Psychiatric Disorders: how can fish help us?

Psychiatric disorders result from a brain malfunction. Disruption of particular genes seem to account for behavioural abnormalities. However, this association between gene and disorder has to be demonstrated and the mechanism by which these genes act understood. **Dr. Héctor Carreño**, a post-doc in the Department of Neurosciences, Psychology and Behaviour, and tells us how his research with the small vertebrate zebrafish hopes to shed light onto this connection.

Zebrafish in Neuroscience

Zebrafish have been used as an animal model for more than three decades and yet most people are still surprised when I say, 'I work in Neuroscience with fish'. They usually think, 'Why? Fish and humans are very different, aren't they?' The answer is yes, at least at first glance, but there are many similarities to mammals that have established zebrafish as a remarkable model in virtually all fields of biomedical research. In the last few years zebrafish have also been used as a model to understand the genetic basis of psychiatric disorders.

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There are some biological and technical advantages that have made this little fish very popular among the scientific community. Due to its reduced size, we can keep large stocks of different experimental groups at a relatively low cost. A single female can spawn hundreds of eggs per week that develop very quickly outside of the mother, permitting the study of developmental processes. You can literally watch the first cell divisions occurring in a few minutes, see how the eyes and tail appear after a few hours, and observe the heart beating in a freely moving animal 24 hours after fertilisation.

At molecular, cellular and physiological levels, zebrafish have much in common with other vertebrates, including humans. Its genome has been sequenced and we know that it is remarkably similar to humans. In the central nervous system, which is formed by the brain and the nerves, the main brain regions, types of neurons, cell machinery, molecules and the way they work are similar in zebrafish and humans. The same type of signaling molecules, such as dopamine or serotonin, are active in the both species.

In addition to these features, zebrafish display a large set of complex behaviours which have made it highly suitable to model human diseases affecting the brain, including psychiatric disorders.

From Human Disease to Fish Models

Psychiatric disorders are a mixture of diseases that affect all aspects of mental function. Unfortunately the treatments available are limited, in part because we know little about the genetic alterations and neurobiology underlying them. Novel and powerful DNA sequencing techniques allow researchers to link genetic variants to human traits, but this association and the pathways and mechanisms altered by these mutations need to be examined in animal models first. This is where fish play a prominent role. Over 80% of the genes associated with human diseases have also been identified in zebrafish. We can use several genetic tools to induce mutations in the genes we want to examine, and assess the alterations that may occur in behaviour. When these alterations mimic some aspects of a specific human disorder, the insight we glean from studying the fish brain is invaluable in understanding the mechanisms leading to the disease. We just require reliable means to measure behaviour in these mutants.

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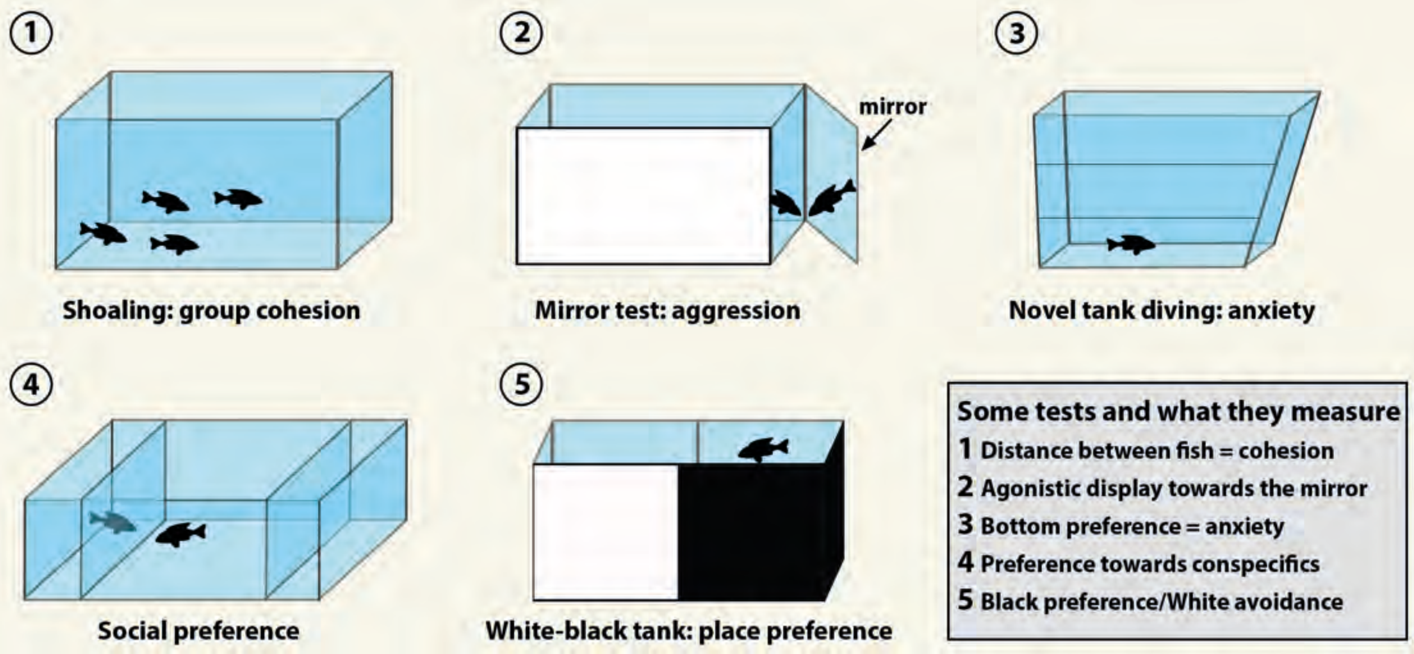
Zebrafish species belonging to the minow family of the order Cypriniformes



