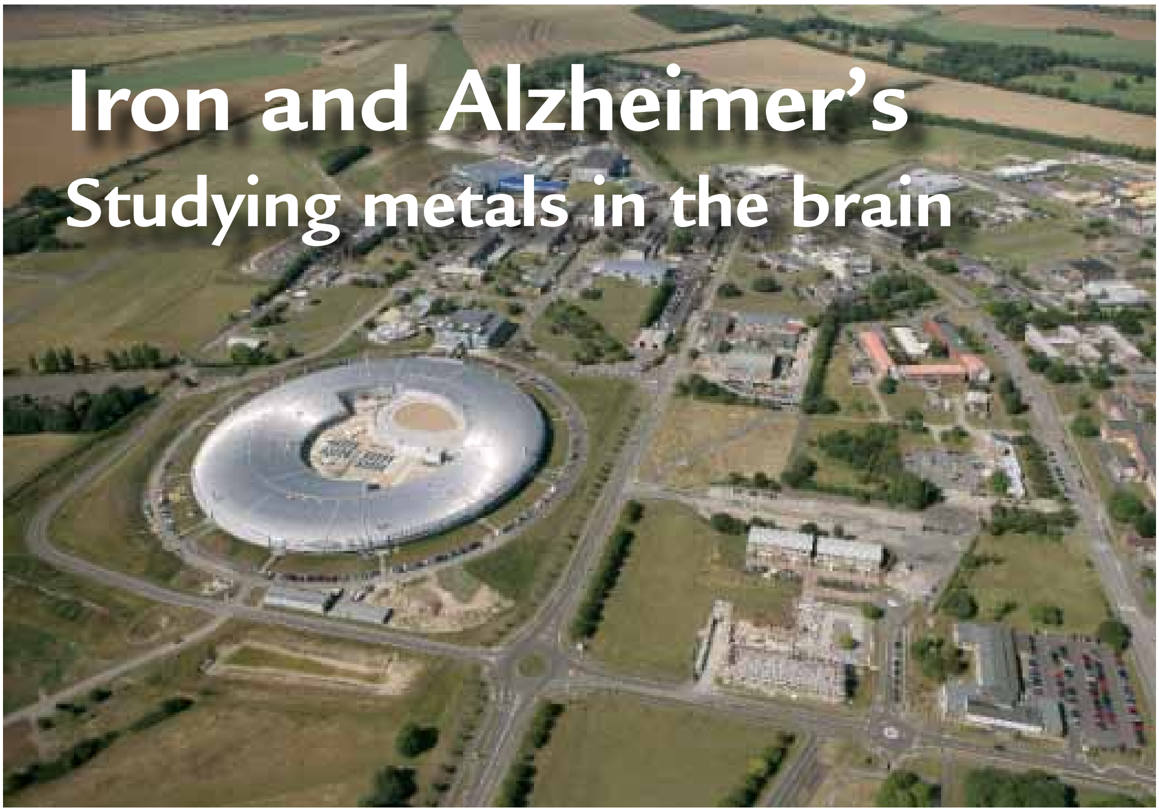
Iron and Alzheimer's
Studying metals in the brain



The Diamond Light Source is housed in this giant circular building in Oxfordshire.

Mary Finnegan

Key words

Alzheimer's disease

synchrotron radiation

X-ray fluorescence

*Could the presence of iron in brain cells help to explain why some people suffer from Alzheimer's disease, a form of dementia? **Mary Finnegan** of the University of Warwick hopes to shed some light on this question.*

The most common elements in the body are oxygen, carbon, hydrogen and nitrogen, but other elements including many metals are found at lower concentrations and are just as important. A well known example is iron which is a key component of haemoglobin in the blood, essential for transporting oxygen around the body. But iron is also important in cells, acting as a catalyst for many chemical reactions. Copper, zinc, potassium, manganese, magnesium and many others are also critical for our bodies to stay healthy. Most of these metals are at such low levels in the body, around 1 in a million or even 1 in a billion atoms, that they are known as trace metals.

Although these metals are essential, they can be harmful if the levels become too high or they are in a dangerous chemical form. To be used properly by the body iron needs to switch between the more reactive Fe^{2+} and the less reactive Fe^{3+}. However too much reactive Fe^{2+} can be toxic to cells and so proteins are used to transport iron around the body and store it as the less reactive Fe^{3+}.

If the way that metals are managed by the body is disrupted, this can cause disease. For example, subtle changes in iron and other metals have been observed experimentally in diseases such as Alzheimer's disease, and these changes may be involved in the disease process.

