Module:
Techniques in Neuroscience

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

Topic 2
Model organisms
– Part 3 of 3

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Slide 3:

So, we now have heard about yeast, we have heard about the worm and we have heard about the fruit fly; and we went all the way from genes, to proteins, to cell types, tissues, and organs, and behaviour and disease. Now, let's make a leap. Now, we are leaving the invertebrates and go to vertebrates. Now, let's have a look at zebrafish.

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So here you see some zebrafish, you are familiar with them, I guess. Some of you may have like an aquarium at home, and those are, of course, fishes, they live in water, but the important point here is that they are vertebrates. In terms of evolution, they are much closer to us than insects or worms.

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Again, here is the phylogenetic tree of animals. We have spent some time looking at nematoda and arthropoda, now we are looking at the chordates. We belong to chordates, we have a chorda dorsalis, we are vertebrates and we are even mammals. But with the fish, we are now looking at the vertebrate, at an animal model that is a vertebrate.

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The interesting thing with the zebrafish is that you can do functional studies, in vivo, of course, in vertebrates, and some of them are illustrated here.

So, at the top left corner, you can look at the naturalistic behaviour. Of course, you can look at affection or aggression, you can also do high speed behaviour tracking. You can develop computational models, of course,

that you could do with other animals as well, but it still is a vertebrate. Then bottom left, you can do functional imaging and this is interesting. You can image the activity of an entire brain. You can manipulate circuits and of course you can do genetics, and this is what we will have a closer look at now.

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Another great advantage of the zebrafish is that its embryo is transparent. You can basically look at it while it develops. It's so transparent that if you, for example, use a method a bit similar to what I showed you with *Drosophila*, where you visualise the gene expression or you visualise the gene circuitry, you can see how it develops and how it behaves – and this is very fast. In 72 hours, a zebrafish is hatching, and it can almost live for up to three months.

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So, here are several images that illustrate how powerful the zebrafish can be. This is whole brain imaging of a behaving animal. In the sea, you see one of these zebrafish, and then consecutively, you see with different colours the activity of individual neurons in the brain. A high resolution is shown in D, again you see the different colour, and the more reddish or yellowish it is, the highly active this neuron is and in E you can see specific regions that are active. It doesn't matter what kind of behaviour this fish is currently doing, this is just meant to illustrate how powerful it is. Imagine you can see the entire landscape when there is a thunderstorm, you will immediately understand where you are. This is similar to when you can do whole brain functional imaging. It tells you which neurons and circuits are active while the animal is performing a certain behaviour.

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So, let's look into an example that illustrates the power of zebrafish, and that is the mutation in a gene, cntnap2a. Why is this gene of interest? This has been found to be related to autism in humans. What people did is they induced mutations in this gene in zebrafish, as shown in A, and then looked at the behaviour of the zebrafish. You can see the setup: you have a video camera, you have a tracking computer and then you have white and infrared LED lights and you can follow the activity of the fish. In B, you now see the activity pattern of a fish over the time of 70 hours. Now the interesting thing is that it's divided in night and day, and when you compare the two activity patterns - blue for the wild-type and red for the mutant fish - it immediately jumps out that the mutant fish is much more active at nighttime, which is a kind of hyperactivity. This is enormously interesting because remember, the autism spectrum disorder also has some hyperactivity components in it, and this is quantified in Z, where you see the average activity. The mutant is much more active than the wild-type.

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So, this study into this autism-related gene has been used to carry out a screen - a screen to identify suppresses of this defect. Remember, these fish show hyperactivity at night. Now, this is something you can easily screen for, and what has been done here in this study is they used a collection of psychoactive compounds which is shown in B, left bottom. They applied that to the zebrafish and then did the quantitative behavioural profiling, and with that, they actually identified that estrogenic phenotypic suppressors were able to suppress this hyperactivity. But, one should note here that, of course, autism is not just hyperactivity, and it would be sure to say that the zebrafish all of a sudden now is an autistic zebrafish. Always keep in mind that especially human diseases are multi-factorial and have multiple phenotypes. But, it is of course of great interest to study a specific phenotype, and how you might be able to suppress it, as has been shown here.

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So, again, we had an example where we went from a gene all the way to its relation to disease that was in the zebrafish. Now the next one would be the lamprey, but actually I just placed the image here, so that you look up what are lampreys. They are interesting in evolutionary terms and I have just put them here to give you a little bit of a quiz.

But, the next one is we are now looking at the mouse.

