THE FOUNDATIONS OF OUR BEHAVIOUR

By Michael Simm

In order to explain behaviour – be it in humans or animals – researchers are looking from the genome to the connectome. They are trying to understand how genes affect the function of the neurons that underlie complex behaviour such as obtaining food or selecting a mate. Cutting-edge technology, including optogenetics, is helping in this research.

In a dark-but-casual suit and with the charisma of a great entertainer, Sebastian Seung enters the stage. The professor at Massachusetts Institute of Technology (MIT) speaks insistently to the audience, while on the screen behind him flicker four letters that symbolize the age of genetics: A, T, C and G. The sequence of these letters seems both endless and accidental, but nowadays every child learns at school that this code represents genetic information: construction manuals for biomolecules, rules for growth and development, and – over and over again – weak spots and 'misprints' that make us susceptible to a plethora of diseases.

Seung, however, is not a geneticist. Nor does he believe that people's destinies lie in their genes, as Nobel Prize winner James Watson once put it in a moment of exuberance, soon after he and Francis Crick had worked out the structure and functioning of the DNA molecule.

Seung has a different kind of belief. So he gets his audience to chant: 'I am not my genes.' Once more, but louder this time: 'I am not my genes.' The lab head in the Department of Brain and Cognitive Sciences at MIT leads a team of some 20 highly gifted scientists from very different disciplines and his idea is: 'I am my connectome.' It is to explain what this means that he has come here.

Just as the sum of a living organism's genes constitute its genome, the connectome corresponds to the sum of the connections between its nerve cells. They define, as Seung is firmly convinced, the human personality with all its peculiarities, whether strengths or weaknesses: I am my connectome, so my connectome defines my 'self'.

As early as the 1960s, Nobel Prize winner Eric Kandel showed in experiments with the sea slug *Aplysia* that experiences and learning processes alter the strength of connections between nerve cells. Fifty years later, most scientists are convinced that our experiences are also reflected in what are called 'neural circuits' – in the number and kind of the nerve cells (neurons) involved and in the strength of the connections between these cells. By now, hundreds of laboratories are using state-of-the art technologies in attempts to establish the relation between the processes taking place in these circuits and phenomena such as perception, cognition and behaviour. It is exactly these issues that scientists from all over the world will discuss at the 103rd International Titisee Conference 'Genetic Analysis of Neural Circuits', held by the Boehringer Ingelheim Fonds from 23–27 March 2011.

The most recent successful large-scale scientific project has been the deciphering of the human genome with its three billion 'genetic letters' and some 30,000 genes. It took ten years and three billion dollars to complete the first draft, an achievement that the then US president, Bill Clinton, honoured in a celebration broadcast live from the White House. But the challenges presented by the human genome project, or even landing humans on the moon, pale in comparison to the task that neurobiologists are facing: the human brain contains an estimated 100 billion nerve cells, and each of those neurons is thought to have up to 10,000 synapses, i.e. contacts with its neighbouring cells. The fact that 'our →

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brains look like spaghetti', as Seung explains to his listeners with a picture of a huge plate full of pasta, does not make things any easier. The human brain may well be one of the most complex structures in the known universe.

Up to some 50 years ago, it would have seemed rather frivolous to most researchers to even think of trying to study the activation of a few small neural circuits in detail. Back then there were hardly any researchers who could have even imagined the kind of technology required to establish a connection between the states of excitation of these neural circuits and complex behaviours, such as obtaining food, selecting a mate, or maintaining social relationships.

Yet one attempt to map every connection of every nerve cell has already succeeded – albeit 'only' with a tiny model organism, the nematode *Caenorhabditis elegans*. Like the telephone network of a village, the tiny worm's connectome is made up of some 7,000 connections between its 302 nerve cells. Establishing this network by studying the transparent *C. elegans* under the microscope probably has ruined the eyesight of numerous doctoral candidates who undertook that work during the 1970s and 1980s. Then the field of connectomics ran up against the limits not only of what a human being could bear, but also of what technology could deliver.

It is at the limits of what is technically possible that Seung stands today. Working with his collegues Jeff Lichtmann and Kenneth J. Hayworth of the Harvard Center for Brain Science, Seung has already extended these bounds several times. For instance, a cutting tool developed by Hayworth at a cost of several million dollars allows the scientists to slice the brains of mice into thin sections no more than three nanometers wide. They now use a largely automated computer-guided process to reassemble these serial sections into tiny three-dimensional blocks of nerve tissue. Indispensable for this are special electron microscopes developed by Winfried Denk and Heinz Horstmann at the Max Planck Institute of Biomedical Research in Heidelberg. They make it possible to identify and mark those interconnected neurons that would constitute a particular neural circuit. Last but not least, software developed by two of Seung's students, Viren Jain and Srini Turaga, also helps in this Herculean task. Based on the principle of artificial intelligence these programmes are capable of learning to recognize the synapses that connect particular nerve cells based on the examples given by the researchers, so that eventually a great deal of that work can be performed without further human input.

