Alzheimer's Disease

– Rex M Heyworth PhD

Alzheimer's disease is named after Dr. Alois Alzheimer (pictured above, left), the doctor who, in 1906, first described the affliction. And pictured on the right is the first patient he examined.

Alzheimer's disease is the most common cause of dementia primarily among older adults. While we do not know know precisely, it is estimated that in 2019 nearly 50 million people worldwide have Alzheimer's (or a related dementia). Alzheimer's disease is characterised by memory loss, language problems, a loss of thinking skills and unpredictable behaviour.

What is the cure for the disease? Unfortunately, despite the expenditure of billions of dollars, there is still no cure. And although treatments are available, most only reduce symptoms of the disease.

Part of the reason for no cures is that what happens in the brains of Alzheimer's disease patients is not fully understood. This is despite many years of research and thousands of research articles. Adding to the frustration is that many of these articles are frustratingly contradictory, reflecting this lack of understanding.

This article is primarily an entry-level look at the subject. But a caveat. 'Entry-level' does not mean either 'easy' or 'simplistic'. And another caveat. This is without doubt the most difficult project I have done and it has taken about nine months to do!! It is not just the subject itself that has made it difficult but the fact that even the experts in the field cannot agree on many things.

So, read this article to get key ideas and an initial understanding. The summary might be a useful place to start. And you may need to read the project several times. Then perhaps, do your own reading as well! The reference list is well-stocked with links you might refer to.

Contents

* *Note on the glossary*: Many terms in the project may be unfamiliar to the reader. Some are defined in the text. These and many others are included in the glossary.

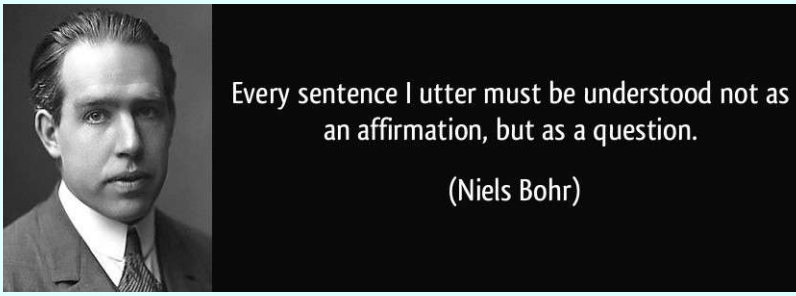
Alzheimer's Disease: Introduction

Alzheimer's disease (also known as just **Alzheimer's** or **AD**) is an affliction that affects many millions of people around the world. It is one of several *neurodegenerative* conditions (that is, those involving the loss of brain cells, specifically neurons), others being Parkinson's disease and Amyotrophic Lateral Sclerosis (ALS also known as Lou Gehrig's disease). In this project, we look just at Alzheimer's disease.

For more on dementia and dementia types, go to:
https://www.medicalnewstoday.com/articles/142214.php

But first – a caveat

I begin with the quote by Niels Bohr (1885 – 1962), the Danish physicist, famous for his work on the structure of atoms. This was a caution he would give his students each year to be wary of *dogmatism* and not just accept everything they heard or read.



Why do I include this caution?

Because there is a lot about Alzheimer's disease that is either not known or poorly understood. This was made clear to me in researching on the disease. Many hypotheses, yes, but no coherent explanation. There has been a lot of research but many of the findings do not always agree. I found relatively few articles that could give a good overall explanation. Therefore, much of what I have written here may be right or may be wrong, and sometimes may need to be taken with a grain of salt!

Some scientists say certain findings regarding Alzheimer's disease (such as the amyloid hypothesis, which is discussed later) have begun to take on the trappings of *dogma*. Because dogmatism is a bad idea in general, and a bad idea in science in particular, these researchers are rather pleased to see the growing number of alternative hypotheses to help us better understand and treat Alzheimer's.

So, read this article to get key ideas and an initial background, but do your own reading as well!

What is Alzheimer's disease?

Alzheimer's disease is an irreversible, progressive brain disorder that slowly destroys memory and thinking skills and eventually, the ability to carry out the simplest tasks. In most people with the disease – who get it later in life – symptoms first appear in their mid-60s. Early-onset Alzheimer's occurs between a person's 30s and mid-60s but is very rare. Alzheimer's disease is the most common cause of dementia (that is, brain diseases that cause a long-term and often gradual decrease in the ability to think and remember that is severe enough to affect daily functioning) among older adults. Alzheimer's disease is the most common cause of dementia among older adults.

The discovery of Alzheimer's disease

The disease is named after Dr. Alois Alzheimer (1864 – 1915), the German doctor who first described it. In 1906, Dr. Alzheimer carried out an autopsy on the brain of a 51-year-old woman named Auguste Deter who had died of an unusual mental illness. Her symptoms had included memory loss, language problems,


Auguste Deter

and unpredictable behaviour.

Dr. Alzheimer found many abnormal clumps of brain tissue (now called *amyloid plaques*) and tangled bundles of fibres (now called neurofibrillary, or *tau tangles*). (More about these soon.) However, what he discovered was not accorded the status of a disease until several decades later. Alois Alzheimer himself also noticed other patients with the *same* symptoms but with *none* of the damaged brain tissue, and still others *with* the brain changes but *no* symptoms of the disease. So, there were problems with the disease right at the beginning!!

Can Alzheimer's disease be defined?

Scientists today still don't yet fully understand what causes Alzheimer's disease. Causes probably include a *combination* of age-related changes in the brain, along with genetic, environmental, and lifestyle factors.

Older age itself does *not* cause Alzheimer's, but is the most important known risk factor for the disease.

Because of the problems above (and others), no *precise* definition of Alzheimer's disease is available. Instead, *characteristics* of the disease are given. The nearest we might come to a definition is:

Alzheimer's disease is an irreversible, progressive brain disorder that slowly destroys memory and thinking skills and, eventually, the ability to carry out the simplest tasks.

What then are characteristics of Alzheimer's disease?

Alzheimer's-type dementia is *characterised* by the gradual loss of important mental functions. Memory problems are typically one of the first signs of Alzheimer's, though initial symptoms can vary from person to person. A decline in other aspects of thinking, such as finding the right words, vision/spatial issues, and impaired reasoning or judgment, may also signal the very early stages of Alzheimer's disease. People with Alzheimer's have trouble doing everyday things like driving a car, cooking a meal, paying bills and the ability to plan. They may ask the same questions over and over, get lost easily, lose things or put them in odd places, and find even simple things confusing. As the disease progresses, some people become worried, angry, or violent (as did August Deter, Alzheimer's first patient).

Mild cognitive impairment (MCI) is a condition that can be an early sign of Alzheimer's disease, but not everyone with MCI will develop the disease.

Over time, a person with Alzheimer's gradually loses his or her ability to live and function independently. Ultimately, the disease is fatal.

Note: Have you ever forgotten a name or an appointment from time to time, and then recall it later on? Or go to the kitchen and completely forget why you went there? If so, you probably do <u>not</u> have Alzheimer's disease. This is one thing, but it's quite another when someone forgets such things frequently and does not remember them later, to the point that the forgetfulness starts to interfere with his or her daily activities, which is characteristic of Alzheimer's disease.

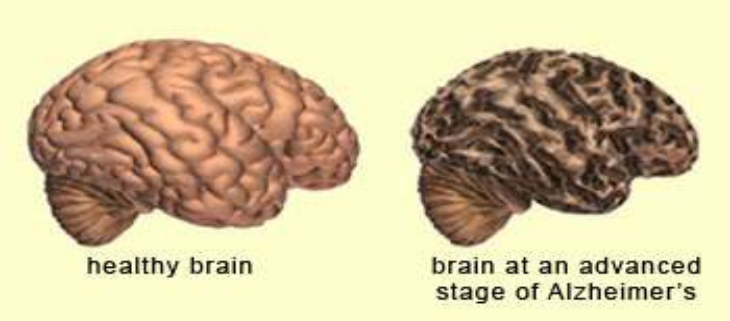
Changes in the brain: A creeping disease

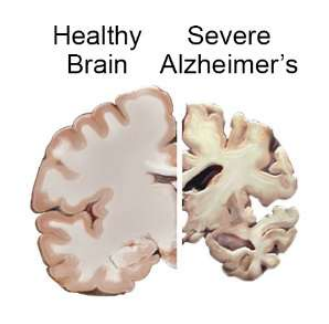
As mentioned, Alzheimer's disease is a *neurodegenerative* process, meaning a disease in which a part of the brain and nervous system deteriorates irreversibly.

A characteristic feature in the brain is the loss of connections between nerve cells (neurons).

The main *macroscopic* effect (that is, what we see just by looking) observed in people with Alzheimer's is atrophy of the cortex – the thin layer of grey matter whose myriad convolutions (folds) that give the brain its characteristic appearance [atrophy = decrease in size or wasting away of a body part or tissue]. This degeneration is essentially limited to the cortex (the outer layer of the brain) and does not affect other structures the way Parkinson's and Huntington's diseases do. By the final stages of Alzheimer's, this process – called brain atrophy – is widespread, causing significant loss of brain volume.

The images (below) compare the sizes of a healthy brain and one with Alzheimer's disease and show this atrophy.





As a result of this brain atrophy, over the course of 10 years or so, people with Alzheimer's can lose as much as 8 to 10% of their brain *mass*, whereas in healthy people, the loss over 10 years is only about 2%. The Alzheimer's brain also can lose close to *50% loss* of the neurons in the cortex.

This damage initially appears to take place in the *hippocampus*, the part of the brain essential in forming new memories. *Short-term memory* loss is therefore one of the first symptoms of Alzheimer's disease.

Hippocampus: (*hippo-* = horse) The two small seahorse-shaped structures in the centre of the brain; one on the left side and one on the right (the white parts in the diagram). They are important for the formation of memories.

The atrophy gradually extends into the front and sides of the brain and then to the whole cerebral/brain cortex. As the disease progresses to deeper parts of the brain, *long-term memories* are also lost. It later affects the areas in the cerebral cortex responsible for language, reasoning, and social behaviour.

By the final stage of Alzheimer's, brain damage is widespread, and brain tissue has shrunk significantly.

The brain typically shrinks to some degree in healthy ageing but, surprisingly, does not lose neurons in large numbers. In Alzheimer's disease, however, damage is widespread, as many neurons stop functioning, lose connections with other neurons, and die. Alzheimer's disease disrupts processes vital to neurons and their networks, including communication, metabolism, and repair.

How is Alzheimer's disease diagnosed?

To diagnose Alzheimer's, doctors may:

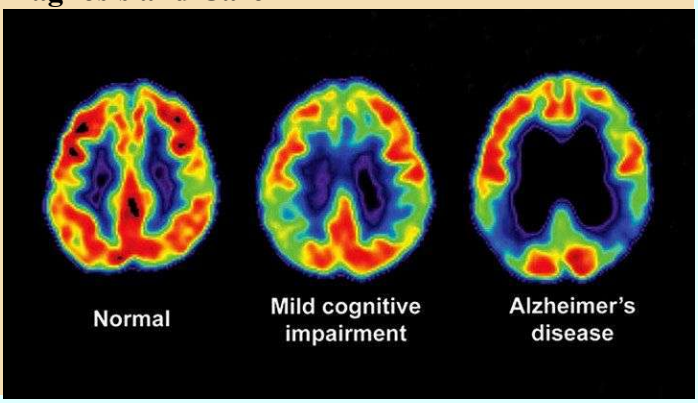
1. Ask the patient, a family member or friend, questions about overall health, use of prescription and over-the-counter medicines, diet, past medical problems, ability to carry out daily activities, and changes in behaviour and personality.
2. Conduct tests of memory, problem solving, attention, counting ability and language
3. Carry out standard medical tests, such as blood and urine tests, to identify other possible causes of the problem.
4. Perform brain scans to rule out other possible causes for observed symptoms.

It's important to note that Alzheimer's disease can be definitively diagnosed *only* after death, by linking clinical measures with an examination of brain tissue in an autopsy.

Early accurate diagnosis is beneficial for several reasons. Beginning treatment early in the disease process may help preserve daily functioning for some time, even though the underlying Alzheimer's process cannot be stopped or reversed.

PET Scans May Drastically Change Alzheimer's Diagnosis and Care

The images (right) were made using positron emission tomography (PET) scans and compare different brains. They clearly show the brain atrophy that occurs s the disease progresses.
For more on this topic, go to:
https://www.everydayhealth.com/alzheimers-disease/amyloid-pet-scans-may-drastically-change-alzheimers-diagnosis-care-study-finds/



Alzheimer's disease in the population

Alzheimer's disease presently affects approximately 13% of people over the age of 65 and 45% over the age of 85, with an estimated number of at least 50 million Alzheimer's disease patients around the world. Recent estimates indicate that Alzheimer's disease may rank third, just behind heart disease and cancer, as a cause of death for older people.

Due to an increasing elderly population, Alzheimer's disease has become one of the greatest health issues of this century and by 2050, according to some estimates, the number of Alzheimer's disease patients will rise to about 152 million people.

The total estimated worldwide cost of dementia was US$818 billion in 2015, which represented 1.09% of global GDP. Worldwide dementia *care* is estimated to cost upwards of US$1 trillion.

Types of Alzheimer's disease

There are two types of Alzheimer's disease: Early-onset AD (EOAD) – also called familial Alzheimer's – which is often in a family and can be inherited. The vast majority however is late-onset Alzheimer's disease (LOAD) – also called sporadic Alzheimer's. Both types have a genetic component. Early-Onset Alzheimer's disease represents less than 10 percent of all people with Alzheimer's.

Some differences between Late-Onset and Early-Onset Alzheimer's disease are shown in the table below:

Late-Onset Alzheimer's	Early-Onset Alzheimer's
Signs first appear in a person's mid-60s	Signs first appear between a person's 30s and mid-60s
Most common type	Very rare
May involve a gene called APOE ε4	Usually caused by gene changes passed down from parent to child

Sporadic Alzheimer's disease can affect adults at any age, but usually occurs after age 65 and is the most common form of Alzheimer's disease. Familial Alzheimer's disease is a very rare genetic condition, caused by a mutation in one of several genes.

How long can a person live with Alzheimer's disease?

The time from diagnosis to death varies – as little as 3 or 4 years if the person is older than 80 when diagnosed, to as long as 10 or more years if the person is younger.

Although treatment can help manage symptoms in *some* (not all) people, currently there is no cure for this devastating disease.

Kinds of Cells in the Brain

As Alzheimer's is a disease affecting the brain and thus the cells in the brain, it is necessary to have a reasonably good understanding on these cells and their functions. Here, some basic ideas of brain cells are discussed. For a more detailed exposition, refer to my earlier project entitled "*Transmission of Nerve Impulses*".

Here is one link to the project ***Transmission of Nerve Impulses***:
https://drive.google.com/file/d/1uS-XrmON_EA6LVc4ZgHgqK6ap2iFf1qp/preview

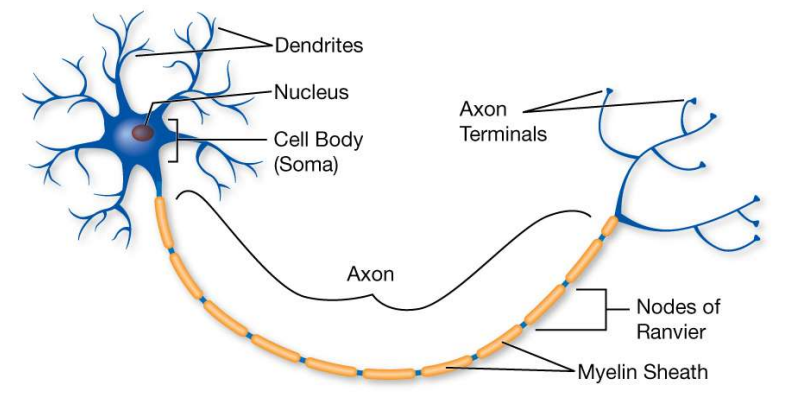
Brain tissue, also referred to as brain parenchyma, is made up of *cells* and *fluids*. Cells in the brain are grouped into two main categories – neural cells (neurons) and glia (non-neurons). Between the brain cells are spaces called the *extracellular* space or *interstitial* space. These spaces are filled with fluid called interstitial fluid (ISF), which is about 99% water.

Note: Extracellular space is *commonly* equated with interstitial space and this is the usage in this project. But not everybody equates them as you may find out if you read articles. So beware! Also, compare *extracellular* space with *intracellular* space, the latter being the space *inside* cells [*intra-* = inside].

We now turn to the cells inside the brain.

Neurons

The healthy human brain contains tens of billions of neurons with estimates ranging from 85 billion to 200 billion or even 1000 billion (=1 trillion, which is 1 followed by 12 zeroes!). Too large to comprehend!



Neurons pass chemical and electrical signals (also called *impulses* or *messages* – we will use these words interchangeably) along the neural pathways in the brain. Neurons come in many shapes and sizes. Their shapes and connections help them carry out specialised functions, such as storing memories and controlling muscles.

The diagram shows a typical neuron and its components. Key components for our purposes are the dendrites, soma, axon and axon terminals. (You don't need to worry about the other terms.)

- Dendrites (from the Greek *dendon* = tree): The branches of neurons that *receive* signals from other neurons. The signals go into the cell body (or soma) and then along the axon.
- Soma or cell body (from the same Greek word meaning *body*): The bulbous portion of a neuron containing the cell nucleus.
- Axon: The long threadlike part of a neuron along which impulses are conducted from the soma to the end of the neuron.
- Axon terminal: The very end of a branch of a nerve's axon. As we will see shortly, the signal passes from here to the next neuron.

Biological processes in neurons

The function and survival of neurons depend on several key biological processes:

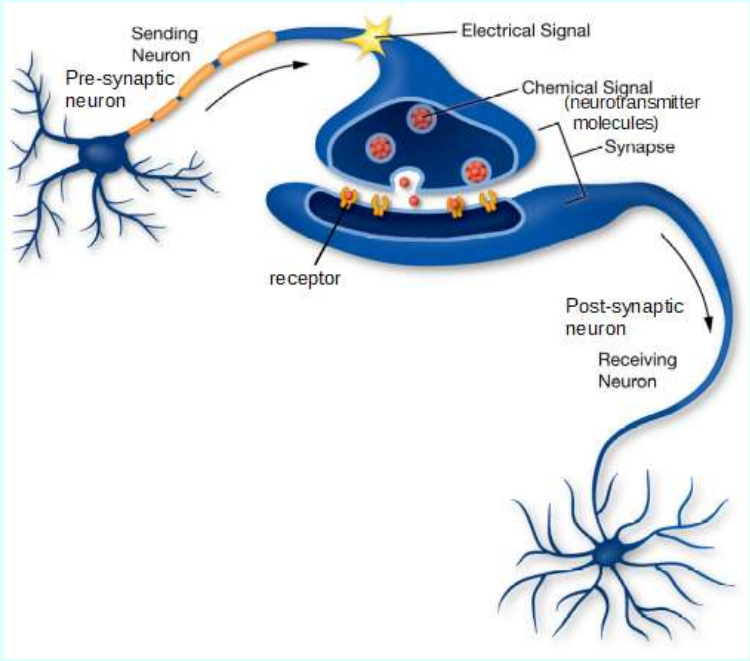
1. Communication. Neurons are constantly in communication with neighbouring neurons. This is done by the signals/impulses/messages that pass from one neuron to the next.
2. Repair, remodelling, and regeneration: Because neurons can live for a long time (unless, as we shall see, you have Alzheimer's), neurons must constantly maintain and repair themselves. Neurons also continuously adjust/change, or 'remodel' themselves depending on how much stimulation they receive from other neurons.

How neurons communicate

Signals travel from one neuron to the next. They first travel along the axon to the end of the axon terminals. They then cross a gap to the next neuron. This gap is called a **synapse** (or synaptic gap).

Two kinds of signals are involved – *electrical* and *chemical*. Electrical signals travel down axons. Chemical signals travel from the presynaptic (or pre-synaptic) neuron (the *sending* neuron) to the postsynaptic (or post-synaptic) neuron (the *receiving* neuron) across the synapses using molecules called **neurotransmitters**. On the dendrites of the post-synaptic neuron are **receptors** that receive the neurotransmitters.

Look at the diagram. Neurotransmitters (the small red dots in the diagram) collect in vesicles (containers). When an electrical signal arrives at an axon terminal, this signal causes the vesicles to move down and release their neurotransmitters into the synapse (synaptic gap). The neurotransmitter molecules move across the gap to the receptors. The neurotransmitters bind to the receptors and cause them to open, allowing ions (present in the extracellular fluid around the neurons) to enter through the receptor.



Once the ions have entered the next neuron, the signal becomes electrical again and travels down the axon to the synapses at its axon terminals. This process continues from neuron to neuron throughout the brain.

One sending neuron can connect to *several* receiving neurons, and one receiving neuron can connect to several different sending neurons. One neuron may have as many as 10 000 synaptic connections with other neurons. (Only one such connection is shown in the diagram here.)

Note that electrical signals/messages along axons are *fast* whereas neurotransmitter/chemical signals across the synapse are slow. (This is the same in everyday life – electricity in wires moves very fast whereas physical objects, even the fastest of them, are much slower.)

As we shall see later, Alzheimer's disease disrupts this communication between neurons, resulting in loss of functions, especially memory, and the death of neurons.

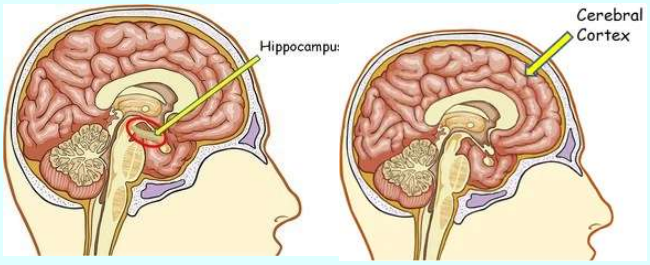
Neurons can live a long time

Unlike many other kinds of cells in the body, which are relatively short-lived, neurons have evolved to live a long time – more than 100 years in humans. As a result, neurons must constantly maintain and repair themselves. Adult brains can even generate new neurons—a process called *neurogenesis*.

Effects of Alzheimer's disease on neurons

The brain does not normally lose neurons in large numbers. In Alzheimer's disease, however, damage is widespread, as many neurons stop functioning and lose connections with other neurons as the brains of Alzheimer's disease patients now have significantly fewer synapses.

Alzheimer's disrupts the processes above which are vital to neurons and their networks. When neurons lose their ability to communicate, they die.



At first, Alzheimer's disease typically destroys neurons and their connections in the hippocampus, the part of the brain important for learning and memory.

Alzheimer's disease later affects areas in the cerebral cortex (the outer layer of of the brain) responsible for language, reasoning, and social behaviour. Eventually, many other areas of the brain are damaged.

Over time, a person with Alzheimer's gradually loses his or her ability to to think, remember, make decisions and function independently. Ultimately, the disease is fatal.

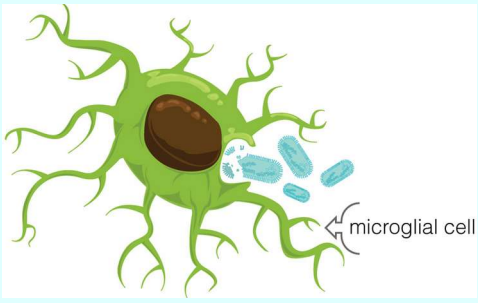
Glial cells (also called glia and neuroglia)

All the other cell types in the brain, collectively known as **glia**, are also key to healthy brain function. Glial cells are by far the most numerous cells in the brain, outnumbering neurons by about 10 to 1.

The term *neuroglia* means 'nerve glue'. Their function is primarily to surround and support the function and health of neurons. Together, glial and blood vessel cells regulate the delicate balance within the brain to ensure that it functions at its best.

There are several types of glia in the brain. These include: **microglia**, **astrocytes**, and **oligodendrocytes**.

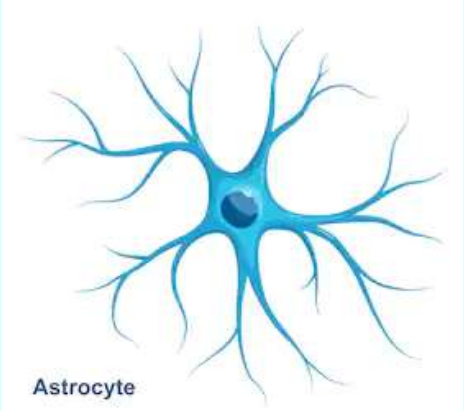
Microglia



Microglia protect neurons from physical and chemical damage and are responsible for clearing foreign substances and cellular debris from the brain. They also eat/engulf foreign invaders (bacteria and viruses) then display the chewed up parts on their cell surface to signal for help (from the astrocytes – see next).

This engulfing of various materials is the main role of microglia, a process known as *phagocytosis* (*phag-* = to eat/devour + ~~*o*~~ + *-cyt-* = vessel/cell + *-osis* = abnormal condition).

Astrocytes



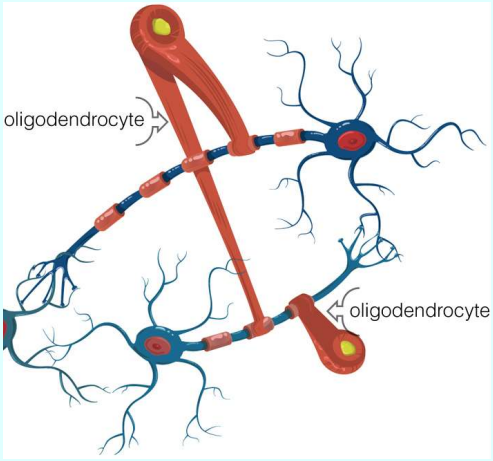
Astrocytes (from Greek *astron* = star + cyte from *kytos* = cavity, but also means cell) are star-shaped glial cells and are the most abundant glial cells. They keep neurons healthy by holding them in place and supplying them with nutrients.

Astrocytes are also signalled by the microglia to clear away the build-up of the debris left behind from their phagocytosis. But over time, in people with Alzheimer's disease, microglial cells fail to clear away this debris.

Astrocytes can also communicate with neurons and modify the signals the neurons send and receive. That means astrocytes are much more involved than we once thought in both the processing of information, and the signalling at the synapse. Further, astrocytes can alter how a neuron is built by directing where to make synapses or dendrites.

Knowing more about astrocytes will also shed light on diseases in which communication between astrocytes and neurons is altered, including Alzheimer's disease.

Oligodendrocytes



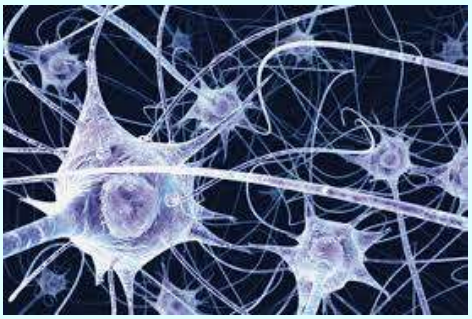
Oligodendrocytes (from Greek, meaning '*cells with a few branches*'), or oligodendroglia, are a special type of neuroglia whose main functions are to provide support and insulation to axons of neurons.

Oligodendrocytes do this by wrapping tightly around axons to form a cover called the myelin sheath (the cylindrical brown parts). A single oligodendrocyte can wrap around 50 axons (those in the diagram are only wrapping around one and two respectively).

Having myelin sheaths speed up the electrical signals that travel down an axon. Without them, a signal would travel down an axon 30 times slower!

More reading: Much of the information here, including the pictures, came from the following website. You may find it interesting reading.
https://learn.genetics.utah.edu/content/neuroscience/braincells/

Neurons and Memory



Memories are formed in the brain. But what do we mean by a memory? How does the brain store memories?

First, there are two kinds of memories – short-term memory (STM) and long-term memory (LTM). Short-term memory typically lasts between 15 and 60 seconds. Any memory that can be recalled after that length of time is a long-term memory. Unlike short-term

memories, long-term memories are more permanent and have a 'permanent' physical presence in the brain.

In the brain, memory is a large network of many inter-connected neurons (such as that shown in the diagram). When a new long-term memory is formed, neurons make new physical connections and synapses with each other. And, as mentioned earlier, that could mean up to 10 000 or more connections between neurons.

Note: The image above representing inter-connections between neurons is also used in the project "**Transmission of Nerve Impulses**".

