Biomedical Sciences Tutorial 3 (Neuro) Summary

Tutorial discussions were based on the following paper:

Till SM et al (2015). **Conserved hippocampal cellular pathophysiology but distinct behavioural deficits in a new rat model of FXS.** *Hum Mol Genet.* 24(21):5977-84.

Background:

- What is the aim of the paper?

A mouse model of Fragile X Syndrome exists. The aim of the paper was to describe a new rat model of the same disease and compare it to findings from the mouse model.

The paper is a good example of recent research into Fragile X Syndrome coming from Edinburgh.

- What is a "rat model"? If we already have a mouse model for a disease, why do we need a rat model as well?

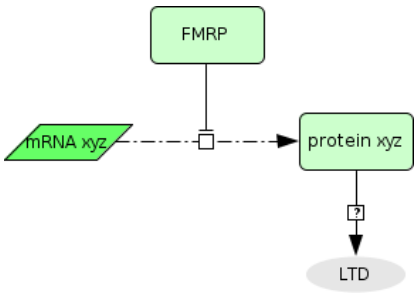
A rat model is a transgenic rat that has a genetic deficiency which is the same as (or similar to) the genetic deficiency in a known human disease. It can be used to investigate disease mechanisms and learn more about possible treatments.

Although mice and rats look similar, they are quite different from each other and separated by a large evolutionary distance (of over 12 million years). So "a rat is not just a large mouse". Therefore, just because we observe a certain phenotype in a mouse model, it does not mean it has to be there in a rat model. If we observe something in both mice and rats, the chances are greater that it generalises to other species (including humans) as well.

Someone brought up the point that humans are closer to rats than to mice in evolutionary terms. I did a quick online search and could not find evidence for this. The common ancestor of both rats and mice split from the human ancestor around 75 to 80 million years ago, so our evolutionary distance from both rats and mice is about the same. It is true though that rats share more physiological properties and cognitive abilities with humans than mice do. Therefore, we would expect rat models to be more "human-like" than mouse models. Also, apparently, they can ride skateboards! (See here: http://theconversation.com/animals-in-research-rats-16634)

- Can you draw a diagram of what happens at a cellular level in Fmr1 knockout rats, as compared to wildtype animals?

The Fmr1 gene encodes a protein called FMRP (Fragile X Mental Retardation Protein). This protein supresses translation for some proteins. We do not know all of those proteins, but we know that they probably play a role in long-term depression (LTD). In the knockout, FMRP is not expressed, and there is increased transcription of those proteins, and therefore increased LTD.



Synaptic phenotype:

- The text says that "loss of FMRP is associated with subtle alterations in dendritic spines of pyramidal neurons." Looking at figure 2, what are those subtle alterations?

They seem to be very subtle indeed. Figure 2B' and 2B'' look quite similar – it is difficult to see the difference between wildtype and mutant with the naked eye. Figure 2C shows the number of protrusions per 10 µm (so, essentially spine density) for both basal (closer to the soma) and apical (further away from the soma) dendrites. There seems to be a slight, but statistically significant difference for apical dendrite, with knockouts showing a slightly higher spine density. There is no difference for basal dendrites.

The mean spine head diameter (MSHD) does not seem to be different between wildtype and knockout mice, according to figure 2D. Authors claim that the relative frequency data indicates a small, but significant difference between wildtype and mutant. This is, however, hard to tell.

In short, if we look at morphological differences between mutant and wildtype, there are few and they are very subtle indeed.

Would we have expected this? If FMRP supresses protein translation, then no FMRP would mean more protein, and we might therefore expect larger (or more) spine heads to accommodate these increased protein levels. This is not happening though. The key lie in the sort of proteins that are more likely to be expressed in the knockout, and in their function. The functional consequences of knocking out FMRP are more important than differences due to just increased protein levels.

Plasticicity:

- The authors used the mGluR agonist DHPG to induce long-term depression both in wildtype and knockout rats. What were the results?

Long-term depression is a long-term reduction in the strength of synaptic response. (The opposite effet is called long-term potentiation, or LTP). High-frequency stimulation of a synapse induces LTP, while low-frequency stimulation induces LTD. LTD can also be induced using mGluR agonists, that is, agents that activate mGluR receptors.

The results are somewhat inconclusive. The text says that there is a significant difference in LTD between wildtype and knockout animals. The legend to figure 3A says there is no significant difference. The right part of figure 3A seems to suggest there is a significant difference (but again, not a difference that is big in magnitude). It is hard to tell.

- What is the difference in long-term depression between wildtype and Fmr1 knockout when a protein synthesis inhibitor is used? How would you explain this finding?

When cycloheximide is used to inhibit protein synthesis, LTD is disrupted in the wildtype. This is because long-term depression is dependent on new protein synthesis in the wildtype. In the mutant, we still get LTD though. This is probably because the proteins involved in LTD induction are already overexpressed (because there is no FMRP), and this increased level is enough to induce LTD, even if no new protein is expressed.

