Recording from the brain

In the earliest days of philosophy and science, there was a belief that we could make progress in understanding the brain by thinking about thought, by a complex process of structured introspection. It is likely that this still has a role in our broad subject, but it is equally clear that the fact of science, the facts we can discover by observing the dynamics of neural matter, are vital to understanding the brain. The tools of neuroscience were for a long time very crude; as we saw, the main approach was to study patients while they were alive and then dissect them after they died. Now, though, we have a diversity of approaches to recording the dynamics of neural matter.

The ideal, obviously, would be to record all the voltage changes for all the neurons in the brain and, probably, the different levels of different chemical. That isn't possible and any approach to recording from the brain is a compromise between spatial and temporal resolution and between the degree of intervention: at one extreme ***in vitro*** experiments are done on slices of brain that have been removed from an animal, typically one that has been killed as part of the experiment, these experiments can record the voltage inside the neuron an sub-millisecond resolutions. At the other extreme *electroencephalography* is completely noninvasive, you might end up with conductive gel on your hair but beyond that, there is no ill-effect or annoyance to the subject, but the data are noisy and reflect a blurry average of the activity of many synapses.

This document is intended only as a quick tour of some recording techniques: the speed at which the field is advancing and the vast diversity of approaches means any description is only very partial and slightly out-of-date. I will only mention ways of recording brain activity, but this has been paralleled by technologies to distinguish different cells, for example, using dyes that transport backwards through a synapse allowing researches to see what cell is connected to which and technologies, such as optogenetics and Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), which can switch on or off specific cell populations, allowing researchers to work out what different cells are contributing to network activity.

In vitro electrophysiology

In **in vitro** electrophysiology a small piece of the brain is removed and placed in a dish where it is kept alive by careful control of chemical composition and oxygen level of the fluid the slice is bathed in. An electrode is then used to record from the cell; this electrode is often a thin glass tube filled with salty water. Some approaches puncture a neuron's cell membrane with this electrode, and measure the voltage *inside* the cell (*intracellular* recording). Intracellular recordings can be used, for example, to measure the size of an PSP, the dendritic voltage change at a synapse. Another class of approaches, **patch clamp**, does not puncture the cell. Instead, the tip of a glass electrode is suctioned onto the cell's exterior, making a seal (Fig. 1). A patch-clamp recording can measure the ionic currents flowing through membrane-bound ion channels and receptors inside the patched portion of membrane.

Slices are usually made in a way that preserves as much of the neurites (the axons and dendrites) as possible; this is easier in some parts of the brain than others, the hippocampus for example lends itself to *in vitro* approaches because it has a very laminar structure and a lot of what we know about synapses was discovered in hippocampus. However, the key point is that these neurons are not in their natural environment, they will have damaged neurites, the chemical and temperature environment is not normal and their input is nothing like what it would be in the brain.

One nice approach, which has been used a lot in studying the retina, is to lay the slice directly only an array of electrodes. This does not give **intracellular data**: the electrodes record from outside the cell, giving **extracellular data**, so the data are spike times rather than voltages, but the density of electrodes that are possible does give a complete picture of the network activity, albeit in this weird context.

In vivo electrophysiology

The *vitro* in *in vitro* means glass, referring to the glass dish the brain slice is placed in. In contrast, the *vivo* in *in vivo* means living and *in vivo* electrophysiology is performed using living animals, either anaesthetised or awake and behaving. To do this an electrode is placed in the animals brain; in the anesthetized preparation a hole is cut in the brain, *trepanation*, and the electrode is inserted through that, or, in the awake behaving preparation, a *head stage*

