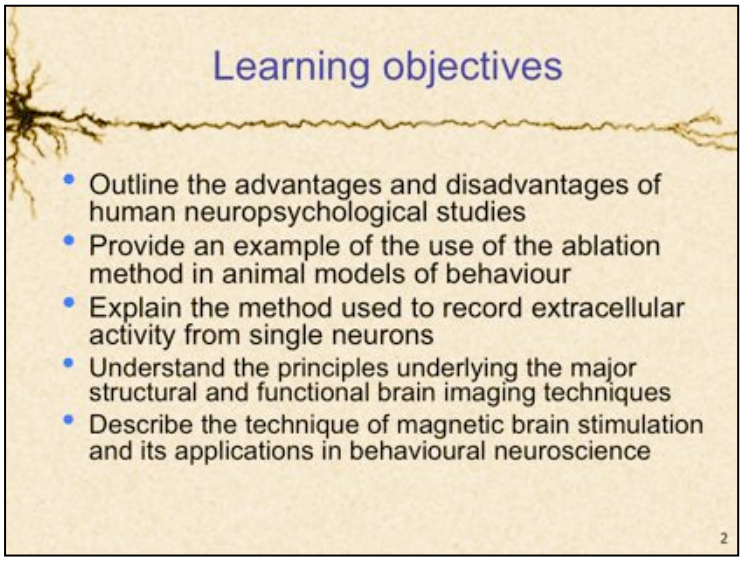
Mind, Brain & Behaviour 1
Behavioural Neuroscience

Methods for studying the living brain


Learning objectives
Outline the advantages and disadvantages of human neuropsychological studies
Provide an example of the use of the ablation method in animal models of behaviour
Explain the method used to record extracellular activity from single neurons
Understand the principles underlying the major structural and functional brain imaging techniques
Describe the technique of magnetic brain stimulation and its applications in behavioural neuroscience
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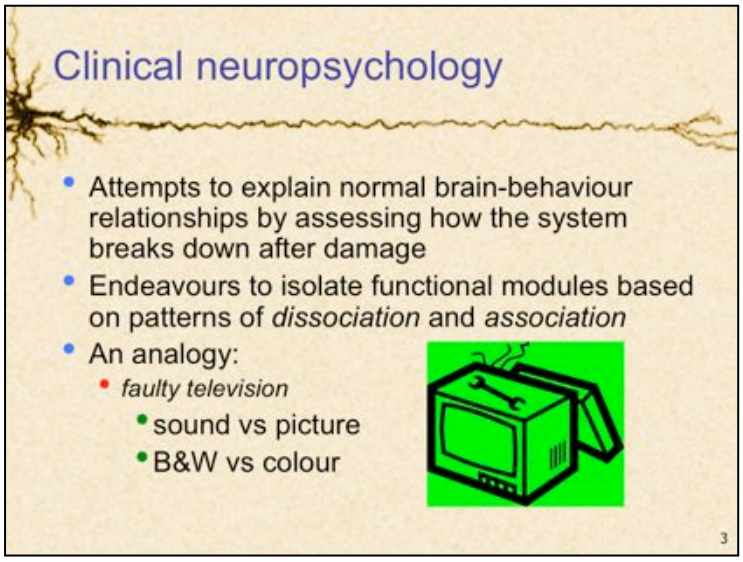
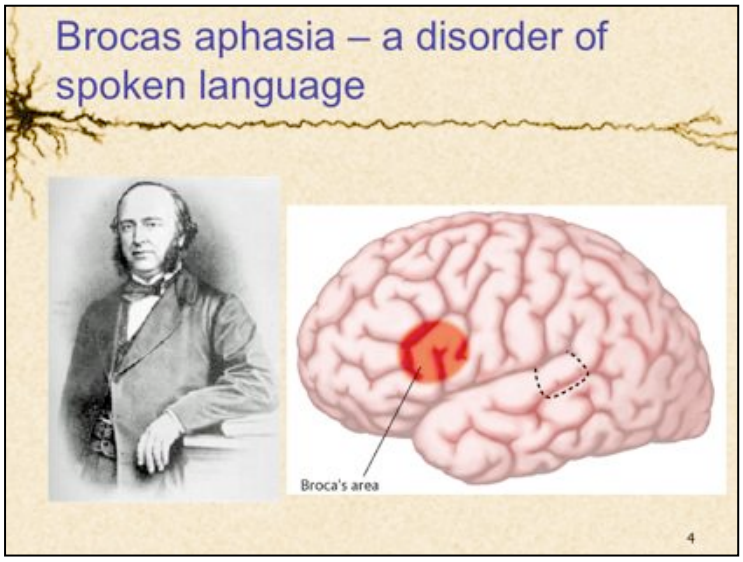


As we saw in Lecture 1, **clinical neuropsychology** is a discipline closely allied with Behavioural Neuroscience.

In addition to helping with the diagnosis, management and treatment of patients with neurological disorders, an important goal of clinical neuropsychology is to explain normal brain-behaviour relationships by assessing how the system breaks down after damage.

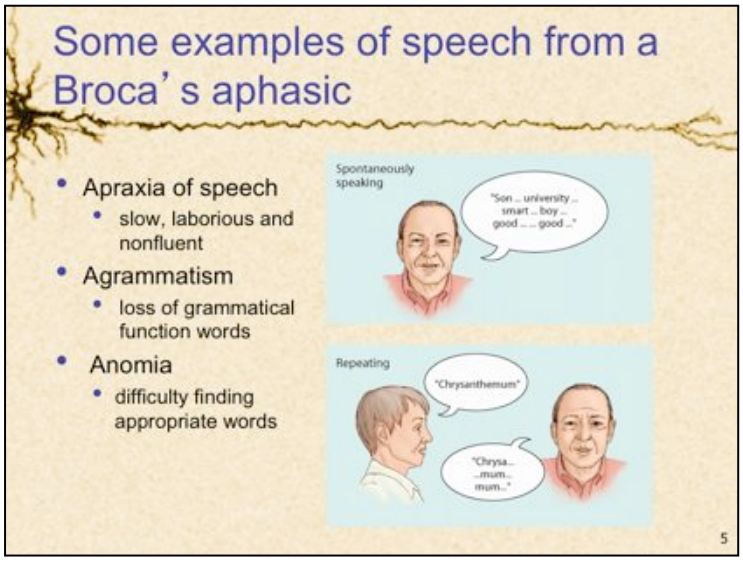
A key principle underlying the approach of clinical neuropsychology is to establish patterns of **association** and **dissociation** in the behaviour of brain damaged individuals. This provides a way to describe the **functional modules** of perceptual and cognitive processing that have been affected by the damage, and those that have been spared.

Consider an analogy with faulty television sets. We may encounter a TV that no longer displays a picture, but in which the sound remains normal, and another TV that has the reverse problem – a normal picture but no sound. Without needing to open up the sets to examine their electrical components, we can deduce that picture and sound must be controlled by separate processes, because either function can be damaged in isolation, leaving the other intact.



