SCAFFOLDING 2

THE COLOR OF BRAIN: BRAINBOW MICE AND CONECTOME

A central aim of neuroscience is to map neural circuits, in order to learn how they account for mental activities and behaviors and how alterations in them lead to neurological and psychiatric diseases. The modern part of this saga started in 1873 at Psychiatry Hospital of the University of Pavia, Italy, where Dr Camillo Golgi applied silver staining to visualize brain cells. He initially named this procedure *la reazione nera* (from Italian, "the black reaction"), later known as Golgi method. The method was further developed by Santiago Ramón y Cajal in Madrid, Spain, and in 1905 they both were awarded Nobel prize for their great contribution to visualizing the whole neuronal morphology expressing long processes, axons and dendrites.

Recently, in a seminal paper published on 1 November 2007 in *Nature*, Jeffrey Lichtman and colleagues at Harvard University Medical School provided a novel tool for staining brain cells, using a set of fluorescent protein transgenic mice whose neurons have the potential to display every color of the rainbow, hence brainbow mice (1,2) (Fig. 1, 2). Such large-scale circuit reconstruction, which has been called connectomics, may soon be possible, owing to numerous advances in technologies for neuroimaging. Remarkably, the Human Conectome Project is now in progress, whose goal is to map, store, analyze, and visualize neural circuitry (2-4).

To develop Brainbow mice (type 1 and type 2), the Harvard researchers used the Cre/loxP site-specific recombination system. The *cre* gene encodes a viral enzyme that recognizes a specific 34-base pair DNA sequence called loxP, which is usually present in pairs that are located close to-

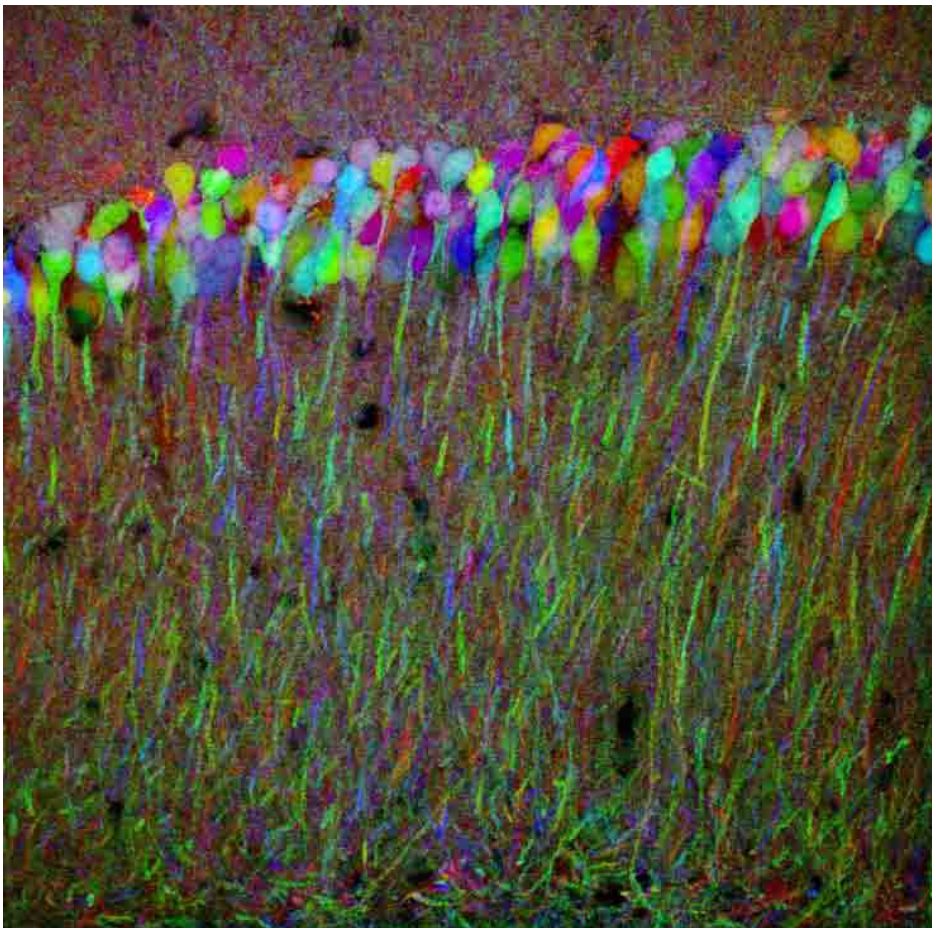

Figure 1. *Using confocal microscopy to generate three-dimensional reconstructions, this multi-color view traces the neural circuitry in the inner granule layer of the cerebellum. From reference 1.*

