Project Notes:

Project Title: **Developing a Predictive Model to Predict the Progression of Alzheimer's Disease via a Neural Apoptotic Marker**

Name: **Rishab Nair**

Note Well: There are NO SHORT-cuts to reading journal articles and taking notes from them. Comprehension is paramount. You will most likely need to read it several times, so set aside enough time in your schedule.

Contents:

Knowledge Gaps:

This list provides a brief overview of the major knowledge gaps for this project, how they were resolved and where to find the information.

Knowledge Gap	Resolved By	Information is located	Date resolved
Potential markers of frayed neuron endings			
Proof of Concept: Biological AI Predictive Model			

Literature Search Parameters:

These searches were performed between 08/19/23 and XX/XX/2024.
List of keywords and databases used during this project.

Database/search engine	Keywords	Summary of search
Google	Alzheimer's Disease	Decently successful, majority of articles are found within the National Library of Medicine
National Library of Medicine	Nerve Endings	Found an article regarding injury of the brain and its "healing"

Tags:

Tag Name	
#introduction	#neurodegenerative
#dementia	#tauopathies
#aggregation-of-toxic-proteins	#synucleinopathies
#polyglutamine	#classification-of-neurodegenerative-diseases
#treatment	#overview
#key-information	#experiment
#background-info	#protein-correlation
#basic-schematics-of-the-brain	#potential-marker
#cell-death	#caspase-function
#labwork	#processes
#neurons	#main
#cs	#AI-learning

#predictive-AI	#competitor

Article #1 Notes: Toxic Proteins in Neurodegenerative Disease

Source Title	Toxic Proteins in Neurodegenerative Disease
Source citation (APA Format)	Taylor, J. P., Hardy, J., & Fischbeck, K. H. (2002). Toxic proteins in neurodegenerative disease. *Science (New York, N.Y.), 296*(5575), 1991–1995. https://doi.org/10.1126/science.1067122
Original URL	https://pubmed.ncbi.nlm.nih.gov/12065827/
Source type	Science Journal
Keywords	Neurodegenerative diseases, proteins, plaques, tangles, inclusions
#Tags	#neurodegenerative #dementia #aggregation-of-toxic-proteins
Summary of key points + notes (include methodology)	Many neurodegenerative disorders, including Alzheimer's, Parkinson's, and Huntington's, have shown one major similarity– an aggregation and accumulation of abnormal proteins. These malformations are caused by a mistake in protein folding, and these defective proteins cause harm when bypassing the body's defenses and aggregating. These may then cause plaques, tangles, inclusions(which are caused by the body's response to an excess of toxic proteins), and other bodies of malformed proteins which would affect the brain, causing these diseases.
Research Question/Problem/ Need	How do proteins play a role in neurodegenerative diseases?

Important Figures

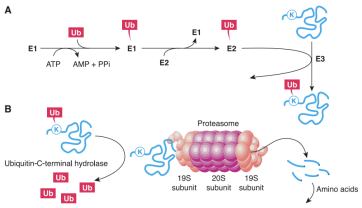


Fig. 1. The ubiquitin-proteasome system. (A) Proteins targeted for degradation are identified by covalent linkage to ubiquitin. Selective ubiquitination is accomplished by a series of enzymes (E1, E2, and E3) that constitute the ubiquitin ligase system. (B) Ubiquitinated substrates are recognized, unfolded, and degraded in an energy-dependent manner by the proteasome.

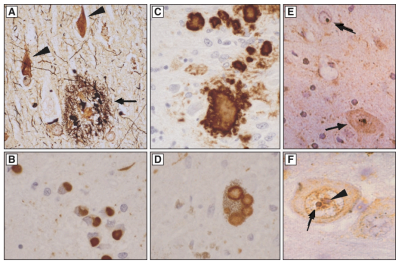


Fig. 2. Aggregation of misfolded proteins in microscopically visible inclusions or plaques in various neurodegenerative diseases. (A) Alzheimer's disease. Arrowhead, intracellular neurofibrillary tangles; arrow, extracellular amyloid plaque. (B) Fibrillar tau inclusions in Pick's disease. (C) PrP^{Sc} amyloid deposition in prion disease. (D) Multiple Lewy bodies in a nigral neuron in Parkinson's disease. (E) Neuronal intranuclear inclusions of mutant ataxin-3 in Machado-Joseph's disease. (F) Higher power micrograph of nuclear inclusion of mutant ataxin-3, demonstrating that it is distinct from the nucleolus. Magnification, ×40. [(A) to (D) courtesy of D. Dickson; (E) and (F) courtesy of H. Paulson.]

Table 1. Features of neurodegenerative disorders characterized by aggregation and deposition of abnormal protein.

Disease	Protein deposits	Toxic protein	Disease genes	Risk factor
Alzheimer's disease	Extracellular plaques	Aβ	APP* Presenilin 1† Presenilin 2†	apoE4 allele
	Intracellular tangles	tau		
Parkinson's disease	Lewy bodies	α-Synuclein	α-Synuclein* Parkin† UCHL1†	tau linkage
Prion disease	Prion plaque	PrP^{Sc}	PRNP*	Homozygosity at prion codon 129
Polyglutamine disease	Nuclear and cytoplasmic inclusions	Polyglutamine-containing proteins	9 different genes with CAG repeat expansion*	
Tauopathy	Cytoplasmic tangles	tau	tau*	tau linkage
Familial amyotrophic lateral sclerosis	Bunina bodies	SOD1	SOD1*	

*Pathogenic mutations are associated with a toxic gain of function. †Pathogenic mutations are associated with a loss of function.

VOCAB: (w/definition)

Aggregation: the formation of a number of things into a cluster.
Inclusion: a person or thing that is included within a larger group or structure

Cited references to follow up on

Aguzzi, A. and Raeber, A.J. (1998), Neurodegeneration: Of (transgenic) Mice and Men. Brain Pathology, 8: 695-697. https://doi.org/10.1111/j.1750-3639.1998.tb00195.x

Refolo, L.M., Eckman, C., Prada, C.-M., Yager, D., Sambamurti, K., Mehta, N., Hardy, J. and Younkin, S.G. (1999), Antisense-Induced Reduction of Presenilin 1 Expression Selectively Increases the Production of Amyloid β42 in Transfected Cells. Journal of Neurochemistry, 73: 2383-2388. https://doi.org/10.1046/j.1471-4159.1999.0732383.x

Follow up Questions

- What may be some factors which may heighten or lower one's risk of forming these toxic proteins?
- Would there be a way to "unfold and refold" these proteins to limit the damage done by them?
- As many of the failed protein foldings are caused by genetic factors, are there any preventative measures one with the faulty gene could take to limit toxic proteins?

Article #2 Notes: Genetic Classification of Primary Neurodegenerative Disease

Source Title	Genetic Classification of Primary Neurodegenerative Disease
Source citation (APA Format)	Hardy, J. (1998). Genetic Classification of Primary Neurodegenerative Disease. *Science*, *282*(5391), 1075–1079. https://doi.org/10.1126/science.282.5391.1075
Original URL	https://www.science.org/doi/pdf/10.1126/science.282.5391.1075?casa_token=zuruNOqak1UAAAAA:lGmtBOJsTkDM4A5K06ndl3pGjgJ6Fg5EW6mZNU59WrGw3MGD8GU-4jX2X9-Y1BVb7LMJFCU_xkQxTA
Source type	Science Journal
Keywords	Phenotypic classification, progress of research, Tauopathies and Synucleinopathies, Polyglutamine diseases
#Tags	#tauopathies #synucleinopathies #polyglutamine #classification-of-neurodegenerative-diseases
Summary of key points + notes (include methodology)	The first “golden age” in neurodegenerative disease research focused on the outcome and therefore classified various diseases together as such. However, as research progressed, a newer, refined understanding regarding the cause of these diseases was obtained, which in turn caused confusion when using the phenotypic classifications for these diseases. A newer classification was then put into place; the two main groups consisted of those that were polyglutamine diseases and those that were tauopathies and synucleinopathies.
Research Question/Problem/ Need	As research has progressed, a greater understanding of the cause of neurodegenerative diseases has been obtained. With this understanding, what is the most beneficial classification for these diseases?

Important Figures

Disease	Linkage	Gene	Mutations	Pathology	Transgenic (comment)	Ref.
Prion	Ch20	Prion	Mainly missense	PrP plaques, sometimes T or LB; classically associated with spongiform changes	+ (no T or LB)	(36)
AD	Ch21	APP	Missense around Aβ, increase Aβ42	Amyloid plaques and T, may see LB	+ (no T or LB)	(37)
	Ch14	PS1	Mainly missense, increase Aβ42	Amyloid plaques and T	+ (no plaques T or LB)	(38)
	Ch1	PS2	Missense, increase Aβ42	Amyloid plaques and T	+ (no plaques T or LB)	(39)
PD	Ch4q	α-synuclein	Missense	LB	Not reported	(33)
	Ch2	Not identified	Not known	LB (and T?)	Not reported	(40)
	Ch4p	Not identified	Not known	LB	Not reported	(41)
FTD	Ch17	Tau	Missense and splice	T, sometimes with "unusual periodicity"	Not reported	(15)
	Ch3	Not identified	Not known	Not reported	Not reported	(42)
ALS	Ch21	SOD	Mainly missense	Lewy-like bodies	+ (motor neuron disease, inclusions, cell loss)	(43)
SBMA*	X	AR	Polyglutamine	Nuclear inclusions	+ (no phenotype)	(44)
HD	Ch4	Huntingtin	Polyglutamine	Nuclear inclusions	+ (inclusions, movement disorder, cell loss)	(45)
DRPLA	Ch12	Atrophin 1	Polyglutamine	Nuclear inclusions	Not reported	(46)
SCA1	Ch6	Ataxin 1	Polyglutamine	Nuclear inclusions	+ (ataxic, inclusions, cell loss)	(47)
SCA2	Ch12	Ataxin 2	Polyglutamine	Not reported	Not reported	(48)
SCA3/MJD	Ch14	Ataxin 3	Polyglutamine	Nuclear inclusions	+ (ataxic, cerebellar atrophy)	(49)
SCA4	Ch16	Not identified	Not known	Not reported	Not reported	(50)
SCA5	Ch11	Not identified	Not known	Not reported	Not reported	(51)
SCA6	Ch19	CACNL1A4	Polyglutamine	Not reported	Not reported	(52)
SCA7	Ch3	SCA7	Polyglutamine	Nuclear inclusions	Not reported	(53)

*SBMA is technically not autosomal dominant but it is probably dominant in its cellular mode of action.

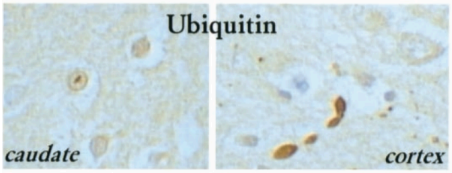


Fig. 1. Ubiquitin immunostaining of Huntington's disease neurons reveals intranuclear inclusion bodies in select neurons in the caudate nucleus and dystrophic neuronal processes in the neocortex. These lesions have been shown to contain huntingtin.

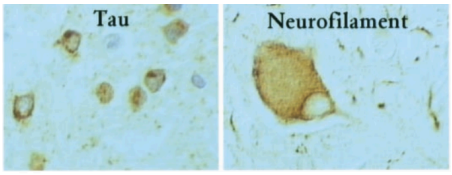


Fig. 2. FTDP-17 has characteristic neuronal (and glial) tau immunoreactivity, such as perinuclear filamentous aggregates in small nonpyramidal neurons in layer II of the cortex. Ballooned neurons, which are best demonstrated with antibodies to phosphorylated neurofilament epitopes, are commonly found in lower layers of cortex in FTDP-17.

VOCAB: (w/definition)

Tauopathies: neurodegenerative disorders characterized by the deposition of abnormal tau protein in the brain
Synucleinopathies: a group of neurodegenerative disorders characterized by neuronal or glial inclusions, or both, composed of aggregated α-synuclein
Polyglutamine: group of neurodegenerative disorders caused by expanded cytosine-adenine-guanine (CAG) repeats encoding a long polyQ tract in the respective proteins.

Cited references to follow up on

https://www.nejm.org/doi/full/10.1056/NEJM199603213341202
https://www.nature.com/articles/31508

Follow up Questions

- Do medications that work on one neurodegenerative disease in a group also work on its groupmates?
- As research progresses even further, would it be possible that this classification could be altered yet again?
- Now that research has looked at phenotypic properties as well as proteins, what would be the next direction for research regarding these diseases?

Article #3 Notes: Comprehensive Review on Alzheimer's Disease: Causes and Treatment

Source Title	Comprehensive Review on Alzheimer's Disease: Causes and Treatment
Source citation (APA Format)	Breijyeh, Z., & Karaman, R. (2020). Comprehensive Review on Alzheimer's Disease: Causes and Treatment. *Molecules (Basel, Switzerland)*, *25*(24), 5789. https://doi.org/10.3390/molecules25245789
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7764106/
Source type	Journal Article
Keywords	Alzheimer's, Cures, Treatment, Background, Effects, Comprehensive, Explanation, neurodegeneration; β-amyloid peptide; tau protein; risk factors; disease-modifying therapy; chaperons; heat shock proteins
#Tags	#introduction #treatment #overview #key-information
Summary of key points + notes (include methodology)	This article serves as a comprehensive overview of dementia with all of its known information regarding causes and treatment so far. Proteins such as tau and beta-amyloid mainly serve as the "perpetrator" for this disease. The article continues to talk about various genetic factors which may heighten one's chance of being victim to this disease. The figures below provide a glimpse into the main points of the text. MISCELLANEOUS NOTES I FIND IMPORTANT: - "Synaptic proteins serve as biomarkers for the detection of synapses loss" - Alzheimer's has been classified as a "World Health Concern" - Throughout this article, proteins have been a main focus. This coaxes me to believe that the marker I'm looking for is most likely a protein or protein-related compound. The following notes are done with the aid of Google Bard, an AI service: What is Alzheimer's disease? - Alzheimer's disease is a progressive neurodegenerative disorder that causes the loss of neurons and their connections in the brain. It is the most common cause of dementia in older adults.

Causes of Alzheimer's disease

The exact cause of Alzheimer's disease is unknown, but it is thought to be caused by a combination of genetic and environmental factors. Some of the risk factors for Alzheimer's disease include:

- Age: The risk of Alzheimer's disease increases with age.
- Family history: People with a family history of Alzheimer's disease are at an increased risk of developing the disease.
- Head injury: A history of head injury may increase the risk of Alzheimer's disease.
- Down syndrome: People with Down syndrome are at an increased risk of developing Alzheimer's disease.
- Certain medical conditions: Certain medical conditions, such as diabetes, heart disease, and high blood pressure, may increase the risk of Alzheimer's disease.
- Lifestyle factors: Certain lifestyle factors, such as smoking and obesity, may increase the risk of Alzheimer's disease.

Symptoms of Alzheimer's disease

The early symptoms of Alzheimer's disease are often mild and may be mistaken for normal signs of aging. However, as the disease progresses, the symptoms become more severe and can interfere with daily life. Some of the common symptoms of Alzheimer's disease include:

- Memory loss, especially for recent events
- Difficulty with problem-solving and decision-making
- Confusion
- Disorientation
- Language problems, such as difficulty finding the right words or understanding what others are saying
- Changes in mood and behavior, such as becoming irritable, withdrawn, or aggressive
- Changes in sleep patterns

Neuropathology of Alzheimer’s Disease

- Senile plaques:
    - extracellular deposits of beta-amyloid protein (Aβ) with different morphological forms, including neuritic, diffuse, dense-cored, or classic and compact type plaques
- Neurofibrilary Tangles:
    - abnormal filaments of the hyperphosphorylated tau protein that in some stages can be twisted around each other to form paired helical filament (PHF) and accumulate in neuralperikaryal cytoplasm, axons, and dendrites, which cause a loss of cytoskeletal microtubules and tubulin-associated proteins
- Synaptic Loss
    - causes memory impairment and generally is observed at the early stages of AD

Stages of Alzheimer's disease

	Alzheimer's disease is typically divided into three stages: mild, moderate, and severe. - Mild Alzheimer's disease: People with mild Alzheimer's disease may experience mild memory loss and difficulty with complex tasks. They may also be withdrawn from social activities. However, they are still able to live independently and perform most daily activities. - Moderate Alzheimer's disease: People with moderate Alzheimer's disease have more severe memory loss and difficulty with daily activities. They may also experience changes in mood and behavior. They may need assistance with some daily activities, such as cooking, cleaning, and managing their finances. - Severe Alzheimer's disease: People with severe Alzheimer's disease are completely dependent on others for care. They may have lost the ability to speak, walk, and recognize loved ones. Treatment for Alzheimer's disease There is currently no cure for Alzheimer's disease, but there are treatments that can help with the symptoms. Some of the medications used to treat Alzheimer's disease include: - Cholinesterase inhibitors: These medications help to improve memory and cognitive function by increasing the levels of acetylcholine in the brain. - NMDA receptor antagonists: These medications help to improve memory and cognitive function by blocking the NMDA receptor, which is involved in memory formation. In addition to medication, there are a number of other things that can help people with Alzheimer's disease, including: - Cognitive stimulation: Cognitive stimulation activities, such as puzzles, games, and crafts, can help to improve memory and cognitive function. - Physical activity: Physical activity is important for everyone, but it is especially important for people with Alzheimer's disease. Physical activity can help to improve mood, sleep, and overall cognitive function. - Social interaction: Social interaction is important for everyone, but it is especially important for people with Alzheimer's disease. Social interaction can help to improve mood and cognitive function. Conclusion Alzheimer's disease is a serious and progressive neurodegenerative disorder. There is currently no cure, but there are treatments and other interventions that can help people with the disease live longer and better lives. Ongoing research is being conducted to develop new and more effective treatments for Alzheimer's disease.
Research Question/Problem/ Need	What exactly is Alzheimer’s Disease, and what are its causes and treatments?

Important Figures

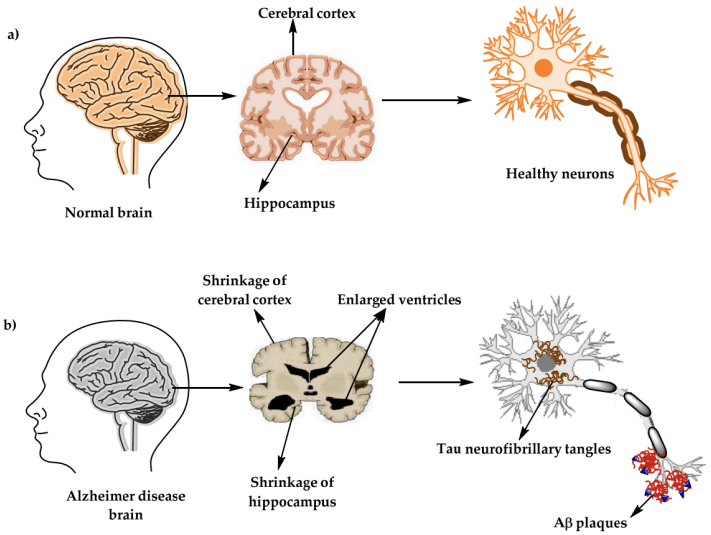


Figure 1. The physiological structure of the brain and neurons in (**a**) healthy brain and (**b**) Alzheimer's disease (AD) brain.

- This is a comparison of a healthy brain to an Alzheimer's patient brain. One can visually understand the physical effects of the disease on the brain through this figure.

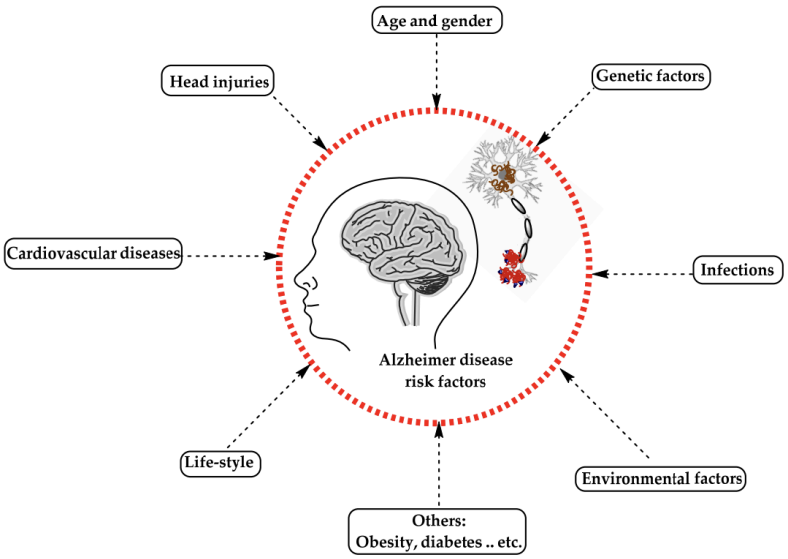


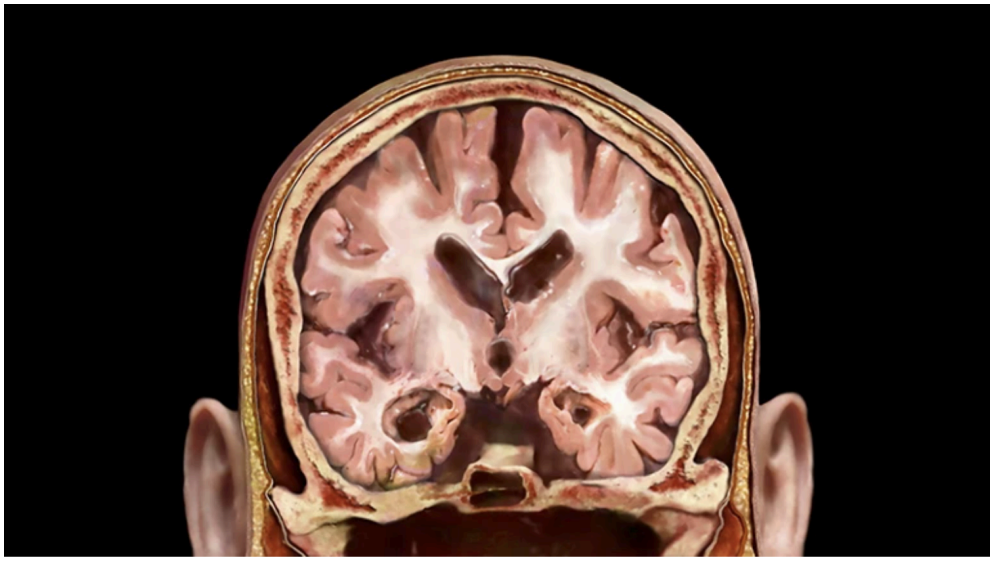
Figure 2. The risk factors for Alzheimer's disease.

- This is a general overview of the risk factors associated with Alzheimer's Disease
    - Cardiovascular Diseases
    - Head Injuries
    - Age and gender
    - Genetic factors
    - Infections

	- Environmental factors - Lifestyle - Other miscellaneous factors: obesity, diabetes, etc.
VOCAB: (w/definition)	Multifactorial: involving or dependent on a number of factors or causes. Hyperphosphorylated: occurs when a biochemical with multiple phosphorylation sites is fully saturated Phosphorylation: the addition of a phosphoryl (PO3) group to a molecule. Antagonists (biochem): a substance that interferes with or inhibits the physiological action of another. Cholinesterase- an enzyme that helps your nervous system work the way it should. It itmmediately breaks down or hydrolyzes acetylcholine (ACh), a naturally occurring neurotransmitter, into acetic acid and choline.
Cited references to follow up on	- Spires-Jones T.L., Hyman B.T. The intersection of amyloid beta and tau at synapses in Alzheimer's disease. Neuron. 2014;82:756–771. doi: 10.1016/j.neuron.2014.05.004
Follow up Questions	- Is there a scan which detects synaptic proteins? If so, how readily available are they? - What exactly defines a "World Health Concern"? - Is there a way to optimize cognitive stimulation? Would this increase the accumulation of the marker I am looking for? - Do plaques accumulate near frayed nerve endings, as they "eat away" at the pathways? Would this be a valid marker to identify?

Article #4 Notes(summer): Dementia Risk Linked to Blood-Protein Imbalance in Middle Age.

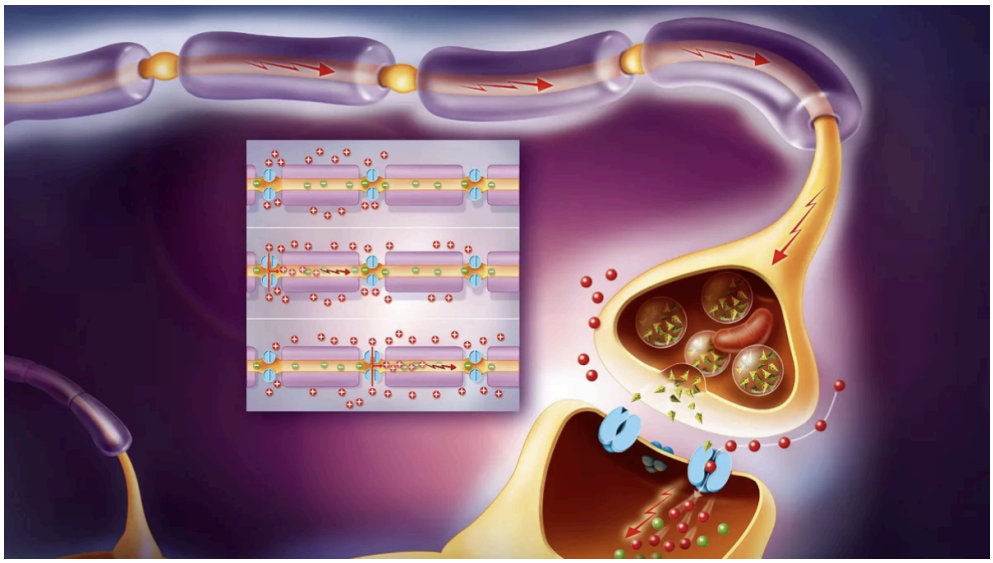
Source Title	Dementia Risk Linked to Blood-Protein Imbalance in Middle Age.
Source citation (APA Format)	Tozer, L. (2023) Dementia Risk Linked to Blood-Protein Imbalance in Middle Age. *Nature*, vol. d41586-023-02374-2, 21 July 2023, www.nature.com/articles/d41586-023-02374-2, https://doi.org/10.1038/d41586-023-02374-2.

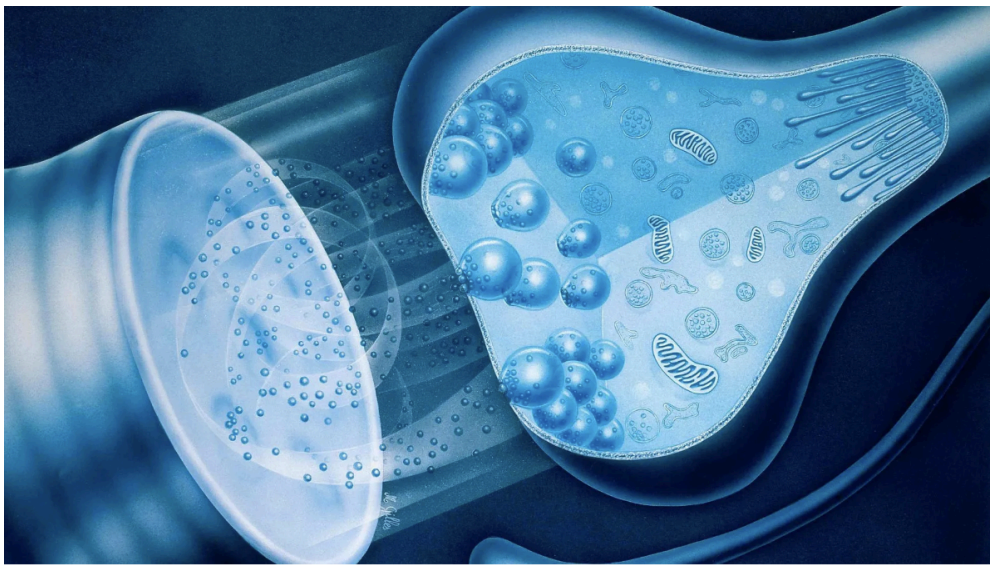
Original URL	https://www.nature.com/articles/d41586-023-02374-2
Source type	Journal article
Keywords	Medical research, Proteomics, Alzheimer's Disease, Brain
#Tags	#experiment #background-info #protein-correlation
Summary of key points + notes (include methodology)	This article was a review on an experiment conducted over the course of 25 years, beginning in 1987. In this experiment, thousands of people's protein levels were compared to their chance of suffering from dementia- which one in five people developed throughout its course. An interesting correlation was noticed here; there were 32 proteins, which if kept dysregulated especially within ages 45 to 60, would leave people with a higher risk of developing dementia. Even more interesting, however, was the fact that some of these proteins weren't even found in the brain: "one of the proteins found with the strongest association with dementia risk — called GDF15 — was not detected in the brain." Using this information, doctors would be able to better understand how dementia, or more specifically, Parkinson's for example, is caused. This could further boost research regarding a potential cure. This could be interesting to take a look at for my project, as this could be a good starting point to work on furthering a cure.
Research Question/Problem/ Need	Do protein levels have a correlation to the risk of suffering from dementia?
Important Figures	 - This was the only visual element the article provided. The caption states: "A slice through the brain of a person with Alzheimer's disease, the most common cause of dementia."

VOCAB: (w/definition)	Onset- the beginning of something, especially something unpleasant Dysregulation- abnormality or impairment in the regulation of a metabolic, physiological, or psychological process Proteome- the entire complement of proteins that is or can be expressed by a cell, tissue, or organism Proteostasis- the dynamic regulation of a balanced functional proteome
Cited references to follow up on	- https://www.science.org/doi/10.1126/scitranslmed.adf5681 - https://alzres.biomedcentral.com/articles/10.1186/s13195-022-01072-x
Follow up Questions	- Were there any prerequistes in order to take part in this study? - Nicholas Seyfried stated that "Mechanisms below the neck could also play a role" in the onset of dementia. What exactly would these mechanisms be?

Article #5 Notes(summer): How do Brain Cells Send Messages?

Source Title	How do Brain Cells Send Messages?
Source citation (APA Format)	Barker, H. (2023, July 25). *How do brain cells send messages?* Livescience.com. https://www.livescience.com/health/neuroscience/how-do-brain-cells-send-messages
Original URL	https://www.livescience.com/health/neuroscience/how-do-brain-cells-send-messages
Source type	Journal Article

Keywords	Neurons, pathways, messages, transmission, action potential
#Tags	#background-info, #basic-schematics-of-the-brain
Summary of key points + notes (include methodology)	This article was about neurons- specifically, how they are used to send messages to the brain. To begin the process of sending these messages, a wave of electricity known as the action potential is triggered in the neuron. This happens when the sense organs activate neurons leading to the brain, opening little tunnels in neuron membranes. Ions then leak into the neuron. The ions travel from the membrane to the axon- the tail-like structure of the neuron. The axons of two neurons separate from each other with a gap known as a synapse. As the electrical impulse gets to the end of an axon, chemicals called neurotransmitters are put into the synapse, which then stick to receptors on other neurons. When enough receptors are switched on, the message consequently gets passed. However, since the connection of neurons is serial in this method, the messages transferred are known as "secret messages". Neurons can also send a mass message. This is done by releasing small protein fragments known as neuropeptides through cell membranes, which float around the entire brain. This connects to my topic as it is quite literally the definition of the function of the brain. I now have a better understanding of the basics, which is important if I want to create a complex project. Every building, no matter how tall, needs a foundation.
Research Question/Problem/ Need	This would also be the title: How do brain cells send messages?
Important Figures	 - This shows an action potential moving down the length of an insulated axon (red arrows). This triggers the release of neurotransmitters from the end of that axon, and activates the receptors of an adjacent neuron

	 - Caption: The synapse (illustrated) is the gap between the outgoing wire, or axon, of one nerve cell and the receiving wire, or dendrite, of the next. Nerve cells send chemical messages across these gaps.
VOCAB: (w/definition)	Action potential- a rapid sequence of changes in the voltage across a membrane Neuropeptides- small proteins produced by neurons that act on G protein-coupled receptors and are responsible for slow-onset, long-lasting modulation of synaptic transmission. Neurotransmitters- chemical messengers that your body can't function without. Their job is to carry chemical signals (“messages”) from one neuron (nerve cell) to the next target cell. The next target cell can be another nerve cell, a muscle cell or a gland. Dendrite- a short branched extension of a nerve cell, along which impulses received from other cells at synapses are transmitted to the cell body.
Cited references to follow up on	None listed
Follow up Questions	(Although at this point I probably know the answers to the following questions, this is what I presume I was wondering at the time) - What in dementia patients inhibits the ability for these electric signals to travel? - Would external shocks influence messages in the brain? - Would it be possible to aid the transmission of electric signals in dementia patients’ brains? - How exactly would memories be stored in this system?

Article #6 Notes: Caspase Functions in Cell Death and Disease

Source Title	Caspase Functions in Cell Death and Disease
Source citation (APA Format)	McIlwain, D. R., Berger, T., & Mak, T. W. (2013). Caspase functions in cell death and disease. *Cold Spring Harbor perspectives in biology*, *5*(4), a008656. https://doi.org/10.1101/cshperspect.a008656 - Correction: McIlwain, D. R., Berger, T., & Mak, T. W. (2015). Caspase functions in cell death and disease. *Cold Spring Harbor perspectives in biology,* 7(4), a026716. https://doi.org/10.1101/cshperspect.a026716
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3683896/ - Correction: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4382736/
Source type	Journal Article
Keywords	Caspase, cell death, disease, endoproteases, apoptosis
#Tags	#potential-marker #cell-death #caspase-function

Summary of key points + notes (include methodology)	Caspases, a type of endoproteases, play a major role in the process of cell death. There are three major types of caspases- Initiator, Executioner, and Inflammation. Each individual caspase plays a different role in the grand scheme of cell death, and have been found to hold connections to certain diseases.
	
	- Key regulators of apoptosis and inflammation
	- Insufficient caspase activation promotes tumorigenesis or infection
	- **Hyperactivation promotes neurodegeneration/inflammatory conditions**
	- Family of genes important for maintaining homeostasis through regulating cell death + inflammation
	  - Endoproteases that hydrolyze peptide bonds in reaction that depends on catalytic cysteine residues in caspase active site
	  - Occurs only after certain aspartic acid residues in substrate
	- Caspase-mediated processing can result in substrate inactivation
	  - may also generate active signaling molecules that participate in ordered processes such as apoptosis and inflammation

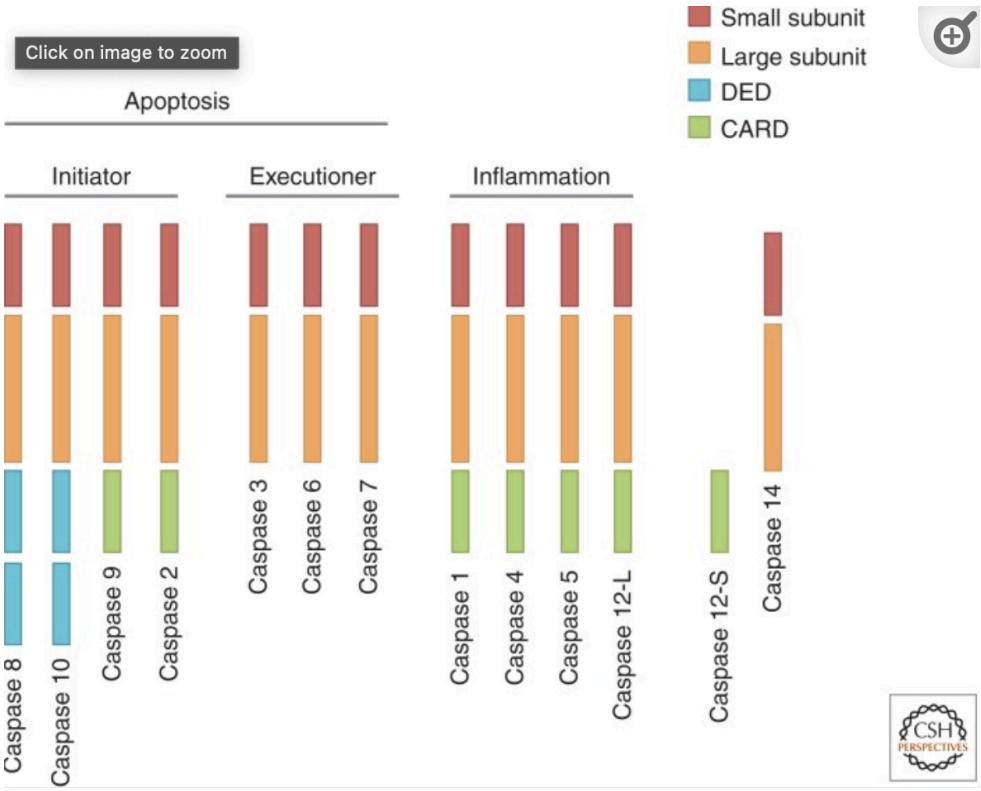
- Due to this, caspases have been broadly classified by known roles in apoptosis(3, 6, 7, 8, 9 in mammals) and inflammation(1, 4, 5, 12 in humans, 1, 11, 12 in mice)
    - 2, 10, and 14 are less easily categorized
    - Caspases involved in apoptosis aresubclassified by their mechanism of action
        - either initiator caspases (caspase-8 and -9) or executioner caspases (caspase-3, -6, and -7).

- Initially produced as inactive monomeric procaspases requiring dimerization + cleavage for activation
    - Assembly into dimers facilitated by various adapter proteins that bind to specific regions in prodomain of procaspase
        - Exact mechanism of assembly depends on specific adapter involved:
            - Different caspases have different protein-protein interaction domains in prodomains
                - Allows them to complex with different adapters
                    - Caspase 1, 2, 4, 5, 9 contain caspase recruitment domain (CARD)
                    - Caspase 8, 10 have death effector domain (DED)
- Apoptosis
    - Programmed cell death
        - Involves controlled dismantling of intracellular components while avoiding inflammation + damage to surrounding cells
            - Initiator caspases activate executioner caspases which subsequently coordinate activities to demolish key structural proteins, activate other enzymes
        - Morphological hallmarks include DNA fragmentation + membrane bleeding
    - Initiator caspases
        - inactive procaspase monomers that are activated by dimerization and not by cleavage
    - Executioner Caspases
        - Inappropriate activation prevented by production as inactive procaspase dimers
            - Must be cleaved by initiator caspases to activate
                - Once activated, a single executioner caspase can cleave and activate other executioner caspases
                    - Leads to feedback loop of caspase activation

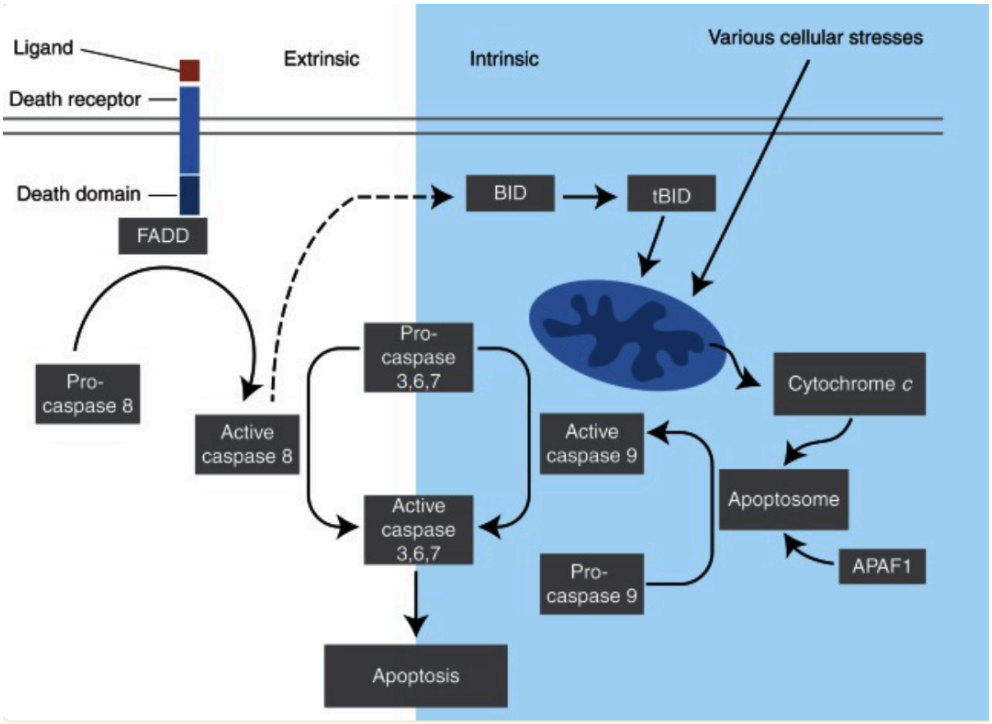
	- Pathways of Apoptosis
	  - [see Important Figures]
	 - Caspase-8 Dual-Role in Apoptosis and Necrosis
	  - tissue-specific deletions of caspase-8 have revealed new roles for this caspase, which appear to be unrelated to apoptosis.
	  - also critical for T-cell homeostasis
	- Inflammation
	 - Inflammatory Caspases
	  - Caspase-1, -4, -5, and -12 comprise the inflammatory subset in humans
	  - caspase-1, -11, and -12 serve the same function in mice
	  - he genes encoding inflammatory caspases are located in close proximity on human chromosome 11 and murine chromosome 9
	  - they may have arisen from gene duplication events
	  - At the protein level, inflammatory caspases, like their proapoptotic counterparts, are produced as inactive procaspases in resting cells
	  - Activates via cellular stimulation
	 - Inflammasome Formation
	  - Resembles apoptosome formation

Research Question/Problem/ Need	How do caspases play a role in cell death and disease?