One of the earliest examples of the contribution of clinical neuropsychology to the understanding of brain function began with the work of Paul Broca, the French physician who we met in Lecture 1.

Recall that in 1861 Broca described two patients who had lost the ability to communicate through speech, even though they could make lip, tongue and mouth movements normally (indicating that they were not suffering from facial weakness or paralysis), and could understand what was said to them. Both patients had lesions in the left hemisphere, located in the posterior part of the inferior frontal gyrus. Broca concluded that this region must be responsible for expressive language. We now call this region of the left hemisphere **Broca's area**, and the language impairment that results from damage to it **Broca's aphasia**.



In fact most people with Broca's aphasia are able to communicate to some degree, but their pattern of speech is slow, laborious and nonfluent. The person with Broca's aphasia still has thoughts and ideas, but cannot express them normally. Broca's aphasia is therefore sometimes called **nonfluent aphasia** or **expressive aphasia**. Patients with Broca's aphasia are aware of their problems and understandably become very frustrated in their attempts to communicate.

Some types of words are easier for a Broca's aphasic patient to say than others. They have particular difficulty with **grammatical function words** (e.g., a, the, some, in, about), so-called because they have important grammatical functions. By contrast, Broca's aphasic patients may be able to say some words that convey meaning, also known as **content words** (e.g., boy, smart, university).

Patients with Broca's aphasia also have some other characteristic problem in producing speech:

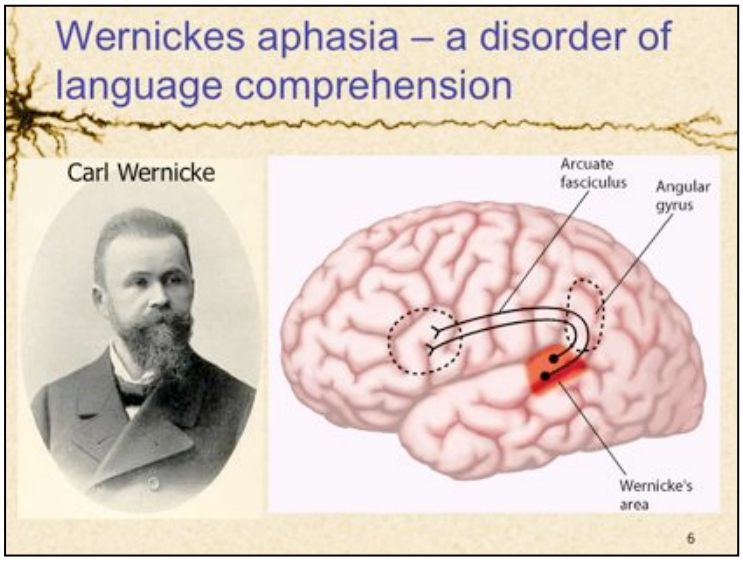
Agrammatism – problems in comprehending or using grammatical constructions (e.g., the use of *–ed* at the end of words)

Anomia ('without name') – a difficulty in finding (remembering) the appropriate word to describe an object

Apraxia of speech – an impairment in the ability to program movements of the tongue, lips and throat required to produce the proper sequence of speech sounds

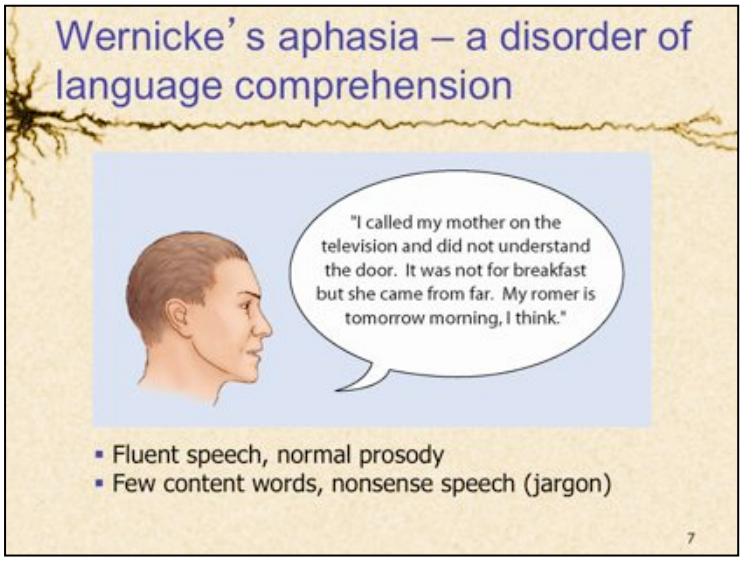
Different patients will exhibit these various problems to differing extents depending on the location and size of their brain lesion.

Since the time of Broca it had been thought that such aphasic patients retained normal comprehension of speech. However, it has become clear that comprehension is also compromised to some extent in these individuals, especially for complex sentences that involve many components. For example the agrammatism of Broca's aphasia may cause difficulties in using word-order to interpret meaning.



Not long after Broca described his cases another physician, **Carl Wernicke**, described some patients who had lost the ability to comprehend speech, even though they were not deaf. These individuals remained fluent in their speech (unlike Broca's cases), though much of what they said was meaningless. These individuals also had damage to their left hemisphere, but in a location posterior to Broca's area, in the **posterior part of the superior temporal gyrus**.

Wernicke proposed that the region damaged in his patients must mediate our ability to comprehend the meaning of words. We now call this region **Wernicke's area**, and the comprehension deficit that follows damage to it is called **Wernicke's aphasia**.

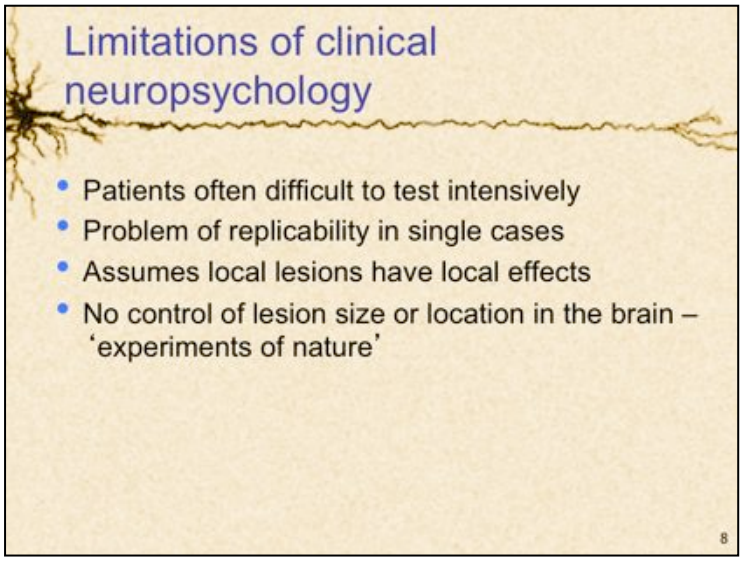


Unlike patients with Broca's aphasia, speech in Wernicke's aphasia is fluent and unlaboured; patients do not hesitate in finding words and have no apparent problem articulating them.

