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**Appendix to the Assembly Journal** Wisconsin,1910      *The Propagation of Neurodegenerative Diseases by Inflammation and Exosomes* Valerie Sackmann,2019-10-16 Alzheimer's disease AD and Parkinson's disease PD are the two most common neurodegenerative diseases with rates increasing along with the ageing global population Despite best efforts we still do not understand the etiopathogenesis of these diseases and there are no effective disease modifying treatments Cognitive deficiencies or motor complications that emerge during AD and PD are thought to be the result of the accumulation of misfolded aggregate prone proteins such as amyloid A and tau or synuclein syn respectively Growing evidence suggests that prefibrillar oligomers of A and syn oA and o syn are key contributors to the progression of these diseases The progressive accumulation of these proteins leads to a gradual spread of pathology throughout interconnected brain regions but the mechanisms by which this spreading occurs are still largely unknown Neuroinflammation has been recognised as an important contributor to neurodegenerative disease It is hypothesised that a pro inflammatory environment initiated by the innate immune system either through activation from A itself or indirectly through neuronal injury signals in AD These phenomena are thought to either cause or accelerate AD such that an anti inflammatory approach may be neuroprotective In paper I we investigated whether different inflammatory environments affected the transfer of oA between neuron like cells in addition to investigating inter and intracellular protein changes This study demonstrated that an anti inflammatory environment reduces the transfer of oA between cells We also provide evidence that these cells begin to take on the phenotype of the inflammatory milieu while also demonstrating that the expression profile of endosomal lysosomal and protein trafficking proteins is altered during these conditions Small extracellular vesicles called exosomes which are key players in cell to cell communication have been proposed to play an influential role in spreading neurodegenerative proteins between cells Exosomes are small membranous vesicles that are formed by the inward budding of multivesicular bodies MVBs These MVBs can then merge with the plasma membrane to be released into the extracellular environment as vesicles which serve as vehicles for transferring proteins lipids and mRNAs between cells The ESCRT dependent pathway is the most understood mechanism underlying exosome biogenesis However exosomes can also be formed through ESCRT independent

pathways including through the hydrolysis of sphingomyelin by neutral sphingomyelinase 2 nSMase2 which produces ceramide Paper II investigated whether exosomes formed through an ESCRT independent pathway plays a significant role in the transfer of *o* syn between neuron like cells As oxidative stress is a common feature in PD brains which in turn dysregulates nSMase2 activity we also tested our model under hypoxic conditions Inhibition of nSMase2 significantly reduced the transfer of *o* syn between cells but also resulted in decreased syn aggregation Hypoxia did not influence *o* syn transfer however it significantly dysregulated the sphingolipid composition which may be important for syn binding to exosomes and exosome communication During AD and PD there is a noted reduction in the effectiveness of autophagy a process critical to cellular proteostasis Recent studies have uncovered shared regulatory mechanisms of exosome biogenesis and autophagy suggesting that they are closely linked Previous findings have shown that inhibition of autophagy in AD mice mediates A trafficking through altering the secretion of A in MVBs To further study this effect we investigated the interplay between autophagy and exosome secretion using ATG7 knock out x APPNL F knock in AD mice in paper III These autophagy deficient AD mice had a reduced extracellular A plaque load but increased intracellular A which was found to be assembled into higher ordered assemblies While exosomal secretion was dysregulated in these mice the amount of A packaged into the exosomes was unchanged Lastly one of the biggest challenges in developing effective treatments for AD is the lack of early diagnosis of living patients As the connection between exosomes and the spread of neurodegenerative proteins is still relatively new there remains a diagnostic potential to be explored with exosomes Paper IV aimed to develop a new diagnostic assay to detect *o*A in exosomes isolated from human cerebrospinal fluid Although exosomal *o*A was readily detected in some of these samples the assay's sensitivity requires additional optimisation before it can be further validated for the clinic In summary the studies presented in this thesis have furthered our understanding of how inflammation autophagy and exosomes contribute to the intercellular transmission of AD and PD associated proteins We have shown that an anti inflammatory approach may slow down the progression of AD through reducing the transfer of *o*A between cells We also provide novel findings relating to the biogenesis of exosomes which in turn affected the ability of exosomes to transmit neurodegenerative proteins between cells and their association with autophagic processes Finally we have investigated the feasibility of exosomes as an early AD diagnostic marker This work has helped to elucidate some of the mechanisms underlying the progression of neurodegenerative diseases which may be useful targets for the investigation of new therapeutic avenues

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## **Table of Contents X App This Month**

1. Understanding the eBook X App This Month
  - The Rise of Digital Reading X App This Month
  - Advantages of eBooks Over Traditional Books
2. Identifying X App This Month
  - Exploring Different Genres
  - Considering Fiction vs. Non-Fiction
  - Determining Your Reading Goals
3. Choosing the Right eBook Platform
  - Popular eBook Platforms
  - Features to Look for in an X App This Month
  - User-Friendly Interface
4. Exploring eBook Recommendations from X App This Month
  - Personalized Recommendations
  - X App This Month User Reviews and Ratings
  - X App This Month and Bestseller Lists
5. Accessing X App This Month Free and Paid eBooks

- X App This Month Public Domain eBooks
- X App This Month eBook Subscription Services
- X App This Month Budget-Friendly Options

6. Navigating X App This Month eBook Formats

- ePUB, PDF, MOBI, and More
- X App This Month Compatibility with Devices
- X App This Month Enhanced eBook Features

7. Enhancing Your Reading Experience

- Adjustable Fonts and Text Sizes of X App This Month
- Highlighting and Note-Taking X App This Month
- Interactive Elements X App This Month

8. Staying Engaged with X App This Month

- Joining Online Reading Communities
- Participating in Virtual Book Clubs
- Following Authors and Publishers X App This Month

9. Balancing eBooks and Physical Books X App This Month

- Benefits of a Digital Library
- Creating a Diverse Reading Collection X App This Month

10. Overcoming Reading Challenges

- Dealing with Digital Eye Strain
- Minimizing Distractions
- Managing Screen Time

11. Cultivating a Reading Routine X App This Month

- Setting Reading Goals X App This Month
- Carving Out Dedicated Reading Time

12. Sourcing Reliable Information of X App This Month

- Fact-Checking eBook Content of X App This Month
- Distinguishing Credible Sources

13. Promoting Lifelong Learning

- Utilizing eBooks for Skill Development

---

- Exploring Educational eBooks
- 14. Embracing eBook Trends
  - Integration of Multimedia Elements
  - Interactive and Gamified eBooks

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