Important Figures

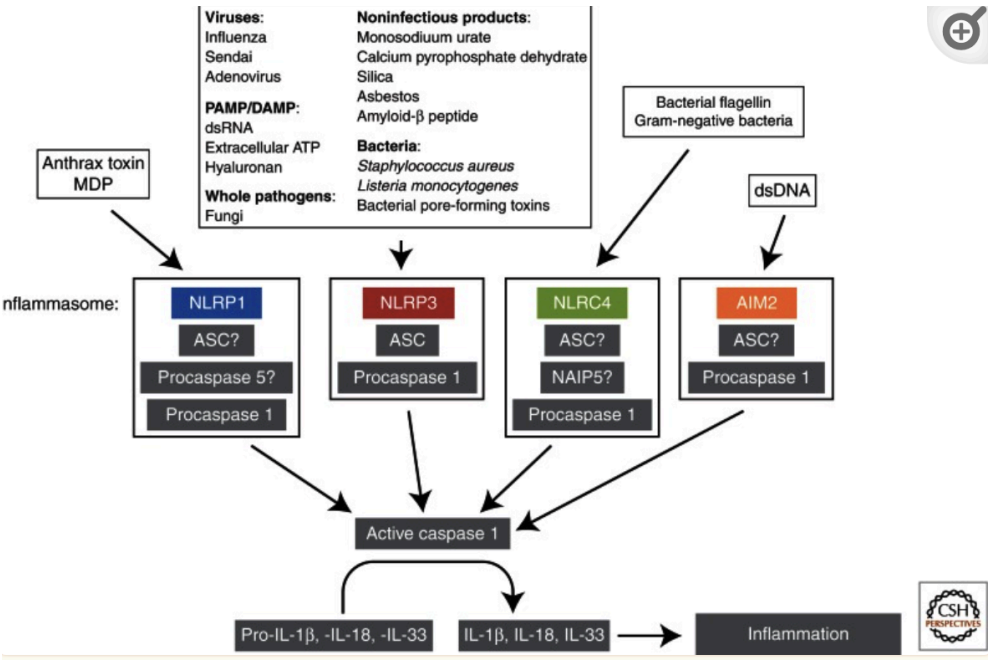


- This figure represents the domain structure of human caspases. The various colors represent a different part of the overall caspase unit.



- This figure shows the pathways of apoptosis.

- The extrinsic pathway is activated by the binding of a ligand to a death receptor.
    - This leads, alongside adapter proteins (FADD/TRADD) to recruitment, dimerization, and activation of caspase 8
        - Caspase 8 can either:
            - Initiate apoptosis directly by cleaving and consequently activating executioner caspase(see Figure 1)
            - activates the intrinsic apoptotic pathway through cleavage of BID to induce efficient cell death
- The intrinsic/mitochondrial apoptosis pathway is activated through various cellular stresses which lead to Cyt C release from mitochondria and formation of apoptosome
    - Apoptosome is comprised of the following:
        - APAF1
        - Cytochrome C
        - ATP
        - Caspase 9
    - Results in activation of Caspase 9
        - Initiates apoptosis by cleaving and consequently activating excecutioner caspases



- This figure shows the signaling+composition of inflammasomes
    - Activation of inflammatory caspases i.e. caspase 1 is achieved through inflammasome formation. Many cellular stimuli are recognized by family of pattern-recognition receptors
        - Engagement of these receptors lead to binding of adapter protein ASC, and recruitment and activation of inactive

	inflammatory procaspase, typically procaspase 1 - Procaspase 1 cleaves pro-IL-1β, pro-IL-18, and pro-IL-33, which facilitates the secretion of these proinflammatory cytokines leading to inflammation.
\]p[VOCAB: (w/definition)	Endoprotease- any of a group of enzymes that catalyse the splitting of polypeptide chains within a molecule - Catalyse- In chemistry, if something catalyses a reaction or event, it causes it to happen Zymogens- an inactive substance which is converted into an enzyme when activated by another enzyme. Ligand- an ion or molecule attached to a metal atom by coordinate bonding. - Coordinate bond- a covalent bond (a shared pair of electrons) in which both electrons come from the same atom. Also known as dative covalent bond Hypoxia- a state in which oxygen is not available in sufficient amounts at the tissue level to maintain adequate homeostasis FADD- Fas-associated death domain protein FADD/Mort1 is a signaling adaptor protein which mediates the activation of caspase 8 during death receptor-induced apoptosis. TRADD- TNFRSF1A Associated Via Death Domain is a protein coding gene. It is considered essential for TNF-like ligand 1A/death receptor 3 signaling BID- An abundant proapoptotic protein of the Bcl-2 family that is crucial for the induction of death receptor-mediated apoptosis in primary tissues such as liver. Inflammasomes- multiprotein complexes that are assembled by pattern-recognition receptors following the detection of pathogenic microorganisms and danger signals in the cytosol of host cells Procaspase- an inactive protease enzyme that is an inactive precursor of caspase Tumorigenesis- A pathologic process that involves the transformation of normal cells to a neoplastic state and resulting in polyclonal or monoclonal neoplastic cell proliferation - Neoplastic- relating to a neoplasm or neoplasia. - Neoplasm- a new and abnormal growth of tissue in some part of the body, especially as a characteristic of cancer. - Polyclonal- produced by, involving, or being cells derived from two or more cells of different ancestry or genetic constitution - Monoclonal- forming a clone that is derived asexually from a single individual or cell.

	- Proliferation- rapid increase in numbers Aspartic acid- a nonessential amino acid(in other words, made from other substances in body) which helps make other amino acids and some nucleotides, and plays a role in energy production in the body + sending chemical signals through nervous system Prodomain- areas in the protein that are essential for its correct folding, usually in the transition of a protein from an inactive to an active state.
Cited references to follow up on	- D'Amelio M, Cavallucci V, Middei S, Marchetti C, Pacioni S, Ferri A, Diamantini A, De Zio D, Carrara P, Battistini L, et al. 2011. Caspase-3 triggers early synaptic dysfunction in a mouse model of Alzheimer's disease. Nat Neurosci 14: 69–76 - Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES 2009. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature 458: 509–513
Follow up Questions	- Are there any brain tests which can scan for caspase activation? - Does neuronal damage from neurodegenerative diseases occur through apoptosis? - Which caspase activation would be closest to the real-time activation of apoptosis– as in what marks "complete death"? From what I've learned, it would have to be one of the executioner caspases - Which animal would be best to test on regarding caspases to reflect the results on humans? The article had mentioned that mice, for example, had different caspases for the same function as humans had.

Article #7 Notes: Peripheral Nerve Trauma: The intersection of amyloid beta and tau at synapses in Alzheimer's disease

Source Title	The intersection of amyloid beta and tau at synapses in Alzheimer's disease
Source citation (APA Format)	Spires-Jones, T. L., & Hyman, B. T. (2014). The intersection of amyloid beta and tau at synapses in Alzheimer's disease. Neuron, 82(4), 756–771. https://doi.org/10.1016/j.neuron.2014.05.004

Original URL	https://www.cell.com/neuron/fulltext/S0896-6273(14)00390-0?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0896627314003900%3Fshowall%3Dtrue
Source type	Journal Article
Keywords	Alzheimer's disease, Amyloid beta (Aβ), Tau, Synapses, Neurodegeneration, Synaptic dysfunction, Cognitive decline
#Tags	#Synaptic-plasticity #Neurofibrillary-tangles #Tau #Amyloid-beta #Alzheimer's
Summary of key points + notes (include methodology)	Notes done with the aid of GhatGPT, an AI service. Looked over manually– corrections and additional information necessary has been added Summary: The article explores the interaction between amyloid beta (Aβ) and tau proteins in Alzheimer's disease, particularly their impact on synapses. It discusses the importance of synapses in memory and learning, highlighting synaptic plasticity mechanisms such as long-term potentiation (LTP) and long-term depression (LTD). The study links Aβ and tau to alterations in synaptic plasticity, leading to synapse dysfunction and loss in Alzheimer's disease. Additionally, it suggests that tau pathology contributes to synapse degeneration in later disease stages. The article also explores the potential roles of Aβ and tau in normal synaptic function. Mechanisms of synaptic dysfunction related to Aβ and tau, such as calcium influx and calcineurin activation, are discussed. Introduction - Alzheimer's Disease (AD) is characterized by specific brain abnormalities. - Amyloid beta (Aβ) accumulates into senile plaques, and tau becomes hyperphosphorylated, forming neurofibrillary tangles. - These features were first described by Alois Alzheimer in 1907, but their exact connection to brain degeneration remained uncertain. - Genetic evidence from familial AD cases strongly supports Aβ accumulation as a causative factor. - Mutations in genes such as amyloid precursor protein (APP) and presenilins 1 and 2, which contribute to Aβ production, cause early-onset AD. Challenges to the Amyloid Hypothesis 1. Plaque Load and Cognitive Impairment - The accumulation of plaques doesn't consistently correlate with cognitive impairments in AD patients. - Some individuals without cognitive impairment have substantial plaque accumulations in their brains. - Reducing plaque load through immunotherapy doesn't necessarily lead to cognitive improvement.

2. Tau and Cognitive Decline
- In contrast, neurofibrillary tangles (tau) strongly correlate with cognitive decline, neuronal loss, and synapse loss.
- Mutations in the tau protein lead to frontotemporal dementia, not AD.

3. Synapse Loss
- Synapse loss is a critical neuropathological feature that significantly correlates with dementia in AD.
- Dysfunctional synapses and impaired synaptic plasticity are key components of AD neurodegeneration.
- Both Aβ and tau contribute to the synaptic dysfunction.

Understanding Synapse Disruption in AD

- Synapses, the connections between neurons, play a crucial role in cognitive function.
- Recent research has focused on how Aβ and tau disrupt synaptic structure and function in AD, contributing to cognitive impairment.
- Additionally, there's a growing understanding of synapses' role in the spread of pathology through the brain.

Role of Aβ and Tau in Synaptic Disruption

- Accumulation of Aβ and hyperphosphorylation of tau contribute to synaptic dysfunction in several ways:
- Disrupting synaptic plasticity.
- Inducing neuroinflammation.
- Altering neurotransmitter release.
- Promoting oxidative stress.
- Initiating apoptotic pathways.

Synaptic Dysfunction and Cognitive Impairment

- Synaptic dysfunction is increasingly recognized as a central player in AD pathogenesis.
- Cognitive deficits in AD are closely linked to the extent of synaptic damage.
- Understanding the precise mechanisms of synaptic disruption by Aβ and tau may provide insights into potential therapeutic targets for AD.

The Role of Synapses in Pathology Spread

- Evidence suggests that damaged synapses may contribute to the propagation of Aβ and tau pathology throughout the brain.
- Understanding the dynamics of this spread could offer new approaches to diagnosing and treating AD.

Synaptic Plasticity

- In a healthy adult brain, synaptic plasticity plays a pivotal role in learning and memory formation.
- Alzheimer's Disease (AD) is primarily characterized by memory loss, which is why it's not surprising that brain areas crucial for memory and synaptic plasticity degenerate.
- The hippocampus and neocortex are vital for learning and memory, with the circuitry connecting them significantly impacted by AD pathology.

Historical Perspective on Synaptic Plasticity

- Ramon y Cajal suggested that changes in the strength of connections between existing neurons, rather than the birth of new neurons, underlie memory formation (Cajal, 1894).
- In 1949, Hebb proposed that synapses between two neurons would strengthen if activated simultaneously and weaken if activated separately (Hebb, 1949).
- Long-term potentiation (LTP) and long-term depression (LTD) are key mechanisms of synaptic plasticity, providing a molecular understanding of synapse strengthening or weakening.

Long-Term Potentiation (LTP)

- LTP is a long-lasting increase in synaptic transmission strength when pre- and postsynaptic neurons activate simultaneously.
- Early-phase LTP involves protein kinase activation, leading to changes in synaptic AMPA receptors (AMPARs), including phosphorylation and enhanced activity.
- Late-phase LTP results from increased calcium levels and persistent kinase activation, leading to transcription factor activation (e.g., CREB) and protein production, contributing to dendritic spine formation.

Long-Term Depression (LTD)

- LTD is the weakening of synaptic strength following a stimulus.
- Mechanisms include AMPA receptor internalization.
- NMDA receptor-dependent LTD, which is particularly affected in AD, depends on calcium influx, calcineurin activation, and caspase activation.
- LTD is thought to play a role in clearing old memory traces and enabling behavioral flexibility, with a potential connection to AD-related synapse degeneration.

Structural Changes in Synaptic Plasticity

- LTP is associated with the formation of new dendritic spines, increased postsynaptic densities, and enlarged spine heads.
- LTD is associated with spine shrinkage and loss, possibly involving

nonapoptotic caspase-3 activation.
- Environmental enrichment can lead to the formation of new dendritic spines and dendritic branches on pyramidal neurons.

Synaptic Changes in Alzheimer's Disease

- AD disrupts normal synaptic function, leads to synapse elimination, and involves the transport of pathological proteins through synapses.
- Upcoming discussions will delve into the neuropathology of AD and its impact on synapses.

Structural Changes in the AD Brain

- Structural changes in the AD brain are categorized as "positive" and "negative" lesions.
- “Positive" lesions include the accumulation of plaques, tangles, neuropil threads, dystrophic neurites, cerebral amyloid angiopathy (CAA), and other deposits in AD patients' brains.
- "Negative" lesions involve massive atrophy due to neuron loss and the degeneration of neurites and synapses (Serrano-Pozo et al., 2011a).
- The characteristic patterns of these lesions in AD provide insights into their relationship with disease progression and symptoms.

Senile Plaques

- Senile plaques were first described by Alzheimer and later found to be primarily composed of the amyloid beta peptide (Aβ) (Glenner and Wong, 1984, Masters and Selkoe, 2012).
- Neuritic or dense-cored plaques consist of a dense Aβ center surrounded by a halo of silver-positive neurites.
- "Diffuse" plaques, with various morphologies, also contain Aβ aggregates (Dickson and Vickers, 2001, Gomez-Isla et al., 2008).

Distribution of Senile Plaque Deposition

- Cross-sectional studies of postmortem human brains reveal that senile plaque deposition begins early in AD and progresses slowly.
- The process starts in the neocortex and advances through the allocortex, diencephalon, striatum, basal forebrain cholinergic nuclei, brainstem nuclei, and cerebellum (Thal et al., 2002).
- In live mouse models with AD-associated mutations, in vivo multiphoton imaging shows rapid coalescence of individual plaques from soluble Aβ, forming within 24 hours.
- Surrounding neurites begin to curve and degenerate within days after plaque formation (Meyer-Luehmann et al., 2008).

Toxic Effects of Dense Plaques

- Dense plaques have toxic effects on the surrounding brain parenchyma, contributing to synapse dysfunction and loss.
- Neurites around plaques often exhibit swollen, dystrophic morphologies and contain phospho-tau aggregates and disrupted cellular components.
- The trajectories of axons and dendrites are disrupted near amyloid plaques in mouse models, potentially affecting synaptic signal integration (Le et al., 2001, Spires et al., 2005, Stern et al., 2004, Urbanc et al., 2002).
- Gliosis and oxidative stress are observed around plaques, likely further contributing to synaptic changes (Ingelsson et al., 2004, McLellan et al., 2003, Serrano-Pozo et al., 2011b).

Neurofibrillary Tangles (NFT) and Their Impact

- Less is known about the impact of NFT on the surrounding neuropil compared to plaques.
- NFT and neuropil threads consist of aggregated tau protein, which is primarily found in axons, stabilizing microtubules (Goedert and Spillantini, 2006).
- In AD, tau becomes hyperphosphorylated, detaches from microtubules, and accumulates in somatodendritic compartments as paired helical filaments and straight filaments (Kidd, 1963, Spillantini and Goedert, 2013, Stoothoff and Johnson, 2005).
- The deposition of tangles follows a hierarchical pattern, starting in the entorhinal cortex and progressing through the hippocampal formation, association cortices, and, in late stages, affecting primary sensory areas (Arnold et al., 1991, Braak and Braak, 1991).

NFT and Cognitive Decline

- NFT deposition in human AD is correlated with cognitive decline and neuronal loss (Arriagada et al., 1992, Duyckaerts et al., 1998, Giannakopoulos et al., 2003, Gómez-Isla et al., 1997).
- Some neurons with NFT die during the disease, supported by the presence of ghost tangles (NFT remaining after neuron death).
- However, the extent of neuronal loss surpasses the number of NFT and ghost tangles in specific brain regions, suggesting that NFT are not a necessary factor for neuron death (Gómez-Isla et al., 1997).
- While NFT are intracellular lesions, their impact on the surrounding environment is less understood, but gliosis in proximity to NFT correlates with disease progression (Serrano-Pozo et al., 2011b).

Synaptic Dysfunction, Synapse Loss, and Relationships to Pathology

- Synapse loss in AD was observed in the early 1990s, with electron microscopy and immunostaining of synaptic proteins.
- Synapse loss is most strongly correlated with dementia, affecting the frontal cortex, temporal cortex, and dentate gyrus of the hippocampus (DeKosky and Scheff, 1990, DeKosky et al., 1996, Masliah et al., 1994, Terry

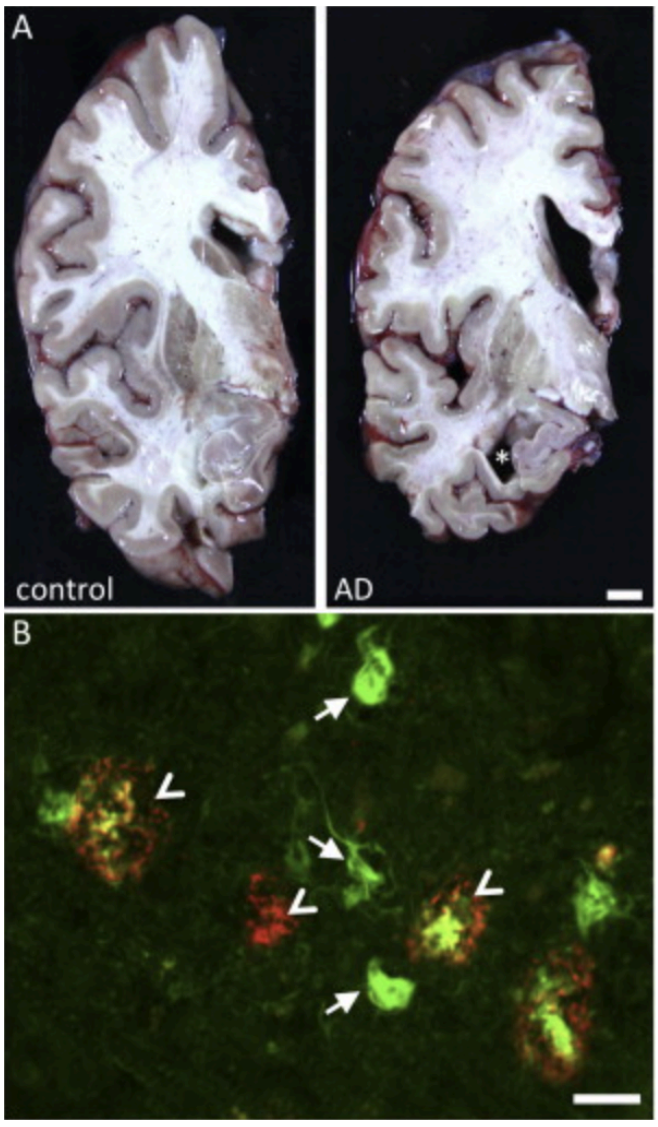
et al., 1991).
- Notably, the entorhinal cortex, severely impacted by AD, does not experience a loss of synapse density in the remaining neuropil, despite significant synapse loss in the dentate gyrus target zone (Scheff et al., 1993).

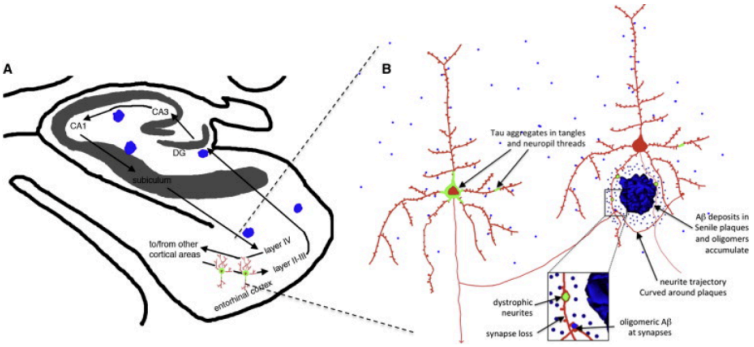
Amyloid Pathology and Synapse Loss
- Amyloid pathology's association with local synapse loss was largely established through animal and cell culture models.
- Soluble forms of Aβ, accumulating around dense plaques, are implicated as more toxic than fibrils.
- Soluble Aβ causes dendritic spine loss, disrupts LTP, and impairs cognition in vivo (Cleary et al., 2005, Shankar et al., 2007, Shankar et al., 2008, Walsh et al., 2002, Walsh et al., 2005).
- Dimeric Aβ is also associated with dementia in human brain (McDonald et al., 2010).
- Studies using advanced imaging techniques show the presence of oligomeric Aβ at synapses in AD mouse brains (Koffie et al., 2009).
- This finding is extended to human autopsy tissue, confirming oligomeric Aβ's presence in both pre- and postsynaptic puncta (Kay et al., 2013).
- Furthermore, an association is noted between increased Aβ at synapses and apolipoprotein E ε4 (apoE4), a gene that increases AD risk, suggesting apoE4 contributes to AD risk by increasing the localization of toxic oligomeric Aβ to synapses (Koffie et al., 2012).

Amyloid and Tau: Contributions to Synapse Degeneration
- Aβ-induced synaptic changes may primarily contribute to early-stage disease, while tau pathology becomes a significant factor in later stages (Hyman, 2011).
- Tau, traditionally associated with axons, plays a role in maintaining the postsynaptic density (PSD), targeting fyn kinase to the PSD and impacting dendritic spines and neurotransmitter receptor composition (Ittner et al., 2010; Hoover et al., 2010).
- Overexpression of P301L tau in mice leads to synaptic alterations and loss (Crimins et al., 2011; Crimins et al., 2012; Crimins et al., 2013; Kopeikina et al., 2013a; Kopeikina et al., 2013b; Rocher et al., 2010).
- Tau has been observed in dendritic spines in mouse models (Kopeikina et al., 2013a), and endogenous tau in dendrites undergoing Aβ-induced spine loss has been noted (Zempel et al., 2010).
- Tau is also observed in postsynaptic terminals of both control and AD cases (Tai et al., 2012).

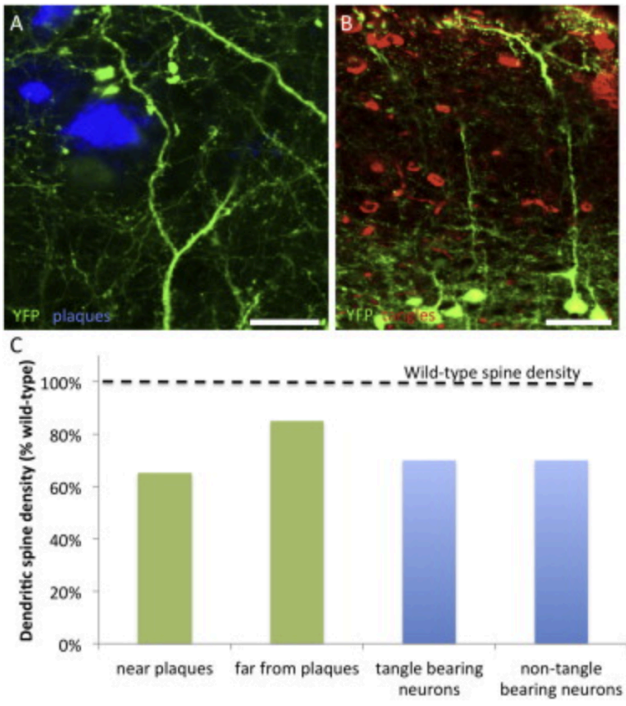
Tau and Aβ's Role in Normal Synaptic Physiology
- Aβ is implicated in developmental synaptic plasticity and is associated with smaller synapse volume in nondemented human subjects (Cao et al., 2012; Kim et al., 2013; Koffie et al., 2012).
- The machinery for Aβ generation is present at the synapse, supporting its normal role (Cirrito et al., 2005; Kamenetz et al., 2003; Li et al., 2013;

	Sheng et al., 2012). - Tau regulates microtubule stability and axonal transport, with evidence that its phosphorylation is involved in synaptic biology during hibernation (Arendt et al., 2003). - Tau may play a role in regulating NMDAR function via interaction with fyn kinase (Ittner et al., 2010; Mondragón-Rodríguez et al., 2012). Mechanisms of Synaptic Dysfunction and Loss - While it is well-established that oligomeric Aβ is toxic to synapses, the exact species and receptors responsible for Aβ's toxic effects are debated (Benilova and De Strooper, 2013). - Aβ is known to cause increased Ca2+ levels in dendrites and dendritic spines, central to synapse dysfunction and loss (Demuro et al., 2005; Hudry et al., 2012; Mattson et al., 1992; Wu et al., 2010; Zempel et al., 2010). - Calcineurin appears crucial in mediating synaptic degeneration downstream of Aβ (Cavallucci et al., 2013; Rozkalne et al., 2011; Wu et al., 2010). - Changes in calcium concentration and activation of calcineurin interfere with normal synaptic plasticity, disrupting LTP and causing internalization of AMPA and NMDA neurotransmitter receptors (Hsieh et al., 2006; Koffie et al., 2011; Snyder et al., 2005; Wang et al., 2004). - Nonapoptotic caspase activation also plays a role in the internalization of synaptic receptors and is induced by Aβ (Chen et al., 2013; D'Amelio et al., 2011; Liu et al., 2010). - Altered calcium dynamics destabilize the cytoskeleton in dendritic spines, allowing spine collapse (Roh et al., 2013).
Research Question/Problem/ Need	What is the effect of Amyloid Beta and Tau proteins at neural synapses in Alzheimer's Disease?

Important Figures	 - "AD brains are characterized by striking atrophy compared to control brains (A). Particularly evident is shrinkage of the cortical mantle and the hippocampus (asterisk shows hippocampal atrophy). Microscopically, AD is defined by deposition of Aβ in senile plaques (arrowheads) and tau in neurofibrillary tangles (arrows). In this micrograph, the fibrillar deposits (both plaques and tangles) are stained green with thioflavine S. Aβ is also immunostained with antibody AW7 (courtesy of Dominic Walsh), illustrating the halo of soluble Aβ around fibrillar plaque cores and the heterogeneous nature of plaques. Scale bars represent 1 cm in (A) and 20 μm in (B)"

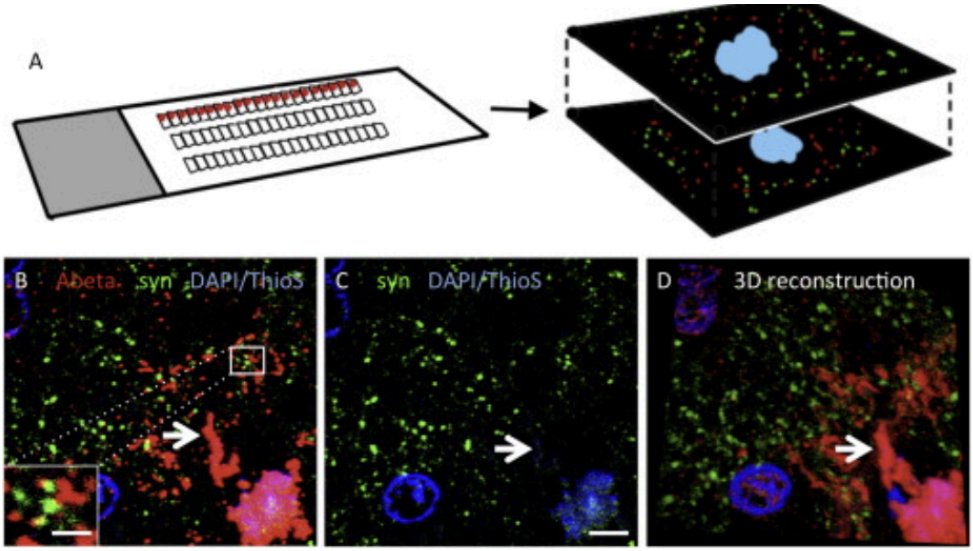


- "The neural circuitry involved in memory including the entorhinal cortex-hippocampal circuitry (A) are severely affected by AD pathology, including the deposition of plaques (blue) and tangles (green) and dramatic neuronal and synapse loss. Along with the dramatic neuronal loss, there are structural changes to remaining neurons in the AD brain that are thought to contribute to neural circuit disruption and cognitive impairments (B), including damage to neurites in the halo of soluble amyloid beta surrounding plaques, tau aggregation in cell bodies and neurites, and synapse loss associated with oligomeric Aβ around plaques. (A) is modified from Gomez-Isla et al., 2008."

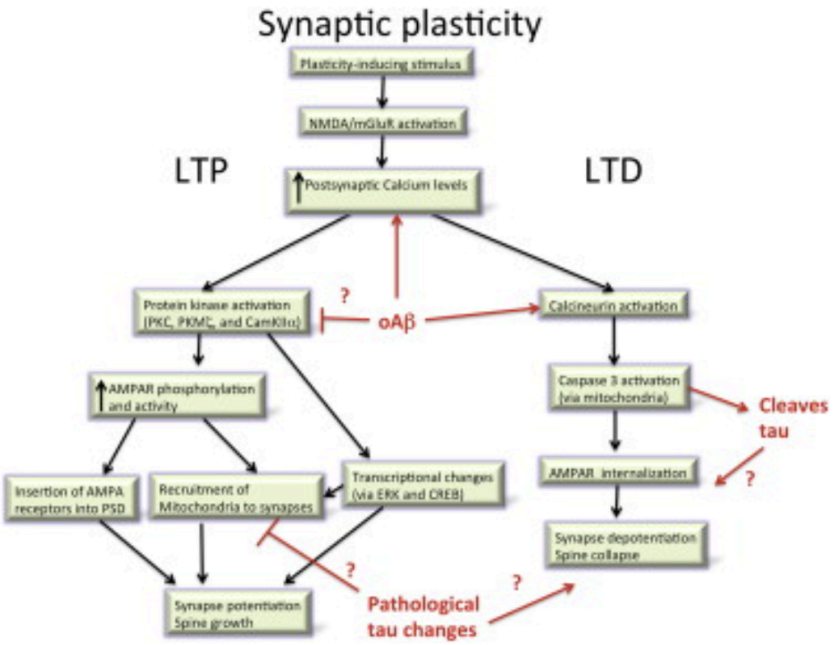


- "Mouse models that exhibit plaque formation or tangle formation exhibit dendritic spine loss. Crossing APP/PS1 mice (A) and rTg4510 mice (B) with YFP overexpressing lines allowed quantification of dendritic spine density on cortical pyramidal neurons (layer II/III). Dense plaques are stained with thioflavine S in (A) and neurofibrillary tangles are stained with PHF1 antibody in (B), while neurons in both panels are filled with YFP due to

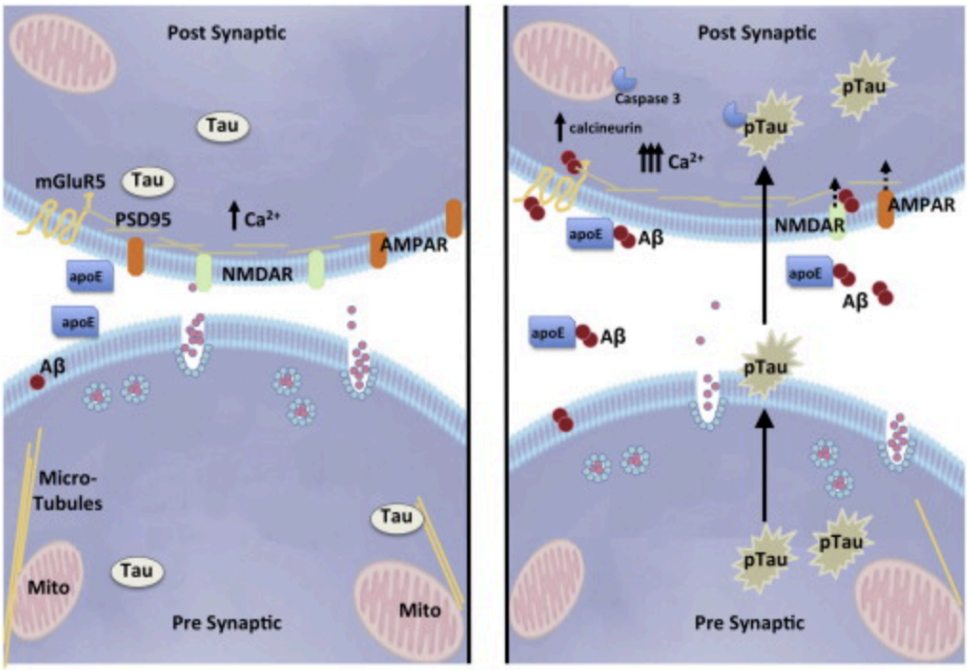
transgenic overexpression. Similar results are found when fluorescent markers are introduced via viral infection of neurons or direct injection of fluorophores. In plaque-bearing mice, dendritic spine loss is most pronounced within 50 µm of plaques, whereas in tangle-bearing mice, the presence of a tangle does not affect dendritic spine density (C). Data in (C) are adapted from Kopeikina et al., 2013a, Kopeikina et al., 2013b, Rocher et al., 2010, Rozkalne et al., 2011, and Spires et al., 2005. Scale bars represent 20 µm in (A) and 50 µm in (B)."



- "The array tomography technique overcomes the axial resolution of light microscopy by physically sectioning resin-embedded brain tissue into ribbons of ultrathin (70 nm) serial sections that are stained with immunofluorescence, imaged with a fluorescent microscope at the same place along the ribbon (red dots), and a three-dimensional data set acquired of multiple markers at synapses (A and D). Using human AD brain tissue (B and C), we observed oligomeric Aβ stained with NAB61 (red) present at a subset of synapses as can be seen in the inset in (B) (presynaptic terminals stained here with synapsin I, green). We also observe a reduction in synapse density in the halo of oligomeric Aβ surrounding the Thioflavin S (ThioS)-positive dense cores of plaques (arrows). Scale bars represent 5 µm in (B and C) and 1 µm in inset in (B). (D) is a reconstruction of a 36 µm × 33 µm × 1.2 µm volume (images from 17 serial sections). (A) is adapted from Micheva and Smith, 2007."



- "Under normal conditions, LTP promotes recruitment of neurotransmitter receptors to active synapses and causes synapse potentiation, stabilization, and growth. LTD conversely results in synapse depotentiation and spine collapse. Both of these processes are affected in animal models of AD, with oligomeric Aβ clearly affecting the calcium and calcineurin pathways involved in these phenomena. Tau overexpression has been observed to affect synaptic function in transgenic models and to be necessary for oligomeric Aβ-mediated synapse dysfunction, but the mechanisms by which pathological forms of tau affect synaptic plasticity are less well understood. It is possible that hyperphosphorylation affects microtubule stability and the transport of mitochondria to synapses, which could affect synaptic function. The cleavage of tau by caspase-3 has also been observed, which could be tied to the nonapoptotic role of caspase-3 in LTD and spine collapse."



Normal synapse **AD synapse**

- "Many studies implicate oligomeric Aβ in synapse dysfunction and loss in models of AD. Aβ may be specifically trafficked to the synapse by apoE4, where it binds to postsynaptic receptors, causes an increase in calcium concentration, calcineurin activation, caspase-3 activation, and downstream internalization of synaptic receptors. Tau has also been implicated in synapse dysfunction downstream of Aβ, and pathological forms of tau (pTau) are transferred through synaptic circuits, although which forms of tau are transported and how they are transported remains to be determined. Figure courtesy of A. Hermann."

VOCAB: (w/definition)

Transsynaptic- occurring or taking place across nerve synapses.

Presenilins- evolutionarily conserved transmembrane proteins that regulate cleavage of certain other proteins in their transmembrane domains.

Neocortex- a part of the cerebral cortex concerned with sight and hearing in mammals, regarded as the most recently evolved part of the cortex.

Potentiation- the increase in strength of nerve impulses along pathways which have been used previously, either short-term or long-term.

Neurite- small processes on developing neurons that ultimately grow out into axons or dendrites under the control of growth stimulating or inhibiting factors from their direct extracellular environment sensed by receptors in the growth cone, the tip of the neurite

Allocortex- one of two types of cerebral cortex defined on the basis of cytoarchitecture and fetal development.

- Cytoarchitecture- the arrangement of cells in a tissue, especially in specific areas of the cerebral cortex characterized by the arrangement of their cells and each associated with particular functions.

Diencephalon- the caudal (posterior) part of the forebrain, containing the epithalamus, thalamus, hypothalamus, and ventral thalamus and the third ventricle.

Striatum- the input module to the basal ganglia, a neuronal circuit necessary for voluntary movement control

- Basal Ganglia- a group of subcortical nuclei responsible primarily for motor control, as well as other roles such as motor learning, executive functions and behaviors, and emotions.
    - Subcortical- relating to or denoting the region of the brain below the cortex

Cholinergic- denoting nerve fibres that release acetylcholine when stimulated. of or relating to the type of chemical activity associated with acetylcholine and similar substances.

Parenchyma- the functional tissue of an organ as distinguished from the connective and supporting tissue.

Gliosis- a fibrous proliferation of glial cells in injured areas of the CNS

Neuropil- the space between neuronal and glial cell bodies that is comprised of dendrites, axons, synapses, glial cell processes, and microvasculature.

- Microvasculature- small vessels; arterioles, capillaries, and venule

Somatodendritic- The region of a neuron that includes the cell body (cell soma) and dendrite(s), but excludes the axon

Entorhinal- of, relating to, or being the part of the cerebral cortex in the medial temporal lobe that serves as the main cortical input to the hippocampus.

Glutaraldehyde- a compound $C_5H_8O_2$ that contains two aldehyde groups and is used as a disinfectant and in fixing biological tissues

- Aldehyde- an organic compound containing the group —CHO, formed by the oxidation of alcohols. Typical aldehydes include methanal (formaldehyde) and ethanal (acetaldehyde).

Apolipoprotein- a protein that combines with a lipid to form a lipoprotein —often used with a letter or letter and number

Tyrosine- : a phenolic amino acid $C_9H_{11}NO_3$ that is a precursor of several important substances

- Phenolic- of, relating to, or having the characteristics of a phenol
    - Phenol- a corrosive poisonous crystalline acidic compound C_6H_5OH present in the tars of coal and wood that in dilute solution is used as a disinfectant

Kinases- any of various enzymes that catalyze the transfer of phosphate groups from a high-energy phosphate-containing molecule (such as ATP) to a substrate

EphB2- a protein coding gene

Proteasome- a highly sophisticated protease complex designed to carry out selective, efficient and processive hydrolysis of client proteins

- Protease- an enzyme which breaks down proteins and peptides.
- Hydrolysis- the chemical breakdown of a compound due to reaction with water

Calcineurin- a serine-threonine specific Ca(2+)-calmodulin-activated protein phosphatase that is conserved from yeast to humans, used in regulating the transcription factor NF-AT during T-cell activation, and in mediating responses of microorganisms to cation stress

- Serine- an amino acid
- Threonine- an essential amino acid
- Calmodulin- a low molecular weight, acidic, calcium binding protein
- NF-AT- Nuclear Factor of Activated T-cells; a family of transcription factors shown to be important in immune response.
- Phosphatase- an enzyme that catalyzes the hydrolysis of organic phosphates in a specified (acid or alkaline) environment
- T-cell- a type of white blood cell called lymphocytes

Microglia- resident cells of the brain that regulate brain development, maintenance of neuronal networks, and injury repair.