The patient will use grammatical function words, and the melody or **prosody** of speech remains normal (the rising and falling tones we use in speech to help convey meaning and emotion). However, the patient with Wernicke's aphasia may use few content words, and often will insert nonsense words or jargon.

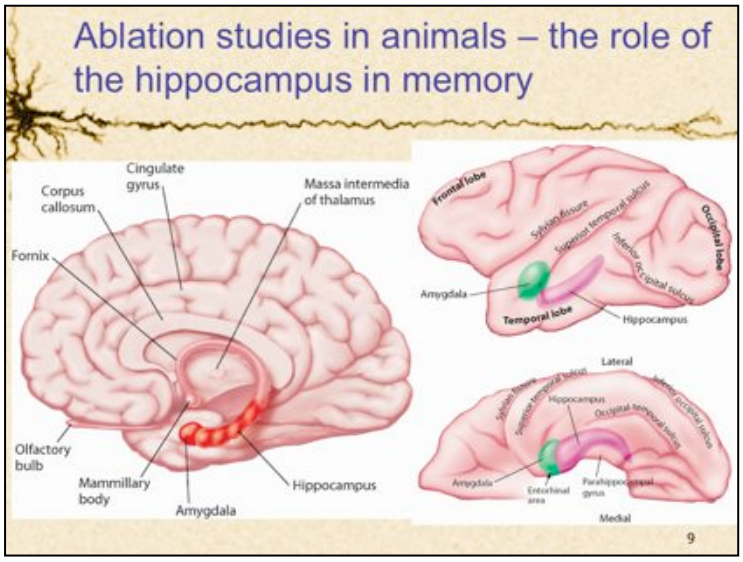
Remarkably, patients with this kind of language disorder are often **unaware** of their impairment, perhaps because their comprehension deficit prevents them from realising that their speech is faulty.

The discovery of two distinct types of language impairment following damage to two different cortical areas of the left hemisphere is a good example of the contribution of clinical neuropsychology to our understanding of normal brain function. The fact that there is a dissociation between processes associated with speech production and those associated with comprehension suggests that these abilities represent distinct functional modules of the mind. These functional modules also happen to be subserved by distinct regions of the brain, though this need not always be the case.



In addition to its many strengths, clinical neuropsychology also has some weaknesses:

1) Patients with brain lesions may have medical problems that prevent them from being tested intensively
2) Since brain lesions are 'accidents of nature', no two patients will ever have identical brain pathology. It is therefore difficult to replicate experimental findings in other cases.
3) A key assumption of the neuropsychological method is that lesions only affect the functions of the tissue that has been destroyed, leaving the functions of other areas unaffected. This is probably never true. Brain lesion can cause swelling in distant brain sites that are not directly destroyed. Moreover, because all areas of the brain are connected with many other areas, damage to one structure will change the inputs and outputs of many other regions.
4) Because human cases are 'experiments of nature', it is not possible to control the size or location of damage. Many patients have extensive lesions or diffuse damage that makes it impossible to link a particular area of the brain with a particular function.



In an attempt to overcome the problem of being unable to specify the location and extent of damage in human neuropsychological cases, some researchers have turned to other animal species.

One approach has been to make small lesions in distinct parts of the brain of these animals and to observe the effects the lesions have on behaviour. This method is called experimental **ablation** (meaning 'to carry away'), and it may be achieved surgically, by using radiofrequency energy, or by injecting toxic chemicals to destroy the neurons in a particular area. All of these procedures are invasive, and so must be carried out according to strict ethical guidelines and, in most cases, under anaesthesia.

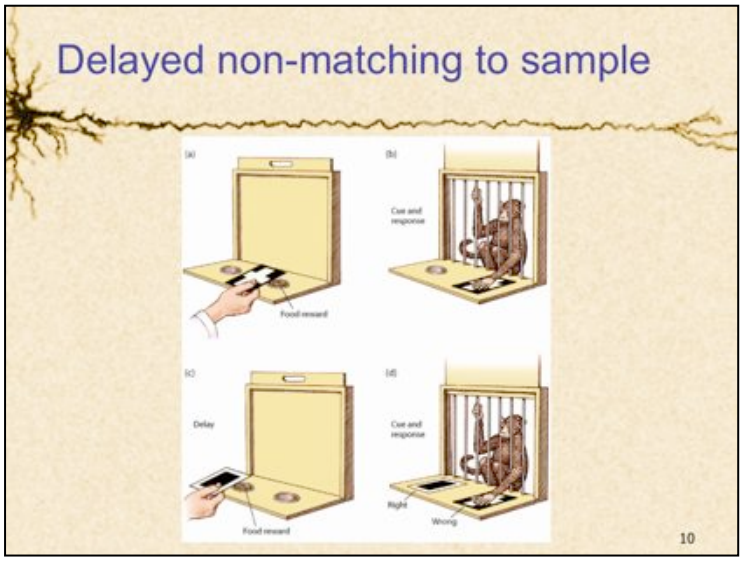
The main advantage of ablation methods in animals is that the location of the lesion can be determined with a fairly high degree of precision.

One example of the usefulness of the ablation method has been in determining the role of parts of the limbic system, and in particular the hippocampus, amygdala and surrounding cortex, in learning and memory.

Recall the case of H.M., the man who had his medial temporal lobes removed bilaterally for relief from epileptic seizures. He became densely amnesic following his operation, which led researchers to postulate that the medial temporal lobe, which includes the hippocampus and amygdala, may be critical for learning and memory.

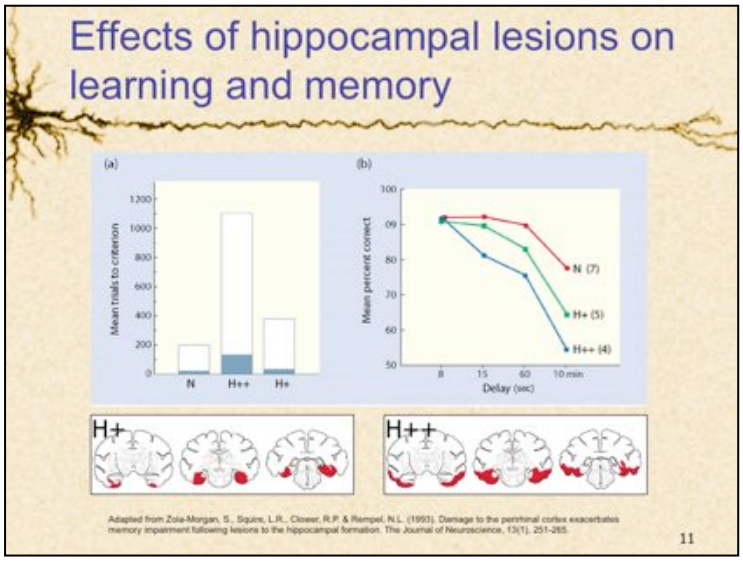
Obviously it would not be ethical to test this hypothesis by conducting more such operations in other humans to see what would happen!

