Dispatches

Neuroanatomy: Decoding the Fly Brain

Despite their relatively small brains, with only about 100,000 neurons, fruit flies show many complex behaviours. Understanding how these behaviours are generated will require a wiring diagram of the brain, and significant progress is being made towards this goal. One study has labelled 16,000 individual neurons and generated a coarse wiring diagram of the whole fly brain, identifying subnetworks that may carry out local information processing.

Johannes Kohl and Gregory S.X.E. Jefferis

Just as genomics set out to sequence an organism's hereditary information base by base, the emerging discipline of connectomics [1,2] aims to determine the complete wiring diagram of the brain, neuron by neuron, synapse by synapse. The human connectome, however, is dramatically more complex than the human genome: the three-dimensional image data of a single human brain at synaptic resolution would occupy orders of magnitude more space than current genome archives. Furthermore, the hardware to acquire such data and the automated software required to analyse it remain huge challenges. As a human, or indeed any mammalian, connectome at synaptic resolution remains far out of reach, a two-pronged strategy seems to be evolving. This includes the development of long-range connectivity maps, aka 'projectomes' [2], using lower resolution imaging techniques, such as the recently funded NIH human connectome project which will use functional magnetic resonance imaging (fMRI) to identify axon tracts connecting different brain regions *in vivo* (http://www.humanconnectomeproject.org). The second approach is to target model organisms with smaller brains that are more experimentally addressable by high-resolution methods. In this vein, two new studies [3,4] have generated projectomes of the *Drosophila* brain by labelling either many single neurons, as reported by Chiang *et al.* [3] in this issue, or axon bundles [4].

Approaches to obtaining a neural wiring diagram can be characterized as either dense or sparse. In a dense reconstruction, all of the neurons within a brain or block of tissue are labelled and imaged. Many neural processes, however, are significantly smaller than the resolution of conventional light microscopy. The only complete connectome available describes the wiring of the 302 neurons in the nervous system of *Caenorhabditis elegans* and was obtained by serial electron microscopy more than 20 years ago [5]. Despite recent advances in optical imaging, electron microscopy remains the only imaging approach that can resolve all the tiny axonal and dendritic processes within a single brain [6]. Nevertheless, electron microscopic reconstruction of a whole fly brain, with 100,000 neurons packed into the volume of a poppy seed, remains a challenge for the medium term.

In a sparse reconstruction, a small fraction of the neurons in a brain are labelled and then information is integrated across many samples, as typified by Cajal's use of the Golgi method. Chiang *et al.* [3] have used a modern version of this approach, summarised in Figure 1. They used a genetic labelling technique called MARCM to disassemble the fly brain [7]. MARCM stochastically labels single cells from a starting population defined by a genetic driver line. The authors used nine lines covering most known neurotransmitter types in the fly brain. They dissected over a million brains, and then carried out high-resolution confocal imaging of 16,000 single cells (75% from females). Although still substantially fewer than the 100,000 neurons in a single brain, they have likely identified the majority of anatomical cell types. Breaking a neural circuit into so many pieces naturally requires a means to put everything back together. They used image registration — more specifically global affine registration — to map all collected neurons back onto a common reference brain [8–10]. Of course registration cannot overcome the fact that these data originated from different brains and therefore the predictions about connectivity remain tentative.

Perhaps the most important achievement of this new study [3], and a clear parallel with genomics, is the creation of a comprehensive, interactive database (www.flycircuit.tw) of all 16,000 neurons. This allows open access to this information, as well as the opportunity to upload new data for analysis. Each neuron has a summary web page with information about its expression phenotype, projection pattern and putative birth time. *Drosophila* neuroscientists of all stripes are likely to spend many hours poring over this resource when it goes live and it has the potential to become a community information hub.

Although these single cell data are extremely rich, Chiang *et al.* [3] concentrated on trying to extract some global principles of circuit organisation. They started by identifying what they termed local processing units (LPUs), brain regions that have populations of spatially restricted local neurons that are connected with other LPUs by long range projection neurons. They identified 41 LPUs, of which all but two correspond to gross neuropil regions of the fly brain. By tracing axons of the projection neurons, they identified connections between different LPUs, thereby obtaining a brainwide wiring diagram. Further analysis of this connectivity matrix identified four clusters of densely interconnected LPUs that appear to be specialised for processing different sensory information. One immediate prediction is that the downstream targets of the mechanosensory neurons in the antennal mechanosensory and motor centre that sense sound, wind and gravity lie in the caudal ventrolateral protocerebrum. Such predictions require functional confirmation but remain invaluable, as almost 75% of the central fly brain is of unknown function [4].

Pereanu *et al.* [4] used a very different approach to construct a coarse wiring diagram of the fly brain. They divided the brain into 41 neuropil compartments separated by glial borders and then labelled the axon

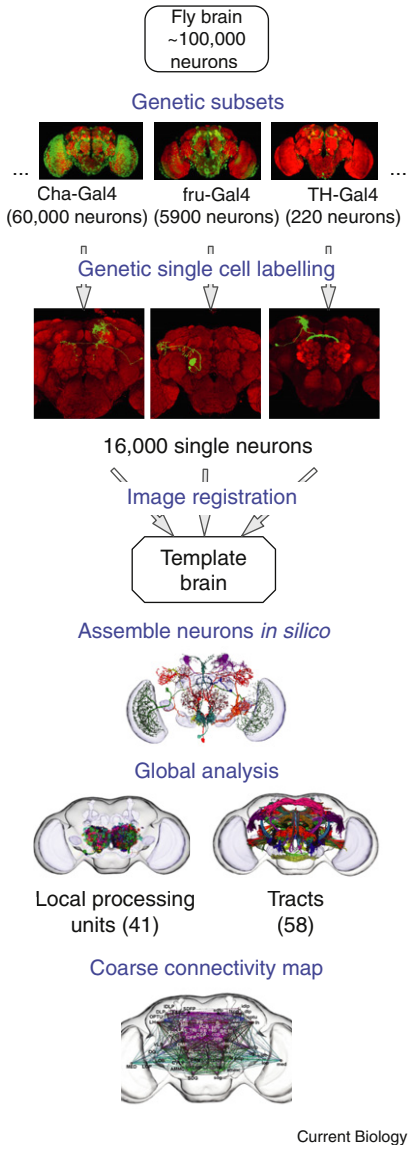

Figure 1. Mapping the fly brain.