Anterograde- directed forward in time: of or denoting a type of amnesia involving inability to remember any new information.

FTD- Frontotemporal dementia; a group of disorders that occur when nerve cells in the frontal and temporal lobes of the brain are lost.

P301L- tau mutation most frequently observed in patients with frontotemporal dementia with parkinsonism linked to chromosome 17

rTg4510 Line- a mouse model of tauopathy overexpressing P301L mutant human Tau in the forebrain

Glutamate- an excitatory neurotransmitter with several types of receptors found

	throughout the central nervous system AMPA- α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; glutamate-gated ion channels, present in a wide range of neuron types and in glial cells. Their main role is to mediate fast excitatory synaptic transmission, and therefore, they are critical for normal brain function.
Cited references to follow up on	Berger Z. Roder H. Hanna A. Carlson A. Rangachari V. Yue M. Wszolek Z. Ashe K. Knight J. Dickson D. et al. Accumulation of pathological tau species and memory loss in a conditional model of tauopathy. J. Neurosci. 2007; 27: 3650-3662 Bero A.W. Yan P. Roh J.H. Cirrito J.R. Stewart F.R. Raichle M.E. Lee J.-M. Holtzman D.M. Neuronal activity regulates the regional vulnerability to amyloid-β deposition. Nat. Neurosci. 2011; 14: 750-756
Follow up Questions	The article had a section on follow-up questions; here are a few of them below: - Which forms of Aβ and tau are toxic? Although strong evidence does show that soluble forms of these are toxic at synapses, the exact forms of these species are still unknown - What are the molecular mechanisms leading to synapse loss? - Is there reason to study extracellular tau, as it is recognized as a normal species, increased after neuronal activity, rather than simply a marker of neuronal damage? To add on to them, here's one of my own: - Would extracellular tau be able to be used as a marker for frayed nerve endings?

Article #8 Notes: In Vivo and In Vitro Determination of Cell Death Markers in Neurons

Source Title	In Vivo and In Vitro Determination of Cell Death Markers in Neurons
Source citation (APA Format)	Naniche, N., Sau, D., & Piera Pasinelli. (2011). In Vivo and In Vitro Determination of Cell Death Markers in Neurons. *Methods in Molecular Biology*, *793*, 9–21. https://doi.org/10.1007/978-1-61779-328-8_2

Original URL	https://link.springer.com/protocol/10.1007/978-1-61779-328-8_2
Source type	Journal Article
Keywords	Mitochondria, Cytochrome C, Immunofluorescence, Western blot, ELISA
#Tags	#potential-marker #labwork #processes #cell-death #neurons
Summary of key points + notes (include methodology)	Summary: The following summary was written with the aid of Google Bard, an AI service. Mitochondria are essential organelles that play a critical role in cellular metabolism and energy production. However, mitochondria also play a key role in programmed cell death, or apoptosis. During apoptosis, mitochondria release a number of proteins into the cytosol, including cytochrome c (Cyt C). Cyt C then binds to other proteins to form a complex called the apoptosome, which activates caspases, a family of proteases that trigger apoptosis. Measurement of Cyt C release can therefore be used as a marker of both mitochondrial integrity and cell death. A number of different methods can be used to measure Cyt C release, which provide a comprehensive assessment of the relationship between mitochondrial viability and activation of an intracellular cell death process. Notes: The following notes were done independently. - Stresses the importance of mitochondria - Life and death processes - Metabolic pathways - Energy production - Calcium storage - production/regulation of reactive oxygen species(ROS) - Regulation of apoptosis - Intact mitochondria required for healthy cellular metabolism and regualtion of mitrochonsdrial transmembrance potential(DΨm) - If and when outer membrane loses integrity: - Mitochondrial ionic+metabolic homeostasis is disrupted, leading to deathly consequences for cell - To maintain metabolic viability of cell, integrity must be maintained - Cell death can also happen through mitochondria triggering the

release of apoptogenic proteins into cytosol
- Done through permeabilization of Mitochondrial Outer Membrane(MOM)
  - decreased ATP production
  - Change in mitochondrial membrane conductances
  - Increases in cytosolic calcium labels
  - Increase in ROS production
  - Eventual cell death

- Cytochrome C
  - Normally resides within intermembrane space (IMS) of mitochondria
  - Binds to apoptotic protease-activating factor 1 (APAF1) upon cytosolic localization
    - Leads to oligomerization of APAF1
    - Leads to formation of "apoptosome"
      - Serves as platform for dimerization+activation of initiator caspase 9
        - Cleaves+activates executioner caspases 3+7, causing cell death

- Neurons' dependency on mitochondria-provided energy
  - Highly dependent
    - Sensitive to mitochondrial dysfunction

  - **Cyt C from mitochondria can be doubly useful tool to define time window between decrease in energy supply, commitment to death**
    - Represents valuable marker for mitochondrial integrity- released into cytosol when mitochondrial integrity is challenged
      - Released when mitochondria is damaged

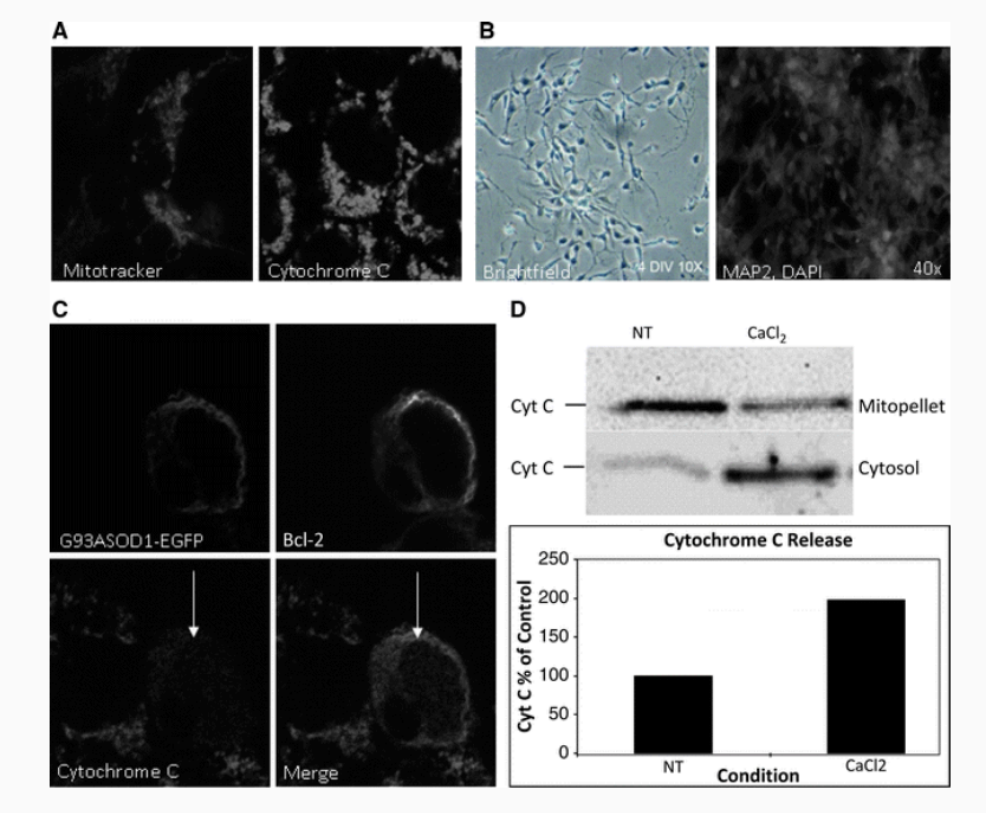
    - Highly soluble

    - Plays crucial role in cell death mechanisms, released in response to toxic stimuli

    - "Cyt C release from mitochondria is, therefore, a good marker of cell death, especially as associated with mitochondrial toxicity."

- Methods to detect Cyt C behavior
  - Cell Culture and Transfection

	- Cell and Spinal Cord Mitochondrial Isolation - Cell and Spinal Cord Lysis - ELISA - SDS–Polyacrylamide Gel Electrophoresis - Western Blotting - Immunofluorescence - NSC34 cells - a hybrid cell line generated by the fusion of mouse embryonic motor neuron-enriched spinal cord cells with mouse neuroblastoma N18TG2 - The rest of the article goes in depth with the methods used. Refer to Figure for some of these results
Research Question/Problem/ Need	What highlights and determines cell death in specifically neuron cells?

Important Figures	 - The caption is as follows: "Determining Cytochrome C (Cyt C) release in cell lines. (a) Nontreated NSC34 cells were stained with MitoTracker (left panel) or probed for Cyt C (right panel) for observation of intact, healthy mitochondria. Comparison of Cyt C with known mitochondrial stain gives same intact, punctate expression pattern across the cell. (b) NSC34 cells were transfected with a toxic protein (amyotrophic lateral sclerosis-linked mutant SOD1-EGFP) to induce release of Cyt C. In the transfected (cell with arrow), Cyt C displays a diffuse staining across the cell, indicating release. Conversely, nontransfected cells retain punctate staining, indicating intact mitochondria and no release into the cytosol. (c) Cortical neurons were stained for neuronal marker MAP2 and nuclear marker DAPI. (d) Mitopellet and cytosolic fractions of nontransfected (NT) and positive control (CaCl2) NSC34 cells were run on Western blot and probed for Cyt C to determine amount retained in mitochondria and released into cytosol. Cytosolic fraction was also analyzed by ELISA; representative blot shown in lower panel."
VOCAB: (w/definition)	Cytochrome- any of a number of compounds consisting of heme bonded to a protein. Cytochromes function as electron transfer agents in many metabolic pathways, especially cellular respiration. - Heme- an iron-containing compound of the porphyrin class which forms the nonprotein part of hemoglobin and some other biological molecules. - Porphyrin- any of a class of pigments (including heme and chlorophyll) whose molecules contain a flat ring of four linked

heterocyclic groups, sometimes with a central metal atom
- Heterocyclic- denoting a compound whose molecule contains a ring of atoms of at least two elements (one of which is generally carbon).

Immunofluorescence- a technique for determining the location of an antigen (or antibody) in tissues by reaction with an antibody (or antigen) labeled with a fluorescent dye.
- Antigen- a toxin or other foreign substance which induces an immune response in the body, especially the production of antibodies.

Caspase- any of several intracellular proteases that have a cysteine residue at their active site, that cleave substrate proteins at specific aspartic acid residues, and that are involved in the initiation and mediation of apoptosis.
- Cysteine- a sulfur-containing amino acid which occurs in keratins and other proteins, often in the form of cystine, and is a constituent of many enzymes.
  - Cystine- an amino acid that is found in digestive enzymes, in the cells of the immune system, in skeletal and connective tissues, skin, and hair
- Protease- an enzyme which breaks down proteins and peptides.

Immunolabeling- a biochemical process that enables detection and localisation of an antigen to a site within a cell, organ or tissue.

Apoptosome- the adaptor protein complex that mediates the activation of an initiator caspase at the onset of apoptosis
- Protein Complex- a molecular machine that consists of several proteins (nucleic acids and other molecules) that bind each other at the same place and time

Dimerization- the process of joining two molecules or ions by bonds

ELISA- (enzyme-linked immunosorbent assay)- a plate-based assay technique designed for detecting and quantifying soluble substances (i.e. peptaides, proteins, antibodies, and hormones) also known as EIA(Enzyme Immunoassay)

Neuroblastoma- a cancer that starts in certain very early forms of nerve cells, most often found in an embryo or fetus

MitoTracker- cell permeable probes that contain a mildly thiol-reactive chloromethyl moiety for mitochondrial labeling.
- Thiol- also called mercaptan, any of a class of organic chemical compounds similar to the alcohols and phenols but containing a sulfur atom in place of the oxygen atom
  - Phenol- a colorless to light-pink, crystalline solid with a sweet,

	acrid odor - Acrid- having an irritatingly strong and unpleasant taste or smell. In vivo- (of a process) performed or taking place in a living organism. In vitro- (of a process) performed or taking place in a test tube, culture dish, or elsewhere outside a living organism.
Cited references to follow up on	- https://www.pnas.org/doi/abs/10.1073/pnas.95.26.15763 - https://journals.sagepub.com/doi/abs/10.1177/1073858404271087?casa_token=kxPCajtCzPwAAAAA:AVtknmqodolsVhuNrHhXqEWw7-EwMn-H2icBB7V6tH7cSd2AwAwBRq-T6epD1iSWh95_j4GAnWbGgA
Follow up Questions	- Could Cyt C be a potential marker? Refer to bolded note - How can we be sure that this would correlate to human bodies? After all, the cells used were from mice. - Although the article is named "in Neurons", Cyt C sounds like it may be in any cell mitochondria. Is this just a misunderstanding based on the way they phrased it, or is Cyt C present in many cells? - Is there a scan that would detect Cyt C? In this article, only experiments to isolate the compound were used.

Article #9 Notes: PATENT- Passive immunization treatment of Alzheimer's disease

Source Title	Passive immunization treatment of Alzheimer's disease
Source citation (APA Format)	Schenk, Dale B. Passive immunization treatment of Alzheimer's disease. United States US6761888B1, filed May 26, 2000, and issued July 13, 2004. https://patents.google.com/patent/US6761888B1/en.
Original URL	https://patents.google.com/patent/US6761888B1/en
Source type	Patent
Keywords	Antibodies, Immunoglobulins, Immune serum: e.g. antilymphocytic serum, beta-amyloid

#Tags	#competitor #treatment
Summary of key points + notes (include methodology)	Notes done with the aid of GhatGPT, an AI service. Looked over manually– corrections and additional information necessary has been added. The invention encompasses a comprehensive array of methods targeting the prevention, treatment, and detection of diseases linked to amyloid deposits of Aβ in the brain, notably Alzheimer's disease, Down syndrome, and cognitive impairment. It primarily revolves around employing antibodies, Aβ fragments, and immunogenic agents to specifically bind to distinct epitopes within the Aβ peptide. These methods involve administering antibodies that induce a clearing response against amyloid deposits via various routes and dosages, including encoding antibody chains in patients through polynucleotides. Fragments of Aβ or analogs are utilized to trigger an immunogenic response against selective epitopes within Aβ, potentially treating Aβ-associated diseases. Additionally, the invention provides diagnostic kits containing antibodies for detecting amyloid deposits, highlighting a multi-faceted approach that spans therapeutic, preventive, and diagnostic strategies to combat diseases related to Aβ accumulation in the brain. Treatment and Prevention Methods: - Disease Scope: Addresses diseases associated with amyloid deposits, specifically Alzheimer's disease, Down's syndrome, and cognitive impairment. - Antibody Targeting: Detailed discussion on using antibodies that specifically bind to various segments of Aβ, indicating different regions like residues 1-10, 1-6, 1-5, 1-7, 3-7, 1-3, 1-4, and more. - Administration Strategies: Covers diverse administration routes (intraperitoneal, oral, subcutaneous, intracranial, intramuscular, topical, intranasal, or intravenous) and dosage ranges (0.0001 to 100 mg/kg). Highlights the use of sustained release compositions and repeated dosing over an extended period (at least six months). - Alternative Administration Approach: Discusses administering polynucleotide-encoded antibodies, enabling in vivo antibody production within patients' bodies, and emphasizes monitoring administered antibody levels in the patient's bloodstream. Use of Aβ Fragments and Analogues: - Immunogenic Response: Details on using fragments or analogs of Aβ to trigger an immune response against specific epitopes within Aβ, notably focusing on N-terminal segments and heterologous polypeptides. - Adjuvants to Enhance Response: Mentions the use of adjuvants like aluminum hydroxide, aluminum phosphate, MPL™, QS-21 (Stimulon™), or incomplete Freund's adjuvant to boost immune responses to Aβ

	fragments or peptides. Screening and Diagnostic Methods: - Antibody Activity Screening: Methodologies for screening antibodies to determine their efficacy in treating diseases linked to Aβ deposits. Specifically, testing binding with polypeptides containing specific segments of Aβ, indicating potential efficacy. - Clearing Activity and Phagocytosis: Describes methods for screening antibodies' abilities to induce a clearing response against antigen-associated biological entities, involving phagocytic cells bearing Fc receptors. - Diagnostic Kit Development: Creation of diagnostic kits utilizing antibodies to detect amyloid deposits in patients' brains, potentially employing paramagnetic labels and nuclear magnetic resonance tomography for detection. - These detailed points underline the diverse approaches and methodologies outlined in the patent for combatting diseases related to amyloid deposits in the brain, especially Alzheimer's disease. The patent focuses on utilizing antibodies, fragments, immunogenic responses, and diagnostic tools for prevention, treatment, and detection.
Research Question/Problem/ Need	How can targeted antibody-based therapies effectively prevent, treat, or detect diseases associated with amyloid deposits in the brain, particularly Alzheimer's disease?

Important Figures

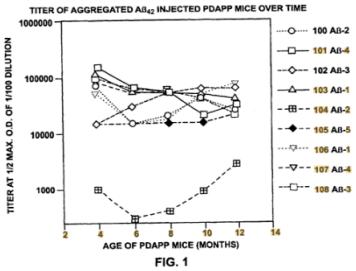


FIG. 1

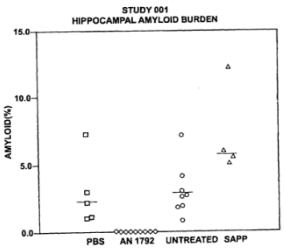


FIG. 2

FIG. 1: Antibody titer after injection of transgenic mice with Aβ1-42.
FIG. 2: Amyloid burden in the hippocampus. The percentage of the area of the hippocampal region occupied by amyloid plaques, defined by reactivity with the Aβ-specific monoclonal antibody 3D6, was determined by computer-assisted quantitative image analysis of immunoreacted brain sections. The values for individual mice are shown sorted by treatment group. The horizontal line for each grouping indicates the median value of the distribution.

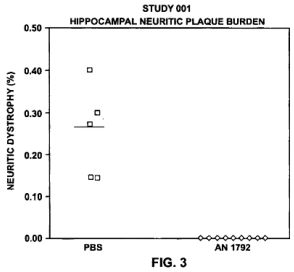


FIG. 3

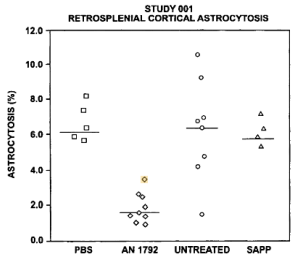


FIG. 4

FIG. 3: Neuritic dystrophy in the hippocampus. The percentage of the area of the hippocampal region occupied by dystrophic neurites, defined by their reactivity with the human APP-specific monoclonal 8E5, was determined by quantitative computer-assisted image analysis of immunoreacted brain sections. The values for individual mice are shown for the AN1792-treated group and the PBS-treated control group. The horizontal line for each grouping indicates the median value of

the distribution.

FIG. 4: Astrocytosis in the retrosplenial cortex. The percentage of the area of the cortical region occupied by glial fibrillary acidic protein (GFAP)-positive astrocytes was determined by quantitative computer-assisted image analysis of immunoreacted brain sections. The values for individual mice are shown sorted by treatment group and median group values are indicated by horizontal lines.

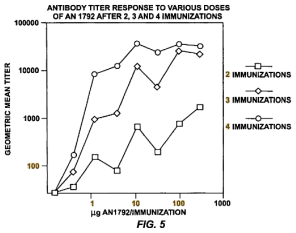


FIG. 5

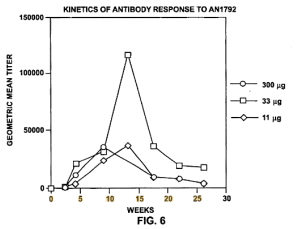


FIG. 6

FIG. 5: Geometric mean antibody titers to Aβ1-42 following immunization with a range of eight doses of AN1792 containing 0.14, 0.4, 1.2, 3.7, 11, 33, 100, or 300 µg.

FIG. 6: Kinetics of antibody response to AN1792 immunization. Titers are expressed as geometric means of values for the 6 animals in each group.

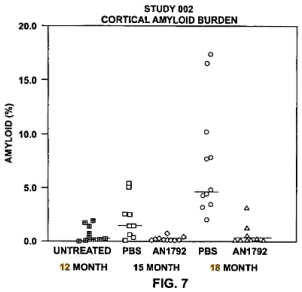


FIG. 7

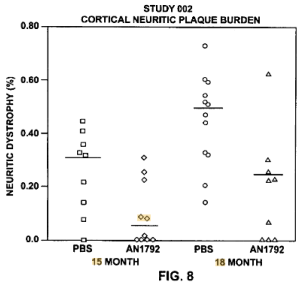


FIG. 8

FIG. 7: Quantitative image analysis of the cortical amyloid burden in PBS- and AN1792-treated mice.

FIG. 8: Quantitative image analysis of the neuritic plaque burden in PBS- and AN1792-treated mice

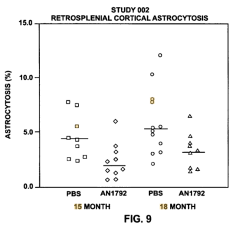
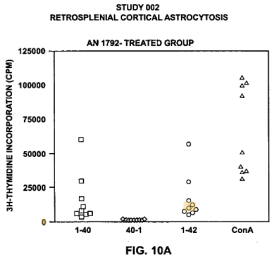


FIG. 9: Quantitative image analysis of the percent of the retrosplenial cortex occupied by astrocytosis in PBS- and AN1792-treated mice.



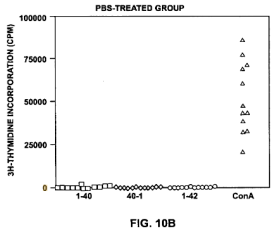
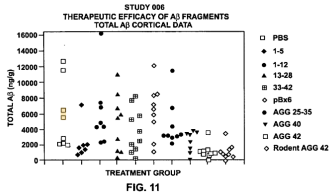


FIG. 10: Lymphocyte Proliferation Assay on spleen cells from AN1792-treated (FIG. 10A) or PBS-treated (FIG. 10B).



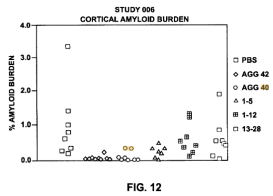


FIG. 11: Total Aβ levels in the cortex. A scatterplot of individual Aβ profiles in mice immunized with Aβ or APP derivatives combined with Freund' adjuvant.
FIG. 12: Amyloid burden in the cortex was determined by quantitative image analysis of immunoreacted brain sections for mice immunized with the Aβ peptide conjugates Aβ1-5, Aβ1-12, and Aβ13-28; the full length Aβ aggregates AN1792 (Aβ1-42) and AN1528 (Aβ1-40) and the PBS-treated control group.

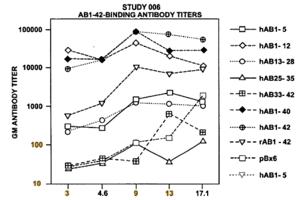


FIG.13

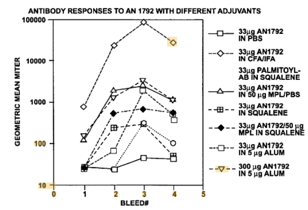


FIG.14

FIG. 13: Geometric mean titers of Aβ-specific antibody for groups of mice immunized with Aβ or APP derivatives combined with Freund's adjuvant.
FIG. 14: Geometric mean titers of Aβ-specific antibody for groups of guinea pigs immunized with AN1792, or a palmitoylated derivative thereof, combined with various adjuvants.

CORTEX

PBS CONTROL		UNTREATED CONTROL	
624-165	272	764-181	3470
625-166	1802	785-182	171
626-167	62	766-183	91
633-168	4696	767-184	6692
634-169	3090	768-185	1353
671-170	2417	771-186	1153
672-171	2840	772-187	3800
829-172	3320	780-188	3740
830-173	1833	843-189	163
831-174	416	844-190	122
792-175	126	845-191	427
793-176	2559	846-192	2674
794-177	289	887-193	453
732-178	179	888-194	2996
733-179	1329	889-195	1075
734-180	5665		
MEDIAN	1817	MEDIAN	1153
p VALUE (M-W)		p VALUE (M-W)	
MEAN	1931	MEAN	1825
ST. DEV.	1718	ST. DEV.	1769
% CV	89	% CV	97
p VALUE (t TEST)		p VALUE (t TEST)	
	n=16		n=15

FIG. 15A

CORTEX

2 mg ALUM 100 µg AN1528		50 µg ALUM 100 µg AN1528	
660-083	295	643-105	385
661-084	3180	644-106	2640
662-085	2480	645-107	2403
633-086	3014	654-108	1741
664-087	5870	655-109	3053
665-088	5978	656-110	5990
693-089	1620	678-111	3360
694-090	35	679-112	1230
695-091	3400	704-114	2680
697-092	2630	705-115	78
698-093	983	706-116	1290
699-094	5327	729-117	3180
701-095	1862	730-118	1833
702-096	1849	731-119	4590
703-097	2239	736-120	1112
739-098	806	737-121	1653
740-099	5303	757-122	992
741-100	459	758-123	4692
800-103	154	808-124	785
801-104	852	809-125	244
		810-126	32
MEDIAN	2051	MEDIAN	1741
p VALUE (M-W)		p VALUE (M-W)	
MEAN	2407	MEAN	2140
ST. DEV.	1913	ST. DEV.	1659
% CV	79	% CV	78
p VALUE (t TEST)		p VALUE (t TEST)	
	n=20		n=21

FIG. 15B

CORTEX

25 µg QS21 100 µg AN1528		CFA/IFA 100 µg AN1792	
615-128	1257	539-068	693
616-129	361	640-069	508
617-130	1008	641-070	440
536-131	3290	642-071	467
637-132	2520	690-072	42
638-133	3880	691-073	2491
744-134	627	692-074	121
745-135	58	795-075	137
746-136	2610	796-076	822
747-137	1509	797-077	475
769-138	1788	748-087	600
770-139	988	749-079	78
773-140	1199	750-080	1267
774-141	339	751-081	1351
775-142	402	761-082	69
776-143	537		
840-144	1119		
841-145	194		
821-146	1259		
822-147	5413		
823-148	2233		
MEDIAN	1199	MEDIAN	475
p VALUE (M-W)		p VALUE (M-W)	0.0481
MEAN	1552	MEAN	637
ST. DEV.	1364	ST. DEV.	655
% CV	88	% CV	103
p VALUE (t TEST)		p VALUE (t TEST)	0.0106
	n=21		n=15

FIG. 15C

CORTEX

5 µg THIMEROSAL/PBS 10 µg AN1792		2 mg ALUM 100 µg AN1792	
635-149	1337	610-001	432
669-150	4644	611-002	1012
670-151	6335	612-003	3607
673-152	3700	613-004	508
674-153	2750	620-005	465
676-154	1687	621-006	16
681-156	185	622-007	28
682-157	8031	623-008	217
683-158	3450	708-009	2738
754-159	157	709-010	927
755-160	6857	710-011	1609
756-161	482	716-012	1608
805-162	524	784-014	3890
806-163	397	785-015	1614
807-164	234	786-018	285
		787-017	3102
		788-018	1617
		789-019	1474
		815-020	424
		816-021	1375
		817-022	2323
MEDIAN	1687	MEDIAN	1375
p VALUE (M-W)		p VALUE (M-W)	0.5000
MEAN	2718	MEAN	1394
ST. DEV.	2685	ST. DEV.	1166
% CV	99	% CV	84
p VALUE (t TEST)		p VALUE (t TEST)	0.2650
	n=15		n=21

FIG. 15D

CORTEX

50 µg MPL 100 µg AN1792		25 µg QS21 100 µg AN1792	
646-023	2002	627-045	91
647-024	147	628-046	3397
648-025	1304	631-049	3702
649-026	34	632-050	1776
650-027	980	667-052	1832
724-028	1282	668-053	3023
726-030	1966	686-054	189
727-031	733	687-055	891
720-032	2563	688-056	240
721-033	5563	689-057	110
802-034	113	712-059	3311
803-035	671	825-061	1009
804-036	51	826-082	18165
811-037	613	827-063	73
812-038	332	828-064	78
813-039	1454	837-065	1051
814-040	2441	838-066	270
833-014	742	839-067	371
834-042	40		
836-044	807		
MEDIAN	774	MEDIAN	950
p VALUE (M-W)	0.1710	p VALUE (M-W)	0.4076
MEAN	1192	MEAN	2199
ST. DEV.	1299	ST. DEV.	4187
% CV	109	% CV	190
p VALUE (t TEST)	0.1506	p VALUE (t TEST)	0.8131
	n=21		n=18

FIG. 15E

FIGS. 15 A—E: Aβ levels in the cortex of 12-month old PDAPP mice treated with AN1792 or AN1528 in combination with different adjuvants. The Aβ level for individual mice in each treatment group, and the median, mean, and p values for each treatment group are shown.
FIG. 15A: The values for mice in the PBS-treated control group and the untreated control group.
FIG. 15B: The values for mice in the AN1528/alum and AN1528/MPL-treatment groups.
FIG. 15C: The values for mice in the AN1528/QS21 and AN1792/Freund's adjuvant treatment groups.
FIG. 15D: The values for mice in the AN1792/Thimerosol and AN1792/alum treatment groups.
FIG. 15E: The values for mice in the AN1792/MPL and AN1792/QS21 treatment

groups.

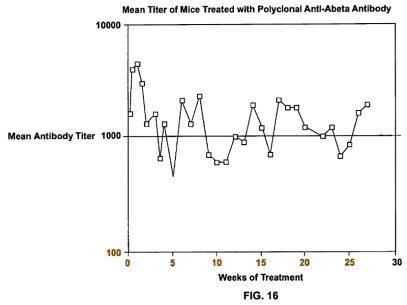


FIG. 16: Mean titer of mice treated with polyclonal antibody to Aβ.

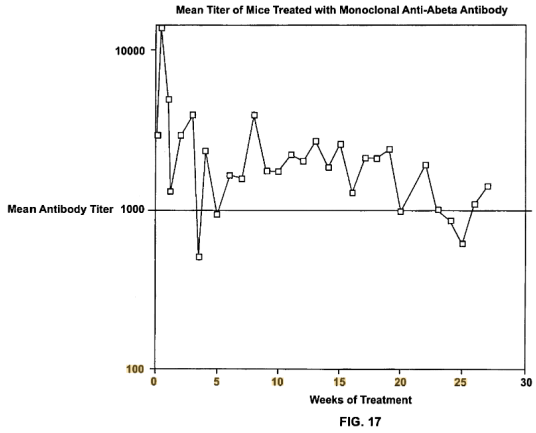


FIG. 17: Mean titer of mice treated with monoclonal antibody 10D5 to Aβ.

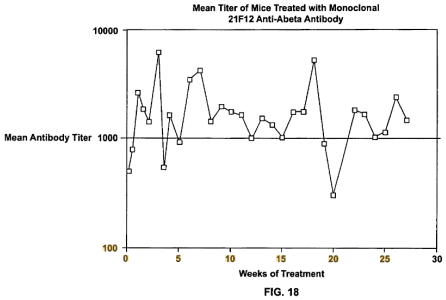


FIG. 18: Mean titer of mice treated with monoclonal antibody 2F12 to Aβ.

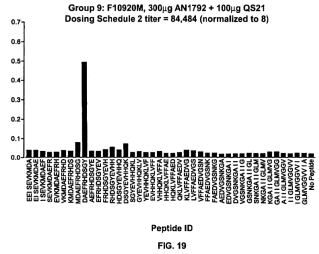
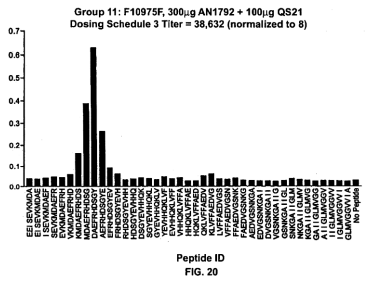


FIG. 19: Epitope Map: Restricted N-terminal Response. Day 175 serum from cynomolgus monkeys was tested by ELISA against a series of 10-mer overlapping peptides (SEQ ID NOS:1-41) covering the complete AN1792 sequence. Animal number F10920M shows a representative N-terminal restricted response to the

	peptide DAEFRHDSGY (SEQ ID NO:9) which covers amino acids 1-10 of the AN1792 peptide which was used as immunizing antigen.  FIG. 20: Epitope Map: Non-restricted N-terminal response. Day 175 serum from cynomolgus monkeys was tested by ELISA against a series of 10-mer overlapping peptides (SEQ ID NOS:1-41) covering the complete AN1792 sequence. Animal number F10975F shows a representative non-restricted N-terminal response. Reactivity is seen against the two peptides N-terminal and one peptide C-terminal to the peptide DAEFRHDSGY (SEQ ID NO:9) which covers amino acids 1-10 of the AN1792 peptide.
VOCAB: (w/definition)	Amyloidogenesis- the formation or growth of amyloid structures Immunoglobulins- antibodies Prophylactic- intended to prevent disease. Transgenic- relating to or denoting an organism that contains genetic material into which DNA from an unrelated organism has been artificially introduced. Astrocytes- a subtype of glial cells that make up the majority of cells in the human central nervous system (CNS)
Cited references to follow up on	https://patents.google.com/patent/US4666829A/en https://patents.google.com/patent/US4713366A/en
Follow up Questions	- What specific stage does this treatment become usable in? - Similar to article 8, how can we be sure that this would correlate to human bodies? After all, the tests used were from mice. - Does the research still hold true? According to Google, the patent expired in 2020. - The article mentioned beta-amyloid is visible in Down Syndrome as well. Does it cause Down Syndrome-linked dementia?

Article #10 Notes: PATENT- Method of treating Alzheimer's disease

Source Title	Method of treating Alzheimer's disease
Source citation (APA Format)	Davis, Bonnie. Method of treating Alzheimer's disease. United States US4663318A, filed January 15, 1986, and issued May 5, 1987. https://patents.google.com/patent/US4663318A/en.
Original URL	https://patents.google.com/patent/US4663318A/en
Source type	Patent
Keywords	Galantamine, Intracerebroventricular, Acid addition, treatment, Cognitive function enhancement
#Tags	#competitor #treatment
Summary of key points + notes (include methodology)	There was a summary given by the patent itself: A method for treating Alzheimer's disease and related dementias which comprises administering to mammals, including humans, an effective Alzheimer's disease cognitively-enhancing amount of galanthamine or a pharmaceutically-acceptable acid addition salt thereof. A radioactively-labeled form of the molecule may also serve as a diagnostic test for Alzheimer's disease. Notes done with the aid of GhatGPT, an AI service. Looked over manually– corrections and additional information necessary has been added. - Novel method for treating Alzheimer's disease via galanthamine or its acid addition salts to enhance cognitive function. - Background: - Galanthamine's properties: - Anticholinesterase effects established in studies. - Observed effects: - Increased plasma cortisol and ACTH in anesthetic patients. - Induction of θ-rhythm on EEG in rabbits. - Enhanced short-term memory in dogs. - Antagonistic effect on scopolamine-induced amnesia in rats. - Alzheimer's Disease Context:

- Disease burden causing distress to patients and caregivers.
- Lack of effective treatments leads to high societal costs.

- Objective: Improve cognitive function in Alzheimer's patients.

- Summary of the Invention:
- Administering galanthamine or its pharmaceutically-acceptable acid addition salts to mammals (including humans) for treating Alzheimer's disease and related dementias.
- Potential use of a radioactively-labelled form of galanthamine for Alzheimer's diagnosis.

Detailed Description of the Invention:
- Forms of Administration: Oral, subcutaneous, intravenous, or intracerebroventricular via an implanted reservoir.
- Chemical Forms: Hydrobromide, hydrochloride, methylsulfate, or methiodide forms of galanthamine or its salts.
- Solubility: Generally sparingly soluble in water at room temperature; necessitates aqueous suspension for injectable compositions, possibly with suspension aids.
- Dosage Ranges: Varying dosages based on administration method and body weight:
  - Injections: 5-1,000 mg per day.
  - Oral: 10-2000 mg per day.
- Oral Formulations: Tablets, capsules, sustained-release preparations containing galanthamine hydrobromide.
- Animal Model for Alzheimer's Disease: Specific lesion in subcortical nucleus in rats to simulate Alzheimer's disease and assess drug efficacy.
- Specific Formulations for Treatment: Tablets, capsules, parenteral solutions, and oral liquid formulations with specified galanthamine hydrobromide concentrations.
- Consideration for Side Effects: Reports of cardiac arrhythmias suggest the potential use of propantheline bromide for management.

- Claims:
1. A method of treating Alzheimer's disease and related dementias which comprises administering to a patient suffering from such a disease a therapeutically effective amount of galanthamine or a pharmaceutically-acceptable acid addition salt thereof.
2. A method according to claim 1, wherein the administration is parenteral at a daily dosage of 5-1,000 mg of galanthamine or a pharmaceutically-acceptable acid addition salt thereof.
3. A method according to claim 2, wherein said dosage rate is 50-300 mg per day.
4. A method according to claim 1, wherein said administration is oral and is in the range 10-2000 mg per day.
5. A method according to claim 4, wherein said dosage rate of 100-600 mg per day.
6. A method according to claim 1, wherein galanthamine is administered at a

	dosage rate of 0.1 to 4 mg/kg body weight of a patient, parenterally. 7. A method according to claim 1, wherein galanthamine is administered intracerebroventricularly via an implanted reservoir at a dosage rate of 0.01 to 5.0 mg/kg day. This invention proposes a comprehensive method of treating Alzheimer's disease using galanthamine through various administration routes and dosage ranges, acknowledging potential side effects and management strategies.
Research Question/Problem/ Need	The central aim of the patent is to propose and protect a method for treating Alzheimer's disease and related dementias by administering galanthamine or its pharmaceutically-acceptable acid addition salts.
Important Figures	None in this patent
VOCAB: (w/definition)	Galantamine- used to treat mild to moderate dementia (memory loss and mental changes) that is a sign of Alzheimer's disease. Galantamine will not cure Alzheimer's disease, and it will not stop the disease from getting worse. Intracerebroventricularly- a route of administration for drugs via injection into the cerebral ventricles so that it reaches the cerebrospinal fluid (CSF). Subcutaneous- situated or applied under the skin. Parenterally- situated or occurring outside the intestine
Cited references to follow up on	https://patents.google.com/patent/WO1988008708A1/en https://patents.google.com/patent/US4897388A/en
Follow up Questions	Have there been any clinical trials conducted to validate the effectiveness of galanthamine in treating Alzheimer's disease? What were the observed outcomes or improvements in cognitive function in patients undergoing this treatment? Are there any specific subgroups of Alzheimer's patients that responded more positively to this treatment?

Article #11 Notes: Artificial Intelligence in Biological Sciences

Source Title	Artificial Intelligence in Biological Sciences
Source citation (APA Format)	Bhardwaj, A., Kishore, S., & Pandey, D. K. (2022). Artificial Intelligence in Biological Sciences. Life *(Basel, Switzerland)*, *12*(9), 1430. https://doi.org/10.3390/life12091430
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9505413/
Source type	Journal Article
Keywords	artificial intelligence, biotechnology, agriculture, medicine, crop yield, life science
#Tags	#AI #Replication #Healthcare #Agriculture #Sustainability #Optimization #Biotech
Summary of key points + notes (include methodology)	Notes done with the aid of GhatGPT, an AI service. Looked over manually–corrections and additional information necessary have been added The article discusses the transformative impact of AI, particularly in replicating human-like intelligence and decision-making abilities, within the context of Industry 4.0. It highlights how AI's object recognition and decision-making capabilities can be applied to various fields, including medical research, agriculture, and bio-based industries, to improve sustainability. In the medical field, AI can aid in early disease prediction and precise personalized medicine, potentially saving lives and reducing healthcare costs. Precision farming, another area benefiting from AI, involves optimizing agricultural practices, reducing production costs, and meeting the food demand of the growing global population. The article emphasizes how AI can address challenges in these sectors and enhance efficiency in the production of biotechnological products. Definition and Roots of Artificial Intelligence (AI) - AI refers to machines simulating higher organism intelligences. - AI has roots in philosophy, mathematics, computing, psychology, and biology. - John McCarthy coined the term "artificial intelligence" in 1956. - Alan Turing is considered the father of AI and initiated the concept of self-learning machines. - Turing's "The Turing Test" and paper "Computing Machinery and Intelligence" were pivotal in AI history.

- Early AI milestones include Arthur Samuel's work and the creation of IBM's Deep Blue.