Instead, why not use a closely related animal species (in this case macaque monkeys), surgically remove the medial temporal structures, and perform careful behavioural tests of the effects of the removal on learning and memory?



One experimental task that is often used to test learning and memory in monkeys is called the **delayed non-matching to sample task**.

The monkey is separated from two food wells by an opaque screen. Initially the experimenter hides a food reward in one of the wells by covering it with a card with a distinctive symbol. The screen is raised and the monkey is shown the correct response, which allows him to retrieve the reward. The screen is then lowered again and a food reward hidden beneath a card with a *different* symbol to that used in the first trial. After a delay, during which the monkey must remember the two symbols, the screen is raised again, and the monkey must choose which of the two cards the reward is hidden beneath. After just a few trials a normal monkey will learn the simple rule of the task: after the delay the food will always be hidden under the card that was NOT rewarded on the first trial (hence the task's odd name 'non-matching to sample').



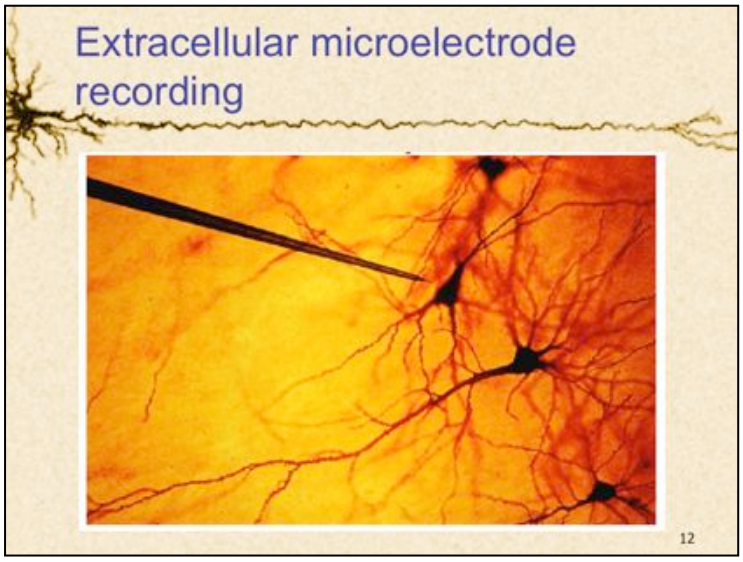
Monkeys with surgical ablation of the medial temporal lobes bilaterally take much longer to learn the non-matching to sample task than unoperated control monkeys, and this impairment is greater for monkeys whose lesions are more extensive (H++) than those with relatively smaller lesions (H+).

In addition, monkeys with surgical ablation of the medial temporal lobes make relatively more errors in their choices of food well as the delay between them learning the correct response and having to make their choice increases, compared with normal monkeys. This impairment of memory is also greater for monkeys with more extensive lesions.

The results of this study illustrate the use of the ablation method to answer an important question about the role of a particular region of the brain in a specific domain of cognition (learning and memory).

But ablation methods also have their limitations. For example, the experimental lesion might still affect distant structures in the brain, just as is the case for the naturally occurring lesions in humans. Also, there are many important aspects of cognition that are difficult or impossible to test in other species (e.g., language and complex reasoning).

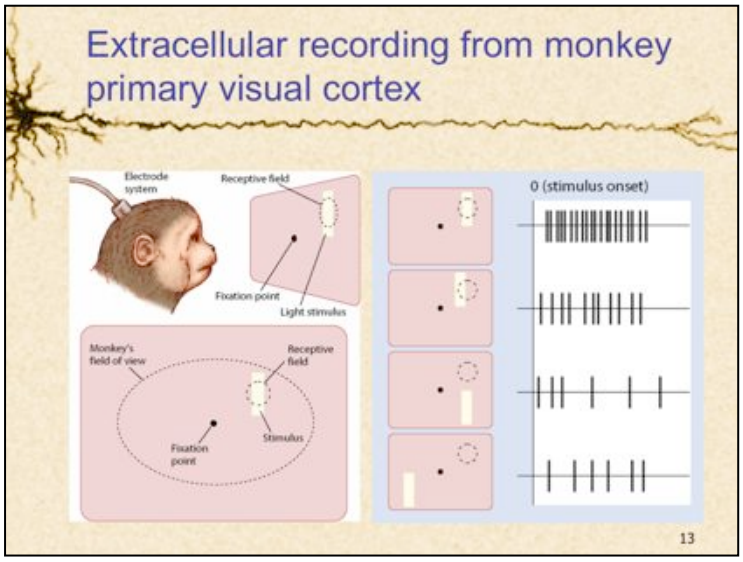
Zola-Morgan, S., Squire, L.R., Clower, R.P. & Rempel, N.L. (1993). Damage to the perirhinal cortex exacerbates memory impairment following lesions to the hippocampal formation. The Journal of Neuroscience, 13(1), 251-265.



Another technique used in animals involves recording from single neurons using a fine **microelectrode** that is sensitive enough to record electrical potentials. This technique is sometimes called **single-unit recording** (a unit refers to a single neuron). In order to lower an electrode into position the animal must first be anaesthetised and undergo surgery to remove a small window in the skull and meninges.

In some cases the recording of electrical potentials is done while the animal remains under anaesthetic, but this approach is obviously limited in scope since the animal is unable to perform any meaningful task. In other cases the electrode is mounted on the skull using dental acrylic (a kind of plastic), the wound is surgically closed, and the animal is allowed to recover. With this approach the electrode remains in place over an extended period, and many recordings can be taken while the animal is fully conscious and performing a particular task.

The electrical signal recorded by a microelectrode is very small and so must be amplified many times. In **extracellular recording**, the microelectrode is located in the extracellular space. One limitation of this method is that it is not possible to guarantee that the electrical signal recorded by the electrode is from a single neuron; it could in fact come from several. One way around this limitation is to place a microelectrode inside the cell body of a neuron. Given how tiny a single neuron is, such **intracellular recording** is technically very difficult and few laboratories have the facilities to accomplish it.

