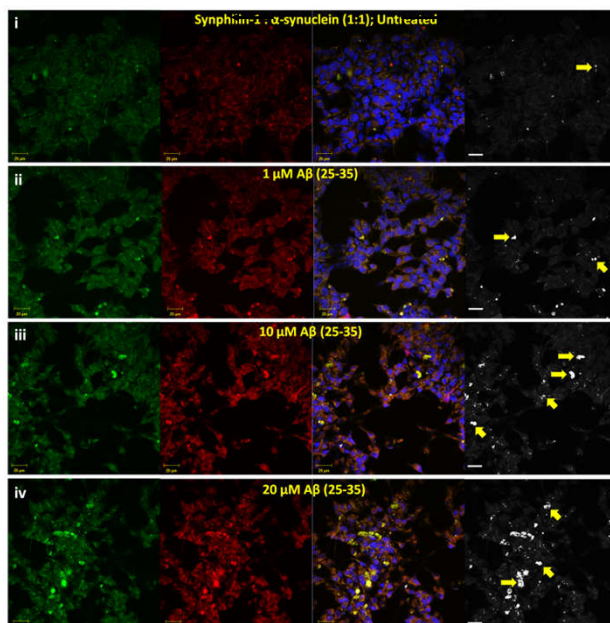


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Lewy-body like Aggregates



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Amyloid beta (25-35) links Alzheimer's and Parkinson's

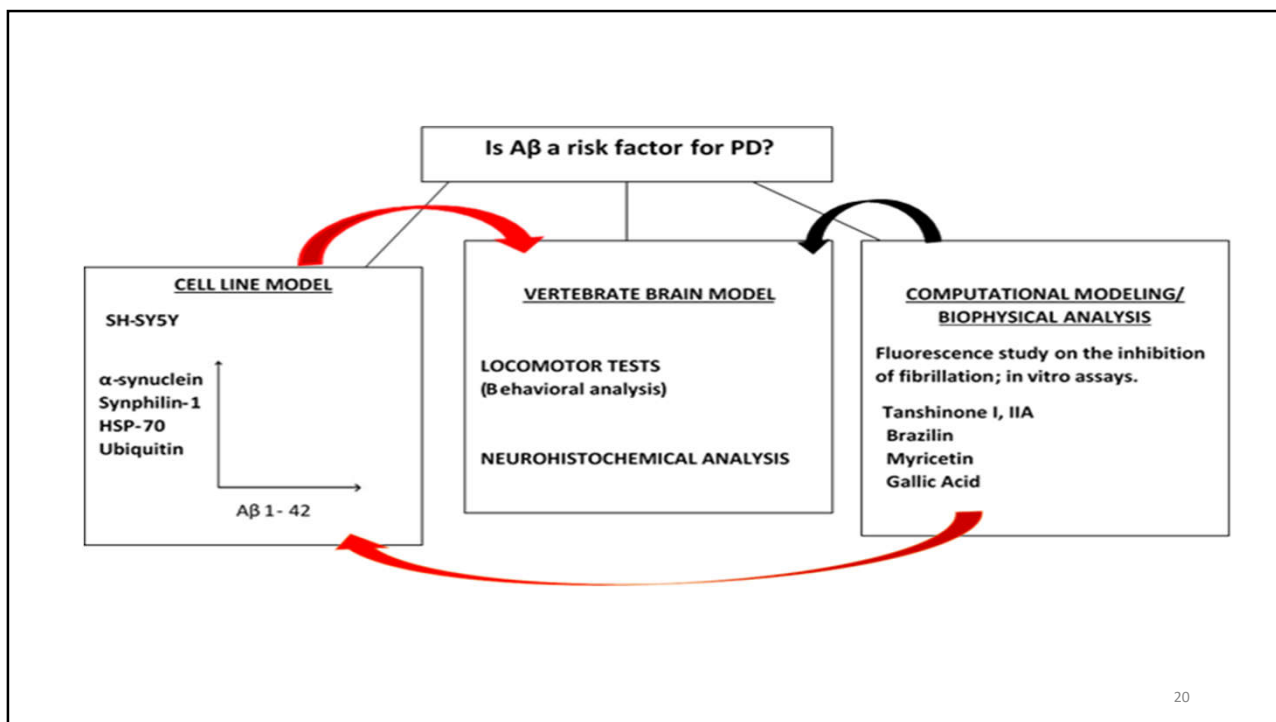
November 16, 2016
 Volume 7, Issue 11
 Pages 1469-1619

