ABSTRACT: While amyloid proteins such as amyloid β (Aβ), α-synuclein, tau, and lysozyme are known to be prion-like; emerging data have revealed that they are also able to seed the misfolding of prion-like proteins differing in sequence. In the present study, we have developed a tool designed to test neurohistochemical outcomes associated with the entry of an amyloid protein into heterotypic neurons, i.e., neurons that do not express the invading amyloid and, instead, endogenously express amyloids differing in sequence. The stereotaxic introduction of Aβ into the rodent tegmental area of the mid-brain revealed that the foreign amyloid had infiltrated into nigral neurons. Furthermore, Aβ was found colocalized with α-synuclein, an amyloid endogenous to the substantia nigra and differing in sequence relative to Aβ. Disruption of α-synuclein status in the substantia nigra is associated with Parkinson’s disease onset and progress. In addition to the study findings, a significant inroad to future neurodegenerative research was made via the stereotaxic introduction of the foreign amyloid. This technique limits the presence of confounding neurometabolic variables that may be prevalent in transgenic animal models of cross-toxicity and, thereby, better addresses the role of individual neuronal factors in cross-toxicity. Finally, the data from this work may help reconcile the high frequency of clinical comorbidity seen in neurodegenerative diseases.

1. INTRODUCTION

Amyloidosis refers to diseases that occur when misfolding-prone proteins, called amyloids, transform from their soluble monomers to toxic aggregates and build up within cells expressing them. The aggregation phenomenon interferes with normal cellular, tissue, and organ function and can lead to cell death and associated pathologies.

Today, we know that the prion protein shares its prion-like tendencies with a number of other proteins including amyloid β (Aβ), α-synuclein (α-syn), tau, and mutant Huntingtin (mHTT).1–10 Like the prion protein, these amyloid-forming proteins are able to spontaneously convert into toxic particles from their soluble monomers. Furthermore, the toxic particles, which are oligomeric or proto-fibrillar in nature, can serve as seeds (templates) for the continued soluble-to-toxic conversion of their monomeric counterparts.11 The seeds then propagate from the neurons that they originated in, to neighboring neurons and beyond through a number of different mechanisms.12–17 There is a clear correlation between the clinical progress of the neurodegenerative syndrome and the spreading of the seed associated with the said syndrome (Scheme 1).18

Of interest is the sequelae of events, and consequences thereof, that arise when an amyloid seed encounters a neuronal domain that does not constitutively express its soluble monomeric counterparts. Can the invading seed corrupt cellular homeostasis in such neurons? Particularly, the scenario becomes relevant and interesting if such neurons constitutively express soluble amyloids that differ in sequencing from the infiltrating amyloid. It becomes relevant to understand whether the amyloid that is foreign to the host neuron (heterotypic neurons) can “hijack” it using the host neuronal amyloid as an “accomplice”. That is can the invading amyloid drive the soluble-to-toxic conversion of the local amyloid and induce “cross-toxicity”? The cross-toxic notion is not new and has been experimentally observed (Scheme 2; Table 1).19,20 In vitro, the tau-dependent cytoskeletal framework has been found to be disrupted because of interactions between the amyloid and α-synuclein.21–23 In an unrelated finding, tau phosphorylation and its distribution have been found to be influenced by...
Scheme 1. Diagram Showing the Spread of Amyloidoenic Pathological Protein through Different Brain Regions, as Seen in AD & PD Shown Here. Adapted from ref 18

mHTT.24 The interactions between α-synuclein and Aβ have been characterized as having a number of scenarios that reveal overlap at the protein product level, the genome-wide level, and clinical crossover.25–42

There is an unmet need to unequivocally examine the possibility that amyloid-driven cross-toxicity drives cross-pathology in the vertebrate brain.43–45 Although a number of preclinical trials using transgenic animals have addressed this scenario, they fall short due to a number of reasons. For example, a transgenic mouse designed to explore the role of Aβ in promoting α-synuclein-aggregation-dependent outcomes would not be able to insulate the experimental variable to Aβ alone. Furthermore, there would be no firm knowledge of when the invading amyloid enters neurons that do not endogenously express it. There is no control over the “concentration” of the invading amyloid. There is a need for a more controlled experimental setup, which restricts the variables to primarily the invading amyloid and overcomes gaps associated with an amyloid dose and amyloid “time-of-entry” into the heterotypic neuron.