Alzheimer's disease

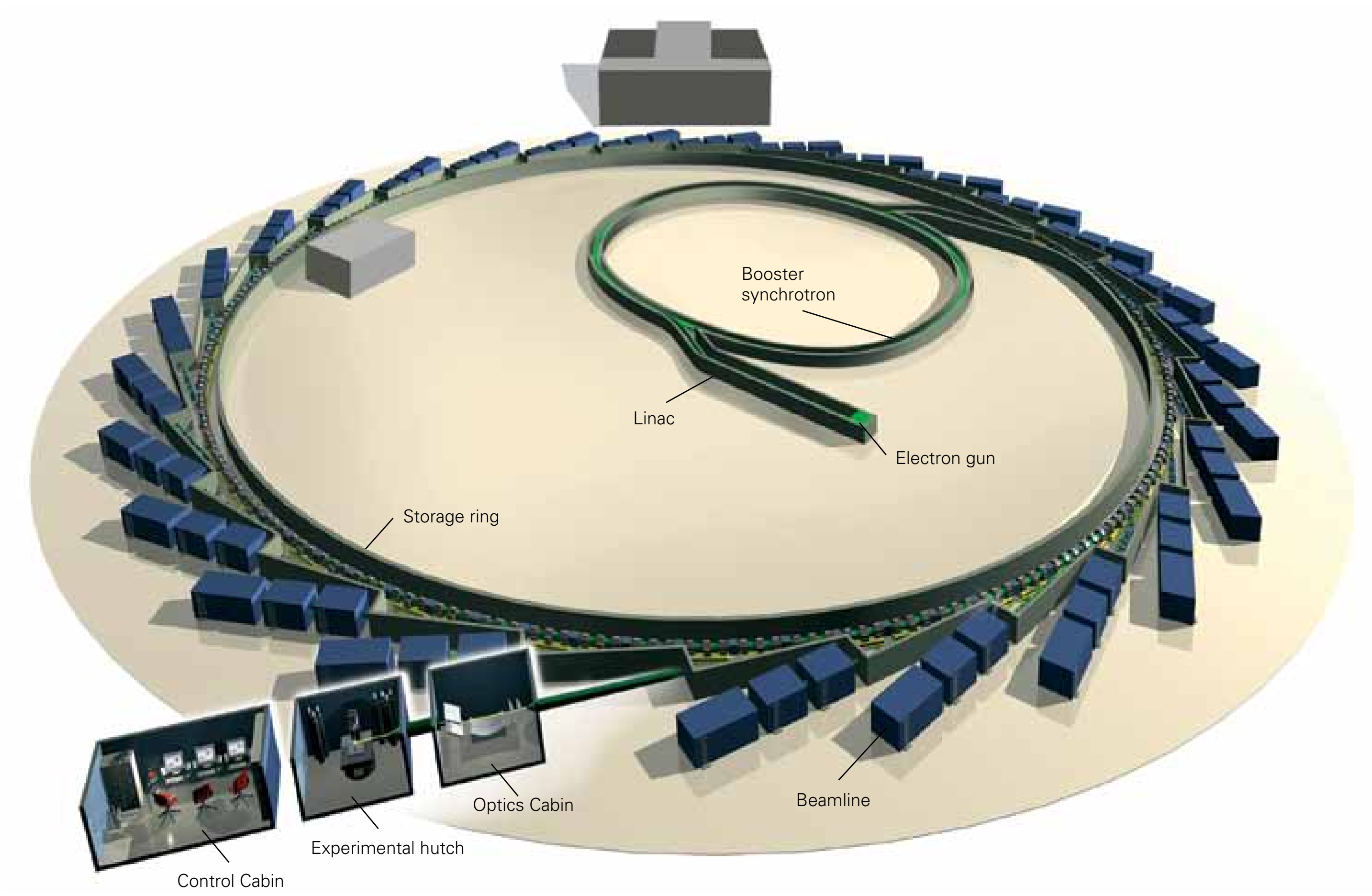
Alzheimer's disease affects around 465 000 people in the UK and the number of sufferers is growing as our population ages. It is a form of dementia that causes cell death in the brain and leads to memory loss and mood swings. Many people with Alzheimer's disease become unable to look after themselves. There is currently no cure for the disease and although we know many details about what is happening in the brain in Alzheimer's disease, the underlying causes – why some people get it and others don't – are not well understood.

Researchers have found small changes in the quantity of iron in some of the regions of the brain affected by Alzheimer's disease and also changes in the quantity of proteins that regulate iron. There has also been some work to observe what happens to other metals, such as copper and zinc, in the diseased brain compared to normal healthy brains of the same age.

However it is very difficult to measure changes in trace metals in the body as the quantities are so small. Traditional methods involve taking a block of tissue, dissolving it and using a spectrometer to measure the total concentration of the different metals in that block. However this does not provide any information about where the changes in the metals occur or what form the metal is taking. Is there more iron, copper, and/or zinc in the cells that are dying? Is there more reactive, and therefore more toxic, Fe^{2+} present in Alzheimer's disease than in people with healthy brains?