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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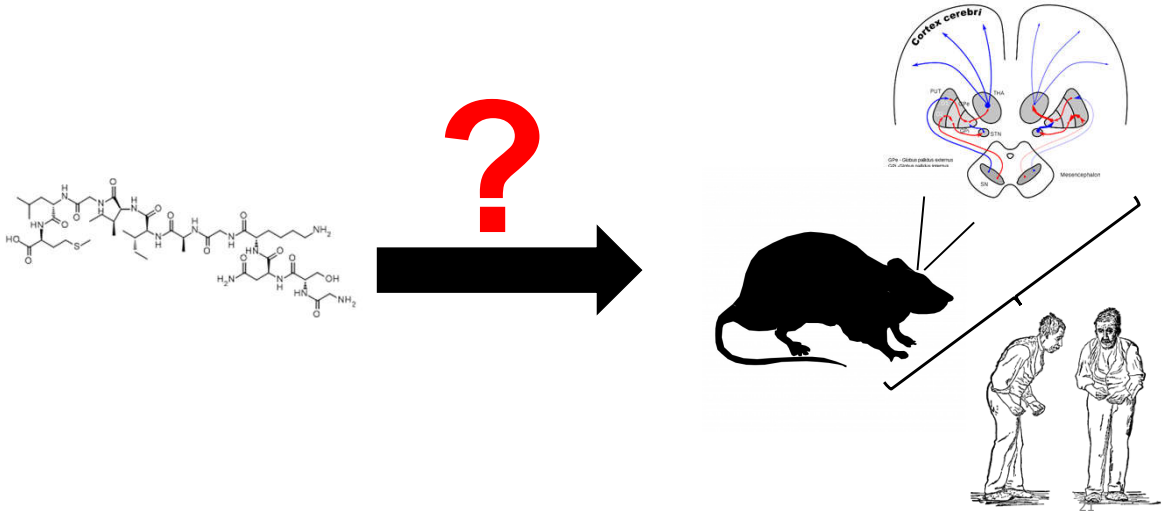
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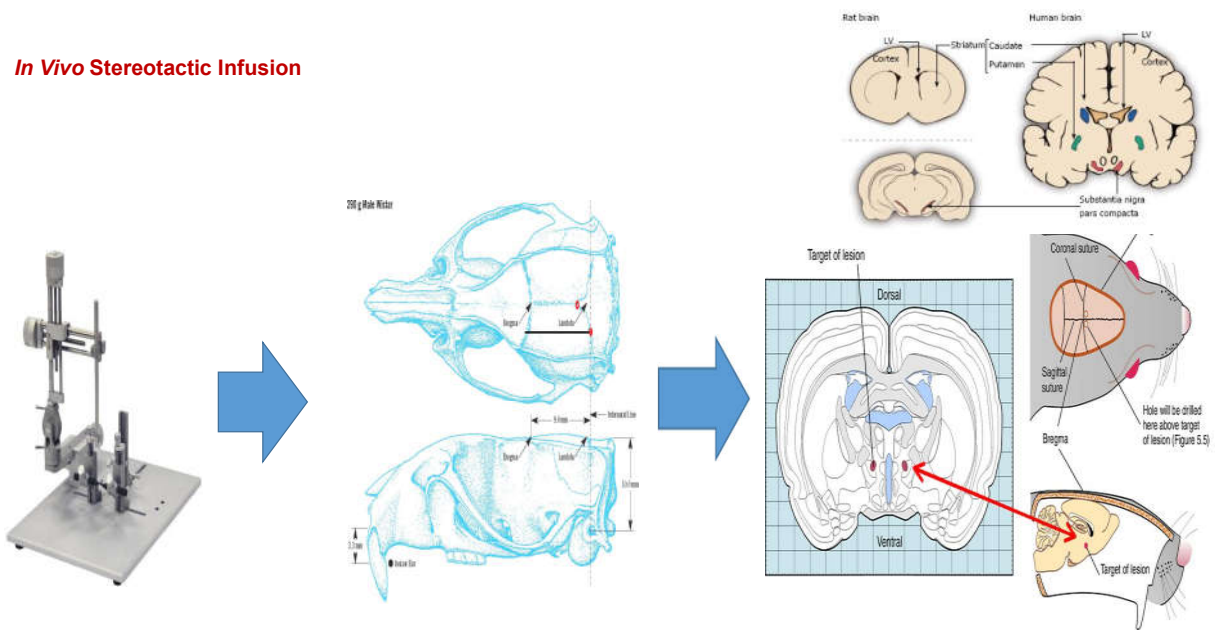
In vitro assays

Hypothesis: amyloidogenic seeds can provoke cross-pathology.



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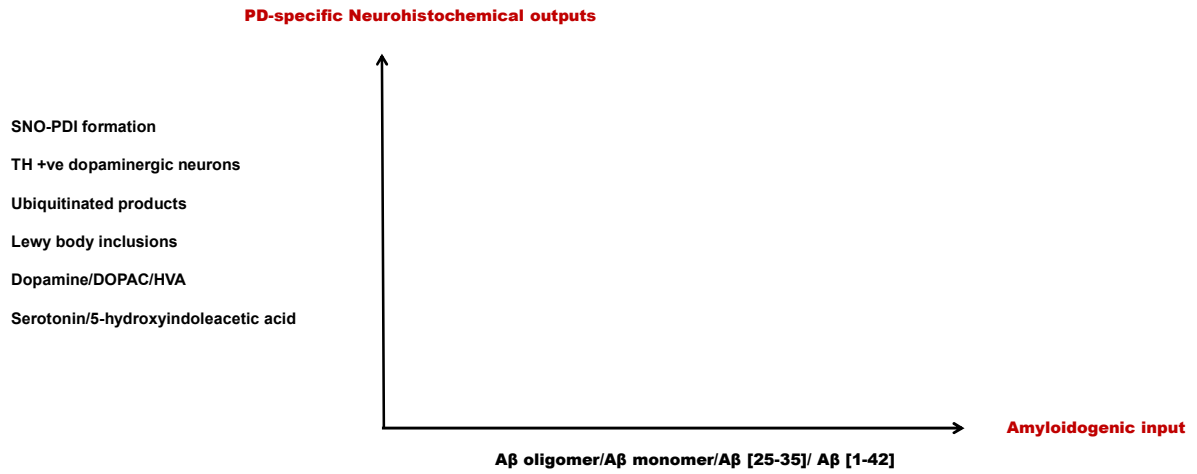
In Vivo Stereotactic Infusion



