acid, dendritic cells produce more IL-12. They also found that IL-15 and retinoic acid promote the development of pro-inflammatory T_H17 effector cells by enhancing production of the IL-12-related cytokine IL-23, which, along with TGF-β and IL-6, favours their differentiation. In the presence of IL-15, therefore, retinoic acid seems to act as a co-factor for production of IL-12-family cytokines by dendritic cells, establishing a new and unanticipated function for this metabolite in mucosal immunity (Fig. 1).

A telling aspect of this study emerged when DePaolo and co-workers[1] studied mice that expressed the human MHC class II molecule HLA-DQ8, which can present gluten-derived peptides to mouse T cells. When fed gluten, these animals developed oral tolerance — not immunity. This is consistent with findings[5–7] in humans that the great majority of individuals who carry the *HLA-DQ2* or *HLA-DQ8* susceptibility alleles do not develop coeliac disease. Only in gluten-fed mice expressing both HLA-DQ8 and high levels of IL-15 were features of early coeliac disease detected. What's more, administration of retinoic acid to these animals augmented the disease induced by feeding them gluten alone. This finding warns us that treatment of coeliac-disease patients with retinoids could well promote, rather than quell, disease, drawing possible parallels with a report[8] of patients in whom inflammatory bowel disease developed or was exacerbated following treatment of their acne with retinoids.

Apart from providing insights into the mechanisms underlying coeliac disease, DePaolo and colleagues' work should help to focus future studies on several unanswered questions. What causes the upregulation of IL-15 in patients with coeliac disease? And does the abnormal expression of this cytokine cause or just aggravate the disorder? Consistent with the present study, a gene encoding a subunit of IL-12 has been identified as a risk factor for coeliac disease[9] whereas, intriguingly, the gene that encodes IL-15 has not. And although the mouse model[1] shows many features of early coeliac disease, it does not exhibit villous atrophy, which is characteristic of more advanced disease in humans and is thought to be mediated by IL-15 activity. So why does it not show atrophy? And what are the implications of this observation for the hypothesis that IL-15 promotes the damage to the intestinal epithelium in coeliac disease?

As for retinoic acid, how exactly does it amplify the pro-inflammatory effects of IL-15 on dendritic cells? DePaolo *et al.* show that retinoic acid enhances activation of the JNK signalling cascade. How does it do this, and is abnormal production of retinoic acid a component of coeliac disease?

Irrespective of the answers to these questions, this study[1] re-emphasizes the fact that the IL-15 pathway is an attractive target for new therapies for coeliac disease. Whereas most autoimmune diseases are difficult to control because the offending antigen is a constituent of normal tissues and thus cannot be removed, in coeliac disease simple exclusion of dietary gluten is often palliative. Nevertheless, in a large subset of patients, a gluten-free diet does not completely control disease, necessitating other interventions. Abrogation of IL-15 expression or signalling in the intestinal tissues may well stem the adverse immune responses to gluten, as well as providing a means to re-establish oral tolerance in these patients, whether married to efforts to target retinoic acid or not. ■

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1. DePaolo, R. R. *et al. Nature* **271,** 220–224 (2011).
2. Coombes, J. L. *et al. J. Exp. Med.* **204,** 1757–1764 (2007).
3. Sun, C. M. *et al. J. Exp. Med.* **204,** 1775–1785 (2007).
4. Mucida, D. *et al. Science* **317,** 256–260 (2007).
5. Jabri, B. & Sollid, L. M. *Nature Rev. Immunol.* **9,** 858–870 (2009).
6. Kagnoff, M. F. *J. Clin. Invest.* **117,** 41–49 (2007).
7. Meresse, B., Ripoche, J., Heyman, M. & Cerf-Bensussan, N. *Mucosal Immunol.* **2,** 8–23 (2009).
8. Reddy, D. *et al. Am. J. Gastroenterol.* **101,** 1569–1573 (2006).
9. Hunt, K. A. *et al. Nature Genet.* **40,** 395–402 (2008).

NEUROSCIENCE

Towards functional connectomics

To understand the brain, the thousands of synaptic connections made by each of billions of neurons should be mapped and related to neuronal function. First steps towards this formidable goal are now reported. SEE ARTICLES P.177 & P.183

H. SEBASTIAN SEUNG

Neurons are classified into cell types, which are traditionally defined by location and shape[1]. Rules of synaptic connection that depend on cell type, although important, are not sufficient for understanding neuronal function, because cells of the same type can have diverse functions. In this issue, Bock *et al.*[2] and Briggman *et al.*[3] report an exciting and pioneering approach to finding rules of connection between neurons that depend on their functional properties as well as their cell type.

In the 1970s and 1980s, researchers[4] painstakingly compiled the 'wiring diagram' — now also known as the connectome[5] — of the worm *Caenorhabditis elegans*. They imaged extremely thin slices of this organism using serial electron microscopy, virtually reconstructed each neuron by finding all its cross-sections in the images, and found every synaptic connection between neurons.

Serial electron microscopy has been revived and reinvented in recent years, raising hopes of determining the connectomes of mammalian brains[6]. Briggman *et al.* and Bock *et al.* applied improved versions of this method to the mouse retina and primary visual cortex, respectively. They also achieved a new feat — imaging the activity of neurons in a functioning network using two-photon microscopy and calcium-sensitive indicators before mapping their connectivity. Combining serial electron microscopy with two-photon microscopy is not just a technical tour de force. It is also a way of directly studying the relationship of a neuron's function to its connections — an example of what might be called 'functional connectomics'.