This is where the Diamond Light Source can help. Diamond is the UK's synchrotron and scientists can use the special X-rays produced at Diamond to detect metals in biological tissues.

Fe^{2+} and Fe^{3+} are iron ions, atoms which have lost 2 or 3 electrons. Fe^{2+} can be harmful.



This schematic diagram of Diamond shows the beam lines radiating out from the large accelerator storage ring.

The 'light' produced by the Diamond accelerator is not just visible light. It covers much of the electromagnetic spectrum, from infra-red to X-rays.

Specimen thicknesses are measured in micrometres (μm); 1 μm = 10^{-6} m

X-ray energies are measured in electron-volts (eV); 1 eV = 1.6×10^{-19} J

Diamond Light Source

A synchrotron is a particle accelerator designed to produce very bright light. There are four steps to producing the synchrotron light at Diamond:

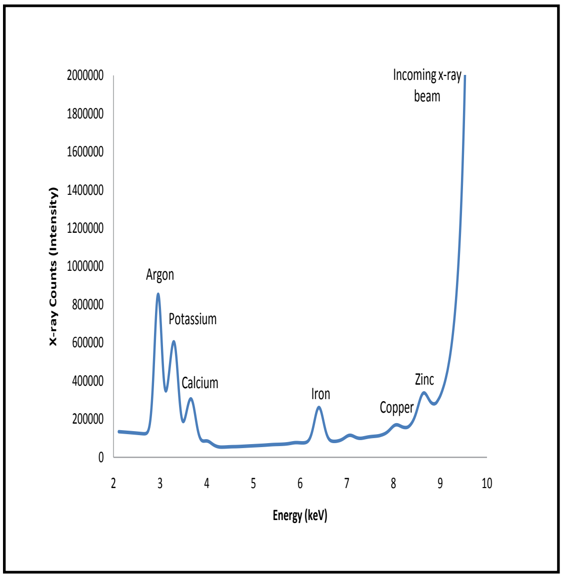
- Electrons are produced by an electron gun, and a linear accelerator (linac) uses high voltages to accelerate the electrons.
- These electrons are fed into a small booster synchrotron and accelerated until they are travelling close to the speed of light.
- The electrons are now passed into the storage ring of the main synchrotron. The storage ring is not actually a circle, but a pentagon made up of straight lines. At the junction of these straight sections are bending magnets which change the direction of the electrons and cause them to give off energy in the form of light.
- This light can be channelled out of the storage ring, at points called beamlines, and used for experiments.

Different bending magnets mean that Diamond can produce light with a range of wavelengths from infra-red, to visible, to ultra-violet to X-rays. The synchrotron light is extremely bright – up to a 100 billion times brighter than the sun – and can be tuned to a very narrow range of wavelengths (energy) at each beamline, depending on what experiments are to be carried out.

How synchrotron light can 'see' metal in tissues

To detect metals in biological tissues scientists can make use of a phenomenon called X-ray fluorescence. X-ray fluorescence occurs when high energy X-rays lose some of their energy as they strike atoms in the sample. The atoms emit lower-energy X-rays. The useful thing is that each element emits X-rays of a specific energy so, by measuring the energies of these X-rays, we can find out which elements are present.

Post mortem brain tissue, left to medical research by someone who had Alzheimer's disease, is cut into thin sections of around 30 μm thick and X-ray fluorescence is used to look for metals in the tissue. The beam of X-rays is tuned to an energy of 10 000 eV and focused to a square spot size of between 60 and 3 μm. The X-ray beam scans across the sample and a detector collects the X-rays emitted at each point. The detector can detect X-rays across a wide energy range and so produces a spectrum of emitted energies. The spectrum has peaks where there is an element fluorescing at that energy. For example, iron fluoresces at an energy of 6403 eV so if there are X-rays detected at this energy we know iron is present. By looking at how the intensity (brightness) of the X-rays at 6403 eV changes across the sample, the variation in iron concentration can be observed.



The X-ray fluorescence spectrum of brain tissue has peaks which show which metal elements are present.