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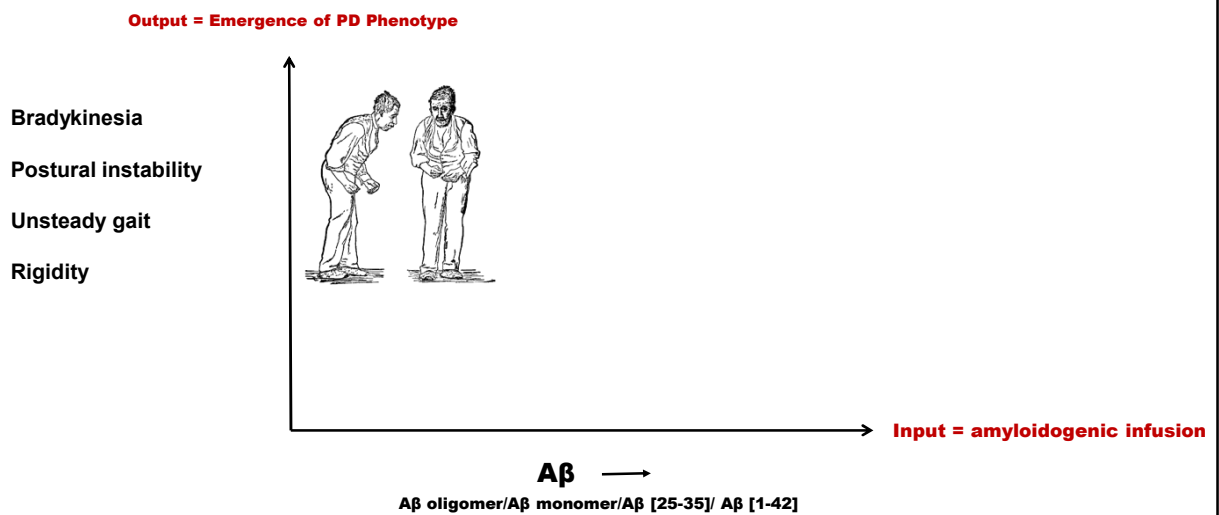
Neurohistochemistry of nigral neurons



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Qualitative Behavioral Aberrations



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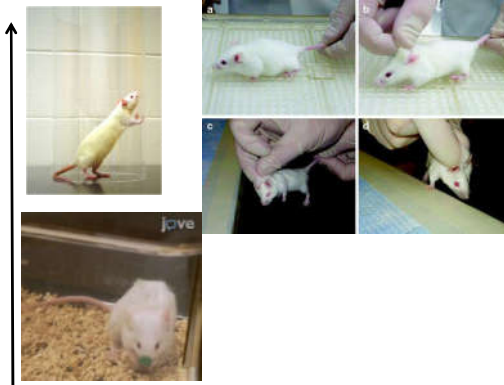
Quantitative Locomotor Deficits

Output = Emergence of PD Phenotype

Single Akinesia Test
(Cylinder Test)

Forelimb Placement

Adhesive Removal Test
(Sticky Dot Test)