Components and Techniques of AI

- AI is self-aware, logical, and capable of learning from experience.
- Algorithms based on machine learning (ML) and deep learning (DL) are fundamental in developing intelligent systems.
- AI can be categorized into Narrow or Weak AI and Artificial General Intelligence or Strong AI.
- Weak AI automates tasks, such as Tesla's autopilot or voice assistants.
- Strong AI simulates human cognitive qualities and emotions, though it's still in early development.
- AI techniques include rule-based systems and artificial neural networks (ANNs).
- AI systems share characteristics of knowledge representation, learning, implicit or explicit rules, and search methods.
- Rule-based AI relies on predefined instructions, while ML extracts rules from training data.

AI in Various Fields

- AI has made its way into biology, aiding in areas like protein structure identification and drug discovery.
- Advances in information technology and biology have generated vast amounts of data.
- Computational resources and big data are closely linked.
- AI can efficiently store and process large volumes of raw data.
- AI applications in biology include genomic data analysis and medical image processing.
- AI-based tools automate complex production processes and drug development.
- AI's role in analyzing big data is crucial for biotechnology and bioinformatics.

AI in Medicine and Biotechnology

- AI has significant potential in drug discovery and novel therapeutic molecule development.
- Machine learning aids in disease diagnosis and precision medicine.
- AI contributes to disease detection, diagnosis, and prediction.
- AI-based biomarkers assist in patient diagnosis, treatment response, and survival prediction.
- AI advances improve the efficiency and reliability of epidemiological models.

Title: Structured Notes on the Application of AI in Precision Medicine, Healthcare, and Agriculture

Precision Medicine in Healthcare

- Precision medicine considers individual differences in genetics, ecology,

	and lifestyle. - Metabolic, physical, physiological, and genetic makeup influence drug responses. - AI is enabling the transition to personalized medicine by collecting and analyzing vast amounts of patient data. - AI-driven predictions assist in selecting optimal therapeutic molecules. - AI helps visualize drug effects on DNA, RNA, and proteins. - AI systems like IBM Watson personalize treatment plans based on medical history and genetic data. - Personalized medicine reduces treatment costs and minimizes drug side effects. - AI simplifies gene editing, radiography, and drug management. - AI improves electronic health records with clinical decision support systems. - AI contributes to the development of theoretical models of disease pathophysiology. - AI applications in cardiology, dermatology, and oncology outperform human diagnosis. - AI can detect diseases early, improving prognosis and treatment. AI in Pharmaceutical Industry - AI accelerates drug discovery and reduces the need for clinical trials. - AI may forecast the onset of genetically predisposed diseases. - AI-based platforms like "Open Targets" explore drug-disease relationships. - AI contributes to quantitative structure-activity relationship (QSAR) studies for drug development. - Natural language processing (NLP), ML, and robotic process automation enhance medical practices. - AI models have FDA approval for various medical applications. - AI systems, such as IDx-DR, assist in the diagnosis of diseases with high accuracy. - Clinical trials demonstrate the efficacy of AI and ML models in medical decision making. - AI contributes to cost-effective medical treatment and drug selection. AI in Agriculture - AI is essential in addressing the challenges of modern agriculture. - Agriculture faces obstacles like pest infestations, inefficient use of resources, and inefficient harvesting. - AI in agriculture involves soil management, water assessment, precise mapping, and yield prediction. - Drones and robots equipped with AI improve monitoring and harvesting. - AI processes drone-collected data for decision-making in agriculture. - AI predicts environmental variables that affect crop yields. - Machine vision and high-throughput phenotyping accelerate crop improvement. - AI-based biosensors detect diseases in crop plants.

	- AI-driven drone technology is used for plant disease detection. - AI aids in early disease detection in crop plants, reducing product loss. - AI-enhanced biosensors and drone technologies improve plant health monitoring. - AI simplifies the identification of genetic targets and the design of synthetic promoters for improving agronomic traits in plants. - AI-based agricultural forecasting and prediction enhance crop productivity. - Image datasets analyzed using AI algorithms like ANN and genetic algorithms predict crop yields with high accuracy. - AI models effectively predict greenhouse crop yields using RNN and TCN algorithms. - Prior yield datasets play a crucial role in accurate future crop productivity predictions. - AI-driven approaches aim to reduce the use of harmful agro-chemicals for sustainable agriculture. - Remote sensing assisted control systems (RSCS) utilize AI and ML for optimizing resource management. - AI technology reduces overuse of herbicides through smart sprayers. - Integrating image-based features with omics data can identify traits related to stress tolerance and climate resilience in crops. - AI enables farmers to increase productivity and quality with fewer resources. - AI models can handle multi-omics datasets and predict complex traits under various environmental conditions. AI in industrial Biotechnology - AI and ML are applied in industrial biotechnology for the efficient processing and manufacturing of chemicals, medicines, and biochemistry-related products. - ML and AI-based technologies aid in the design and testing of pharmaceuticals, reducing time to market. - AI technologies, IoT, ML, and robotics improve efficiency and product quality in biotechnology processes. - AI models help optimize growth conditions for strains in bioengineering processes. - AI models are used to scale up and optimize bioprocesses for enzyme production on a pilot scale. - Biofuel production benefits from AI predictions and optimization using AI-based approaches. - AI is utilized for forecasting biomass feedstock properties, bioenergy end-uses, and bioenergy supply chains. - AI models predict and maximize biofuel production through torrefaction and pyrolysis. - Systems metabolic engineering employs AI to optimize microbial strains for long-term chemical production. - AI and ML techniques influence biotechnology businesses to adopt AI-based systems.

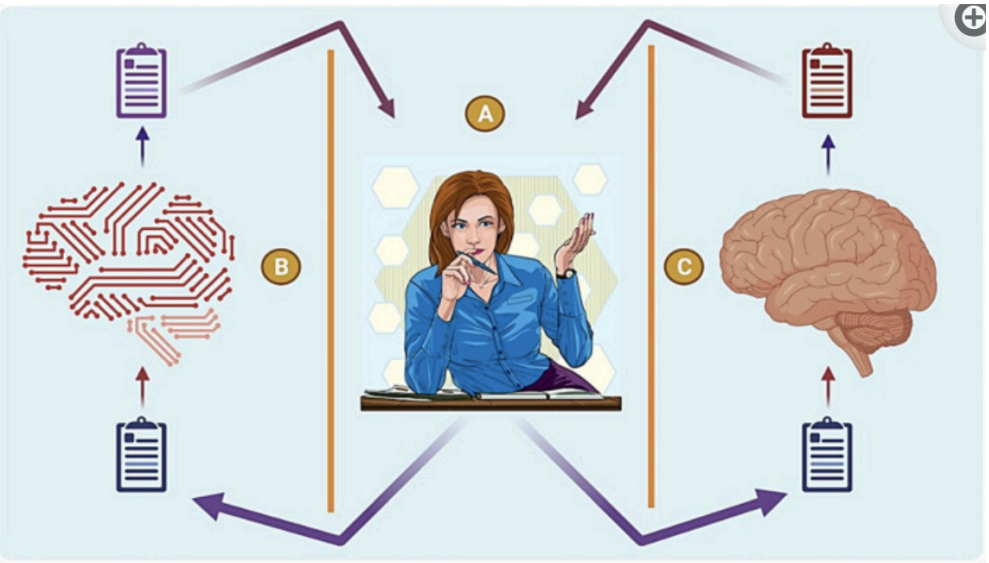
- AI technology is applied to assess the quality traits and aroma of beer in the brewing industry.

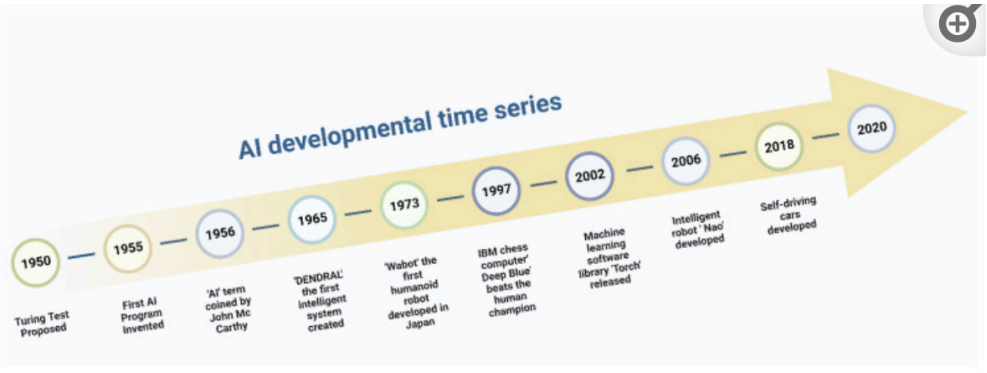
Challenges and Considerations

- The widespread adoption of AI-based technologies in genetics, agriculture, and biotechnology is still a work in progress.
- AI models have the potential to reinforce biases present in training data.
- Privacy, security, and data handling are significant challenges in digital agriculture.
- The cost and regulatory barriers exist for implementing AI-based technologies in agriculture, such as AI-driven drones.
- Data mining methodologies need to be adapted for geographically scattered agricultural data.
- Protocols for adopting AI algorithms and assessing dataset size are required in industrial biotechnology.
- Challenges include a lack of comparative studies across AI-ML designs and issues related to the black-box nature of some AI models.
- Inefficient data integration and the need for a skilled workforce are hurdles in AI-based biotechnology.
- Extensive datasets and relative studies are necessary for real-time monitoring and control of bioreactors and bioprocesses.

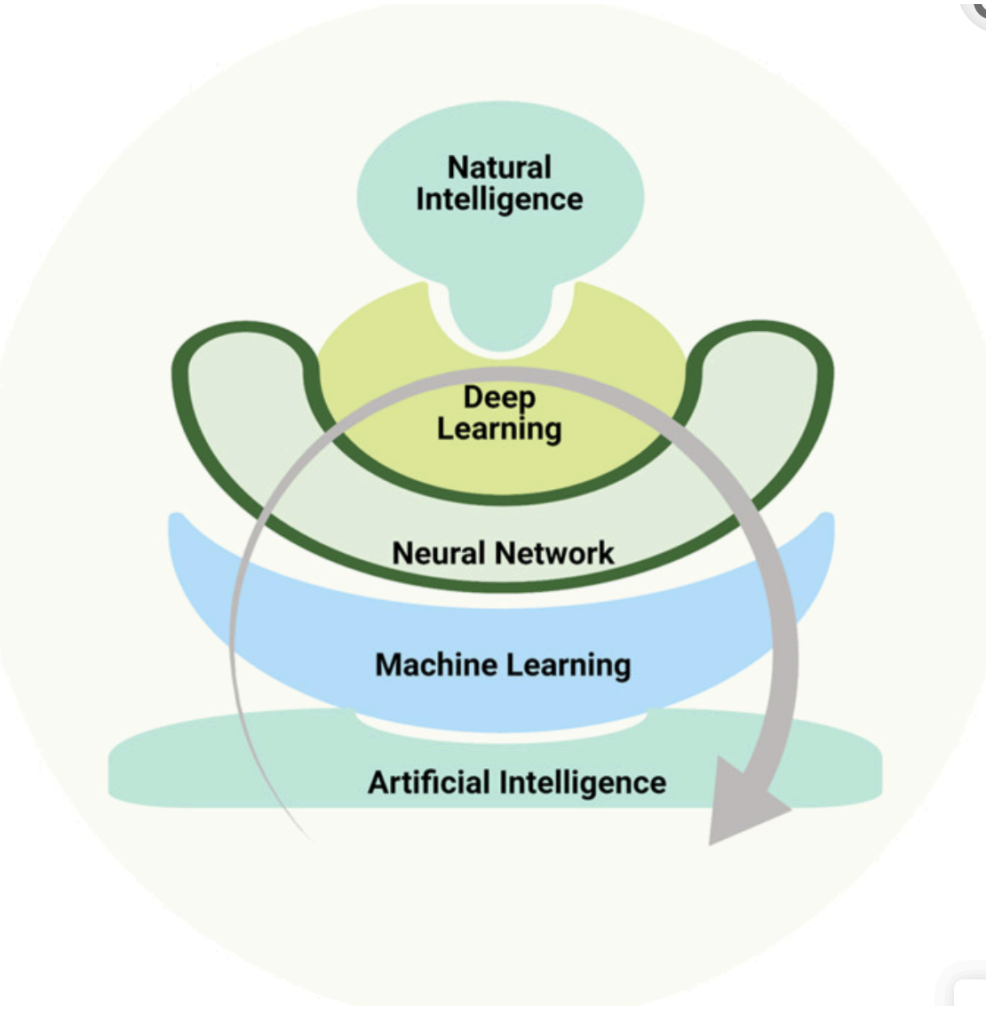
Conclusions

- Industry 4.0 showcases the ability of machines to mimic the intelligence of living systems, particularly human intelligence.
- A key feature of biological systems is their ability to recognize objects and make decisions.
- AI has made significant progress in replicating these cognitive and perceptual abilities, enabling machines to recognize objects and make decisions akin to biological systems.
- AI's potential extends to various sectors, including medical research, agriculture, and bio-based industries.
- In medical science, AI can be harnessed for early disease prediction, precise treatment, and personalized medicine, even during asymptomatic conditions.
- This has the potential to save lives and reduce medical costs significantly.
- In agriculture, AI-driven efficient algorithms and programs enable precision farming, optimizing practices like soil management, water analysis, and modeling fertilizer, pesticides, and crop management.
- AI can revolutionize agricultural practices to meet the increasing food demands of the world's growing population.
- Large-scale production often faces challenges due to numerous variable factors that increase costs.
- AI-based programs and computer models are becoming highly efficient in optimizing conditions for maximum output while minimizing costs.
- This efficiency applies to various sectors, including agriculture, medicine, biotechnology, and lifestyle products.
- For instance, AI-driven optimization of bio-enzyme production is a notable

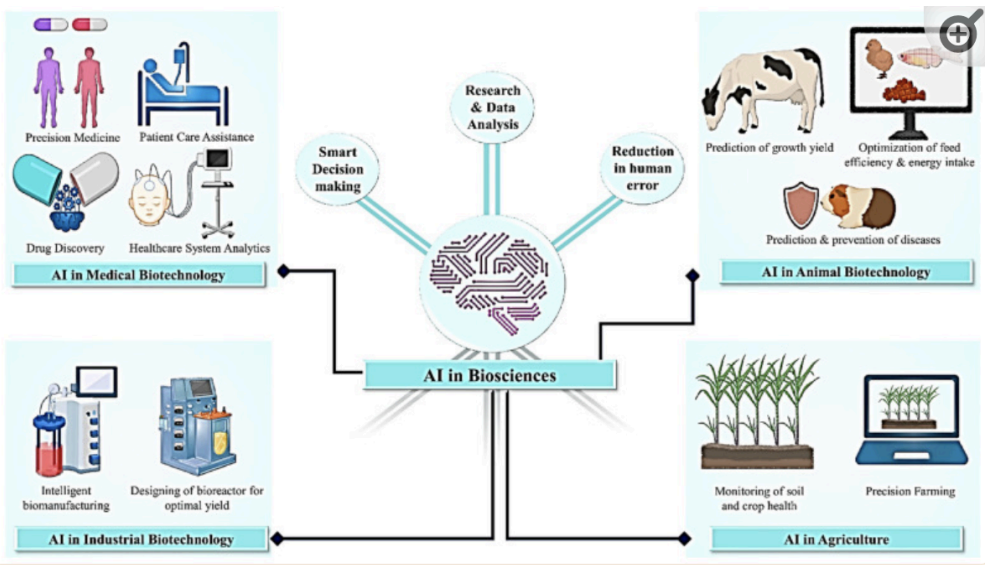
	success story, illustrating how AI can reduce production costs in the biotech industry. - The application of AI in various fields offers the potential to transform industries and improve efficiency, saving both lives and resources. - The reduction of production costs, facilitated by AI, is a significant step towards overcoming challenges in large-scale production and ensuring sustainability. - As AI continues to evolve, it is poised to play a pivotal role in shaping the future of Industry 4.0 and beyond.
Research Question/Problem/ Need	How can AI's ability to replicate biological intelligence benefit various industries?
Important Figures	 - "Alan Turing designed the Turing Test in 1950. This test includes three participants, a human interrogator, an intelligent machine and another human who we can call A, B and C, respectively. A is not aware of the identity of B and C, and A can send and receive response in only the form of text messages from B and C. A may ask B and C, a variety of questions, and based on their response, if A is unable to distinguish which one of B and C is a computer, then computer B may be considered as intelligent with thinking ability. If a human interrogator A could not distinguish the difference between another human and a computer, then the computer must be intelligent enough to be considered human. This test simply is to figure out whether or not a machine has ability to think."



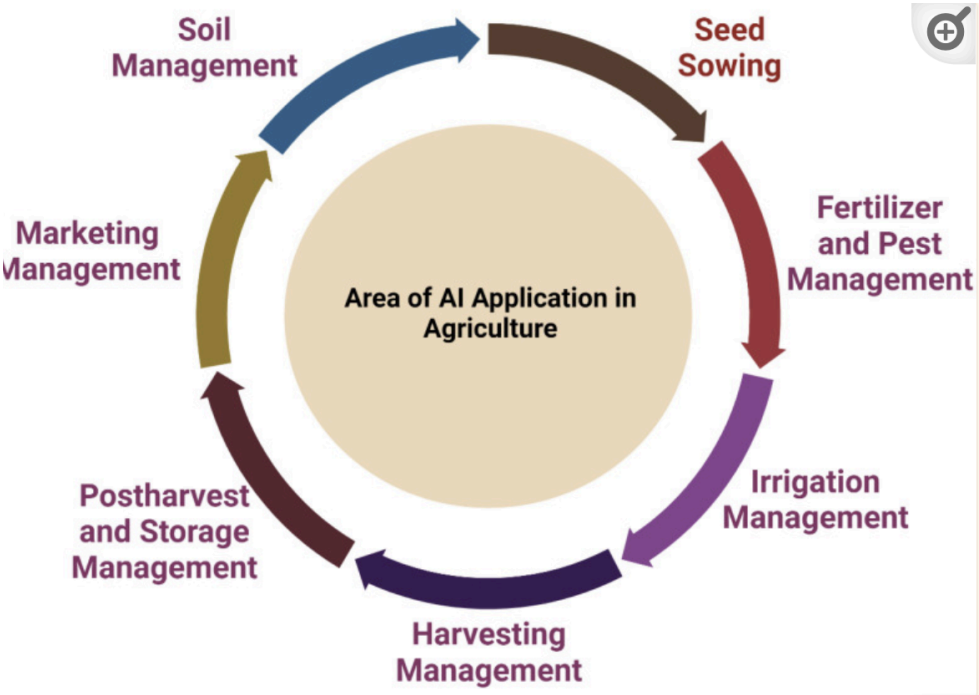
- A timeline with the important breakthroughs in the evolutionary path of artificial intelligence and its application in various fields



- Schematic representation of major components of artificial intelligence and the continuous learning process with the help of natural intelligence to make smarter machines.



- A conceptual model that illustrates the application possibilities of artificial intelligence in the disciplines of health, agriculture, animal science and industrial biotechnology.



- Agricultural fields where artificial intelligence (AI) could have a positive impact.
-

VOCAB: (w/definition)	ANN- Artificial Neural Network Epidemiology- the branch of medicine which deals with the incidence, distribution, and possible control of diseases and other factors relating to health.

	Adenoma- a benign (noncancerous) tumor Polyp- a projecting growth of tissue from a surface in the body, usually a mucous membrane Atrial Fibrillation- an irregular and often very rapid heart rhythm
Cited references to follow up on	- Oliveira A.L. Biotechnology, Big Data and Artificial Intelligence. Biotechnol. J. 2019;14:1800613. doi: 10.1002/biot.201800613. - Costa F.F. Big Data in Biomedicine. Drug Discov. Today. 2014;19:433–440. doi: 10.1016/J.DRUDIS.2013.10.012.
Follow up Questions	- What specific AI technologies are being used in medical research, agriculture, and bio-based industries to replicate human-like intelligence and decision-making capabilities? - How are potential issues related to data privacy and ethical concerns addressed when integrating AI into medical diagnosis and precision farming? - What are some examples of successful AI applications in optimizing production processes for bio-enzymes and other biotech products?

Article #12 Notes: Predictive Modelling in Clinical Bioinformatics: Key Concepts for Startups

Source Title	Predictive Modelling in Clinical Bioinformatics: Key Concepts for Startups
Source citation (APA Format)	Pais R. J. (2022). Predictive Modelling in Clinical Bioinformatics: Key Concepts for Startups. Biotech *(Basel (Switzerland))*, *11*(3), 35. https://doi.org/10.3390/biotech11030035
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9397027/
Source type	Journal Article

Keywords	predictive modelling, clinical bioinformatics, mathematical models, diagnostics, prognostics, clinical applications
#Tags	#main #cs #AI-learning #predictive-AI

Summary of key points + notes (include methodology)	Clinical bioinformatics is a newly emerging field, and the data used to make decisions in this field can in turn become a little overwhelming. This is why mathematical modelling, via a computer model, must be used. This article serves as a precursor for one who would want to create a model for a medical problem.
	
	- Clinical bioinformatics
	  - Newly emerging field
	  - Applies bioinformatics techniques for:
	  - Identification of diseases
	  - Discovery of biomarkers
	  - Therapy decision
	  - Mathematical modelling
	  - Part of bioinformatics analysis pipelines
	  - Fundamental step to extract clinical insights from genomes, transcriptomes, proteomes, metabolomes
	  - Considered advantageous for improving diagnostics and prognostics of diseases
	  - Known as precision medicine
	  - has the potential to enable more personalized and effective medicine due to individual data
	  - Next Generation Sequencing (NGS) and Mass Spectrometry (MS) technologies made characterization of genomes and quantification of proteomes from patients' biological samples with reasonable accuracy and scalability possible
	  - compatible with its application in the clinical point-of-care
	  - **data from these technologies is too complex to be humanly handled and interpreted by clinicians**
	  - Fundamental for providing humanly readable and clinically relevant genomics and proteomics interpretations from NGS and MS techniques
	  - considered as a fundamental bridge between clinicians and 'omics' technology
	  - potential to facilitate the automation of data analysis and opens the possibility for "big data" processing
	  - Branch is expected to play a central role in facilitating the identification of genetic diseases,the discovery of novel biomarkers, characterization of pathogens and enable a more

informed decision for the therapeutical strategy to follow

- **Predictive Modelling**
  - researchers have been proposing the combination of predictive modelling approaches with bioinformatics for improving current practices in disease identification, therapeutics and prognostics
    - shown advantageous to unlock full potential of high-throughput technologies as solutions for large population screening of multiple disorders and precision medicine
  - Basic explanation: any mathematical abstraction of a system which generates a prediction of an unknown system component/property based on known system components
    - For biomedical systems of interest, the components would be:
      - Biological relevant and measurable or observational entities (dependent variables), which are the inputs of the model.
      - Relational factors between variables with or without biological meaning (parameters), which can be estimated empirically or based on data fitting methodologies.
      - Unknown clinical entities or properties of interest for prediction (dependent variables), which are the outputs of the model.
  - These can be "translated" as a conjunction of mathematical equations, graphs containing processes with gate decisions or even more complex mathematical objects
  - should be as accurate as possible in descriptions of the system and validated against enough data in an unbiased and independent manner
  - **Always a certain degree of uncertainty associated with any prediction generated by any model**
  - predictive models should be seen as insights that enable a clinician to make a better informed and supported decision
- **[see figure 1 below for most frequently used clinical modelling techniques]**
- most straightforward application of predictive models is the capacity of generating a prediction for the future
  - can be applied in the clinical context for the generation of disease prognostics for example for predicting:
    -
  - Successful examples include the modelling efforts conducted for predicting impact and control of SARScov2 transmission effects and control during the COVID-19 outbreak
    - Statistical and Ordinary Differential Equations based models were successfully used for predicting expected peaks of infected, hospitalized and the timings by which

the peaks occurred
        - simulations from Susceptible and Infected ODE models also predicted useful information for the decision of implementing controlling measures that minimize the total of deaths in a certain region
    - Also useful for detection of diseases in an early stage, in particular, if current diagnostic methods fail and the treatment efficiency benefits from early detection
        - Example: poor detection rates of 40% observed during ovarian cancer screening programs
            - does not show symptoms up to later stages and by then the treatment success is largely compromised, but some statistical and machine learning models have successfully combined multiple biomarkers resulting in surprisingly high sensitivities (>90%) and reasonable specificities (> 80%) which largely outcompete the sensitivities and specificities obtained under current screening programs
    - Generation of insights when current diagnostic methodologies are too invasive and put health of testing subjects at risk
        - Example: detection of genetic diseases in prenatal screening by amniocentesis and pre-implantation embryo testing using post-freezing PGT-A next-generation sequencing techniques
            - the procedures for conducting genetic testing are too invasive, compromising the viability of pregnancy and embryo survival during implantation
                - Machine learning models have been quite successful in predicting aneuploidies from indirect data such as embryo secretome in culture media and urine
                - predictive models from mass spectral patterns of secretome have rendered sensitivities very close to the diagnostic level with reasonably tolerable false positive rates, enabling affordable and non-invasive testing
    - Sequence-based prediction of pathogenic genetic variants (Single Nucleotide Polymorphisms, insertions or deletions)
        - Helps with identifying rare genetic diseases
- **Choosing the correct modelling framework**
    - **first gather the available data, available knowledge of the system we want to model and access which is the best suitable modelling framework for that particular case**
        - **Admittedly very tedious and theoretical, but pays off**

- **Saves time later on by preventing reaching dead ends where models do not describe the systems, cannot be validated or their performances are simply not different from flipping a coin**

- Machine-learning is a very powerful approach
  - Ideal for building disease classifiers with yes/no outcomes
- Statistical models
  - conservative modelling approaches used in clinical contexts
  - relies on the choice of a theoretical statistical model and requires the estimation of its parameters with data
  - Often combined with machine learning algorithms for data fitting-based parameter estimation
  - offer an estimated probability of having or not a particular disease
    - advantage over classification models in particular for the scenarios that best describe “gray” zones of uncertainty making them more realistic than the yes/no classification models
  - depends on sample size numbers but often do not require large datasets as in machine learning approaches
  - imple and has a straightforward implementation in laboratory software tools.
- **Deterministic frameworks**
  - **Examples: ODE-based and logical modelling**
  - **Powerful quantitative and discrete modelling approaches (logical)**
  - **by far more descriptive in comparison to statistical and machine learning**
  - **rely on the laws of chemistry, physics, biochemical circuits and mathematics, making them more realistic and robust for finding drug targets and predicting therapeutic effects**
  - **this approach does not rely on sample sizes but requires extensive literature knowledge including knowing parameters and relational laws**
  - **huge investment of effort and time-consuming in comparison to the other frameworks as are more complex in terms of variables, and development and require an huge in-depth knowledge of the system**
  - **may take years to develop and depend on the availability of existing literature data or the capacity to estimate them experimentally**
  - **kinetic models are always preferable to logical as they are more accurate descriptions of the systems and provide a quantitative assessment**
  - **only advantage of choosing logical modelling is in the**

	case where the kinetic parameters (e.g., rate constants of the processes) are unknown This article does have a section titled “Challenges of Clinical Bioinformatics: The Business Perspective”, however I found this to be unrelated to the information I’m looking to receive. If necessary, I will come back for more notetaking.
Research Question/Problem/ Need	Would it be possible to use predictive modeling in clinical bioinformatics?
Important Figures	Table 1 Often used modelling techniques in clinical bioinformatics and their main characteristics. **Modelling Technique** / **Description** / **Application** / **Requirements** Statistical / Scoring and probability functions that assumes a distribution shape or behaviour. / Continuous Quantification / Data for parameter estimation. Depend on sample size. Kinetic / Solving of systems of nonlinear differential equations. Do not assume any behaviour. Instead relies on rate laws of processes such as chemical reactions. / Binary Classification / Requires reported or estimated kinetic parameter. Do not depend on sample size. Logical / Solving of logical equations based on predefined rules for each component. Assumes asynchronous or synchronous update schemes. / Binary Classification / Requires relational knowledge of its components. Do not depend on sample size. Regression / Fitting of an assumed mathematical equation on data. Often are used models that describe a particular assumed data behaviour such as linear, polynomial, exponential, and logistic. / Binary Classification / Data for model fitting. Depend on sample size. Random Forests / Supervised machine leaning algorithm based on averaging multiple generated decision trees. / Binary Classification / Data for model training and validation. Requires large datasets Support Vector Machines / Supervised machine leaning algorithm based on clustering algorithms such as principal component analyses. / Binary Classification / Data for model training and validation. Requires large datasets Neural Networks / Supervised machine leaning algorithm based on defining a set of neuron and layers as model components. Assumes all possible relational interactions between neurons. / Binary Classification / Data for model training and validation. Requires large datasets - Generally self-explanatory: this table gives a general overview on the most popular modeling techniques in medical contexts, their description, application, and requirements
VOCAB: (w/definition)	Transcriptomes- the full range of messenger RNA, or mRNA, molecules expressed by an organism Metabolomes- the scientific study of the set of metabolites present within an organism, cell, or tissue - Metabolite- a substance formed in or necessary for metabolism Bioinformatics- the science of collecting and analyzing complex biological data such as genetic codes. Big Data- extremely large data sets that may be analyzed computationally to reveal patterns, trends, and associations, especially relating to human behavior and interactions. Precision Medicine- innovative approach that uses information about an individual's genomic, environmental and lifestyle information to guide decisions related to their medical management Prognostics- an advance indication or portent of a future event - Portent- a sign or warning that something, especially something momentous or calamitous, is likely to happen.

- (in article, used for the prediction of contracting a disease)

Proteomes- the entire complement of proteins that is or can be expressed by a cell, tissue, or organism.

Ordinary Differential Equations- an equation which consists of one or more functions of one independent variable along with their derivatives.

Amniocentesis- the sampling of amniotic fluid using a hollow needle inserted into the uterus, to screen for developmental abnormalities in a fetus.

- Amniotic- relating to the amnion
  - Amnion- the innermost membrane that encloses the embryo of a mammal, bird, or reptile.

Secretome- the global study of proteins that are secreted by a cell, a tissue or an organism

Spectral Patterns- one of a series of linear images formed by a spectrograph or similar instrument and corresponding to a narrow portion of the spectrum of the radiation emitted or absorbed by a particular source.

- Spectrograph- sometimes called a spectroscope or spectrometer — breaks the light from a single material into its component colors the way a prism splits white light into a rainbow.

Single Nucleotide Polymorphisms- a genomic variant at a single base position in the DNA

SIFT- scale-invariant feature transform; a computer vision algorithm to detect, describe, and match local features in images, invented by David Lowe in 1999.

Fathmm (incorrectly(?) spelled Fathmn in article)- Functional Analysis through Hidden Markov Models (v2.3); A high-throughput web-server capable of predicting the functional consequences of both coding variants, and non-coding variants in the human genome.

- Hidden Markov Model- a statistical model that was first proposed by Baum L.E. (Baum and Petrie, 1966) and uses a Markov process that contains hidden and unknown parameters
  - Markov process- a random process indexed by time, and with the property that the future is independent of the past, given the present.

Phanter- ??

Polyphen-2- a tool which predicts possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations.

Neural Networks- a method in artificial intelligence that teaches computers to process data in a way that is inspired by the human brain

Recurrent Neural Networks- a type of artificial neural network commonly used in

	speech recognition and natural language processing. Recurrent neural networks recognize data's sequential characteristics and use patterns to predict the next likely scenario. R Packages- collections of functions, data, and compiled code in a well-defined format, created to add specific functionality in the statistical programming language known as R Black Box Models- device, system, or object which produces useful information without revealing any information about its internal workings. Hemoglobinopathy- genetic disorders which are characterized by the inheritance of either an abnormal hemoglobin as in sickle cell disease or an insufficient production of hemoglobin chains as in thalassemia. - Hemoglobin(I know this definition, but I think it is still beneficial to note down)- the protein contained in red blood cells that is responsible for delivery of oxygen to the tissues SaaS- Software as a Service; allows users to connect to and use cloud-based apps over the Internet. Examples include email, calendaring, office tools, etc.
Cited references to follow up on	- 62. Tng S.S., Le N.Q.K., Yeh H.-Y., Chua M.C.H. Improved Prediction Model of Protein Lysine Crotonylation Sites Using Bidirectional Recurrent Neural Networks. J. Proteome Res. 2022;21:265–273. doi: 10.1021/acs.jproteome.1c00848. - Simon R. Genomic Biomarkers in Predictive Medicine: An Interim Analysis. EMBO Mol. Med. 2011;3:429–435. doi: 10.1002/emmm.201100153.
Follow up Questions	- Which model would be the best fit for compiling different FMRI* scans for a general understanding of the progression of Alzheimer's? - HIPAA laws would definitely hinder the accumulation of data needed for models. Would this affect the accuracy of models? - Where are some databases for "big data" for Alzheimer's? - If data needs to be accumulated manually, what would be considered "enough" for a model? * I'm still not sure what marker I'll be using, so FMRI may not be the scan I end with

Article #13 Notes: Cell survival following direct executioner-caspase activation

Source Title	Cell survival following direct executioner-caspase activation
Source citation (APA Format)	Nano, M., Mondo, J. A., Harwood, J., Balasanyan, V., & Montell, D. J. (2023). Cell survival following direct executioner-caspase activation. *Proceedings of the National Academy of Sciences*, *120*(4), e2216531120. https://doi.org/10.1073/pnas.2216531120
Original URL	https://www.pnas.org/doi/10.1073/pnas.2216531120#data-availability
Source type	Research Article
Keywords	Caspase, cell death, apoptosis, experiment, proof by data
#Tags	#caspase-function #potential-marker #cell-death #experiment

Summary of key points + notes (include methodology)	In order to answer the question "Can cells recover from direct caspase activation without pro-survival stress responses induced by drugs?", Nano's team engineered a HeLa cell line to express caspase-3, an executioner caspase, combining it with what is known as a quantitative caspase activity reporter. High caspase activity levels unsurprisingly killed all cells while low levels kept the cells alive. However, caspase activity doses fit to kill 15 to 30% of cells still let 70 to 85% to live. With these doses, the rate, peak level, or the total amount of caspase activity wasn't able to accurately predict cell death vs. survival. To conclude, it was determined that cells were in fact able to "survive direct executioner-caspase activation, and variations in cellular state modify the outcome of potentially lethal caspase activity".
	
	- Apoptosis
	  - Form of programmed cell death
	  - Required for tissue homeostasis
	  - Balance between cell survival, multiplication, and death allows for proper development and health of multicellular organisms
	  - Excessive cell death occurs in degenerative diseases, ischemia, and immune disorders
	  - Survival of abnormal cells can directly support cancer development and recurrence
	  - Cancer cells can also use apoptosis as a weapon, by triggering T cell death
	  - Initiated by death-inducing ligands or by intrinsic damage
	  - Culminates in activation of caspases

- Dismantles cell by cleaving numerous substrates
- Apoptosis is onsidered irreversible after activation + Mitochondrial Outer Membrane Permeabilization (MOMP)

- Recent results state cells can survive apoptotic stimuli in what is known as fractional killing, failed apoptosis, or ananstasis
  - Anastasis is defined as survival from transient stimulus which activates executioner-caspase + would be lethal if sustained
    - observed in multiple cell types in vitro + in vivo
      - Mouse neurons
      - Drosophilia tissues
    - Molecular mechanisms supporting cell survival include proteins such as Snail, Akt1, and dCIZ1
- Small molecules commonly used to induce apoptosis are pleiotropic
  - staurosporine is a broad-spectrum kinase inhibitor
  - ethanol induces many kinds of cellular stress and damage
  - The question that arises: can cells recover from direct executioner-caspase activation in the absence of concomitant pro-survival stress responses?
    - Team engineered a system to study effects of caspase-3 activation
      - high doses of caspase uniformly led to cell death and low doses to cell survival, while intermediate caspase activity was compatible with either outcome
        - Conclusion: heterogeneities in cell states determine the outcome of exposure to levels of caspase activity that are potentially lethal
          - cellular stress is one factor that influences the response to intermediate caspase activity
          - results indicate that, in addition to mechanisms such as genetic drug resistance, anastasis may be a source of tumor repopulating cells and recurrence after apoptosis-inducing cancer treatments.
- Experiment - Figure 1: “Precise Control of Apoptotic Stimulation with Photoactivatable CaspaseLOV.”
  - Assessed whether cells can recover from direct

executioner-caspase activation
- Generated a stable, monoclonal HeLa cell line lacking caspase 3
  - To titrate caspase activity, a special type of caspase-3 known as CaspaseLOV was used
    - CaspaseLOV uses the blue light-sensitive light-oxygen-voltage (LOV) domain to cage cleaved and activated caspase), under the control of a doxycycline (DOX)-inducible promoter
  - **For monitoring caspase activation, genetically encoded caspase-3 activity sensor GC3AI would be used (GFP-based, caspase-3-like, protease activity indicator), kept in the dark state until cleaved by caspases**
- Live cell imaging confirmed GC3AI cleavage upon caspase activation, generating fluorescent GC3AI (cGC3AI)
- DOX accelerated caspase activation, and blue light further increased the rate
- 75 ng/mL DOX was the lowest concentration inducing consistent GC3AI activation with reliable separation between dark and illuminated cells, so 75 ng/mL DOX was used for all subsequent experiments.
- Light did accelerated the appearance of cGC3AI+ cells, but both dark and illuminated samples reached about ~97% cGC3AI+ cells by 8 h 30 min post-DOX treatment. On the other hand, the untreated controls reached ≤4%.
- GC3AI activation was followed by cell death, as judged by cell rounding, shrinkage, and detachment from the substrate
- CaspaseLOV expression increased from 2 h post-DOX, and cleavage of a validated downstream target of endogenous caspase (poly (ADP-ribose) polymerase, PARP) increased from 5 h post-DOX
- **It was concluded that DOX-inducible caspaseLOV efficiently induces cell death through direct effector caspase activation.**

- Experiment - Figure 2: Can cells undergo anastasis following direct caspase activation?
  - transiently exposed caspaseLOV cells to DOX, plus or minus blue light illumination
  - removed DOX(wash) after five hours and monitored cell survival for at least 20 h
  - 82% of cells activated GC3AI after 5 h illumination, and fraction of cGC3AI+ cells continued rising even after wash, reaching 94% at 10 h 30 min post-wash
  - caspaseLOV activation was slower in the dark
    - 58% of cells activated GC3AI by 5 h, and 92% by 10 h 30 min post-wash
  - with the continued increase in cGC3AI+ cells over time, caspase expression persisted after DOX washout
  - **20 h post-treatment, 51% of cells activating GC3AI still exhibited normal morphology, consistent with cell survival after direct**

caspase activation

- Some cGC3AI+ surviving cells underwent mitosis and thus were neither quiescent nor senescent and contributed to repopulating the well
- cGC3AI was detectable in living cells at even later time points
- Surviving cells contained fewer mitochondria, but retained normal mitochondrial potential
    - Suggests they had specifically lost non-functional mitochondria
    - Surviving cells had a small but significant increase in DNA double strand breaks
        - Did NOT detectably expose phosphatidylserine, which only labeled dead cells
        - Surviving cells were not simply completely resistant to death: the dying fraction increased with increasing concentration or duration of DOX treatment.
- Illumination, including light used for imaging, caused more cells to die
- the population that died could be rescued by the pan-caspase inhibitor Q-VD-Oph (Q-VD), confirming that the death we observed was caspase-dependent
- **It was concluded that direct effector caspase activity sufficient to kill many cells is nevertheless compatible with survival of others.**

- Experiment - Figure 2: Are Anastasis Frequencies Similar in Cells with Induced or Spontaneous Caspase Activity?
    - Absence of DOX meant cells did not express caspaseLOV
        - Although lacking caspase-3, they were able to undergo apoptosis in response of staurosporine
            - This was presumably mediated by caspase-7
    - In the absence of DOX, spontaneous GC3AI activation was rare (<5%) in both dark and illuminated cells
    - **anastasis occurred in ~50% of untreated cells that spontaneously activated cGC3AI+, similar to the frequency for DOX-treated cells**
    - **It was concluded that the probability of anastasis is similar whether caspase is activated spontaneously or artificially and that heterogeneities in the cell population determine life or death in response to potentially lethal caspase levels.**
- Experiment - Figure 3: Surviving Sister Cells Can Have Higher Caspase Levels than Their Dying Siblings.
    - To address whether some feature of caspase dynamics, rather than dose, at the single-cell level determined apoptosis vs. survival, long-term live imaging was conducted.
        - an mCherry marker was used to normalize the cGC3AI signal

- Cell line turned out to be less sensitive to DOX (~21% survival after 24 h of DOX without washout, vs. ~1.6% in parental cells).
- responded well to apoptotic stimulation, since ~24% of the population died after DOX treatment followed by washout + most cells activated GC3AI over time
- Imaging began 30 min post-DOX and every 4 min thereafter, plus or minus blue light
- Cells were washed and imaged overnight after 5 h of DOX
- DOX plus blue laser stimulation promoted cell death

- The fraction of mitotic cells was similar between treated and untreated samples (42% vs. 44%, respectively), confirming that anastatic cells could multiply
  - >98% of daughters from the same mitosis shared the same fate and and exhibited similar cGC3AI dynamics
  - However, 33 sister cell pairs with opposite fates were found, and in 5/33 cases, the surviving sister exhibited a higher level of caspase than its dying sibling
- **It was concluded that the difference between death and survival does not only depend on a specific caspase activity threshold, even in closely related cells, and suggest that additional factors contribute to the outcome when cells experience intermediate caspase activation.**

- **MAIN** Experiment: Caspase Activity Dynamics Does Not Fully Predict Cell Fate.
  - Gentle live imaging was carried out using low laser power to further probe the relationship between caspase activity, GC3AI cleavage, and commitment to apoptosis
  - correlative live and fixed confocal microscopy was used to control for possible differences in levels of **total GC3AI (tGC3AI**)
  - **tGC3AI was measured and surviving cells with average (medium expressors) or high expression levels were selected**
  - Change in caspase activity over time was measured, and cells that lived were compared to those that died.
  - A **logistic regression analysis** was run to address the effectivity of caspase-3 activity in predicting death, where surviving cells were assigned 0, and dying cells were assigned 1
    - These values were plotted against cGC3AI fluorescence
      - If all dying cells had high and all surviving cells had low cG3AI levels, 0s and 1s would segregate completely, indicating that cGC3AI (and consequently caspase-3) is a perfect predictor of fate
        - By contrast, it was found that the fold

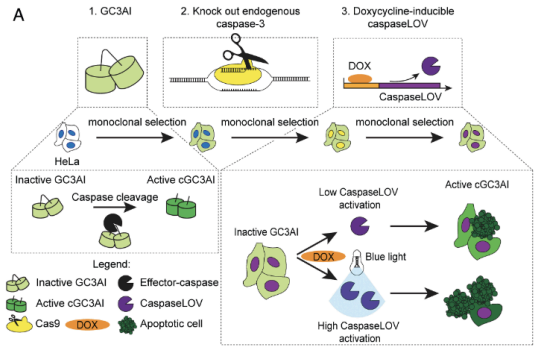
change in caspase activity predicted the fates of only 2% of cells, or 11% when the analysis was restricted to medium expressors

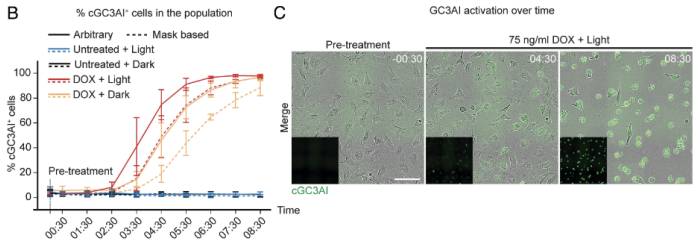
- Roux et al. (2015) reported that the maximal rate of caspase-8 activation predicts cell death with >80% accuracy in cells treated with the death-inducing ligand Trail.
- the rate of caspase-3 activation predicted cell fate better than maximal fold change.
- However, when the rate at which cGC3AI is produced was plotted, the predictive power was only 16%, or 24% when the analysis was restricted to medium expressors
- Dying cells tended to reach GC3AI maximal fold change earlier than surviving cells, while time at which the rate of caspase activity peaked was similar for dying and surviving cells
- the area under the curve (AUC) of cGC3AI in dying and surviving cells to asswss whether total accumulated caspase activity accounts for apoptotic commitment
    - Similar to maximal rate, fold change, and caspase activity timings, the area under the curve was insufficient to explain cell fate choices
- **It was concluded that overall, faster, stronger, or longer caspase activation did not necessarily lead to cell death.**
- To check whether the early phase of apoptotic stimulation told a different story, it was tested again while restricted to this phase
    - even when considering only the first 100 time points of imaging (up to 428 min post-treatment), caspase dynamics did not reliably predict survival vs. death
    - maximal cGC3AI fold change had higher predictive power when considering only its early values (Tjur's R2 = 0.02 vs. 0.15), consistent with faster cGC3AI accumulation in dying cells
    - an inversion in the logistic relationship between cell survival and AUC was found
        - Total AUC was moderately larger in surviving cells because they accumulated cGC3AI for longer.
        - Initial AUC was slightly larger in dying cells, indicating they accumulated cGC3AI faster
- **Direct effector caspase activation is compatible with cell survival. In addition, they show that surviving cells somehow tolerate total levels of caspase activity comparable to those in dying cells.**
- A combination of the caspase dynamics parameters was also tested to check whether it would better predict cell fate
    - This combined resulting numerical factor was known as "caspase dynamics score"
    - when combined, the time derivative, the cGC3AI maximal, and the AUC performed **worse** than the individual

	parameters in predicting whether cells lived or died - When the score was calculated only on the initial dynamics of cGC3AI, its predictive power did not exceed 30%. - **It was concluded that additional factors influence life vs. death fates in response to intermediate levels of caspase activity** *There was more information regarding stress increasing the predictive power of the rate of caspase activation. However, this is an external factor which would most likely not be present in Alzheimer's. If otherwise, I will come back to this article.*
Research Question/Problem/ Need	"Can cells recover from direct activation of executioner-caspase, or is survival a consequence of drug-induced stress responses that occur together with death signaling? And, to what extent do caspase signaling levels and/or dynamics determine cell fate?"