Here, we develop a model to test molecular outcomes associated with the entry of an amyloidoenic vector (Aβ) into a vertebrate heterotypic neuronal assembly, viz., tegmental area of the mid-brain. Neurobiochemical analysis is used to validate the entry (via endocytosis) of the vector into an exemplar heterotypic host (nigral neurons). Furthermore, we assay for the impact of the AD-associated Aβ on a heterologous nigral-resident amyloid (α-synuclein).46,47 The implications for the role of amyloids in driving cross-toxic outcomes are discussed.

2. RESULTS

2.1. Correct Needle Placement. Nissl staining was used to observe the cannula tract into the rodent tegmental area of the mid-brain. Figure 1A shows a section from the Rat Brain Atlas by George Paxinos & Charles Watson, whereas Figure 1B indicates the site of stereotactic inoculation into the rat brain.48 This method of staining demonstrates the effectiveness of the injection into a locus that does not endogenously express Aβ. It also facilitates postmortem neurobiochemical assays by identifying a region of interest expressing amyloids different in sequence (for example, the substantia nigra pars compacta (SNpc)).

2.2. Impact of Aβ Presence in Heterotypic (nigral) Neurons. Thioflavin-S (Th-S) is commonly used to detect amyloid-like fibrils such as plaques and tangles in tissues. Figure 2A depicts the Th-S fluorescence results from nigral neurons, indicating that the foreign amyloid (Aβ) has been successfully introduced into heterotypic neurons.

We examined the impact of Aβ1–42 and Aβ25–35 on local amyloid proteins 6 months after its infusion. This immunofluorescence assay confirms the colocalization of the nigral-resident amyloid, α-synuclein, with Aβ, as observed in the figure (see Figure 2C,D).

### Table 1. Cross-Amyloid Network for the Aβ Peptide

| amyloid proteins that enhance the risk of AD in mice or in vivo | amyloid proteins that decrease the progression of AD in mice | amyloid proteins that promote Aβ fibrillation in vitro | amyloid proteins that suppress Aβ fibrillation in vitro | unknown |
|---------------------------------------------------------------|----------------------------------------------------------|--------------------------------------------------|--------------------------------------------------|---------|
| tau protein                                                   | tau                                                     | apolipoprotein A-I                                | APOAI                                            | Alys    |
| α-synuclein                                                  | α-syn                                                   | cystatin                                         | Acys                                             | SOD     |
| insulin                                                      | AIns                                                    | transthyretin                                     | ATTR                                             | AL      |
| prion-protein                                                | Prp                                                     | tau protein                                      | prion-protein                                    |         |
| fibrinogen-α                                                 | Afb                                                     | apolipoprotein A-I                                | APOAI                                            |         |
| islet amyloid polypeptide                                     | IAPP                                                    | cystatin                                         | Acys                                             |         |

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3. DISCUSSION

It has been previously demonstrated that a truncated peptide of Aβ1–42 corrupts homeostasis in a model heterotypic cell line. The peptide was found to be cross-toxic in vitro as it promoted protein disulfide isomerase S-nitrosylation (S-NO-PDI formation), drove the upregulation of HSP-70, and increased ubiquitinated debris. The truncated amyloid altered the soluble α-synuclein status via its colocalization with PDI and via the formation of Lewy-like neurites in a dose-dependent manner. Here, we developed a model to translate the question of amyloid cross-toxicity. We addressed whether an invading amyloid corrupts the local machinery in heterotypic neurons by disrupting the homeostasis of a resident amyloid differing in sequence. Our model introduces Aβ, the invading amyloid, into the rodent tegmental area of the mid-brain. We then assayed the substantia nigra, which does not express Aβ, for the corruption of cellular homeostasis by the invading amyloid. The disruption of α-synuclein, an amyloid that is endogenous to the substantia nigra, was observed via its colocalization with the endocytosis of Aβ from the mid-brain.