Input = amyloidogenic infusion

A β →

A β oligomer/A β monomer/A β [25-35]/ A β [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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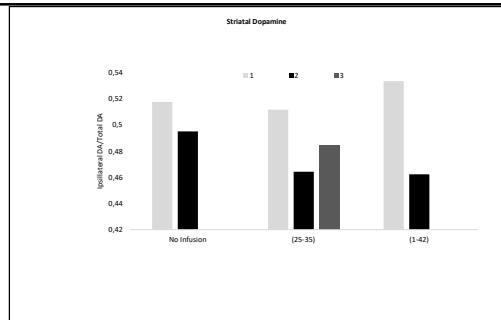
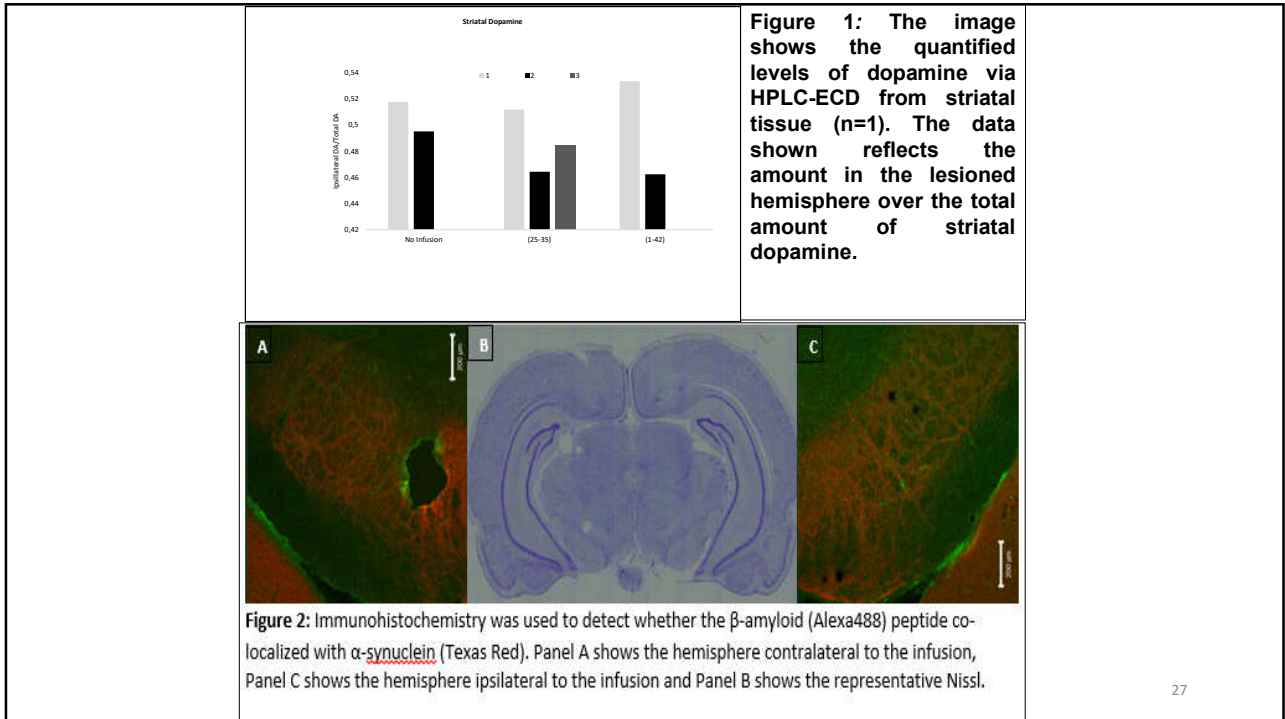
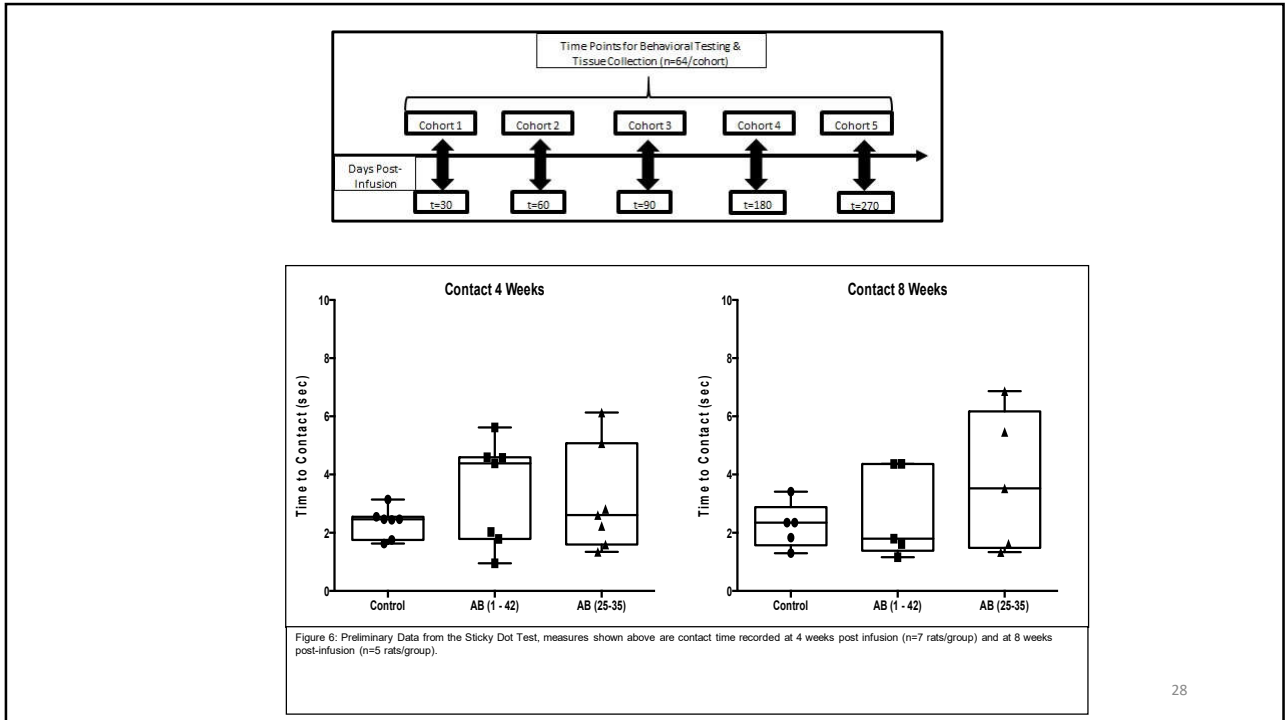


Figure 1: The image shows the quantified levels of dopamine via HPLC-ECD from striatal tissue (n=1). The data shown reflects the amount in the lesioned hemisphere over the total amount of striatal dopamine.



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.



Findings....