New memories appear to reside in the hippocampus for a while. But as more memories are formed, the neurons that represent a specific memory migrate further into the cortex. As a result, memories are stored throughout the brain. It's a bit like the internet, which is made of information spread all across the planet and accessed via countless connections.

The more we develop and use our memories, the '*stronger*' (more permanent) this network of connections becomes which allows us to more easily recall the memory in the future.

Long-term potentiation (LTP)

[Meanings: long-term = long-lasting; potentiation = making more effective. So LTP = making our memories more long-lasting.]

Making a memory permanent (or almost so) is called memory consolidation. The process of consolidation involves a phenomenon called long-term potentiation, or LTP. LTP occurs when the same group of neurons in a network repeatedly 'fire' together which is another way of saying the memory becomes 'stronger' (more permanent) and easier to recall. [To 'fire' just means that a signal passes from one neuron to the next.]

Note that the brain doesn't *grow* new neurons to store memories. It just *strengthens* connections between *existing* neurons by means of LTP.

There are a number of ways in which LTP can occur. The best-known mechanism involves receptors known as the NMDA receptor and the acetylcholine receptor. (We look at these receptors next.)

Long-term depression (LTD)

LTD may be described as the *inverse* mechanism to long-term potentiation. It happens if the number of neurons in a network decreases or if the number of *signals* between neurons decreases. The neural networks, instead of firing often, now fire less frequently which can can decrease memory. Alzheimer's disease kills neurons, and this decrease in the number of neurons also leads to LTD.

However, the *precise* role that LTD plays in memories has yet to be determined. One speculation is that in the hippocampus, one of the areas of the brain active in memory, LTD is thought to return synapses that have been strengthened by LTP to a 'weaker' level so that they will be available to store *new* information and perhaps even to cause superfluous memories to be forgotten. The forgetting generated by LTD is probably just as active a process as LTP in forming memories. We don't know for sure.

Another term: The ability of the connections, or synapses, between neurons to change in strength, and for lasting changes to occur in the efficiency of synaptic transmission, is known as synaptic plasticity or neural plasticity, and it is one of the important foundations of memory and learning. [The word *plasticity* comes from *plastic* because plastic is easily shaped or moulded as are neurons.]

Neuron receptors and memory

There are several kinds of neuron receptors. Two play important roles in human learning and memory formation, as well as in Alzheimer's disease. They are:

1. N-methyl-D-aspartate receptor receptors (abbreviated **NMDA** receptor or **NMDAR**),
2. **Acetylcholine** receptors (abbreviated **AchR** or **AChR**). Also called *cholinergic* receptors.

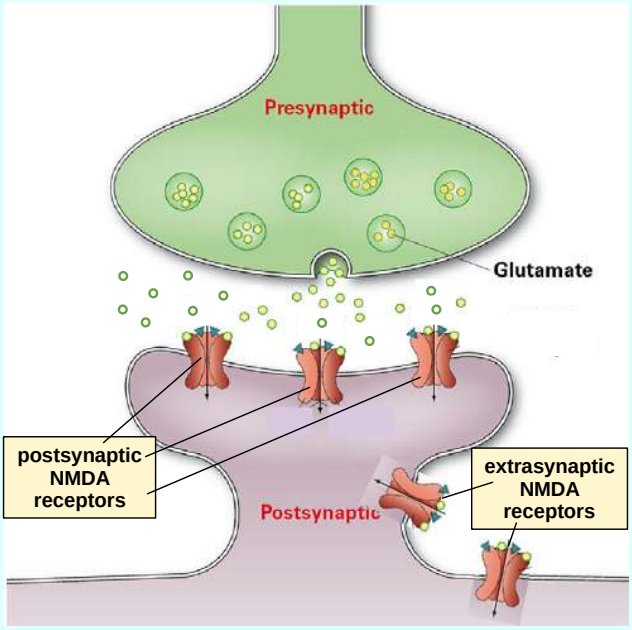
As mentioned earlier (page 7), these receptors receive signals across the synapse from a previous neuron and are believed to play an important role in learning and *memory* formation.

Below is a (relatively) simple explantation of how they work.

1. NMDA receptors

NMDA receptors are neurotransmitter receptors that are located in the *postsynaptic* membrane of a neuron. Refer to the diagram which shows the condition in a normal healthy brain.

Note: As well as NMDA receptors, the postsynaptic membrane contains acetylcholine (and other) receptors. But to simplify the discussion here, only NMDA receptors are shown. (Keep this note in mind!)



NMDA receptors are located in two positions:

(i) Postsynaptic (or sometimes just synaptic) NMDA receptors, at the 'top' of the dendrites in a neuron – just three are shown in this diagram;

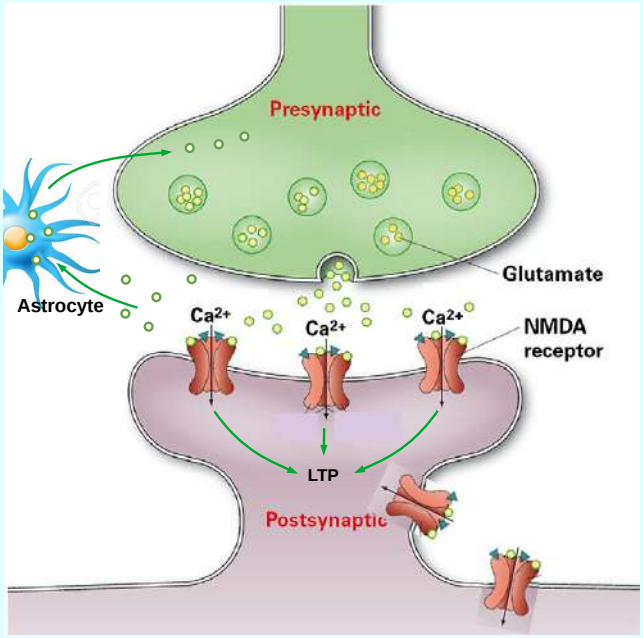
(ii) Extrasynaptic NMDA receptors, at the sides of dendrites – just two are shown in the diagram. [*extra-* = outside of, beyond; so extrasynaptic = beyond/away from the synapse.]

The neurotransmitter for NMDA receptors is glutamate. It is the most common neurotransmitter in the brain.

Briefly, here is what happens (and refer to the diagram on the next page):

1. The glutamate molecules are released into the synapse and move across the gap to the NMDA receptors in the postsynaptic neuron and bind to these receptors.
2. This binding causes a channel in the receptors to open, which allows calcium ions Ca^{2+} (and a few sodium ions Na^{+}) present in the space around neurons, to flood into the postsynaptic neuron.
3. The calcium ions activate a series of steps that results in long-term potentiation (LTP).

4. LTP results in a strong electrical signal moving down the axon in the postsynaptic neuron.
5. Excess glutamate enters nearby astrocytes and from there returns to the presynaptic neuron where it is used again (recycled).



AMPA receptors: There is subtype of NMDA receptor called the AMPA receptor, which are located near these NMDA receptors (but not shown in the diagram). The influx of calcium ions through the NMDA receptors initiates a mechanisms that causes more AMPA receptors (lurking inside the postsynaptic area) to be inserted into the neuron's membrane. AMPA receptors also respond to glutamate and allow even more calcium ions to enter resulting in even stronger LTP. So more receptors results in a better memory.

Long-term depression and these receptors: The same neurotransmitter (glutamate) and the same receptors (NMDA) are involved in both LTP and LTD. But in LTD, the influx of calcium ions is smaller. It is also believed that the number of AMPA receptors *decreases* during LTD. These AMPA receptors would be removed from the surface of the postsynaptic membrane and placed in reserve inside: in short, the opposite of what happens in LTP, when additional receptors are inserted into the membrane to develop memory.

Caveat: The description above is *very* simplified but serves the purpose of this project.

2. Acetylcholine/cholinergic receptors (AChR)

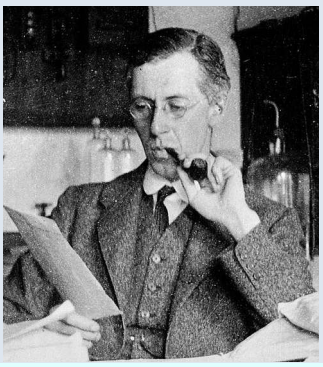
These receptors also play an important role in human learning and memory formation, as well as in Alzheimer's disease.

There are actually two kinds of AChR – nAChR and mAChR. The one discussed here in nAChR. We will not go into the differences.

An acetylcholine receptor responds to the binding of the neurotransmitter acetylcholine (ACh). Acetylcholine is one of the most important neurotransmitters in the human body. Like glutamate, it is important for learning and memory, especially short-term memory. Individuals with Alzheimer's disease have low levels of this neurotransmitter.

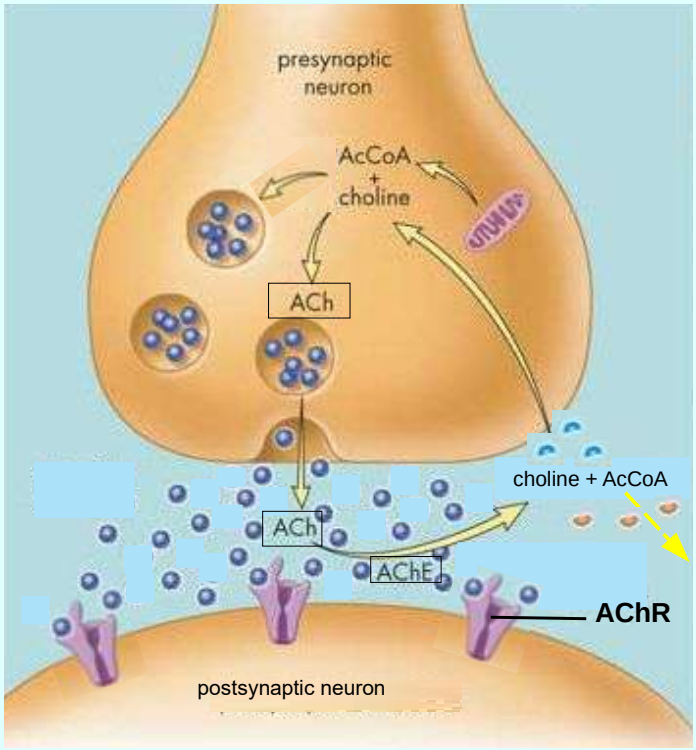
Notes:

The name acetylcholine is derived from its structure. It is a chemical compound made up of acetyl CoA and choline. (Acetyl CoA is itself made from acetic acid – what gives vinegar its sour taste!).



Discovery: Acetylcholine was the first neurotransmitter to be identified. It was discovered by Henry Hallett Dale in 1914 (pictured, right, in 1918), and its existence was later confirmed by Otto Loewi. Both individuals were awarded the Nobel Prize in Physiology/Medicine in 1936 for their discovery.

How it works in the normal brain: Refer to the diagram. (Again, only the receptors needed for the explanation are shown. But remember that NMDA and other receptors are present.)



1. Like glutamate, the acetylcholine neurotransmitter is produced in the *presynaptic* neuron. It is made from two substances – choline and acetyl CoA (AcCoA) and stored in vesicles.
2. The acetylcholine is released into the synapse and moves across the gap to the acetylcholine receptors (AChR).
3. The ACh neurotransmitter binds to and opens the receptor. Sodium ions (Na^+) and potassium ions (K^+) flood/diffuse in, again resulting in LTP and memory. A new electrical signal is generated which travels down the axon.
4. Floating round in the synapse between the neurons is an enzyme called acetylcholinesterase (AChE or AchE) that *rapidly* breaks down/degrades the neurotransmitter once its job is done, to form its original components, that is, acetylcholine → choline + acetyl CoA. This *rapid* break-down of ACh prevents accumulation of too much neurotransmitter. (Note: The rapid break-down is important when we discuss the effects of Alzheimer's disease later.)
5. The choline ( ) is taken back into the presynaptic neuron and recycled to make more acetylcholine. The acetyl CoA ( ) is *not* recycled but is broken down and removed from the brain. *Note*: This is *unlike* the glutamate neurotransmitter, *all* of which is recycled and used again.

Note on names of enzymes: In biology, the names of *most* enzymes (but not all) end in -ase and indicate the substance they act on. Thus acetylcholinesterase above is an enzyme that acts on acetylcholine (but this name has an extra 'ster'!).

ACh neurotransmitter and ageing

During the course of the *normal* ageing process, concentrations of the ACh neurotransmitter (and other neurotransmitters) tend to *decrease*, perhaps by as much as 50% between young adulthood and old age. This impairs the ability to think and perform memory tasks, resulting in the occasional lapses of short-term memory that many elderly individuals tend to experience from time to time.

These memory lapses are because the fewer ACh neurotransmitter molecules result in weaker LTP due to fewer sodium ions entering postsynaptic neurons.

This normal, non-debilitating decline in memory, referred to as Age-Associated Memory Impairment (AAMI), or benign senescent forgetfulness, should not be confused with Alzheimer's disease, in which levels of ACh can drop by up to 90 percent.

ACh and muscles

As well as being involved with brain neurons for learning and memory, acetylcholine is also found in all motor neurons, where it stimulates muscles to contract and move. From the movements of the stomach and heart to the blink of an eyelash, all of the body's movements involve the actions of this important neurotransmitter.

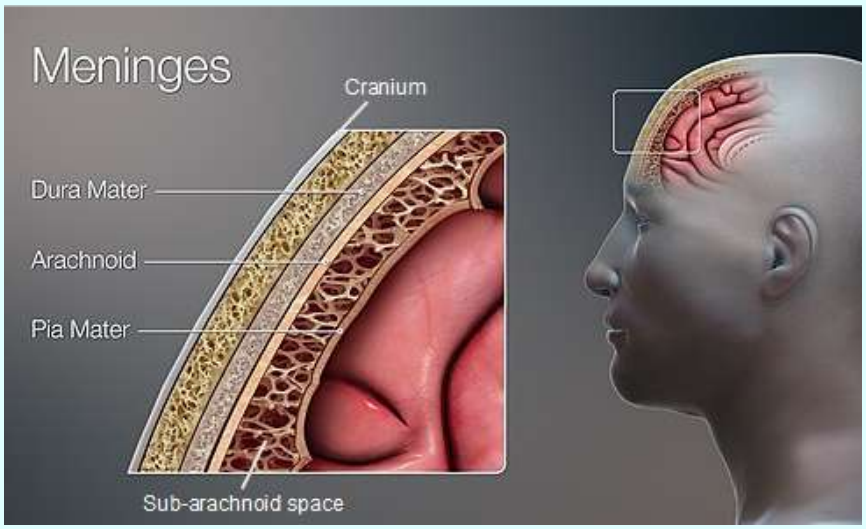
The venom of a black widow spider (pictured) acts by causing the release of acetylcholine. When people are bitten by a black widow, their acetylcholine levels rise dramatically, leading to a dramatic increase in muscle contractions/movements, spasms, paralysis, and even death.

The Brain and Brain Blood Vessels

The Brain

Note: *In this section, we just mention points about the brain that are related to our discussion of Alzheimer's disease.*

The brain sits within the cranium, that is, the part at the top of the skull that encloses just the brain but not the bones of the face or jaw.



Brain meninges (see diagram)

Below the cranium are three layers/membranes. These are called – from the inside to the outside (diagram right):

- the pia mater,
- the arachnoid mater, and
- the dura mater, closest to the cranium.

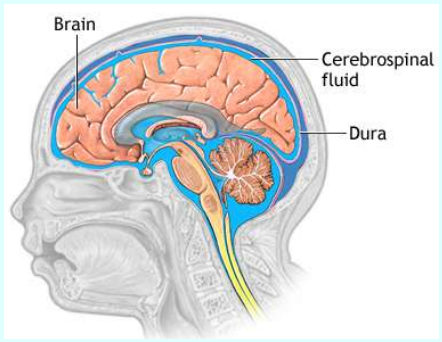
Tip: To remember them, think of PAD – for Pia, Arachnoid and Dura.

Together, these three membranes are called the **meninges** (from the Greek, plural of meninx meaning *membrane*). The meninges envelop both the brain and the spinal cord.

(In the diagram, the whitish layer on the outside is the bone of the cranium. The skin and hair on the cranium are not shown.)

Terminology: Pia mater (Latin: *tender mother* because it is a very delicate or tender membrane). Arachnoid mater (Greek: *spidery mother*, because of its spider-web like appearance – cf. picture). Dura mater (Latin: *tough mother* because it is a thick, durable membrane (the same dura- as in durable!). The arachnoid and pia mater together are sometimes called the leptomeninges (lepto- = Greek for *thin*).

Sub-arachnoid space (SAS): This is a space underneath the arachnoid. It is filled with a fluid known as the *cerebrospinal fluid*. In the diagram (right), the CSF is shown in blue and is in the SAS.



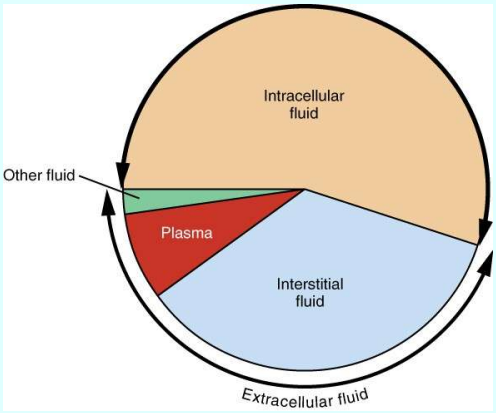
Fluids in the brain

There are four kinds of fluids in the brain:

1. Blood plasma: The fluid part of the blood, that is, excluding blood cells (~10% of brain fluid)
2. Intracellular fluid (ICF): This is a fluid inside brain cells (intra- = *inside*) (60-68% of brain fluid).
3. Interstitial fluid (ISF): This is a fluid between brain cells (inter- = *between*). Also called extracellular fluid (extra- = *outside*) (12-20% of brain fluid).

4. Cerebrospinal fluid (CSF): (~10% of brain fluid).

Note: Different sources give slightly different percentages.



Cerebrospinal fluid (CSF)

As mentioned above, CSF is the fluid that fills the sub-arachnoid space. CSF is a clear solution consisting of about 99% water with several dissolved ions formed in the brain from blood. The total volume of cerebrospinal fluid is approximately150 ml.

Etymology: Cerebrospinal fluid = cerebro- + spinal. cerebro- = originally from Latin *cerebrum* ('brain, skull') + spine, so cerebrospinal = relating to the brain and spine.

Functions of the CSF

CSF is a liquid. Its functions include:

1. Protection: To cushion a blow to the head and lessen the impact. (But for some contact sports, it is not able to provide enough cushioning!)
2. Buoyancy: Because the brain is immersed in fluid, the weight of the brain is reduced from about 1 400 g to about 50 g. Therefore, pressure at the base of the brain is reduced. (Cf. Swimming – the body feels almost weightless due to the buoyancy of the water. Picture here is of me swimming.)
3. Excretion of waste products: The CSF takes potentially harmful substances out of the brain. (Much more on this function later – see the *glymphatic system* on page 45.)

Formation of CSF from the blood

Refer to both the text, and to the diagram on next page.

The majority of CSF (~80%) is produced in parts of the brain known as ventricles, which are located in the middle and lower parts of the brain. A ventricle is just a chamber or cavity. (The heart also has two ventricles – but for blood, *not* CSF!!)

There are four interconnected ventricles in the brain: two *lateral ventricles* (also referred to as the *first* and *second ventricles*), one in each side of the brain, and below these are a *third ventricle* and a *fourth ventricle*.

Cerebrospinal fluid is produced within these ventricles by a specialised membrane called a **choroid plexus** located in the walls of the ventricles. *Ependymal* cells (which are one type of glial cell) surround many tiny blood capillaries in each choroid plexus and filter the blood to remove blood cells to make CSF. The majority of CSF is produced from within the two *lateral* ventricles.

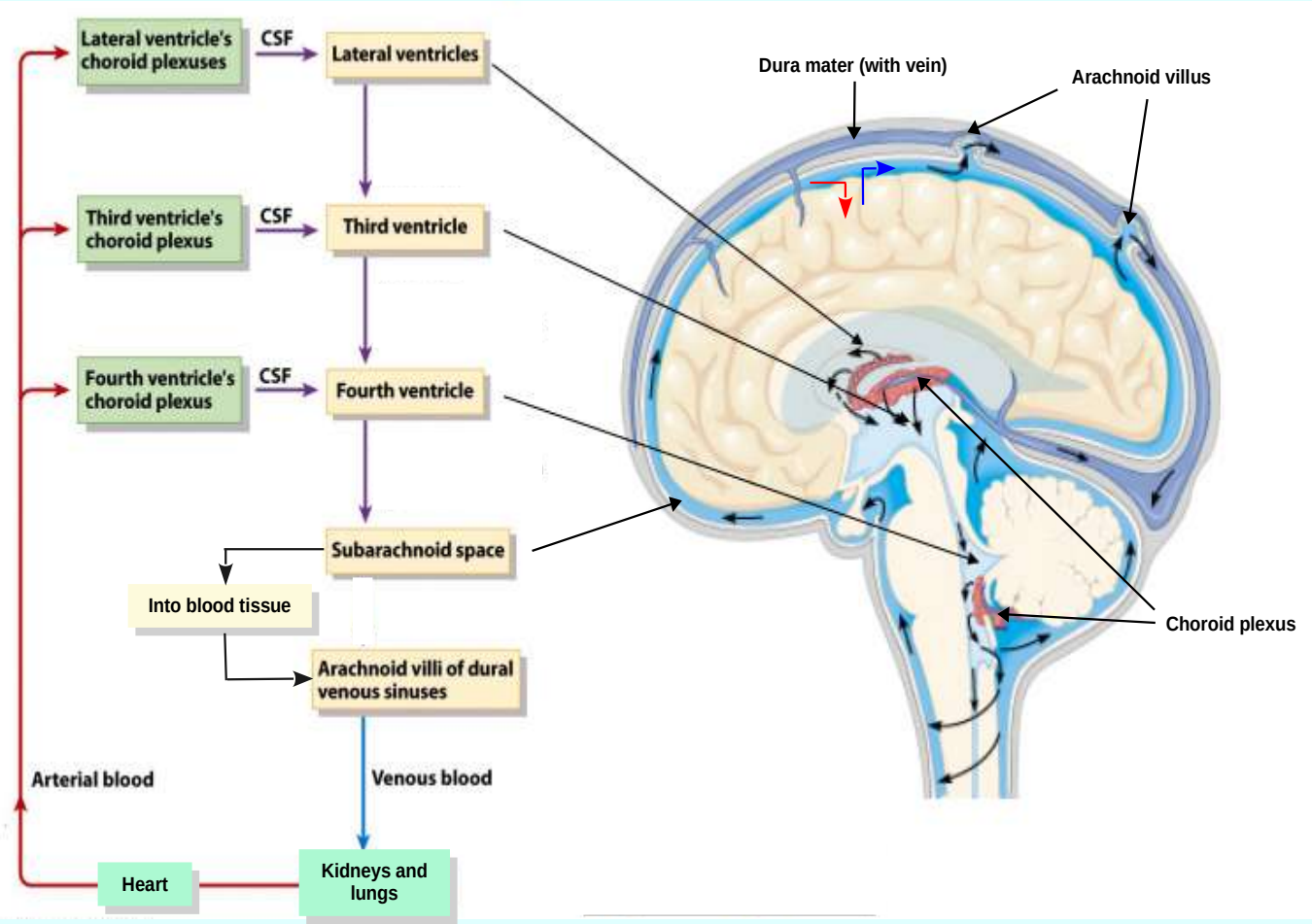
CSF produced in the lateral ventricles flows into the third ventricle and then the fourth ventricle where more CSF is produced. From the fourth ventricle, the flow of CSF splits: a small volume passes into the central canal of the *spinal cord*, while most enters and fills the **subarachnoid space**, which, remember, is part of the meninges surrounding the outer part of the brain, between the arachnoid mater and the pia mater.

Reabsorption of CSF into the bloodstream

As we will learn in a later section, the CSF enters the blood tissue and flushes out / clears waste products, including beta-amyloid. This 'dirty' CSF returns to the subarachnoid space and then drains through the arachnoid granulations/villi into spaces called **sinuses** then into blood *veins* located in the *dura mater*. It flows through these veins, around the outer brain and into larger veins (the jugular veins) that leave the head and neck and join the rest of the body blood circulation and flow to the liver and kidneys from where wastes are removed.

Although, as mentioned earlier, the total volume of cerebrospinal fluid is approximately 150 ml, it is *produced* at a rate of 450 ml daily. This is because 300 ml is removed from the brain into the body circulation daily. Thus the CSF in the brain replaces itself three times a day (i.e. 450 = 150 x 3).

Pathway of CSF flow: Summary



Notes on the diagram:

1. The dura mater layer actually has a vein running through it, into which the returning CSF enters. The diagram does *not* show this vein.
2. Choroid plexuses are shown in brown-red. They are present in all four ventricles.
3. The diagram may give the appearance that the CSF just moves around the subarachnoid space then out again into the dura mater. But it actually enters the brain tissue first at *many* points (small red arrow shows just one point of the many) to flush out waste then re-enters the subarachnoid space (blue arrow shows just one such point). This is discussed later (pages 21 – 23).

CSF circulation

A brief video showing where the CSF is produced, its movement in the SAS and its removal. Note that it does not include the entry and exit of the CSF into and out of the brain tissue.

https://www.youtube.com/watch?v=SDMO4vYkqdg

Blood Vessels in the Brain

There are many blood vessels in the *brain*. Put end-to-end, they would stretch for about 640 km (400 miles), which is the distance from Hong Kong to Southern Taiwan.

Some blood vessels enter the brain from arteries that come up the neck and into the brain. Others dive down into the brain from the meninges layers around the outside of the brain.

Blood–Brain Barrier

But there is is one very important difference between the blood vessels in the brain and those in the rest of the body.

In the late 19th century (in 1885), the German physician Paul Ehrlich (1854 – 1915) (pictured, right) injected a blue dye into the bloodstream of a mouse. To his surprise, the dye left the blood vessels and infiltrated *all* tissues/cells of the body and turned them blue *except* those in the brain and spinal cord. There seemed to be some sort of barrier preventing the dye from leaving the blood vessels and crossing into the brain.

A similar experiment (left), done in 1909, with blue dye after being injected into a *rat*. [Picture is not of the 1909 rat!]

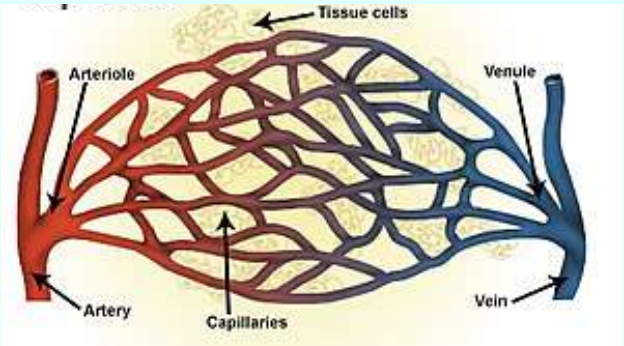
However, it wasn't until the 1960s that researchers could use microscopes powerful enough to determine more about the structure of what is now called the **blood–brain barrier (BBB)**.

In order to understand Alzheimer's disease, we must know about the structure and function of the BBB.