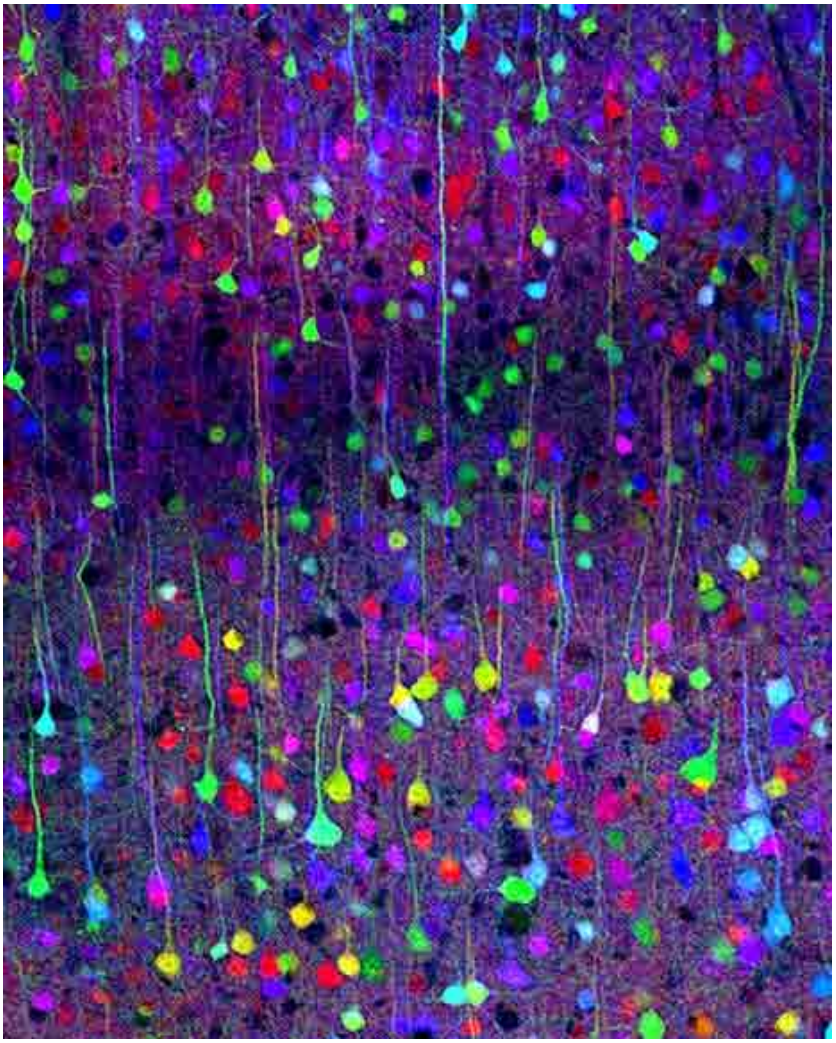

Figure 2. *Using confocal microscopy to generate three-dimensional reconstructions, this multi-color view illustrates the brain cortical layers. From reference 1.*

gether on the chromosome. When a Cre molecule binds to its target sequence, a process called recombination occurs. The method is complex and time-consuming - it involves breeding at least two different strains of mutant mice created from embryonic stem cells transfected with specially designed DNA constructs. One strain is derived from stem cells containing the *cre* gene under the control of a regulatory DNA sequence (the promoter) which drives expression in a specific tissue or cell type. The other is derived from cells in which the gene of interest has been replaced with a construct containing the same gene flanked by loxP sites. The two mouse strains are then mated, and recombination occurs in some of the offspring but not others.

Because the integration sites have multiple copies of the transgene, each neuron may express one of a wide variety of possible fluorescent protein combinations, and therefore emit a distinctive hue. Depending on the type of analysis used, over 89-166 colors may be distinguished (cf. 5).

REFERENCES AND NOTES

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3. Sporns O, Tononi G, Kotter R. 2005. The human connectome: A structural description of the human brain. *PLoS Comput Biol* 1:245-251.
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5. Between November of 1881 and July of 1890, Vincent van Gogh painted almost 900 paintings. One of them is shown herein. The vivid tones of van Gogh's palette demonstrate that the Master, has, long time before brainbow, achieved the beauty of mixing the colors.

Vincent van Gogh's ***Willows at Sunset*** is an
oil on cardboard (12-1/2x13-1/2 inches) housed
in the Kröller-Müller Museum in Otterlo, Netherlands.

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