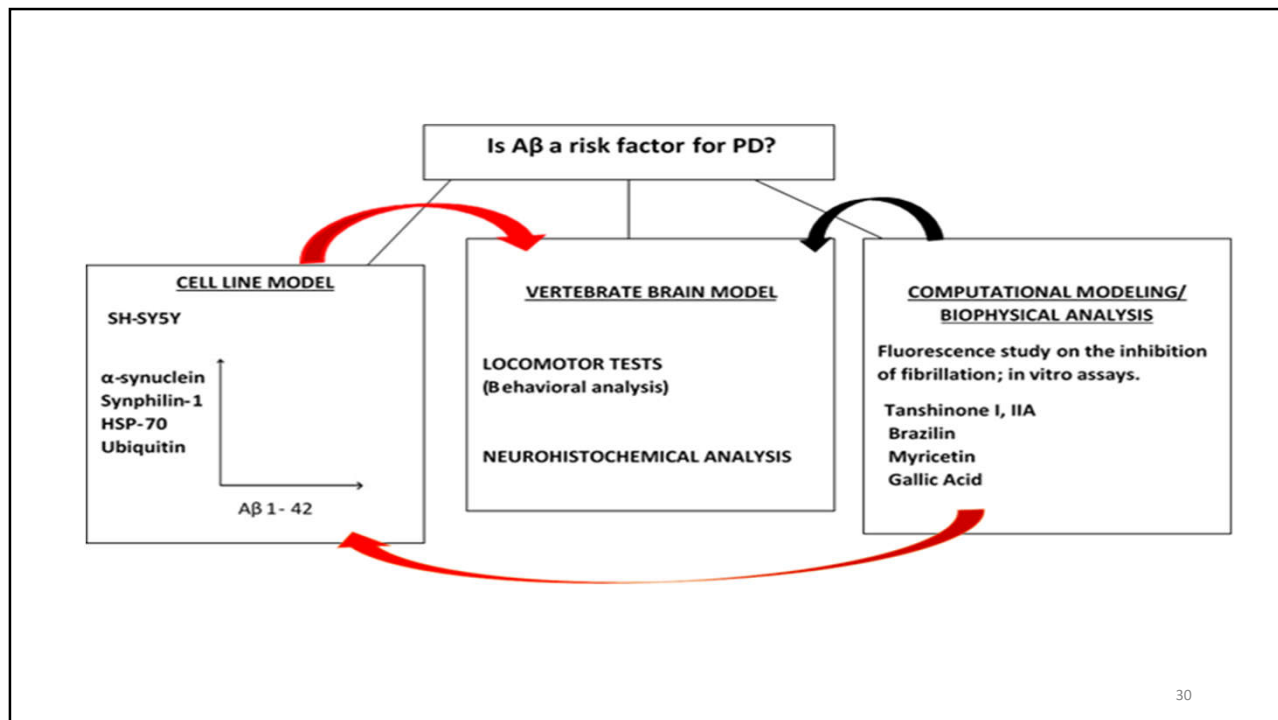
In Vitro

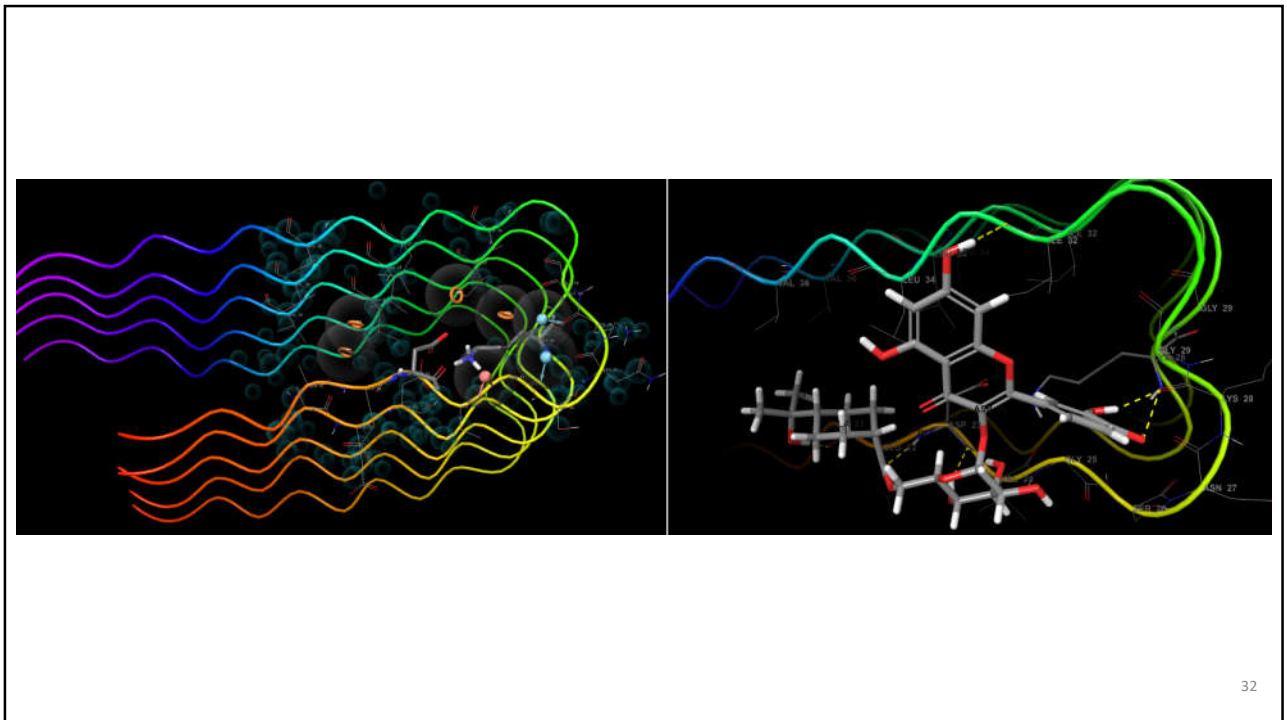
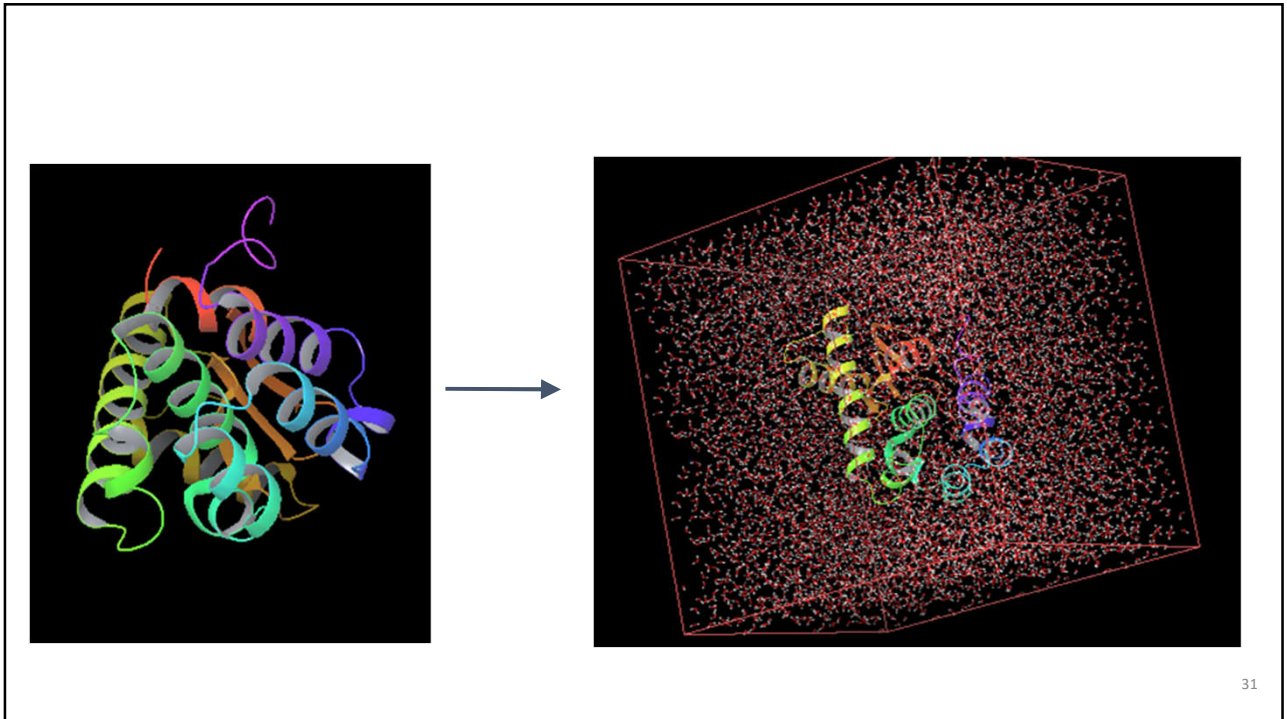
Our findings suggest that A β [25-35] initiates a cascade of physico-chemical events *in vitro* that provokes the Parkinsonian phenotype:

- 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) A β dependent co-localization of α -syn with PDI (perinuclear)
- 6) co-localization of A β (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 A β -associated infiltration of the vertebrate nigral mass.