Single-cell labelling is performed on driver lines that label subsets of the ~100,000 neurons in the fly brain. Individual neurons are extracted from confocal stacks, registered onto a template brain and then reassembled into a common reference space. This information is used to identify both local processing units (LPUs) and fibre tracts. Finally, a coarse connectivity map is constructed that predicts information flow between LPUs.

bundles connecting different compartments. They identified a total of 125 unique connections. Even such coarse wiring diagrams propose many novel connections between brain areas. One nice point of the Pereanu *et al.* [4] study is that they were able to use a simple behavioural paradigm to validate one novel connection and identify and manipulate a set of neurons that actually make up the connection.

The impressive effort started by Chiang *et al.* [3] may eventually identify and roughly map every neuron in the fly brain. There is no doubt that anatomical constraints on how information flows in such an experimentally tractable nervous system provide an important advance for neuroscience in general. But data of this kind also introduce new challenges. Initial comparison of the two new maps is complicated by practical issues such as the lack of standardised brain nomenclature. Another challenge is integration of large image data sets like that of Chiang *et al.* [3] with other brain-wide mapping studies, such as recent work on sex circuits in flies [11,12]. Raw image data are crucial for such analysis and Chiang *et al.* [3] have taken the key step of making all their original image data (though not their analysis) available for download by other groups. Of course neuroanatomical maps of any resolution must be translated into functional connectivity.

The scale of new anatomical studies, combined with genetic approaches to monitor and manipulate neurons in *Drosophila*, suggest that it will be a key model system in trying to understand how behaviour is encoded in neural circuits. Finally, our ability to decode more complex brains like our own will depend critically on our ability to acquire, store and comprehend vast amounts of data. But in the light of the stunning advances in genomic sequencing technology over the last decade maybe we can start to dream of a 'thousand-dollar connectome' sooner than expected.

References

1. Sporns, O., Tononi, G., and Kötter, R. (2005). The human connectome: A structural description of the human brain. PLoS Comput. Biol. *1*, e42.
2. Lichtman, J.W., and Sanes, J.R. (2008). Ome sweet ome: what can the genome tell us about the connectome? Curr. Opin. Neurobiol. *18*, 346–353.
3. Chiang, A.S., Lin, C.Y., Chuang, C.C., Chang, H.M., and Hsieh, C.H. (2011). Three-dimensional reconstruction of brain-wide wiring networks in Drosophila at single-cell resolution. Curr. Biol. *21*, 1–11.
4. Pereanu, W.S., Ahammad, P., Jenett, A., Myers, E.W., and Truman, J.W. (2010). Investigating neural circuitry using a compartment-level connectivity map of the adult Drosophila brain. PLoS Biol., in press.
5. White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The structure of the nervous system of the nematode Caenorhabditis elegans. Philo. Trans. R. Soc. Lond. B. *314*, 1–340.
6. Helmstaedter, M., Briggman, K.L., and Denk, W. (2008). 3D structural imaging of the brain with photons and electrons. Curr. Opin. Neurobiol. *18*, 633–641.
7. Lee, T., and Luo, L. (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron *22*, 451–461.
8. Rein, K., Zockler, M., Mader, M.T., Grubel, C., and Heisenberg, M. (2002). The Drosophila standard brain. Curr. Biol. *12*, 227–231.
9. Jefferis, G.S.X.E., Potter, C.J., Chan, A.M., Marin, E.C., Rohlfing, T., Maurer, C.R.J., and Luo, L. (2007). Comprehensive maps of Drosophila higher olfactory centers: spatially segregated fruit and pheromone representation. Cell *128*, 1187–1203.
10. Lin, H.H., Lai, J.S.Y., Chin, A.L., Chen, Y.C., and Chiang, A.S. (2007). A map of olfactory representation in the Drosophila mushroom body. Cell *128*, 1205–1217.
11. Yu, J.Y., Kanai, M.I., Demir, E., Jefferis, G.S.X.E., and Dickson, B.J. (2010). Cellular organization of the neural circuit that drives Drosophila courtship behavior. Curr. Biol. *20*, 1602–1614.
12. Cachero, S., Ostrovsky, A.D., Yu, J.Y., Dickson, B.J., and Jefferis, G.S.X.E. (2010). Sexual dimorphism in the fly brain. Curr. Biol. *20*, 1589–1601.

Division of Neurobiology, MRC Laboratory of Molecular Biology, Cambridge, CB2 0QH, UK.
E-mail: jefferis@mrc-lmb.cam.ac.uk

DOI: 10.1016/j.cub.2010.11.067

Chromatin Reprogramming: Gender Equality during *Arabidopsis* Germline Differentiation

Large-scale histone H3 reprogramming during male germline differentiation is conserved between animals and plants. A new report now shows that histone H3 reprogramming also occurs in the female germline of the flowering plant *Arabidopsis thaliana*.

Yannick Jacob and Robert A. Martienssen

Unlike mammals, the hermaphrodite *Arabidopsis thaliana* does not set aside its male and female germlines early during embryogenesis. Instead, they differentiate late during sporophytic development from floral tissues. As a consequence, epigenetic