These results are of particular relevance because the disruption of α-synuclein status is associated with the presence of Parkinson’s disease. Our findings then suggest that an invading amyloid can hijack heterotypic neuronal cells via an endogenously expressed amyloid that differs in sequence from its own. It can drive cross-toxic outcomes.

4. CONCLUSIONS

We have modeled a scenario mimicking the entry of amyloid seeds into heterotypic neurons. The proposed work bridges a gap in preclinical transgenic animal models designed to address the possibility of amyloid cross-toxicity using animal models. Such work often presents with confounding variables and lack experimental control. For example, using a translational animal model, it is not possible to unequivocally determine whether a single neurometabolic factor triggers cross-pathology. It is also near impossible to control the timeline and dose of the invading amyloid as it “metastasizes” to neighboring neurons.20 By contrast, our stereotaxic method facilitates knowledge of “time-zero” and thereby establishes a robust longitudinal timeline post-infusion. It limits confounding neurometabolic factors. It permits dosing of the invading amyloid. It can be used to deliver the invading amyloid “chronically” via an osmotic pump.

An important outcome of this model is the potential paving of a detailed behavioral, cellular, and molecular roadmap of the trajectory to amyloid-induced cross-pathology. Our translational model may help reconcile the incidence of comorbidities seen across neurodegenerative diseases differing in origin and evolution. As discussed in the Introduction, there is already ample evidence of neurometabolic factors potentially crossing over to exert cross-toxic outcomes. Our study can easily be applied to addressing the role of crossover factors in triggering seemingly unrelated neurodegenerative pathologies. For example, the role of α-synuclein in dementia with Lewy bodies (DLB), or its interactions with tau to impact AD can be tested by introducing α-synuclein into cortical neurons where it is not endogenously expressed. Thus, our methodological inroad is likely to propel inquiry into Parkinson’s disease with dementia, DLB, and multisystem atrophy, all of which may share pathophysiological underpinnings.
5. MATERIALS AND METHODS

5.1. Chemicals and Reagents. The following reagents were sourced commercially as high purity reagents (>99%): hexafluoroisopropanol (HFIP) (Fisher Scientific, ICN15124580); Ajf25–35 (Ana Spec AS-24448); rodent Ajβ1–42 (Genscript, RP10013); Thioflavin-S (Sigma-Aldrich; 1326-12-1), and anti-α-synuclein (Cell signaling Technology; C81H6, 2647). Secondary antibodies were obtained from Abcam (Alexa Fluor-488, ab150077; Texas red, ab6787). Other chemicals were sourced as follows: thionin acetate (Cat#: 229840250; Acros Organic) and cytoseal TM-60 (Thermo scientific 8310-16).

5.2. Amyloid-β Preparation. Monomeric Aβ was prepared by dissolving lyophilized Aβ (25–35; 1–42 [rodent]) in HFIP to solubilize the peptide and dissociate any preformed aggregates. The stock solution of the monomer was then freeze-dried and stored for future use. Oligomeric Aβ(1–42) was prepared by dissolution in dimethyl sulfoxide (DMSO) followed by the fractionation of monomeric and oligomeric form by gel filtration chromatography. The aforementioned step was always performed immediately prior to its use for experiments to ensure sample freshness. The size (monomer, oligomer, fibril) of Aβ in solution was periodically measured using dynamic light scattering (DLS) after preparation.