Important Figures

- Figure 1



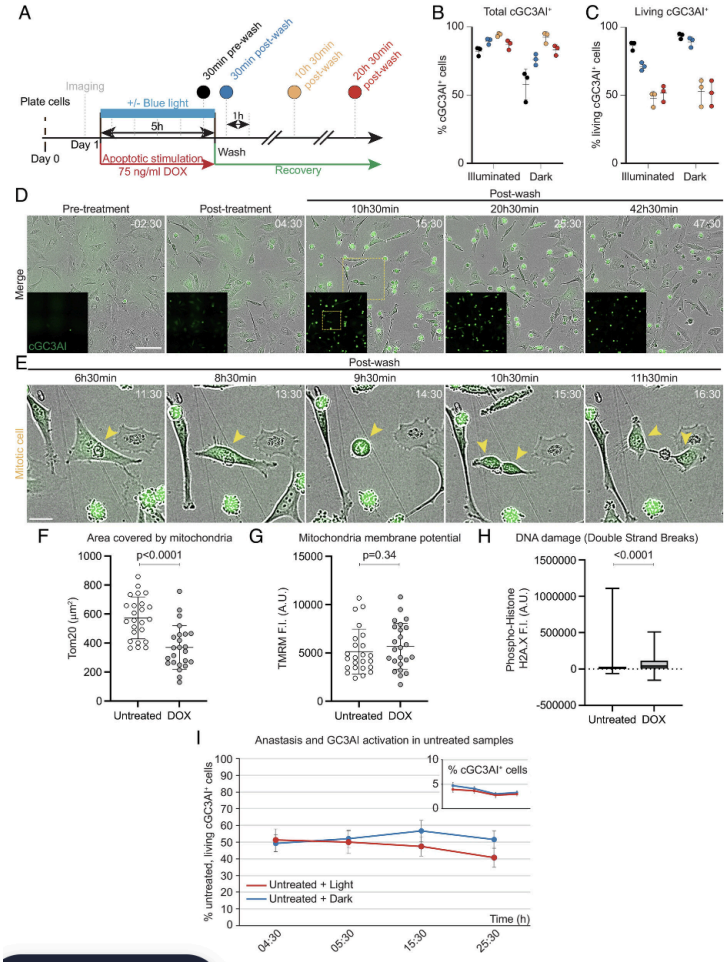


- A: This was a schematic representation of how caspaseLOV cells were generated. GC3AI **(GFP-based, caspase-3-like, protease activity indicator)** was stably introduced in HeLa cells via lentiviral transduction. Endogenous caspase-3 was removed using CRISPR-Cas9, and cells were then transduced with DOX-inducible caspaseLOV. After each “change”, the cells underwent monoclonal selection and validation. Then, GC3AI was cleaved, activated by effector caspases. DOX induces caspaseLOV expression and GC3AI activation, and blue-light illumination enhanced caspaseLOV activity.
- B: This line chart indicated the percentage of GC3AI+ cells over time in caspaseLOV expressing cells in response to different treatments. It was figured out that GC3AI was inactive in the absence of DOX, yet maximal when 75 mg/mL DOX treatment was combined with illumination, delayed in the dark. The dotted lines signify threshold-based identification of GC3AI positivity+manual counting, which the solid lines signify arbitrary (eye-based) estimation of GC3AI positivity+manual counting. Automated quantifications provided a rapid overview of cGC3AI increase over time. Manual counting confirmed the automated analysis and allowed quantification of both cGC3AI+ and cGC3AI− cells. By 8 h 30 min post-treatment 98% of DOX-treated, illuminated cells and 97% of non-illuminated cells are cGC3AI+ (n = 412/420 illuminated cells, n = 497/515 non-illuminated cells, n = 3 independent experiments), compared to ≤4% of untreated controls (n = 11/464 cGC3AI+ illuminated cells, n = 9/459 cGC3AI+ non-illuminated cells; n = 3 independent experiments). Time post-treatment is in

hh:mm. Error bars = Standard Deviation.

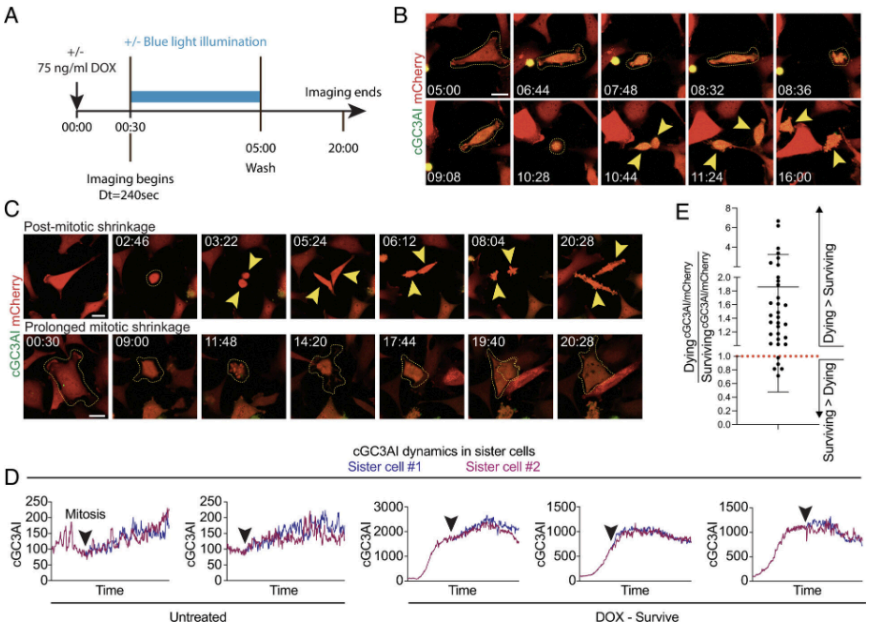
- C: Images from a timelapse series of HeLa cells stably encoding the effector caspase sensor GC3AI (green) and DOX-inducible caspaseLOV. Cells were treated with 75 ng/mL DOX and exposed to blue light. Over time, cells undergo sensor activation and shrinkage, indicative of cell death. Insets show cGC3AI. (Scale bar, 100 µm). Time is in hh:mm.

- Figure 2



- A: Experimental design of recovery experiment. Apoptotic stimulation was carried out for 5 h with 75 ng/mL DOX, ±blue light illumination, and was then removed to allow cells to recover. After a pre-treatment acquisition, the sample was imaged hourly, starting 30 min post-treatment.
- B: Dot plot indicating the percentage of total cGC3AI+ cells at different times after apoptotic stimulation (75 ng/mL DOX ± blue light illumination). By the end of 5 h treatment (30 min pre-wash, black), 82% of illuminated cells activated GC3AI (n = 2,421/2,954 cells). The fraction of cGC3AI+ cells increased over time, reaching 94% 10 h 30 min post-wash (n = 3,104/3,296 cells, yellow). In the dark, 58% of cells activated GC3AI by 5 h (n = 837/1,437 cells,

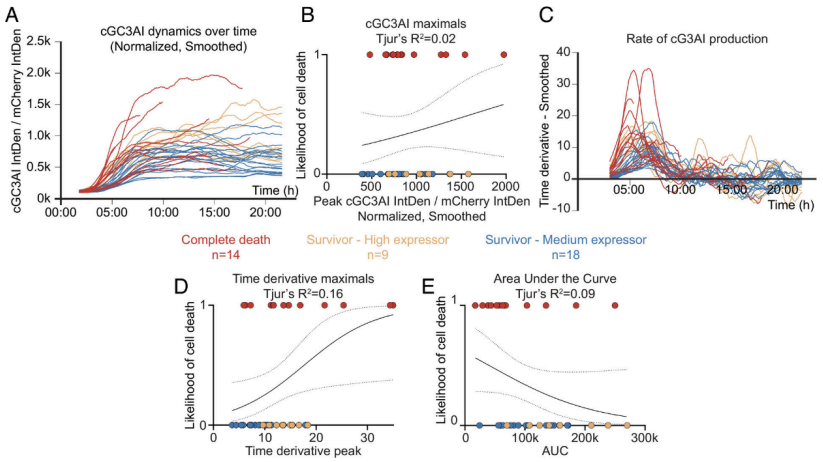
	black) and 92% by 10 h 30 min post-treatment (n = 1,438/1,556 cells, yellow). - C: Dot plot indicating the percentage of living cGC3AI+ cells at different times after apoptotic stimulation (75 ng/mL DOX ± blue light illumination). 51% of cGC3AI+ cells survive up to 20 h 30 min post-wash (n = 1,615/3,169 illuminated cGC3AI+ cells and 706/1,390 non-illuminated cGC3AI+ cells, red). Error bars = SD. Each dot represents one experiment (n = 3). - D: Images from a timelapse series of caspaseLOV-overexpressing cells during a recovery experiment. GC3AI is in green and in the Insets. (Scale bar, 100 µm). Time post-treatment is in hh:mm. Yellow square outlines the mitotic cell magnified in E. - E: cGC3AI+ cell undergoing mitosis. (Scale bar, 25 µm). - F: Quantifications of mitochondrial area in cGC3AI- untreated cells and cGC3AI+ anastatic cells. Statistical significance: unpaired t test. Error bars = Standard Deviation. - G: Quantifications of mitochondria activity in cGC3AI- untreated cells and cGC3AI+ anastatic cells. TMRM Fluorescence intensity (F.I.) is shown in Arbitrary Units (A.U.) Statistical significance: Mann–Whitney test. Error bars = Standard Deviation. - H: Quantifications of DNA damage in cGC3AI- untreated cells and cGC3AI+ anastatic cells. Statistical significance: Mann–Whitney test. Error bars = Standard Deviation. - I: Line chart indicating the frequency of spontaneous anastasis in untreated cells exposed to light (red) or kept in the dark (blue). The frequency is robust and similar to what observed for DOX-treated cells 10 to 20 h after treatment (Fig. 2C). Inset shows the percentage of untreated cells experiencing spontaneous caspase activation, for the same time points shown in the main graph. Error bars = SD. More than 2,000 total cells and more than 75 cGC3AI+ cells were quantified for every time point. n = 3 independent experiments. - Figure 3



- A: Experimental design of long-term live imaging experiment. After 30 min of apoptotic stimulation (75 ng/mL DOX), cells were imaged ±blue laser illumination every ~240 s (see Methods). Controls were not stimulated with DOX but were illuminated to control for phototoxicity.
- B: Stills of a caspaseLOV cell undergoing mitosis after DOX treatment. GC3AI is in green. Stable mCherry expression (red) was used to normalize cGC3AI fluorescence. Yellow dotted lines outline the parental cell boundaries. Yellow arrowheads indicate daughter cells. The cell undergoes several brief shrinkage events. Time post-treatment is in hh:mm. Time 05:00 is the first acquisition post-washout. (Scale bar, 25 µm).
- C: Representative examples of shrinkage. Time post-treatment is in hh:mm. (Scale bar, 25 µm). Top panels show daughter cells that undergo shrinkage after cell division. Yellow dotted lines outline the parental cell. Yellow arrowheads indicate daughter cells. Bottom panels show a parental cell undergoing a first round of abortive mitosis (notice the darker mCherry signal in the center of the cell, where the metaphase plate is). The cell blebs and attempts mitosis again, this time generating two superimposed cells that fuse in a single daughter. Yellow dotted lines outline cell boundaries.
- D: Line graphs of representative cGC3AI dynamics in surviving sister cells (expressed as cGC3AI IntDen/mCherry IntDen and normalized). Note that y axes are not equivalent. Time is from beginning to end of imaging (~20 h). Black arrowheads indicate mitosis.
- E: Dot plot chart showing the cGC3AI/mCherry ratio in dying versus surviving sister cells. A ratio greater than 1 (red dotted line)

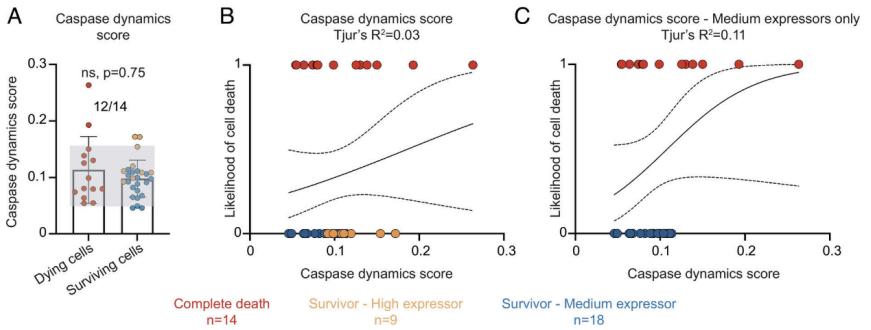
represents higher caspase activity in the dying sister, a ratio of 1 represents equal activity between sisters, and a ratio lower than 1 represents higher caspase activity in the surviving sister. In 5/33 couples of siblings analyzed, the surviving sister exhibited higher levels of caspase than its dying sibling.

- Figure 4



  - Relationship between caspase activity and commitment to cell death. Data points are color-coded by fate or according to the level of total GC3AI (tGC3AI) measured by immunostaining (red = dying cells; orange = surviving cells with high tGC3AI; blue = surviving cells with average levels of tGC3AI). n = number of cells analyzed for each condition (from three independent experiments). Dotted lines represent the 95% confidence interval (C.I.). Tjur's R2 evaluates the goodness of fit.
  - A: Line graph showing the dynamics of caspase activation over time in dying vs. surviving cells. Dynamics were obtained as percentage fold change of the ratio cGC3AI/mCherry over time after normalization. Before representation, data were smoothed using a moving average of 20. Smoothed data were used for further analysis.
  - B: Logistic regression between the maximum fold change recorded for cGC3AI (denoted "maximal" or "peak") and the likelihood of cell death (0 = alive, 1 = dead)
  - C: Line graph showing the smoothed time derivative of curves in A.
  - D: Logistic regression between the maximum of the time derivative (denoted maximal or peak) and the likelihood of cell death (0 = alive, 1 = dead)
  - E: Logistic regression between the area under the curve (AUC) of curves in A and the likelihood of cell death (0 = alive, 1 = dead)

- Figure 5



- Caspase dynamic score in dying (red) and surviving cells (orange = surviving cells with high tGC3AI; blue = surviving cells with average levels of tGC3AI). n = number of cells analyzed for each condition (from three independent experiments).
- A: Dot plot chart and histogram showing the death score of dying and surviving cells. Error bars = SD. Lack of statistical significance (P = 0.75) was assessed with Mann–Whitney test. Gray shading helps visualize dying and surviving cells with overlapping death scores. The numbers on top indicate the fraction of dying cells with death scores overlapping with surviving cells.
- B: Logistic regression between the death score and the likelihood of cell death (0 = alive, 1 = dead) considering all surviving cells
- C: Logistic regression between the death score and the likelihood of cell death (0 = alive, 1 = dead) considering medium expressors only.
- Dotted lines represent the 95% C.I. Tjur's R2 evaluates the goodness of fit.

VOCAB: (w/definition)

Anastasis- cellular recovery from the brink of apoptotic death

Staurosporine- a natural product for anti-cancer treatment originally isolated in 1977 from the bacterium Streptomyces staurosporeus. It was the first of over 50 alkaloids to be isolated with this type of bis-indole chemical structure

- Bis-indole- consists of two monomeric indole alkaloid units as their obligate constituents
  - Indole- a crystalline organic compound with an unpleasant odor, present in coal tar and in feces.
  - Obligate (biology)- restricted to a particular function or mode of life

Concomitant- naturally accompanying or associated.

Transient- lasting only for a short time; impermanent

Drosophila- a genus of flies, belonging to the family Drosophilidae, whose members are often called "small fruit flies" or pomace flies, vinegar flies, or wine flies, a reference to the characteristic of many species to linger around overripe or

rotting fruit.

Snail (protein)- also known as SNAI1, this is a protein that in humans is encoded by the SNAI1 gene. Snail is a family of transcription factors that promote the repression of the adhesion molecule E-cadherin to regulate epithelial to mesenchymal transition during embryonic development.

- E-cadherin- a tumor suppressor protein
- epithelial-mesenchymal transition- a biologic process that allows a polarized epithelial cell, which normally interacts with basement membrane via its basal surface, to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype
    - Epithelial- relating to or denoting the thin tissue forming the outer layer of a body's surface and lining the alimentary canal and other hollow structures.
    - Mesenchymal- unspecialized cells
- SNAI1 gene- silencing gene, inhibits expression of properties that are associated with the malignant phenotype of MCF-7 cells and reverses the epithelial-mesenchymal transition process by regulating relevant target gene E-cadherin.
    - MCF-7- a human breast cancer cell line with estrogen, progesterone and glucocorticoid receptors
        - Estrogen- any of a group of steroid hormones which promote the development and maintenance of female characteristics of the body. Such hormones are also produced artificially for use in oral contraceptives or to treat menopausal and menstrual disorders.
        - Progesterone- a steroid hormone released by the corpus luteum that stimulates the uterus to prepare for pregnancy
        - Glucocorticoid- any of a group of corticosteroids (e.g. hydrocortisone) which are involved in the metabolism of carbohydrates, proteins, and fats and have anti-inflammatory activity.
            - Corticosteroids- any of a group of steroid hormones produced in the adrenal cortex or made synthetically.

Akt1- a protein-coding gene (AKT Serine/Threonine Kinase 1)

dCIZ1- a protein-coding gene found in the fly species D. melanogaster, also known as the common fruit fly

Pleiotropic- multiple traits expressed by a single gene

Heterogeneity- the quality or state of being diverse in character or content

Monoclonal- forming a clone that is derived asexually from a single individual or

cell

Endogenous- growing or originating from within an organism.

DOX(Doxycycline)-induction- a system which is widely used to control gene expression in mammalian cells

- Doxycycline- a broad-spectrum antibiotic of the tetracycline group, which has a long half-life in the body.
    - Tetracycline- any of a large group of antibiotics with a molecular structure containing four rings.

GFP- Green Fluroescent Protein, exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range

Lentiviral Transduction- an efficient method for the delivery of transgenes to mammalian cells

- Lentiviral- any of a genus (Lentivirus) of retroviruses (such as the HIVs and SIV) that cause slowly progressive often fatal human and animal diseases (such as AIDS)
    - Lentivirus- a genus of single-stranded RNA viruses (as HIV and SIV) of the family Retroviridae that cause progressive often fatal human and animal diseases
    - Retroviruses- any of a family of RNA viruses that have an enzyme (reverse transcriptase) capable of making a complementary DNA copy of the viral RNA, which then is integrated into a host cell's DNA. The family includes a number of significant pathogens, typically causing tumors or affecting the function of the immune system, e.g. HIV.
- Transduction- the action or process of converting something and especially energy or a message into another form

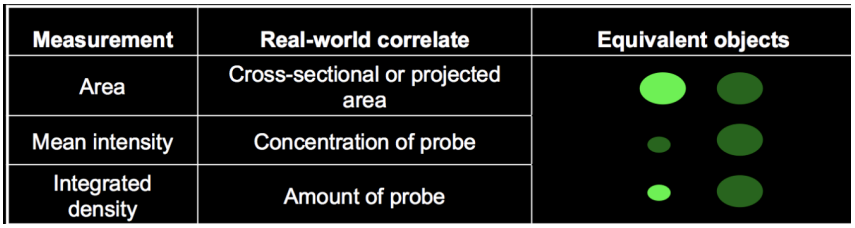
Tetramethylrhodamine Methyl Ester Perchlorate (TMRM)- a cell-permeant dye that accumulates in active mitochondria with intact membrane potentials

Unpaired T-Test- a statistical procedure that compares the averages/means of two independent or unrelated groups to determine if there is a significant difference between the two.

Mann-Whitney Test/Mann-Whitney U Test= a non-parametric test that can be used in place of an unpaired t-test. It is used to test the null hypothesis that two samples come from the same population or whether observations in one sample tend to be larger than observations in the other.

Quiescent- in a state or period of inactivity or dormancy.

Senescent(of a cell)- no longer capable of dividing but still alive and metabolically active.

	Phosphatidylserine- a type of phospholipid. It exists in brain cell membranes Phototoxicity (photoirritation)- a toxic response that is elicited after the initial exposure of skin to certain chemicals and subsequent exposure to light, or that is induced by skin irradiation after systemic administration (oral, intravenous) of a chemical substance. In the lab setting, this regards the damage done to cells mCherry- a bright red monomeric fluorescent protein created by rounds of directed evolution of DsRed. - DsRed- a brilliantly red fluorescent protein IntDen- the product of Area and Mean Gray Value  Helpful table, found in this forum: https://forum.image.sc/t/intden-vs-rawintden/5147/3 Confocal Microscopy- using a microscope which produces a point source of light and rejects out-of-focus light. This provides the ability to image deep into tissues with high resolution, and optical sectioning for 3D reconstructions of imaged samples. Fold Change- a measure describing how much a quantity changes between an original and a subsequent measurement
Cited references to follow up on	- D. R. Green, The coming decade of cell death research: Five riddles. *Cell* 177, 1094–1107 (2019). - S. Elmore, Apoptosis: A review of programmed cell death. *Toxicol. Pathol.* 35, 495–516 (2007).
Follow up Questions	- As this focused on caspase-3, would caspase-7 give a different outcome? - If not caspase-3, are there any better predictors of cell death? - Could the modifications of GC3AI have affected the experiment in any way? - Would the results reflect all cell types?

Article #14 Notes: Neuronal cell death mechanisms in Alzheimer's disease: An insight

Source Title	Neuronal cell death mechanisms in Alzheimer's disease: An insight
Source citation (APA Format)	Goel, P., Chakrabarti, S., Goel, K., Bhutani, K., Chopra, T., & Bali, S. (2022). Neuronal cell death mechanisms in Alzheimer's disease: An insight. *Frontiers in Molecular Neuroscience*, *15*. https://doi.org/10.3389/fnmol.2022.937133
Original URL	https://www.frontiersin.org/articles/10.3389/fnmol.2022.937133/full
Source type	Journal Article
Keywords	Neuronal Death, Apoptosis, Necrosis, Necroptosis, Ferroptosis, Autophagy, Microautophagy
#Tags	#Alzheimer's-Research #Neurodegeneration-Study #Cell-Death-Mechanisms #Aging-And-Neurological-Health #Neuronal-Loss-Pathways #Alzheimer's-Pathogenesis #Neurodegenerative-Processes
Summary of key points + notes (include methodology)	The following notes were written with the aid of ChatGPT, an AI service. Alzheimer's disease overview: - Chronic neurodegenerative disease affecting elderly people, characterized by memory and cognitive impairment. - Considered the sixth leading cause of death in the United States. - Global impact: Affects over 46 million people, societal loss of $818 billion. - Disease progression: Begins in preclinical stages, leads to dependency on caregivers, and typically results in death within 4–8 years post-diagnosis. - Brain changes: Include amyloid beta peptide 42 (Aβ42) plaques, neurofibrillary tangles, neuronal loss, synaptic degeneration, and reactive gliosis. Factors contributing to Alzheimer's disease: - Aging: Primary risk factor, with prevalence doubling every 5 years after 65. - Genetic and modifiable risk factors also play a role. - Pathogenesis mechanisms involve mitochondrial dysfunction, oxidative stress, protein accumulation, and inflammatory responses. Neuronal death in Alzheimer's disease: - Contrasts with minimal neuronal loss in non-pathological brain aging. - Neuronal loss begins in preclinical stages, progresses through prodromal

phases, affecting various brain areas.
- Correlation between neuronal loss, synaptic degeneration, and cognitive decline.
- Studies in transgenic AD models show varying degrees of neuronal loss, less pronounced compared to other pathologies.

Cell death types:

- Apoptosis: Regulated cell death with morphological changes, triggered by death ligands or mitochondrial injury. Involves caspases and chromatin fragmentation.
- Necrosis and necroptosis: Unregulated and regulated cell death respectively, with defined signaling pathways. Necroptosis involves RIP kinases and MLKL.
- Ferroptosis: Iron-dependent cell death caused by ROS accumulation and lipid peroxidation, prevented by iron-chelators and antioxidants.
- Autophagy: Degradation mechanism for damaged components; debated if it serves as a cell death pathway.

Relationship to Alzheimer's disease:

- Unclear which specific cell death pathways predominate in AD neuronal loss.
- Apoptosis, necrosis, necroptosis, ferroptosis, and autophagy all have implications but their roles in AD pathogenesis require further investigation.-

Microautophagy:
- Involves direct engulfment of autophagic substrates by lysosomal membrane invagination.
- Degradation of captured cargo by hydrolytic enzymes.

Chaperone-mediated autophagy (CMA):

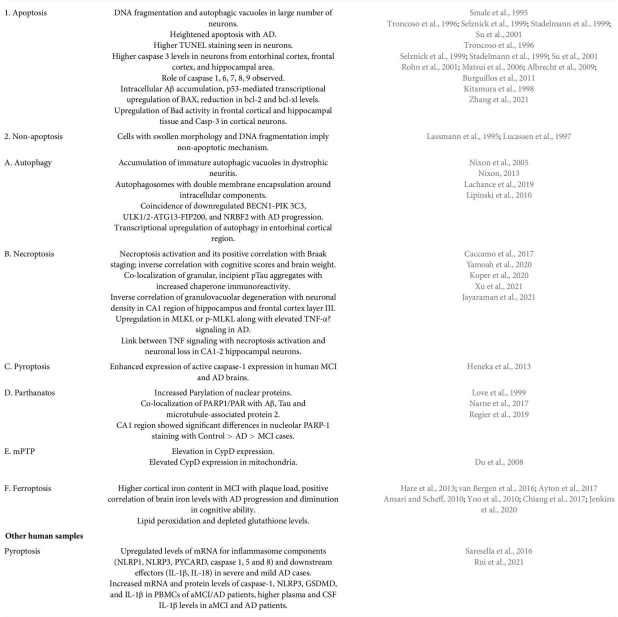
- Distinct from macroautophagy.
- Removes specific proteins with KFERQ-like motif using molecular chaperones like hsc70.
- Facilitates translocation of substrate proteins through lysosomal membranes via LAMP2A receptor.

Pyroptosis:

- Lytic cell death associated with inflammation.
- Involves inflammasomes, activation of inflammatory caspases (e.g., caspase-1/4/5/11), and release of inflammatory cytokines (IL-1β, IL-18).
- Can occur independently or through inflammasome-dependent mechanisms.

Neuronal Death:

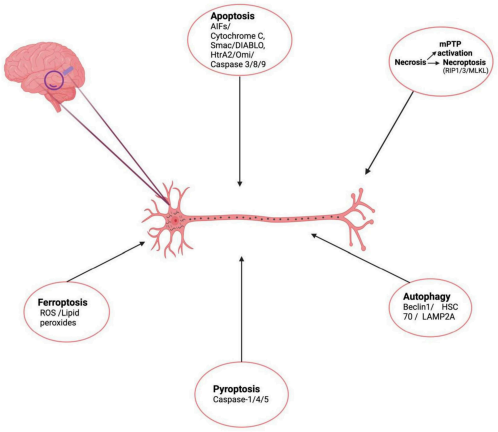
- Apoptosis plays a role in neural development but is restricted in mature neurons.
- Mature neurons inhibit cell death pathways, but triggers for reversal are

	unclear. - Neurodegenerative disorders show signs of neuronal de-differentiation and cell cycle re-entry. Neuronal Death Mechanisms: - Metabolic changes in neurons may trigger de-differentiation and cell death. - Evidence suggests re-entry into the cell cycle in neurodegenerative diseases like Alzheimer's. Cell Cycle & Neurodegeneration: - In Alzheimer's, markers indicate a return to immature states in neurons. - Various drugs and inhibitors targeting cell cycle components show promise in treatment. Types of Neuronal Death in Alzheimer's: - Apoptosis shows mixed evidence in post-mortem studies. - Necroptosis is implicated in early stages of AD before Aβ aggregation.
Research Question/Problem/ Need	What are the molecular and pathological mechanisms underlying neurodegenerative diseases like Alzheimer's and associated neuronal dysfunctions?
Important Figures	 **Post-mortem studies in AD brain** 1. Apoptosis — DNA fragmentation and autophagic vacuoles in large number of neurons. Heightened apoptosis with AD. Higher TUNEL staining seen in neurons. Higher caspase 3 levels in neurons from entorhinal cortex, frontal cortex, and hippocampal area. Role of caspase 1, 6, 7, 8, 9 observed. Intracellular Aβ accumulation, p53-mediated transcriptional upregulation of BAX, reduction in bcl-2 and bcl-xl levels. Upregulation of Bad activity in frontal cortical and hippocampal tissue and Casp-3 in cortical neurons. — Smale et al., 1995; Troncoso et al., 1996; Selznick et al., 1999; Stadelmann et al., 1999; Su et al., 2001; Troncoso et al., 1996; Selznick et al., 1999; Stadelmann et al., 1999; Su et al., 2001; Rohn et al., 2001; Matsui et al., 2006; Albrecht et al., 2009; Burguillos et al., 2011; Kitamura et al., 1998; Zhang et al., 2021 2. Non-apoptosis — Cells with swollen morphology and DNA fragmentation imply non-apoptotic mechanism. — Lassmann et al., 1995; Lucassen et al., 1997 A. Autophagy — Accumulation of immature autophagic vacuoles in dystrophic neurites. Autophagosomes with double membrane encapsulation around intracellular components. Coincidence of downregulated BECN1-PIK 3C3, ULK1/2-ATG13-FIP200, and NRBF2 with AD progression. Transcriptional upregulation of autophagy in entorhinal cortical region. — Nixon et al., 2005; Nixon, 2013; Lachance et al., 2019; Lipinski et al., 2010 B. Necroptosis — Necroptosis activation and its positive correlation with Braak staging; inverse correlation with cognitive scores and brain weight. Co-localization of granular, incipient pTau aggregates with increased chaperone immunoreactivity. Inverse correlation of granulovacuolar degeneration with neuronal density in CA1 region of hippocampus and frontal cortex layer III. Upregulation in MLKL or p-MLKL along with elevated TNF-α? signaling in AD. Link between TNF signaling with necroptosis activation and neuronal loss in CA1-2 hippocampal neurons. — Caccamo et al., 2017; Yamoah et al., 2020; Koper et al., 2020; Xu et al., 2021; Jayaraman et al., 2021 C. Pyroptosis — Enhanced expression of active caspase-1 expression in human MCI and AD brains. — Heneka et al., 2013 D. Parthanatos — Increased Parylation of nuclear proteins. Co-localization of PARP1/PAR with Aβ, Tau and microtubule-associated protein 2. CA1 region showed significant differences in nucleolar PARP-1 staining with Control > AD > MCI cases. — Love et al., 1999; Narne et al., 2017; Regier et al., 2019 E. mPTP — Elevation in CypD expression. Elevated CypD expression in mitochondria. — Du et al., 2008 F. Ferroptosis — Higher cortical iron content in MCI with plaque load, positive correlation of brain iron levels with AD progression and diminution in cognitive ability. Lipid peroxidation and depleted glutathione levels. — Hare et al., 2013; van Bergen et al., 2016; Ayton et al., 2017; Ansari and Scheff, 2010; Yoo et al., 2010; Chiang et al., 2017; Jenkins et al., 2020 **Other human samples** Pyroptosis — Upregulated levels of mRNA for inflammasome components (NLRP1, NLRP3, PYCARD, caspase 1, 5 and 8) and downstream effectors (IL-1β, IL-18) in severe and mild AD cases. Increased mRNA and protein levels of caspase-1, NLRP3, GSDMD, and IL-1β in PBMCs of aMCI/AD patients, higher plasma and CSF IL-1β levels in aMCI and AD patients. — Saresella et al., 2016; Rui et al., 2021 Cell death pathways responsible for neuronal death in postmortem AD brains with evidence from various studies.

Experimental studies in AD models

1. Apoptosis	Improvement of behavioral alteration and cognitive decline in 5X-FAD mice after Bad loss.	Zhang et al., 2021
2. Autophagy	Autophagosomes accumulation in neuronal dendrites in PS-1/APP double transgenic mice before amyloid plaque formation. Accumulation of immature AVs in hippocampal neurons of AD mice before actual synaptic and neuronal loss.	Yu et al., 2005 Sanchez-Varo et al., 2012
3. Necroptosis	Reduction in cell loss after lowering necroptosis activation. Co-localization of granular, incipient pTau aggregates in pR5 tau transgenic mouse neurons with increased chaperone immunoreactivity. Upregulated levels of MLKL or p-MLKL (necroptosis marker).	Caccamo et al., 2017 Yamoah et al., 2020 Xu et al., 2021
4. Pyroptosis	Amyloid-β induced upregulation of NLRP1 inflammasome, NLRP1-mediated caspase-1-dependent 'pyroptosis.' Protection of $Nlrp3^{-/-}$ or $Casp1^{-/-}$ mice from spatial memory loss and other AD associated sequelae. $A\beta_{1-42}$ induced pyroptosis, increased cell permeability and LDH release, Upregulated cellular GSDMD and p30-GSDMD expression, NLRP3 inflammasome and GSDMD-cleaved protein caspase-1 inflammatory factors levels. Inflammasome-mediated pyroptosis induced by hyperphosphorylated tau.	Tan et al., 2014 Heneka et al., 2013 Han et al., 2020 Li Y. et al., 2020
5. Parthanatos	PARP-1 activation at early stages of amyloid deposit. Crossing of PARP-1 [−/−] mouse with AD transgenic mouse prevented synaptic damage, cognitive dysfunction and microglial activation. p53, PARP-1, NF-κB, Bax overexpression, decreased Bcl-2 levels in $A\beta_{1-42}$ treated group while upregulation in Bcl-2 and downregulated PARP-1, NF-κB, p53, and Bax levels with NA treatment. Aβ42 induced hippocampal neurotoxicity by oxidative stress-mediated PARP1 activation, TRPM2 activation, Ca^{2+} influx and mitochondrial dysfunction.	Martire et al., 2013 Kauppinen et al., 2011 Turunc Bayrakdar et al., 2014 Li and Jiang, 2018
6. mPTP	Co-localization of extracellularly applied Aβ with mitochondrial markers in inner mitochondrial membrane. Increased Cyp D levels in AD mouse model with overexpression of mutant human form of APP. Inhibition of ABAD-Aβ interaction, *in vitro* and *in vivo*, protected against aberrant mitochondrial and neuronal function, improved spatial learning/memory. Increased CypD expression in cortical mitochondria with translocation to IMM, Ca^{2+} dyshomeostasis and mitochondrial swelling. Mitochondria derived from CypD-deficient mAPP mouse model ($mAPP/Ppif^{-/-}$) exhibited reduction in mitochondrial swelling as well as permeability transition after induction by Ca^{2+}. Aβ accumulation provoked Ca^{2+} overload in mitochondria via MCU complex. Loss of efflux from MCU led to mCa^{2+} overload, promoted superoxide generation, metabolic dysfunction and neuronal cell death.	Hansson Petersen et al., 2008 Du et al., 2008 Yao et al., 2011 Hung et al., 2010 Du et al., 2008; Yao et al., 2011 Calvo-Rodriguez et al., 2020 Jadiya et al., 2019
7. Ferroptosis	Spatial learning and memory function deficits co-related with lipid peroxidation, ERK activation and increased neuroinflammation.	Hambright et al., 2017

Cell death pathways responsible for neuronal death in experimental studies on AD models with evidence from various studies.