Structure of the BBB

As everywhere in the body, arteries from the heart start off large then become smaller arteries called arterioles. These become even smaller and are called capillaries. Similarly, for veins returning blood to the heart: So:

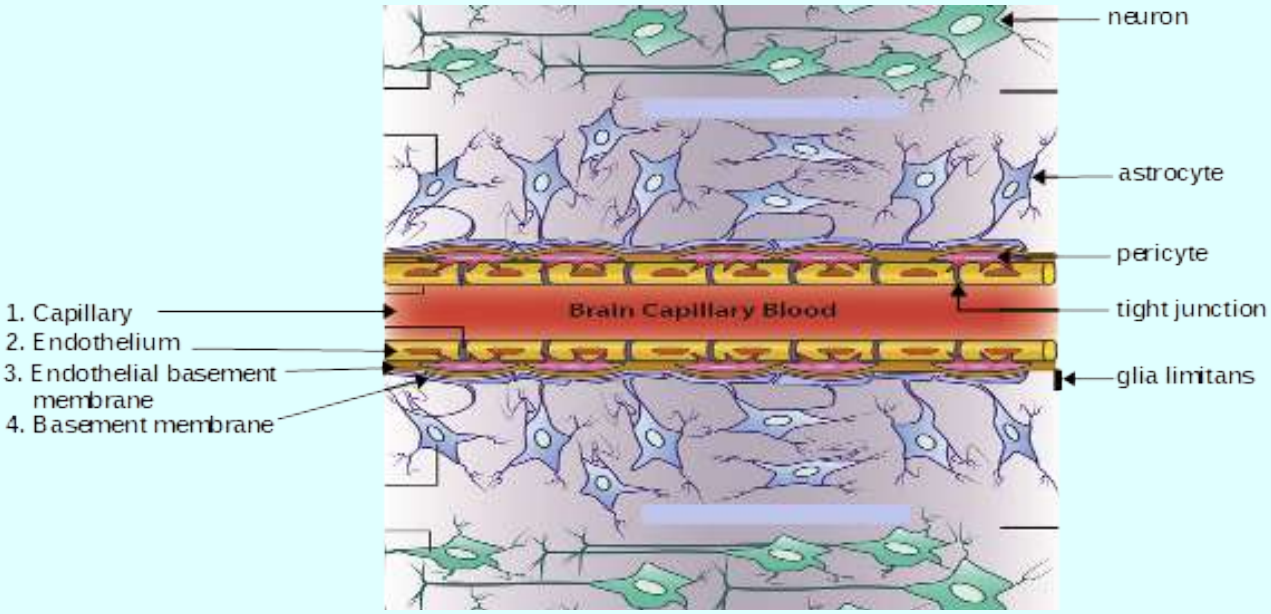
arteries→ arterioles → capillaries→ venules (small veins) →veins.



In the body, but *not* the brain, it is from the capillaries that substances enter or leave the tissue of the *body*.

But, the capillaries in the *brain* have this blood-brain barrier that can prevent many substances from *freely* entering (or leaving) the brain. This differs from capillaries in the rest of the body which *do* let substances through (though we do not need to discuss this here).

The diagram below shows the *basic* structure of the BBB in a brain *capillary* (but is the same in the larger blood arteries and veins). Let us start from the inside and work outwards.



1. **Capillary:** On the inside along which blood flows.
2. **Endothelium** (walls of the capillary): The walls are made up of cells called endothelial cells. Endothelial cells line the interior of *all* blood vessels in the body, but in brain capillaries they are wedged extremely close to each other, forming so-called **tight junctions** that prohibit free movement of molecules across the BBB. Some larger molecules, such as glucose, can gain entry (see below). These tight junctions are a key structure of the blood–brain barrier. (The junctions between the endothelial cells in blood vessels elsewhere in the body are *not* tight and so allow substances through.)
3. **Endothelial basement membrane**: This is a layer that surrounds the endothelium. In it are embedded cells known as pericytes (shown in pink). One role of pericytes is in wound healing. A deficiency of pericytes can cause the blood–brain barrier to break down thus allowing substances to cross from the capillaries into the brain. (Pericytes line the capillaries *throughout* the whole body and not just in the brain.)
4. **Basement Membrane** (or parenchymal basement membrane): This is a second membrane (not too clear in the diagram). As the diagram also shows, astrocytes surround the blood vessels in the brain. These astrocytes have protrusions/extensions (called endfeet) that secrete this second membrane. The astrocytic endfeet plus the basement membrane are called the **glia limitans**.

The whole entity of these four parts is collectively referred to as the **neurovascular unit (NVU)**.

Why does the brain need a BBB?

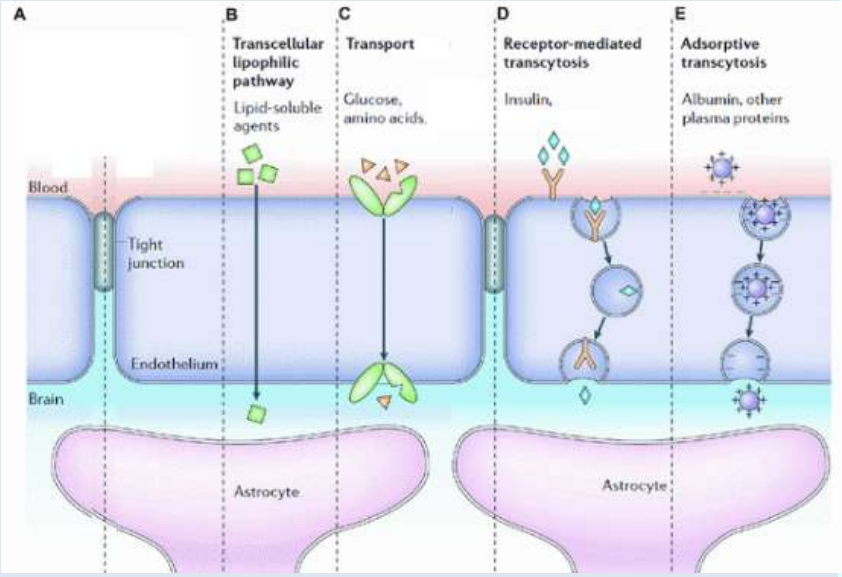
The BBB has an important function in protecting the brain from the entry of harmful substances such as toxins and bacteria. However, it *does* allow necessary substances to cross this barrier, such as oxygen, glucose and amino acids, from the blood into the brain. (Glucose is needed for energy, oxygen for respiration and amino acids for building proteins.)

The blood-brain barrier also helps to maintain the volume of the brain fluid, since the brain is located in a rigid skull. This it does by limiting the movement of salts and water from the blood into the brain.

How do substances cross the BBB?

The BBB is *semi-permeable*; that is, it allows *some* materials to cross, but prevents others from crossing.

The diagram (right) shows how how some substances *do* (or *don't*) cross the BBB.



A: Tight junctions do <u>not</u> allow *any* substances to cross the BBB.

B: Small substances that are lipid-soluble (i.e. soluble in fat-like liquids) cross by passive diffusion into the brain, for example, oxygen (O_2), alcohol and caffeine, and carbon dioxide (CO_2) to leave. Many drugs also enter via this route. This is possible as the membranes of cells are made from a fat-like material.

C (Active) Transport: Special 'transporters', which act like special doors that open only for particular molecules, such as glucose and amino acids.

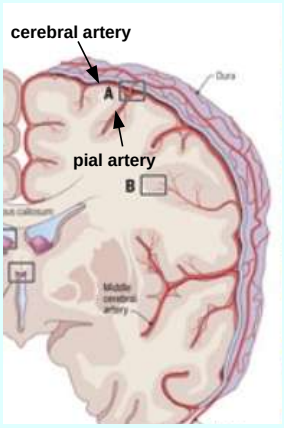
D and **E** Transcytosis (Receptor-mediated transport): Specific receptor-mediated endocytosis and transcytosis result in transport of certain proteins, such as insulin, into the brain. These molecules link up to receptors on the surface of the brain and are 'escorted' through. Many drugs also enter via this route.

Therefore substances such as oxygen, glucose and amino acids, which are needed by *every* cell in the body, are able to get to the brain cells. In a person with Alzheimer's, a faulty blood-brain barrier can prevent glucose (and perhaps oxygen) from reaching the brain, meaning the person will not get enough energy, which glucose provides.

Blood vessels from the *outer* brain

Here, we discuss more on how CSF flows round the brain. But in order to do that, we need to *first* need to talk more about the blood vessels in the brain that allow this to happen.

Look back at the *meninges* layers in the brain cranium – the dura mater, the arachnoid mater (and its subarachnoid space) and the pia mater (the inner of the three). All three are involved in the circulation of the blood from the surface of the brain. And, as you know by now (hopefully!), the subarachnoid space is filled with CSF.

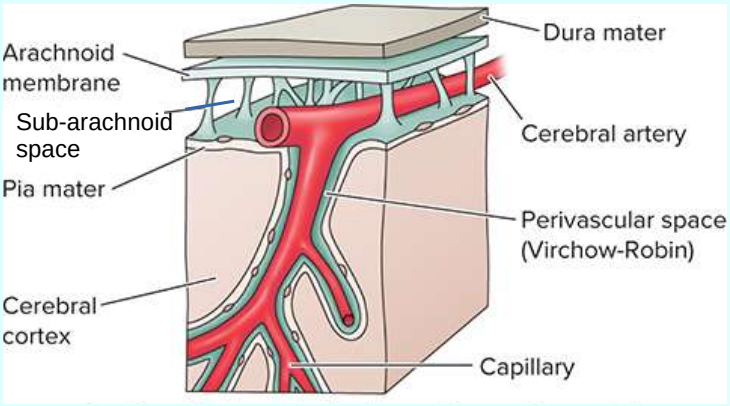


Blood vessels enter/leave the brain from the SAS

As you read the text below, refer to the diagrams on these pages.

An artery, called the **cerebral artery** passes along the *subarachnoid space*. Smaller arteries, called **pial arteries** (red colour = taking blood in), branch off the cerebral artery and dive down/penetrate/enter into brain tissue (the diagram right shows just a few of the many such arteries, e.g. at area 'A').

Similarly, **pial veins** (blue colour = taking blood out) *leave* the brain tissue and join the **cerebral vein**, also located in the subarachnoid space (*not* shown in the above diagram).



The pial arteries that dive down into the brain are surrounded by a **perivascular space (PVS)**. This PVS is filled with CSF, simply because, as the pial artery dives/penetrates down into the brain, it takes with it the pia mater layer. The same too for the pial veins that return to the cerebral vein in the subarachnoid space. The diagram (right) may help to show this. This diagram would be the same for the returning veins if you *imagined* 'Cerebral artery' changed to 'Cerebral ''vein' and coloured blue instead of red. (Refer also to the diagram on page 17.) These PVS are unique to these arteries and veins in the outer brain.

Terminology and history

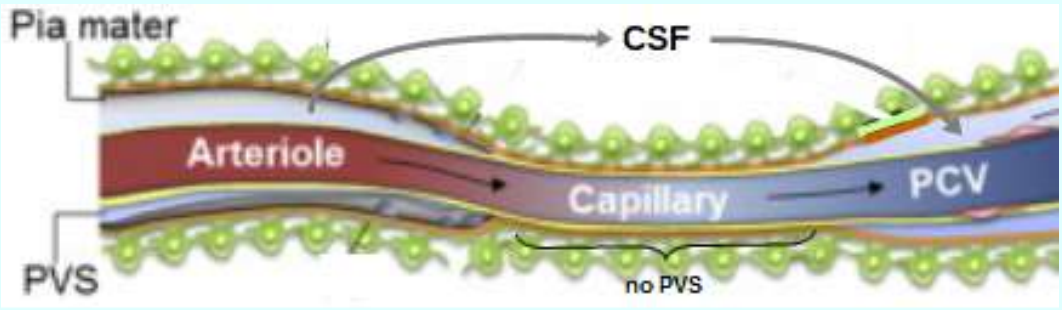
[perivascular = peri- from Greek *around* + -vascular = from Latin *vessel*) So, perivascular = *a vessel around (blood) vessels*.]

These spaces were first described in 1849. In 1851, Rudolph Virchow (1821 – 1902, pictured right) was the first to provide a *detailed* description of these microscopic spaces surrounding these brain vessels. Charles-Philippe Robin confirmed these findings in 1859. The spaces were called *Virchow-Robin spaces* in their honour.

In the current literature, the terms 'perivascular' and 'paravascular' (para- = from Greek *next to*, *beside*) are each used, sometimes interchangeably, to refer to the PVS. Virchow–Robin space is also a term used to refer to the this space. No consensus currently exists within the field on this terminology. For *simplicity*, we use the single term 'perivascular space' (PVS) to indicate the CSF-filled space surrounding these blood vessels.

The capillaries

As the pial artery penetrates further into the brain and forms the smaller arterioles, the PVS becomes narrower and narrower until, at the level of capillaries, it *disappears* (or is extremely small). The PVS then re-appears in the venules and the veins that leave the brain and re-enter the subarachnoid space. This is shown in the diagram below. *Note*: PCV = post-capillary venule.



What happens to the CSF in the PVS?

Because the *capillaries* have no PVS, the CSF cannot continue its movement. Instead, it flows *into* the brain, moves through the brain tissue and then re-enters the PVS in the venules (see the arrows in the diagram above). This is good because, as we will see shortly, as the CSF moves through the brain tissue, it flushes/clears out β-amyloid and other wastes into the veins for removal from the body.

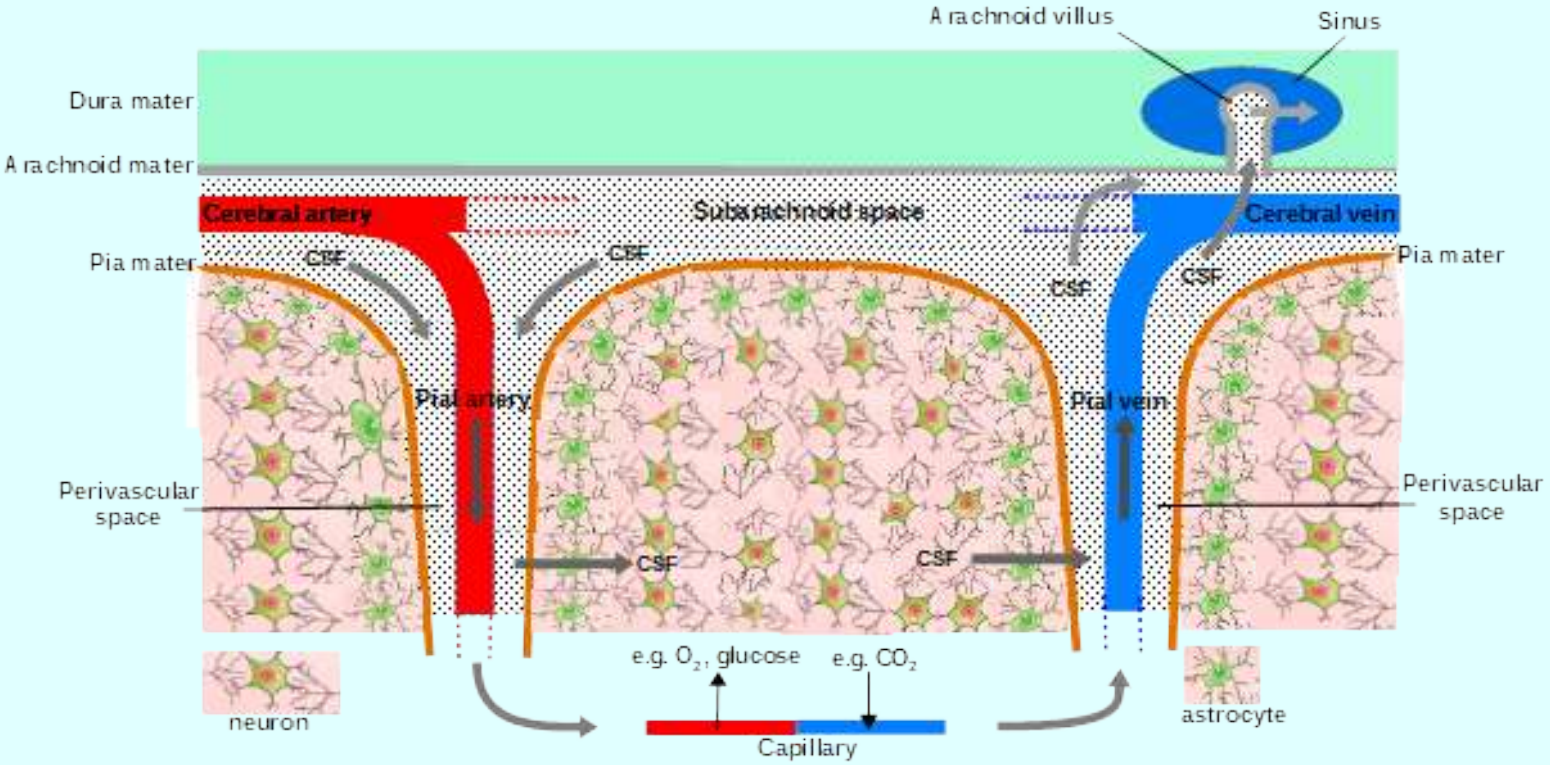
Exit of CSF from the brain parenchyma

Now that the CSF is back in the venous PVS, how is it (plus its waste) removed? The CSF re-enters the subarachnoid space (from where it came). But then, it enters small protrusions called **arachnoid villi** (or **arachnoid granulations**), which push from the SAS into the dura mater (see diagram below). These arachnoid villi drain into blood-filled spaces in the dura mater called **sinuses**. From these sinuses, the blood – with its CSF – flows back around and out of the brain and connects with a major vein, called the **jugular vein**. Veins take the blood towards the *heart* to be pumped to the lungs for re-oxygenation and to the liver and kidneys for removal of other wastes. The CSF itself, being mainly water anyway, is also removed, which is why the choroid plexuses must continually producing CSF (see page 17 again).

Note on sinuses: There are many sinuses in the brain with different names. For example, there are the *superior sagittal sinus*, the *occipital sinuses*, the *straight sinus*, the *transverse sinuses* and the *sigmoid sinuses*, each with slightly different functions which we need not go into! All very complex!

Summary diagram

The diagram below is *my* attempt to combine most of what we have discussed about the arteries and veins in the outer brain. Study it and read the text again to try to get a good understanding of what is rather a complex and difficult subject. Note that there are some things that are *not* shown in the digram. These include the BBB in arteries and veins, the basement membranes and other cells that are found in the brain such as microglia oligodendrocytes (look back at pages 8 – 9.)



Causes of Alzheimer's disease: plaques and tangles

Now, with all that background, back to Alzheimer's disease!!

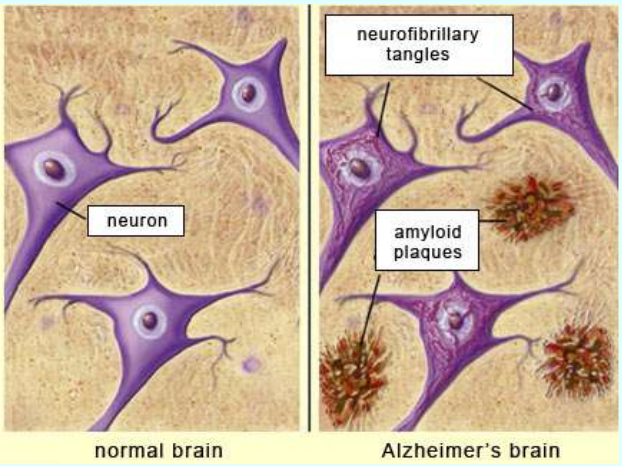
Scientists don't yet fully understand what causes Alzheimer's disease in most people. The causes probably include a combination of age-related changes in the brain, along with genetic, environmental, and lifestyle factors.

Primary hypotheses

Alzheimer's is generally associated with two types of lesions throughout the cerebral cortex:

1. Amyloid plaques (also called *senile* plaques), which are found *between* the neurons (some accumulate on axons and dendrites in neurons), and
2. Neurofibrillary (or tau) tangles, which are found *inside* neurons.

Look at the diagram, right. The brains of a lot of people with Alzheimer's disease (but not all) contain many amyloid plaques and tangled bundles of neurofibrillary tangles.



Both amyloid plaques and neurofibrillary tangles are build-ups of protein molecules that also occur as part of the *normal* ageing process, but in people with Alzheimer's-type dementias, the amounts of these proteins that build up are far greater.

This led many researchers (but not all) to hypothesise that plaques and tangles are the prime suspects in damaging and killing nerve leading to Alzheimer's disease and are the cause of personality and cognitive decline that occurs over time.

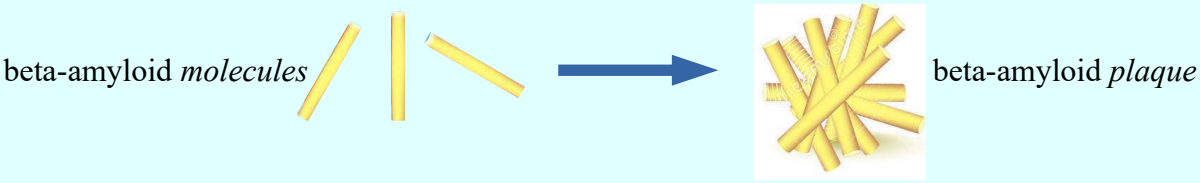
We will look briefly at each hypothesis in turn then together. First, the amyloid hypothesis.

The amyloid hypothesis

Amyloid plaques were the first of these two types of lesions to be described. The plaques consist of small, dense deposits of **beta-amyloid** (or β-amyloid, or Aβ, whichever you prefer). Beta-amyloid is a short protein molecule, made up of 36 – 43 amino acids (or 37 – 49 depending on source you read!!). Beta-amyloid molecules are *chemically* adhesive, so that they gradually 'stick' to one another and accumulate or clump to form plaques.

Note: Beta-amyloid molecules are *chemically* adhesive/sticky, not sticky in the sense that glue is. Their stickiness is due to chemical bonds that bind the molecules to each other.

The diagram below illustrates, in a *simple* way, this clumping.



Aβ was first isolated from the brains of AD patients in 1984. One year later, it was identified as the core of plaques observed in the brain tissue of AD patients. The amyloid hypothesis was first posited in 1992, and postulates that it is the accumulation of Aβ peptides in the brain that results in AD over time. Deposits of Aβ molecules and plaque are mainly observed in the region of the hippocampus and the neocortex in the brain.

Note on proteins and peptides: Both consist of amino acids joined together to form chains. Proteins contain many amino acids, often hundreds. A peptide is a *short* chain of amino acids, the shortest having just two amino acids. (One amino acid by itself is an amino acid *not* a peptide.)

It is believed that the 42-amino-acid beta-amyloid, usually written as Aβ42 (i.e. a peptide with 42 amino acids) is the most dangerous variety and that aggregation of this molecule poisons and kills neurons.

The amyloid cores of plaques are surrounded by microglial cells (the brain's scavenger cells), which seem to limit the growth of Aβ plaques. But how microglia do this remains controversial. Also, microglia *depletion* does not seem to affect plaque *numbers*, but does cause a significant increase of plaque *size* over time.

The neurofibrillary tangles hypothesis

Neurofibrillary tangles are also due to a protein, this one called the tau protein ('tau' stands for '*t*ubulin *a*ssociated *u*nit', rhymes with 'wow'). It is found *inside* the neurons rather than outside (as with beta-amyloid). It was first observed in 1961, under an electron microscope. Like beta-amyloid protein molecules, tau protein molecules are present in the neurons normally.

This protein too becomes problematic when it undergoes certain chemical changes to form fibres with a very small diameter which have a propensity to agglutinate (stick together) and wind around one another, instead of remaining straight, and get tangled *inside* the neuron to form the neurofibrillary tangles (NFTs) (illustrated, right). This eventually leads to the death of the neurons. That is:

tau proteins → tau fibres (straight) → neurofibrillary tangles → death of neurons

Neurofibrillary tangles are found in people with Alzheimer's.

Most researchers believe that Alzheimer's is expressed (i.e. shown/revealed/displayed) first following the build-up of amyloid plaques. But other researchers think that it is actually expressed with the appearance of neurofibrillary tangles.

Notes on tau and NFTs:

1. The formation of neurofibrillary tangles is not specific to Alzheimer's. It occurs in other dementias and neurodegenerative diseases, such as numerous Parkinsonian diseases.
2. Neuroscientists now believe that after age 75, this tau problem appears even in the *normal* human brain, especially in parts of the brain that are closely involved in long-term memory, such as the hippocampus.
3. What causes tau protein to turn toxic? A number of recent studies have suggested there my be some everyday triggers, such as gum disease or an infection, or different conditions, such as 'leaky gut', in

which microbes and other particles leak from the digestive system into the nervous system. Scientists still do not understand the tau protein very well.

4. Many neurons that have tangles inside of them are still alive, while nearby neurons have been killed off. This is important to remember when considering treatments for tau. Targeting the tangles (NTFs) might kill these still functioning neurons. So, if you aim to clear all the tangles, you may be asking for trouble. A better approach would be to prevent the tau build-up from happening in the first place.
5. Tau was long thought to be a secondary actor in Alzheimer's. Amyloid plaque was thought to build up first, largely outside of neurons, followed by tau tangles which clog their insides. But research has found that people can continue to function well with amyloid plaques in their brain. It is only when toxic tau *starts* to *spread* that people begin confusing 'breakfast' with 'baseball', forgetting not just where they left their keys but how to use them or that they even have keys at all.

A combined hypothesis

For over a decade, the amyloid plaque hypothesis dominated research on Alzheimer's disease. Numerous findings began to cast doubt on this *single* hypothesis which led to alternative explanations, the best known being the *neurofibrillary tangles hypothesis* that is damaging and killing nerve cells.

Further research then suggested that Alzheimer's-related brain changes may result from a complex interplay among *both* abnormal tau and beta-amyloid proteins (and several other factors, which we will not worry about). It appears that abnormal tau accumulates, as mentioned, in specific brain regions involved in memory, especially the hippocampus. Beta-amyloid clumps into plaques between its neurons.

With the combined hypothesis, many researchers believe that Alzheimer's is expressed:
1. *first* following the build-up of amyloid plaques, and
2. *then* tau comes into play forming tangles inside neurons.

As the level of beta-amyloid reaches a tipping point, there is a rapid spread of tau throughout the brain. So, there is believed to be some connection between the two. What is less clear is the relative importance of each of them in the development of Alzheimer's disease.

The atrophy of the cortex that is seen in Alzheimer's-type dementia also seems to depend on the *amount* of neurofibrillary degeneration and build-up of amyloid plaques in the brain. The extent of this atrophy generally correlates with the extent of the cognitive losses observed.

Caveat: But even now, much about amyloid plaques and neurofibrillary tangles remains unknown. Scientists do not know exactly the precise role plaques and tangles play in Alzheimer's disease. As one brain scientist put it ironically, "At this point, we know almost everything about Alzheimer's, except for the role of amyloid plaques and neurofibrillary tangles."

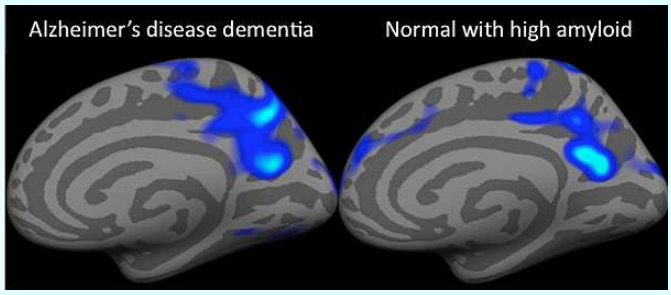
Problems with these hypotheses

Although the amyloid hypothesis on the origin of Alzheimer's has been so predominant, that is starting to change. More and more researchers are beginning to voice *alternative* explanations. To justify their explorations off the beaten path, these scientists point out that the quarter-century history (and counting)

of research based on the amyloid hypothesis is littered with failures, with little tangible progress to show for it.

Here is a brief summary of the flaws that these researchers see in the amyloid hypothesis:

1. First, very little correlation has been observed between the extent of cognitive deficits in Alzheimer's patients and the quantity of amyloid plaques in their brains. This represents a major anomaly in this paradigm that has dominated Alzheimer's research since the start of the 1990s, if not longer.
2. The second perceived flaw is that amyloid plaques are also found in the brains of *normal* individuals, something that the defenders of the amyloid hypothesis are hard-pressed to explain. In fact, 'normal' brains, especially in normal *older* individuals without Alzheimer's disease, can sometimes contain *more* amyloid plaques than the brains of patients with severe cases of Alzheimer's. Hence some have argued that the criteria for diagnosing 'Alzheimer's disease' are too vague and that Alzheimer's cases may be nothing more than special cases of normal ageing (rather than a distinct disease).
   The images (right) show similar amyloid plaque (blue areas) in an Alzheimer's patient (left) and in a normal older individual (right). Look similar, don't you think?



3. But the overall picture remains unclear. For example, normal people can have only moderate levels of beta-amyloid plaque deposits but at the same time have fairly extensive neurofibrillary tangles. And then again, cases have been reported of patients with lots of tau but no beta-amyloid deposits.
4. Some researchers even directly question the harmfulness of amyloid plaques and neurofibrillary tangles, arguing that they may in fact represent a defensive response by the brain to harmful processes that precede them, such as oxidative stress, inflammation, and dysfunctions in the cellular cycle.

The amyloid hypothesis, some authors say, has begun to take on the trappings of dogma. Because, as mentioned earlier, dogmatism is a bad idea in general, and a bad idea in science in particular, many researchers are rather pleased to see the growing number of alternative hypotheses to help us better understand and treat the condition known as Alzheimer's.