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So, this is the house mouse, *Mus musculus*. It's a mammal with social behaviour which is illustrated in this picture here. What is interesting for the mouse is that it's so close to us. 90 per cent of its genes are homologous to the human genes. It has a social behaviour and it has various aspects that are of interest for us to study.

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Now, this brings me ultimately back to the initial picture I showed you: the fly, the mouse and the human brain. We said why would we use animals? We wanted to study causes, mechanisms and pathways, and ultimately we want to go from molecule to mind. Now this is really prevalent in the mouse: it's a genetic model organism, it's very close to humans - 90 per cent of the genes are homologous, it's a small brain and we can do those studies in the mouse.

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To illustrate the power of the mouse, I would like to briefly introduce their study in Parkinson's disease.

Now, what is Parkinson's disease? This is shown on the right-hand side, there is a Parkinsonian patient. They typically have the shuffling gait and this tremor and rigidity and the bradykinesia. What causes these motor phenotypes is the loss of the nigrostriatal pathway which is shown on the left-hand side. A region in the central brain, which is called the substantia nigra pars compacta, is rich of dopaminergic neurons. These dopaminergic neurons, via the nigrostriatal pathway, innovate the striatum, which is composed of the putamen and the caudate. In Parkinson's disease, there is a degenerative loss of those dopamine neurons in the SNPT which leads to a degeneration of this nigrostriatal pathway. As a consequence, dopamine is no longer present in the striatum.

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To understand what the loss of the nigrostriatal pathway does, we need to have a look at a bit of a more complex network which is called the basal ganglia. On the left-hand side, you see an image that depicts the different components. We have the cortex at the top in green, you have activating activity to the striatum. The striatum then sends inhibiting - in red - connections to GPi SNR. These are the output nuclei of the basal ganglia. It's the substantia nigra pars reticulata, not to be confused with the pars compacta and the internal segment of the globus paladis- those are inhibited by the striatum. The striatum also inhibits the GPE - an intrinsic nucleus which is the external segment of the globus paladis - which in turn inhibits the subthalamic nucleus, and the subthalamic nucleus then can activate the output nuclei. So, this is a very complex interplay that is related to inhibition and excitation. The SNC as reported beforehand is a neuromodulator, because it sends dopaminergic projections onto the striatum. What is important here is that this basal ganglia output is inhibiting the thalamus, which has an effect on the movement.

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Now, what happens in Parkinson's disease here is, as I said, is a degeneration of the nigrostriatal pathway, and dopamine is no longer available in the striatum. Now, this is illustrated on the right-hand side where you see S and C and a big cross. So, dopamine no longer reaches the striatum. This has tremendous consequences on the striatum and what it in turn does to the GPe and the GPi/SNR.

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To illustrate what the effects are, this is best shown in a mouse model where targeted manipulation of a neural circuitry has been done.

This is a landmark paper by Anatol Kreitzer's lab in San Francisco. It's a bit complex, but let's have a look at the principles. In A, you see what is called an adenovirus that has been modified with ChR2-YFP. What is that? ChR2 stands for channelrhodopsin. This is a protein that, when expressed in neuron and activated by a light pulse, will overactivate the neuron. So, in effect, it's a trick with which you can buy a remote control, actually with the switch of light, activate a neuron. Now, in order to direct this trick into the basal ganglia, what these people did is they injected the virus into the basal ganglia of the mouse. Now, to be even more specific, they injected it with a modification that made sure that in one case, this channelrhodopsin gene was only expressed in the D1 pathway, shown in C, or in the D2 pathway as shown in D- and you may appreciate that these are different regions of the basal ganglia. Why this is important, we will see in a minute.

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So, what does it mean, D1 and D2? Let's have a look at this scheme in Parkinson's disease. We are looking on the right-hand side and there's a red inhibitory connection from the striatum to the output nucluei GPi/SNR. This direct connection is also called the direct pathway. Now, we also have another connection from the striatum to the GPI, but it goes via the GPe and the STN. There's an inhibitory connection to the GPe, and that in turn is inhibiting the STN. This is called the indirect pathway, or D2. They differ because they express different dopamine receptors. So, that is, dopamine coming from the SNC is innervating the striatum, but it has different functions depending on whether it binds to the direct D1 receptor, or the indirect D2 receptor.

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This knowledge has now been made use by Anatol Kreitzer and colleagues in order to activate channelrhodopsin in D1 or D2, and they made use of that also in a Parkinsonian model.