Schematic representation of cell death mechanisms responsible for neuronal death in AD. (Created with BioRender.com.) Apoptosis – initiated inner mitochondrial membrane changes leads to release of cytochrome c, AIF, Smac/DIABLO, HtrA2/Omi, activates effector caspases and causes apoptotic cell death. Necroptosis – is a form of RCD involving RIPK1, RIPK3, necrosome formation and MLKL activation. Another form of necrosis mPTP leading to rupture of outer membrane and non-specific release of intermembrane space proteins into cytosol, causing cell death. Autophagy – another cell death mechanism mediated by Beclin 1, HSC70, LAMP2A leads to formation of LC3II autolysosomes causing cell death. Pyroptosis – lytic form of cell death associated with inflammasomes formation, activation caspase-1/4/5 Gasdermin-D cleavage and release of inflammatory cytokines is also responsible for neuronal cell death in AD. Ferroptosis – Iron-dependent mode of cell death, induced by diverse triggers involves ROS, lipid peroxide accumulation depleted glutathione levels.

VOCAB: (w/definition)

Pyroptosis- a highly inflammatory mode of regulated cell death which has evolved as a way of removing intracellular pathogens and has a distinct morphology which

	depends on the formation of plasma membrane pores resulting in cell explosion. Microautophagy- involves the direct uptake of cargo through invagination of the lysosomal membrane Necroptosis- a programmed form of necrosis, or inflammatory cell death Glutathione- a substance made from the amino acids glycine, cysteine, and glutamic acid
Cited references to follow up on	Abbott, A. (2018). Is' friendly fire' in the brain provoking Alzheimer's disease? Nature 556, 426–428. doi: 10.1038/d41586-018-04930-7 Acosta-Cabronero, J., Betts, M. J., Cardenas-Blanco, A., Yang, S., and Nestor, P. J. (2016). In vivo MRI mapping of brain iron deposition across the adult lifespan. J. Neurosci. 36, 364–374. doi: 10.1523/JNEUROSCI.1907-15.2016 Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G. M., et al. (2000). Inflammation and Alzheimer's disease. Neurobiol. Aging 21, 383–421. doi: 10.1016/s0197-4580(00)00124-x
Follow up Questions	- How do early intraneuronal amyloid accumulations trigger inflammatory signaling pathways, contributing to neurodegeneration in Alzheimer's disease? - What are the specific mechanisms and therapeutic potential of targeting neuronal cell death pathways like ferroptosis and apoptosis in managing neurodegenerative disorders? - How do metabolic alterations and dysfunctional molecular interactions impact the pathogenesis and progression of Alzheimer's disease, focusing on insulin receptor dysfunction, metabolic reprogramming, and gene expression changes?

"How would this project be used for your future endeavors?"

- PLAN A: Creating artificial tissue to aid with the restoration of human independent function, if not memories as a whole, using the information gathered by the AI model

- PLAN B: Reversing the AI model from FINAL STAGES → INITIAL STAGES to INITIAL STAGES → FINAL STAGES: "Timeless Model"

- **Rely less on notes**
- **Understand data and overall conclusions better- relevancy to project**

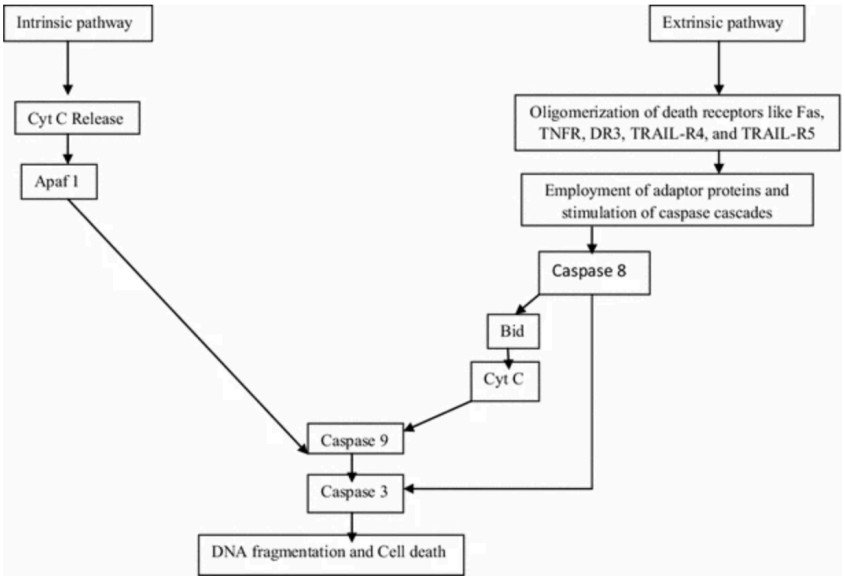
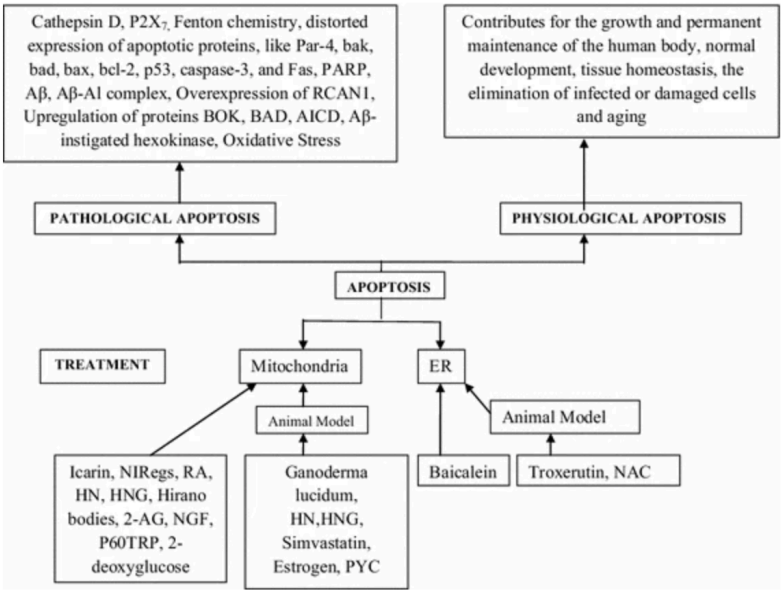
Article #15 Notes: Apoptosis in Alzheimer's Disease: An Understanding of the Physiology, Pathology and Therapeutic Avenues

Source Title	Apoptosis in Alzheimer's Disease: An Understanding of the Physiology, Pathology and Therapeutic Avenues
Source citation (APA Format)	Obulesu, M., Lakshmi, M.J. Apoptosis in Alzheimer's Disease: An Understanding of the Physiology, Pathology and Therapeutic Avenues. *Neurochem Res 39*, 2301–2312 (2014). https://doi.org/10.1007/s11064-014-1454-4
Original URL	https://link.springer.com/article/10.1007/s11064-014-1454-4
Source type	Journal Article
Keywords	Apoptosis, Treatment, Peptides, Flavonoids
#Tags	#Molecular-Mechanisms #Treatment-Strategies #Caspases #Neuroprotective-Peptides #Flavonoids
Summary of key points + notes (include methodology)	The following notes were written with the aid of ChatGPT, an AI service. The text outlines the intricate involvement of apoptosis, a programmed cell death mechanism, in neurodegenerative diseases like Alzheimer's Disease (AD), offering a comprehensive array of potential treatment avenues. Notably, peptides such as Humanin and its derivative [Gly14]-humanin exhibit antiapoptotic properties by interfering with proapoptotic factors like Bax and ASK1, while flavonoids like baicalein and natural compounds like icariin, sulforaphane, and troxerutin demonstrate promising neuroprotective effects by mitigating ER stress-induced apoptosis and reducing oxidative damage. Moreover, nutrition-based approaches like caloric restriction, along with natural substances such as Ganoderma lucidum spore and simvastatin, show potential in protecting against neurodegeneration. Despite challenges in translating preclinical findings into clinical success, understanding apoptosis pathways and exploring various compounds underscore the need for further research in developing effective therapeutic strategies against neurodegenerative disorders. Introduction to Alzheimer's Disease (AD):

	AD is a neurodegenerative disorder affecting the elderly, posing a significant threat to healthcare systems due to the increasing number of patients and associated costs. Etiology involves oxidative stress, Aβ plaques, NFTs, metal-mediated neurotoxicity, gene mutations, neuroinflammation, tau hyperphosphorylation, and apoptosis. Apoptosis Overview: Apoptosis is a biological process leading to programmed cell death upon specific stimuli. Major cellular changes include cell volume contraction, nuclear condensation, plasma membrane blebbing, nuclear fragmentation, and eventual cell fragmentation. Physiological apoptosis is essential for growth, maintenance, and nervous system development by eliminating improperly connected neurons. Apoptosis Mediators and Processes: Apoptosis involves a multitude of mediators like p21, p38, MAPK, p53, caspases, Bcl-2 family proteins, and apoptosis-inducing factors. Genetically-mediated programmed cell death is essential in development, tissue homeostasis, and aging. The balance between pro-apoptotic and anti-apoptotic Bcl-2 family members maintains mitochondrial integrity. Pathological Apoptosis in AD: Cathepsin D, a brain-expressed lysosomal protease, contributes to cellular apoptosis, implicating its role in AD pathogenesis. P2X7, a purinoreceptor, triggers cell death by necrosis or apoptosis, leading to cellular and mitochondrial morphological changes. Oxidative stress-induced hydroxyl radicals via poorly liganded iron contribute to cell death pathways, including NF-kB system changes. Specific Mechanisms and Triggers in AD: Cyclin B1 accumulation, stimulated by NMDAR activation, induces neuronal apoptosis, observed in degenerative brain areas in AD and stroke. Caspases, particularly caspase-3, are pivotal in the initiation and execution of apoptosis in AD. Two primary apoptosis pathways: intrinsic (mitochondrial-dependent) and extrinsic (death receptor-mediated), both converging at caspase-3 activation. RCAN1 in Alzheimer's Disease (AD): Overexpression of RCAN1-1 in neurons of the hippocampus and cortex aggravates neuronal apoptosis in AD brains. Increased RCAN1-1 activates caspase-9 and caspase-3, worsening neuronal apoptosis induced by dexamethasone and Aβ. Knockout of caspase-3 attenuates the neurotoxicity of RCAN1-1, suggesting its mediation through caspase-3. Additional copies of RCAN1 on chromosome 21 contribute to AD and Down

	Syndrome (DS) pathogenesis. Beta-Arrestin 1 (ARRB1) and Neuronal Apoptosis: ARRB1 interaction with APLP1 induces neuronal apoptosis, belonging to a protein family including APP. Role of TNF-α in Apoptosis: TNF-α family ligands induce apoptosis in the CNS through cytochrome-c release and caspase-9 activation. Proapoptotic proteins like HIP-1, DAPK1, and Pycard exacerbate caspase-9 in TNF-α stimulated cells. TNF-α activation leads to FasL expression via NFAT activation, promoting apoptosis through caspase-3. Interleukins and Apoptosis: Increased IL-1b and TNF-α levels induce neuronal death and apoptosis. HN binds to cytokine receptors, inhibiting neuronal cell death and dysfunction. AICD and Apoptosis: AICD and CTs relocate to the nucleus, inducing apoptosis through various cellular events and interactions with other proteins. AICD interactions with p53 and c-AbI regulate neuronal apoptosis. Metabolism and Apoptosis: Hexokinase (HK) role in curbing mitochondrial ROS production prevents apoptosis. Normal glucose levels protect brain cells from apoptosis under stress conditions. Other Factors Involved in Apoptosis: Retinoic acid (RA) mediates crucial biological processes including apoptosis via arachidonic acid release. ER stress and calcium homeostasis alterations contribute to ER stress-mediated apoptosis. Animal models confirm the involvement of P2X7 and p53 in apoptosis linked to AD. Humanin (HN): Demonstrates neuroprotective effects by inhibiting Bax, ASK1, and JNK, thus attenuating apoptosis in neuronal cells. Acts through binding to cytokine receptor complexes involving CNTFR, WSX-1, and gp130. Specifically binds to Bax, preventing its translocation to mitochondria, thereby inhibiting apoptosis. [Gly14]-humanin (HNG) has higher protective properties against Prion-peptide-induced apoptosis. Other Peptides: C-terminal peptide of γ-enolase (γ-Eno) downregulates Bax and upregulates Bcl-2, reducing caspase-3 activation. Peroxiredoxin 6 protects cells from Aβ-induced neurotoxicity.

	Flavonoids: Baicalein: Mitigates ER stress-induced apoptosis, ROS accrual, and unfolds protein responses. Exhibits both anti- and pro-apoptotic activities depending on conditions and cell types. Other Flavonoids: Icariin protects against H2O2-induced neurodegeneration by inhibiting caspase-3 and p53. Troxerutin inhibits ER stress-induced apoptosis and caspase activation. Sulforaphane (SUL) ameliorates Aβ25–35-provoked cytotoxicity and apoptotic features. Nutrition: Caloric Restriction (CR) and Resveratrol: Augment Sirtuin1 (SIRT1) levels, which are decreased in AD, showing potential neuroprotective effects. Other Nutritional Compounds: Germinated brown rice extract demonstrates neuroprotective potential against oxidative stress. Polyphenols like magniferin and morin decrease active caspase-3 neurons via glutamate receptor activation. Natural Compounds: Various compounds from natural sources like Ganoderma lucidum spore, simvastatin, and 2-Arachidonoylglycerol show neuroprotective effects against neurodegeneration. Preclinical Versus Clinical Studies: Despite extensive studies in animal models, human brain studies have shown ambiguous results regarding apoptosis in neurodegenerative diseases. Various attempts targeting Aβ accumulation and tau protein phosphorylation have failed in clinical trials due to side effects or limited efficacy. Conclusion: Understanding apoptosis mechanisms in animal models is crucial for developing effective therapies. Identification of molecular targets like NFAT, YAP/Bax pathway may offer potential therapeutic strategies. Several compounds and natural substances show promise but require further validation through rigorous studies.
Research Question/Problem/ Need	Can a deeper understanding of apoptosis provide insights into the physiological mechanisms, pathological changes, and potential therapeutic avenues for Alzheimer's disease?

Important Figures	**Fig. 1**  Intrinsic and extrinsic pathways of apoptosis and various factors involved in the mechanism **Fig. 2**  Both physiological and pathological apoptosis and various therapeutic avenues targeting apoptosis in both mitochondria and ER in AD
VOCAB: (w/definition)	Immunoreactivity- Measure of the response of cells or tissues to antibodies.

	Neurotoxicity- Damage or harmful effects on the nervous system, often due to toxic substances. Neuroprotection- Strategies or interventions to protect neurons from damage or degeneration. Flavonoids- Compounds found in plants with potential health benefits, including antioxidants. ERK (Extracellular Signal-Regulated Kinase)- Protein involved in cell growth and differentiation.
Cited references to follow up on	Obulesu M, Rao Dowlathabad Muralidhara (2010) Animal models of Alzheimer's disease: an understanding of pathology and therapeutic avenues. Int J Neurosci 120:531–537 Obulesu M, Somashekhar R, Venu R (2011) Genetics of Alzheimer's disease: apo E and presenilins instigated neurodegeneration. Int J Neurosci 121:229–236
Follow up Questions	Is the main goal to solely alleviate symptoms, or reverse them? What is the overall budget of this endeavor?

Article #16 Notes: Apoptotic Pathways and Alzheimer's Disease: Probing Therapeutic Potential

Source Title	Apoptotic Pathways and Alzheimer's Disease: Probing Therapeutic Potential
Source citation (APA Format)	Sharma, V.K., Singh, T.G., Singh, S. et al. Apoptotic Pathways and Alzheimer's Disease: Probing Therapeutic Potential. *Neurochem Res 46*, 3103–3122 (2021). https://doi.org/10.1007/s11064-021-03418-7
Original URL	https://link.springer.com/article/10.1007/s11064-021-03418-7#citeas
Source type	Journal Article
Keywords	Apoptosis, Neurodegeneration, Pathology, Therapeutics, Neurons
#Tags	#ApoptosisMechanisms #NeurodegenerationPathways #TauProteinPathology #NeuronalLoss #TherapeuticResearch
Summary of key points + notes (include methodology)	The following notes were written with the aid of ChatGPT, an AI service.

The intrinsic process of apoptosis serves as a crucial regulator of cell death and survival, maintaining cellular balance, but its aberrant function contributes significantly to various disorders, notably in Alzheimer's disease (AD). AD, a progressive neurodegenerative condition characterized by hallmark features like amyloid-beta plaques, tau protein tangles, inflammation, and neuronal loss, involves an abnormal apoptotic cascade in vulnerable brain regions. This dysregulated cascade interacts with cellular organelles, signaling pathways (PI3K/AKT, JNK, MAPK, mTOR), and trophic factors, leading to neuronal loss—a pivotal event preceding AD progression. Understanding the diverse apoptotic mechanisms in AD offers insight into potential therapeutic targets (restoration of apoptotic balance, modulation of caspases, TRADD, RIPK1, FADD, TNFα) crucial for developing treatments targeting neuronal fate in AD.

- Dementia Overview:
  - Neurodegenerative pathology impacting 46 million, expected to double by 2040.
  - Pharmacoeconomic burden: Approx. US$818 billion, likely to increase.

- Alzheimer's Disease (AD):
  - Accounts for 70–80% of dementia cases, affecting around 50 million globally(2019).
  - Associated with behavioral, psychiatric, and cognitive abnormalities.
  - Etiological factors: Stress, sedentary lifestyle, infections, inflammation, concurrent illnesses.

- Understanding AD Mechanisms:
  - Mechanistic explanation remains unclear despite discovery over a century ago.
  - Current theories: Cholinergic hypothesis, inflammatory hypothesis, energy depletion hypothesis, oxidative stress hypothesis.

- Pathological Markers, Symptoms, and Treatment Challenges:
  - AD characterized by impairments in memory, spatial orientation, speech, leading to vital function loss.
  - Treatments focusing on Aβ show limited success, severe side effects.
  - Controversial FDA approval of aducanumab in 2021 due to insufficient data on efficacy.

- Role of Apoptosis in AD:
  - Neuronal loss attributed to apoptosis, correlated with pathological markers.
  - Aβ and neurofibrillary tangles involved, but synaptic dysfunction, neuronal loss significant in disease progression.
  - Apoptosis represents a target to delay cell demise, activate neuroprotective mechanisms.
  - Factors: apoptotic and antiapoptotic factors, caspases, TNF-α, Bcl2, Bax, Aβ, reactive oxygen species.

- Apoptosis Origins and Observations:
  - Term ''apoptosis'' originates from Greek, referring to cell disintegration.
  - First observed by Karl Vogt in 1842, named by Kerr, Wyllie, and Currie in 1972.

- Apoptosis Functionality:
  - Maintains homeostasis, responds to environmental changes, aging, and stress.
  - Involves pro-apoptotic and anti-apoptotic mechanisms for cell survival.

- Characteristics of Apoptosis:
  - Cell shrinkage, surface perturbations, nuclear and chromatin changes.
  - Formation of apoptotic bodies without inciting inflammation.

- Major Players in Apoptosis:
  - Apoptosis binding protein R (Apaf1), apoptotic protease activating factor-1, apoptotic initiating factor (AIF), caspase family proteins [21].

- Apoptosis Process:
  - Involves cell rounding, cytoplasmic changes, nuclear modifications, DNA laddering, and apoptotic body formation.
  - Contrasted with irreversible necrosis, which causes inflammation and affects neighboring cells.

Oxidative Stress in Alzheimer's Disease (AD)
- Oxidative stress is crucial in AD pathogenesis due to elevated reactive oxygen species (ROS) levels.
- Elevated ROS levels result in blood-brain barrier permeability, synaptic signaling disruption, and neuronal loss.

Role of ROS in AD
- Normal ROS levels are essential for cellular functions but lead to apoptotic neuronal death in AD when elevated.
- Oxidative stress-induced lipid peroxidation exacerbates apoptotic pathways and modifies membrane transporter properties.

Impact on Antioxidant Defense
- The compromised antioxidant defense mechanism in the hippocampus triggers apoptotic signals and mitochondrial dysfunction.
- Caspase-6, colocalized with Aβ plaques and NFTs in AD brains, correlates with attention deficits and neurodegenerative changes.

Caspase-6 Function in AD
- Aberrant activation of caspase-6 via upstream activators escalates apoptotic cascades, indicating its role as an upstream modulator of AD.
- Caspase-6 exists between static and active conformations, impacting substrate recognition and activity, suggesting its role in apoptosis.

Pathways and Apoptosis
- JNK pathway overactivation in AD mediates Aβ aggregate formation, neuronal loss, and protein dysfunction.
- ERK1/2 pathway inhibits apoptosis by interacting with various triggers and

activating antiapoptotic signaling.

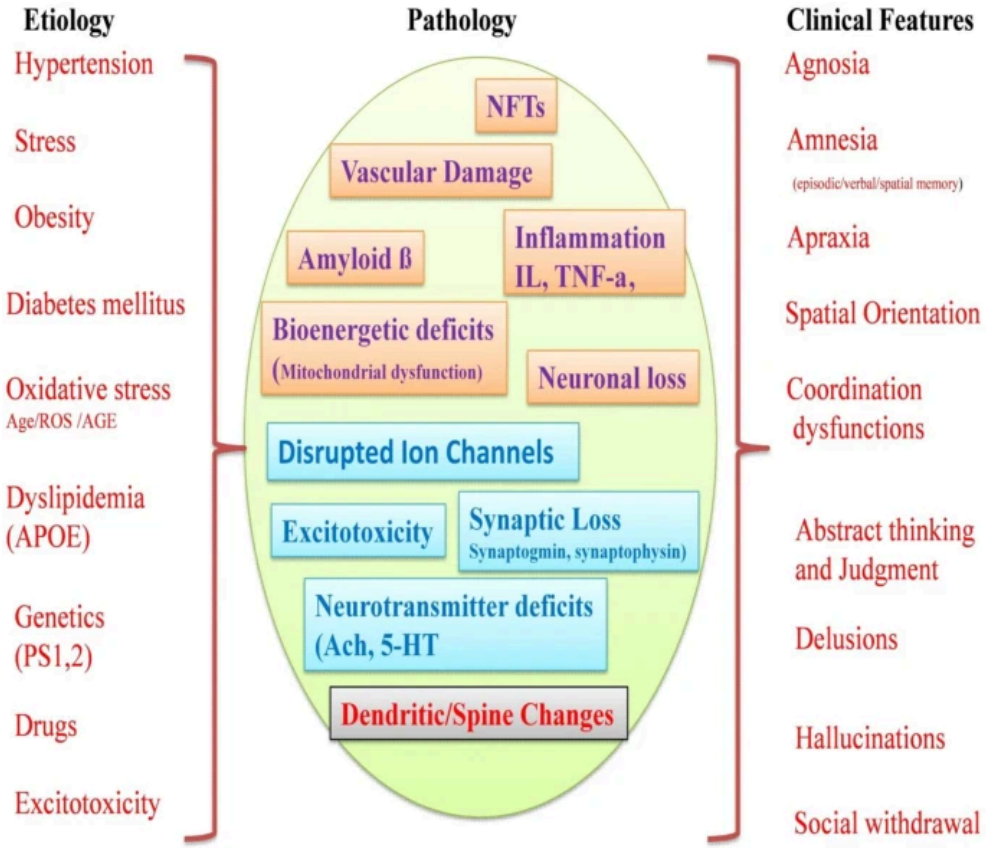
Antiapoptotic Mechanisms
- The PI3K/AKT pathway's antiapoptotic effects involve phosphorylation of factors inhibiting apoptotic pathways and promoting cell survival.
- Neurotrophins like BDNF and NGF activate survival mechanisms through Akt and PI3K pathways, promoting cell endurance.

mTOR Signaling and Apoptosis
- mTOR signaling influences memory formation and protein synthesis but hyperactivation in AD leads to Aβ and NFT accumulation, contributing to neuronal death.
- The JAK-STAT pathway regulates apoptosis and inflammation in AD, inducing neuronal death through specific signaling.

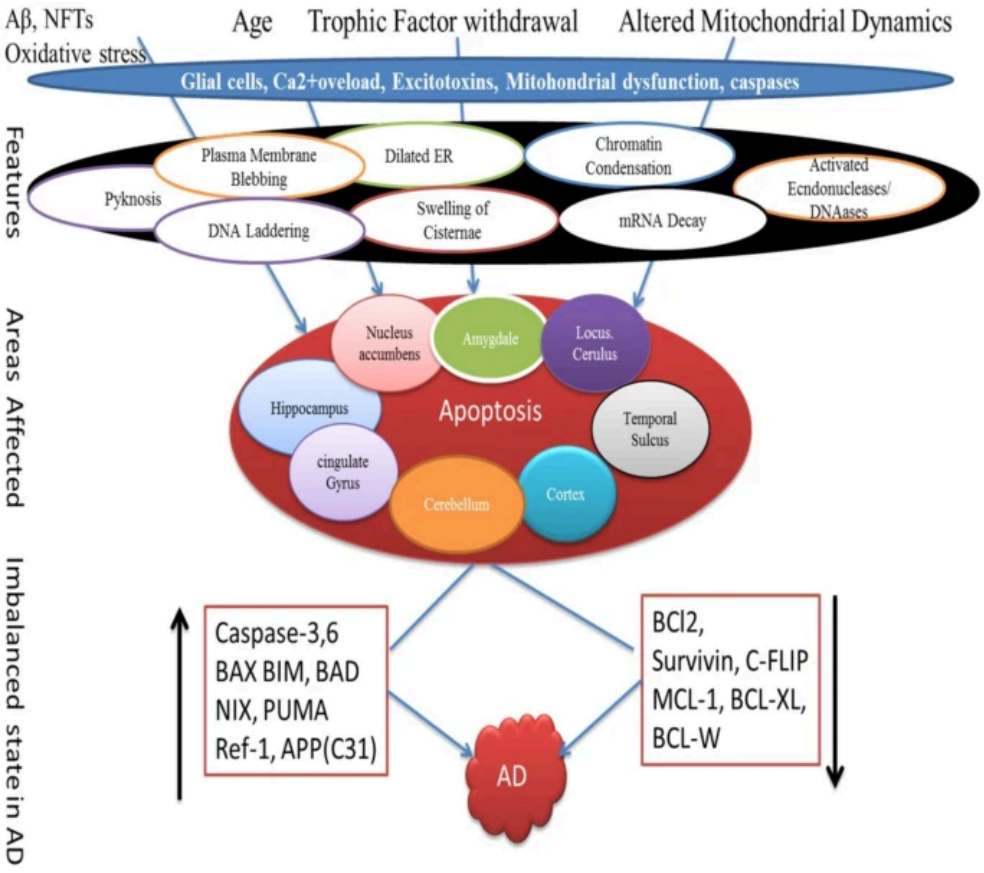
Other Factors in AD Pathology
- Elevated ceramide levels contribute to Aβ cluster formation, oxidative stress, and apoptotic signaling, leading to neuronal death.
- ER stress by Aβ oligomers induces apoptotic signaling via specific pathways, activating JNK and inducing mitochondrial dysfunction.
- Dysregulated Wnt signaling affects synaptic plasticity and transmission, making it a potential therapeutic target against Aβ toxicity and neuronal loss.

Poly ADP-Ribose Polymerase and Sirtuin in AD
- Aβ-induced neuronal loss in the AD brain leads to overexpression of PARP, activating in response to oxidative stress and Aβ accumulation.
- PARP activation causes mitochondrial dysfunction-mediated neuronal death by ATP depletion, inflammatory mediator activation (NF-κB), and increased oxidative stress.
- NF-κB increases p53 and apoptotic protein expression (Bax, Bcl2), leading to neuronal death via PARP-1-associated DNA fragmentation.
- Overactivation of PARP-1 causes NAD+ depletion, inhibiting SIRT1, resulting in bioenergetics impairment-induced neuronal death in AD.
- Aggravated oxidative stress and DNA damage decrease SIRT1 activity, favoring amyloidogenic pathway processing, leading to neurotoxic Aβ excess.
- Inhibiting PARP-1 and regulating SIRT activity may target Aβ-mediated mitochondrial dysfunction in AD.

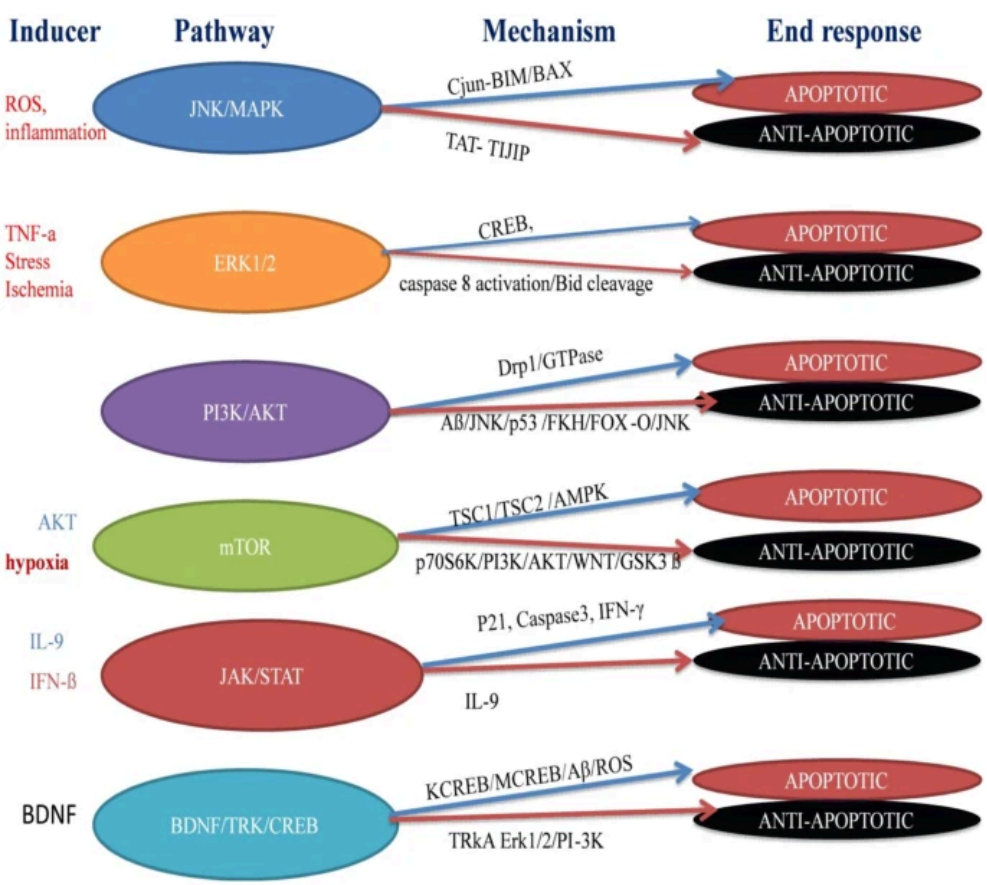
Apoptosis and Epigenetics: AD
- Epigenetic mechanisms (DNA methylation, histone modifications, miRNA regulation) control cellular metabolic pathways.
- Changes in methylation patterns (hypomethylation or hypermethylation) affect gene expression, contributing to neuroinflammation, apoptosis, and Aβ production.
- DNA methylation regulates cell cycle components (P16, P21, P27, P53, RB1, cyclin B2, ARF protein product) and apoptosis pathways in AD neurons.
- miRNA alterations (miR-181c, miR-20a) impact apoptotic factors and Aβ

	formation, highlighting their complex roles in AD progression. - Reduced genomic methylation with age is linked to abnormal cell cycle events (p53-dependent apoptosis) and early epigenomic involvement in AD disease processes. Conclusion and Future Perspective - Apoptosis, a tightly regulated process, is crucial for cell number maintenance, but imbalance leads to pathological implications in AD. - Balancing pro-apoptotic and antiapoptotic signals is vital for cell survival. - Research aims to understand apoptotic neuronal death mechanisms in AD, targeting apoptotic factors as a potential preventive strategy. - Future studies may explore genomics, microRNA, nucleotide polymorphism, and genetic variants' roles in apoptosis for AD treatment.
Research Question/Problem/ Need	"What are the intricate apoptotic mechanisms contributing to neurodegeneration in Alzheimer's disease, and how can the identification of specific targets within these pathways guide potential therapeutic interventions?"
Important Figures	 The Multidomain Alzheimer's Etiopathology. AD is associated with multiple etiology which alters the optimum status of neurotransmission, bioenergetics, and

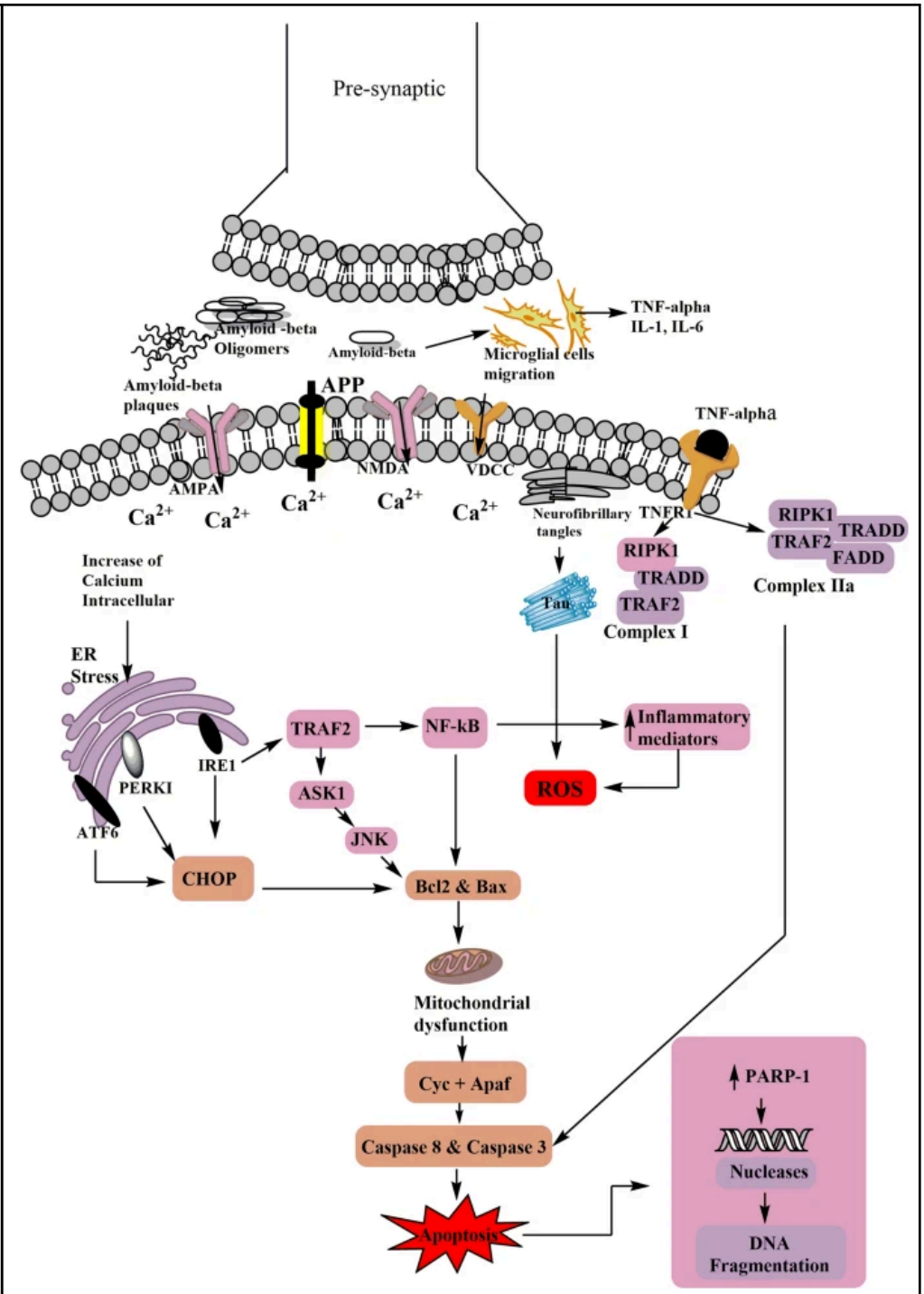
protein aggregation and clearance mechanisms. The clinical features of AD also include neuropsychiatric changes, behavioral alterations, and severe cognitive deficits. ROS Reactive Oxygen species; AGE advanced glycation end products; PS1 Presenilin 1; NFTs Neurofibrillary tangles; Aβ Amyloidβ; Ach Acetylcholine; 5-HT 5 hydroxytryptamine



The Dysfunctional apoptotic domain in AD. Various etiological factors (Aβ, NFTs, Oxidative stress) instigate an abnormal apoptotic cascade that affects diverse brain regions (cerebral cortex, hippocampus) with simultaneous activation of proapoptotic factors and decline in the anti-apoptotic mechanism. Aβ Amyloidβ, Bcl-2 The B-cell lymphoma 2, BCl-XL Bcl-XL (extra long), Mcl-1 myeloid cell leukemia-1, APP Amyloid precursor protein

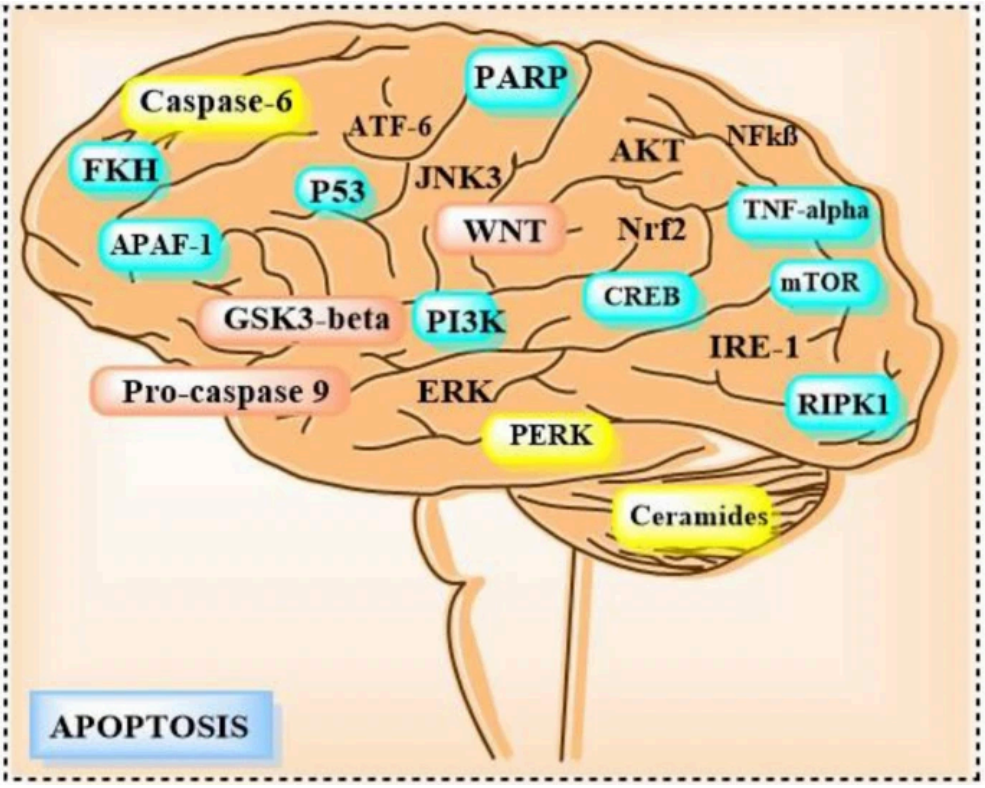


Mechanistic implications of various pathways in apoptotic and antiapoptotic response. Apoptosis implicates various pathways which have a dual role (apoptotic/antiapoptotic) depending upon the stage and status of activation. BAX Bcl-2 associated X protein, TAT–TIJIP Tat cell transporter sequence–Truncated Inhibitor of JNK Interacting Protein, CREB cAMP response element-binding protein, Drp1 Dnm1 in yeast, GTPase guanosine triphosphatase, Aβ β-amyloid peptide, JNK c- jun N-terminal kinase, FKH/FOXO factor Forkhead/forkhead box transcription factors, TSC1 Tuberous sclerosis protein 1, AMPK AMP-activated protein kinase, PI3K Phosphoinositide 3-kinases, AKT Protein kinase B (PKB), WNT Wingless-related integration, GSK3B Glycogen synthase kinase 3 beta, IFN-γ Interferon-gamma, IL-9 Interleukin 9, Erk1 extracellular-signal-regulated kinase, Trk tyrosine kinases, BDNF Brain-derived neurotrophic factor, JAK/STAT Janus kinase/signal transducers and activators of transcription, mTOR Mammalian Target of rapamycin, MAPK mitogen-activated protein kinase



An illustration of intracellular pathways mediated neuronal death in Alzheimer's disease. The abnormal aggregation of β-amyloid peptide (Aβ) in AD activates the intracellular downstream signaling of apoptosis by hyperactivation of metabotropic receptors inducing excitotoxicity by disrupting Ca2+ homeostasis. The increased intracellular calcium influx elicits mitochondrial dysfunction and endoplasmic oxidative stress-mediated neuronal death. In response to the prolonged endoplasmic stress, the unfolding to proteins initiates the activation of

IRE1/ PERK1/ ATF6 as an adaptive mechanism activating pro-apoptotic proteins mediated neuronal apoptotic death. The IRE1 also activates TRAF2 associated inflammatory signaling by activating NF-kB and increasing the expression of pro-inflammatory mediators playing a significant role in mitochondrial oxidative stress with increased expression of BCL2, BAX pro-apoptotic protein activating caspase-8 than caspase-3 mediated neuronal apoptotic death by up regulation of PARP1 causing DNA fragmentation. In response to the β-amyloid peptide (Aβ) toxicity, there is a migration of glial cells as a defense mechanism also leads to the initiating RIPK1/TRADD/ TRAF2 (complex I) cascade activating the NF-kB increasing ROS mediated neuronal apoptotic death or the TNFR1 also directly activates the RIPK1/TRADD/TRAF2/FADD (complex IIa) initiating the caspase-8 mediated neuronal apoptotic death. Aβ β-amyloid peptide, NF-kB nuclear factor kappa-B, IRE1 Inositol-requiring enzyme 1, PERK1 Protein kinase RNA-like endoplasmic reticulum kinase, ATF6 Activating transcription factor 6, TRAF2 tumor necrosis factor receptor-associated factor 2, BCL2 B-Cell CLL/Lymphoma 2, BAX Bcl-2-associated X protein, PARP1 Poly (ADP-ribose) polymerase 1, RIPK1 Receptor-interacting serine/threonine-protein kinase 1, TRADD TNFR1-associated death domain protein, FADD Fas-associated protein with death domain



Feasible targets to modulate Apoptotic cascade in AD. APAF-1 Apoptotic protease activating factor 1, GSK-3β Glycogen synthase kinase 3 beta, mTOR Mammalian Target of rapamycin, ATF Activating transcription factor, PARP Poly (ADP-ribose) polymerase, CREB cAMP response element-binding protein, NF-kB nuclear factor kappa-B, IRE1 Inositol-requiring enzyme 1, PERK1 Protein kinase RNA-like endoplasmic reticulum kinase

VOCAB: (w/definition)	Signal transduction - the process in which binding of an extracellular messenger to the cell surface receptor is translated into changes in biochemistry, cell biology, and gene transcription that make it possible for the cell to respond to the information that was received Excitotoxicity - cell death resulting from the toxic actions of excitatory amino acids. Neurotrophins - a family of proteins that induce the survival, development, and function of neurons Autophagy - a23n intracellular degradation process that allows cells to recycle damaged intracellular components to generate energy and provide building blocks to create new cellular structures.
Cited references to follow up on	Bhute S, Sarmah D, Datta A, Rane P, Shard A, Goswami A, Borah A, Kalia K, Dave KR, Bhattacharya P (2020) Molecular pathogenesis and interventional strategies for Alzheimer's disease: promises and pitfalls. ACS Pharmacol Transl Sci 3(3):472–488. https://doi.org/10.1021/acsptsci.9b00104 Sharma VK, Singh TG (2020) Navigating Alzheimer's disease via chronic stress: the role of glucocorticoids. Curr Drug Targets 21(5):433–444. https://doi.org/10.2174/1389450120666191017114735
Follow up Questions	How does the dysregulation of the PI3K/AKT signaling pathway impact apoptotic cascades in Alzheimer's disease? What are the specific epigenetic modifications influencing the expression of apoptotic genes in AD, and how do they contribute to neuronal loss? Can targeting specific inflammatory mediators, such as TNFα, alter the apoptotic pathways associated with neurodegeneration in Alzheimer's disease?