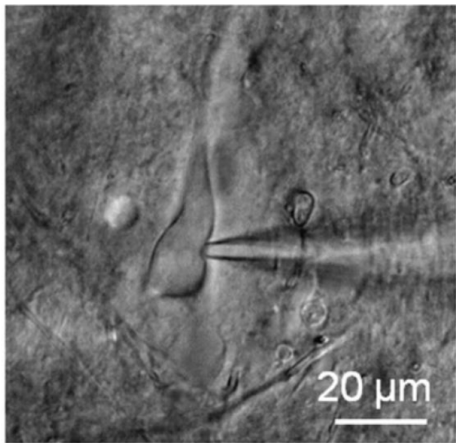

Figure 1: **Patched cell**: here an electrode has been *patched clamped* onto a cell to record its internal voltage. [figure from Ding, S., Mattam, S. G. & Zhou F. M. (2011)]

is mounted on the head covering and sealing up a hole in the skull. In the past the electrode was moved until the activity it recorded showed it was near a neuron; these days though silicon probes can be used. Silicon probes are made using processes similar to those used to manufacture silicon integrated circuits and have hundreds of recording sites; in this case the experimenter will rely on at least some of the recording sites being near the neurons.

In an **in vivo** experiment the electrode is outside the neuron so only the spikes are recorded and typically, spikes from more than one neuron are mixed together. A set of algorithms called **spike sorting** are used to assign spikes to different neurons. The accuracy of spike sorting is often debated. High-density silicon probes can help improve accuracy, since spikes are often detected at different amplitudes by electrodes at multiple locations. This provides a richer set of features that spike sorting can use to distinguish spikes from nearby neurons.

The advantage of **in vivo** electrophysiology is that you can record from the brain in a more natural state; however, the need to tether the electrode to the recording equipment in the awake behaving preparation limits the experiments that can be performed. Furthermore, the data recorded gives spikes, not voltages and spiking activity rather than synaptic activity. Although the

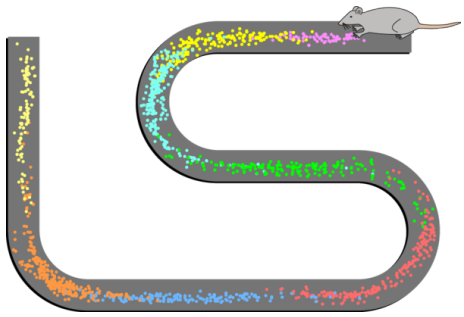

Figure 2: **Place cells**. Here each color of dot corresponds to a different neuron and the dots are placed on the maze in the location the rat was in when a spike in one of the eight neurons was fired. It shows clear evidence of place cells, with different cells firing in different locations. [figure from wikipedia]

number of neurons that can be recorded has increased a huge amount over the last few years, it is still a long way from recording whole brain activity. It can also be difficult to decide exactly what sort of neuron you are recording from, although different cells have different firing properties they are not always distinct enough to be distinguishable from their spike trains. It is an invasive procedure, there are animal welfare concerns and, except in very limited circumstances related to medical treatment, it is not possible to record from humans. Nonetheless *in vivo* electrophysiology has been one of the main tools of neuroscience over the last 50 years and has been the source a lot of the progress that has been made, see as just one example Fig. 2

Calcium imaging

Although, for simplicity and for historical reasons, the story we tell about neuronal dynamics focuses on the movement of sodium and potassium ions, in fact, in mammalian brains calcium ions play a big role in spiking, synaptic signalling, and plasticity.

Over the last decade, it has become possible to measure calcium ion concentrations in neurons—using either genetic tools to make neurons produce calcium-sensitive fluorescent proteins, or special dyes to introduce into neurons. These chemicals bond with calcium and emit photons. This really is incredible; it is also very useful, using two-photon microscopy it is possi-

ble to measure neuronal activity. This allows a large number of neurons to be imaged and recorded and, often, allows the same cells to be recorded from over a long period or even across successive days; since the recording is linked to an image, it is often possible to identify cell type and to study development. Huge numbers of neurons can be recorded.