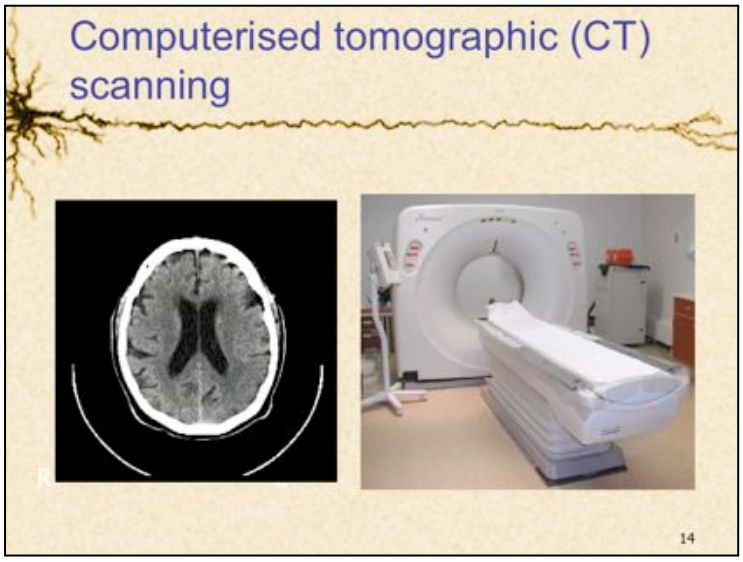


The aim of most experiments that involve single-unit recording is to determine the manipulations that will produce the most consistent change in a neuron's firing rate. (Recall from Lecture 2 that neurons convey information primarily through the rate at which they produce action potentials or **spikes**.) All neurons have a baseline or **resting rate** of spontaneous spiking activity, so the goal of single-unit recording is to look for alterations in this baseline level of activity that are correlated with a particular stimulus or task. Both increases and decreases in the baseline rate of firing of a neuron are informative.

Single-unit recordings have been made in virtually all regions of the brain, but one region that has been a particular focus is the **visual cortex**. Single neurons in this region respond to different visual properties, such as shape or colour, or whether the stimulus is moving or stationary. One particularly important attribute is the **location** of the stimulus. Most neurons in the visual cortex only respond to stimuli that appear in a limited region of space, an area known as the neuron's **receptive field**. Thus, some neurons may respond most vigorously when a light bar appears in the top right corner of the visible field, whereas others may respond best when the bar appears in the lower left corner.

Receptive fields of neurons in the primary visual cortex tend to be fairly small, whereas those in 'higher' areas (e.g., in regions of association cortex) can be much larger (often encompassing the whole of the left or right visible field).

In primary visual cortex, adjacent neurons tend to have overlapping receptive fields. A representation of external space is reflected in a continuous map across the cortical surface, yielding a topographic (**retinotopic**) representation of space. Damage to a small region of visual cortex results in blindness for the corresponding location of the visual field.



Studies of humans and animals with brain lesions, and experiments involving single-unit recordings, have yielded many significant insights into the relationship between brain and behaviour.

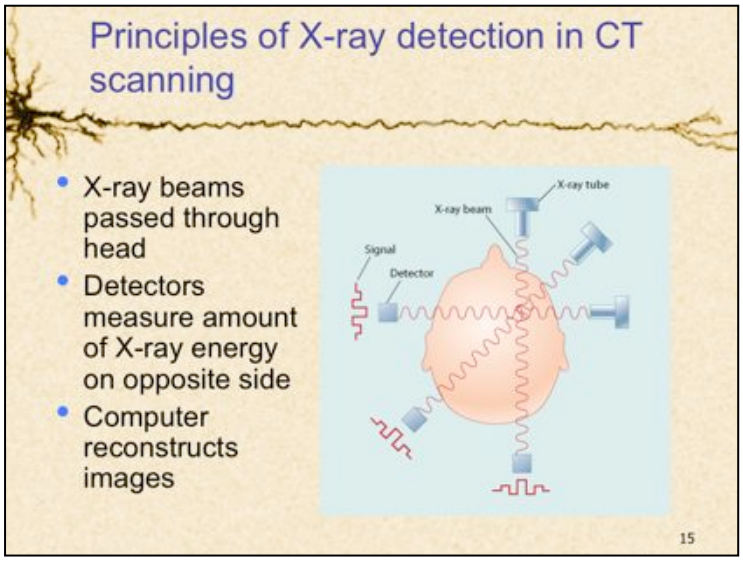
In the last few years, some of the most profound advances in the field of Behavioural Neuroscience have come from experiments that have provided images of the living human brain.

These techniques can be divided into two broad categories. **Structural brain imaging** techniques provide a static 'snapshot' of the **anatomy** of brain, very much like an X-ray is used to reveal whether a bone is broken. **Functional brain imaging** techniques reveal which areas of the brain are **metabolically active**, either at rest or as a person is performing a particular cognitive task.

We shall consider the main structural imaging techniques first.

Computerised tomographic (CT) scanning was first introduced commercially in 1983. It is used widely in medical settings, not just for providing images of the brain but for assessing the integrity of all other parts of the body as well. In the field of neurology, CT scanning is used to delineate the site and extent of brain injury or disease. For example, CT scanning may be used to show a suspected cerebral tumour, a region of damage caused by a stroke (rupture or blockage of an artery in the brain), or brain atrophy (shrinkage) due to Alzheimer's disease.

Here a CT scan shows the brain of an adult with a stroke-induced lesion in the left temporoparietal region, which caused a fluent (Wernicke's) aphasia. The flickering time-lapse shows the lesion 'evolving' over a 5 day period.

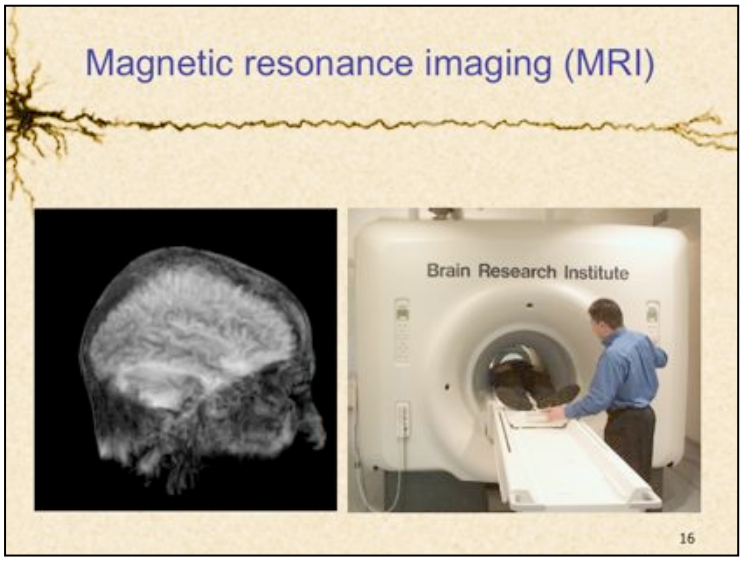


CT scanning is based on the same principles as X-ray imaging, except that whereas a conventional X-ray shows 3D structures as a flat 2D image, CT scanning allows a series of 2D ‘slices’ of to be reconstructed into 3D space.

In CT scanning, a highly focused X-ray beam is passed through the head. A radiation detector located directly opposite the beam picks up the amount of X-ray energy that emerges from the other side of the head. The X-ray source and the detector can be rotated around the head, thus allowing X-rays to be passed through the head from all angles.

The technique is based on the principle that tissues of different density absorb different amounts of X-ray energy. Bone, for example, absorbs a relatively large amount of X-ray energy, and is thus said to be radio-opaque. Cerebrospinal fluid, on the other hand, absorbs relatively little X-ray energy because it is a liquid. All other tissues have absorption characteristics that lie somewhere between these two extremes. A computer analyses the amount of energy in the detectors as they rotate around the head, and uses this information to reconstruct an image.