Many molecules are certainly involved in the development of Alzheimer's, and new candidates are announced regularly in the media. For example, in 2009 and 2010 alone, there was much discussion of leptin, a hormone that the body's fat cells produce after meals and that causes appetite to decrease. In one study, the subjects who had the *lowest* levels of leptin were shown to be more likely to develop Alzheimer's (see also '*Some alternative hypotheses*' #4 next.)

Some alternative hypotheses

The cause of most Alzheimer's cases is largely unknown. Although it is characterised mostly by the formation of amyloid plaques in the brain, there are other competing hypotheses regarding the cause of

the disease. A number of alternative hypotheses have been suggested, including non-Aβ hypotheses. Here are brief comments on four such alternatives.

1. A non-Aβ hypothesis: The tau hypothesis

As already mentioned, this hypothesis postulates that tau protein forms neurofibrillary tangles. These tangles lead to the disintegration of *microtubules* (small tubes) in neurons and lead to the death of the neurons. (More about this hypothesis will be discussed soon).

2. The cholinergic hypothesis

Earlier (pages 11ff.), we discussed acetylcholine/cholinergic receptors (AChR) and mentioned that these receptors, besides playing an important role in human learning and memory formation, also play a role in Alzheimer's disease.

It has been proposed that AD is caused by the reduced synthesis of the neurotransmitter acetylcholine, or by the initiation of large-scale aggregation of amyloid plaque. (How, we will not go into here.)

Some currently available drug therapies are based on this hypothesis (see page XX)

3. Alzheimer's may be caused by brain's defence against infection

Alzheimer's disease has long been linked to the accumulation of sticky plaques of beta-amyloid proteins in the brain, especially as people get older, but their function has remained unclear. Recent research (reported in 2018) has found that beta-amyloid can kill microbes. When researchers injected bacteria into the brains of mice bred to be able to develop plaques much as *humans* do, the mice developed amyloid plaques overnight.

This suggested that microbial *infection* could be triggering the formation of plaques that cause Alzheimer's disease. Somehow, bacteria, viruses or other pathogens may be crossing the blood-brain barrier and getting into the brain and the brain may be responding by using beta-amyloid to trap and kill them. But if these plaques aren't cleared away fast enough, they may then lead to tau tangles causing neurons to die and the progression towards Alzheimer's disease.

The researchers point out that it is not the microbes themselves that causes the damage to in the brain but the beta-amyloid. But critics of the idea claim that such infections and physical hallmarks of Alzheimer's disease coincide simply because both are common in aged brains.

If certain infections can lead to Alzheimer's disease, this suggests that antimicrobial or antiviral drugs might have therapeutic value. (Refer later to treatment of Alzheimer's disease on pages XX.)

4. Obesity and leptin resistance leads to Alzheimer's Disease

Leptin and obesity, especially in middle age, has also been linked to Alzheimer's disease (AD). In the last 15 years, obesity and Alzheimer's disease risk have been related.

Leptin is known to be neuroprotective. For example, it is able to lower the levels of Aβ peptide produced in the brain. Moreover, leptin is believed to enhance the removal of harmful Aβ by promoting its clearance. An important target of leptin protection is the hippocampus, where it has a role in the *synaptic plasticity* process, in memory preservation/consolidation.

Obesity is known to induce leptin and insulin resistance. But when leptin-resistance takes place due to middle-age obesity, the above protective effects of leptin are blocked and the risk of AD is increased. And a 2019 report suggests that leptin resistance is linked to an increased risk of Alzheimer's disease.

To learn more bout leptin and obesity, you my like to refer to the earlier project ***Pure, White and Deadly: Part I*** which deals with leptin and obesity. Here is one link to the project: https://drive.google.com/file/d/1iCbUDuzAg7Ztq4xrsimuBlVZdGJOWpAe/preview

Structure and Production of Amyloid Plaques and Tau Tangles

Despite the above problems with hypotheses, let us now look more closely into the hypotheses related to beta-amyloid, amyloid plaques and tau tangles.

Structure of Aβ plaques

Aβ monomer

As seen, amyloid plaques are made of beta-amyloid *molecules* (also called beta-amyloid *monomers* or *Aβ monomers*) clumped together. (The ▬ shown earlier is an Aβ monomer.) [Monomer – from the Greek: mono- = *one*; - *mer* = part.]

Aβ monomers are made up of small molecules called *amino acids* joined together into chains. An Aβ monomer can have 37 – 49 amino acids in its chain. One important Aβ monomer has 42 amino acids, and is called, naturally enough, the Aβ42 monomer. The diagram (right) shows this monomer, with each circle representing an amino acid. There are 42 circles – count them! (The key elements of an amino acid are carbon (C), hydrogen (H), oxygen (O), and nitrogen (N), although other elements are found in some amino acids. But as this is not a chemistry course, we will not go into details about the structure of an amino acid.)

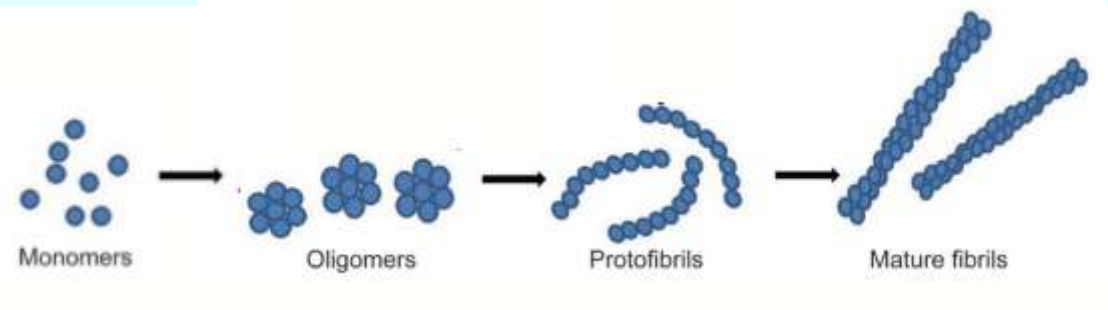
The Aβ42 monomer is thought to be especially toxic. In the Alzheimer's brain, abnormal levels of this naturally occurring peptide (that is, a short protein, remember) clump together to form plaques that collect between neurons and disrupt cell function.

Deposits of Aβ monomers are observed in the region of the hippocampus and the neocortex (the outermost portion of the brain involved in 'higher' functions such as perception, cognition, memory, reasoning/thinking and language).

Aβ was first isolated from the brain vessels of AD patients in 1984 and the amino acid sequence of the Aβ monomers determined. One year later, it was identified as the core of amyloid/senile plaques observed in the brain tissue of AD patients.

From Aβ monomers to Aβ plaques

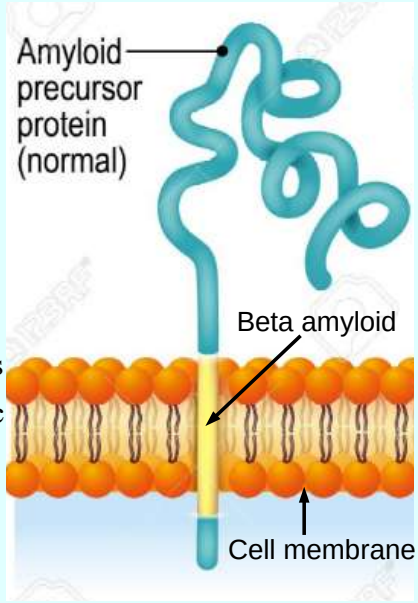
The earlier diagram (page 24) seemed to suggest that the clumping occurs in one step. But it actually involves several steps. The diagram (below) shows the proposed pathway for the conversion. For simplicity, the 42 amino acid monomers in this diagram are shown as blue dots (●) rather than yellow lines (as on page 24).



1. Monomers to oligomers: (from the Greek: olig- = *a few*; - *mer* = part) Dimers, trimers, and tetramers are, for instance, oligomers composed of two, three, and four monomers, respectively. (The oligomers in the diagram are heptamers, as they have seven monomers – *hepta-* = seven.) Aβ oligomers are *soluble* and may spread throughout the brain.
2. Oligomers to protofibrils: (from Latin: *fibrilla* = small fibre) The oligomers rapidly form protofibrils (from Greek *proto-* = first;), which are straighter, longer, *insoluble* structures.
3. Protofibrils to mature/amyloid fibrils: These single length protofibrils then align to form amyloid (or mature) fibrils, characterised by rod-like structures.
4. Fibrils to amyloid plaque: Many fibrils clump together and cluster around neurons forming lesions that are characteristic of Alzheimer's disease (not shown in this diagram).

Basic components in Aβ production

The surfaces of many cells throughout the body, but especially concentrated in the synapses of neurons (both presynaptic and postsynaptic neurons), contain a protein called **amyloid precursor protein** (APP).



APP is known as a *transmembrane* protein (*trans-* = through/across), because it moves from the inside of the neuron (in a place called the endoplasmic reticulum (ER) – for those interested!) through the membrane of the presynaptic and postsynaptic terminals. That is, it begins life *inside* the neurons and is transported to the membranes of the synaptic terminals.

APP consists of three component parts represented by the colours in the diagram. The whole thing (blue curly bit + yellow bit + small blue bit) is the APP. The part in yellow is *beta-amyloid* which anchors the APP in the cell membrane.

APP is produced in several different forms, ranging in size from 695 to 770 amino acids. The most abundant form in the brain is called APP695 (that is, a chain of 695 amino acids joined together) and is produced mainly in neurons.

The primary function of APP is not known, though it has been implicated as a regulator of synapse formation and in neural growth and repair.

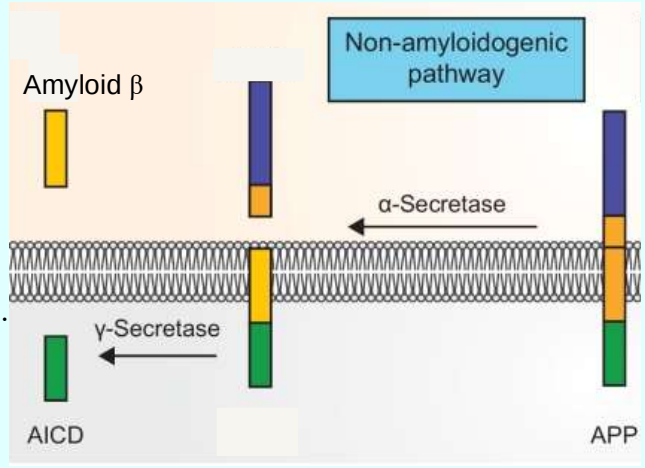
APP is best known as the precursor molecule which generates beta amyloid (Aβ).

Explanation for Aβ formation/production

Human APP is cut into its three component parts by enzymes, termed secretases. (Remember – names of most enzymes end in -ase.) This cutting occurs in two ways, one of which occurs in *normal*, healthy people; the other is *abnormal*. These two alternative pathways are called the non-amyloidogenic pathway and the amyloidogenic pathways respectively.

[*Word analysis*: amyloidogenic = amyloid + -o- + genic (= from Greek: *to produce*). So, amyloidogenic = to produce amyloid; non-amyloidogenic = not to produce amyloid (at least not the harmful form)]

Non-amyloidogenic pathway: a normal process



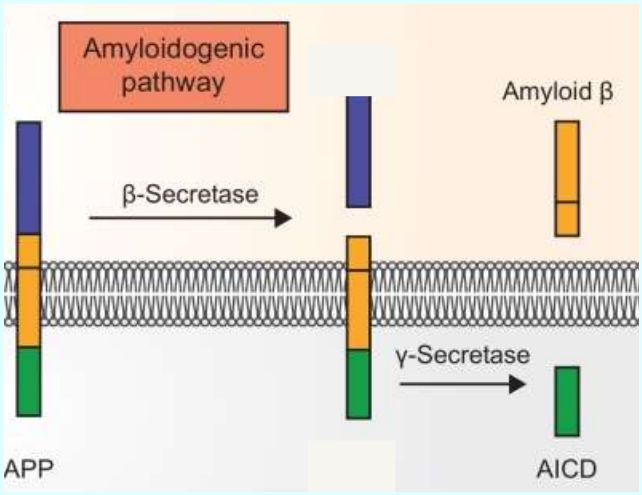
The non-amyloidogenic pathways is normal and not harmful, and is the principal pathway under healthy conditions. There are two enzymes that do the 'cutting': these are α-secretase (alpha-secretase), followed by γ-secretase (gamma-secretase). This is carried out at the membranes of the presynaptic and postsynaptic neurons.

In healthy brains, the vast majority of APP (>90%) is processed by this pathway. The amyloid produced is the Aβ40 monomer (i.e. 40 amino acids), which is then removed into the extracellular space (i.e. the space outside the neuron). (From Latin *extra* = outside.)

The AICD (APP intracellular domain) protein formed can be transported to the nucleus of the neuron, where it functions as a transcriptional factor, that is, it 'reads' and interpret the genes of the DNA to ensure that neurons function correctly (but don't worry about this).

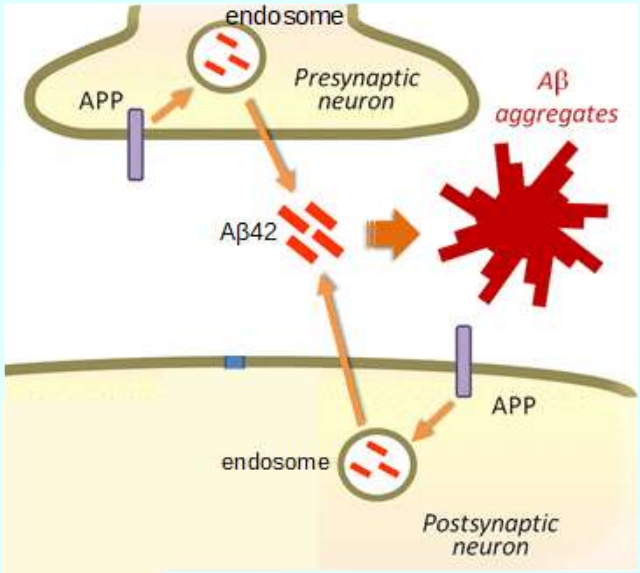
Aβ40 is believed to be *not* toxic and carries out essential jobs in brains cells including inhibiting the formation of Aβ42 oligomers, which *are* toxic. However, the question of toxicity is still not settled and some recent experimental evidence suggests that Aβ40 may promote atherosclerosis in the body's arteries.

Amyloidogenic pathway: an abnormal process



In this pathway, the two secretases that do the 'cutting' are β-secretase (beta-secretase), followed by γ-secretase, again in both presynaptic and postsynaptic neurons (refer to the diagram, right.)

However, *unlike* the non-amyloidogenic pathway, which is carried out at the *membrane*, the amyloidogenic pathway mainly occurs in *endosomes* inside the synapses [endosome = a compartment/space inside a cell, sometimes filled with fluid.]



Refer now to the (very simplified) second diagram (right).

The APP molecules are first transported inside the synapses to the endosomes. There, the APP forms Aβ42 and AICD using the two enzymes.

The Aβ42 monomers produced are removed from the endosomes to the extracellular (outside the cell/synapse) space as shown in the diagram. The Aβ42 monomers then *aggregate* first into oligomers then fibrils and finally plaques/aggregates, leading to Alzheimer's disease.

The AICD, still inside the synapse, is transferred to the nucleus of the neuron, as before.

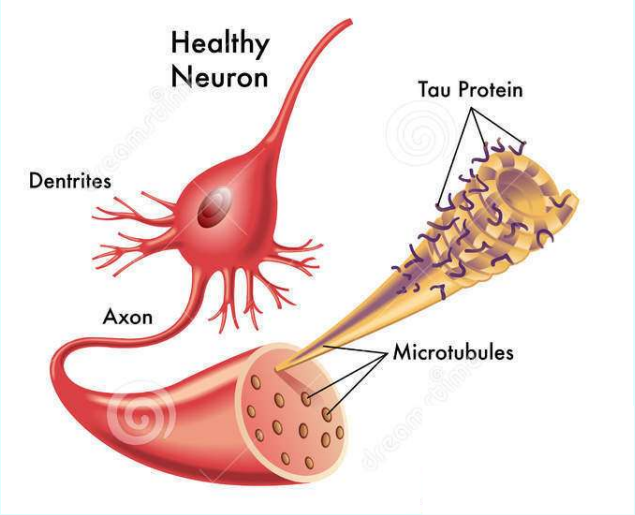
As mentioned above, Aβ42 monomers are significantly more neurotoxic than Aβ40 and form amyloid fibrils much more rapidly than Aβ40.

Note: Amyloid deposits are also found in the blood vessels of the brain, in the retina, and in glial cells. The latter have various support functions in the brain (for example, as seen earlier, they help to form the BBB), but it is unclear whether this plays a role in the development of Alzheimer's disease.

Extension: **Alzheimer's: Synthetic protein blocks toxic beta-amyloid**
In a 2019 paper (not yet published at the time I finished this project), scientists write about having designed a *synthetic* peptide (i.e. a small protein – remember!), which has only 23 amino acids, that can bind to small clumps of beta-amyloid in its early stage, which is the most harmful stage, and block them from forming larger clumps (plaques). The researchers say that the findings could lead to treatments that clear away toxic beta-amyloid in its early forms. They also see potential for using the peptide as the basis of a test for diagnosing Alzheimer's disease before symptoms emerge. These notes are taken from the link below:
https://www.medicalnewstoday.com/articles/324985

Production of Tau Tangles

To understand how neurofibrillary tangles (may!) occur, it is necessary to be reminded about neuron anatomy. Look back at page 6 and focus on the soma, axon and dendrites.



In the *axons* of healthy neurons are many specialised filaments called microtubules that transport nutrients and other essential materials from the cell body (soma) to the tip of the axon.

Tau proteins

Proteins, known as tau proteins, promote the assembly of these microtubules then bind to them, keeping them parallel in the axons, which contributes to their stability and the proper functioning of the neuron.

Alterations in the *amount* or the *structure* of tau protein can affect its role as a stabiliser of microtubules as well as some of the processes in which it is implicated.

Notes

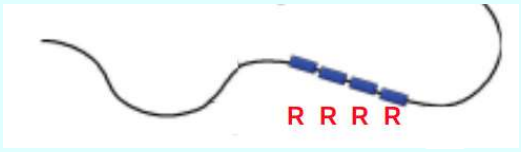
1. The tau proteins (or τ proteins, after the Greek letter with that name) are a group of six similar highly-soluble proteins (called *isoforms*).
2. Tau proteins also occur at very low levels in astrocytes and oligodendrocytes but are present in greatest extent in the axons of neurons.
3. Tau protein was discovered in the mid-1970s.

Structure of tau

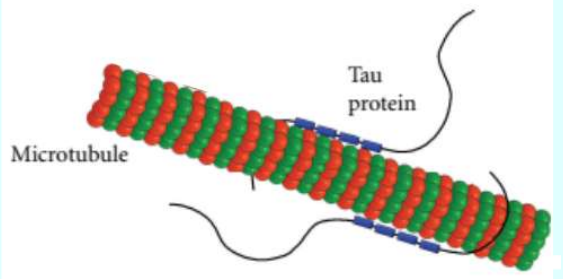
Tau are protein molecules, that is, they are made up of chains of amino acids. The six forms of tau, range from 352 to 441 amino acids.

The tau protein is distinguished by the repetition of a particular group of amino acids, designated R for 'repeat', in its amino acid sequence. It is this repetition that enables the tau protein to *bind* to the microtubules.

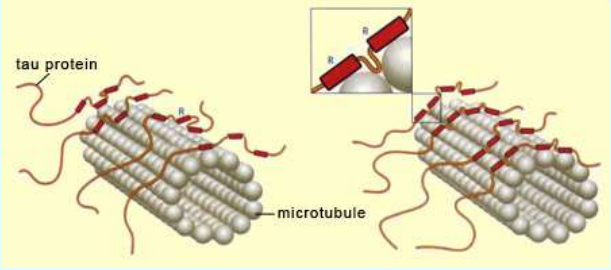
Three of the six isoforms/variants of the tau protein repeat this group of proteins three times, while the other three isoforms repeat it four times. Hence these two groups are referred to as 3R variants and 4R variants, respectively. The longest of the six isoforms is a 4R variant (shown as the four **blue** boxes in the diagram, right).



In its normal function, the tau protein binds to the microtubules through these three (or four in case of the longest isoform) repeat units. The second diagram shows a 4R variant binding to a microtubule. Because 4R tau proteins have one additional binding site, they stabilise the microtubule filaments inside the neurons more effectively/strongly than the 3R variants do, thus encouraging longer, more rigid axons.



The third picture, showing 3R and 4R variants binding microtubules seems to suggest that not all the R units actually needs to bind. But in a normal mature neuron, practically *all* the R units in the tau protein are bound to the microtubule (I think this is correct!).



Terminology:

1. Tau protein belongs to a group of proteins referred to as Microtubule-Associated Proteins (MAPs).
2. The technical term for a repeat unit R is tubulin binding domain.

Phosphorylation – a second process

The microtubules are stabilised *first* by the binding of the repeat units on the tau protein molecules as discussed above.

The binding of tau protein to the microtubules also requires a *second* process called phosphorylation of the tau protein. The stabilisation of the microtubules can be controlled even more finely by the degree of phosphorylation.

Phosphorylation is a process whereby a phosphate group ($-PO_4^{3-}$ containing phosphorus and oxygen) binds to three *kinds* of amino acids of a tau protein (note: three *kinds*, not just three). But these three kinds are abundant in the tau protein. The tau protein has numerous sites where phosphorylation can occur, The longest of the six variants of tau protein (441 amino acid) holds about 80 (85 or 88 depending on which article you read!) potential phosphorylation sites. The tau

protein in the diagram is shown (above right), merely for simplicity, with three phosphate groups (pink balls); in reality there will be more than three, up to perhaps 80.

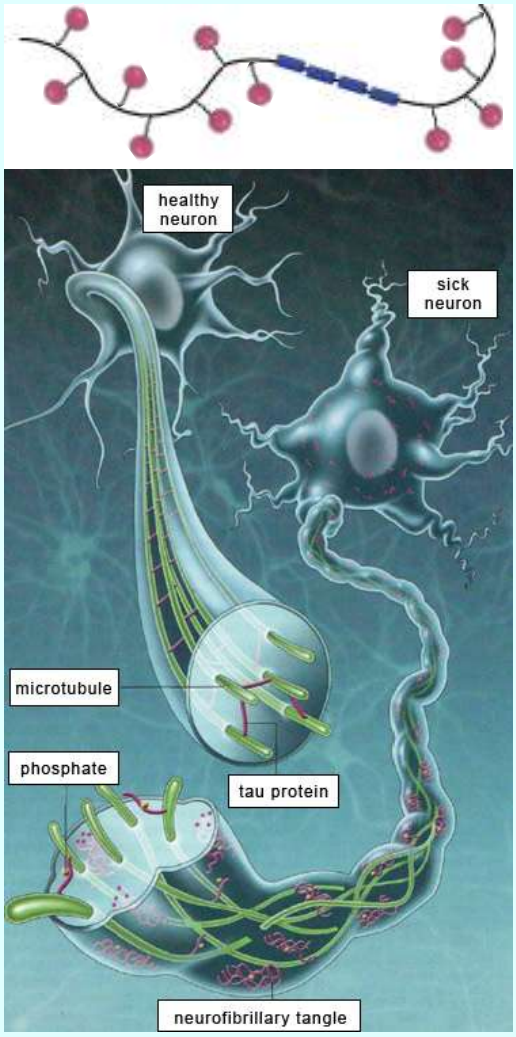
Equilibrium

Phosphate groups are attached to the tau proteins while, at the same time, they are also removed, that is, *both* phosphorylation and *de*phosphorylation occur at the same time. Eventually, an equilibrium is reached with the number being added balancing/equalling the number being removed. Phosphorylation and *de*phosphorylation depend on the balance between the actions of two types of enzymes: protein **kinases** (which *add* phosphate groups) and **phosphatases** (which *remove* them). Stabilisation of microtubules by the tau protein is regulated by these kinases and phosphatases and in a normal, healthy, tau protein, the amount of phosphorylation is just right to keep the microtubules stable.

Note: Phosphorylation with its counterpart, dephosphorylation, is critical for many cellular processes in biology and not just for tau proteins.

Question: (Wiki) says that, rather than the addition of a phosphate group ($-PO_4^{3-}$), it is actually a phosphoryl group ($-PO_3^{2-}$) that is added. Who is correct? *I* think the Wiki article is correct and that the chemistry involved is rather more complicated than suggested in most articles. But I will stick to *phosphate* in this project.

Abnormal phosphorylation: Hyperphosphorylation



Abnormal phosphorylation of tau is known to contribute to Alzheimer's disease. The tau protein accepts more phosphate groups than is normal. This excess phosphorylation of the tau protein is called hyperphosphorylation [hyper- = from Greek *over*, *above*, *more than*, *in excess*]. The diagram (right) shows one such possible hyperphosphorylated tau protein.

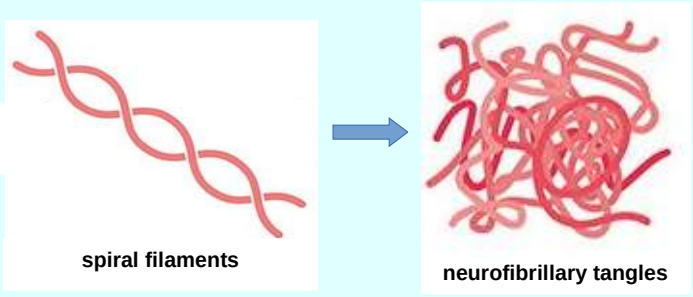
There are several serious consequences of abnormal phosphorylation:

First: The *abnormally* phosphorylated tau protein chains detach both themselves and other *normal* tau completely from microtubules. Certain chemical changes then occur that cause the tau molecules to become 'sticky'. They then start sticking to one another instead of sticking to the microtubules.

Second: Without tau protein molecules to *stabilise* them, the neurons' microtubules no longer remain straight and begin to disintegrate and disrupt the axonal transport of the materials essential to the neuron's survival and so ends up killing the neurons. And loss of neurons is very strongly correlated with the seriousness of the cognitive deficits displayed by people with Alzheimer's. Further, the nerve endings at the very tip of the axon

(the synapses) also degenerate as a result of this lack of materials, causing them to die. As a result, communication with the following neurons is cut off completely.

Third: Tau protein molecules that are thus detached and deactivated by excess phosphorylation then wind around one another to form pairs of spiral filaments (right). These filaments in turn bind to one another and get tangled inside the neurons to produce the neurofibrillary tangles (NFTs) that gradually disrupt the functioning of the neurons until they are destroyed, and are reported to be responsible for neuron death.



Abnormal tau, like beta-amyloid, accumulates especially in the neurons in the hippocampus and other specific brain regions involved in memory.

A brief note: Only about 60% of the abnormally hyperphosphorylated tau in the brain of AD patients actually forms spiral filaments and NFTs. The remaining 40% or so just remains in the neurons.

Helping tau protein to stick to microtubules

There is a naturally-occurring protein in neurons that provides *neuroprotection*. It is called *activity dependent neuroprotective protein* (ADNP). A short section of this, a peptide of just eight amino acids, known as NAPVSIPQ (NAP for short) has highly potent neuroprotective activity. It is thought to help tau protein attach more strongly to microtubules and so less likely to detach, but its exact mechanism of action is not clear. For some reason, it seems to prefer binding to Tau3R rather than Tau4R.

Cause of hyperphosphorylation and NFTs: Beta-amyloid?

Just what causes the increased phosphorylation of the tau protein remains an open question.

One hypothesis is that beta-amyloid initiates a series of events that leads to the death of neurons. The presence of beta-amyloid forms substances called *free radicals*, and that they cause the neuron's cell membrane to deteriorate. Calcium ions (Ca^{2+}) and beta-amyloid fragments then penetrate the neuron more readily and over-activate the kinase enzymes which control the addition of phosphate groups to tau proteins.

But which form of beta-amyloid? Early studies suggested that it was the beta-amyloid plaques that cause neuron to die. But recent research (2017) suggests that, rather than the larger beta-amyloid plaques, it is the small, *soluble* oligomers of beta-amyloid may be more toxic. Researchers now believe that these small, soluble beta-amyloid oligomers may be more toxic for the neuron synapses in regions of the brain that underlie memory (especially the hippocampus).