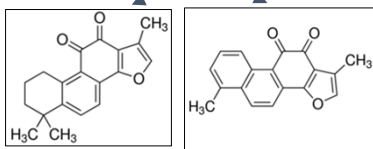




Phytochemicals



Salvia miltiorrhiza

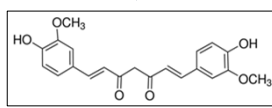


Tanshinone IIA

Tanshinone I



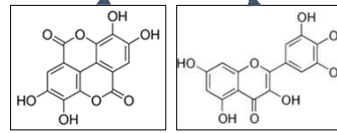
Curcuma Longa



Curcumin



Family:Rosaceae Rubus

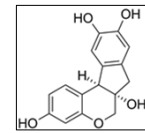


Ellagic acid

Myricetin



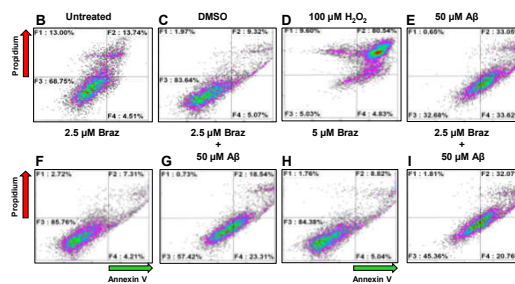
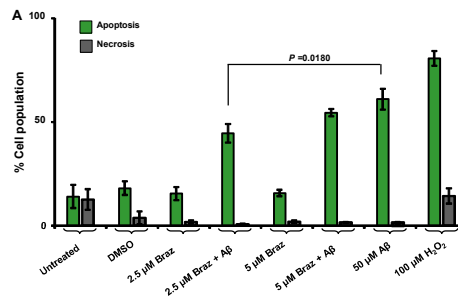
Caesalpinia sappan