However, in spite of these aids, Seung's digitized mini-blocks of tightly packed nerve cells, which currently measure six micrometers along their edges, at most, are still several orders of magnitude short of an entire brain – even if it were only that of a fly.

That is why Barry Dickson of the Institute of Molecular Pathology in Vienna is using a different set of methods to come to grips with the fruit fly *Drosophila melanogaster*. 'Our goal is to understand how information processing in defined neural circuits generates complex animal behaviours,' he says. The Australian is particularly interested in the fly's mating behaviour – in other words, in sex.

Here, as with other kinds of behaviour, the brain receives a variety of signals from the body and from the world via its sensors. These signals must be integrated and weighted against each other, as well as against previous experience. From such information, the brain of the male fly 'decides' whether or not to court a female. Dickson is not alone in believing that when a male fly dances around the female of his choice and vibrates his wings in a kind of 'love song', when she holds back and he exerts himself, and when the courtship ritual finally leads to mating, the same general principles are involved – at the level of cells and circuits at least – as with other

OPTOGENETICS – A RECIPE

Take the standard equipment of a modern molecular biology lab and a handful of technically experienced staff from very different disciplines. Add genetic instructions for light-responsive proteins from green algae and archaebacteria, as well as synthetic genes that cause activated nerve cells to glow. Implant these genetic constructs with the help of selected viruses and a stereotactical guidance system precisely in the region of brain to be examined in a mouse or a rat. Then insert a fibre-optic probe into the same region and, at the surface of the skull, carefully connect it to a miniaturised laser controlled by a signal generator, as well as to a high-speed camera for detecting altered optical signals.

This method for scientists wanting to examine the neural circuits of the brain with the help of optogenetics reads like a cordon bleu recipe. The whole procedure can be completed in only 4 to 5 weeks, promise the authors led by Karl Deisseroth in 18 pages of instructions, which they have recently made available to the scientific community in the journal *Nature Protocols*.

Their laboratory at Stanford University's Department of Bioengineering, has since received requests for the necessary reagents from more than 700 labs all over the world. Deisseroth has not only given his name to the technique selected by *Nature* as the Method of the Year in 2010; he is also firmly persuaded that it 'will open up new country for the study of biology'.

Further information:

ZHANG F ET AL. (2010) Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. **Nat. Protoc. 5 (3), 439–56** representatives of the animal kingdom, up to and including humans.

'These are complex decisions, made by complex brains,' explains Dickson. At least with flies, unlike humans, a wide range of powerful genetic tools are available for identifying the neurons involved and manipulating them. Molecular genetics and electrophysiology are combined with sophisticated computer analyses and with optogenetics, which uses beams of light to measure and manipulate neural circuits (see box).

Dickson's team has investigated the complex interaction between hereditary factors and nerve cells especially intensively in the case of a gene named 'fruitless'. Although its genetic information is identical in the male and female, their cells produce different blueprints from it. If a male is induced to make female blueprints from fruitless, he behaves more like a female. Dickson's team has also carried out changes in the other direction, imposing upon female flies the courtship behaviour of the opposite sex.

The differing behaviour of males and females is apparently dictated by the 2,000 nerve cells in which blueprints of fruitless are read and whose 'circuit diagram' has now been mapped completely in three dimensions. These fruitless neurons are differentiated, moreover, into around 100 types. One of the next tasks that Dickson and his team want to tackle, therefore, is to specifically manipulate sub-populations of cells in the circuit. The goals are to find out which role each of the different types of nerve cells play in courtship behaviour, which biochemical and electric signals are processed there and whether differences between male and female flies can be attributed to any of these. The scientists have already found eleven anatomical differences in the fruitless 'circuit diagram': sub-types of nerve cells that are present either exclusively or predominantly in the male, or whose branching patterns look different between males and females.

The initial processing of sensory signals and the generation of motor outputs appear to be organized very similarly in both sexes of *Drosophila*. Thanks to new technology, however, Dickson's team may have pinpointed the reason why males and females nevertheless behave differently. The key is in the dimorphic neural circuits in the higher brain that couple sensory input to motor output. The sex differences in these circuits may explain why males and females do different things when stimulated by the same sensory stimuli.

The fly brain contains approximately 150,000 nerve cells at most; our own has at least 700,000 times that many. The number of connections in the human brain has been estimated at about one trillion. Given this fact, is it not fanciful to make inferences from flies to humans? 'Of course people are vastly more complex than flies,' answers Dickson diplomatically, 'but some of the general principles of neuronal function are likely to be shared between humans and flies. Our aim is to help elucidate these conserved principles.'