There are many constraints on calcium fluorescence imaging. For a start, calcium concentrations inside neurons change more slowly than the dynamics that produce spikes, so complicated **deconvolution** algorithms are needed to deduce spike times from luminescence. In addition, one must be able to record the emitted fluorescence. Experiments that record large numbers of cells (hundreds, thousands) therefore typically use a *head-fixed* preparation. For a rodent, the head of animal stands on a wheel or ball, free to move, but with its head fixed under the microscope and with a monitor in front of it: of this is rather grandly called a virtual reality experiment, but that amounts to little more than linking the picture on the monitor to the movement of the wheel or ball. Another, very impressive, example, involves the developing zebrafish, hatchling zebrafish respond to optical flow, the movement, or apparent movement, of the river bed. Since hatching zebrafish are transparent, a huge fraction of its neurons can be recorded at once. New methods of long-term calcium imaging using implanted microendoscopes and fibre optics are also under development.

The glory of calcium imaging is that you can see the activity of neurons spread across the piece of the brain that is being imaged, so rather than including an image here I urge you to go and look at videos on youtube.

Electroencephalography

In electroencephalography (EEG) electrodes are placed on the scalp, usually of human participants and these are used to record the electrically activity in the brain. The story behind the discovery of EEG is amusing, you can read about it by looking up Hans Berger on wikipedia; in short, he wanted to explain what he believed was the real phenomena of telepathy. It is surprising that EEG works, the biggest electrically signals in the brain come from the activity in synapses, if all the synapses were in random directions these signals would all cancel out; it turns out that, particularly in cortex, there is some bias in the orientation in synapses, leading to an overall electrical field that can be measured at the scalp.

Needless to say, EEG signals are incredibly noisy, they mix together a plethora of neural activity and average a huge number of tiny electrical fields. The spatial resolution is terrible. However, the key advantage of EEG is two fold, it has good temporal resolution and it is easy to do, it is non-invasive, portable and relatively inexpensive. It was, for a long time, an important diagnostic tool; when I had childhood migraine for example, I was given an EEG; these days I would probably have an MRI. It has been of considerable help in studying epilepsy and lead to the discovery of sleep stages, the distinction between slow wave and REM sleep. I use EEG data in my own work on language since linguistic processing happens at short temporal scales and the only useful experimental subjects are humans. There is an example EEG trace, from a medical case report, in Fig. 3.

Magnetoencephalography (MEG), which detects magnetic fields rather than electrical ones produces better data, basically the magnetic fields aren't screened in the same way by the brain fluid; but MEG is much more expensive, the instruments used to measure magnetic field require superconductors and, thus, need to be cooled. There is optimism that a new technology, optically-pumped magnetometry, will lead to a cheaper and less cumbersome version of MEG.

Magnetic resonance imaging

In magnetic resonance imaging (MRI) an oscillating magnetic field is used to shift atoms between different energy states, this causes them to release radio-frequency electromagnetic radiation, which can in turn be detected. By changing the oscillations, different sorts of things can be imaged; a structural scan can be used to get a very detailed image of the brain. This is very important in diagnostics, the brain can't be x-rayed; since neural matter has the same density as the fluid around it, by design of course to protect the brain, it is invisible in x-rays. As recently as the 1970 the only way to image the brain if a tumour was suspected was to introduce an air bubble into the skull and then x-ray the brain while trying by head movements to get the bubble in the right place. Another type of scan, Diffusion-Tensor Imaging (DTI), can find tracts of neuronal axons, showing how the brain is connected, see Fig. 4.

For our purposes, the most important type of MRI is functional MRI. In fMRI the radio frequency pulses are tuned to distinguish hemoglobin that is bound