On a typical CT scan, bone appears as white, CSF appears black, and the grey and white matter of the brain appear as different shades of grey.

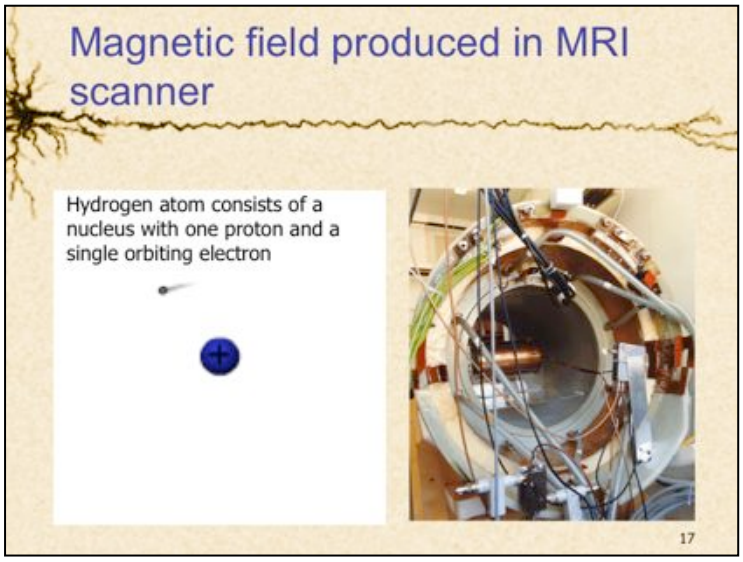


One limitation of CT scanning is that the images do not differentiate well between grey and white matter. This is because these two tissue types absorb very similar amounts of X-ray energy. Another limitation is that CT scans have a relatively poor **spatial resolution** (ability to discriminate fine structure). Using CT scanning it is not possible to discriminate two structures that are closer than about 5 mm.

Although CT scans are still widely used in clinical practice, for most purposes they have been superseded by **magnetic resonance imaging (MRI).**

Instead of using X-rays, MRI exploits the magnetic properties of brain tissue. The individual is placed in a tube that creates an extremely powerful magnetic field, up to several thousand times more powerful than the earth's magnetic field. Magnetic field strength is measured in tesla. The earth's magnetic field is about 1/1000 tesla, whereas the field created by an MRI scanner is between 1.5 and 4 tesla.

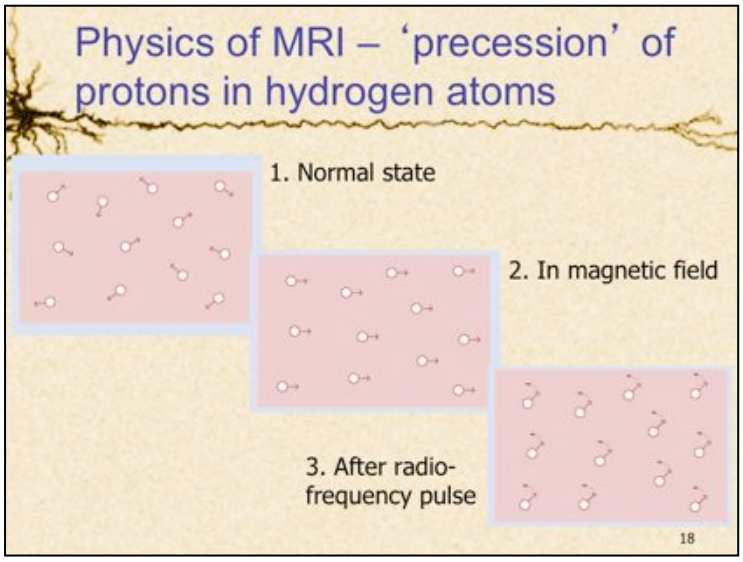
As can be seen in this example, MR scans provide a much more detailed reconstruction of the brain than CT scans. How?



The principle behind MRI is the enormous superconducting magnet inside the scanner itself. The magnet is constantly cooled with liquid helium to prevent it melting the entire machine and anyone located inside it!

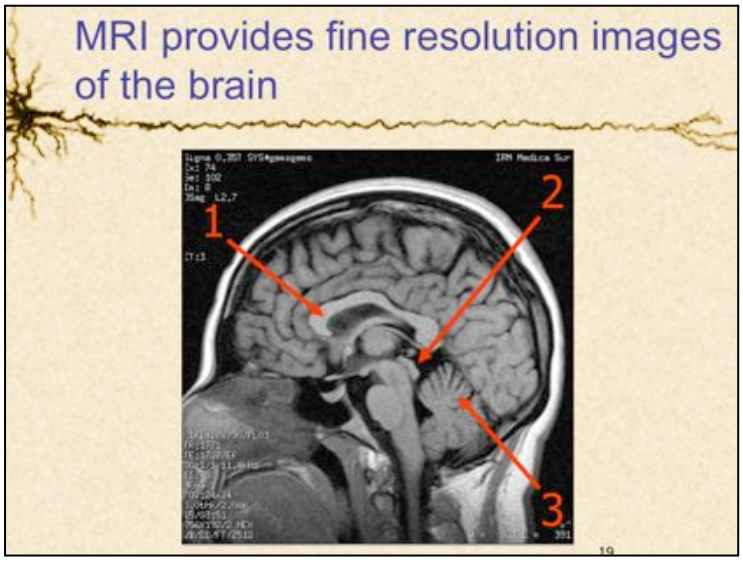
MRI is sensitive to the behaviour of **protons in the nuclei of hydrogen atoms**. Hydrogen is pervasive in all living tissue.

Protons in the nucleus of hydrogen atoms have a characteristic motion ('spin'). This motion creates a tiny magnetic field.



In the earth's very weak gravitational field, the orientation of protons is random. When the body is placed in the strong magnetic field of an MRI scanner, however, the protons align in a direction that is parallel with the magnetic field. Because the protons are now all spinning around a common axis, the magnetic field they produce is strong enough to be measured.

During MRI scanning, a brief **radio-frequency (RF) pulse** is passed through the head. The energy in the RF pulse perturbs the axis of spin of the protons. Upon termination of the RF pulse, the protons gradually relax back into the orientation of the static magnetic field. This synchronised relaxation of the protons produces energy that is picked up by detectors surrounding the head. The outputs of these detectors are used by a computer to reconstruct a 3D image, which reflects the distribution of protons and other magnetic agents in the tissue.



MRI provides images with a high spatial resolution. It can discriminate structures down to around 1 mm, allowing clear visualisation of the cortex and the pattern of gyri and sulci. MRI also provides excellent differentiation between grey and white matter, because the density of protons is much greater in grey matter.