5.3. Animals and Surgical Procedures. Male Lewis laboratory rats (N = 79) were purchased from Hilltop Lab Animals, Inc. At the onset of the study, rats were 2–5 months old (young adults). Animals were pair-housed under standard housing conditions, in an IVC rack system on a 12/12 h light–dark cycle (dark: 0800–2000), at a temperature of 22 ± 3 °C with constant relative humidity. Rats were fed standard rodent chow and water ad libitum. Prior to experiments, rats were habituated for a 7-day period. All procedures were approved by the Institutional Animal Use and Care Committee (IACUC) at The University of Texas at El Paso.

5.4. Stereotaxic Infusion. Rats underwent standard stereotaxic surgical procedures under aseptic conditions to introduce Aβ(1–42) (100 μM), Aβ(25–35) (100 μM), or vehicle into the SNpc. Animals were sedated with 3% inhalant isoflurane and then maintained throughout the surgery with 2–3% inhalant isoflurane. A unilateral infusion to the rodent striatum area of the mid-brain was performed using the coordinates as per the Paxinos & Watson Atlas. All groups received a 2 μL infusion of the respective substance at appropriate concentrations. After being placed in the proper coordinates, the syringe was allowed to sit for 2 min prior to starting infusion and after the infusion. Rats were allowed to recover for 14 days postsurgery. Personnel performing the surgeries were blinded to the solution being infused.

Animals were divided into three infusion groups: vehicle, Aβ(25–35), and Aβ(1–42) groups. They were further divided into groups by sacrificial time point; with a time point at 3 months (n = 9), 6 months (n = 16), 9 months (n = 16), and 12 months (n = 38). Evaluations of animals’ health and appearance were performed every other day to monitor animal’s welfare and possible symptoms.

5.5. Histology. At the experimental time points post-infusion, rats were euthanized by administering 100 mg/kg, i.p., of sodium pentobarbital and perfused with 0.9% of saline followed by 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS). Brains were collected, cryoprotected in a sucrose solution, and frozen. Finally, frozen tissue specimens were cut in 30-μM-thick coronal sections on the Leica CM 1950 cryostats. Tissue slices were placed into an ethylene glycol antifreeze solution for storage until future use. 5.5.1. Nissl Staining. Nissl staining is a standard technique used to reveal anatomical subdivisions of the brain. Sections were mounted and dehydrated in ascending alcohol concentrations (from 0% ethanol/100% water up to 100% ethanol/0% water). Xylene was then used for defatting of tissue. Sections were then rehydrated in the reverse order followed by thionin immersion. Sections were then dehydrated once again and then sealed with Cytoseal. Images were then taken using an inverted fluorescence phase microscope (Keyence BZ-X710) with a 5x objective.

5.5.2. Colocalization of α-Synuclein and Aβ. Once the site of infusion was determined by Nissl staining, a tissue section collected from the same series was further processed via immunostaining to detect the presence of colocalized aggregates of the endogenously expressed α-synuclein and the exogenous Aβ. First, selected tissues were removed from their cryopreservative solution and rinsed three times with phosphate-buffered saline (PBS) for 5 min every wash. Tissues were then subsequently incubated in a blocking solution (0.25% goat serum and 0.1% Triton X-100) solution for 2 h. Following blocking, tissues were incubated with anti-α-synuclein (mouse) antibody (1:1000) overnight at 4 °C. The following day, tissues were washed again, as previously described, then incubated in a fluorescent-labeled secondary antibody, Anti-Mouse Alexa 555 (1:2000), for 2–4 h at room temperature on a shaker protected from light. Following secondary antibody incubation and rinsing, tissue sections were co-stained with 1% aqueous thioflavin-S for 8 min at room temperature and then washed two times with 80% ethanol for 3 min, then washed for 3 min with 95% ethanol, followed by three washes with distilled water. The tissues were then mounted onto glass slides and sealed using sodium bicarbonate mounting media. The images of the tissue sections were then taken with a confocal microscope, LSM Zeiss 700, with a 40x magnification objective.