(Note that the bars on top of the graph on the left sides of figures 3A and 3B indicate the time point and duration of cycloheximide and DHPG treatment. If the cycloheximide treatment lasted longer, we would of course expect LTD to be disrupted in the knockout as well, because the excess protein that is there because FMRP is knocked out would ultimately degrade, and no new protein would be made.)

In summary, the LTD phenotype is similar to what we already know from the mouse model of Fragile X Syndrome: mGluR-dependent LTD is elevated in the knockout, and this elevation seems to be independent of new protein synthesis (since it can be observed even if protein synthesis is blocked during LTD induction).

Behaviour:

- How would you summarise the results shown in figures 4 and 5?

Rats underwent a spatial learning task, which included reversal (the animal has to learn one thing and then "unlearn" it to learn something else). Two versions of such a task were used: In the first version (figure 4), the rat learns one position of a hidden platform in a water maze for several day, and the position is then changed, and the rat learns again. In the second version (figure 5), the position of the hidden platform is changed (and has to be re-learned) every day. The parameter recorded were the path length, i.e. how far the rat travels before it reaches the platform, and the number of target crossings, i.e. how often the rat crosses the platform. In both tasks, there seemed to be no differences between wildtype and Fmr1 knockout rats, indicating that spatial memory is unimpaired.

Knockout animals showed faster swimming speed than wildtype animals. Interestingly, human Fragile X Syndrome is associated with hyperactivity, so this could be a behavioural parallel between humans and rats.

- OR, OP, OC, and OPC tasks all assess whether a rat can spot a novel, unfamiliar object. They work by first letting a rat getting acquainted with a specific space and set of objects, and then changing the setup and measuring whether the rat spends more time exploring the novel object. Using figure 6A, explain the difference between OR, OP, OC and OPC tasks.

These are quite complex cognitive tasks. The diagrams in figure 6A show learning and testing situations as circles with the testing situation being the last (rightmost) circle, and the novel object in that circle indicated with an arrow. Shading of the circles indicates context (i.e. the type of cage that the rat was in during learning and/or testing). Different objects are indicated with different geometrical shapes.

- In OR (Object Recognition) tasks, the rat gets time to explore two objects (learning phase). In the testing phase, one object is replaced by a new, unfamiliar object.

- In OP (Object Place), the rat again learns to identify two objects. In the testing phase, a familiar object appears in an unfamiliar place.

- In OC (Object Context), the rat learns an association between objects and context: It sees a particular type of object in a particular type of cage, and another type of object in another. The

novelty during testing consists of seeing an object in a novel context.

- The most complex paradigm, OPC (Object Place Context) combines OP and OC: The rat learns different object-place pairing in two different contexts. The novel element during testing is an object-place pairing that has not appeared in the same context before. Of all the four tasks, this is the most complex, and it involves the hippocampus.

What is measured in these tasks is a discrimination index. This is computed from the times the rat spends exploring the novel and the familiar objects during testing (see Materials and Methods part of the paper for a formula). Basically, 0 means that the rat spends the same amount of time exploring the novel object and the familiar objects, in other word: it does not seem to recognise the novel object as novel. 1 would mean it spends all of its time exploring the novel object, -1 would mean it spends all of its time exploring the familiar object. A number greater than zero means that the rat spends more time exploring the novel object than exploring the familiar object. Wildtype rats spend more time exploring the novel object in all of the four tasks, indicating that they have successfully learned during the training stage and now recognise what has changed in testing. Knockout rats seem to do just as well as wildtype on the OR task. They do a bit less well, but still pass the OP and OC tests. On the OPC test, however, the discrimination index is not significantly different from zero, indicating that the rat does not recognise the novel object as novel. In other words, it has not learned the association between object, place, and context during training.

This suggests, that Fmr1 knockout rats show deficiencies in a quite complex hippocampal learning task, but perform OK in simpler cognitive tasks, as well as spatial reversal learning tasks. This is different from mouse models, where Fmr1 knockouts show impaired spatial reversal learning.

Discussion:

- The discussion makes the point that although rats and mice with Fmr knockouts share similar cellular phenotypes, the behavioural phenotypes are different. How can this be explained?

As we said earlier, rats aren't just bigger mice. A similar cellular phenotype can give rise to different behavioural phenotypes, because behaviour is a complex function that involves a variety of learning and other cognitive processes. The paper refers to “species-specific differences associated with ethologically relevant tasks”. What that means is that rats and mice have a different evolutionary history, live in different environments, and therefore behave differently. They might have different strategies for learning the same kind of task, depending on how often they need it in their natural environment. Therefore, a learning task disrupted in one species might not be disrupted in the other.

Does this mean we cannot learn anything from knockout experiments in a different species? The molecular and cellular phenotype seems to be pretty well conserved. This is important because most medical and pharmacological interventions actually target molecular signalling pathways, so there is a good chance of getting medically relevant information from mouse and rat models. In terms of behaviour, it is less easy to generalise from an animal model to a different animal model, let alone to humans.