Trans-neuronal spread of tau

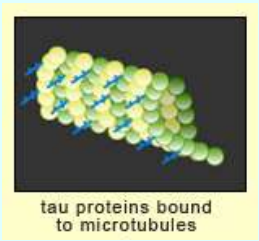
Recent research (2018) also suggests that hyperphosphorylated tau protein (that is, the damaged/abnormal tau) may spread like an infection, rather than remaining in each neuron. The idea is that it can start in one neuron and move across synapses to other neurons eventually killing these neurons too.

This spread of tau could have implications for clinical care if drugs can be developed that attack tau inside synapses or when it comes outside, locking it up inside affected cells early, before it can spread.

Summary of current belief

The current (and widely-held) idea is that beta-amyloid is responsible for neuron death in cases of Alzheimer's disease – either directly, or by giving rise to tau hyperphosphorylation, in which the protein tau is bent into neurofibrillary tangles that disrupt nutrient supply to brain cells, eventually killing them. That is:

1. Normal tau proteins bind to microtubules in axons; the tau stabilises the microtubules.
2. Beta-amyloid (probably oligomers rather than plaques) causes excess phosphorylation of the tau proteins. These abnormal hyperphosphorylated tau proteins detach from the microtubules, causing the microtubules to disintegrate.
3. The free tau proteins aggregate to form filaments then NFTs leading to neuron death and Alzheimer's disease.

1. 

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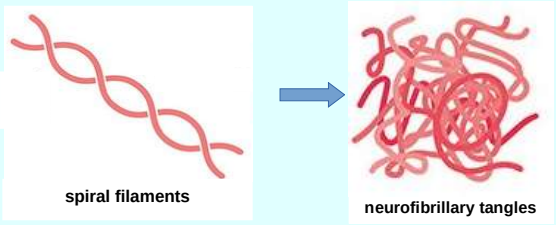
3. 

Having said all this, scientists still don't know for sure exactly what is causing neuron death in Alzheimer's disease!

Receptors and Alzheimer's Disease

As well as Aβ oligomers and plaques, and NFTs, Alzheimer's disease can also result from synaptic receptors that do not function normally. Here we will look at the two kinds of receptors introduced earlier – NMDA receptors and Acetylcholine receptors.

NMDA receptors

Earlier (pages 11 – 12), we saw that in the *normal* brain, glutamate neurotransmitter molecules cross the synapse and bind to NMDA receptors on the receiving neuron, which leads to LTP. Excess glutamate molecules are recycled by astrocytes.

We also saw that there are two kinds of NMDA receptors on the receiving neuron – *postsynaptic* and *extrasynaptic*. In the brain of the Alzheimer's patients, both these kinds of receptors are affected. This is due to Aβ42 oligomers, which are elevated in the brains of patients with the disease. And, in addition, the Aβ42 oligomers block astrocytes from recycling excess glutamate molecules (shown by the **X** in the diagrams on the next page).

We now look at how Aβ42 oligomers are believed to affect postsynaptic and extrasynaptic NMDA receptors in people with Alzheimer's disease.

First the postsynaptic NMDA receptors.

(a) Postsynaptic NMDA receptors – endocytosis

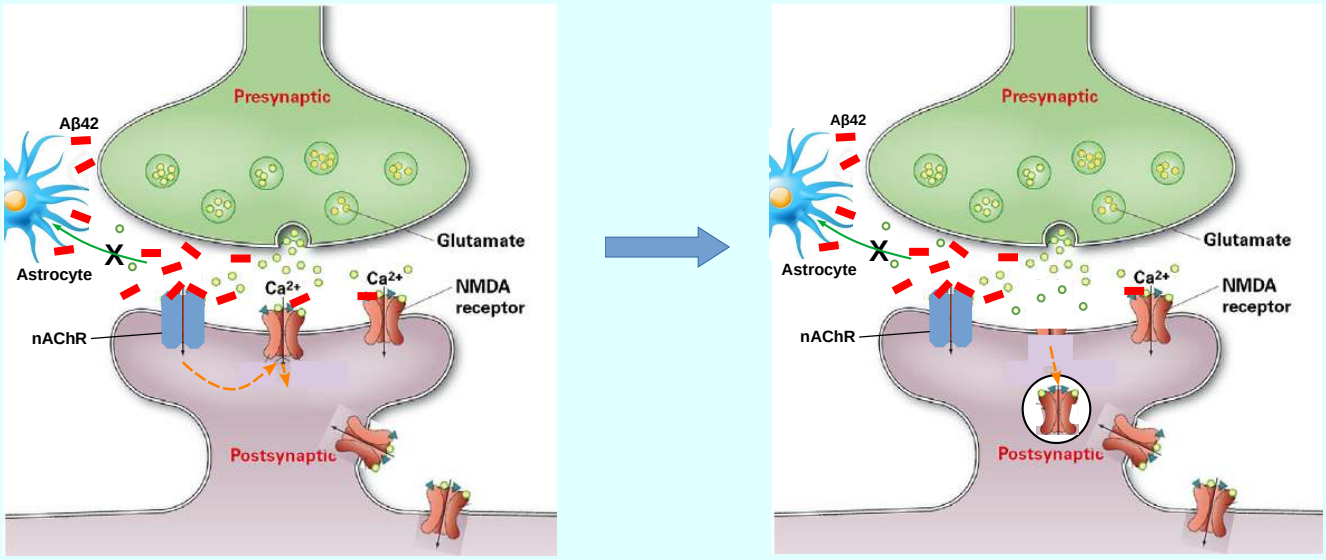
We have seen that NMDA receptors play a role in learning and memory (known technically as long-term potentiation or LTD). So, fewer receptors would effect learning and memory. This is what is believed to happen. NMDA receptors are removed from the *postsynaptic* surface and internalised by *endocytosis* then broken down. This is a result of the high levels of Aβ42 oligomers present in the Alzheimer's brain.

How this happens in the *abnormal* brain is shown in the diagrams below. (Again, the diagrams are very simplified and show just a few of the many receptors present on the surface of the postsynaptic neuron.)

1. The endocytosis of NMDA receptors begins with the acetylcholine receptors (nAChR). Aβ42 oligomers bind strongly to the surface of these nAChR.
2. This binding allows calcium ions (Ca^{2+}), present in the space around neurons, to flood into the postsynaptic neuron. This initiates a series of (complex!) chemical reactions inside the neuron (just shown by the dotted lines) that result in the absorption/internalisation of the NMDA receptors into the cell body by *endocytosis*, thus reducing their availability at the synapse.
3. As there are now fewer NMDA receptors present, this reduces glutamate transmission and so *decreases* the ability to form to learn and remember things. (Remember, this is known as long-term depression, LTD). Some researchers believe that the Aβ42 oligomers also bind to the NMDA receptors themselves (shown in the diagrams by the red bars ▬ bound to the receptors) and aid their internalisation.
4. The internalised receptors are then degraded/broken down into smaller bits and removed.

The internalisation of postsynaptic NMDARs means fewer calcium ions enter, leading to LTD.

This explantation was first proposed way back in 2005 but unlike many other hypotheses on the causes of Alzheimer's disease, still seems to be accepted.



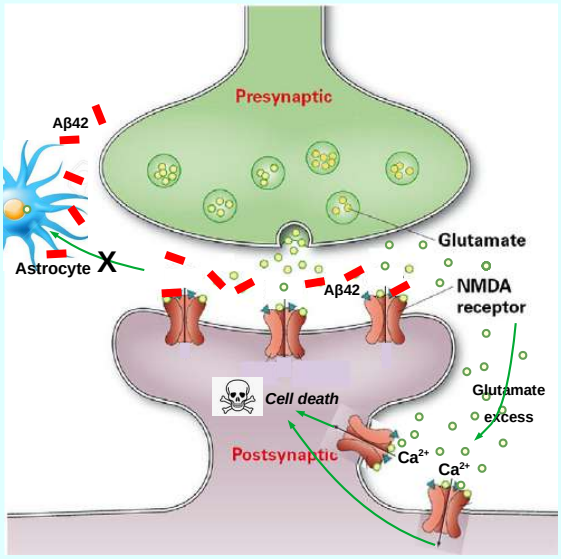
(b) Extrasynaptic NMDA receptors – cell death

As already mentioned, in the normal brain, astrocytes recycle excess glutamate neurotransmitter molecules. But in the diseased brain, Aβ42 oligomers block this process.

All the extra unused glutamate now floating around is believed, by some scientists at least, to affect the *extrasynaptic* NMDA receptors.

Here is an explanation:

1. In a normal brain, astrocytes *recycle* the excess glutamate neurotransmitter molecules. In the diseased brain, Aβ42 oligomers *block* this recycling process. The result is an accumulation of excess glutamate neurotransmitters.
2. The excess glutamate molecules drift towards the extrasynaptic NMDA receptors and bind with them.
3. Because they are NMDA receptors, calcium ions (Ca^{2+}) enter. But this time, the calcium ions block/inhibit the pathways leading to LTP and activate *other* pathways that causes the neuron to shrink in size.
4. The more this condition persists, the more the activated pathologic pathways lead to neuron degeneration and cell death.



These excess glutamate molecules become harmful because they overstimulate healthy extrasynaptic NMDA receptors making the glutamate toxic to the neurons causing them to become damaged or to die. (This phenomenon is called *excitotoxicity*.)

Extension note:

Accumulating evidence suggests that tau as well as Aβ work together in affecting NMDA receptors, independently of their accumulation into plaques and tangles. Remember that tau protein is present in neurons and in tau protein, hyperphosphorylation of tau protein occurs. The overstimulation of extrasynaptic NMDA receptors induces tau hyperphosphorylation and cell death.

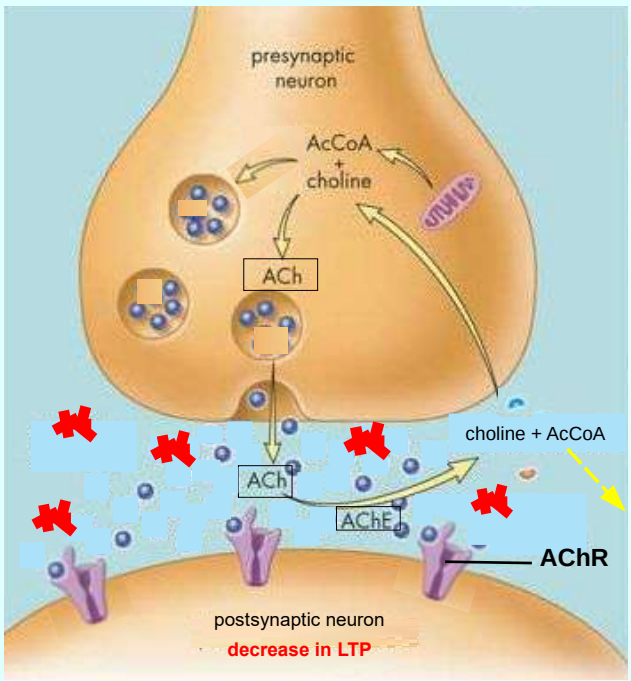
However, clear evidence of this is still deficient.

Acetylcholine receptors [also called cholinergic neurons]

Neurons that use acetylcholine as a neurotransmitter are especially affected by Alzheimer's resulting in a slowing of mental faculties.

It was mentioned earlier (page 13) that in *normal ageing*, the *production* of acetylcholine (and other neurotransmitters) is reduced, perhaps by as much as 50% between young adulthood and old age. This means fewer ACh neurotransmitter molecules reach the receptors resulting in less LTP, which impairs our ability to think and perform memory tasks.

Refer to the diagram (right) and compare this with that for the younger brain on page 13. Note the fewer ACh produced and stored in the vesicles and the fewer present in the synapse.



Alzheimer's Disease and acetylcholine drop

As already discussed, acetylcholine and glutamate are two neurotransmitters that play a role in Alzheimer's disease. In the brains of Alzheimer's disease, levels of ACh (acetylcholine) can drop by up to 90 percent. The leads to the gradual death of cholinergic neurons. There must be another reason for this dramatic decrease.

Some research suggests that beta-amyloid plaques (in diagram) may be a reason for these very low levels of ACh because they *increase* the activity/speed of the enzyme acetylcholinesterase (AChE), which breaks down ACh (see page 13 again). Too much of the AChE enzyme has the overall effect of decreasing ACh levels, which contributes to the characteristic memory and language loss symptoms of Alzheimer's disease.

Drugs and substances that interrupt acetylcholine function have been investigated. See 'Treatment' later (page XX).

Extension notes

1. *Tau protein again*:
   As well as beta-amyloid plaques, neurofibrillary degeneration is also believed to be a cause for the dysfunction and death of cholinergic neurons. (Oh dear it is getting more complicated!)

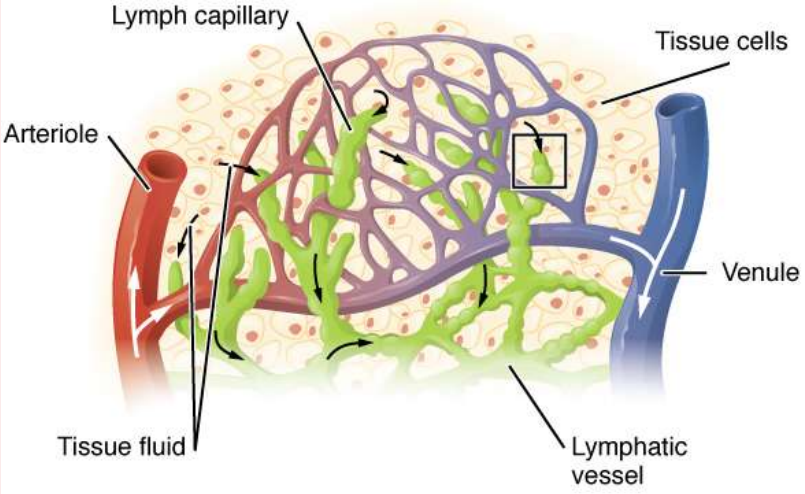
2. *Pesticides can affect acetylcholine function*:
   Many agricultural and household pesticides (including insecticides) combine with acetylcholinesterase (AChE) – the enzyme that breaks up excess acetylcholinesterase. This causes acetylcholine (ACh) to build up and leads to poisoning. The pesticides can enter the body through skin absorption, inhalation and on food. Signs and symptoms include tiredness, dizziness and blurred vision (in mild cases); headache, sweating, tearing, drooling, vomiting, (in moderate cases); abdominal cramps, diarrhoea, staggering walk, slow heartbeat, breathing difficulty, and possibly death (in severe cases, after continued daily absorption).

   You can learn more about cholinesterase and pesticides from the following website:
   http://pmep.cce.cornell.edu/profiles/extoxnet/TIB/cholinesterase.html

Clearance of Amyloid from the Brain

Introductory note: Clearance of waste from the body

The body's peripheral system (that is, everything except the brain) has a special system called the lymphatic system which works with the blood system to clear waste from the body.



The peripheral system

The *blood system* consists of a network of blood arteries and veins. Arteries carry oxygen and nutrients to all cells in the body. Veins carry some wastes (just smaller molecules such as CO_2) away from cells.

The lymphatic system is an extensive network of lymph vessels that traverse the body and contain a fluid called *lymph* to clear away from tissues toxins, waste and other unwanted materials, including larger molecules (e.g. proteins) and also bacteria and other pathogens too large to enter veins. These lymph vessels eventually empty their wastes into *large* veins which carry them to the liver and kidney for breakdown and elimination from the body. The cleared fluid then re-renters the blood system.

The brain

The brain produces metabolic waste at a higher rate than any other organ, making transport of waste substances out of the brain (waste clearance) a critical cerebral process. Waste clearance failure from the brain, including the removal of amyloid-β peptides (Aβ), is a central feature of neurodegenerative diseases.

The brain has a blood system but *not* a lymphatic system. How then does the brain remove wastes?

There is a waste removal system that appears to be unique to the brain. Various methods of this system are discussed below primarily focusing on the clearance of amyloid-β plaques.

Clearance of Amyloid Plaques from the Brain

Because the amyloid-β peptide (Aβ) is still best known as a molecule to causes Alzheimer's disease, there is a need to remove/clear it from the brain.

In the brain of a *normal healthy* person, the production of Aβ is usually counterbalanced by several processes which clear it from the brain and so can be cleared/removed before it can cause any damage.

But *impaired* Aβ clearance from the brain occurs in people with Alzheimer's disease. Even small defects in Aβ clearance can be sufficient to cause Aβ accumulation leading to cell toxicity. And many data clearly suggest that a decreased Aβ clearance is more responsible for the development of Alzheimer's disease rather than increased Aβ synthesis.

Five ways to remove Aβ

Under *normal* physiology, Aβ is cleared from the brain by *five* pathways. These include both enzymatic and non-enzymatic pathways.

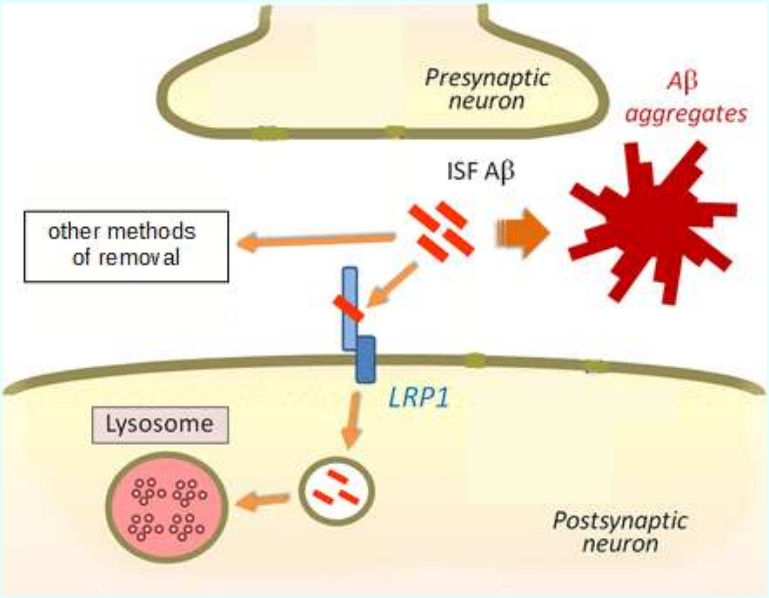
The five pathways are:

1. Destruction/degradation in postsynaptic neurons.
2. Proteolytic enzymes (i.e. degradation of proteins by enzymes).
3. Uptake by microglial cells.
4. Aβ transport across the blood-brain barrier (BBB).
5. Elimination via the glymphatic system.

The first four are more established methods. The fifth is relatively new and still controversial but promising. We now look at each of these in greater detail.

1. Destruction/degradation in postsynaptic neurons

The diagram (right) shows a model of neuronal Aβ clearance in post synapses. (Refer to an earlier similar diagram on page 32 on the *production* of Aβ). Aβ, as has already been explained, aggregates in extracellular space of the brain as oligomers then plaques (the diagram shows just plaques).



In the membranes of postsynaptic neurons are another kind of receptor known as LDL receptor-related protein 1 (**LRP1**). The diagram shows just *one* such receptor. They are endocytic receptors, that is, receptors that take substances, including Aβ, into the neuron (endo- = *inside*; -cytic = *cell*). Once there, the Aβ undergoes lysosomal degradation in lysosomes. The lysosomes break down the Aβ into smaller harmless fragments that are less neurotoxic and more easily cleared.

Note on lysosomes: A lysosome is a cell structure that contains digestive enzymes which break down excess or worn-out cell parts. Lysosomes may be used to destroy invading viruses and bacteria. If the cell is damaged beyond repair, lysosomes can even help it to self-destruct.

The LRP1 pathway is *very* important as any disturbance to it seems to be sufficient to induce Aβ accumulation in spite of it also being eliminated by the other methods.

Question: Aβ is formed *throughout* the neuron. But LRP1 are just in the post-synapses. Does Aβ from other parts of the neuron, such as the soma, drift to the postsynaptic area for clearance or is it cleared by other methods?

2. Destruction/degradation by proteolytic enzymes (proteases)

Terminology: Proteolytic enzymes (proteo- = *protein*, -lytic = *split/break*). Protease = an enzyme that breaks down proteins into smaller polypeptides or single amino acids. Proteases that break down Aβ are known as Aβ-degrading enzymes (ADE).

The enzymatic clearance involves several *proteases* present in the intercellular fluid. These enzymes cleave the full-length Aβ (e.g. Aβ42), producing smaller fragments that are less neurotoxic and more easily cleared (though some of the smaller fragments, such as Aβ22 to Aβ35, have similar toxicity and aggregation property as the full-length Aβ40 or Aβ42 forms!!).

Many Aβ-degrading enzymes (ADE) implicated in the clearance process have been identified in the brain. These include: neprilysin (also known as NEP) or insulysin (IDE), insulin degrading enzyme (IDE) (IDE degrades a broad range of substances, including insulin).

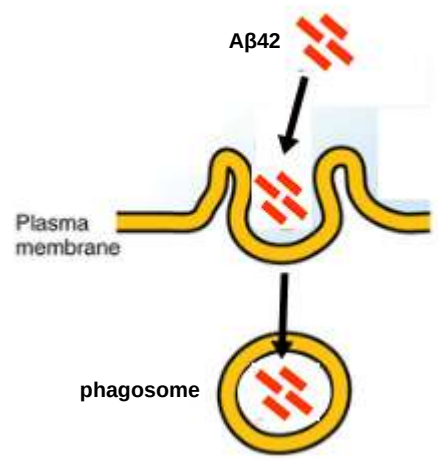
Over-production of NEP and/or IDE *decreases* Aβ level by around 90% and relieves amyloid pathology. But, *deletion* of NEP or treatment with an NEP inhibitor/blocker leads to *increased* levels of Aβ. Interestingly, mice that have had their brain neprilysin removed show both Alzheimer's-like behavioural impairment and amyloid-beta deposition in the brain, providing strong evidence for the association of amyloid-beta with the Alzheimer's disease.

3. Uptake by microglial cells

Glial cells include microglial cells and astrocytes. (Look back at pages 8 – 9.) Microglial cells are the immune cells of the brain – they patrol the brain and clear it from cellular waste products and debris to keep it healthy.

In the normal healthy brain: Microglia also play an essential role in clearance of Aβ through their ability to take up and degrade the *shorter* forms of Aβ by *phagocytosis* (phag- = to eat/devour + ~~o~~ + -cyt- = vessel/cell + -osis = abnormal condition).

Definition of phagocytosis: The process by which a cell uses its membrane to engulf/ingest a large particle or even a microorganism (such as a bacterium), giving rise to an internal compartment (called a phagosome). It is one type of endocytosis. (Digram, right)



The ingested material is then digested/broken up. As well as Aβ, bacteria, dead tissue cells, and small mineral particles are all examples of objects that may be phagocytised.

Astrocytes are then signalled to help clear the build-up of the excess debris left behind from the phagocytosis.

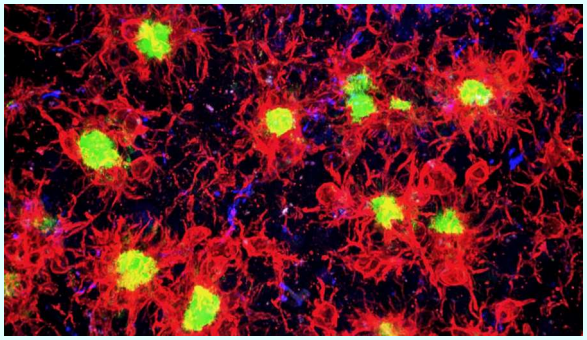
Clearing the Aβ thus prevents the formation of amyloid plaques in the brain. While this process does not ultimately prevent the progression of AD, it certainly does delay it.

In the already diseased/Alzheimer's brain: Here the news is not so good! As we age, microglia become steadily less efficient at the clearance process. Also, the microglia start to act differently. They tend to become over-activated in response to stimulation by the larger amounts of Aβ produced. Over-activated

microglia are a common feature of Alzheimer's disease. And even worse, after amyloid plaques begin forming, microglia fail to clear away the plaques.

Continual, prolonged microglia activation results in neuronal damage and even death of the neurons they are meant to protect.

Astrocytes are then signalled to help clear the build-up of plaques and other cellular debris left behind. These microglia and astrocytes collect around the neurons but fail to perform their debris-clearing function. In addition, they release chemicals that actually damage the neurons they are meant to protect.



In the image (right), microglia, shown in red, surround and engulf the amyloid-beta plaques, shown in green, in a brain with Alzheimer's disease. (Not easy to see the green parts, but they are in the yellow parts.)

Certainly not good news!

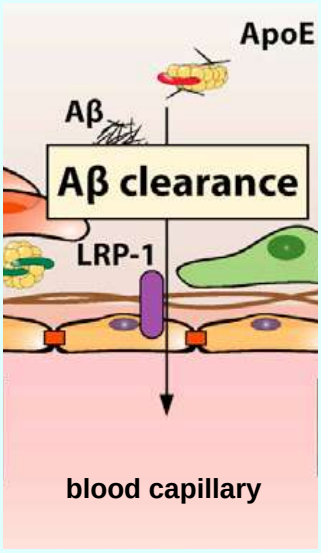
No microglia, no plaques!

Recently (2019), scientists investigating the effects of microglia on amyloid plaques, decided to see what would happen in the *absence* of microglia. With mice, they used a drug that blocks the action of microglia. This blocking essentially eliminates the microglia from the brain. They found that in areas without microglia, plaques *didn't* form! However, in places where microglia survived, plaques *did* develop. So, you don't have Alzheimer's without plaques!! So it seems that microglia are a necessary component in the *development* of Alzheimer's!! (But of course, no microglia also mean no removal of brain debris or pathogens, such as bacteria which would result in other diseases.)

(This is another example showing that we still have a long way to go in fully understanding Alzheimer's disease.)

4. Aβ transport across the BBB

Aβ can be transported out of the brain into the blood across the blood brain barrier (BBB). A little revision: The BBB, as already discussed, separates the circulating blood in the blood vessels from the liquids in the brain. The BBB is composed of thin, flat *endothelial* cells in the capillaries. The BBB also includes a thick basement membrane, smooth muscle cells and astrocytic end-feet. This barrier restricts the diffusion of most materials into and out of the brain.



The LRP1 receptor – the same one found in neuron synapses in Method 1 above – is also found in brain capillary endothelium cells and also plays a role in Aβ clearance by transporting from the brain to the blood *not* Aβ plaque, but the smaller, soluble oligomers of Aβ which are very neurotoxic.

To do this, the Aβ is first attached to a protein called ApoE (don't worry about the details of this); this then passes through the BBB via the LRP1 into the bloodstream for eventual degradation in the liver (see diagram on previous page).

Many studies have shown that dysfunction in the BBB contributes to the accumulation of neurotoxic materials in the brain, suggesting that this method of clearance is quite important.

Note: ApoE has a form called ApoE4, which can be inherited. ApoE4 also *slows* Aβ clearance across the BBB by shifting clearance from LRP1 to another receptor (called VLDLR), which has a much slower rate of removal. (ApoE4 also damages the tight junction of the BBB thereby disrupting the integrity of the barrier.)

5. Elimination via the glymphatic system

In the past, it was thought that the *majority* of extracellular Aβ was believed to be cleared by the BBB (Method 4 above). However, recent studies have suggested that the glymphatic system, involving the flow of cerebrospinal fluid (CSF) through the brain, contributes to a larger portion of extracellular Aβ clearance than previously considered.

As mentioned earlier (pages 15 – 16), there are four kinds of fluids in the brain:
1. Blood plasma.
2. Intracellular fluid (ICF), *inside* brain cells.
3. Interstitial fluid (ISF), *between* brain cells.
4. Cerebrospinal fluid (CSF).

The glymphatic system (also called the perivascular circulation) is a waste removal system unique to the brain. β-amyloid is one of the important wastes cleared by the glymphatic system. Interestingly, the glymphatic system functions mainly during sleep and is largely disengaged during wakefulness (more on this point later). Dysfunction of this glymphatic waste clearance system has been demonstrated in Alzheimer's disease.

What's in a word? Meaning of glymphatic:
glymphatic = g + lymphatic = glial + lymphatic
This movement of brain waste was entitled the glymphatic system based on its similarity to the lymphatic system in the peripheral tissue (that is, all parts of the body except the CNS, remember) and on the important role played by of glial cells (microglia and astrocytes) to clear away harmful debris.