Brazilin

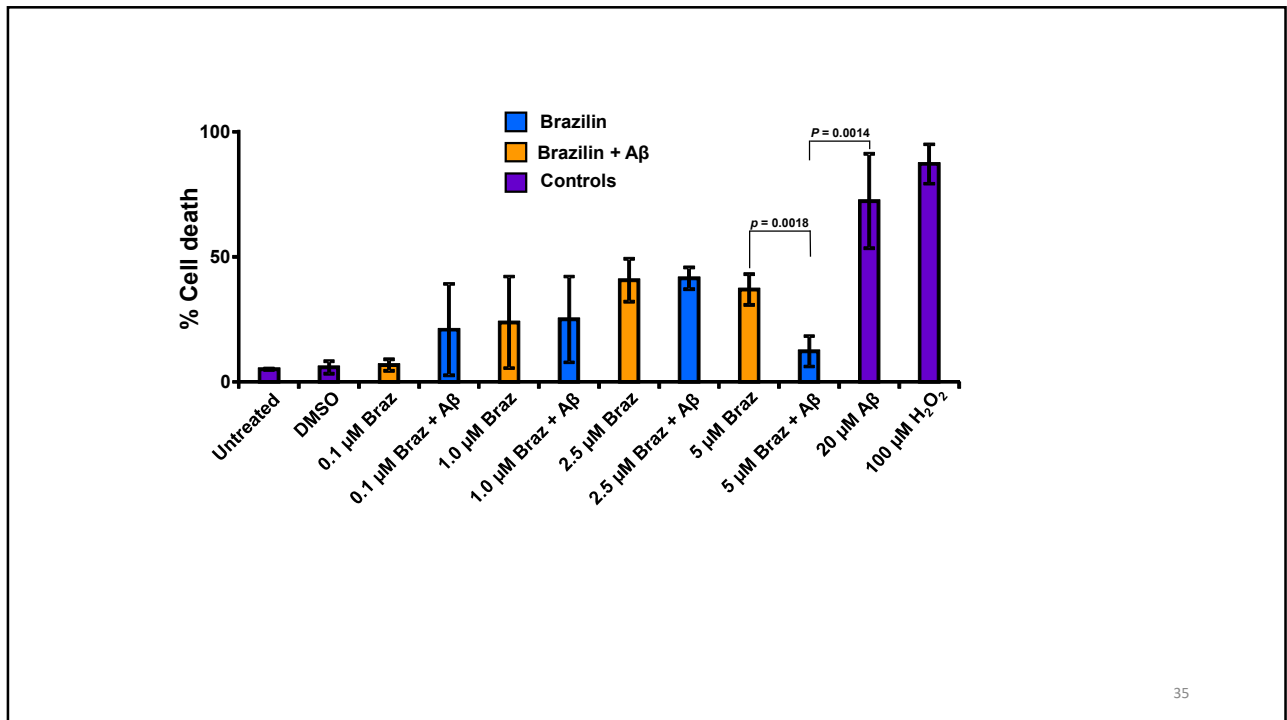
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Nanoscope Portrait of an Amyloidogenic Pathway Visualized through Tip-Enhanced Raman Spectroscopy

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ABSTRACT: Insights into understanding the process by which amyloid proteins become toxic have been hampered by the lack of experimental techniques that adequately resolve the process. Recently, tip-enhanced Raman spectroscopy, with its unique capability to spectroscopically image and chemically identify reactive moieties with atomic precision, was used to obtain high-resolution mapping of the mid-to-isolectric continuum of amyloid beta. This technique opens the door for studying the toxic aggregation pathway of alpha amyloid protein and space effects devoted to prophylactic and therapeutic interventions in neurodegenerative and protein-misfolding related disorders.

KEYWORDS: Amyloidogenesis, amyloid beta, tip-enhanced Raman spectroscopy, toxic amyloids, oligomers, protofibrils, fibrils

The conversion of soluble amyloid proteins to their toxic oligomers and smaller aggregates, known as protofibrils, is a key event that transitions neurons, proteins into pathogenic conformations (Scheme 1). It is a conformation associated with both the onset and progress of both neurodegenerative and non-neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), mutant Huntingtin's disease (mHTT), hepatitis, amyloidosis, and type II diabetes. In these neurodegenerative, oligomeric and protofibrillar of the beta-amyloid protein, such as amyloid beta (Aβ), tau, α-synuclein, mutant Huntingtin protein, human lysozyme, and late-onset polyglutamine (ApoE) during the severity of the disease is critical to neuron death or plaque build. Therefore, a detailed chemical and morphological molecular (with conformational precision) of the structure by which soluble, neurotoxic, amyloid-precursor sites that transition in progression to underlying not only how pathology events and progress but also to emerging prophylactic and therapeutic genes against their respective disorders.