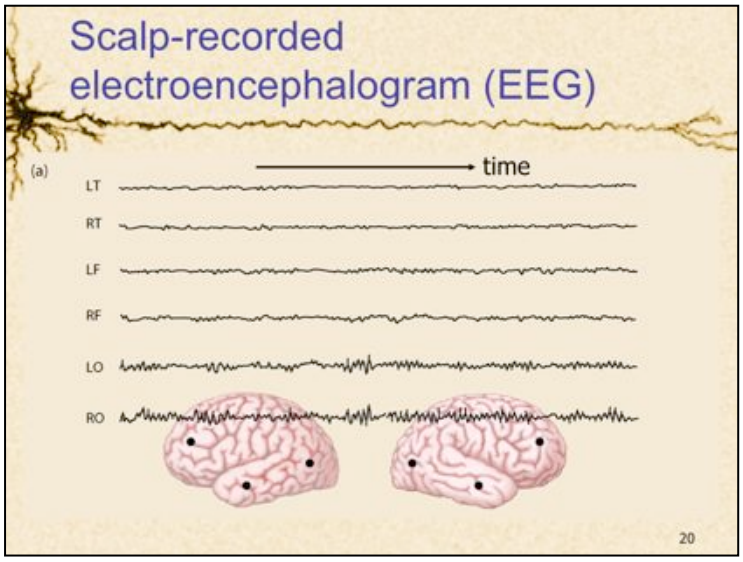
Can you identify the structures indicated by the arrows (1-3)?

Clues:

1 – see Slide 6, Lecture 5

2 – see Slide 10, Lecture 5

3 – see Slide 12, Lecture 5



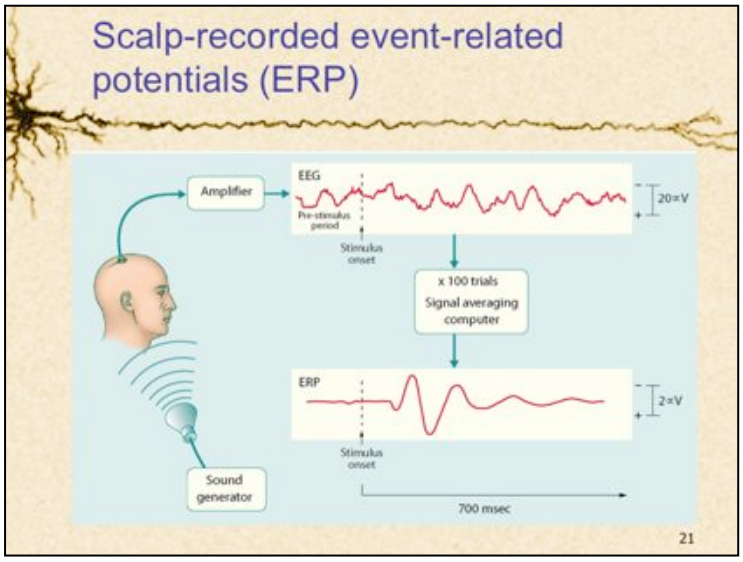
Structural brain imaging techniques such as CT and MRI are particularly useful for detecting brain abnormalities, but they reveal nothing about the underlying function of brain areas. Given the limitations of studies of the effects of brain lesions in humans and animals, it would clearly be useful to have brain imaging techniques that told us something about brain **function** as well as structure.

In recent years several methods have been devised to map the brain regions that are selectively active during performance of perceptual, cognitive and motor tasks. These are known as **functional brain imaging** methods, and we shall consider some of them here.

Functional brain imaging methods measure the electrical or metabolic activity from local areas of the brain. The assumption underlying these methods is that when an area is involved in a particular function or task, it will become more active than when it is not involved.

Electroencephalography (EEG) measures the weak electrical signals produced by the brain. This is achieved by placing electrodes at one or more points on the surface of the scalp. The electrical signals detected by the electrodes are sent to an amplifier and displayed on a computer monitor or printed onto paper. The characteristic waveforms indicate the strength and rhythmicity of electrical activity in the brain.

EEG has good temporal resolution (i.e., it can discriminate very brief events in time), but poor spatial resolution. With more electrodes the spatial resolution can be improved, but it still difficult to determine precisely from which area of the underlying brain the signal has come.

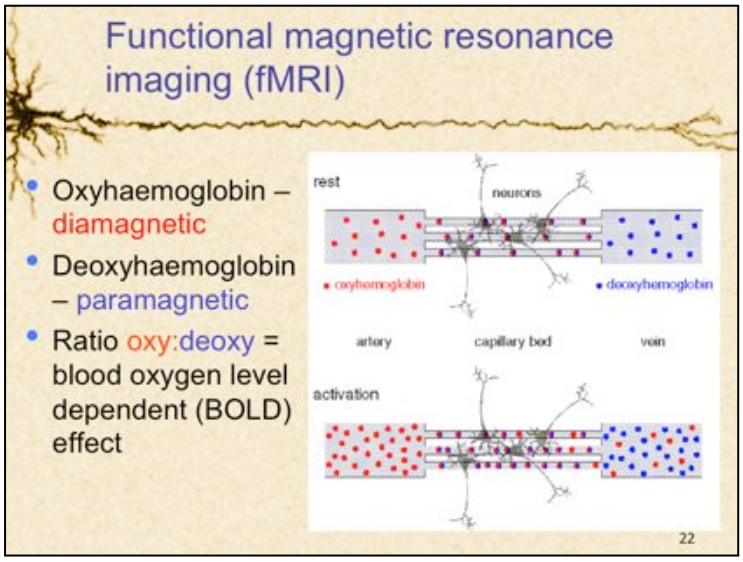


A simple modification of the EEG can reveal details about the brain's **evoked response** to a stimulus. Rather than examining a single continuous trace of electrical activity recorded from a scalp electrode, many such traces can be recorded relative to an external event. For example, we might record the pattern of electrical activity from an electrode for a few hundred milliseconds after a brief sound is played. We can repeat this sound many times over, recording the electrical response from the brain for just a few hundred milliseconds each time.

Although the signal recorded in an EEG is very 'noisy' (i.e., has lots of peaks and troughs), by aligning the electrical trace from many presentations of the auditory event any noise (which by definition is random) gets cancelled out, leaving just the electrical trace specifically associated with the sound. The resulting waveforms are known as **event-related potentials (ERPs)**, and they provide a snapshot of the electrical activity in a region of the brain associated with the processing of a stimulus or the preparation of a response.

As with the EEG, ERPs have a very good temporal resolution, but a fairly poor spatial resolution.

Clinically the ERP can be very useful in diagnosing neurological conditions. For instance, in multiple sclerosis the ERP associated with the presentation of a visual test pattern is delayed, reflecting the slower conduction of electrical signals in visual areas due to demyelination of axons. Similarly, in cases where a tumour is growing in an auditory area of the brain, the timing and amplitude (size) of the auditory ERP are abnormal.