Examples of zebrafish tests

Recording zebrafish: fishing for answers!

In 2016 alone, more than 300 papers on zebrafish behaviour were published in peer reviewed scientific journals. Here in Leicester, my research combines neurochemistry and molecular biology using a platform of behavioural tests to study novel mutant lines and search for novel compounds affecting behaviour. In this platform we use a number of different setups, cameras and automated tracking systems to gather as much information as possible.

"Will Norton's lab combines neurochemistry and molecular biology using a platform of behavioural tests to study novel mutant lines and search for novel compounds affecting behaviour."

We can measure the cohesion of groups of fish in a large tank as a readout of social behavior, quantifying the distance between the individuals in a shoal.

We can quantify aggression levels using the mirror induced stimulation protocol, in which we place a mirror outside the experimental tank and record the reaction of the fish towards its own reflection. Fish are smart, but like the majority of vertebrates they do not recognise their own reflection, and they attack thrashing the tail, erecting the fins and biting against the mirror as if an intruder were present.

The fish react to novel or threatening environments with anxiety. To measure behavior we can use the novel tank diving test. When we put a fish in this setup, the first reaction we observe is swimming exclusively in the bottom third of the tank, where they alternate between zig-zagging movements and freezing, both being indicative of stress. After a few minutes they get used to to the new tank, become bolder, and increasingly explore the rest of the tank reaching the top third.

Another way to investigate social behaviour is measuring the preference of the fish for another, since they prefer company. If we put a fish in a tank half white and half black, we will see a clear preference for the black side, which can also be used as a readout of anxiety induced by white avoidance. These are only some examples of the type of tests we can do in the lab, and the usual responses we see in animals, but there are many more tests, which we can perform to examine sleep, impulsivity or learning.

Recording a large number of individuals, collecting different behavioural outcomes, and carrying out thorough statistical analysis enables us to determine the type of alteration in behaviour, comparing the performance of fish carrying mutations in specific genes with the fish expressing the most commonly found gene used as a reference.

