

NEUROSCIENCE

Neuroscience in a virtual world

Using a virtual reality setup and a deep window into the brain, researchers can image the activity of neurons as mice navigate virtual environments.

The study of how our brains help us navigate the world has fascinated neurobiologists for many decades. The encoding of spatial information in the brain is the job of, among others, neurons in the hippocampus called 'place cells'. Each place cell fires, or becomes active, when we are in a certain place in our local environment. Neighboring place cells fire at different locations such that the entire space that surrounds us is 'mapped' by the activity of these cells.

To study the neural basis of navigation, animals such as rats and mice can be placed in a small box decorated with landmarks and the activity of their place cells can be recorded, using electrodes, as the animals move around the space. However, using electrodes one cannot sample highly dense populations of neurons, and it is hard to know with exactitude which cells are being recorded at a given time.



Virtual reality setup. Photograph of the microscope and virtual reality setup (top) and view from one end of the virtual reality track (bottom). Reprinted from *Nature Neuroscience*.

Imaging techniques offer some advantages. The use of two-photon microscopy and fluorescent neuronal activity reporters, which can be genetically encoded, allows

neuroscientists to monitor the activity of many identified neurons at once. If one uses tiny microscopes, which rats and mice can carry while they move around the space, neuronal activity can be monitored while the animals are involved in the relevant behaviors.

Whichever method is used, a box, no matter how ornamented, is hardly representative of the ever-changing environments that surround us. As an alternative, David Tank and his team at Princeton University devised a way to bring changing sceneries to a stationary mouse. In their setup, mouse heads are kept still and two-photon microscopy or electrodes are used to record neuron activity in awake mice while they run on a Styrofoam ball, as one would do on a treadmill. To study neural circuit dynamics involved in navigation, the mice are then surrounded by a wraparound screen that displays a virtual environment that can be dynamically controlled using modified videogame engines, a contribution of graduate student Forrest Collman in Tank's laboratory.

In 2009, when the group first reported the virtual technology setup (Harvey *et al.*, 2009),

STEM CELLS

iPS CELL ABERRATIONS

Gene expression profiles identify chromosomal aberrations in human induced pluripotent stem cell lines.

The potential of human induced pluripotent stem cells (hiPSCs) for research, drug development and, eventually, cell-based therapy, is now widely acknowledged, but there are many hurdles that remain to be crossed. One relatively unexamined question about these cells is whether, like human embryonic stem cells, they may have a propensity for acquiring genetic aberrations.

In collaborative work conducted by researchers at the Hebrew University in Jerusalem, the Cedars-Sinai Medical Center in Los Angeles and the University of California, Los Angeles, a group led by Nissim Benvenisty isolated embryonic stem cells from human embryos determined by preimplantation genetic screening to have trisomies of specific chromosomes. The scientists noticed during the course of this work that these trisomic lines had expression levels well above normal for genes on the trisomic chromosomes. This mirrored observations that have been made in other systems, notably in yeast and in cancer cells, and it led the scientists to the idea that a meta-analysis of gene expression profiles of hiPSCs might identify aneuploidies and other aberrations in the genome.

The researchers combined microarray-derived gene expression profiles for pluripotent stem cell lines from 18 independent studies,

with 38 human embryonic stem cell lines and 66 hiPSC lines (some of them subclones, in the latter case). They then statistically examined normalized expression levels of about 12,000 genes in each sample, and identified regions on the chromosomes for which expression was unusually high or low. These regions were candidates for harboring a genetic change, a duplication or a deletion.

For a subset of the samples, the researchers could compare the results of the gene expression analysis with either karyotyping or higher-resolution array-based studies of copy-number variation. In all cases for which data were available on comparable samples, the results matched. However, especially for smaller, subchromosomal changes, the number of samples was not large enough to draw conclusions about the sensitivity of the approach. "For trisomies and chromosomal arms, [using gene expression is] very sensitive and specific," says Yoav Mayshar, first author on the paper reporting the study, "but for small changes, to really know the limits, we'd need a larger dataset."

One of the strengths of using gene expression profiles to identify genetic aberrations is that the data are readily available. "Ideally we would do the same experiment using [single-nucleotide polymorphism] arrays or some such approach," says Mayshar, "but this was really the only feasible way to get our hands on data

postdoc Chris Harvey used whole-cell electrophysiological recordings to monitor the activity of hippocampal place cells as the mice navigated through a virtual linear track. The group could monitor place cells firing at specific locations in the trajectory and identified critical signatures in their activity patterns. As Tank explains, “the natural progression was then to put imaging and virtual reality together,” which the group now shows (Dombeck *et al.*, 2010).

Given that the hippocampus is located deep in the brain, this was not a trivial task. Postdoc Dan Dombeck adapted a surgical procedure to expose a portion of the hippocampus and create a chronic hippocampal ‘window’. To facilitate imaging the same neurons over several weeks, they monitored their activity using the genetically encoded calcium sensor GCaMP3.