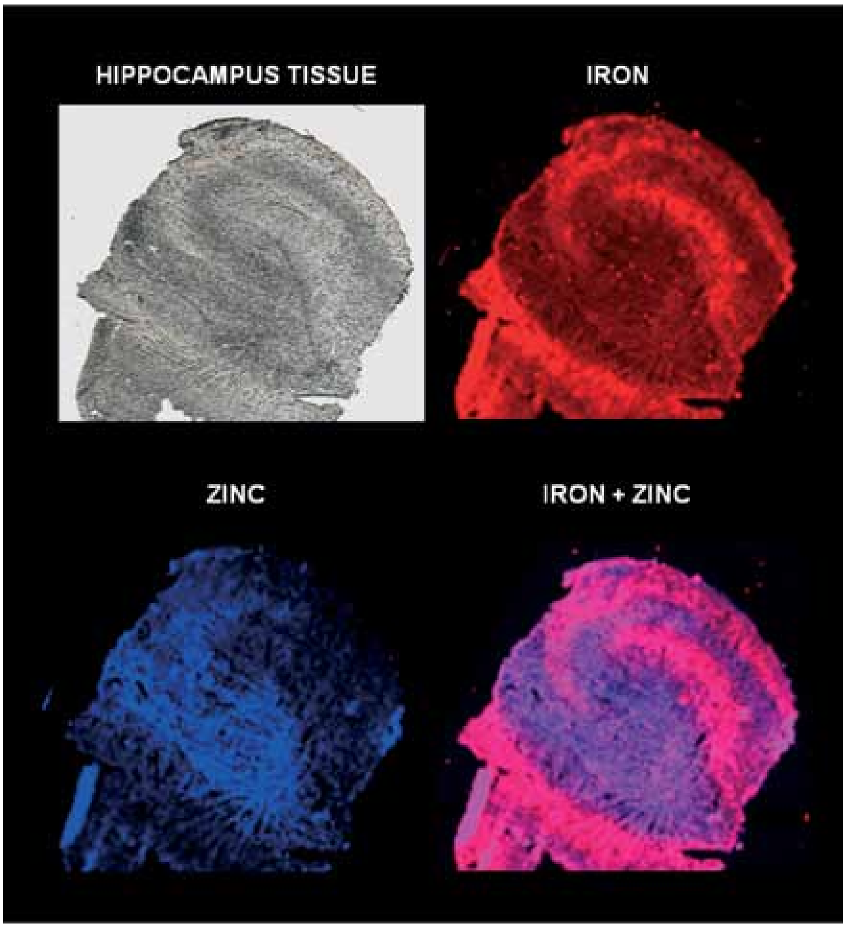
Sample preparation

The way the samples are prepared is very important as this technique is very sensitive and any contamination on the sample could give false results or 'shine' much more brightly than trace metals in the tissue, masking their signal. The tissue must be cut in a very clean environment with a non-metal knife. The section is mounted onto a slide made of quartz – glass slides cannot be used for most of these measurements as glass often contains randomly distributed inclusions of the metals being examined.

The elements that can be detected by this technique depend on the energy of the X-ray beam focused on the sample. With an electron beam of 10 000 eV, zinc, copper and iron can be detected. Maps of these different elements can be created to compare the distribution of the metals in the tissue. For example, in X-ray fluorescence maps from the hippocampus, a region of the brain important in memory that is badly affected in Alzheimer's disease, does the iron distribution vary across the cell population in the same way zinc does?

Choosing the spot size is also important. With a bigger spot size a larger area of tissue can be mapped, but many cell bodies in the brain are around 10 μm in diameter so choosing a smaller spot size may allow the metals to be pin-pointed to specific cells. Experimental time at Diamond is very limited for the scientists who get to use it, maybe only a few days a year, so it is important that experiments are planned carefully!

X-ray fluorescence mapping will detect atoms of an element no matter what chemical form it is in and no matter what other elements it is bound to. However, at Diamond the way the tissue absorbs X-rays can also be measured to provide information about what form a metal is in. For example is iron in the safer Fe^{3+} form that we expect the body to store it in? Or is there evidence for the more toxic Fe^{2+}?



These 'maps' show the distribution of iron and zinc in a section of brain tissue from a person who had Alzheimer's disease.

Understanding Alzheimer's

Without the intensity of synchrotron X-rays it would be extremely difficult to detect these metals while maintaining the spatial information. By studying the changes in iron and other metals in Alzheimer's disease compared to people who died with a healthy brain, researchers hope to improve understanding of the disease that will hopefully lead to better treatment and ways of diagnosing Alzheimer's disease.

Looking at iron in the Alzheimer's diseased brain is not the only example of how synchrotron X-rays are being used in medical research. Some other examples include looking at metal changes in other brain diseases, such as Parkinson's disease and looking at inflammation around metal implants such as hip replacements.

Mary Finnegan is studying for a PhD in biomedical engineering at the University of Warwick.

Look here!

Health Q&A video at *www.youtube.com/watch?v=MTZyUlfBrlA*

The Alzheimer's Society funds Mary's research: *www.alzheimers.org.uk*

More about how Diamond works: *www.sep.org.uk/catalyst/articles/catalyst_16_1_255.pdf*