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Author Contributions

G.H. conceptualized, designed, and performed experiments and analyzed the data. L.M. conceptualized, designed, and performed experiments. A.N.S. and S.A.C. contributed with and analyzed the data. L.M. conceptualized, designed, and performed experiments. M.N. contributed to the peptide preparation and helped with the surgical preparation of animals in this project. M.N. acknowledges support from NIH 1SC3 GM111200 01A1 for this work. The authors gratefully thank Armando Varela and the staff members of the Cellular Characterization and Biorepository Core Facility of the Border Biomedical Research Center at The University of Texas at El Paso (UTEP). This facility was supported by the Grant nos. 2G12MD007592, 5G12MD007592, and SU54MD007592 from the National Institute on Minority Health and Health Disparities (NIMHD), a component of the National Institutes of Health (NIH). Finally, the authors would like to thank Les and Harriet Dodson Endowment and the President’s Office for financial support.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Guo, J. L.; Lee, V. M. Cell-to-Cell Transmission of Pathogenic Proteins in Neurodegenerative Disease. Nat. Med. 2014, 20, 130–138.
(2) Peng, C.; Gathagan, R. J.; Covell, D. J.; Medellin, C.; Stieber, A.; Robinson, J. L.; Zhang, B.; Pitkin, R. M.; Olfemni, M. F.; Luk, K. C.; Trojanowski, J. Q.; Lee, V. M.-Y. Cellular Milieu Imparts Distinct Pathological α-Synuclein Strains in α-Synucleinopathies. Nature 2018, 557, 558–563.
(3) Jucker, M.; Walker, L. C. Prion-like Mechanisms in Alzheimer Disease and Other Neurodegenerative Disorders. Annu. Neurol. 2011, 70, 532–540.
(4) Walker, L. C. Prion-like Mechanisms in Alzheimer Disease. Handb. Clin. Neuro. 2018, 153, 303–319.
(5) Olsson, T. T.; Klementieva, O.; Gouras, G. K. Prion-like Seeding and Nucleation of Intracellular Amyloid-β. Neurobiol. Dis. 2018, 113, 1–10.
(6) Sarnataro, D. Attempt to Untangle the Prion-Like Misfolding Mechanism for Neurodegenerative Diseases. Int. J. Mol. Sci. 2018, 19, No. 3081.
(7) Strang, K. H.; Croft, C. L.; Sorrentino, Z. A.; Chakrabarty, P.; Golde, T. E.; Giasson, B. I. Distinct Differences in Prion-like Seeding and Aggregation between Tau Protein Variants Provide Mechanistic Insights into Tauopathies. J. Biol. Chem. 2018, 293, 2408–2421.
(8) Mudher, A.; Colín, M.; Dujardin, S.; Medina, M.; Dewachter, I.; Naini, S. M. A.; Mandelkow, E. M.; Mandelkow, E.; Buée, L.; Goedert, M.; Brion, J. P. What Is the Evidence That Tau Pathology Spreads through Prion-like Propagation? Acta Neuropathol. Commun. 2017, 5, 99–119.
(9) Zhang, Z.; Nie, S.; Chen, L. Targeting Prion-like Protein Spreading in Neurodegenerative Diseases. Neural Regener. Res. 2018, 13, 1875–1878.
(10) Pearce, M. M. P.; Kopito, R. R. Prion-like Characteristics of Polyglutamine-Containing Proteins. Cold Spring Harbor Perspect. Med. 2018, 8, No. a024257.
(11) Gupta, S.; Jie, S.; Colby, D. W. Protein Misfolding Detected Early in Pathogenesis of Transgenic Mouse Model of Huntington Disease Using Amyloid Seeding Assay. J. Biol. Chem. 2012, 287, 9982–9989.
(12) Törnqvist, M.; Michaels, T. C.; Sanagavarapu, K.; Yang, X.; Meisl, G.; Cohen, S. I.; Knowles, P. J.; Linse, S. Secondary Nucleation in Amyloid Formation. Chem. Commun. 2018, 54, 8667–8684.