Memory, too, probably works no differently in fruit flies and mice than it does in people. So Michael Häusser of the Wolfson Institute for Biomedical Research at University College London attracted a lot of attention when he presented proof of a long-held supposition: in learning, individual memories are encoded in the form of specific groups of activated neurons, which together form a network.

In his experiment, Häusser had taught mice to associate a particular audio tone with a mild electric shock that followed it, so that subsequently, the rodents stiffened in apprehension as soon as they heard the tone. Beforehand, the researchers had implanted a synthetic gene into the brains of the animals. It is switched on only in activated cells, where it is translated into a light-responsive protein. A green fluorescent protein was simultaneously activated as a 'reporter' when the researchers directed blue laser light along an optical fibre into a particular brain structure, the hippocampus, and then activated the neurons that are involved in the formation of memory. The mice then showed a startle reflex even without hearing the feared acoustic signal.

'With only a flash of light, we succeeded in reactivating the animals' memory,' comments Häusser, who is amazed at the efficacy of optogenetics. Around two million neurons lie in the gyrusdentatus, the region of the hippocampus that Häusser →

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FROM THE LAB TO THE PATIENT

Photo: Engine Images/Fotolia

He is chiefly celebrated as a bioengineer and as the pioneer of optogenetics: the man who in only a few years achieved a major breakthrough in this new and powerful method of investigating neural circuits. As a psychiatrist, however, Karl Deisseroth is also deeply rooted in medical practice. At least once a week, the 39-year-old sees patients who suffer from depression, schizophrenia and other mental disorders hardly understood up to now. ‘Only gradually are we getting beyond the point at which we think of psychiatric illnesses as biochemical problems that originate in an imbalance between certain transmitters,’ says Deisseroth. Believing this concept to be clumsy and imprecise, he rather supposes that the causes could lie in the ways in which specific neural circuits are connected with each other. In optogenetics (see box ‘Optogenetics – a recipe’), therefore, Deisseroth recognizes a new tool for clarifying the function of these neural circuits and for clearing up disorders by using targeted stimulation.

Deep brain stimulation with implanted electrodes is, indeed, an established treatment for patients in the advanced stages of Parkinson’s disease and, furthermore, there have recently been reports of success in treating depression with this method. Charles Nemeroff of Emory University School of Medicine, for example, has written that it induced ‘sudden ease’ in his patients. They spoke of the ‘disappearance of the emptiness’ and a ‘sudden lightening of the room, with sharper details and more intensive colours’. These approaches, however, are too inexact and are associated with too many side effects, thinks Deisseroth. He compares the stimulation of entire brain regions or neural nodes (ganglia) via electrodes with ‘a conductor who encourages the whole orchestra at once, instead of bringing the flutes to the fore and subduing the timpani. Electrodes are quick, but stupid.’

That it can be done differently has already been demonstrated by Herbert Covington in experiments with mice, performed in the lab of the famous psychiatrist Eric Nestler at Mount Sinai School of Medicine in New York. Using an optogenetic technique that involved submitting stressed rodents to flashes of light, he achieved an effect similar to that of antidepressants. The animals, which up to then had refused social contact, reverted to behaviour normal for their species after Covington and his colleagues had stimulated the neurons of the prefrontal cortex.

Garret Stuber and Antonello Bonci, too, have implanted an optogenetic switch in the brains of mice. At the Ernest Gallo Clinic and Research Center of the University of California in San Francisco, they used it to study a neural circuit involved in regulating addictive behaviour.

In addition, the researchers focused on an anatomical pathway deep in the brain, extending along specific nerve fibres from the amygdala to neurons in the nucleus accumbens, located in the forebrain. Presented with the opportunity to push a button with their noses and thus to generate a beam of light that activated those neurons in the nucleus accumbens, the test animals began frantically stimulating themselves. The experiment therefore confirmed that the region targeted by the optical actuator plays a central part in addictive behaviour.

With the help of optogenetics, Deisseroth concludes, psychiatry is moving towards becoming a network science, interpreting complex brain functions (including behaviour) as the properties of a system, properties that arise from the electrochemical dynamics of cells and neural circuits. ‘As a doctor’, he adds, ‘I find this development fascinating.’

investigated. Approximately 200,000 neurons are activated when the mice get anxious and learn to associate the sound signal with the upcoming electric shock. Interestingly, though, activating fewer than 100 neurons with the laser – and in some experiments a mere 20 – was enough to make the mice recollect the lesson learned and to achieve ‘total recall’.

Sebastian Seung also likes the concept of total recall and so he invites his listeners to join him for a fascinating game of the mind: The ultimate test of his theory ‘I am my connectome’, Seung suggests, might be an attempt to read out all of the memories contained in a human brain. Maybe even a dead one. ‘We laugh at people who have their bodies frozen after death, hoping that the medicine of the future will give new life to them, somehow resurrecting their identity. But who knows? Maybe one day they’ll be standing at our graves, smiling.’ ◄

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