Article #17 Notes: PATENT- Method of treating Alzheimer's disease

Source Title	Method of treating Alzheimer's disease
Source citation (APA Format)	Malluche, H. Method of treating Alzheimer's disease. United States US4663318A, filed January 15, 1986, and issued May 5, 1987. https://patents.google.com/patent/US4663318A/en.

Original URL	https://patents.google.com/patent/US4897388A/en
Source type	Patent
Keywords	Vitamin D3, Calcitriol, treatment, Topical administration
#Tags	#treatment #VitaminD3 #competitor
Summary of key points + notes (include methodology)	The following notes were written with the aid of ChatGPT, an AI service. The patent details a method for treating Alzheimer's disease by administering biologically active vitamin D3 or vitamin D2 materials orally, parenterally, or topically. The dosage depends on the route of administration and the patient's condition. While vitamin D materials have historically been used for various calcium metabolic disorders, recent studies suggest a correlation between serum parathyroid hormone levels and cognitive decline in Alzheimer's patients, indicating a potential avenue for treatment. The patent elaborates on specific materials, dosages, and methods of administration, emphasizing the potential benefits for patients suffering from Alzheimer's disease. Field of Invention - Method of treating Alzheimer's disease using biologically active vitamin D3 and D2 materials. Background - Alzheimer's disease (AD) affects approximately 1.5 million individuals over 65 in the U.S. - Currently, the cause and treatment for AD remain unknown. Summary of the Invention - Treatment method involves administering biologically active vitamin D3 or D2 materials orally, parenterally, or topically, depending on the route of administration, patient's condition, and disease stage. Detailed Description - Administering calcitriol (Roche Laboratories' ROCALTROL®) is an especially preferred oral product for managing hypocalcemia in chronic renal dialysis. - Topical application methods include applying the biologically active vitamin D3 or D2 materials directly or utilizing their provitamins or previtamins on the skin. Example - Improvement observed in an Alzheimer's patient after administering 0.5 mcg of calcitriol daily for 7 days. Dosage and Administration - Dosage ranges from 0.25 mcg/day to 2 mcg/day for oral administration, adjusted based on the patient's condition and discretion of the physician.

	Conclusion - Various biologically active vitamin D2 and D3 materials, their provitamins, and previtamins can be utilized topically or orally for treating Alzheimer's disease. These notes maintain the content's structure and organization while using normal text headings.
Research Question/Problem/ Need	How can biologically active vitamin D3 and D2 materials be effectively employed to treat Alzheimer's disease and mitigate its progressive deterioration?
Important Figures	None given
VOCAB: (w/definition)	Calcitriol - hormonally-active, synthetic vitamin D analog prescribed to treat hypocalcemia, osteoporosis, and the prevention of corticosteroid-induced osteoporosis Cholecalciferol - secosteroid produced by the skin, also known as D3 Hypercalcemia - a condition in which the calcium level in your blood is above normal Ergosterol - a component of fungal cell membrane, serving the same function that cholesterol serves in animal cells
Cited references to follow up on	https://patents.google.com/patent/US20130330428A1/en https://patents.google.com/patent/US20100105646A1/en
Follow up Questions	How does the administration of biologically active vitamin D3 or D2 materials affect serum calcium levels and what implications does this have for Alzheimer's disease treatment? Can the application of these materials topically impact their transport and regulation in the bloodstream, and how is the danger of excessive dosages minimized through skin-controlled transport? In what ways does the administration route (oral, parenteral, or topical) and the specific biologically active material impact the effectiveness of treating Alzheimer's disease with vitamin D3 or D2 compounds?

Article #18 Notes: Caspases, Apoptosis, and Alzheimer Disease: Causation, Correlation, and Confusion

Source Title	Caspases, Apoptosis, and Alzheimer Disease: Causation, Correlation, and Confusion
Source citation (APA Format)	Roth, K. A., Caspases, Apoptosis, and Alzheimer Disease: Causation, Correlation, and Confusion, *Journal of Neuropathology & Experimental Neurology*, Volume 60, Issue 9, September 2001, Pages 829–838, https://doi.org/10.1093/jnen/60.9.829
Original URL	https://academic.oup.com/jnen/article/60/9/829/2916226
Source type	Journal Article
Keywords	Neuronal apoptosis, Caspase activation, Neurodegeneration, Molecular pathways, Cell death
#Tags	#NeurodegenerativeDisorders #ApoptosisMechanisms #CellularPathways #CaspaseInhibition #NeuronalDysfunction
Summary of key points + notes (include methodology)	The following notes were written with the aid of ChatGPT, an AI service. Extensive neuronal loss observed in Alzheimer's disease (AD) has led to speculation about the involvement of dysregulated apoptotic death pathways in its pathogenesis. Caspases, a family of cysteine proteases, have been implicated in regulating neuronal cell death triggered by amyloid β (Aβ) exposure in various studies. However, uncertainty remains regarding the direct role of caspase-dependent neuronal apoptosis in AD development. Contradictory experimental findings and the absence of clear evidence for morphologically identifiable apoptotic neurons in most AD brains have led to a revised hypothesis. This revised view suggests that molecular events linked to apoptosis might induce neuronal dysfunction in AD without necessarily causing neuronal death. This shift in perspective diminishes the functional significance of the term "apoptosis-associated" and diverts attention away from investigating the specific molecular triggers that prompt this response in AD neurons. Overall, while the involvement of caspases in AD remains inconclusive, targeting caspases for inhibition holds potential for prolonging neuron survival. Such an approach might allow other agents, targeting upstream events in AD pathology, to potentially delay

	or reverse primary disease processes. Apoptosis is a type of cell death - It is a natural process that occurs in organisms and is characterized by specific changes in the cell's structure and function. - These changes include condensation and fragmentation of the cell's genetic material (chromatin), shrinkage of the cell, and the formation of small structures called apoptotic bodies. Apoptosis in Caenorhabditis elegans - In the nematode Caenorhabditis elegans, apoptosis is regulated by a specific pathway involving four molecules: EGL-1, CED-9, CED-4, and CED-3. - EGL-1 is a pro-apoptotic molecule that initiates the process, while CED-9 is an anti-apoptotic molecule that inhibits apoptosis. - CED-4 and CED-3 are involved in the activation of apoptosis, with CED-3 being the key molecule that commits the cell to undergo apoptotic cell death. Mammalian counterparts of the C. elegans apoptotic pathway - In mammals, there are homologues (similar molecules) of the C. elegans apoptotic pathway. - The pro-apoptotic Bcl-2 family members, anti-apoptotic Bcl-2 family members, Apaf-1-like molecules, and the caspase family are the mammalian counterparts of EGL-1, CED-9, CED-4, and CED-3, respectively. - These molecules play similar roles in regulating apoptosis in mammalian cells. Caspase activation and cytological features of apoptosis - In both C. elegans and mammalian cells, caspase activation is responsible for the characteristic changes in cell structure and function that define apoptosis. - Caspases are a family of enzymes that play a central role in the execution of apoptosis. - The activation of caspases leads to the condensation and fragmentation of chromatin, membrane blebbing, cell shrinkage, and the formation of apoptotic bodies. Apoptotic cell death in normal nervous system development - Apoptosis has long been recognized as an important process in the normal development of the nervous system. - Studies in mice with targeted gene disruptions have helped to identify the specific molecular pathways that regulate naturally occurring cell death during nervous system development. Apoptotic cell death in disease processes - Apoptotic cell death can also be involved in disease processes in adult organisms. - Too little or too much cell death can cause significant pathology. - For example, in neoplasia (abnormal growth of cells), there may be insufficient cell death, while in hypoxic-ischemic tissue damage (lack of oxygen and blood

supply), there may be excessive cell death.

Apoptotic cell death in neurodegenerative diseases
- Neurodegenerative diseases are characterized by the selective loss of neurons.
- It has been speculated that apoptosis may play a role in the development of these diseases.
- Some authors have suggested that apoptosis is proven to be involved in neurodegenerative diseases, including Alzheimer's disease (AD).
- The loss of hippocampal neurons through apoptotic cell death is a prominent feature of AD.

Review of evidence for apoptotic death in AD
- The manuscript aims to review the evidence for apoptotic cell death in AD.
- It specifically focuses on the possible role of caspases, the key enzymes involved in apoptosis, in the pathogenesis of AD.

Extent of neuron death in the AD brain
- There is clear evidence of significant neuron death in the brain of individuals with Alzheimer's disease (AD).
- This means that a large number of neurons are dying in the AD brain.
- The term "neuron" refers to a specialized cell in the nervous system that transmits information through electrical and chemical signals.
- The term "neuron death" refers to the loss or destruction of these neurons.

Lack of neuron replacement
- Except for rare cases, the neurons that die in the AD brain are not replaced.
- This means that new neurons are not generated to replace the ones that have died.
- The term "neuron replacement" refers to the process of generating new neurons to replace the ones that have been lost.

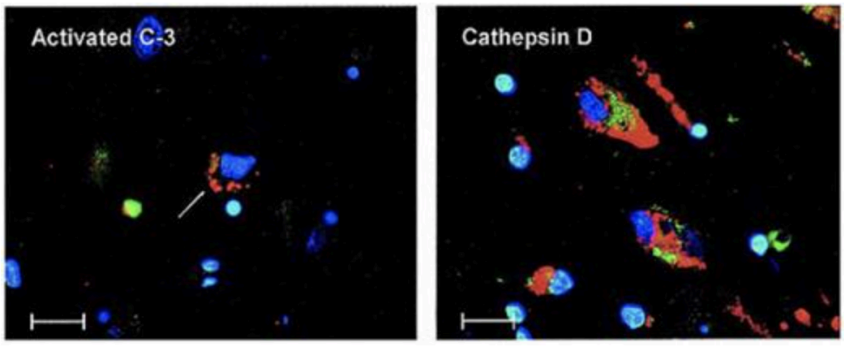
The role of neuron death in causing dementia
- The text suggests that the death of neurons may be responsible for the development of dementia in AD.
- Dementia refers to a decline in cognitive function, including memory loss, thinking skills, and problem-solving abilities.
- The term "neuronal dysfunction" refers to abnormal functioning of neurons, which can lead to cognitive impairments.
- The term "precedes" means that neuronal dysfunction may occur before neuron loss.
- This means that the dysfunction of neurons may happen before the actual death of neurons.
- The term "neurological symptoms" refers to the symptoms related to the nervous system, such as memory loss and cognitive decline.

Neuropathological hallmarks of AD
- Four key features are commonly observed in the brains of individuals with AD.

	- These features are referred to as "neuropathological hallmarks" because they are characteristic signs of the disease. - The four hallmarks are: mature senile plaques, neurofibrillary tangles, decreased synaptic density, and neuron loss. - Senile plaques and neurofibrillary tangles are abnormal protein deposits that can be easily identified in brain sections of individuals with AD. - The term "synaptic density" refers to the number of connections between neurons, known as synapses. - The term "neuron loss" refers to the death or loss of neurons. Correlation between hallmarks and AD symptoms - The text mentions that the density of senile plaques is only weakly correlated with the symptoms and progression of AD. - This means that the presence of senile plaques does not strongly predict the severity or progression of the disease. - Similarly, although neurofibrillary tangles are more closely correlated with AD symptoms, they affect only a small number of neurons and cannot fully explain all the neuronal dysfunction and death observed in AD. - Several studies have shown that both decreased synaptic density and neuron loss are highly correlated with clinical dementia in AD. - This means that a decrease in the number of synapses and the death of neurons are strongly associated with the development of dementia in AD. The "chicken and egg" dilemma - The text mentions a "chicken and egg" dilemma in understanding the relationship between synapse loss and neuron death in AD. - This dilemma arises because synapse loss is expected to occur as a result of neuron death. - On the other hand, neuron death may be a consequence of decreased support from the synapses. - The term "post-synaptic neurotrophic support" refers to the support and nourishment provided to neurons by the synapses. - This means that the loss of synapses can lead to the death of neurons, and the death of neurons can also result in the loss of synapses. - The text discusses the relationship between synaptic changes, neuron loss, and cell death in Alzheimer's disease (AD). - Several studies suggest that synaptic changes, such as loss of synapses and neuronal atrophy, occur before frank neuron loss in AD. - Rueger et al. proposed that synaptic loss and neuronal atrophy, rather than cell death itself, are important factors in AD. - Neuronal atrophy without significant cell loss can occur in various regions of the AD brain, including the nucleus basalis of Meynert. - The levels of synaptophysin, a protein related to synapses, were found to be decreased in hippocampal neurons with neurofibrillary tangles (a hallmark of AD) but without evidence of cell death. - Decreased synaptophysin protein expression is observed in the early stages of AD, indicating altered synaptic protein expression before neuronal death.

	- Ultrastructural examination of tangle-bearing neurons in AD suggests a gradual progression of cellular dysfunction rather than rapid cell death. - Several studies have shown decreased glucose metabolism in the temporal cortex of AD patients early in the disease, supporting the concept that dysfunction precedes cell death. - The resolution of the debate between synapse loss and cell death in AD is important for understanding the disease's causes and developing therapeutic strategies. - Evidence for apoptotic death in AD brain includes the detection of fragmented DNA, patchy neuron loss, increased expression of pro-apoptotic molecules, experimental data showing the effects of Aβ (a protein associated with AD) on apoptosis, and the detection of activated caspases (enzymes involved in apoptosis) in AD brain. - The text discusses the sequence of events in Alzheimer's disease (AD) and the importance of synaptic changes and neuronal atrophy in the disease process. - Studies have shown that synaptic changes, such as loss of synapses and neuronal atrophy, occur before frank neuron loss in AD. This suggests that synaptic changes may be an early event in the disease. - Rueger et al. proposed that synaptic loss and neuronal atrophy, rather than cell death itself, are important factors in AD. This means that the changes in synapses and neurons may be more relevant to the disease process than actual cell death. - Neuronal atrophy without significant cell loss can occur in various regions of the AD brain, including the nucleus basalis of Meynert. This means that the neurons may shrink or degenerate without actually dying. - The levels of synaptophysin, a protein related to synapses, were found to be decreased in hippocampal neurons with neurofibrillary tangles (a hallmark of AD) but without evidence of cell death. This suggests that changes in synaptic proteins may occur before neuronal death. - Decreased synaptophysin protein expression is observed in the early stages of AD, indicating altered synaptic protein expression before neuronal death. This further supports the idea that synaptic changes occur before cell death in AD. - Ultrastructural examination of tangle-bearing neurons in AD suggests a gradual progression of cellular dysfunction rather than rapid cell death. This means that the dysfunction of neurons may worsen over time, leading to cell death, rather than an abrupt cell death. - Several studies have shown decreased glucose metabolism in the temporal cortex of AD patients early in the disease, supporting the concept that dysfunction precedes cell death. This means that the brain's ability to use glucose for energy is reduced before cell death occurs. - The resolution of the debate between synapse loss and cell death in AD is important for understanding the causes of the disease and developing therapeutic strategies. Understanding whether synapse loss or cell death is the primary event in AD can guide research and treatment approaches. - Evidence for apoptotic death in AD brain includes the detection of fragmented DNA, patchy neuron loss, increased expression of pro-apoptotic molecules, experimental data showing the effects of Aβ (a protein associated with AD) on apoptosis, and the detection of activated caspases (enzymes involved in apoptosis)

	in AD brain. This means that there are multiple lines of evidence suggesting that apoptosis (programmed cell death) occurs in the brains of individuals with AD. - Caspase Function and Activation: - Caspases serve diverse roles, including involvement in apoptotic cell death pathways. - Different caspases show varying degrees of participation in neuronal apoptosis pathways. - Involvement of Specific Caspases: - ICE-like caspases (e.g., caspase-1, caspase-12) might indirectly impact neuronal apoptosis through inflammatory responses. - The role of initiator caspases (e.g., caspase-2, caspase-8, caspase-9) in AD brain remains inconclusive. - Effector caspases (e.g., caspase-3, caspase-6, caspase-7) show inconsistent expressions and activities in AD, lacking a clear association with AD pathogenesis. - Caspase-3 in AD Brain: - Reports on caspase-3 activation in AD brains are contradictory. - Limited and inconsistent caspase-3 activation challenges its role in widespread neuronal death in AD. - Interpretation of Activated Caspase-3 Immunoreactivity: - Caspase-3 immunostaining observed in some AD cases might indicate autophagic involvement rather than direct apoptosis, especially in granulovacuolar degeneration. - Complexity in Studying Caspase Involvement: - Diverse findings underscore the complexity and difficulties in studying caspase activation and apoptosis in AD brains. - Conclusions on Caspase Activation in AD: - Overall evidence supporting widespread neuronal apoptosis through caspase activation in AD is inconclusive and remains an active area of investigation.
Research Question/Problem/ Need	"Is caspase-dependent neuronal apoptosis a key factor in the pathogenesis of neurodegenerative diseases?"

Important Figures	**Fig. 1.**  Immunohistochemical detection of activated caspase-3- and cathepsin D-like immunoreactivity in AD brain. Activated caspase-3 (C-3)-like immunoreactivity (left panel) was detected in hippocampal pyramidal neurons possessing histological features of granulovacuolar degeneration. Activated caspase-3-like immunoreactivity (red; indicated by an arrow) appeared confined to the granulovacuolar structures, and these positive cells lacked apoptotic nuclear features. No specific staining was observed associated with neurofibrillary tangle-bearing neurons, senile plaques, or abnormal neurites. Cathepsin D-like immunoreactivity (right panel; red) was abundantly present throughout the cell body and proximal processes of virtually all pyramidal neurons in the AD hippocampus. Immunoreactive activated caspase-3 and cathepsin D were detected with cyanine-3 tyramide signal amplification (red). Autofluorescent material, largely lipofuschin, was visualized with a fluorescein filter set (green) and nuclei were labeled with Hoechst 33,258 (blue). Scale bar: 20 µm
VOCAB: (w/definition)	Neuritic- Pertaining to the processes or structures related to neurons, particularly their extensions or projections. Etiologic- Referring to the cause or origins of a disease or condition. Synaptosomes- Small vesicles containing a high concentration of synapses isolated from nerve endings
Cited references to follow up on	Wellington CL Hayden MR. Caspases and neurodegeneration: On the cutting edge of new therapeutic approaches. *Clin Genet* 2000;57;:1–10 Gervais FG Xu D Robertson GSet al. . Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-β precursor protein and amyloidogenic Aβ peptide formation. *Cell 1999*;97:395–406
Follow up Questions	How might the dysregulation of certain molecular pathways other than apoptosis or caspase activation contribute to neuronal dysfunction in neurodegenerative disorders? What are the primary methodologies or techniques utilized to discern early-stage neuronal dysfunction before the occurrence of significant cell death in neurodegenerative diseases? In the context of potential therapeutic interventions, what challenges exist in targeting upstream events that trigger the "apoptosis-associated" response in

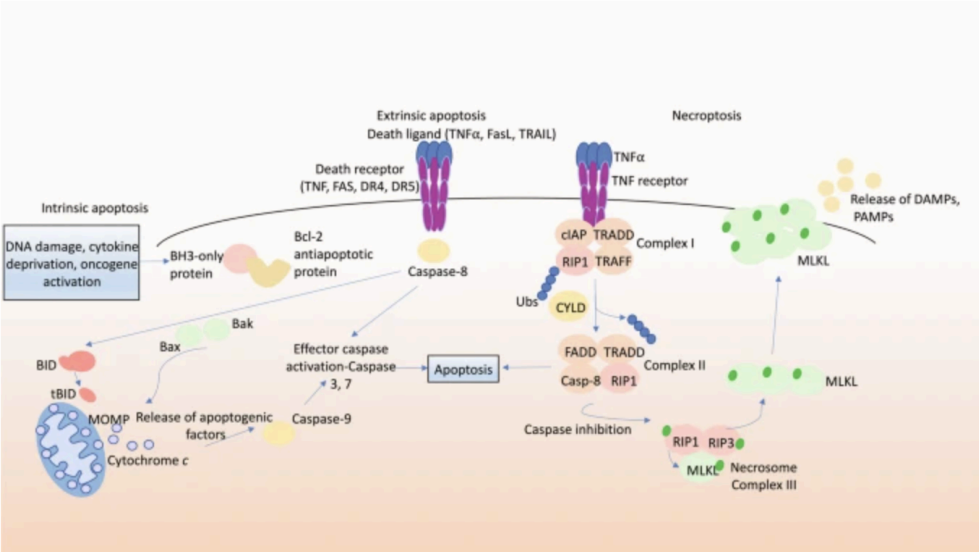
	neurons without directly inhibiting or altering the apoptotic cascade?

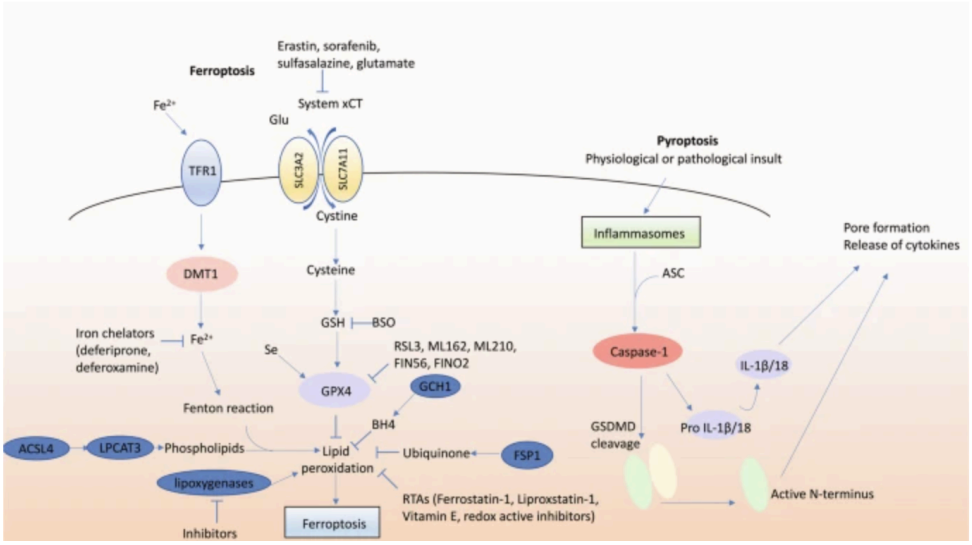
Article #19 Notes: Molecular mechanisms of cell death in neurological diseases

Source Title	Molecular mechanisms of cell death in neurological diseases
Source citation (APA Format)	Moujalled, D., Strasser, A. & Liddell, J.R. Molecular mechanisms of cell death in neurological diseases. *Cell Death Differ* 28, 2029–2044 (2021). https://doi.org/10.1038/s41418-021-00814-y
Original URL	https://www.nature.com/articles/s41418-021-00814-y
Source type	Journal Article
Keywords	Neurodegeneration, PCD, Therapeutics, Pathogenesis
#Tags	#apoptosis #mechanisms #general #cell-death
Summary of key points + notes (include methodology)	The following notes were written with the aid of ChatGPT, an AI service. The orchestrated signaling of programmed cell death (PCD) is integral during normal neuronal development, shaping the central nervous system (CNS) in a precise spatial and temporal manner. However, abnormalities in various PCD pathways, including apoptosis, necroptosis, pyroptosis, ferroptosis, autophagy-associated cell death, and unprogrammed necrosis, are evident in the pathogenesis of neurological diseases. These pathways can be activated by cellular stress and inflammatory processes, leading to neuronal loss observed in diseases like ALS, Alzheimer's, Parkinson's, and Huntington's. Conversely, inadequate activation of PCD contributes to brain cancers, impacting their response to treatments. Current therapies for these conditions have limited effectiveness, prompting a need for deeper investigations into disease origins. Given the dysregulation of PCD pathways in brain-related diseases, potential therapeutic agents capable of either inhibiting or inducing PCD may hold key roles in future treatment strategies. Developing such therapies necessitates rigorous preclinical studies using animal models that accurately mimic human disease conditions. This review comprehensively discusses the roles of various PCD and unprogrammed cell death processes in neurodegenerative diseases and brain cancers, highlighting

	the interaction between different cell death signaling pathways and their contributions to disease progression. Additionally, it outlines pharmacological agents targeting pivotal players in these pathways that have advanced into clinical trials. Apoptosis Pathways: Intrinsic Pathway: Regulated by BCL-2 protein family, BH3-only proteins, liberation of BAX and BAK, MOMP, and activation of caspase cascade. Death Receptor Pathway: Activation via TNFR superfamily members, intracellular death signaling complex formation, and downstream caspase activation. Amyotrophic Lateral Sclerosis (ALS): Molecular processes: Oxidative stress, excitotoxicity, mitochondrial dysfunction. Molecular associations: Mutant SOD1 with anti-apoptotic proteins, altered expression of BCL-2 family members, caspases, and BH3-only proteins. Huntington's Disease (HD): Observations in mouse models: Increased levels of pro-apoptotic proteins BIM and BAX, significant effects of Bim loss on disease phenotype. Parkinson's Disease (PD): Mechanism: Neuronal death primarily via the intrinsic apoptosis pathway. Genetic associations: Mutations in genes related to mitochondrial function intersecting with apoptosis pathway, observations of increased caspase-3 and BAX in PD patients. Glioblastoma Multiforme (GBM): Resistance mechanisms: Higher expression of BCL-2, BCL-XL in GBM cells, potential markers for therapy resistance. Therapeutic potential: RNA interference-mediated reduction in BCL-2/XL causing caspase-dependent cell death. Necroptosis Mechanism: Activation via blockage of caspase-8, involving RIPK1, RIPK3, MLKL, causing plasma membrane lysis, release of DAMPs/PAMPs, and inflammation. Disease Associations: AD: Evidence of necroptosis exacerbating cognitive deficits, potential therapeutic implications with RIPK1 inhibition. Stroke: Preclinical evidence showcasing neuroprotection via necroptosis inhibition, lacking clinical validation. Cancer Implications: Contradictory roles: Necroptosis either promoting or inhibiting tumor growth based on expression levels of RIPK1, RIPK3, MLKL. GBM and Lung cancer: Higher expression of necroptosis signaling proteins correlating with adverse prognosis, suggesting potential therapeutic strategies. Autophagy: Overview: Autophagy is a cellular process crucial for degrading molecules and organelles, playing a role in maintaining cellular and tissue balance. Functions: It aids in protein turnover, organelle degradation, and provides cells with nutrients during stress. Role in Diseases: Implicated in various diseases like ALS, Alzheimer's (AD),

	Parkinson's (PD), Huntington's (HD), and glioblastoma (GBM). Neurodegenerative Diseases: In ALS, mutations in autophagy-related genes are linked to familial forms of the disease. In AD, defective autophagy affects Aβ turnover. PD, HD, and GBM also show associations with dysregulated autophagy. Therapeutic Potential: Enhancing autophagy might offer neuroprotective outcomes, especially in AD, but its role is complex and context-dependent in other diseases. Ferroptosis: Characteristics: Iron-dependent necrotic cell death involving lipid peroxidation leading to cellular damage. Mechanisms: Involves molecules like ACSL4, LPCAT3, GPX4, and regulators like system xCT and iron-disrupting stimuli. Implications: Implicated in various diseases including neurodegenerative disorders like AD, PD, ALS, and glioma. Therapeutic Potential: Several inhibitors, including deferoxamine and deferiprone, have shown promise in preclinical models and ongoing clinical trials for AD, PD, and ALS. Pyroptosis: Nature: Inflammatory cell death involving caspase-1 activation by inflammasomes leading to IL-1β and IL-18 release. Implications: Observed in various neurodegenerative diseases like AD, PD, ALS, and multiple sclerosis. Role in Diseases: Associated with microglia and oligodendrocytes in disease models, suggesting a role in pathology. Necrosis: Characteristics: Unprogrammed, unregulated cell death involving cell swelling, membrane integrity loss, and triggering an inflammatory response. Disease Implications: Seen in various pathological conditions, including neurodegenerative diseases like AD, PD, and stroke. Interplay with Other PCD Pathways: Interplay of various programmed necrotic pathways like necroptosis, ferroptosis, and pyroptosis may drive the pathology of neurological diseases. Therapeutic Implications: Targeting Cell Death Pathways: While targeting apoptosis has shown limitations, inhibitors of necroptosis, pyroptosis, and apoptosis effectors could hold promise. Clinical Trials and Potential Treatments: Trials involving RIPK1 inhibitors, sodium phenylbutyrate, Taurursodiol, and Bcl2l12 siRNAs show promise in various diseases like AD, PD, ALS, and GBM. Conclusion: Complexity and Promise: The pathogenesis of neurological diseases involves multiple cell death mechanisms, demanding a multifaceted therapeutic approach.
Research Question/Problem/ Need	How do aberrations in programmed cell death pathways, including apoptosis, necroptosis, pyroptosis, ferroptosis, autophagy-associated cell death, and unprogrammed necrosis, contribute to the pathogenesis of neurodegenerative diseases and brain cancers, and what are the potential therapeutic implications of

	targeting these pathways?
Important Figures	 Intrinsic apoptosis signalling: in response to growth factor deprivation, DNA damage or oncogene activation, BH3-only pro-apoptotic proteins are induced transcriptionally or post-transcriptionally. The BH3-only proteins bind to and inhibit the anti-apoptotic BCL-2 proteins. This leads to release and activation of the effectors of cell death, BAX and BAK, which then oligomerise and promote mitochondrial outer membrane permeabilisation (MOMP), leading to release of apoptogenic factors, including cytochrome c and Smac/Diablo. This initiates a cascade of caspase activation, cleavage of hundreds of cellular proteins and consequent cell demolition. Death receptor induced (extrinsic) apoptosis signalling: stimulation of death receptors (members of the TNFR family with an intracellular death domain) by their cognate ligands (e.g. stimulation of FAS by FASL) results in adaptor protein (FADD, TRADD) mediated recruitment and activation of the initiator caspase, caspase-8 (in humans also caspase-10), which can then activate downstream effector caspases (caspases-3, -7), resulting in cell demolition (see above). The death receptor induced apoptosis pathway can connect to the intrinsic apoptotic pathway by proteolytic activation of the pro-apoptotic BH3-only protein BID (to generate tBID) by caspase-8. Necroptosis: the best characterised pathway triggering necroptosis is via TNFR1 stimulation. Upon binding of TNFα to TNFR1, cIAPs, RIPK1, TRAFs and TRADD are recruited to the intracellular part of TNFR1, forming the TNFR1 signalling complex I, which activates NFkB and AP1 transcription factors and thereby stimulates cell survival and proliferation. Upon deubiquitylation of RIPK1 by CYLD, RIPK1 can bind to TRADD, FADD and caspase-8 forming complex II which can drive caspase-8 mediated apoptosis (see above). In the absence of caspase-8 (genetic ablation or pharmacological inhibition) and the absence or inhibition of cIAP1/2, the necrosome (complex III) is formed where RIPK3 can phosphorylate MLKL, promoting its translocation to the plasma membrane where MLKL causes cell lysis, resulting in lytic cell death with release of DAMPs and PAMPs.

	 Ferroptosis is potentiated by iron which can be imported by TFR1 and DMT1, and requires the presence of susceptible arachidonic acid-containing phospholipids generated by ACSL4 and LPCAT3. Ferroptosis is endogenously inhibited by GPX4 which requires GSH as a substrate, by ubiquinone which is reduced by FSP1, by BH4 which is generated by GCH1, and by other endogenous RTAs such as vitamin E. Ferroptosis is promoted by disruption of cysteine supply via inhibition of the glutamate/cystine antiporter system xCT (by compounds such as erastin, sorafenib, sulfasalazine or glutamate), or impaired GPX4 activity due to insufficient glutathione or selenium or by direct inhibition (e.g. RSL3, ML162, ML210, FIN56, FINO2). Ferroptosis inhibitors include iron chelators (such as deferiprone and deferoxamine), RTAs (including ferrostatin-1 and liproxstatin-1) and lipoxygenase inhibitors. Pyroptosis can be activated in response to physiological or pathological insults which result in activation of inflammasomes, such as the NLRP3 inflammasome, in which the adaptor ASC is recruited, resulting in activation of caspase-1. Caspase-1 proteolytically processes pro-IL-1β and pro-IL-18 into the bio-active forms of these cytokines. Caspase-1 can also cleave GSDMD, and the N-terminal fragment of GSDMD is recruited to the plasma membrane where it causes pore formation, cell swelling and plasma membrane rupture. BH4 tetrahydrobiopterin, BSO Buthionine sulfoximine, DMT1 divalent metal transporter 1, GCH1 GTP cyclohydrolase-1, GPX4 glutathione peroxidase 4, GSH glutathione, RTAs radical trapping antioxidants, Se selenium, System xCT glutamate/cystine antiporter, TFR1 transferrin receptor 1, NLRP3 NLR family pyrin domain-containing 3.
VOCAB: (w/definition)	Ferroptosis- A type of cellular demise characterized by an iron-dependent cascade leading to oxidative damage of lipids within cell membranes, ultimately resulting in cell death. Necroptosis- A regulated form of cell death sharing similarities with necrosis, occurring due to specific external triggers and involving a programmed series of intracellular events culminating in cellular rupture and the release of intracellular

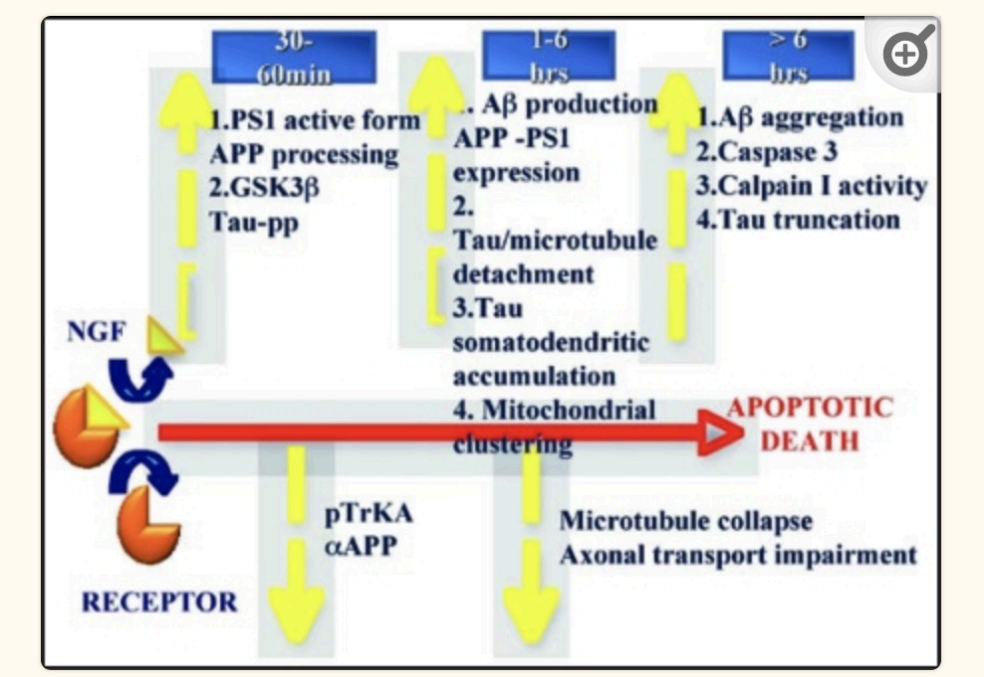
	contents. Interplay- The dynamic interaction or mutual influence among various elements or pathways, in this context, possibly referring to the complex relationships and mutual effects between different cell death signaling pathways and their roles in disease progression. Excitotoxicity- The process where nerve cells are detrimentally affected by excessive stimulation from neurotransmitters, leading to an influx of calcium ions into cells and initiating harmful biochemical reactions, often resulting in cell damage or death in certain neurological conditions.
Cited references to follow up on	Huang DC, Strasser A. BH3-Only proteins-essential initiators of apoptotic cell death. *Cell. 2000*;103:839–42. Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature. 2006*;443:787–95.
Follow up Questions	Could a combination of therapies targeting multiple cell death pathways provide a more effective approach in treating neurodegenerative diseases or brain cancers? What are the future prospects and challenges in the development of therapies that focus on modulating PCD pathways for treating neurological disorders and brain cancers? What reliable biomarkers associated with aberrant PCD pathways could enable early diagnosis and intervention in neurological diseases?