(13) Masnata, M.; Cicchetti, F. The Evidence for the Spread and Seeding Capacities of the Mutant Huntington Protein in In Vitro Systems and Their Therapeutic Implications. Front. Neurosci. 2017, 11, No. 647.
(14) Okuzumi, A.; Kurosawa, M.; Hatano, T.; Takanashi, M.; Nojiri, S.; Fukuhara, T.; Yamanaka, T.; Miyazaki, H.; Yoshinaga, S.; Furukawa, Y.; Shimogori, T.; Hattori, N.; Nukina, N. Rapid Dissemination of Alpha-Synuclein Seeds through Neural Circuits in an in-Vivo Prion-like Seeding Experiment. Acta Neuropathol. Commun. 2018, 6, 96.
(15) Feiler, M. S.; Strobelt, B.; Freischmidt, A.; Helferich, A. M.; Kappel, J.; Brewer, B. M.; Li, D.; Thal, D. R.; Walther, P.; Ludolph, A. C.; Danzer, K. M.; Weishaupt, J. H. TDP-43 Is Intercellularly Transmitted across Axon Terminals. J. Cell Biol. 2015, 211, 897–911.
(16) Duyckaerts, C.; Seilhean, D.; Sazdovitch, V.; Plu, I.; Delatour, B.; Potier, M. C. Seeding and Propagation of Lesions in Neurodegenerative Diseases: A New Paradigm. Bull. Acad. Natl. Med. 2015, 199, 809–819.
(17) Oueslati, A.; Ximerakis, M.; Vekrellis, K. Protein Transmission, Seeding and Degradation: Key Steps for α-Synuclein Prion-like Propagation. Exp. Neurobiol. 2014, 23, 324.
(18) Weickenmeier, J.; Kuhl, E.; Goriely, A. Multiphysics of Prionlike Diseases: Progression and Atrophy. Phys. Rev. Lett. 2018, 121, No. 158101.
(19) Luo, J.; Wärländer, S. K. T. S.; Gräslund, A.; Abrahams, J. P. Cross-Interactions between the Alzheimer Disease Amyloid-β Peptide and Other Amyloid Proteins: A Further Aspect of the Amyloid Cascade Hypothesis. J. Biol. Chem. 2016, 291, 16485–16495.
(20) Narayan, M. The Era of Neurodegenerative Metastasis. ACS Chem. Neurosci. 2019, 10, 3346–3348.
(21) Spires-Jones, T. L.; Attens, J.; Thal, D. R. Interactions of Pathological Proteins in Neurodegenerative Diseases. Acta Neuropathol. 2017, 134, 187–205.
(22) Josephs, K. A.; Murray, M. E.; Whitwell, J. L.; Tosakulwong, N.; Weigand, S. D.; Petrucelli, L.; Liesinger, A. M.; Petersen, R. C.; Parisi, J. E.; Dickson, D. W. Updated TDP-43 in Alzheimer’s Disease Staging Scheme. Acta Neuropathol. 2016, 131, 571–585.
(23) Uchikado, H.; Lin, W. L.; DeLucia, M. W.; Dickson, D. W. Alzheimer Disease with Amygdala Lewy Bodies: A Distinct Form of α-Synucleinopathy. J. Neuropathol. Exp. Neurol. 2006, 65, 685–697.
(24) Irwin, D. J.; Grossman, M.; Weintraub, D.; Hurtig, H. L.; Duda, J. E.; Xie, S. X.; Lee, E. B.; Van Deerlin, V. M.; Lopez, O. L.; Koller, J. K.; Nelson, T. P.; Jicha, G. A.; Woltjer, R.; Quinn, J. F.; Kaye, J.; Leverenz, J. B.; Tsuang, D.; Longfellow, K.; Trojanowski, J. Q. Neuropathological and Genetic Correlates of Survival and Dementia Onset in Synucleinopathies: A Retrospective Analysis. Lancet Neurol. 2017, 16, 55–65.
(25) McAleese, K. E.; Walker, L.; Erskine, D.; Thomas, A. J.; McKeith, I. G.; Attens, J.; TDP-43 Pathology in Alzheimer’s Disease, Dementia with Lewy Bodies and Ageing. Brain Pathol. 2017, 27, 472–479.
(26) Aarsland, D.; Litvan, I.; Salmon, D.; Galasko, D.; Westerlund, C.; Larsen, J. P. Performance on the Dementia Rating Scale in Parkinson’s Disease with Dementia and Dementia with Lewy Bodies: Comparison with Progressive Supranuclear Palsy and Alzheimer’s Disease. J. Neurol., Neurosurg. Psychiatry 2003, 74, 1215–1220.
Diseases.