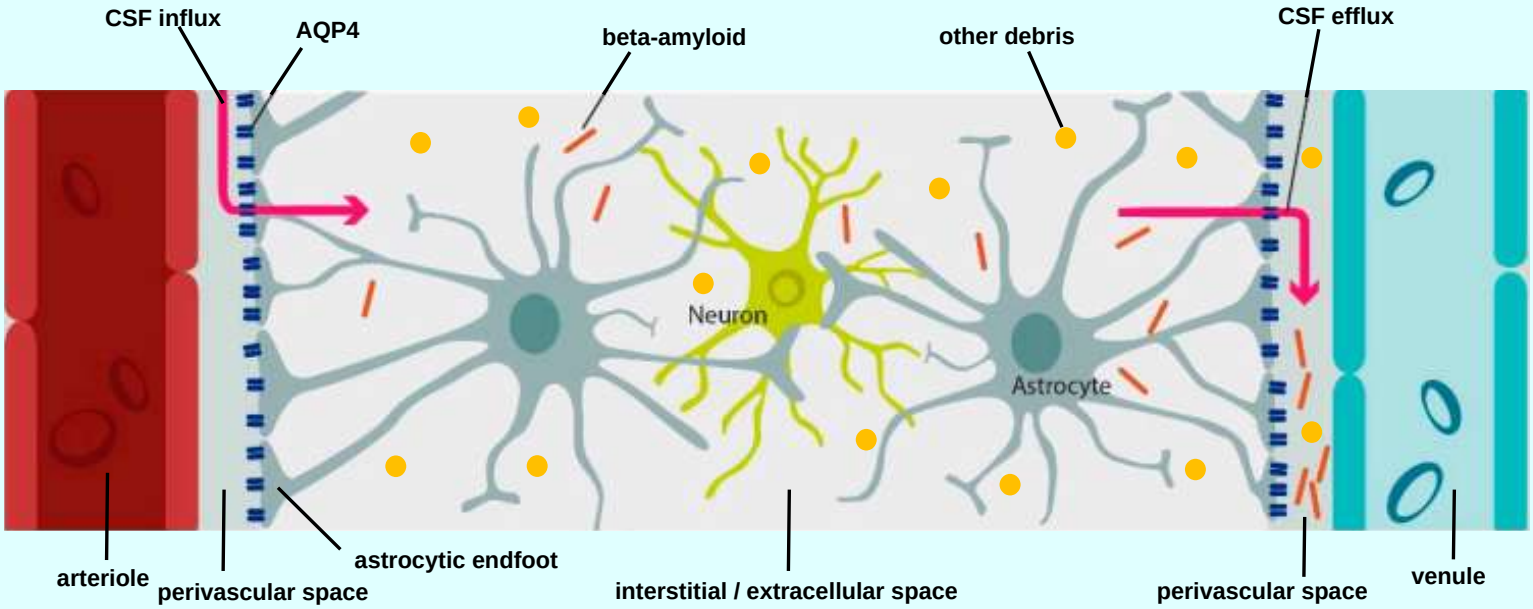
The hypothesised steps in the glymphatic system

Before proceeding, look back at the earlier discussion on the blood vessels in the outer brain, the perivascular space and the flow of CSF around the brain (pages 21ff.).

Summary of how the clearance happens:
1. CSF flows from the subarachnoid space of the outer brain into the perivascular spaces of the arterioles into the brain.
2. From the perivascular spaces in the arterioles, the fluid moves *into* the brain interstitial/extracellular space (also called the *interstitium*). This is facilitated by astrocytic endfeet comprising the perivascular wall.

3. The fluid flows across the interstitial space, dissolving or carrying waste molecules in the flow.
4. CSF efflux of wastes into the perivascular space of the venules for transport out of the brain.

Now in greater detail. As you read, refer to the diagram below.



Step 1: From the outer brain

From the subarachnoid space, CSF is driven into the perivascular (VR) spaces (partly by the pressure exerted by the heart contractions on the blood in the arteries).

Step 2: From the arteriole PVS to inside the brain parenchyma

The CSF then crosses the perivascular wall into the brain tissue. How this occurs in not known for certain, but does involve *gaps between* the endfeet of the astrocytes and also may involve small channels on the endfeet known as AQP4 channels (i.e. **Aq**uaporin **P**-4 channels) (see diagram). AQP4 are present *throughout* astrocytes but their density is greatest in the endfeet. They are known to constantly transport water between the PVS and the astrocyte cell, swelling or shrinking the endfeet, which adjusts the gap size between the endfeet. This is thought to promote or restrict CSF flow through the PVS. A decrease in the number of astrocytic endfeet (known as *AQP4 depolarization*) occurs in many brain injuries and may result in BBB breakdown. This may be particular relevant for the ageing brain and Alzheimer's disease.

Note on diagram above: The two red arrows suggest that the CSF influx and efflux goes *through* the AQP4 in the astrocytic endfeet. This is incorrect; they should be shown going through the *gaps between* them.

Step 3: Movement of CSF through the extracellular space

As cerebrospinal fluid flows through the brain, it collects beta-amyloid and other debris (small coloured bars and dots) produced by brain cells. The movement of these substances probably occurs by two mechanisms: diffusion and advection. Diffusion is always occurring in a fluid. (Diffusion, as you probably already know, is the movement of a substance from an area of high concentration to an area of low concentration, such as happens when salt or sugar dissolve in water.) Advection is the transport of substances by bulk flow which requires an external driving force, probably the 'push' from the CSF that enters. However, some researchers claim that diffusion alone is adequate to describe transport in the interstitial/extracellular space.

Notes:

1. Bulk flow is the movement of substances in large amounts carried long in a liquid; the blood circulation is an example. The transport of solids by the flow of a river is another example of advection (picture).
2. If you read articles (online) on this topic, you may come cross the term convection. In the field of brain transport, convection is the combination of both diffusion *and* advection.

Step 4: From the brain parenchyma out to the venule PVS

The CSF carries the interstitial wastes from the brain parenchyma into the perivascular space around the venules (small veins). From there, the wastes are transported out of the brain via drainage pathways in the sinuses of the dura mater and into the lymphatic vessels for removal of the wastes from the body. (Look back at page 23 for sinuses of the dura mater.)

Glymphatic activity decreases sharply during ageing

There appears to be an age-related decline in glymphatic function in older brains and especially people with late-stage AD. Also, the number of AQP4 channels in the astrocytic endfeet *decreases* with age. This suggests that there is a decline in glymphatic function, which might be in part be attributable to a dysregulation of water transport, which occurs through the AQP4 channels.

Because the glymphatic system removes beta-amyloid, the failure of the glymphatic system in ageing might contribute to an accumulation of hyperphosphorylated proteins and tau tangles resulting in a viscous circle perhaps escalating the progression of Alzheimer's disease and cognitive dysfunction.

The glymphatic system: Some unanswered questions

The glymphatic hypothesis leaves a few questions unanswered:

1. What actually drives advective flow in the arteriole perivascular space?
2. What provides the pressure for flow across the interstitium? It remains unclear if transport in the interstitium is diffusion-only transport or a combination of advective and diffusive transport.
3. What is the precise role of the AQP4 water channels in the glymphatic system?

Sleep, Exercise and Alzheimer's Disease

Here, we will link some of the ideas from the last few pages related to Alzheimer's disease with the two lifestyle factors of sleep and exercise.

Sleep and memory

It is well-known that sleep enhances memory consolidation/storage, that is, long-lasting memories. This consolidation occurs during the so-called deep sleep (during the third and fourth stages of the five stages of the sleep cycle). Deep sleep is needed in order to feel refreshed on waking up in the morning.

Memory formation and maintaining memories in the brain is due to LTP, which increases the strength of the synapses between the neurons. And as discussed earlier (pages 11ff.), both NMDA and acetylcholine receptors are involved in the process. Research has also shown that substances that block NMDA receptors appear to prevent LTP that would result in the enhancement of memories.

Sleep triggers the LTP process causing the synapses in the brain to strengthen, which prompts the strengthening or modification of memories or prompt the emergence of new ones.

People with Alzheimer's should try to get a good sleep to help preserve remaining memories and perhaps slow down the loss of memory, which is characteristic of Alzheimer's. Further, sleep helps as it clears wastes from the brain that cause Alzheimer's (see next).

Sleep and clearance of brain debris

Sleep also plays an important role in the removal of wastes from the brain.

We saw earlier that β-amyloid is one of the important wastes cleared by the glymphatic clearance system and that the activity of this system *increases* during sleep. In the sleep state the glymphatic activity is dramatically enhanced, but is suppressed when *awake*. Experiments have shown the CSF influx in the *awake* state is reduced by 90% (at least in mice!).

The production of beta-amyloid and other debris when neuronal activity is highest. While asleep, the brain clears itself of these (and other) neurotoxic waste products produced during wakefulness, and a good sleep can ward off Alzheimer's. (This particular proposal has become mainstream in popular culture!!!)

And the more you use your brain during the day, the more it needs to rest while asleep. Also women tend to use more of their actual brain than men leading to a greater need for high-quality deep sleep.

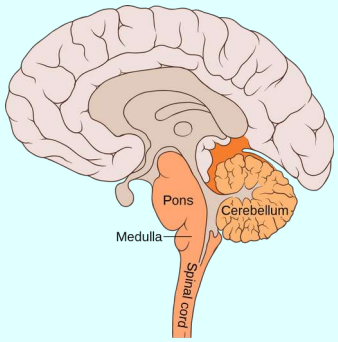
It has also been discovered that people who who not get this deep sleep have higher levels of the brain tau tangles which, as we have seen, are linked to brain damage and cognitive decline.

REM sleep and sleep disorders

Rapid eye movement sleep (REM sleep)

REM sleep is the fifth and last stage of the sleep cycle. *Normal* REM sleep is distinguished by random/rapid movement of the eyes, accompanied with *less* muscle movement (even paralysis) of the rest of the body's muscles and the propensity of the sleeper to dream vividly.

REM Sleep *Disorder* is a disease that occurs during the REM sleep stage. People who have REM Sleep Disorder still have rapid eye movement but lose the skeletal/muscle paralysis, which enables their muscles to physically act out their dreams. Punching, kicking, leaping, and running from the bed during dream enactment are frequent manifestations and usually correspond with what the person is dreaming.



REM sleep and sleep disorder occur in an area located at the back of the brain stem called the pons (picture, right).

In 1965, two French scientists found that removing the pons from a cat's brain prevented it becoming paralysed when in REM. Instead of lying still, the cats walked around and behaved aggressively due to the lack of muscle paralysis. Other cats showed movements identical to actual predatory attacks, as if they were chasing mice in their dreams. Similar dream activity has been seen in dogs.

Research has found that seniors who have sleep REM Sleep Disorder are more likely to develop Alzheimer's and have a faster rate of cognitive decline than those who had healthy sleep habits.

Acetylcholine and its receptors cause normal REM sleep and sleep disorder

Acetylcholine is involved in *promoting* REM sleep, and also plays a large role in the *sleep disorder* mentioned above. Remember that acetylcholine is also found in all *motor* neurons where it stimulates muscles to contract. From the movements of the stomach and heart to eye movements, all of the body's movements involve the actions of this important neurotransmitter.

For *normal* REM sleep, it is the acetylcholine and the activation of its acetylcholine receptors that produces rapid eye movement, and skeletal paralysis. During this REM sleep, the release of acetylcholine is significantly higher than waking levels.

The reasons for REM sleep *disorder* are *less* well understood but are thought to arise from damage to or degeneration of parts of the brain stem that cause REM sleep paralysis thus releasing neurons from their normal source of inhibition.

The onset of Alzheimer's disease happens when some regions of the brain have depleted or very low levels of acetylcholine. Loss of these cholinergic neurons (i.e. neurons with acetylcholine/cholinergic receptors) has been hypothesised to cause the cognitive deterioration observed in Alzheimer's disease.

Sleep, Alzheimer's and prominent personalities

With ageing, sleeping pattern alter. Elderly folk often wake unrested because their sleep time and sleep efficacy is reduced. In Alzheimer's disease patients, these alterations are even more pronounced and may further aggravate cognitive decline. Re-establishing an effective sleep-wake cycle in these patients still remains an unresolved challenge, partly because sleep physiology is quite complex.

Margaret Thatcher (1925-2013) (pictured, right), was British PM from 1975 to 1990. She died in 2013 at age 87 from Alzheimer's, having first showed signs of the illness aged 75.

She is famously said to have slept for only four hours a night. But did her lack of sleep cause her Alzheimer's? It has also been commented that the intense mental stimulation of being Prime Minister may have protected Thatcher from the earlier onset of dementia.

Contrast Margaret Thatcher with Ronald Reagan (1911-2004), US President from 1981 to 1989. In August 1994, at the age of 83, Reagan was diagnosed with Alzheimer's disease, and died from the disease in 2004 at age 93. Reagan, in contrast to Thatcher seemed to get plenty of sleep and was famous for his love of midday naps (though some family members denied that he napped in the White House). But he still developed Alzheimer's.

Their Alzheimer's may have been just due to old age rather than lack of sleep!

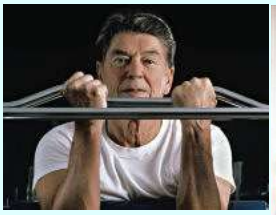
Extension: Tau, more than Aβ, affects sleep early in Alzheimer's
Mounting evidence suggests a link between β-amyloid and *disrupted* sleep. Does the same hold true for tau? Yes, according to recent research. If interested, open the following link:
https://www.alzforum.org/news/research-news/tau-more-av-affects-sleep-early-alzheimers

Exercise and Alzheimer's disease

We have discussed five method for clearing amyloid plaques from the brain. Studies published in 2018 and 2020 demonstrated that exercise, and especially cardiorespiratory exercise – walking briskly, running, biking, swimming and just about any other exercise that gets your heart pumping – is not only good for the body, but for brain health, particularly in grey matter and total brain volume – regions of the brain involved with cognitive decline and ageing and involved in Alzheimer's disease.

Exercise helps clear away harmful debris in the brain. Although the brain takes up only 2 percent of the body's mass, it uses about 20 percent of the body's total energy in order to work efficiently. And when brain neurons consume high amounts of energy, they spit out a lot of debris, including amyloid beta, that floats around the brain.

So then, exercise along with a good sleep, may be just what the brain needs to clear away debris and function at its best. Maybe, but compare Thatcher and Reagan again.



Contrasting Thatcher and Reagan again: I could not find anything to suggest that Margaret Thatcher ever did much, if any, exercise. But she was known *not* to be a lover of sport. Ronald Reagan, on the other hand, was a fitness nut. He had exercised extensively throughout his life and was a lifeguard during his teenage summers. Even while in office, he continued to exercise. But, in spite of all the exercise, he still developed Alzheimer's!

So:

- Thatcher – very little sleep + little exercise → developed Alzheimer's!
- Reagan – a lot of sleep + a lot of exercise → developed Alzheimer's!

Maybe they both got Alzheimer's because they were old. Or in the case of Reagan, who died from Alzheimer's six years later than Thatcher, perhaps exercise did help. But that is just surmising; it is very difficult to draw conclusions based on so little information.

'How to Stay Fit: The President's Personal Exercise Program'

In 1983, Reagan wrote an article about his fitness regime emphasising the importance of a healthy lifestyle. If you would like to learn more about this, go to the following link:

https://parade.com/116185/ronaldreagan/16-reagan-fitness/


The President's personal exercise program

How To Stay Fit

BY RONALD REAGAN

Treatment of Alzheimer's Disease

Despite many years of research and the expenditure of billions of dollars, no drugs for Alzheimer's disease have been able to cure or to reverse the progression of Alzheimer's disease. Existing Alzheimer's disease drugs only help *lessen* or stabilise the symptoms of Alzheimer's disease for a limited time; they are unable to stop or reverse disease progression. Despite intensive research efforts towards AD over several decades, researchers have *not* successfully translated promising disease-modifying strategies identified in laboratories into clinical application.

Only a few of the many drugs developed to improve the symptoms of Alzheimer's disease have actually been approved by the (US) FDA. These include the following:

- Four so-called acetylcholinesterase inhibitors (AChEi) – tacrine (approved in 1993), donepezil (approved in 1996), rivastigmine (approved in 2007), and galantamine (approved in 2001). These drugs act to enhance/improve the action of acetylcholine receptors, which become less effective in old age or with Alzheimer's disease. Note: Rivastigmine has a *dual* effect, being also an alpha-secretase inhibitor (see below) as well as an acetylcholinesterase inhibitor.
- Memantine, which involves the NMDA receptor, was approved in the US in 2003. Memntine and several similar drugs are discussed in this section.
- The last drug (I think) approved by the Food and Drug Administration for therapeutic Alzheimer's disease treatment was a combined drug named namzaric in 2014 (see page 62).

Any effective treatment should affect those processes which mainly contribute to the neuron decay and Alzheimer's disease. Current research and treatments target the following areas:

- (A) Preventing the formation of Aβ monomers from amyloid precursor protein (APP).
- (B) Inhibition of Aβ changing to plaque.
- (C) NFTs – Treatments involving tau.
- (D) Treatments for receptors that are linked with Alzheimer's disease.
- (E) Lifestyle factors.

Here, we will discuss some of the research and potential treatments to illustrate the different approaches, together with some of the failures! As you read, note that many treatments come from substances found in plants.

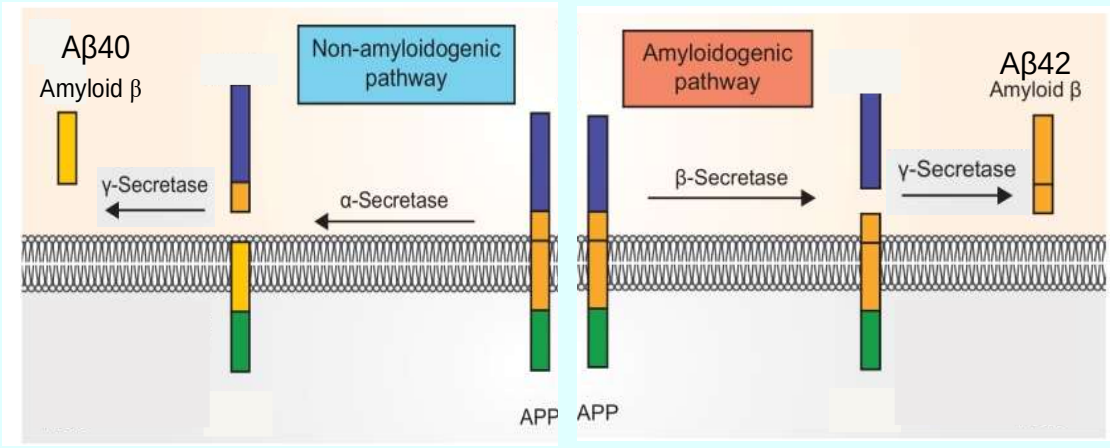
(A) Preventing the formation of Aβ monomers from amyloid precursor protein (APP)

The amyloid precursor protein (APP) is where it all begins as this involves the *formation* of beta-amyloid.

In the *non-amyloidogenic* pathway, which is the *normal* and *non*-harmful process, *alpha* secretase and *gamma* secretase are the two enzymes involved, and form beta-amyloid from APP, especially the non-harmful (or less harmful) Aβ40. (Look back at pages 31 – 32.)

In the *amyloidogenic* pathway, which is the *abnormal* and more *harmful* process, *beta* secretase and *gamma* secretase, the two enzymes involved, form beta-amyloid from APP, including the Aβ40 monomer and the very toxic and harmful Aβ42 monomer.

Revision of the two pathways:



If the enzymes that form beta-amyloids from APP are inhibited (stopped from working), so the argument goes, no Aβ is formed and Alzheimer's disease might be prevented. So, drug companies have tried to make drugs that inhibit beta-secretase and gamma-secretase, two secretases involved in these processes.

- Beta-secretase (BACE) initiates the production of Aβ42 in the *amyloidogenic* pathway, so its inhibition provides a valid target for the AD. Making beta-secretase inhibitors has been tough. Part of the problem has been to get them across the blood-brain barrier from the blood into the brain. As of 2018, several are in different phases of clinical trials. However, one was discontinued due to lack of finding a positive clinical effect.

- Gamma-secretase inhibitor/blocker. Gamma secretase is involved in *both* pathways, so blocking it should prevent the formation of *both* Aβ40 and Aβ42. One drug, called semagacestat was designed to reduce both Aβ40 and Aβ42 production by blocking the enzyme γ-secretase. But when tested on people, it performed worse than the placebo, which led to the trials being stopped. But scientists found that some gamma-secretase inhibitors, *including* semagacestat, do not function as *true* beta-amyloid inhibitors, but instead cause an *accumulation* of the toxic beta-amyloid monomers inside neurons! Another problem is that gamma-secretase acts on lots of other proteins in the brain besides APP, so blocking it will have bad effects on the entire brain.

- Alpha-secretase *stimulator*. There is also the enzyme called alpha-secretase, which is the first enzyme involved in the *non*-amyloidogenic pathway, which is the *normal* pathway and forms the *non*-harmful (or perhaps less harmful) Aβ40. So, using a drug that both *increases/stimulates* the action of this enzyme and at the same time decreases the action of beta-secretase which forms harmful Aβ42, should increase the amount of 'good' beta-amyloid and decrease the amount of 'bad' beta-amyloid. One alpha-secretase stimulator that seems to work is rivastigmine (sold under the trade name *Exelon* among others; pictured, right). It directs APP processing *away* from beta-secretase and *towards* α-secretase. Investigations (reported in 2020) have been done with rats and mice and in human postmortem (i.e. after death) brain tissue studies. It does seem to work but has some side effects including abdominal pain, diarrhoea, dizziness, headache, nausea, vomiting, weight loss, and anorexia.

Natural beta-secretase inhibitors

Alternative therapies for AD sourced from natural products are being investigated. One study (reported in 2019) focused on three substances (called flavonoids) from *Boesenbergia rotunda*, commonly known as *Chinese keys*, *Finger root* or *Chinese ginger*, a medicinal and culinary herb from China and Southeast Asia. In English, the root has traditionally been called *Finger root*, because the shape of the root resembles that of fingers growing out of a centre piece (pictured).

The results of the study showed that one substance in the root led to quite strong beta-secretase inhibition. This provides the first evidence that these flavonoids from *B. rotunda* may be considered as promising AD preventative agents through inhibition of Aβ formation. But read the caveat below!

Caveat: Inhibiting the action of beta-secretase (BACE) shifts the balance from the harmful amyloidogenic pathway towards the non-harmful *non*-amyloidogenic pathway. But no effective intervention based on this approach has been developed, apart from rivastigmine which is still under investigation. Also, it is possible that having only the *non*-amyloidogenic pathway might also disturb neuron function or cause other problems. And unfortunately, some studies have shown that activating/increasing the action of alpha-secretase can promote cancer. So the patient would go from the frying pan into the fire! So, we will have to wait and see if rivastigmine succeeds.

(B) Inhibition of Aβ changing to plaque

Early treatments for Alzheimer's investigated, naturally enough, the prevention of amyloid plaques. But we now know that *plaques are not the major problem*. It has been known for a couple of decades that amyloid plaques don't correlate with cognitive impairment. The amyloid *oligomers* are a greater problem. So investigations switched from to trying to prevent *plaque* formation to preventing Aβ monomers changing into oligomers. No oligomers should, of course, mean no subsequent plaque. (Remember: Soluble amyloid oligomers, rather than insoluble fibrils and plaques, are the neurotoxic forms of amyloid responsible for the onset and progression of AD.)

Several drugs have been designed to target amyloid oligomers, but none has yet really succeeded in improving outcomes for people living with Alzheimer's disease. Here we discuss some drugs to inhibit Aβ monomers changing to oligomers and how well they have fared.

Tramiprosate

Taurine is an amino acid found naturally in some seaweeds (pictured). A modified form of this amino acid is known as *tramiprosate*.

Tramiprosate acts by enveloping the Aß42 protein monomers thereby preventing them from changing into the toxic soluble amyloid oligomers (and subsequently into plaques).

Initially, this drug did not seem to be effective, but a study in cognitive impairment done in 2018 *did* show positive benefits. Tramiprosate reduced both Aβ40 and Aβ42 monomer fractions in the brain by 25

to 30% (and also caused a 30% reduction in brain plaque deposition formed from the monomers that remained).

It is also thought that tramiprosate can also reduce the phosphorylation of tau through inhibition of the relevant kinase (see later).

The seaweed is now promoted as a herbal remedy (but not a cure!) for Alzheimer's disease. There are claims, but no conclusive evidence, that it lowers neuron death and loss, and also increases the number of healthy neurons inside the brain.

China Approves 'Seaweed Drug' for Treatment of Alzheimer's

In 2019, China awarded conditional approval to a seaweed-based drug for treating Alzheimer's disease.

The approval of the seaweed drug, called *Oligomannate* (pictured, right) however is preliminary. It is only allowed in China and could be pulled during continuing safety trials. The researchers identified a unique sugar in the seaweed which *might* account for the relatively low occurrence of Alzheimer's among elderly people who regularly consumed the plant. The second picture shows tablets of the new drug made from this sugar in the seaweed.

For more on this subject, visit the websites below:
https://www.beingpatient.com/seaweed-drug-china/
https://www.inkstonenews.com/science/alzheimers-drug-oligomannate-approved-china/article/3036238

Solanezumab

As mentioned, it is the beta-amyloid *oligomers* that can kill neurons and not the plaques. Solanezumab is an *antibody* drug against soluble Aβ oligomers thus to preventing plaque formation. The drug is for people with *mild* cognitive impairment caused by Alzheimer's disease.

Definition: *An antibody is a protein produced mainly by white blood cells in the blood that is used by the immune system to destroy disease-causing agents, especially harmful substances (such as Aβ oligomers) and harmful bacteria and viruses* (and also tau, as we see below).

The drug is orally administered and so enters the bloodstream and then the brain. It binds to individual amyloid oligomers and carries them away (in the CSF, I think). Early trials failed. One possible reason is because antibodies are generally far too big to actually cross the BBB and get into the brain.

More encouraging (perhaps!) were results from two other solanezumab studies which suggested some mental decline in AD patients with *milder disease* but fell short of clinically significant improvement (but patients with milder AD do have fewer functional deficits anyway).

But another study is underway. It is using participants who do not have memory loss but have evidence of amyloid beta build-up in the brain. This study aims to assess whether solanezumab can slow the progression of memory problems associated with beta-amyloids. The estimated completion date of this study is July 2022. (In early 2020, it was announced that solanezumab had failed in a trial of patients with dominantly inherited Alzheimer's disease. But whether this is the same study I cannot determine.)

Curcumin

Curcumin is another natural substance, a bright yellow chemical found in some plants (pictured, right). It is the principal substance in **turmeric** (*Curcuma longa,* pictured below right), a member of the ginger family. It is sold as a herbal supplement, as a cosmetics ingredient, a food flavouring, as a food colouring and is a mainstay of traditional Chinese and Indian herbal medicines.

Curcumin has been shown to inhibit the formation of plaques from oligomers (and even to break down amyloid-beta *plaques* that are already there), at least in *mice*. Mice that consumed curcumin-based diets for five months had lower levels of amyloid beta protein – a hallmark of Alzheimer's disease – in their brains compared with controls, one study showed. Similar results from earlier research in 2008 at the Chinese University of Hong Kong (university crest shown, left) found that a food supplement containing curcumin helped prevent build-up of these amyloid plaques.

But similar results have *not* been found in *human* clinical studies most likely because of curcumin's relatively low solubility and bioavailability. [*Bioavailability is the extent to which a substance is available where it is needed in the body, in this case in the brain.*]

However, a fresh approach treating healthy and mild AD people, combined with new curcumin formulations that increase bioavailability in the brain is renewing optimism concerning curcumin-based therapy.

Another feature of curcumin is that it seems to *kick-start the cleansing process* to remove beta-amyloid from the brain that otherwise can take up to 18 hours of fasting for the body to begin by itself.

Notes:

1. Other animal studies have suggested that curcumin can reduce tau protein clumping in the brain, slowing cognitive deterioration. Such clumping of tau protein leads to Alzheimer's disease in humans.
2. Curcumin has also been shown to influence acetylcholinesterase activities (see below).

Aducanumab

Aducanumab, like solanezumab, is an antibody but as well as targeting oligomers, also targets beta-amyloid *fibrils*.