Article #20 Notes: Apoptosis and in vitro Alzheimer disease neuronal models

Source Title	Apoptosis and in vitro Alzheimer disease neuronal models
Source citation (APA Format)	Calissano, P., Matrone, C., & Amadoro, G. (2009). Apoptosis and in vitro Alzheimer disease neuronal models. *Communicative & integrative biology, 2*(2), 163–169. https://doi.org/10.4161/cib.7704
Original URL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2686374/
Source type	Journal Article
Keywords	apoptosis, caspase, amyloid beta, tau protein, neurotrophin, Alzheimer disease
#Tags	#neurons #apoptosis #caspases

Summary of key points + notes (include methodology)	The following notes were written with the aid of ChatGPT, an AI service. The text offers a comprehensive overview of Alzheimer's Disease (AD) pathogenesis, focusing on the interplay between apoptotic events, amyloid beta (Aβ) accumulation, tau pathology, and neuronal death. It outlines the neuropathological hallmarks of AD, emphasizing the significance of Aβ and tau proteins in triggering and progressing the disease. The "amyloid cascade hypothesis" is highlighted, showcasing the pivotal role of Aβ production in affecting downstream tau metabolism. The text discusses the involvement of apoptosis in AD, citing multiple triggers such as oxidative stress, Aβ accumulation, metabolic impairment, and altered protein processing. It delves into the roles of caspases, particularly their involvement in amyloid precursor protein (APP) processing, Aβ generation, and tau modifications. Furthermore, it details the significance of experimental models like Cerebellar Granule Cells (CGC) and NGF(Nerve Growth Factor)-deprived PC12 cells, illustrating their relevance in studying apoptosis, amyloidogenesis, tau alterations, and caspase-mediated neuronal death. The text underscores the complex relationship between NGF deprivation, amyloidogenic pathway activation, tau alterations, and apoptotic death in vitro, shedding light on potential therapeutic strategies targeting these pathways. Alzheimer's Disease (AD) Pathology Overview: - Characteristics of AD: - Late-onset and sporadic neurodegenerative disorder affecting cognitive functions. - Presents with progressive global cognitive decline, including memory loss, orientation difficulties, and reasoning impairments. - Neuropathological Hallmarks: - Synaptic loss and dysfunction, reduced neuronal metabolism, senile plaques (SPs), and neurofibrillary tangles (NFTs) are characteristic features. - SPs consist of aggregated amyloid beta (Aβ), abnormal neurites, and glial cells. - NFTs comprise intracellular accumulations of Paired Helical Filaments (PHF) primarily composed of abnormally phosphorylated tau. Role of Amyloid Beta (Aβ) and Tau in AD: - Amyloid Cascade Hypothesis: - Posits that Aβ production serves as the initial factor impacting downstream tau metabolism in AD. - Genetic mutations or environmental factors alter Aβ processing, leading to an increased Aβ42/40 ratio or propensity for aggregation. - Impact of Aβ on Neuronal Function:

	- Aβ accumulation triggers neuronal loss or dysfunction through oxidative stress, altered mitochondrial metabolism, Ca++ imbalance, etc. - Aβ induces caspase-mediated tau cleavage, hyperphosphorylation, and subsequent aggregation. Apoptosis in AD: - Apoptosis Contribution: - Emerging evidence suggests apoptosis may contribute to the onset and progression of AD due to various stimuli and cellular stressors. - Presence of apoptotic markers observed in AD brain tissue, although direct causation remains debated. - Caspase Involvement: - Activation of caspases and their association with pathological markers like senile plaques, neurofibrillary tangles, and altered protein expression levels in AD brain tissue. - Altered ratios of pro-apoptotic (e.g., Bax, Bak, Bad) and anti-apoptotic (e.g., Bcl-2, Bcl-xL) proteins in post-mortem brains of AD patients. - Caspases and APP/Tau: - Caspases play a role in amyloid precursor protein (APP) processing, Aβ generation, and tau protein cleavage. - Specific caspase inhibition exhibits protective effects against Aβ-induced cell death in experimental models. Experimental Models and AD Pathology: - Cerebellar Granule Cells (CGC) Model: - Establishes a link between apoptosis, amyloidogenic processes, tau alterations, and neuronal death. - Offers insights into amyloidosis, tau hyperphosphorylation, and mitochondrial dysfunction in AD-related pathology. - NGF-Deprived PC12 and Hippocampal/Cortical Neuronal Models: - NGF imbalance triggers amyloidogenic pathway activation, tau modifications, and apoptosis. - Links NGF deprivation to altered APP processing, tau phosphorylation, and neuronal death, hinting at potential therapeutic avenues. Complexity of Interactions: - Caspases and Pathological Cascade: - Interplay between caspases, Aβ, tau alterations, and apoptosis forms a complex cascade of events. - Sequential relationship between apoptosis, Aβ production, and tau modifications remains intricate and context-specific.

Research Question/Problem/ Need	Is there a possible involvement of apoptosis in AD pathogenesis, and how useful is ad hoc devised in vitro approaches to study how caspase(s), amyloidogenic processing and tau metabolism might reciprocally interact leading to neuronal death?
Important Figures	 Schematic representation of the apoptotic mechanisms by which the interruption of NGF signaling affects APP processing and Tau metabolism in hippocampal neurons.
VOCAB: (w/definition)	Proteolytic- Relating to or involving the breakdown or digestion of proteins into smaller peptides or amino acids by enzymes called proteases. Neurotransmitter- A chemical substance produced by neurons that transmit signals across synapses to other neurons, muscles, or glands, allowing for communication within the nervous system. Amyloidogenesis- The process by which proteins misfold and aggregate to form insoluble fibrillar structures known as amyloid, which is associated with various neurodegenerative diseases, including Alzheimer's disease. Neurotrophin- A group of proteins that support the growth, survival, and differentiation of neurons in the nervous system. Neurotrophins, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), play crucial roles in neuronal development and maintenance.

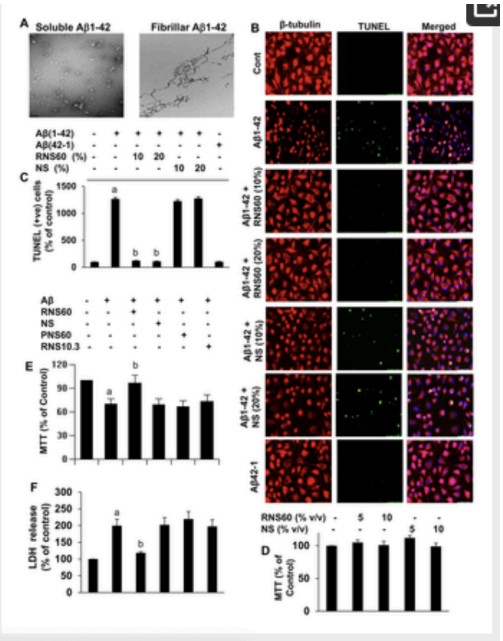
Cited references to follow up on	Selkoe DJ. Alzheimer's disease: genes, proteins and therapy. *Physiol Rev.* 2001;81:741–766.
Follow up Questions	What precise molecular targets or signaling pathways could be manipulated to prevent or halt the apoptotic cascade triggered by Aβ accumulation or tau modifications? How can we design or refine therapeutic interventions that selectively reduce Aβ levels without affecting caspase(s) activity to discern their independent roles in neurodegeneration? In what ways can in vitro models, particularly those mimicking NGF-deprived cultures, be further refined to better replicate the complex molecular and cellular interactions seen in Alzheimer's disease pathology? Are there other models or experimental setups that could complement or enhance our understanding of apoptosis, Aβ production, tau alterations, and their interconnections in neurodegenerative processes?

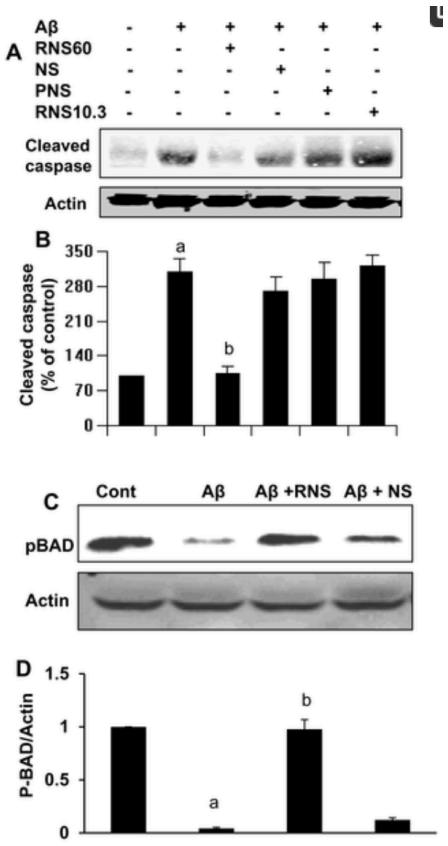
Article #21 Notes: A Physically-Modified Saline Suppresses Neuronal Apoptosis, Attenuates Tau Phosphorylation and Protects Memory in an Animal Model of Alzheimer's Disease

Source Title	A Physically-Modified Saline Suppresses Neuronal Apoptosis, Attenuates Tau Phosphorylation and Protects Memory in an Animal Model of Alzheimer's Disease
Source citation (APA Format)	Modi, K. K., Jana, A., Ghosh, S., Watson, R. L., & K. Pahan. (2014). A Physically-Modified Saline Suppresses Neuronal Apoptosis, Attenuates Tau Phosphorylation and Protects Memory in an Animal Model of Alzheimer's Disease. *PLOS ONE*, *9*(8), e103606–e103606. https://doi.org/10.1371/journal.pone.0103606
Original URL	https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0103606
Source type	Journal Article
Keywords	Amyloid-β (Aβ), Hippocampus, Behavioral tests, Neuronal apoptosis, Tau phosphorylation, Memory, Learning, Neuroprotection

#Tags	#RNS60 #salinetherapy #innovativemedicine #noninvasive #potentialdrug
Summary of key points + notes (include methodology)	The following notes were written with the aid of Google Bard, an AI service. This study investigates the effects of a physically-modified saline (RNS60) on neuronal apoptosis, tau phosphorylation, and memory in an animal model of Alzheimer's disease (AD). RNS60 is a saline solution containing oxygenated nanobubbles that is generated by subjecting normal saline to Taylor-Couette-Poiseuille (TCP) flow under elevated oxygen pressure. The authors found that RNS60 treatment suppressed neuronal apoptosis and tau phosphorylation in SHSY5Y neuronal cells and 5XFAD transgenic mouse model of AD. RNS60 also reduced the burden of amyloid-β (Aβ) in the hippocampus and protected memory and learning in the 5XFAD mice. ● Background: ● ● Alzheimer's disease (AD) is a neurodegenerative disorder characterized by neuronal loss, amyloid-β (Aβ) plaques, tau tangles, and memory decline. ● Current treatments for AD are limited and mainly focus on managing symptoms. ● Study Hypothesis: ● ● A physically-modified saline (RNS60) can protect neurons, reduce Aβ burden, and improve memory in an animal model of AD. ● Methods: ● ● RNS60 preparation: Normal saline was subjected to Taylor-Couette-Poiseuille (TCP) flow under elevated oxygen pressure to generate oxygenated nanobubbles. ● Cell culture studies: SHSY5Y neuronal cells were treated with Aβ and then with RNS60. Neuronal apoptosis and tau phosphorylation were measured. ● Animal model: 5XFAD transgenic mice, a well-established model of AD, were administered RNS60. ● Outcome measures: Neuronal apoptosis, tau phosphorylation, Aβ burden in the hippocampus, memory and learning performance in behavioral tests. ● Results: ● ● Cell culture studies: RNS60 treatment significantly suppressed Aβ-induced neuronal apoptosis and tau phosphorylation in SHSY5Y cells. ● Animal model: RNS60 administration reduced neuronal apoptosis and tau phosphorylation in the hippocampus of 5XFAD mice. ● RNS60 treatment also decreased the burden of Aβ in the hippocampus. ● In behavioral tests, RNS60-treated mice showed significantly better performance compared to untreated mice, indicating improved memory and learning. ● Conclusions:

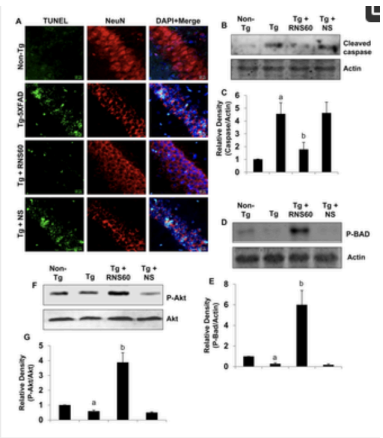
	● ● RNS60 treatment has neuroprotective effects, reducing neuronal apoptosis and tau phosphorylation in both cell and animal models of AD. ● RNS60 also reduces Aβ burden and improves memory and learning in 5XFAD mice. ● These findings suggest that RNS60 may be a promising therapeutic candidate for AD. ● Additional Notes: ● ● The mechanism of action of RNS60 is not fully understood, but it may be related to its physical properties and its interaction with neuronal membranes. ● Further studies are needed to confirm the safety and efficacy of RNS60 in humans before it can be used in clinical trials. ● Potential benefits of RNS60: ● ● Non-invasive and potentially less expensive compared to existing AD medications. ● May have fewer side effects due to its simple saline composition. ● Could potentially be used for early intervention to prevent or delay the progression of AD. ● Limitations of the study: ● ● Only one animal model was used, further testing in other models is needed. ● The long-term effects of RNS60 treatment are not known. ● Future directions: ● ● Investigate the mechanism of action of RNS60. ● Conduct clinical trials to test the safety and efficacy of RNS60 in human patients with AD. ● Explore the potential use of RNS60 for other neurodegenerative diseases. ● Overall, this study provides promising evidence that RNS60 may be a novel therapeutic approach for AD with the potential to improve memory and protect neurons. ●
Research Question/Problem/ Need	Can a physically-modified saline solution called RNS60 protect neurons, reduce tau phosphorylation, and improve memory in an animal model of Alzheimer's disease?

Important Figures	 "Figure 1. RNS60 strongly inhibits fibrillar (Aβ1–42)-induced apoptosis in SHSY5Y neuronal cells. A, Morphology of soluble and fibrillar form of (Aβ1–42) peptides was examined by transmission electron microscopy. B, SHSY5Y neuronal cells were either pretreated with different concentrations of RNS60 or NS for 1 h in Neurobasal medium containing 2% B27-AO followed by insult with 1 μM fibrillar Aβ1–42 for 6 h. Apoptotic events were detected by TUNEL. C, Digital images were collected under bright-field setting using a 20× objective. TUNEL-positive neurons were counted manually in four different images of each of three coverslips. Values obtained from the control group served as 100%, and data obtained in other groups were calculated as percent of control accordingly. Results are mean ± S.D. of three different experiments. ap<0.001 vs control; bp<0.001 vs Aβ1–42. D, Cells were treated with different concentrations of RNS60 and NS for 24 h followed by monitoring cell viability by MTT assay. Results are mean ± S.D. of three different experiments. Cells preincubated with 10% RNS60, NS, RNS10.3, and PNS60 for 1 h were stimulated by Aβ. After 24 h of stimulation, cell viability was monitored by MTT (E) and LDH release (F). Results are mean ± S.D. of three different experiments. ap<0.01 vs control; bp<0.01 vs Aβ1–42."



"Figure 2. RNS60 suppresses fibrillar (Aβ1–42)-induced apoptotic signaling pathway in SHSY5Y neuronal cells.

A, Cells preincubated with 10% RNS60, NS, RNS10.3, and PNS60 for 1 h were stimulated by Aβ. After 4 h of stimulation, cells lysates were analyzed for cleaved caspase by Western blot. Membranes were stripped and reprobed with anti-β-actin antibody. B, Bands were scanned and results presented as protein expression relative to Actin. Results are expressed as mean ± SD of three different experiments. ap<0.001 vs control; bp<0.001 vs Aβ1-42. C, Cells were pretreated with 10% v/v RNS60 or NS for 1 h in followed by exposure to Aβ (1 μM). After 3 h of challenge, cell lysates were prepared and analyzed by Western blotting with antibodies against phospho-BAD. Membranes were stripped and reprobed with anti-β-actin antibody. D, Bands were scanned and results presented as protein expression relative to Actin. Results are expressed as mean ± SD of three independent experiments. ap<0.001 vs control; bp<0.001 vs Aβ1-42."

	 "Figure 3. RNS60 treatment inhibits neuronal apoptosis in vivo in the hippocampus of Tg5XFAD mice. Tg mice (5 months old) were treated with RNS60 and NS (300 µl/mouse/2d) via i.p. injection and after 2 months of treatment, hippocampal sections were double-labeled for TUNEL and NeuN (A). Results represent analysis of two hippocampal sections of each of five mice per group. Tissue lysates were analyzed for cleaved caspase 3 (B&C), phospho-BAD (D&E) and phospho-Akt/total Akt (F&G) by Western blot. Bands were scanned and results presented as relative density (C, E & G). Results represent mean ± SEM of four mice per group. ap<0.001 vs non-Tg; bp<0.001 vs Tg."
VOCAB: (w/definition)	Neuroprotection - Any strategy or treatment that protects neurons from damage or death. RNS60, the physically-modified saline studied in the paper, is being investigated for its potential neuroprotective effects in Alzheimer's disease. Oxygenated Nanobubbles - Microscopic bubbles in RNS60 formed by subjecting saline to Taylor-Couette-Poiseuille flow under elevated oxygen pressure. Synaptic Plasticity - The ability of synapses, the junctions between neurons, to change their strength and function.
Cited references to follow up on	Martin JB (1999) Molecular basis of the neurodegenerative disorders. N Engl J Med 340: 1970–1980. Lee VM, Goedert M, Trojanowski JQ (2001) Neurodegenerative tauopathies. Annu Rev Neurosci 24: 1121–1159.
Follow up Questions	Does RNS60 target specific neuronal populations or pathways, or does it have broader effects? Can its action be further refined for targeted therapy? How efficiently do oxygenated nanobubbles deliver oxygen to neurons? Can this delivery be optimized for deeper brain regions? What specific inflammatory pathways are affected by RNS60?

Article #22 Notes: The modular systems biology approach to investigate the control of apoptosis in Alzheimer's disease neurodegeneration

Source Title	The modular systems biology approach to investigate the control of apoptosis in Alzheimer's disease neurodegeneration
Source citation (APA Format)	Alberghina, L., Colangelo, A.M. The modular systems biology approach to investigate the control of apoptosis in Alzheimer's disease neurodegeneration. *BMC Neurosci* 7 (Suppl 1), S2 (2006). https://doi.org/10.1186/1471-2202-7-S1-S2
Original URL	https://bmcneurosci.biomedcentral.com/articles/10.1186/1471-2202-7-S1-S2#citeas
Source type	Journal Article
Keywords	Apoptosis, modular systems, control
#Tags	#cell-death #solution #investigation
Summary of key points + notes (include methodology)	The following notes were written with the aid of Google Bard, an AI service. The complex puzzle of apoptosis in Alzheimer's disease (AD) can be tackled by dissecting it into manageable modules, thanks to the modular systems biology approach. This approach sheds light on two key hypotheses: the dwindling support of neurotrophic factors like NGF and BDNF, and the cascade of damage triggered by amyloid plaque buildup and tau tangles. However, the path to neuronal death isn't linear – various insults, from disrupted microtubule transport to oxidative stress, converge and interact, adding complexity to the picture. To untangle this web, the proposed model envisions interconnected modules, each focusing on specific aspects like neurotrophic support or oxidative stress. By understanding how these modules interplay, we gain valuable insights into the mechanisms behind AD, potentially paving the way for targeted therapeutic strategies that address the disease at its core. ● Unveiling the Puzzle: ● ● Modular Approach: Decodes the intricate apoptosis process by breaking it down into manageable units for clearer understanding. ● Neurotrophic Factor Hypothesis: Reduced NGF and BDNF support weaken

	neurons, leading to potential death. ● Amyloid Cascade Hypothesis: Aβ plaque buildup and tau tangles trigger a cascade of events, ultimately harming neurons. ● Convergence of Insults: Diverse factors like microtubule issues, metabolic imbalances, and oxidative stress all contribute to neuronal decline in AD. ● Building the Model: ● ● Modular Network: Interconnected modules, like neurotrophic, amyloid, and oxidative stress, work together to influence neuronal fate. ● Neurotrophic Module: Regulates NGF and BDNF signaling, vital for neuronal health and survival. ● Amyloid Cascade Module: Investigates Aβ plaque formation and tau tangle development, potential contributors to neuronal damage. ● Excitotoxicity Module: Explores how excessive glutamate stimulation can harm neurons. ● Oxidative Stress Module: Unravels the role of free radicals and oxidative damage in neuronal decline. ● Mitochondrial Dysfunction Module: Delves into the impact of malfunctioning mitochondria on neuronal health. ● Inflammatory Module: Examines the potential role of inflammation in AD progression. ● Unlocking the Benefits: ● ● Clarity from Complexity: Provides a structured framework to understand intricate apoptotic pathways in AD. ● Toward New Therapies: Informs the development of targeted therapeutic strategies for AD by focusing on specific modules. ● A Promising Path: Opens doors for further research into AD mechanisms and potential interventions.
Research Question/Problem/ Need	How can the modular systems biology approach be used to better understand the complex network of factors contributing to neuronal apoptosis in AD?

Important Figures

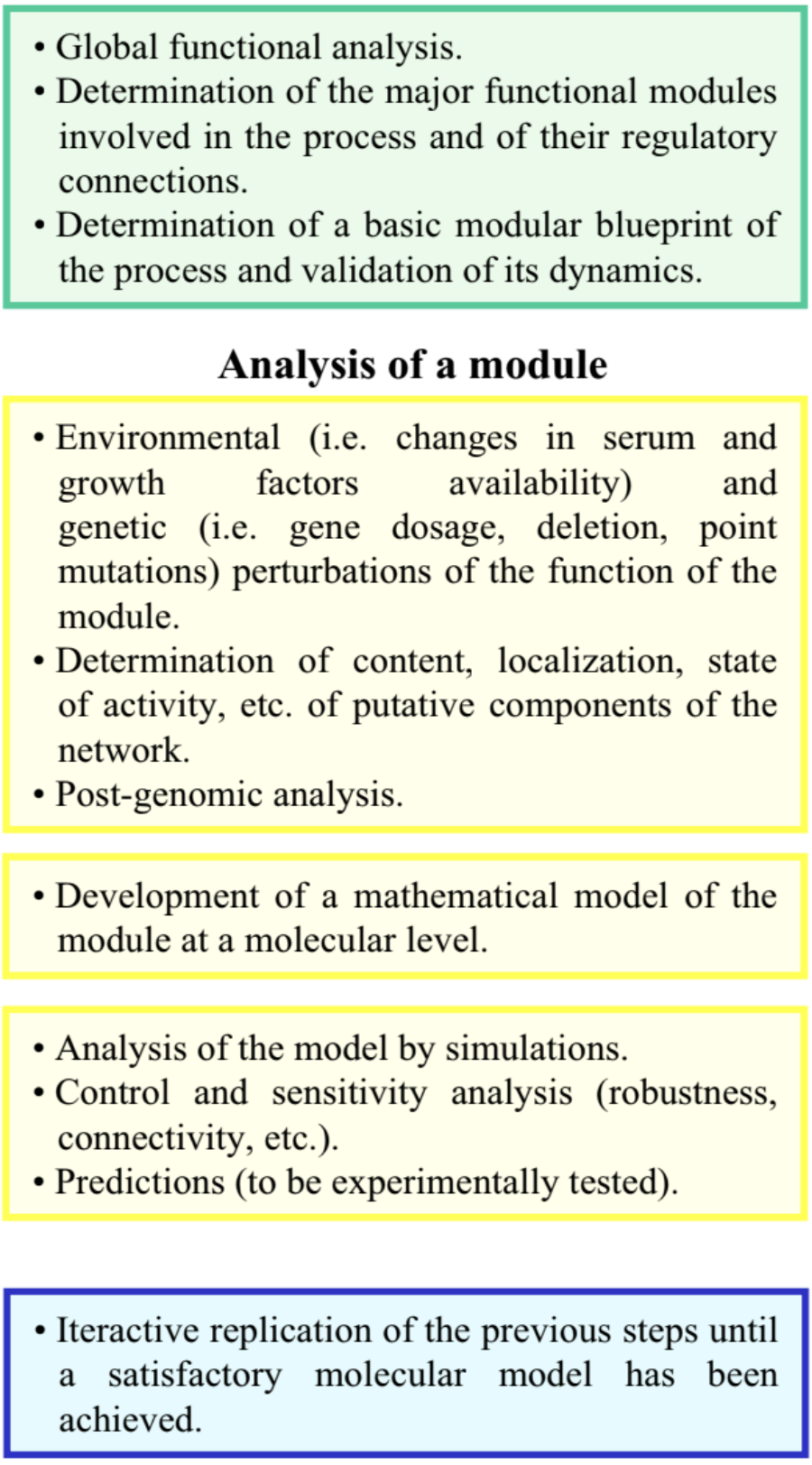


Figure 1
Scheme of the iterative roadmap of computational and experimental approaches applied in modular systems biology.

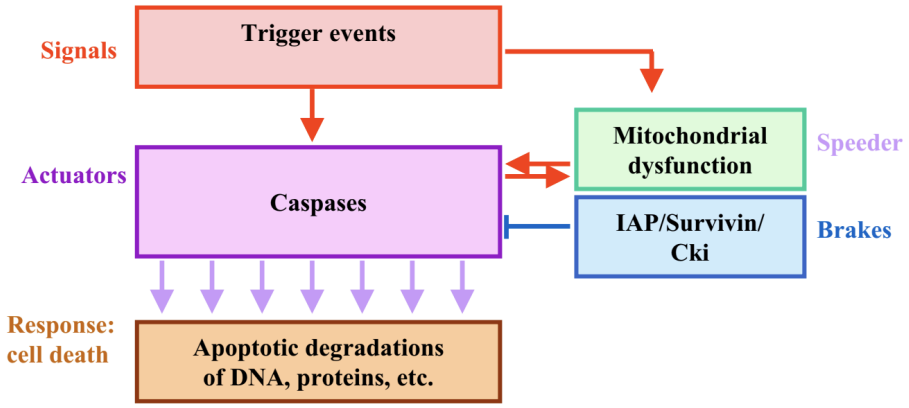


Figure 2
The basic blueprint of neuronal apoptosis showing the different modules and how they are interconnected by positive and negative feed-backs.

Trigger events

- Aβ neuritic plaques and NFTs formation
- Decreased availability of NGF/alteration of NGF processing
- Alteration of trkA/p75 receptors expression
- Impairment of M1/M3 mAchR signaling
- Oxidative stress
- Excitotoxicity
- Alteration of Ca^{2+} homeostasis
- Production of pro-inflammatory cytokines (TNF-α and IL-1β)
- Decreased trophic support (IGF-1/Wnt signaling/GSK3β)
- Impairment of the proteasome system
- Vascular factors (hypercholesterolemia and decreased glucose metabolism)
- Genetic mutations (APP, PS1/2, APOE ε4 allele)

Figure 3
A list of the many genetic and environmental events that contribute to the activation of apoptotic cascades in AD neurodegeneration.

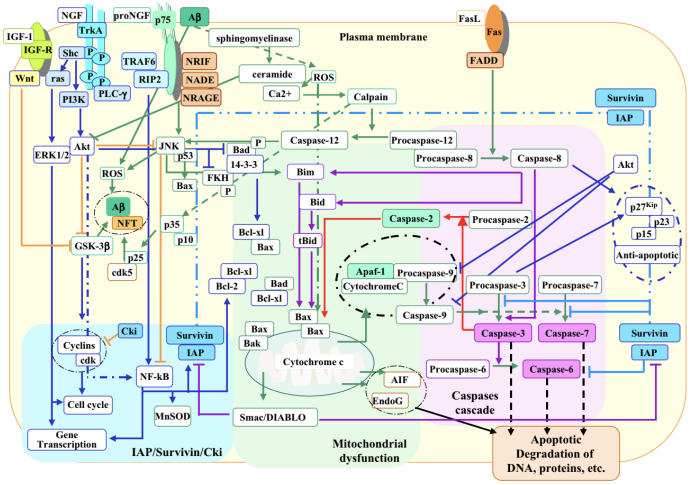


Figure 4
A molecular model of neuronal apoptosis organized in submodules. The central part of the model (green panel) represents the mitochondria submodule and all the molecules that regulate its function as "sensor" of the cellular stress responses (yellow panel). Dark blue and green arrows indicate survival and apoptotic pathways, respectively; orange arrows indicate inhibitions. Red arrows indicate the positive feed-back, while in purple and light blue are indicated the cross-talk between extrinsic/intrinsic apoptotic cascades and the survival regulatory control by IAPs, respectively. More information is the text.

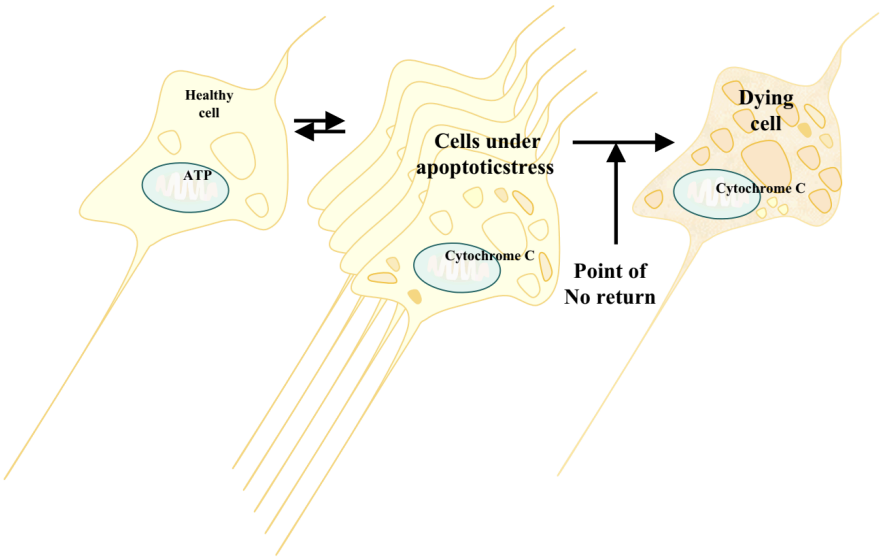


Figure 5
A drawing exemplifying the active "stand-by" status of cells exposed to the various stressful events involved in AD apoptosis. Neuronal death occurs when the cell(s) reaches a point of "No-return", where an overwhelming mitochondria pressure is no longer compensated by the many protective pathways that the cell(s) has adopted.

VOCAB: (w/definition)

Neurotrophic factors - Molecules that promote the survival and differentiation of neurons.

Excitotoxicity - A process that occurs when neurons are exposed to excessive glutamate, an excitatory neurotransmitter.

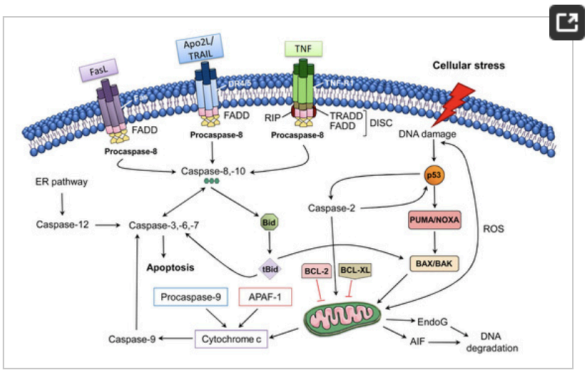
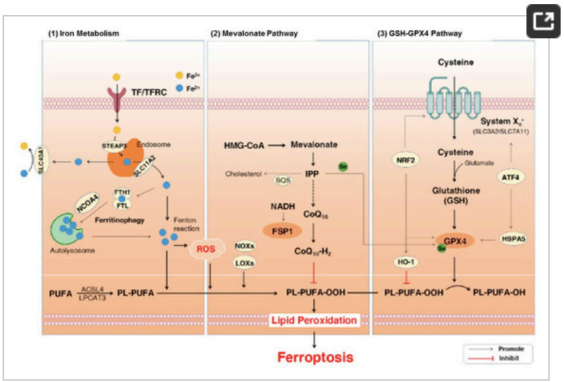
Brain-derived neurotrophic factor (BDNF) - A neurotrophic factor that is involved in the survival and function of a variety of neuronal populations.

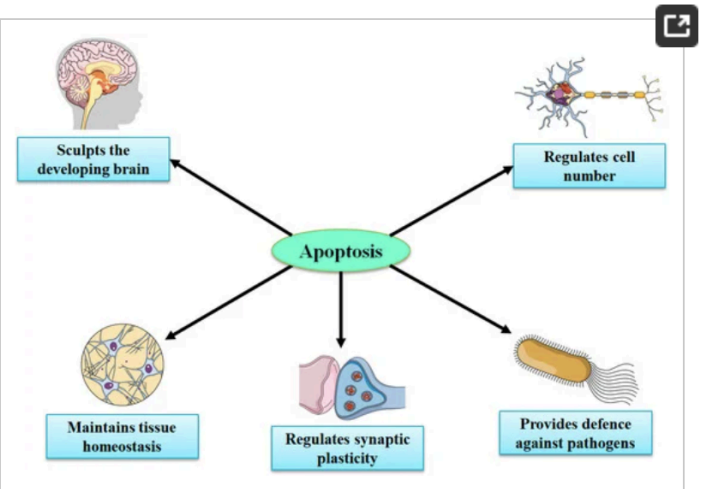
	Mitochondrial dysfunction - Impaired function of mitochondria, the organelles responsible for cellular energy production.
Cited references to follow up on	Scott SA, Mufson EJ, Weingartner JA, Skau KA, Crutcher KA: Nerve growth factor in Alzheimer's disease: increased levels throughout the brain coupled with declines in nucleus basalis. J Neurosci. 1995, 15: 6213-6221. Yatin SM, Varadarajan S, Link CD, Butterfield DA: In vitro and in vivo oxidative stress associated with Alzheimer's amyloid β-peptide (1–42). Neurobiol Aging. 1999, 20: 325-330. 10.1016/S0197-4580(99)00056-1.
Follow up Questions	Can the modular systems biology approach be integrated with other computational and experimental techniques to enhance our understanding of AD? How can the modular systems biology approach be applied to study the role of microRNAs (miRNAs) in AD pathogenesis? How can the modular systems biology approach be used to identify genetic risk factors for AD?

Article #23 Notes: Potential of Therapeutic Small Molecules in Apoptosis Regulation in the Treatment of Neurodegenerative Diseases: An Updated Review

Source Title	Potential of Therapeutic Small Molecules in Apoptosis Regulation in the Treatment of Neurodegenerative Diseases: An Updated Review
Source citation (APA Format)	Dailah, H.G. Potential of Therapeutic Small Molecules in Apoptosis Regulation in the Treatment of Neurodegenerative Diseases: An Updated Review. *Molecules* 2022, *27*, 7207. https://doi.org/10.3390/molecules27217207
Original URL	https://www.mdpi.com/1420-3049/27/21/7207
Source type	Journal Article
Keywords	Neurodegenerative diseases, Apoptosis, Cell death, Therapeutic small molecules, Neuroprotection, Drug discovery
#Tags	#neurodegeneration #apoptosis #neuroprotection #drugtargets #smallmolecules #braintumorresearch

Summary of key points + notes (include methodology)	The following notes were written with the aid of Google Bard, an AI service. The study, a meticulous exploration of apoptosis and its paradoxical role in neurodegeneration, dissects the intrinsic and extrinsic pathways implicated in neuronal demise. Employing a battery of techniques, from immunoblotting to gene expression analysis, researchers investigate the efficacy of various small molecules in modulating these pathways. Promising candidates like minocycline, an antibiotic with neuroprotective properties, and GAPDH ligands, known to stabilize a crucial metabolic enzyme, demonstrate significant reductions in caspase activity, the executioner of apoptosis. Furthermore, the study delves into the intricate interplay between oxidative stress, mitochondrial dysfunction, and ferroptosis, a novel form of cell death linked to iron dysregulation. By elucidating the molecular underpinnings of these processes, the research paves the way for the development of more targeted therapeutic strategies. A. Apoptosis and Neurodegenerative Diseases: Apoptosis: Defined as programmed cell death, crucial for development and homeostasis. Excessive apoptosis can contribute to neurodegeneration. Pathways of Apoptosis: Intrinsic: Triggered by cellular stress (mitochondrial dysfunction, oxidative stress, DNA damage). Extrinsic: Activated by death ligands binding to cell surface receptors. Ferroptosis: Specific form triggered by lipid peroxide accumulation. Neurodegenerative Diseases Affected: Alzheimer's, Parkinson's, Huntington's, ALS. B. Therapeutic Potential of Small Molecules: Targeting apoptotic pathways to protect neurons. Antioxidants: Scavenge free radicals and reduce oxidative stress. Minocycline: Antibiotic with neuroprotective effects, inhibits damaging enzymes. GAPDH ligands: Stabilize GAPDH to prevent its pro-apoptotic activity. p53 inhibitors: Block pro-apoptotic protein p53. JNK inhibitors: Target JNK signaling pathway involved in cell death. GSK-3 inhibitor: Reduces activity of enzyme linked to neuronal damage. Non-steroidal anti-inflammatory drugs (NSAIDs): May have neuroprotective effects. D2 dopamine receptor agonists: Stimulate dopamine receptors, potentially protecting neurons. FK506: Immunosuppressant with neuroprotective properties. Cell cycle inhibitors: Control cell division, potentially preventing apoptosis. Statins: Cholesterol-lowering drugs with potential neuroprotective effects. PPAR agonists: Activate peroxisome proliferator-activated receptors, involved in various cellular processes. Gene therapy: Emerging approach for introducing therapeutic genes into cells. C. Challenges and Future Directions:

	Developing more effective and targeted small molecules. Understanding the complex interplay of different pathways in neurodegeneration. Personalized medicine approaches based on individual genetic profiles.
Research Question/Problem/ Need	What are the potential therapeutic small molecules in apoptosis regulation for the treatment of neurodegenerative diseases?
Important Figures	 **Figure 1.** The extrinsic and intrinsic pathways of apoptosis [92]. Abbreviations: AIF, apoptosis-inducing factor; APAF-1, apoptotic Protease Activating Factor-1; Apo2L/TRAIL, Apo2 ligand or tumor ne-crosis factor-related apoptosis-inducing ligand; BCL-2, B-cell lymphoma 2; BCL-XL, B-cell lym-phoma-extra-large; Bid, BH3-interacting domain death agonist; DISC, death-inducing signaling complex; DR4/5, death receptor 4/5; EndoG, endonuclease G; ER, endoplasmic reticulum; FADD, FAS-associated death domain protein; FasL, Fas Ligand; PUMA, p53 upregulated modulator of apoptosis; ROS, reactive oxygen species; tBid, truncated Bid; TNF-R1, tumor necrosis factor receptor 1. Figure adapted with permission from Ref [92]. Copyright 2014, Elsevier.  **Figure 2.** The signaling pathways involved in ferroptosis [101]. Abbreviations: ATF4, activating transcription factor 4; CoQ10, coenzyme Q10; Fe3+, ferric cation; Fe2+, ferrous cation; FSP1, ferroptosis suppressor protein 1; FTH1, ferritin heavy chain 1; FTL, ferritin light chain; GPx4, glutathione peroxidase 4; GSH, glutathione; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HO-1, heme oxygenase-1; HSPA5, heat shock protein 70 family protein 5; IPP, isopentenyl pyrophosphate; LOXs, lipoxy-genases; NADH, nicotinamide adenine dinucleotide; NCOA4, nuclear receptor coactivator 4; NOXs, NADPH oxidases; NRF2, nuclear factor erythroid 2-related factor 2; PL-PUFAs, phospho-lipids-containing PUFAs; PUFA-OOHs, hydroperoxides derivatives of PUFAs; PUFAs, polyun-saturated fatty acids; ROS, reactive oxygen species; SQS, squalene synthase; TF, transferrin; TFRC, transferrin receptor. Figure reproduced with permission from Ref [101]. Copyright 2021, Elsevier.

	 **Figure 3.** Important roles of apoptosis in the development of the central nervous system.
VOCAB: (w/definition)	Therapeutic small molecules: Therapeutic small molecules are drugs that are typically made up of fewer than 500 daltons.
Cited references to follow up on	Muddapu, V.R.; Dharshini, S.A.P.; Chakravarthy, V.S.; Gromiha, M.M. Neurodegenerative Diseases—Is Metabolic Deficiency the Root Cause? Front. Neurosci. 2020, 14, 213. Nichols, E.; Szoeke, C.E.I.; Vollset, S.E.; Abbasi, N.; Abd-Allah, F.; Abdela, J.; Aichour, M.T.E.; Akinyemi, R.O.; Alahdab, F.; Asgedom, S.W.; et al. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol. 2019*, 18, 88–106.
Follow up Questions	What are the specific molecular mechanisms by which apoptosis is dysregulated in neurodegenerative diseases? How can we develop more effective therapeutic small molecules to target and regulate apoptosis in neurodegenerative diseases?