TECHNOLOGY

Rainbows in the brain

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How are the millions of brain cells connected to achieve the different facets of neuronal function? Until now, we have been limited to studying only a few dye-filled or fluorescent-protein-expressing neurons at a time. Reconstruction of connectivity from three-dimensional electron microscope sections is cumbersome and limited to the sectioned area. To study connectivity on a large scale, Livet *et al.* have generated a set of genetic constructs, termed *Brainbow*, that colour-tag each individual cell with one random colour from a pool of colours, allowing researchers to trace dendrites and axons of individual neurons and to study the interactions between cells even in densely packed areas of the brain.

The *Brainbow* constructs were designed by combining Cre/Lox-dependent DNA excision or inversion with up to four different fluorescent proteins (XFPs). Upon Cre- recombination, stochastic choice of XFP expression produces up to four different fluorescent colours.

To generate transgenic mice, the expression of *Brainbow* was placed under the control of the *Thy-1* promotor, which drives strong gene expression in a number of neuronal cell types. The authors generated 19 transgenic mouse lines which, surprisingly, expressed up to 100 different colours in neuronal cells. However, it is a well-known phenomenon in transgenic mice that constructs are often inserted in tandem. Indeed, the authors confirmed that the colour diversity was caused by up to 16 tandem repeats of the *Brainbow* construct. Individual cells expressed distinct combinations of the four fluorescent proteins, resulting in the multitude of colours that enabled the authors to distinguish single neurons and their processes within dense cell clusters.

The authors then tested several applications of *Brainbow* expression. First, they studied connectivity in the inner granular layer of the cerebellum. They demonstrated that each postsynaptic granule cell received synaptic inputs from multiple presynaptic mossy fibre neurons, answering a long-standing question in cerebellar circuitry.

Some of the transgenic mouse lines also showed *Brainbow* expression in astrocytes, allowing the study of the interaction of these cells with neurons. For example, the authors demonstrated that multiple cerebellar Bergman glia cells ensheathed the same part of Purkinje-cell dendrites, extensively interdigitating in their target areas.

In time-lapse studies of *Brainbow*-expressing Schwann cells at adult-mouse neuromuscular junctions, the authors showed that the glial sheath is dynamic, moving back and forth on the nerve terminal. These experiments also verified that the intensity and colour of the

labelled cells remained constant over extended periods and that, therefore, *Brainbow* can be used for longitudinal studies of circuits and interactions *in vivo*.

The *Brainbow* constructs presented in this study can be seen as the first generation of a series of new and powerful tools for studying connectivity and the interaction of cells *in vivo*. Choosing different promotors and fluorescent proteins are just two options for the further development of this tool, which is by no means limited to neuroscience applications. Combining the *Brainbow* technology with other methods will without doubt profoundly influence neuroscience research in the future.

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ORIGINAL RESEARCH PAPER Livet, J. *et al.* Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. *Nature* **450**, 56–62 (2007)