A study in 2016(?) showed that people who received the drug had lower levels of amyloid in the brain and a slower decline in memory and thinking skills. Further, those who received higher amounts of the drug and those who took it for a longer time (three years) showed the strongest signs of a benefit.

Further trials are continuing.

Caveats on the use of Aβ oligomer inhibitors: The use of methods such as those above (even if they work!) might *not* be a good approach as newer research showing that Aβ – both monomers and oligomers – can be *protective* as well as harmful. However, the protective effects of Aβ have been little studied. (Most work still considers Aβ as fundamentally bad!).

For example, Aβ monomers and oligomers seem to help in stimulating learning and memory (both short- and long term-memory), act as antioxidants (substances that can protect against cell damage) and reduce the neuron death and calcium ion entry induced by NMDA receptor activation.

This protection may even exist throughout advanced stages of AD so getting rid of it may only make the disease worse. Research into this is ongoing.

Also, there are a *variety* of Aβ species besides Aβ42 and Aβ40 coexisting in the synapses of neurons in the AD brain. It is possible that this mixture of Aβ species that might produce a variety of biological reactions some of which may be beneficial and some harmful. We just don't know yet.

(C) NFTs – Treatments involving tau

Decades of focus on the treatment of beta-amyloid have failed to significantly help patients, as the above examples show. So some researchers are turning more attention from beta-amyloid to the second member of the Alzheimer's duo – tau.

From earlier (pages 33ff.), we saw that the formation of neurofibrillary tangles (NFTs) and its precursor tau oligomers are also hypothesised to cause of neuronal degeneration and death. NFTs consist of hyperphosphorylated tau protein and this reduces microtubule binding causing them to disintegrate.

There are now at least 20 compounds against tau in clinical trials, including nine antibodies (some discontinued) and two vaccine candidates. But no one knows for sure if all this is going to work. Initially, potential anti-tau therapies were based mainly on *inhibition* of kinases or tau aggregation into filaments, or on stabilisation of microtubules, but most of these approaches have been discontinued because of toxicity and/or lack of efficacy.

Here we discuss some such treatments and how they have fared.

Methylene blue

Methylene blue has a history of diverse medical applications stretching over a century. It is used as a dye or staining agent to make certain body fluids and tissues easier to view. The picture (right) shows human cheek cells stained with methylene blue.

Methylene blue (and modified forms) *is* able to cross the blood-brain barrier (BBB), and once in the brain seems to break down the tau *filaments* and so block or reduce the aggregation of tau protein and formation of NFTs.

One such *modified* methylene blue tau aggregation inhibitor is *hydromethylthionine* taken in tablet form (pictured), which is *better* absorbed than methylene blue itself. It has been tested in clinical trials in *mild-to-moderate* Alzheimer's disease and produces reductions in cognitive decline and brain atrophy. While the drug can partially prevent the *accumulation* of tau filaments into tangles, it does *not* remove *existing* neurofibrillary tangles from the brain.

It also seems best to treat patients with this drug during the *early* stages of Alzheimer's. This is because once tau accumulation reaches a certain threshold, Alzheimer's disease is probably inevitable.

Hydromethylthionine seems to be the best hope right now for a disease-modifying drug acting on the tau pathology associated with Alzheimer's disease.

Note: Methylene blue also seems to play a role in decreasing amyloid plaques, which derive from the aggregation of amyloid-β. So, it could have a double benefit.

Davunetide (NAP)

Read again the comment on page 36 on the neuroprotection provided by NAP, which helps prevent tau protein separating from microtubules and forming NFTs. A drug, known commercially as *davunetide* and made using the NAP peptide was shown to improve memory performance in Alzheimer's patients by acting in the same way as the natural NAP to block the formation of the harmful neurofibrillary filaments/tangles. Drug delivery methods include injections, and as a tablet (pictured).

Trials with mice have reported beneficial effects. However, clinical development of davunetide in *humans* was halted after it demonstrated negative results, though research with it still continues.

Note: Beneficial effects of the drug in mice for two other kinds of dementia – *amyotrophic lateral sclerosis* (ALS) and *Parkinson's disease* (PD) – have also been reported as well as for Alzheimer's.

Another caveat

Some scientists think that the Davunetide approach using NAP may be better than trying to attack the tau tangles (NFT) themselves, which might actually be detrimental for brain cells! Many neurons that already have tangles inside them are still alive, even though other nearby neurons have been killed off. Targeting the tangles might kill these still functioning neurons. So a better approach may be to prevent the tau from forming tangles in the first place which the drug davunetide does.

Any effective tau treatment then may need to be delivered *before* the tau filaments develop. The main problem in deciding which anti-tau strategy to adopt is that scientists do not yet fully understand the tau protein very well.

Phosphorylation inhibitors

NFTs consist of hyperphosphorylated tau protein this and tau hyperphosphorylation reduces microtubule binding. This hyperphosphorylation is caused by several protein **kinases**. In one recent study (2018), several kinase inhibitors were developed to prevent this tau hyperphosphorylation. Several such inhibitors have been investigated but either did not reach clinical trials due to toxic effects or showed disappointing results. The presently used phosphorylation inhibitor drugs in AD therapy showed a temporary benefit only in the *early* stage of the disease. A cure of AD is not possible with these drugs, as they allow only a treatment of symptoms. Still, this does seem to be promising approach.

So, most of the approaches so far focusing on Aβ amyloid and tau protein have been disappointing in halting or reducing the effects of Alzheimer's disease. A large part of the problem is that while much is known bout the causes of Alzheimer's disease, much is *still* not understood. What we really need is a better theory!!!

(D) Treatments for receptors that are linked with Alzheimer's Disease

Refer to receptors discussed earlier (pages 11ff.).

Here, we will look at treatments for the following two kinds of receptors involved in Alzheimer's disease which were discussed earlier:

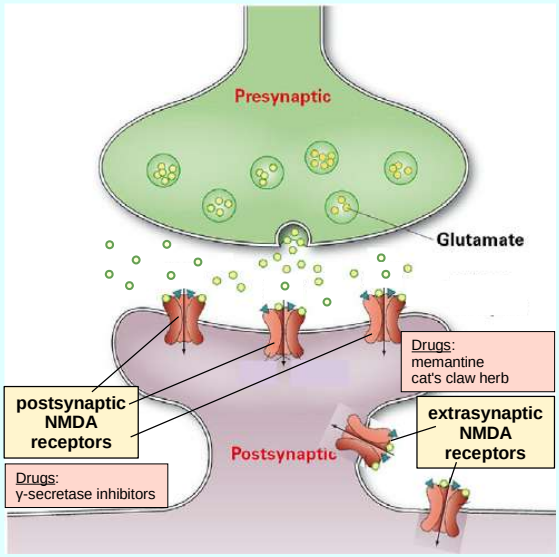
1. NMDA-receptors, and
2. Acetylcholine receptors

Many current medications for AD attempt to alter the action of these two receptors and their synapses. Accumulating evidence suggests that the loss of neuron synapses occurs in the *early* stage of AD and is associated with cognitive decline. Therefore, targeting the factors that affect the synapses might stop or reverse the disease progression.

1. NMDA-receptors

From earlier (pages 38 – XX39 Aβ42 oligomers lead to (a) the *endocytosis* of postsynaptic NMDA receptors, and (b) the production of excess glutamate which results in the *excitotoxicity* of extrasynaptic NMDA receptors. From these effects, there are two approaches to treatment:

(a) Preventing Aβ42 from forming so protecting the postsynaptic neurons, and
(b) Use of NMDA-receptor antagonists to save the extrasynaptic neurons.



Just as a *reminder* as to where these receptors are located, the diagram (right) has been included together with the names of drugs used for treatment at these receptors.

Note: Remember that there are several Aβ species ranging Aβ37 to Aβ49 but the precise Aβ species that target specific types of synapses remain unknown (but is usually assumed to be Aβ42.)

(a) Preventing Aβ42 from forming

Aβ42 oligomers cause *endocytosis* of the *postsynaptic* NMDA receptors. So reducing amyloid-beta oligomers should prevent this from happening.

Reducing the amyloid-beta oligomers is usually done by by treating neurons with a gamma-secretase inhibitor which prevents APP from forming both Aβ42 and Aβ40. One such γ-secretase inhibitor, mentioned earlier, is semagacestat, though it did not fare well in trials on people.

Another γ-secretase inhibitor is DAPT. [For readers with a (very good) chemistry background, DAPT stands for N-[N-(3,5-**D**ifluorophenacetyl-L-**a**lanyl)]-S-**p**henylglycine **t**butyl ester.] In trials, DAPT was found to reduce the levels of beta-amyloid in both mice and humans that have Alzheimer's disease. To date, the clinical experience in humans with AD is that long-term γ-secretase inhibitor treatment designed to produce moderate levels of inhibition of γ-secretase is associated with unacceptable side-effects and dangers (look back at page 53 again for one such danger).

Thus, unless there is an unanticipated breakthrough, γ-secretase inhibition is not likely to be a viable chronic treatment strategy for AD. Furthermore because of these safety issues, it is almost certain that γ-secretase inhibitors will not be suitable for testing in individuals who do not show symptoms but are at risk for AD.

(b) Use of NMDA-receptor antagonists

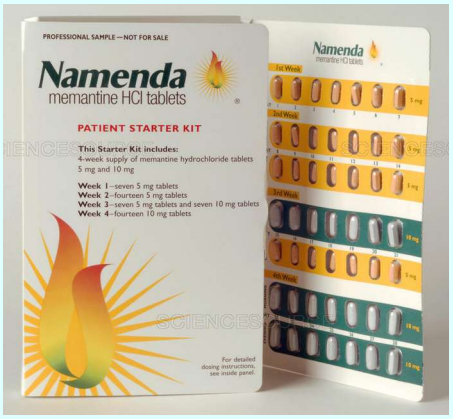
Definitions: *An antagonist is a molecule that blocks the effect that the neurotransmitter normally has on the post-synaptic neuron. Compare a neurotransmitter's agonist which is a molecule that has the same effect on the postsynaptic neuron as the neurotransmitter itself does.*

Glutamate is the neurotransmitter for NMDA receptors. As already mentioned (page 12), in the normal brain, astrocytes recycle unused glutamate neurotransmitter molecules. In the brains of patients with Alzheimer disease, Aβ42 oligomers are elevated and these oligomers *block the recycling process*. Hence excess glutamate accumulates, which is harmful because they overstimulate healthy **extrasynaptic** NMDA receptors making the glutamate toxic to the neurons causing them to become damaged or to die. (This is the phenomenon called *excitotoxicity* mentioned earlier on page 39).

Neuron loss due to glutamate-induced excitotoxicity might therefore be preventable if the NMDARs were partially inhibited/blocked to prevent the action of excess glutamate molecules. Experiments show that this partial blocking could be done using NMDAR antagonists.

Memantine

[Trade name *Namenda* – see picture.]



Memantine is an NMDA receptor antagonist medication to treat *moderate-to-severe* Alzheimer's disease. It was approved for medical use in the United States in 2003. (Recently, an improved derivative of memantine, called *nitromemantine*, has been introduced.)

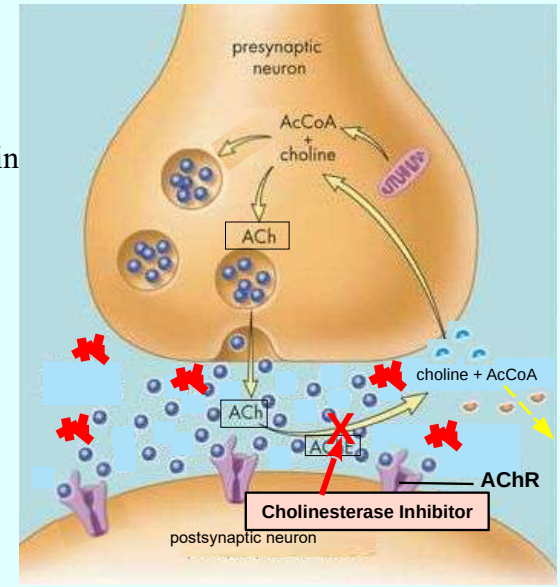
Memantine is believed to work by *partially* blocking NMDA receptors, primarily the **extrasynaptic** rather than the postsynaptic NMDA receptors. This partial blocking *prevents over-activation* of the receptors and the influx of too many calcium ions (Ca^{2+}), while still allowing the transmission of sufficient signals crucial for memory and learning processes. This seems sufficient to prevent neuron excitotoxicity and results in a modest improvement in cognition, mood, behaviour, and the ability to perform daily activities in *moderate-to-severe* Alzheimer's disease. However, there does *not* appear to be any benefit in people with *mild* Alzheimer's disease; they must wait until their condition worsens slightly.

A natural alternative to memantine: Chinese medicinal herbs have shown promising results regarding their ability to target glutamate receptor dysfunction in AD. A key substance in the Chinese medicinal herb *Uncaria rhynchophylla* (pictured, right; English name – *cat's claw herb*), suppresses the Aβ-induced glutamate over-activation of extrasynaptic NMDA receptors and so restores the impaired LTP and spatial memory in AD (at least in mice).

2. Acetylcholine receptors

Remember how the enzyme acetylcholinesterase (AChE) breaks up excess acetylcholine neurotransmitter (ACh) *very rapidly*, which is normally all right, but that in old age or with Alzheimer's, where levels of the neurotransmitter are low and so the enzyme, because of its speed, reduces these to *very* low levels, resulting in less LTP, which impairs the ability to think and perform memory tasks (pages 13, 39 – 40).

The drugs discussed here are called cholinesterase inhibitors. They help to ease the deficit of ACh by *blocking* the action of the enzyme acetylcholinesterase (AChE) thus *increasing* the amount of acetylcholine neurotransmitter (ACh) that remains in the synaptic gap.



Refer to the diagram (right) and compare it with the original diagram (page 13). The cholinesterase inhibitor (represented by the red X) blocks the action of the acetylcholinesterase enzyme (AChE) which allows the acetylcholine neurotransmitter (ACh) to return to its original level. *Note*: The cholinesterase inhibitor does *not* act on the beta-amyloid (I think!) which is why I have still shown these in the diagram (that is, the).

The drugs below are presently on the market for this purpose.

Tetrohydroaminoacridine (THA)

Tetrohydroaminoacridine, marketed in 1993 under the trade name Tacrine or Cognex (shown in picture, right) was the first Alzheimer's drug to be approved by the US FDA. It has been shown to improve memory and language deficits in the *early* stages of Alzheimer's disease. The drug is less effective in later stages however.

Adverse effects included nausea, vomiting, diarrhoea, headaches, dizziness and in a few patients, toxic effects on the liver. However, Tacrine/Cognex was *discontinued* in the US in 2013, due to concerns over the toxic effects on the liver.

Donepezil

A second drug, Donepezil, marketed under the trade name Aricept (among others), also targets the acetylcholinesterase enzyme (AChE) and allows for the same improvements in cognitive function as THA.

Donepezil was approved by the US FDA in 1996 for treatment of *mild, moderate and severe* Alzheimer's disease.

Common side effects include nausea, trouble sleeping, aggression, diarrhoea, feeling tired, and muscle cramps. Occasionally there are more serious side effects including abnormal heart rhythms and difficulty

emptying urine from the bladder. Unlike Tacrine/Cognex, it has not been withdrawn but treatment should be stopped if no significant benefit is seen.

Neither of the above drugs, however, is a *cure* and neither can stop the eventual progression of the disease. The difficulty in finding a cure for Alzheimer's disease reflects the immense complexity of the condition. A deficit in acetylcholine neurotransmitter (ACh) is only one of the many factors contributing to this devastating brain disorder. Nevertheless, cholinesterase inhibitors are one of the few drug therapies that have been proven clinically *useful* (though not a cure) in the treatment of Alzheimer's disease thus validating the cholinergic system as an important therapeutic target in the disease.

Rivastigmine

Rivastigmine (or Exelon) was discussed in Method (A) earlier (page 53) as an alpha-secretase stimulator drug. But it can also work as a cholinesterase inhibitor (like THA and donepezil above), and is currently used as a treatment for Alzheimer's disease symptoms by elevating synaptic acetylcholine (ACh) levels. As of 2015, at least 13 double-blind clinical trials of rivastigmine showed it to be beneficial for mild-to-moderate AD, as compared to placebo.

Note: **Curcumin**, used in Method B 'Inhibition of Aβ monomers changing to oligomers' has also been shown to influence acetylcholinesterase activities so my be useful as a dual-purpose drug.

Notes on these drugs

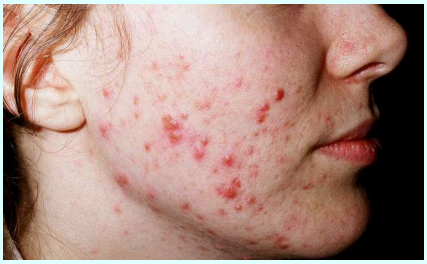
1. Acetylcholinesterase inhibitors, such as those above, can become less effective over time, because the neurons of people with Alzheimer's produce less and less acetylcholine, which the drugs cannot compensate for.
2. **Combining** NMDA-receptor antagonists and acetylcholinesterase inhibitors could offer greater benefits on behaviour and cognition. A combination of memantine and donepezil was approved in 2014 by the United States Food and Drug Administration (FDA) for treatment of *moderate to severe* AD. This combined drug is called Namzaric (one form of namzaric is pictured, right), which is taken orally, and acts on *both* the above receptors, that is, it is an NMDA receptor antagonist and an acetylcholinesterase inhibitor to reduce the levels of both acetylcholine and glutamate. However again, Namzaric also just offers temporary relief from the symptoms of Alzheimer's disease. It does *not* prevent or slow the underlying cause of the disease, nerve cell death. Once again disappointing!

Caveat: Acetylcholine (ACh) is the same neurotransmitter that *motor* neurons of the nervous system release in order to activate muscles to control movement. This property means that drugs, such as THA and donepezil, also affect these motor neuron receptors and can have very dangerous effects on muscles, ranging from paralysis to convulsions.

Infection and Alzheimer's Disease

It was mentioned earlier (pages 25, 28) that certain infections might lead to Alzheimer's disease. If so, this is suggests that antimicrobial or antiviral drugs might have therapeutic value (depending on whether the infection is caused by bacteria or by viruses). Two of the infections that are thought to

be linked to Alzheimer's are oral herpes and pneumonia. One type of herpes virus causes cold sores (picture).



At the moment, there is not enough evidence to know whether treating infections would be a good strategy to treat Alzheimer's or to reduce the risk of the condition. Further research is needed.

However, antibiotics and anti-viral drugs that treat some of these infections are starting to be tested in clinical trials of people with Alzheimer's disease. So far however the results have been inconsistent.

(E) Lifestyle factors: Diet

Lifestyle factors include **exercise** and **sleep** (mentioned earlier) and **diet**. Here we look briefly at diet.

The evidence for diet helping Alzheimer's is not as strong as it is for other interventions such as physical activity, blood pressure, and cognitive training. For a long time, suggestions on how diet can slow down or even cure Alzheimer's disease was the realm of health gurus and the like but less so scientists. But after decades of disappointment, the tide may be turning at last.

In one observational study of 116 cognitively normal adults, those who followed a (so-called) Mediterranean diet had thicker cortical brain regions than those who did not. These brain regions shrink in people with Alzheimer's, so having thicker regions could mean cognitive benefit. A follow-up observational study showed higher levels of beta-amyloid protein – the brain signature of Alzheimer's – in people who did *not* follow the Mediterranean diet closely, compared to those who did.

What is the Mediterranean diet?

There are actually 21 Mediterranean countries, most of which have different diets! So this term must be used carefully and it is better to be specific (to either which country or which foods). According to one source, the main components of the diet should include: The daily consumption of vegetables, fruits, whole grains and healthy fats, weekly intake of fish, poultry, beans and eggs, moderate portions of dairy products and *limited* intake of red meat.

In another study, it was found that eating a daily serving of leafy green vegetables such as spinach and kale was associated with slower age-related cognitive decline, perhaps due to the neuroprotective effects of certain nutrients.

Foods that are naturally high in choline include whole eggs, meats and fish, and whole grains. Studies in laboratory animals and humans suggest that consuming these foods or supplements rich in choline *may* elevate levels of acetylcholine in the brain.

So far however, there is no very strong evidence that eating or avoiding a specific food can prevent Alzheimer's disease or age-related cognitive decline. And few studies have shown a strong link between eating well and a boost in cognition. Overall though, the evidence *suggests*, but does *not* prove, that following a 'Mediterranean' or similar diet might help reduce the risk for Alzheimer's dementia or slow

cognitive decline. To find out more, scientists supported by the U.S. National Institute on Aging (NIA) and other organisations are conducting clinical trials.

More herbal remedies for Alzheimer's Disease

Traditional herbs have long been touted as cures for many diseases, including Alzheimer's. Some have already been mentioned. Here is more on the subject.

Seaweeds

Seaweeds are marine algae. Some contain the amino acid taurine. The modified form of this amino acid is known as tramiprosate, discussed earlier. Chlorella is another kind of seaweed that seems to help Alzheimer's disease. A study showed that among 50 people with Alzheimer's, aged 70-90, taking chlorella extract (pictured) daily for 6 months, 68% experienced either a stabilisation or improvement in cognitive functions.

Curcumin

Curcumin, also discussed earlier, is an extract of turmeric and is widely touted as having many health benefits. There is a significantly lower rate of Alzheimer's disease amongst Asian populations that have diets high in turmeric, but it seems unlikely that turmeric itself is responsible for this. Scientists say that simply eating more curcumin-based curries is unlikely to alleviate Alzheimer's disease because of the spice's limited bioavailability in the brain.

Chinese club moss

In traditional Chinese medicine, Chinese club moss (*Huperzia serrata*) has long been used to sharpen memory. Huperzine A, a substance extracted from Chinese club moss, has been found, in some studies, to act as a *cholinesterase inhibitor* to prevent the breakdown of acetylcholine. However, other studies showed huperzine A failed to improve cognitive function in Alzheimer's patients.

Ashwagandha

From traditional Indian medicine, the herbal plant ashwagandha (scientific name *Withania somnifera*) has been found to inhibit the formation of beta-amyloid plaques in *preliminary* research – at least, it does so in laboratory experiments.

Common sage

There are many varieties of sage. Extracts from common/garden sage (*salvia officinalis*) have shown that in mice and rats and in laboratory investigations, they may be effective for patients with *mild to moderate* Alzheimer's disease by reducing several effects caused by Aβ as well as and having cholinesterase inhibiting properties. While promising, further research is required.

Combining lifestyle factors

Combining lifestyle factors seems to be a more reasonable approach rather than thinking one approach will reverse or cure Alzheimer's disease.

One example is a preliminary study published in September 2014 suggests that the memory losses associated with Alzheimer's can be reversed through an elaborate 36-point treatment program including dietary changes, exercise, intellectual stimulation and other activities that had already been proposed as Alzheimer's prevention factors separately. But in this case, it is the unusual step of *combining* them that may to have curative value.

Comment: Even if such lifestyle factors do work, scientists would still like to find out the *mechanisms* whereby they do work. For example, does eating a particular food act on beta-amyloid, tau or some of the synaptic receptors.

Summary: Main Points

A summary is included as it can be difficult to recall accurately the main points when there is so much information – and that includes me too, and I wrote the article!

What is Alzheimer's Disease?

- Alzheimer's disease is an irreversible, progressive brain disorder that slowly destroys memory and thinking skills and, eventually, the ability to carry out the simplest tasks.
- The disease is named after Dr. Alois Alzheimer (1864 – 1915), the German doctor who first described it.
- Memory problems are, in most people, one of the first signs of Alzheimer's. People with Alzheimer's have trouble doing everyday things. Ultimately, the disease is fatal.
- Characteristic features in the brain are the loss of connections between nerve cells (neurons) and atrophy of the brain cortex.
- Alzheimer's disease presently affects approximately 13% of people over the age of 65 and 45% over the age of 85, with an estimated number of at least 50 million Alzheimer's disease patients around the world.
- There are two types of Alzheimer's disease: Early-onset AD (EOAD) – also called familial Alzheimer's – is often in a family. Most people get late-onset Alzheimer's disease (LOAD) – also called sporadic Alzheimer's.

Kinds of Cells in the Brain

- Cells in the brain are grouped into two main categories – neural cells (neurons) and glia (non-neurons).
- Key components of a neuron are dendrites, soma, axon and axon terminals. Neurons pass chemical and electrical signals along the neural pathways in the brain. Chemical signals travel from the presynaptic neuron to the post-synaptic neuron across the synapses using molecules called neurotransmitters. Alzheimer's disease destroys neurons. Electrical signals travel down axons.
- All the other cell types in the brain, are glial cells. These include: microglia, astrocytes, and oligodendrocytes. One role of microglia is to clear foreign substances and cellular debris from the brain.
- Astrocytes keep neurons healthy by holding them in place and supplying them with nutrients.
- Oligodendrocytes provide support and insulation to the axons of neurons.

Neurons and Memory

- In the brain, memory is a large network of many inter-connected neurons.
- Making a memory permanent is called memory consolidation. The process of consolidation involves long-term potentiation (LTP). Long-term depression (LTD) is the *inverse* mechanism to LTP.
- Two kinds of neuron receptors involved in human learning and memory formation, as well as in Alzheimer's, are NMDA and acetylcholine receptors. The neurotransmitters for these receptors are glutamate and acetylcholine respectively.

The Brain and Brain Blood Vessels

- Below the cranium are three membranes called the meninges.
- There are four kinds of fluids in the brain: Blood plasma, Intracellular fluid (ICF), Interstitial fluid (ISF), and Cerebrospinal fluid (CSF).

- One important function of the CSF is the excretion of waste products from the brain.

Blood Vessels in the Brain

- The blood vessels in the brain have a blood-brain barrier (BBB), which prevents many substances from freely entering (or leaving) the brain (but allows some others, such as oxygen and glucose to do so).
- Blood vessels enter and leave the brain from the subarachnoid space in the meninges layers. Key blood vessels are the cerebral artery and vein, and the pial arteries and veins.
- The pial arteries and veins are surrounded by a perivascular space (PVS) but at the level of capillaries, the PVS disappears (or is extremely small). CSF flows into the brain, moves through the brain tissue and then re-enters the PVS in the venules.

Causes of Alzheimer's disease: plaques and tangles

- Alzheimer's is generally associated with amyloid plaques (senile plaques), found between neurons, and neurofibrillary (or tau) tangles, found inside neurons.
- Amyloid plaques consist of short beta-amyloid (or β-amyloid, or Aβ) molecules clumped together.
- Neurofibrillary tangles are due to the tau protein.
- In people with Alzheimer's, tau proteins change to form tau fibres then neurofibrillary tangles (NFT), leading to the death of neurons.
- Alzheimer's is believed to develop first by the build-up of amyloid plaques between neurons, then tau forming tangles (NFTs) inside neurons.
- The amyloid plaque hypothesis on the origin of Alzheimer's has been dominant. The tau/NFT hypothesis is one alternative hypothesis. Even infection and obesity have been suggested as causes.

Structure and Production of Amyloid Plaques and Tau Tangles

- Amyloid plaques are made of beta-amyloid monomers. An Aβ monomer can have 37 – 49 amino acids in its chain. One important Aβ monomer is the Aβ42 monomer, which is toxic.
- Plaque formation involves several steps: Aβ42 monomer → oligomers → fibrils → plaque.
- Aβ monomers are formed from amyloid precursor protein (APP). There are two pathways: the non-amyloidogenic pathway (involving α-secretase, followed by γ-secretase) which is a normal process, and the amyloidogenic pathway (involving β-secretase, followed by γ-secretase), which is an abnormal process and occurs in Alzheimer's patients. The non-amyloidogenic pathway produces the Aβ40 monomer, while the amyloidogenic pathway produces the harmful Aβ42 monomer.
- There six forms of tau protein molecules ranging from 352 to 441 amino acids in length. Three of the six are 3R variants; the other three are 4R variants.
- Tau molecules bind to and stabilise the neuron's microtubules. The binding of tau protein requires the phosphorylation of the tau protein molecules.
- Hyperphosphorylation of tau is known to contribute to Alzheimer's disease. It is believed to be initiated by beta-amyloid oligomers. The hyperphosphorylated tau protein may spread like an infection, from neuron to neuron, rather than remaining in each neuron.

Receptors and Alzheimer's Disease

- Alzheimer's disease can also result from synaptic receptors that do not function normally.
- With Alzheimer's, Aβ42 oligomers led to the endocytosis of postsynaptic NMDA receptors, while excess glutamate at the extrasynaptic receptors causes neuron to shrink in size and die.

