nature neuroscience gateway

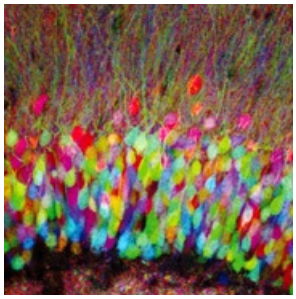
NEUROTECHNIQUES

Brainbow Brite

Neuroscience Gateway (November 2007) | doi:10.1038/aba1794

Researchers trace neurons labeled with nearly 90 different fluorescent hues.

Have you ever tried to sort out the knot of wires connected to your computer? The task is difficult when wires are the same color. Researchers hoping to map the connections between individual neurons have a similar problem. Fluorescent proteins wash populations of neurons in a single color, making them difficult to discriminate. Now Livet *et al*. report a technique that labels neighboring neurons in different colors in a recent article in *Nature*.



Dentate gyrus neurons are mulitcolored in *Brainbow* mice. Image reprinted from *Nature*.

Researchers use combinations of antibodies or transgenes to label different neuronal populations in a handful of fluorescent colors, including green, red, yellow and orange. The authors linked several fluorescent proteins together in a construct called *Brainbow* and devised a strategy to randomly express one per neuron.

The enzyme Cre splices out genetic information located between *loxP* sites. There are variant *loxP* sites, but Cre only splices out genetic information between like sites. The authors alternated *loxP* variants at the 5' end of *Brainbow* and after each fluorescent protein, forcing Cre to 'choose' whether to delete the first or the first several fluorescent proteins. Cre also mediates inversions of genetic material located between *loxP* sites that are facing each other. In *Brainbow-2.0*, Cre inverts tandem pairs of fluorescent proteins arranged between inward-facing *loxP* sites.

The authors expected the fluorescent protein located in the 5' position after excision or inversion to be expressed. *In vitro*, each cell expressing both Cre and *Brainbow* expressed one of the 4 fluorescent proteins in the *Brainbow* construct. However, in mice expressing *Thy1*-driven *Brainbow* and Cre, neurons were labeled in approximately 90 distinct hues, including magenta, blue-green, purple and grey.

How were all of these colors produced? Reverse transcriptase-PCR showed that the mice with colorful brains incorporated multiple copies of the *Brainbow* transgene, so Cre might randomly generate several different *Brainbow* splices per cell. After the final *loxP* site, each *Brainbow* construct contained an *FRT* site, which is the recognition site for Flp recombinase. In *Brainbow* mice, Flp should splice out all but the first *Brainbow* copy. When the authors crossed *Brainbow* mice with Flp-expressing mice, the intermediate hues disappeared, suggesting that tandem *Brainbow* integration produces color variation.

Color did not vary between dendrites and axons of the same neurons. Therefore, neurons could be differentiated without tracing neurites back to their source. In the inner granule layer of

the cerebellum, mossy fibers synapse onto granule cells. The authors used confocal microscopy and three-dimensional reconstruction to examine 93 granule cells, 341 axons and 236 mossy fiber terminals in the inner granule layer. Different colored axons innervated each granule cell dendrite, suggesting that more than one neuron innervates each granule cell.

Although *Thy1* is predominantly expressed in neurons, 4 of the 19 Brainbow lines showed fluorescently labeled glia. Fluorescently labeled Bergmann glia overlapped extensively with Purkinje cell dendrites. Although the arbors of fluorescently labeled astrocytes overlapped, their somas did not.

Therefore, Brainbow offers insight into the relationship between brain cells. The authors also propose using Brainbow for lineage analysis and to track changes in gene expression across populations of neurons.

Debra Speert

1. Livet, J. *et al.* Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. *Nature* **450**, 56–62 (2007). | Article | PubMed | ChemPort |