Understanding the conversion of oligomers and protofibrils to higher-order aggregates a reality process. The network of interactions, between, neurons, oligomers, protofibrils, and neurons that are not yet populated with biochemically tagged species or fixed and contained, both of which need to be identified as such to reveal high-resolution map that will confer from neuron, a neuron can contain protein and an understanding of their (temperature, variations) in structure but also their state of the mid-to-isolectric continuum.

In practice, we limited the conversion of amyloid protein hampered effects to (1) fully in disease onset, (2) delayed disease onset, and (3) disease progression.

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Learnings from Protein Folding Projected onto Amyloid Misfolding

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ABSTRACT: The 1990s saw a revolution in our understanding of the protein folding pathways of both double-strand containing proteins and single conformational folds. High-resolution maps of the folding trajectories, made possible by innovative experimental design, revealed the presence of multiple conformations, their formation and conversion, and the overall of interactions between them that lead to the formation of the folded protein form in solution state. The same level of detail has been found in the amyloid aggregation pathway of protein like proteins, see, concentrated. Nevertheless, a recent development that led to the resolution of interactions in neurodegenerative proteins, without resort to their separation, is likely to not only advance our basic understanding of the atomic- and molecular-level interactions guiding amyloid misfolding but also impact interventional efforts in their associated pathologies.

KEYWORDS: Protein-folding amyloid protein, oligomers, protofibrils, conformational folding, oxidative folding, redox-active amyloid, alpha four receptors, vibrational spectroscopy

The 1990s and the 1980s witnessed an exponential growth in our understanding of the mechanisms by which proteins fold.^{1–3} The efforts of groups led by Bahar, Scheraga, Conlon, Dobson, Fersht, Wickham, Englander, and their academic program generated detailed blueprints of the protein folding pathways of well-studied proteins such as bovine pancreatic trypsin inhibitor, ribonuclease A, cytochrome c, and the ribonuclease D. These efforts provided the structural and functional details of the folding process, and the overall of interactions between them that lead to the formation of the folded protein form in solution state. The same level of detail has been found in the amyloid aggregation pathway of protein like proteins, see, concentrated. Nevertheless, a recent development that led to the resolution of interactions in neurodegenerative proteins, without resort to their separation, is likely to not only advance our basic understanding of the atomic- and molecular-level interactions guiding amyloid misfolding but also impact interventional efforts in their associated pathologies.

The rigorous application of the experimental and computational techniques described above were very key in establishing that the native or native-like conformations could often be accessed by multiple routes.^{4–6} It revealed the presence of co-pathway systems, kinetically trapped intermediates, and dead-end structures. In several cases, the rate constants leading to, and away from, of species populating the folding trajectory were worked out and the overall of interactions between pathways during the manifestation of U to N were mapped.^{7–9}

The past few years have seen a renaissance of such efforts to understand the processes by which proteins amyloid protein aggregate. Amyloid beta (Aβ), α-synuclein, tau, and mutant Huntingtin protein as a few examples of amyloidogenic proteins capable of self-organizing the templated misfolding of other proteins (Scheme 1). These efforts have provided

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The Era of Neurodegenerative Metastasis

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ABSTRACT: The incidence of converging and points across neurodegenerative disorders, leading to comorbidity, has spurred the search for common neurochemical denominators among otherwise distinct pathologies. While recent data have hinted at common factors, and genetic risk factors across the neurodegenerative landscape, we discuss the potential of protein-like amyloid proteins as suspects of not causal, in seeding cross-pathology. While much work remains to be done on how they spread and "fertile" seemingly unrelated neurodegeneration, it is safe to assume that the era of neurodegenerative metastasis is here.

KEYWORDS: Neurodegeneration, amyloid, oligomers, protofibrils, precursor factors, comorbidity, neurodegenerative metastasis

The past few years have seen a renaissance in attempts to comprehend intricacies and nuances within the amyloid disease that drive neurodegenerative disorders. The landmark finding that protein-like behavior, near exclusive to the prion protein, is a hallmark of other proteins including amyloid beta (Aβ), tau, α-synuclein, and mutant huntingtin protein (mHTT) opened efforts to underscore its implications for Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, amyotrophic lateral sclerosis, Parkinson's disease with dementia (PDD), dementia with Lewy bodies (DLB), etc.^{1–3} The trait whereby these proteins can form seeds that act as templates for recruiting their soluble counterparts remained the underpinning of many early studies designed to understand the molecular basis of neurotoxicity in their respective neurodegenerative pathologies.

by neighboring neurons, and seed-templated misfolding of corresponding soluble amyloids in the recipient neuron. An example by which a seed propagates is via neuronal networks. Such networks include the vagus nerve tract to the midbrain, pons, midbrain, cerebellum, and thalamus. Other studies have demonstrated that amyloid seeds released into the extracellular space (synaptic junction) may be internalized by cells in the immediate neighborhood via exosomes or tunneling nanotubes. The latter are membranous in nature and resemble traditional nanotubes with the carbon filament being replaced by polymeric actin. By example, in concert with the progress of Alzheimer's tau-tangles first observed in the transentorhinal and entorhinal regions permeate into the limbic structures and adjoining molecules by contrast PD-associated α-synuclein pathology is first detected in dorsal

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- Ariel Schmid

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- Julieta Aguilera
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- Carolina Melendez
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