Combining virtual reality and high-resolution functional imaging allowed the team to investigate novel aspects of circuit dynamics during navigation. “An important extension for the future is that with imaging we can link neuronal activity to the detailed wiring diagram of synaptic connectivity, which is more difficult to do with electrodes,” says Tank. As a first step, the group optically identified place cells as the cells fired, and studied the correlations between the stimuli the cells responded to and the cells’ anatomical location in the local circuit. Maybe one day virtual worlds will become routine in neuroscience labs as they can bring us closer to our brains’ reality.

Erika Pastrana

RESEARCH PAPERS

Dombeck, D.A. *et al.* Functional imaging of hippocampal place cells at cellular resolution during virtual navigation. *Nat. Neurosci.* **13**, 1433–1440 (2010).

Harvey, C.D. *et al.* Intracellular dynamics of hippocampal place cells during virtual navigation. *Nature* **461**, 941–946 (2009).

from so many different lines, different labs, different passage numbers, different types of reprogramming.” What is more, once a chromosomal region has been identified as aberrant in this way, one already has some information about functional expression changes that may result.

This is not to say that the approach does not have disadvantages as well. It is not as high-resolution as some of the more direct approaches: the smallest change the researchers detected so far was of about ten megabases. Furthermore, it could be confounded by epigenetic effects on gene expression, and it is likely to be less successful than other approaches on heterogeneous samples.

Nevertheless, Mayshar, together with Uri Ben-David and their colleagues, identified abnormalities in about a fifth of the 66 hiPSC lines tested. The data at this stage are probably too limited in scale to suggest that particular reprogramming methods are more or less prone to problems—the analyzed dataset included lines generated using retroviral integration, protein-based reprogramming and episomal vectors—but it remains to be seen with future work on larger numbers of lines whether such patterns emerge.

Natalie de Souza

RESEARCH PAPERS

Mayshar, Y. *et al.* Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. *Cell Stem Cell* **7**, 521–531 (2010).

SEQUENCING

RNA fitness landscapes

Pitt and Ferré-D’Amaré describe an approach to generate fitness landscapes for catalytic RNAs. They incubated a pool of ribozymes with a substrate RNA immobilized on beads; after allowing the RNAs to react, they isolated and deep-sequenced the ribozymes that bound the beads, identifying the fittest variants. The resulting high-resolution map, which yields insights into the fittest genotypes for a particular phenotype, contains 10^7 unique RNA genotypes.

Pitt, J.N. & Ferré-D’Amaré, A.R. *Science* **330**, 376–379 (2010).

CHEMISTRY

Capturing G-quadruplexes

G-quadruplex motifs are four-stranded nucleic acid structures found in genomic DNA that are thought to be associated with telomere maintenance and gene expression, though much remains to be discovered about G-quadruplex function. Müller *et al.* designed a small molecule that selectively binds G-quadruplexes in human cells, so that they can be isolated for further analysis. The small molecule contains a biotin moiety that serves as a handle for capturing G-quadruplexes on streptavidin-coated beads.

Müller, S. *et al. Nat. Chem.* advance online publication 10 October 2010.

SENSORS AND PROBES

Dual-color protein interaction probes

Protein-protein interactions can be detected via protein fragment complementation, whereby two pieces of a split fluorescent or bioluminescent protein are reunited and produce a signal when their fusion partners bind. Villalobos *et al.* describe several pairs of reversible, dual-color complementation fragments based on firefly and click beetle luciferases that use a single substrate, D-luciferin, to produce a bioluminescent signal, allowing dynamic analysis of multiple protein interactions in living cells.

Villalobos, V. *et al. Chem. Biol.* **17**, 1018–1029 (2010).

NEUROSCIENCE

Mapping microcircuitry in the fly brain

The complex patterns of synaptic connections in the brain, known as microcircuitry, form the basis of behavior. Mapping microcircuits across whole brains has been a technical challenge because it requires analyzing complete series of thin sections by transmission electron microscopy (TEM). Cardona *et al.* applied a software package called TrakEM2 to comprehensively reconstruct the neuronal microcircuitry from serial TEM sections of the larval brain of the fly *Drosophila melanogaster*.

Cardona, A. *et al. PLoS Biol.* **8**, e1000502 (2010).

BIOCHEMISTRY

Microscale thermophoresis

The term thermophoresis refers to the directed motion of molecules in solution as a result of temperature gradients; thermophoretic behavior depends on properties such as mass, size and charge. Wienken *et al.* exploited this phenomenon to develop a method to detect protein-protein and protein–small molecule interactions *in vitro* without immobilization. An infrared laser locally heats a solution containing a fluorescently labeled protein of interest; the thermophoretic behavior of the protein changes upon binding, as monitored by fluorescence.

Wienken, C.J. *et al. Nat. Commun.* advance online publication 19 October 2010.