- With acetylcholine receptors, beta-amyloid plaques (probably) cause very low levels of ACh neurotransmitter.

Clearance of Amyloid from the Brain

- The brain produces metabolic waste at a higher rate than any other organ. There is a waste removal system, called the glymphatic system, that is unique to the brain.
- Under normal physiology, Aβ is cleared from the brain by five pathways:

1. Aβ is taken into postsynaptic neurons and broken down in lysosomes.
2. Aβ-degrading enzymes (ADE), present in the interstitial fluid, break down Aβ into fragments.
3. Microglia take up and degrade the shorter forms of Aβ by phagocytosis. Astrocytes then help to clear the build-up of debris that remains.
4. Aβ, with the help of LRP1 receptors, is transported from the brain into the blood across the BBB.
5. Elimination via the glymphatic system. CSF, from the subarachnoid space, enters and flows through the brain by diffusion and advection carrying waste with it and then into the PVS of the venules from where the wastes pass into the sinuses of the dura mater for eventual removal from the body.

Sleep, Exercise and Alzheimer's Disease

- Memory consolidation occurs during the so-called deep sleep. Both NMDA and acetylcholine receptors are involved in this process.
- The production of beta-amyloid and other debris/waste is highest when awake.
- Sleep also plays an important role in the removal of wastes from the brain as the glymphatic activity is enhanced during sleep but is suppressed when awake.
- Seniors who have sleep REM Sleep Disorder are more likely to develop Alzheimer’s and have a faster rate of cognitive decline than those who have healthy sleep habits.
- Acetylcholine receptors and their neurotransmitter acetylcholine cause normal REM sleep, but also can cause sleep disorder in older folks whose levels of acetylcholine are very low, leading to Alzheimer's.
- Cardiorespiratory exercise is good for brain health and helps clear away harmful debris from the brain, and *may* lesson cognitive decline with ageing and Alzheimer's disease.

Treatment of Alzheimer's Disease

- Existing Alzheimer's disease drugs only help lessen or stabilise the symptoms of Alzheimer’s disease for a limited time; they are unable to stop or reverse disease progression.
- Only a few of the many drugs developed to improve the symptoms of Alzheimer's disease have actually been approved by the (US) FDA.
- Current drug research and treatments target primarily the five areas (A) to (E) below.
- (A) Drugs preventing the formation of Aβ monomers from amyloid precursor protein (APP) by inhibiting or stimulating the three enzymes involved in the non-amyloidogenic and amyloidogenic pathways, viz. alpha secretase (stimulated), beta secretase and gamma secretase (inhibited). Results from trials have not been encouraging and some drugs have even been withdrawn.
- (B) Inhibition of Aβ changing to plaque. Several drugs have been designed to target amyloid oligomers, but none has yet really succeeded in improving outcomes for people living with Alzheimer’s disease. Some of these drugs are derived from substances in plants (e.g. seaweeds). Some drugs act as antibodies, some react with Aß42 or tau, some inhibit the formation of plaques from oligomers.

- (C) NFTs – Treatments involving tau. Initially, potential anti-tau therapies were based mainly on inhibition of kinases or tau aggregation into filaments, or on stabilisation of microtubules, but most of these approaches have been discontinued. Current treatments focus on preventing the accumulation of tau filaments into tangles and preventing tau protein separating from microtubules and forming NFTs, with mixed results.
- (D) Treatments for receptors that are linked with Alzheimer's disease, namely, NMDA and acetylcholine receptors. For NMDA receptors, there are two approaches to treatment: Preventing Aβ42 from forming so protecting the postsynaptic neurons, and use of NMDA-receptor antagonists to save the extrasynaptic neurons. For acetylcholine receptors, treatments help to ease the deficit of ACh by blocking the action of the enzyme acetylcholinesterase (AChE) thus increasing the amount of acetylcholine neurotransmitter (ACh) that remains in the synaptic gap.
- Some scientists believe that certain infections might lead to Alzheimer’s disease, though clear evidence is still lacking. If so, antimicrobial or antiviral drugs might have therapeutic value.
- (E) Lifestyle factors – diet. There is some evidence that following a (so-called) Mediterranean diet can decrease levels of beta-amyloid in the brain. Traditional herbs have long been touted as cures for many diseases, including Alzheimer's and some are being scientifically investigated.

Glossary

Here is a list of many of the difficult or specialised words used in this project together with their definitions or meanings, in alphabetical order.

Acetylcholine: The neurotransmitter in acetylcholine/cholinergic receptors.

Acetylcholine/cholinergic receptor: One type of neurotransmitter receptor located in the postsynaptic membrane of a neuron. (The neurotransmitter for these receptors is acetylcholine.)

Acetylcholinesterase: An enzyme that rapidly breaks down/degrades the neurotransmitter acetylcholine.

Advection: The transport of substances in bulk via the movement of a fluid.

Agonist: A molecule that has the same effect on the postsynaptic neuron as the neurotransmitter itself does. (Compare a neurotransmitter's *antagonist*.)

Alzheimer's disease: An irreversible, progressive brain disorder that slowly destroys memory and thinking skills and, eventually, the ability to carry out the simplest tasks.

Amino acid: An organic compound composed of nitrogen, carbon, hydrogen and oxygen (and sometimes other elements). The body needs 20 different amino acids to grow and function properly.

Amyloid beta (Aβ): Peptides of 37 – 49 amino acids that are crucially involved in Alzheimer's disease as the main component of the amyloid plaques found in the brains of people with Alzheimer's disease.

Amyloid plaques (also called senile plaques): Hard, insoluble accumulations of beta amyloid proteins that clump together between the nerve cells (neurons) in the brains of Alzheimer's disease patients.

Amyloid precursor protein (APP): A transmembrane protein which, in the membranes of neuron synapses forms beta amyloid (Aβ).

Amyloidogenic pathway: The process, common in people with Alzheimer's that uses beta-secretase and gamma-secretase to form beta-amyloid from APP, including the very toxic and harmful Aβ42 monomer.

Antagonist: A molecule that *blocks* the effect that the neurotransmitter normally has on the postsynaptic neuron. (Compare a neurotransmitter's *agonist*.)

Antibody: A protein produced mainly by white blood cells in the blood that is used by the immune system to destroy disease-causing agents, especially harmful substances (such as Aβ oligomers) and harmful bacteria and viruses.

Atrophy: Decrease in size or wasting away of a body part or tissue.

Bioavailability: The extent to which a substance is available where it is needed in the body (e.g. in the brain).

Blood–brain barrier (BBB): The semipermeable border of blood vessels in the brain that prevents many substances from freely entering (or leaving) the brain (but allows some others, such as oxygen and glucose to do so).

Cardiorespiratory exercise: Exercise that gets the heart pumping, such as walking briskly, running, biking and swimming.

Cerebral artery: A large artery that passes along the subarachnoid space and channels fresh blood into the brain.

Cerebrospinal fluid (CSF): A clear, colourless body fluid found in the brain and spinal cord.

Convection: In the field of brain transport, convection is the combination of both diffusion and advection.

Cortex (brain): The thin outer layer of the brain whose myriad convolutions (folds) give the brain its characteristic appearance. (Also called the cerebral cortex.)

Deep sleep: The sleep that occurs during the third and fourth stages of the five stage sleep cycle, needed to feel refreshed on waking up in the morning.

Dementia: Brain diseases that cause a long-term and often gradual decrease in the ability to think and remember that is severe enough to affect daily functioning.

Diffusion: The movement of a substance from an area of high concentration to an area of low concentration. Diffusion happens in liquids and gases.

Endocytosis: The process of actively engulfing/taking in molecules into the cell by engulfing them with part of the cell membrane. (Endocytosis includes phagocytosis.)

Endosome: A compartment/space inside a cell, sometimes filled with fluid.

Enzymes: Biological molecules (typically proteins) that speed up the rate of chemical reactions that take place within cells.

Excitotoxicity: The process by which neurons are damaged and killed through the over-activation of receptors by neurotransmitters.

Glial cells: Cells that surround neurons and provide support for and insulation between them. Types of glial cells include microglia, astrocytes and oligodendrocytes.

Glymphatic system (also called the perivascular circulation): A waste removal system unique to the brain that uses the flow of cerebrospinal fluid (CSF) through the brain to flush out wastes.

Hippocampus: The two small seahorse-shaped structures in the centre of the brain; one on the left side and one on the right.

Interstitium: The spaces between cells/tissues in the brain (adjective =interstitial).

Kinases: Protein enzymes which add phosphate groups to tau proteins, that is, cause phosphorylation.

Long-term depression (LTD):The inverse of long-term potentiation, which produces a long-lasting decrease in the strength of the connections between neurons (synaptic strength).

Long-term potentiation (LTP): The process by which synaptic connections between neurons become stronger with frequent activation/use, which makes communication between neurons more likely. LTP underlies learning and memory.

Lysosome: A cell structure that contains digestive enzymes which break down excess or worn-out cell parts. Lysosomes may be used to destroy invading viruses and bacteria. If a cell is damaged beyond repair, lysosomes can even help it to self-destruct.

Meninges: Three layers/membranes located below the cranium that surround the brain (and spinal cord).

Microtubules: Specialised filaments in neurons that transport nutrients and other essential materials from the cell body (soma) to the tip of the axon.

Monomer: A molecule that combines with other monomers to form a larger/longer molecule.

Neurodegenerative: Involving the loss of brain cells, specifically neurons.

Neurofibrillary (or tau) tangles (NFTs): Insoluble twisted fibres, consisting primarily of tau protein, found inside neurons.

Neurogenesis: The process by which new neurons are formed in the brain.

Neurotransmitter: A chemical released from a presynaptic neuron that crosses the synapse and is accepted by a receptor in a postsynaptic neuron.

NMDA receptor: One type of neurotransmitter receptor located in the postsynaptic membrane of a neuron. (The neurotransmitter for NMDA receptors is glutamate.)

Non-amyloidogenic pathway: The normal process that uses alpha-secretase and gamma-secretase to form beta-amyloid from APP, especially the non-harmful (or less harmful) Aβ40.

Oligomer: A molecule that consists of a small number of monomers (usually fewer than five, but occasional higher).

Parenchyma: The functional tissue in organisms [‘functional’ means actually performing something as opposed to ‘structural’ tissues such as bone in animals].

Peptide: A short chain of amino acids. Peptides are distinguished from proteins by their shorter length, although the cut-off number of amino acids for defining a peptide and protein can be arbitrary.

Perivascular space (PVS): A space around the larger arteries and veins in the brain (also called the Virchow-Robin space).

Phagocytosis: The process by which a cell (e.g. microglia, white blood cells or even one-celled organisms, such as an amoeba) uses its membrane to engulf/ingest various materials/particles/debris or even a microorganism (such as a bacterium), giving rise to an internal compartment (called a phagosome). It is one type of endocytosis.

Phosphatases: Protein enzymes which remove phosphate groups to tau proteins, that is, cause dephosphorylation.

Phosphorylation: The (normal) process whereby a phosphate group ($-PO_4^{3-}$ containing phosphorus and oxygen) binds to three kinds of amino acids of a tau protein.

Pial artery: An artery that branches off the cerebral artery and dives down/penetrates/enters brain tissue.

Protease: An enzyme that breaks down proteins into smaller peptides or single amino acids.

REM sleep: The fifth and last stage of the sleep cycle, distinguished by random/rapid movement of the eyes, accompanied with less muscle movement (even paralysis) of the rest of the body's muscles and the propensity of the sleeper to dream vividly.

Secretases: Enzymes that form beta amyloid (Aβ) from APP. There are three secretases – α-secretase, β-secretase and γ-secretase.

Sinus: A hollow sac or cavity in any organ or tissue.

Sub-arachnoid space (SAS): A space underneath the arachnoid membrane.

Synapse (or synaptic gap): The small gap separating neurons. The synapse consists of a presynaptic ending that releases neurotransmitters and a postsynaptic ending that contains receptor sites for these neurotransmitters.

Synaptic plasticity (or neural plasticity): The ability of synapses to strengthen or weaken over time [cf. the word *plastic* which means easily shaped or moulded.]

Tau protein (or t-tau): Proteins that assembly and stabilise microtubules, which contributes to the proper function of neuron.

Tissue: Groups of cells that have a similar structure and act together to perform a specific function.

Website References

The following is a list of websites that I referred to for ideas, text, diagrams or all of these in the preparation of this project. Some of the websites are very good, others marginally so.

Some of the websites are easy to understand; others are *very* difficult or impossible for most readers to comprehend. For this reason, the websites included below are just the easier ones. The difficult ones I have placed in a separate file. Here is the link to this file:

https://drive.google.com/file/d/1iakEVb1t3zEmR7ZdpeMAA9jU9ujS9OT4/preview

In the list below, some links go directly links to websites on the Internet. For others. I downloaded the original websites, highlighted key parts of the texts, then uploaded these files to my cloud storage (these are the links that include in their name: https://drive.google.com). The highlighting should make it easier to identify the important parts in each file. Perhaps focus on the highlighted parts first to get the overall idea of the topic. Alternatively, the link below includes all these highlighted articles (there are 48 of them). From there, you can open any of the files that may interest you.

https://drive.google.com/drive/folders/1HApPMZ1Qb8YSXIa8mBdEoWuBFpred9iQ

The list of files is only approximately in the same sequence as in the project as most contain material that is used in more than one section. Some of the links are also repeated under different headings. Links included within the text of the project are also repeated here.

There are actually millions of websites available on the Internet for the topic of Alzheimer's disease, and while some of these may be very good and have been missed, it is just too time consuming to have to search through so many. I might also add that many of the research articles are just so complex or confusing that I have not included them in the list.

Note: Some files are long and include many topics. In these cases, you may want to do a word search to find a particular topic rather than starting to read from the start of the file.

Alzheimer's Disease: Introduction

What is Alzheimer's disease?
https://drive.google.com/file/d/1XZqPi38oOdH3SJTjI6bonw2dYiGmfEHp/preview
How Is Alzheimer's disease diagnosed?
https://drive.google.com/file/d/1Vs2yHjB99luc72_D6pMHauSYRVeb6-XW/preview
Alzheimer's disease – general
https://www.dementia.org.au/files/helpsheets/Helpsheet-AboutDementia13-AlzheimersDisease_english.pdf
https://www.medicalnewstoday.com/articles/159442#symptoms
Cortical / Brain atrophy in Alzheimer's
https://thebrain.mcgill.ca/flash/d/d_08/d_08_cr/d_08_cr_alz/d_08_cr_alz.html
Alzheimer's disease and the brain (contains good images related to AD)
https://www.alz.org/espanol/about/brain/09.asp
Alzheimer’s disease [Wiki]
https://en.wikipedia.org/wiki/Alzheimer%27s_disease

Age-associated memory impairment
https://human-memory.net/age-associated-memory-impairment/
Alzheimer's disease
https://human-memory.net/alzheimers-disease/
World Alzheimer Report 2018: The state of the art of dementia research: New frontiers
https://www.alz.co.uk/research/WorldAlzheimerReport2018.pdf

Kinds of Cells in the Brain

Kinds of cells in the brain
https://learn.genetics.utah.edu/content/neuroscience/braincells/
Synapses and neurotransmitters
https://thebrain.mcgill.ca/flash/i/i_01/i_01_m/i_01_m_ana/i_01_m_ana.html
Discovery and functions of acetylcholine [2019]
https://www.verywellmind.com/what-is-acetylcholine-2794810
Long-term potentiation (LTD)
https://thebrain.mcgill.ca/flash/i/i_07/i_07_m/i_07_m_tra/i_07_m_tra.html
No microglia, no Alzheimer’s plaques (in mice!)
https://www.futurity.org/microglia-alzheimers-disease-2142612/
Memory consolidation and long-term potentiation (LTD)
https://www.theguardian.com/education/2015/sep/16/what-happens-in-your-brain-when-you-make-a-memory
https://human-memory.net/memory-consolidation/
Discovery and functions of acetylcholine
https://www.verywellmind.com/what-is-acetylcholine-2794810

The Brain and Brain Blood Vessels / CSF / BBB

Endothelial, epithelial and glial brain barriers
https://drive.google.com/file/d/12gtSzJ3PNclvMcoI7rSbNJ6ThAAYnt7N/preview
Spatial model of convective fluid movement
https://drive.google.com/file/d/14esspzuadvLERa8I97P98n6F2DW65qv2/preview
Cerebrospinal-fluid-circulation
https://drive.google.com/file/d/1qzVIVGNfuoABxsRbs9dmclnz_7OsJZMi/preview
What is the blood-brain barrier?
https://qbi.uq.edu.au/brain/brain-anatomy/what-blood-brain-barrier
The vasculature in the central nervous system / meninges / CSF flow
https://drive.google.com/file/d/1DL16X_pQr9tN0RLdUxFTzMq64_7r4PKF/preview
CSF circulation (video)
https://www.youtube.com/watch?v=SDMO4vYkqdg
Figure: The acellular and cellular brain barriers
https://www.nature.com/articles/ni.3666/figures/1
The acellular and cellular brain barriers (includes diagrams similar to ones in the text)
https://www.nature.com/articles/ni.3666/figures/1
How CSF circulates [video]

https://www.youtube.com/watch?v=GgRJnWBnq8o

Meninges

https://www.youtube.com/watch?v=Du22F9CpTHU

CSF, pial arteries,

https://www.nature.com/articles/nrn3114/figures/1

Blood Vessels in the Brain

The neuron and neuroglia

https://www.britannica.com/science/nervous-system/Types-of-neuroglia

The acellular and cellular brain barriers (again)

https://www.nature.com/articles/ni.3666/figures/1

The ventricular system and CSF (for kids!)

https://faculty.washington.edu/chudler/vent.html

The blood-brain barrier (for kids!)

https://faculty.washington.edu/chudler/bbb.html

Causes of Alzheimer's disease: plaques and tangles

The progression of Alzheimer's disease (AB and NFTs)

https://www.brightfocus.org/alzheimers-disease/infographic/progression-alzheimers-disease

What happens to the brain in Alzheimer's disease?

https://www.nia.nih.gov/health/what-happens-brain-alzheimers-disease

What Happens to the brain in Alzheimer's disease?

https://drive.google.com/file/d/1iwDKoh58tVTUzYJF1Z6VIpLYgWq6-eaj/preview

How is Alzheimer's disease diagnosed?

https://drive.google.com/file/d/1Vs2yHjB99luc72_D6pMHauSYRVeb6-XW/preview

Video & transcript: How Alzheimer's changes the brain

https://drive.google.com/file/d/1t5CZiMue-0WjRzlDXZgwCiGfAzOcCRb9/preview

Obesity as a risk factor for Alzheimer’s disease

https://drive.google.com/file/d/1YFiew4DlhF51DTOjO9-orsH2zQJCDKF-/preview

Beta-amyloid and the amyloid hypothesis

https://drive.google.com/file/d/12YoGXULw6YaFXVr9qXSKBCiItnGyhiw2/preview

https://drive.google.com/file/d/1bEAOZfB3MZat1SjE9Oib9YiQlKDj_-zE/preview

Alzheimer's could actually start elsewhere in the body and not the brain

https://drive.google.com/file/d/1NPPbIWBUhGlARVdTb6000wCF8N3cvuyL/preview

Amyloid beta formation

https://drive.google.com/file/d/13IlRmubgO0hZ_YGQh57ijgaiRLjwdU2y/preview

https://drive.google.com/file/d/1H_FJRlo4CbP3GxZbL43tq6PXyFP7dYAV/preview

The amyloid hypothesis on trial (including infections)

https://drive.google.com/file/d/1k8EUjdO898UVuFAYo3MQw9DsqAxhgUpZ/preview

Beta-Amyloid protein

https://thebrain.mcgill.ca/flash/d/d_08/d_08_m/d_08_m_alz/d_08_m_alz.html

Synthetic protein blocks toxic beta-amyloid

https://www.medicalnewstoday.com/articles/324985

Amyloid plaques and neurofibrillary tangles (NFTs)
https://www.brightfocus.org/alzheimers-disease/infographic/amyloid-plaques-and-neurofibrillary-tangles
APP cleavage plus tau protein
https://thebrain.mcgill.ca/flash/i/i_08/i_08_m/i_08_m_alz/i_08_m_alz.html
Video on how Alzheimer's changes the brain (plus notes) [2017]: Click on … → 'Open transcript'
https://www.youtube.com/watch?v=0GXv3mHs9A
Role of oxidative stress in Alzheimer's disease (2016)
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4840676/
We may have been wrong about what kills brain cells in Alzheimer's disease [2018]
https://www.sciencealert.com/we-might-been-wrong-what-kills-brain-cells-alzheimer-s-disease-beta-amyloid-tau-protein-app
Alzheimer's protein may spread like an infection, human brain scans suggest [2018]
https://www.sciencemag.org/news/2018/01/alzheimer-s-protein-may-spread-infection-human-brain-scans-suggest
Diabetes and AD plus insulin
Cognitive losses associated with Alzheimer's / familial form of Alzheimer's / diabetes
https://thebrain.mcgill.ca/flash/i/i_08/i_08_p/i_08_p_alz/i_08_p_alz.html#2
https://thebrain.mcgill.ca/flash/d/d_08/d_08_p/d_08_p_alz/d_08_p_alz.html
The AD brain – plaques, tangles and neurotransmitters
https://www.mentalhelp.net/cognitive-disorders/causes-of-alzheimer-s-disease-continued/
Amyloid plaques and neurofibrillary tangles
https://thebrain.mcgill.ca/flash/d/d_08/d_08_cl/d_08_cl_alz/d_08_cl_alz.html
Infections and Alzheimer's disease
https://www.alzheimers.org.uk/about-dementia/risk-factors-and-prevention/infections-and-dementia

Structure and Production of Amyloid Plaques and Tau Tangles / pathways / receptors
Amyloid beta: structure, biology and structure-based therapeutic development
https://drive.google.com/file/d/1O2feDDwwXDmBl3PsQEVsRXJGn5mwKxS7/preview
Alzheimer's may be caused by brain's sticky defence against bugs [2016]
https://www.newscientist.com/article/2090221-alzheimers-may-be-caused-by-brains-sticky-defence-against-bugs/
Eric Snyder / NMDA receptors / tau protein
https://thebrain.mcgill.ca/flash/a/a_08/a_08_m/a_08_m_alz/a_08_m_alz.html

Clearance of Amyloid from the Brain
Astrocyte networks and cerebral metabolite clearance
https://drive.google.com/file/d/1UhB1TiDCxmc7QkASQd13RtjaHsoAScsm/preview
Solute transport in brain ECS does not support a glymphatic mechanism
https://drive.google.com/file/d/1HZlpoTDSgfpNkO2EfW4ZMJeeyUXNYplv/preview
The glymphatic system for beginners
https://drive.google.com/file/d/1QMZKWoHZ2R885aZzNKibjKhrlVBDx9ce/preview
Fluid Flow and mass transport in brain tissue
https://drive.google.com/file/d/1cMalZuWaT51uuEMMYPenFv68nv7_LImK/preview

Sleep, Exercise and Alzheimer's Disease

Clearing out the junk: Brain waste disposal
https://drive.google.com/file/d/1K0VRCQge7xjceH0PWiQ5FPOs9otC_Xa8/preview

Tau tangles not amyloid plaques drive daytime napping that precedes dementia
https://drive.google.com/file/d/1fN1-Q3_zz4JWw15v1uQVEnhcZBn0pZ-D/preview

Decreased deep sleep linked to early signs of Alzheimer's disease
https://drive.google.com/file/d/1Wr8uNJi1EZdi6xbmriqEFcjoZ5fFa8bV/preview

Exercising and AD
https://drive.google.com/file/d/1FGT2B77ocVQr0t_WCBJW8xorQ4HRQfDj/preview

Tau, more than Aβ, affects sleep early in Alzheimer's
https://www.alzforum.org/news/research-news/tau-more-av-affects-sleep-early-alzheimers

The president's personal exercise program
https://parade.com/116185/ronaldreagan/16-reagan-fitness/

AD destroys neurons that keep us awake
https://www.sciencedaily.com/releases/2019/08/190812075523.htm

How deep sleep may help the brain clear Alzheimer's toxins [2019] plus moving picture
https://www.npr.org/sections/health-shots/2019/10/31/775068218/how-deep-sleep-may-help-the-brain-clear-alzheimers-toxins

Is aerobic exercise the right prescription for staving off Alzheimer's?
https://www.npr.org/sections/health-shots/2019/07/18/743189541/is-aerobic-exercise-the-right-prescription-for-staving-off-alzheimers

REM sleep disorder and Alzheimer's
https://www.alzheimers.net/rem-sleep-disorder-and-alzheimers/

Treatment of Alzheimer's Disease

BACE-1 and γ-Secretase as therapeutic targets for Alzheimer's disease
https://drive.google.com/file/d/1743EqQYaARzBYjNJVgYs3HnMM5lZgwT-/preview

Synaptic dysfunction in Alzheimer's disease – mechanisms and therapeutic strategies
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Aducanumab
https://www.biospace.com/article/a-stunning-surprise-biogen-and-eisai-s-aducanumab-works-in-alzheimer-s/

Aaducanumab clinical trial extension
https://news.joindementiaresearch.nihr.ac.uk/aducanumab-positive-results-alzheimer/

Aducanumab' – A breakthrough or not?
https://dementiasolutions.ca/aducanumab-breakthrough-or-not/

The antibody aducanumab reduces Aβ plaques in Alzheimer's disease
https://www.nextbigfuture.com/2016/09/the-antibody-aducanumab-reduces-plaques.html

A critical appraisal of amyloid-β-targeting therapies for Alzheimer disease
https://www.nature.com/articles/s41582-018-0116-6

PET scans May drastically change Alzheimer's diagnosis and care

https://www.everydayhealth.com/alzheimers-disease/amyloid-pet-scans-may-drastically-change-alzheimers-diagnosis-care-study-finds/
China approves 'Seaweed Drug' for treatment of Alzheimer's
https://www.beingpatient.com/seaweed-drug-china/
https://www.inkstonenews.com/science/alzheimers-drug-oligomannate-approved-china/article/3036238
Lifestyle and diet to prevent AD [2018]
https://www.nature.com/articles/d41586-018-05724-7
Junk food and Alzheimer's: Closer links than once believed
http://www.blog-thebrain.org/blog/2013/12/24/junk-food-and-alzheimers-closer-links-than-once-believed/
Memory loss in Alzheimer's reduced for the first time [2015] (diet etc.)
http://www.blog-thebrain.org/blog/2015/09/01/memory-loss-in-alzheimers-reduced-for-the-first-time/
Therapeutic targets of Alzheimer (acetylcholine and glutamate)
How does acetylcholine affect learning and memory [2014]
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Top 8 herbal remedies for Alzheimer's
http://www.searchherbalremedy.com/top-8-herbal-remedies-for-alzheimers-disease/
Tau-targeting therapies for Alzheimer disease
https://www.nature.com/articles/s41582-018-0013-z
NAP drug blocks formation of Alzheimer's neurofibrillary tangles
https://www.genengnews.com/topics/drug-discovery/nap-drug-blocks-formation-of-alzheimers-neurofibrillary-tangles/
Solanezumab drug trial failure: curtains for the beta-amyloid theory? [2017]
https://www.randombio.com/beta-amyloid-antibody.html
Boesenbergia rotunda/Chinese keys/fingerroot/Chinese ginger,
https://en.wikipedia.org/wiki/Boesenbergia_rotunda
What do we know about diet and prevention of Alzheimer's disease?
https://www.nia.nih.gov/health/what-do-we-know-about-diet-and-prevention-alzheimers-disease
Curcumin
https://alzheimersnewstoday.com/curcumin-plant-extract-possible-alzheimers-treatment/
Did you know one of curcumin benefits includes preventing Alzheimer's?
https://www.healtheuropa.eu/curcumin-benefits-preventing-alzheimers/90826/
Turmeric and dementia
https://www.alzheimers.org.uk/about-dementia/risk-factors-and-prevention/turmeric-and-dementia
The health benefits of huperzine A
https://www.verywellhealth.com/the-benefits-of-huperzine-a-89464
The benefits of ashwagandha; A natural way to fight Alzheimer's
https://www.alzheimers.net/ashwagandha-for-alzheimers/
7 best herbs and spices for brain health
https://www.verywellmind.com/best-herbs-and-spices-for-brain-health-4047818
Herpes and cold sores
https://www.healthline.com/health/skin-disorders/pimple-vs-cold-sore