IMAGING AND VISUALIZATION

Inner glow

The discrete randomization of fluorescent protein expression in neurons creates a Brainbow.

Visualizing neural structures is notoriously tricky. Although neurons vary considerably in morphology, they often are large cells with complicated processes that are delicately interwoven with each other and their target cells. These elaborate structures can be seen when neurons are individually stained, but there are few tools to distinguish between the cells or to follow them during development. Now, Jeff Lichtman, with postdoc Jean Livet, long-term collaborator Joshua Sanes and their colleagues at Harvard University, report the creation of one such tool. In the aptly named 'Brainbow' mice, these researchers genetically randomized the expression of fluorescent proteins in a population of cells, distinctly marking individual neurons to study their behavior.

Lichtman is interested in how neurons decide to connect to develop their higher-order neural structures and how that circuitry changes over time. The neurons vie for these synapses, and so there is, as Lichtman puts it, a "circuit-level competition where you have to think about more than one nerve cell and another, but also how many nerve cells are sorting out their connections simultaneously." It is still unclear, however, what makes one cell better able than another to form a stable synapse on a target cell.

As a first step the researchers wanted to learn more about the cells' interactions. But there are, at present, few methods available to individually label a large number of cells. Most labeling techniques—such as immunohistochemical staining and transgenic expression of fluorescent proteins—are limited to one or, at most, a few colors and cannot show the relationships between many cells. Lipophilic dye combinations label multiple cells with different colors at once, but these compounds do not efficiently diffuse down the length of the axons, severely limiting their value in studying connectivity.

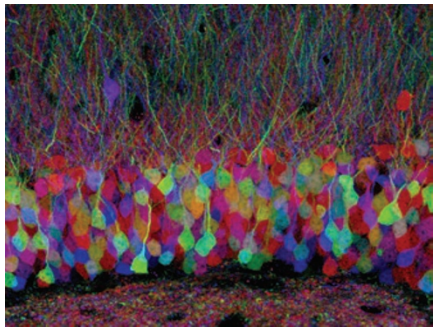

Figure 1 | Combinatorial expression of different-colored fluorescent proteins distinguishes the many neurons of the dentate gyrus. Image courtesy of J. Livet.

So this Harvard group developed a genetic strategy to randomly but discretely paint many cells from the inside using fluorescent proteins. The researchers created constructs that encoded up to four different-colored fluorescent proteins, but only expressed one of them at a time. Using the Cre-*lox* recombination system to rearrange the transgene, however, they physically moved fluorescent protein–coding regions close to or away from the regulatory regions of a neuronal gene, changing which fluorescent protein was expressed. Among the multiply inserted transgenes in each cell, these random recombination events produced a cell-specific array of fluorescent proteins. That mixture gives the cell a unique color (upon excitation by light of the appropriate wavelengths), distinguishing it from neighbors who had recombined their transgenes differently (**Fig. 1**).

This strategy differs from those using standard cellular stains, such as immunohistochemistry, which marks all the cells that express the antibody's target. The Brainbow system "takes any type of cells and splits them up. For the kind of questions that I ask where I've got a bunch of identical motor neurons competing with each other, this is exactly what we want," explains Lichtman. Already he and his colleagues report that glial cells have more intricate cell-cell borders than previous techniques had shown and confirmed the hypothesis that multiple cells can input onto granule-cell neurons.

In their mouse lines, the researchers estimated they could distinguish 90 colors among the Brainbow neurons, whereas their computer reported over 150 distinct shades. These colors are both consistent over the length of the axon, which allows long-distance mapping, and constant over time, which allows long-term studies of cell behavior.

These mice could be used to investigate situations in which individual members of a cell population act differently. For instance, Lichtman hopes the Brainbow mice will shed light on the cellular interactions and competition that result in functional circuits during neural development. Once these patterns are known in normal animals, they can be compared with the neural circuits in disease models to investigate whether these disorders cause neuronal miswiring. For other developmental or inductive pathways, such as the immune response, researchers may find that these constructs can be used to distinguish and follow many individual cells, presenting researchers with a new vantage point into these complicated worlds.

Brainbow mice are a means to let the cells show their differences on their own, without the experimental manipulations of hypothesis-driven research. Lichtman calls this type of approach "inductive or discovery science," when unbiased observations are the primary goal. To have a hypothesis, after all, means that you already think you know how the system works. Brainbow, says Lichtman, "is a tool that lets you see things that you might not otherwise see and sometimes shows you things you didn't expect."

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RESEARCH PAPERS

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