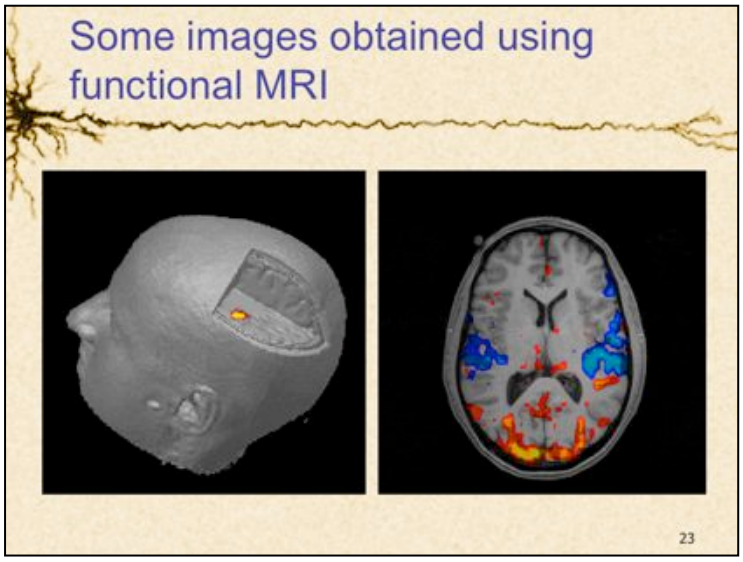


The newest of technique for functional brain imaging is **functional magnetic resonance imaging (fMRI)**. It involves the same hardware and basic physics as structural MRI (considered earlier in this lecture), but is based on a different physiological principle.

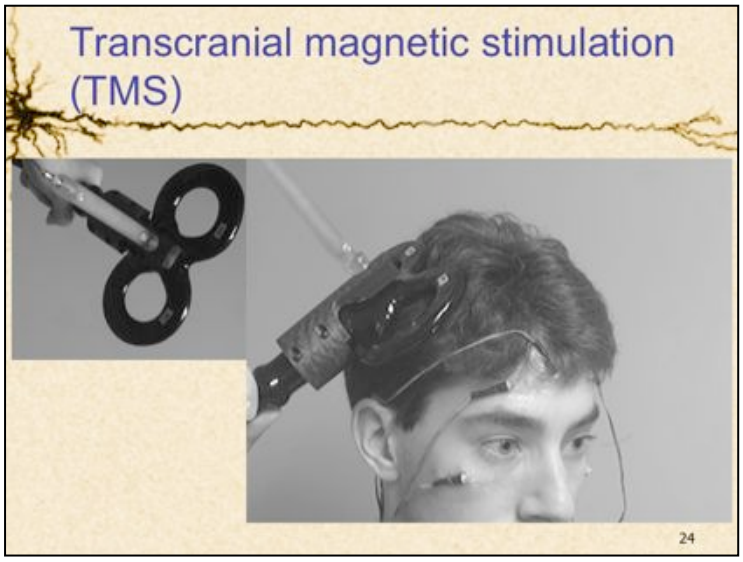
Like PET, fMRI measures brain activity indirectly by measuring changes in cerebral blood flow. The fMRI technique exploits the fact that the **haemoglobin** in blood has magnetic properties. Haemoglobin carries oxygen in the bloodstream. When this oxygen is used up, the haemoglobin is deoxygenated. It turns out that **deoxygenated haemoglobin** is more susceptible to magnetic forces (i.e., more paramagnetic) than **oxygenated haemoglobin**.

During an fMRI scan, detectors measure the ratio of oxygenated to deoxygenated haemoglobin; this is known as the **blood oxygen level dependent effect** (or **BOLD effect**).

Given the metabolic costs associated with neural activity, it might be expected that an active region of brain would show a greater proportion of deoxygenated haemoglobin. However, the capillaries supplying blood to active areas actually provide an oversupply of oxygenated haemoglobin to support the increased activity (more than the active neurons can use), and so there is actually a local **increase** in the proportion of oxygenated haemoglobin.



fMRI produces images with good spatial resolution (around 3 mm). For instance, it is possible to see activity in a very small region of the motor and somatosensory cortex associated with the movement of just a single finger (the left panel in this slide shows an area of activity associated with continuous flexion and extension of the right index finger). fMRI can also be used to image the neural correlates of perception, such as seeing and hearing; or to image much more complicated cognitive tasks, as we shall see in subsequent lectures. The right panel in this slide shows areas of activity associated with listening to speech and music (coded here in blue); and areas associated with viewing of black and white ‘checkerboard’ patterns (coded here in orange).



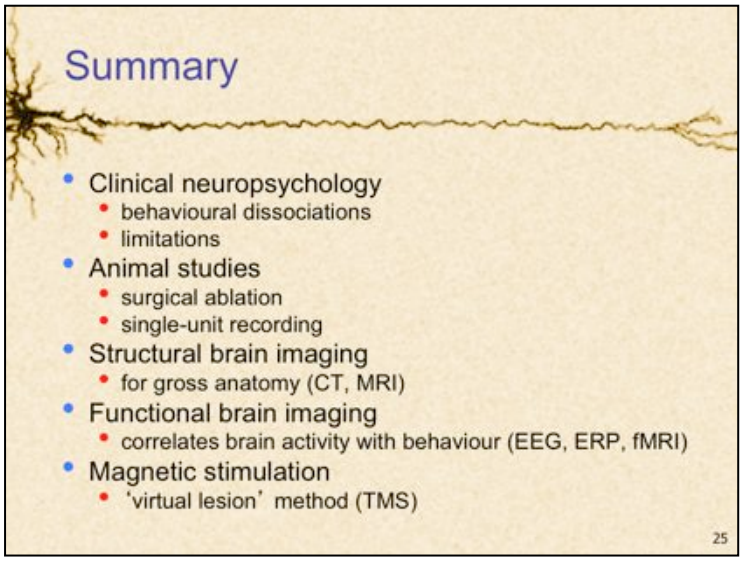
Functional brain imaging techniques provide information concerning the brain areas whose activity correlates with a particular task or stimulus. But correlations do not necessarily imply causation. How can we determine whether a given brain region is actually necessary for a particular task?

One approach might be to examine the behavioural consequences when the area in question is no longer able to contribute. We have already seen that patients with brain lesions provide one way of achieving this. Another, more precisely controlled method is to use **transcranial magnetic stimulation (TMS)**.

In TMS, a coil carrying an electrical current is held over the scalp, and a brief, focal magnetic pulse is generated which activates a small region of cortex (approximately 10 – 15 mm, depending on the size of the coil) underlying the coil. The activation acts like a 'virtual lesion', temporarily disrupting the tissue for a few hundred milliseconds. The technique is painless, and is safe to use in normal healthy participants provided they do not have a history of abnormal electrical discharges in the brain (e.g., epilepsy).

If a particular brain region is critically involved in a task, then TMS of that region should affect performance.

One advantage of TMS is that its effects last for only a very short period (< 500 ms) after the magnetic pulse is discharged. This makes it possible to determine not only which brain areas are involved in a given task, but also **when** those areas are involved.


Summary
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