Now, what have they done? Look at A, there's a control which shows the expression of TH. TH is the rate-limiting enzyme leading to the production of dopamine. In B, you now see, again, a labelling for tyrosine hydroxylase, TH, but obviously, where the white arrow points to, you see a big hole. The dopamine has gone. Why is that? Because they applied 6-OHDA. What's that? This is 6-hydroxydopamine. It's an antagonist of dopamine. Now, what you can see when you compare B to A, they basically have created a region in the basal ganglia where they depleted dopamine- or in other words, it has artificially introduced a Parkinsonian model. In a way, like I showed you before, the nigrostriatal pathway is gone. Why? Because the striatum has no longer the dopamine as a neurotransmitter.

Now, in C, they start to use this trick. They then express channelrhodopsin in this area, where 6-OHDA has been applied, and no dopamine is anymore available, and with that, they can now with a light pulse activate those neurons even though they do not have any dopamine. Now, what does it mean to optogenetically activate D1 or

D2? This is shown in the middle. It shows you the experimental setup. This is the brain of a mouse, and there are some light cables into the brain of the mouse in the striatum. In the middle, you see D1 is activated. In grey, the laser is off. In red, the laser is on. What you can see is if you give a light pulse, these neurons get activated. The D1 neurons are activated and what you can see is that the mouse starts to run around. This is a very powerful proof that the striatal D1 neurons are involved in voluntary movement. The activity of those neurons is causally related to movement initiation and maintenance. Now, look at D2, the green one. When they activate the laser, the mouse immediately stops. So this, so to speak, is the counterpart of D1. When you optogenetically activate D2 neurons, the mouse stops; it abrogates its actions.

Again, this is the most powerful proof so far that the D1/D2 neurons in the striatum are intricately involved in the regulation of voluntary movement. But, the study by Kravitz et al. went one step further, and that's a significant step to understand what happens in Parkinson's disease. They used this setup, the artificial activation of neurons, in order to overcome this movement deficit that is Parkinsonian-like, and that can be induced by 6-OHDA. Look at E - you see the gray, black and white. This is basically the ratio or percentage of time that the mouse has spent ambulating, which is running around, or with fine movement, or freezing. Now, look at the situation in red. This is D1-channelrhodopsin in the 6-OHDA situation. So, in essence, it is the C and D at the top. Now, before you induce any laser, you can see that the mouse spent less ambulating and more freezing. They're basically inactive. Now, in the middle of the red ones, they induced the laser - that is, they activate the D1 neurons - and as shown in this little arena, the mice all of the sudden can run around, and that is even when dopamine is not present in the striatum of these mice. This very powerfully shows that the D1 striatal neurons are absolutely crucial for the phenotype that you see in Parkinsonian phenotypes. It is the loss of activity of those neurons that has here been artificially overcome by channelrhodopsin inactivation, and this is, again, the normalised fine movement velocity that's also shown here in F, which further quantifies how this Parkinsonian phenotype can be overcome. So, what you have here is a study that shows two very, very fundamental aspects: the first one is D1 and D2 have a crucial role in voluntary movement and second, that the activation of D1 can overcome a Parkinsonian phenotype.

Slide 20:

So we've gone all the way now from mutations, inactivations, overexpression of genes, to their relation in disease. What I illustrated was studies in yeast, studies in worm, fruit fly, zebrafish and mouse, and I shall remind you that the majority of these studies are of course not possible in human, except for cell culture or non-invasive studies with written consent, which means we require animal models and studies in animals to have a fundamental understanding of the causes of what these genes do and how this relates to behaviour and disease.

Slide 21:

This final slide brings all together. So, what you see here is a graph which shows the generation time and date, and how these animals evolved, dating back to the last common ancestor. You have *C. elegans* there, which has 21,000 genes, only 302 neurons. Remember *C. elegans* studies about the aging. Then, we have *Drosophila* melanogaster. Now, they are refined to 14,500 genes, with 60 per cent, 65 per cent homology. Then, we looked at the zebrafish, *Danio rerio*, and the mouse. And of course, if you compare that to humans, with an average weight and lifespan by far outsizing those of these animal models.

But, at the same time, when you look at the fact that experiments with humans quite rightly are not possible, except non-invasive ones, it tells you why these studies in animal models are so powerful; and it is the evolutionary conservation of genes, of pathways and of their dysfunction which makes those animal models so powerful in the study of mental health disorders.