Module:
Techniques in Neuroscience

Week 1
Understanding the brain:
Who we study, how and why?

Topic 2
Model organisms
– Part 1 of 3

Dr Frank Hirth
Associate Professor and Reader in Evolutionary Neuroscience, Basic and Clinical Neuroscience, King's College London

Slide 3:

Hello. My name is Frank Hirth, I'm from the department of Basic and Clinical Neuroscience at the Institute of Psychiatry, Psychology and Neuroscience, and today, I'm going to introduce you to animal models within the module Techniques in Neuroscience.

Slide 4:

The aims and objectives of today's lectures are listed here: to gain an understanding why animals are used in research, as models and a means to address a scientific question, both holistic and reductionist; to study *in vivo*- as compared to *in vitro*- the causes, mechanisms and pathways from molecule to mind; to understand why studies on a specific animal can have general application and significance because of evolutionary conservation (and that can be structural, molecular, and functional homology); then to gain knowledge and understanding of how animals are used to study the function of genes, proteins, pathways, circuits, then, of course, the whole brain and behaviour; to gain knowledge and understanding how functional studies in animals are conducted; and then, of course, to be able to name an example of how research using a specific animal species led to insights of general significance, and to know the limitations of such studies.

In the following now, I will guide you through all of these points so that you can have an understanding of the meaning of this.

Slide 6:

Why do we use animal models in neuroscience research? You can see here on these pictures several animals, and as you may note, something which is not an animal.

So on the left-hand side, you see yeast, which is used by bakers, but also to brew beer. You can see a worm, a fruit fly, a zebrafish, a lamprey and mouse. These are the animals we will go through to understand that animal models are used as a means to address a scientific question - this is always the main driver; and then, of course, we use animals for *in vivo* studies, as compared to *in vitro*. Why? Because we can have a better understanding, a more complete understanding. If, for example, we mutate a gene or modify a protein, what does it do? What does it do to the organism? What does it do to the behaviour of it?

What I would like to note right at the beginning here, is, as you will see, there are several vertebrate animals here: a zebrafish, a mouse. You should be aware that you need ethics committee approval and Home Office consent. These are mandatory requirements to work with vertebrates in a laboratory.

Slide 6:

What can we learn from animal models? I said before, we would like to understand causal mechanisms and pathways, and that can go all the way from a molecule to the mind; and as you will see in a few later slides, then of course, there are some animals you will not attribute any mind or consciousness to.

However, there are other animals where this is indeed the case, and as you can see on this slide here, there are three brains indicated: on the right-hand side there's a human brain, then in the middle there's a mouse brain - and those two brains are to scale, you can see the five centimetres - and on the left-hand side, you see a grey dot which is supposed to show a fly brain, but that's cheated actually. If we would stay in scale with five centimetres, you would not even see a fly brain. But as you will later see them, even these very, very tiny brains can tell us something about causes, mechanisms, pathways, all the way from molecule to mind.

Slide 8:

Why do we do research in animals? Or in other words, how does knowledge gained in a specific animal relate to others and humans?

The answer to that is best illustrated when we look at this very, very, famous sketch and drawing from Charles Darwin, which you can find in notebook B, 36. He did that in July 1837 - that was decades before he published *On the Origin of Species.* What he did there was he scribbled a tree, where he tried to illustrate the relationship between species and animals. We call that a phylogenetic relationship. It's like a genealogy: if you think of your granny or your auntie, you're related to them. Now what Charles Darwin, in a strike of genius, actually conceptualised is that animals might be related to each other, and this is the tree of life.

Slide 9:

Now, quite some time later, but based on Darwin's insights, we now have a better understanding how animals relate to each other, and we can depict that in a phylogenetic tree of animals, as you can see here. This is a phylogenetic tree that shows you the parazoa, the radiata, the eumetazoa. What is of interest for us is more towards the right side, where you have the chordates or the arthropods. These are the animals that are often used in laboratories to be studied. What is important here to understand is that they are related, but the relationship can be very remote and long-distance. So for example, we are separate from insects by almost 500 million years. That is a very long time, and you may think, well, what does that tell me? You will see later that even

500 million years of evolution have left a trace, and this trace helps us to understand what we learned, for example, in an insect or a worm can tell us something about ourselves and help us to understand a human disease.