ACS Chem. Biol.

the ER: A New Role for S

Antioxid. Redox

2013

Parkinson

Synuclein Ubiquitination and Novel Therapeutic Targets for

Ther.

2012

No. e55848.

β

That Link

Disease and Alzheimer

Pathophysiology of Neurodegenerative Diseases.

W.; Oh, C.-K.; McKercher, S. R.; Ambasudhan, R.; Okamoto, S.;

Parkinson

Presence in Cellular Inclusions and Lewy Bodies Imply a Role in

Ubiquitylation of Synphilin-1 and -Synuclein by SIAH and Its

D.; Bornemann, A.; Riess, O.; Ross, C. A.; Rott, R.; Engelender, S.

5505.

α

A Powerful Yeast Model to Investigate the Synergistic Interaction of

J. Neurochem.

of ?-Synuclein Aggregation.

(37) Ciaccioli, G.; Martins, A.; Rodrigues, C.; Vieira, H.; Calado, P.

(36) Uversky, V. N. Neuropathology, Biochemistry, and Biophysics of ?-Synuclein Aggregation and Toxicity in Cellular Models of Synucleinopathy. PLoS One 2011, 6, No. e26609.

(35) Badiola, N.; de Oliveira, R. M.; Herrera, F.; Guardia-Laguarta, C.; Gonçalves, S. A.; Pera, M.; Suárez-Calvet, M.; Clarimon, J.; Outeiro, T. F.; Lleó, A. Tau Enhances ?-Synuclein Aggregation and Toxicity in Cellular Models of Synucleinopathy. PLoS One 2013, 8, No. e55848.

(34) Giasson, B. I. Initiation and Synergistic Fibrillization of Tau and Alpha-Synuclein. Science 2003, 300, 636–640.

(33) Goedert, M. Alzheimer’s and Parkinson’s Diseases: The Prion Concept in Relation to Assembled A, Tau, and -Synuclein. Science 2015, 349, No. 1255555.

(32) Liu, C.-C.; Kanekiyto, T.; Xu, H.; Bu, G. Apolipoprotein E and Alzheimer Disease: Risk, Mechanisms and Therapy. Nat. Rev. Neurol. 2013, 9, 106–118.

(31) Tosto, G.; Reitz, C. Genome-Wide Association Studies in Alzheimer’s Disease: A Review. Curr. Neurol. Neurosci. Rep. 2013, 13, No. 381.

(30) Colom-Cadena, M.; Grau-Rivera, O.; Planellas, L.; Cerquera, C.; Morenas, E.; Helgueta, S.; Muñoz, L.; Kulisevsky, J.; Martí, M. J.; Tolosa, E.; Clarimon, J.; Lleó, A.; Gelpi, E. Regional Overlap of Pathologies in Lewy Body Disorders. J. Neuropathol. Exp. Neurol. 2017, 76, 216–224.

(30) Colom-Cadena, M.; Grau-Rivera, O.; Planellas, L.; Cerquera, C.; Morenas, E.; Helgueta, S.; Muñoz, L.; Kulisevsky, J.; Martí, M. J.; Tolosa, E.; Clarimon, J.; Lleó, A.; Gelpi, E. Regional Overlap of Pathologies in Lewy Body Disorders. J. Neuropathol. Exp. Neurol. 2017, 76, 216–224.