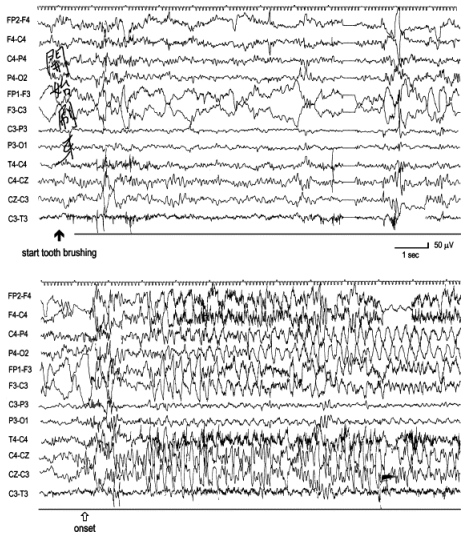

Figure 3: **EEG.** This shows the EEG traces from a woman who has epileptic fits, accompanied by orgasm, when she bushes her teeth; the moment she starts toothbrushing are marked by arrows and the over-synchonized activity typical of epilepsy is seen soon afterwards. [figure from Chuang, Yao-Chung, et al. «Tooth-brushing epilepsy with ictal orgasms." Seizure 13.3 (2004): 179-182.]

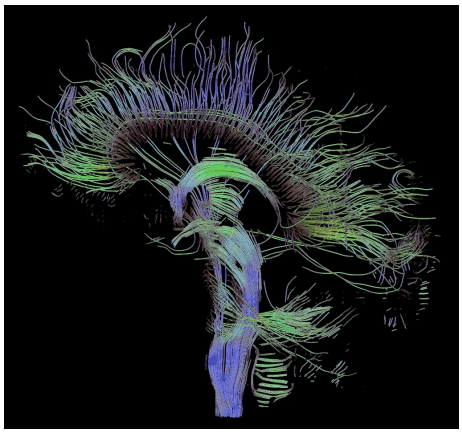

Figure 4: **Diffusion tensor imaging.** DTI allows us to image the connectivity of the brain. [figure from wikipedia, rendered by Thomas Schultz from data from Gordon Kindlmann and Andrew Alexander.]

Figure 5: **MRI machine.** A patient about to enter an MRI machine; the experience is a little claustrophic and very noisy, the magnets make a noise, but not unpleasant. [figure from radiology.ucsf.edu.]

to oxygen from hemoglobin that is not. This gives what is called the BOLD signal, a measure of the level of oxygenated blood in a part of the brain. This is believed to track neuronal activity, so fMRI can show which parts of the brain are active while the participant performs a task: the task of course being constrained by the MRI machine itself, the MRI machine is a huge cylinder with the subject, or patient, slotted into a hole at its centre; if you've never had an MRI the machine will be familiar to you from TV where it is often used as a short hand for 'serious medical stuff', see Fig. 6. The fMRI images have poor temporal resolution, basically the bold signal is giving the average activity of the brain over about three seconds; the spatial resolution, however, is pretty good; each voxel, the area resolved by the machine, is a cubic millimetre.

Summary

All approaches to recording from the brain are a trade-off between how much of the brain is recorded, how good the resolution is, how invasive it is, how much it constrains any behaviour and how much it costs. *in vitro* physiology measures in the voltage inside the cell, but only for brain slices, *in vivo* electrophysiology measures spikes in the living brain, often in awake and behaving animals, the number of cells that can be recorded from is limited but increasing. Calcium imaging records from a huge number of cells, but the experiments are difficult and the behaviour constrained. EEG and fMRI experiments can be used with human participants, the first has poor spatial resolution, the second poor temporal resolution and both are a long long

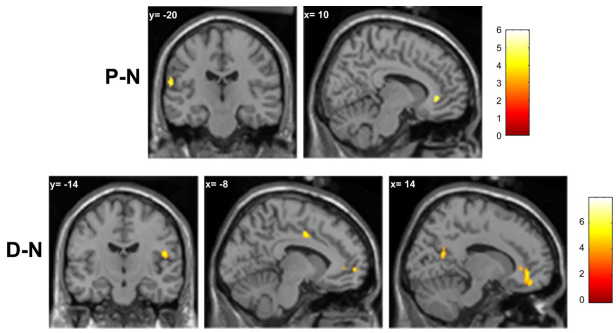

Figure 6: **fMRI images**. An fMRI study to distinguish the parts of the brian responding to disgusting or painful looking images [figure from Benuzzi, Francesca et al. Does It Look Painful or Disgusting? Ask Your Parietal and Cingulate Cortex, Journal of Neuroscience, 28 923-931]

way from recording from individual neurons.