Slide 10:

The phylogenetic tree makes only sense if we understand the very basic principle that was first described by Oken, and then by Charles Darwin. This is the concept of homology, which in a way describes evolutionary conservation. What you see on the top left side are four different appendages. The first one on the left is a human arm. Next to it, it is the leg of a cat. Next to it is the fin of a whale, and finally, you see the wing of a bat. Now, I told you about an arm, a leg, a fin and the wing. They're obviously completely different structures. But, if you now see at the colour code of the bones that make up these structures, you can identify that there are similar colours, and although these bones show a bit difference in their size, length and composition, their relationship to each other is, in all four cases, the same.

And then, Darwin coined very famously what he defines as the 'homologous structures', the relative position or connection in homologous parts, they may differ to almost any extent in form and size, and yet remain connected together in the same invariable order - and this is exactly what you see. You have an invariable order. Compare the human arm with the wing of a bat. They are so different, still the structure and composition are the same. This is what we call structural homology. Now, with the advent of molecular genetics, and especially the discovery of what DNA is made of and how it codes for proteins, the concept of homology could be extended to genes and proteins. What you see on the left lower part is the amino acid code of three different proteins: 'Dm' stands for Drosophila melanogaster, 'otd' is a gene that is absolutely essential for forebrain development, 'Mm' stands for Mus musculus which is the mouse, 'Otx1' and 'Otx2' are homologous genes to otd, because if you look at the letter code, they are almost identical with the exception of three amino acids. These differences seem to be significant, but what we like to point out here is the similarities. These sequences are identical, which in a way gives molecular confirmation to Darwin's hypothesis of homology. These genes must derive from a common ancestor.

Slide 11:

Now having established homology and the concept of evolutionary conservation, it makes a bit more sense why we can study worms, flies or zebrafish to learn about other animals.

Now what can we learn from animal models? It is the knowledge and understanding about the function of a gene and its encoded proteins, and please note that the gene can code for several proteins, which we call different isoforms. We can try to understand how a specific gene and protein interact, we can try to understand a signalling pathway and how it works, we can investigate the formation and specification of cell types, tissues and organs. Consider, for example, a nerve cell, a nervous system and the brain, and then, of course, the circuits and networks in the nervous system and the brain, which are ultimately responsible for our behaviour, and all of the above in relation to disease, how a gene is dysfunctional or a circuit is dysfunctional.

Slide 12:

Now to gain knowledge and understanding, what is usually done in animal models are so-called functional studies - that is, mutate, inactivate or overexpress a gene or protein, find interacting or binding partners of a gene or protein, then screen for enhancers or suppressors of a disease, gene or protein, then epistasis tests and the manipulation of a signalling pathway (this is essentially asking who comes first and next), then the targeted activation and inactivation of neural circuits, where you add the inactivate or overactivate a neuron and then look at the regulation and function of behaviour, and then, again, the dysfunction of the above that may underlie

disease. Again, note please that the majority of these studies are not possible in humans, except for cell culture or non-invasive studies with written consent from the patient.

Slide 13:

To illustrate all of this, I would like to share with you some of the findings we made in the last 10 to 15 years.

Again, on the left-hand side and I'll tilt it, you see the fly brain, the mouse brain and the human brain. Again, of course, you can immediately recognise the differences in size and complexity, but what you see on the right-hand side are kind of bar diagrams of the Drosophila brain and the mouse brain. In yellow is depicted the kind of forebrain and midbrain, and in bluish, gene expression patterns in the hindbrain or ventral nerve cord. What you can see here is that there are some similarities, but also dissimilarities. The interesting point is that these colour codes represent the expression and function of specific genes that are depicted underneath otd, Otx. I told you about the homology and the sequence, then unplug/GbX, Pax2/5/8, Hox1 orthologs. What we found actually is that these genes in a fly and in a mouse, they show structural similarity, they are expressed in similar ways and they seem to have similar functions, which led us to conclude that there are conserved genetic programmes that underlie the formation of an insect and a mammalian brain - and that, even though they are so different in size and composition.

So what you can find here is an underlying theme, like an architectural plan, that is readout; and there are similarities to it, but obviously also dissimilarities, simply because the human brain is so different in size and composition.