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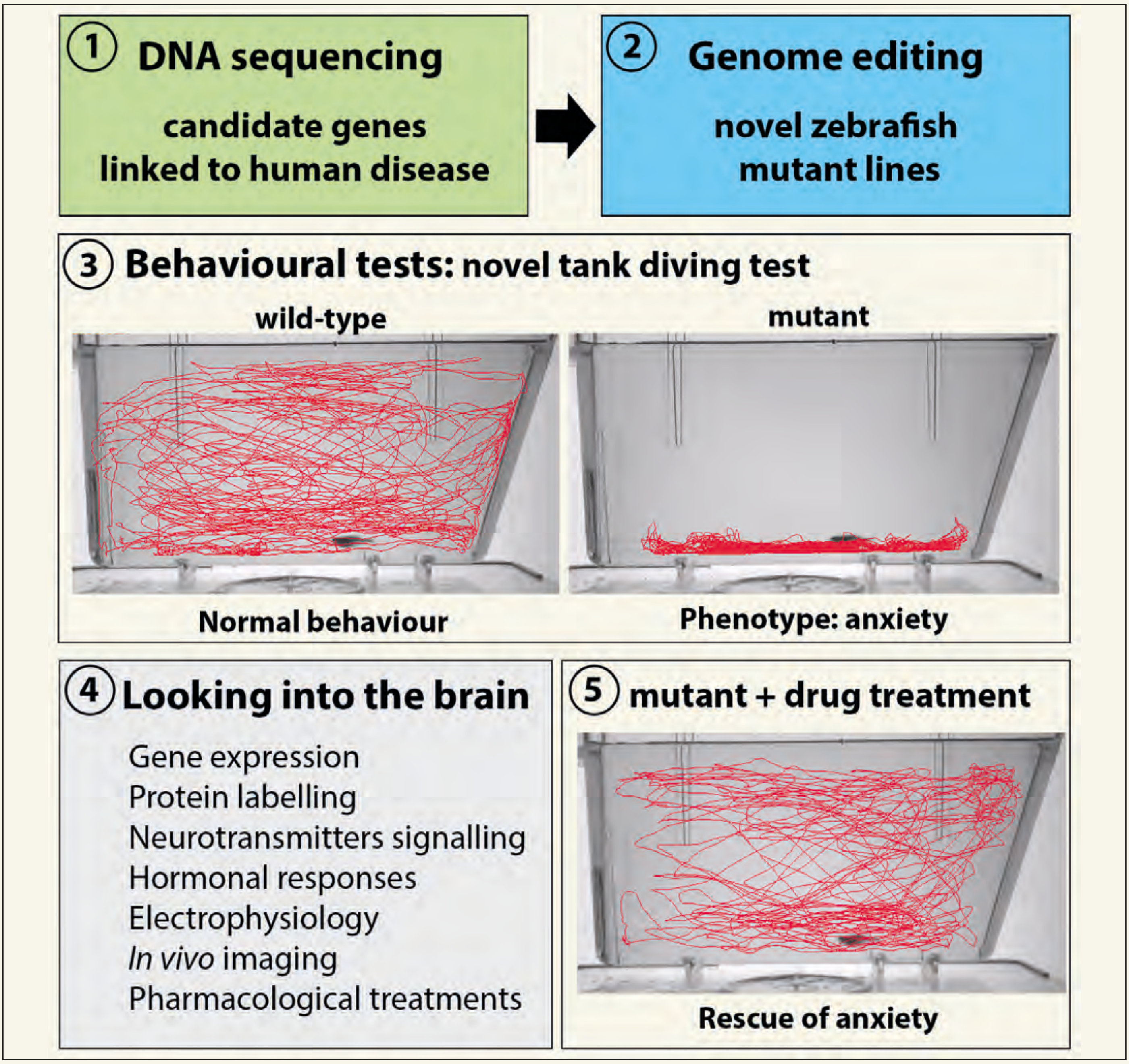
In my research, I am studying the alterations in the behaviour of fish carrying mutations in genes known to be linked to aggression, hyperactivity and depression, to find the changes in the brain underpinning these disorders. These genes have been previously associated with human psychiatric disorders, but the mechanisms by which they operate are not well known.

Below: Schematic of Zebrafish research laboratory



The mystery of the gene...

To determine the mechanisms through which target genes cause disease effects we have to develop mutants for these genes and test them through our behavioural platform. When we find a clear effect of the mutation on behaviour, we use different techniques to look into the brain of these mutants to try and find alterations that could explain the connection between the gene and the psychiatric disorder.

One of the things we can do is slice the brain with an instrument called a microtome and label specific types of neurones to see if they have changed in number or shape. We also split up the brain in different regions, then we mash them up to inject them into a machine that is able to quantify the amount of signaling molecules present in each region with high precision.

To assess the expression and release of some hormones such as estrogens or cortisol we can use commercial kits specially made to detect these molecules, and we can examine the level of expression of virtually any gene using molecular biology techniques. For example we want to test the effect of a particular substance in fish behavior to try and rescue the abnormalities seen in the mutants, we just have to dissolve the compound in the tank water prior to the testing. Thus, we investigate changes in the brain at different levels to try and get an insight about the mechanisms that connect our genes to our behaviour.

With emerging cutting-edge technologies such as genome editing, optogenetics, or calcium imaging, and the development of new and more refined behavioural tests, the approaches to study the brain are always increasing. The knowledge we gain from fish, combined with mice and human data, has the potential to improve our understanding of the cause of human diseases, which is the key to discovering new treatments and to improve the existing ones.

Héctor Carreño is a post-doctoral researcher in Will Norton's lab at the University of Leicester. Héctor focuses on the genetic basis of behaviour by using zebrafish to understand the brain alterations underpinning human psychiatric disorders.