(31) Tosto, G.; Reitz, C. Genome-Wide Association Studies in Alzheimer’s Disease: A Review. Curr. Neurol. Neurosci. Rep. 2013, 13, No. 381.

(32) Liu, C.-C.; Kanekiyto, T.; Xu, H.; Bu, G. Apolipoprotein E and Alzheimer Disease: Risk, Mechanisms and Therapy. Nat. Rev. Neurol. 2013, 9, 106–118.

(33) Goedert, M. Alzheimer’s and Parkinson’s Diseases: The Prion Concept in Relation to Assembled A, Tau, and -Synuclein. Science 2015, 349, No. 1255555.

(34) Giasson, B. I. Initiation and Synergistic Fibrillization of Tau and Alpha-Synuclein. Science 2003, 300, 636–640.

(35) Badiola, N.; de Oliveira, R. M.; Herrera, F.; Guardia-Laguarta, C.; Gonçalves, S. A.; Pera, M.; Suárez-Calvet, M.; Clarimon, J.; Outeiro, T. F.; Lleó, A. Tau Enhances -Synuclein Aggregation and Toxicity in Cellular Models of Synucleinopathy. PLoS One 2011, 6, No. e26609.

(36) Uversky, V. N. Neuropathology, Biochemistry, and Biophysics of -Synuclein Aggregation. J. Neurochem. 2007, 103, 17–37.

(37) Ciaccioli, G.; Martins, A.; Rodrigues, C.; Vieira, H.; Calado, P. A Powerful Yeast Model to Investigate the Synergistic Interaction of -Synuclein and Tau in Neurodegeneration. PLoS One 2013, 8, No. e55848.

(38) Marsh, S. E.; Blurtion-Jones, M. Examining the Mechanisms That Link -Amyloid and -Synuclein Pathologies. Alzheimers. Res. Ther. 2012, 4, 11.

(39) Hashimoto, M.; Misliiah, E. Alpha-Synuclein in Lewy Body Disease and Alzheimer’s Disease. Brain Pathol. 1999, 9, 707–720.

(40) Liani, E.; Eyal, A.; Avraham, E.; Shemer, R.; Szargel, R.; Berg, D.; Bornemann, A.; Riess, O.; Ross, C. A.; Rott, R.; Engelender, S. Ubiquitylation of Synphilin-1 and -Synuclein by SIAH and Its Presence in Cellular Inclusions and Lewy Bodies Imply a Role in Parkinson’s Disease. Proc. Natl. Acad. Sci. U.S.A. 2004, 101, 5500–5505.

(41) Rott, R.; Szargel, R.; Shani, V.; Bisharat, S.; Engelender, S. -Synuclein Ubiquitination and Novel Therapeutic Targets for Parkinson’s Disease. CNS Neurol. Disord.: Drug Targets 2014, 13, 630–637.

(42) Forrester, M. T.; Benhar, M.; Stamler, J. S. Nitrosative Stress in the ER: A New Role for S-Nitrosylation in Neurodegenerative Diseases. ACS Chem. Biol. 2006, 1, 355–358.

(43) Nakamura, T.; Lipton, S. A. Emerging Role of Protein-Protein Transnitrosylation in Cell Signaling Pathways. Antioxid. Redox Signaling 2013, 18, 239–249.

(44) Nakamura, T.; Prakhodko, O. A.; Pirie, E.; Nagar, S.; Akhtar, M. W.; Oh, C.-K.; McKercher, S. R.; Ambasudhan, R.; Okamoto, S.; Lipton, S. A. aberrant Protein S-Nitrosylation contributes to the Pathophysiology of Neurodegenerative Diseases. Neurobiol. Dis. 2015, 84, 99–108.

(45) Conway, M. E.; Harris, M. S-Nitrosylation of the Thioexdoxin-like Domains of Protein Disulfide Isomerase and Its Role in Neurodegenerative Conditions. Front. Chem. 2015, 3, No. 27.