The mammalian retina contains about 50–60 neuronal cell types[1]. Most of what we know about the retina's connectivity is formulated as rules governing these cell types. For example, starburst amacrine cells (SACs) make inhibitory synapses onto direction-selective ganglion cells (DSGCs). Both cell types are involved in computing the direction of movement. Each DSGC is preferentially activated by visual stimuli moving in a particular direction, and different DSGCs have different preferred directions. SACs exhibit functional diversity even within a single cell: each of the dendrites of a SAC functions independently, and each responds selectively to motion in a different preferred direction[7].

Briggman *et al.* (page 183) demonstrate the specificity of SAC–DSGC connections. They show that a DSGC tends to receive many more synapses from a SAC dendrite if their preferred directions are opposite to each other. This supports the hypothesis that DSGCs mostly 'inherit' their direction selectivity from SACs, albeit with an inversion because SACs are inhibitory. By providing this missing piece of evidence, the authors[3] have resolved half

a century of debate over the mechanism of direction selectivity in ganglion cells.

Although a DSGC can receive synapses from SAC dendrites with any preferred direction, the number of synapses tends to be much larger for certain directions. Therefore, methods that merely establish whether a SAC dendrite is connected to a DSGC cannot reveal the specificity of connectivity. It is essential to quantify the strength of interaction by counting the number of synapses involved, as Briggman and co-workers have done.

In the neocortex, most neurons are excitatory and almost all of these are of the pyramidal type. There are also many types of inhibitory neurons and many rules of neocortical connectivity based on cell type[8]. But pyramidal neurons, even in the same cortical location and layer, can differ in their functional properties. For example, a pyramidal neuron in the primary visual cortex is preferentially activated by visual stimuli of one orientation, and the preferred orientations of pyramidal neurons are diverse.

Bock *et al.* (page 177) find that inhibitory neurons receive synapses from pyramidal neurons with a wide range of preferred orientations. This lack of specificity may explain observations[9–11] that inhibitory neurons are untuned, or only weakly tuned, to stimulus orientation: if an inhibitory neuron indiscriminately sums over synaptic inputs from pyramidal neurons of all preferred orientations, then its output would lack orientation selectivity.

The authors[2], however, acknowledge their study's limitations. Even if an inhibitory neuron receives synapses from pyramidal neurons with a wide range of preferred orientations, its summed synaptic input could still be biased towards a particular orientation by more or stronger synapses (see Fig. 5d of ref. 2, for example). Other researchers[12] have reported that one type of inhibitory neuron is sharply tuned to a particular orientation. So although Bock *et al.* have not said the last word on the subject, they have made a step in the right direction.

Neural-network theorists are eager to see the controversies resolved, as they have long speculated that inhibitory neurons receive indiscriminate connections from pyramidal neurons and send back inhibition to prevent runaway excitation[13] or to sharpen response selectivity[14,15]. If this idea is correct, inhibitory neurons have a supporting role in visual computations: they are not primarily responsible for generating selectivity to visual features, but rather they help pyramidal neurons to achieve it.

The two teams[2,3] imaged relatively small volumes of the brain (less than 1% of a cubic millimetre). Furthermore, the volumes were relatively thin — about 50–60 μm — along one dimension. This is not a problem for the retina, which is a thin sheet. (The inner plexiform layer of the retina, which contains the SAC–DSGC connections, is even thinner.) But many cortical axons left the confines of the volume that Bock *et al.* imaged. This limited the size of their sample of connections, and biased it towards nearby neurons.

Why not study larger volumes? Although the present studies benefited from recent inventions, they still required heroic efforts to acquire and analyse terabytes of image data. To tackle larger volumes, the speed of both image acquisition and analysis must be increased. Analysis should be accelerated by augmenting human intelligence — as used in these papers — with artificial intelligence, and progress is being made along these lines[16].

Another limitation of the studies[2,3] is in the fraction of connections mapped within the volume. Mapping a larger fraction would allow the application of sophisticated computational methods for analysing connectivity to understand function[17].

Briggman *et al.* show that DSGCs inherit their direction selectivity from SAC dendrites, whereas Bock *et al.* find that inhibitory neurons in the visual cortex squander their inheritance, discarding the orientation selectivity of their inputs. To understand vision, it will be essential to investigate whether and how connectivity enables a neuron to compute a property that is not already present in any single one of its inputs. Iteration of such connectivity could yield neurons that are selective to more and more complex features, as in many neural-network models of the visual system[18]. It is hoped that functional connectomics will finally succeed in revealing how this happens.

These papers[2,3] have introduced a general approach to relating the structure of neural networks to their function: search for rules of connectivity that depend on functional properties of neurons. Finding such rules will be more arduous than finding connections between brain regions, or rules of connection between neuronal cell types. But it is crucial for testing the claim that "Nothing defines the function of a neuron more than its connections with other neurons"[19]. This battle cry will be heard more often as the nascent field of connectomics matures. ■

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1. Masland, R. H. *Curr. Biol.* **14,** R497 (2004).
2. Bock, D. D. *et al. Nature* **271,** 177–182 (2011).
3. Briggman, K. L., Helmstaedter, M. & Denk, W. *Nature* **271,** 183–188 (2011).
4. White, J. G., Southgate, E., Thomson, J. N. & Brenner, S. *Phil. Trans. R. Soc. Lond. B* **314,** 1–340 (1986).
5. Sporns, O., Tononi, G. & Kotter, R. *PLoS Comput. Biol.* **1,** e42 (2005).
6. Helmstaedter, M., Briggman, K. L. & Denk, W. *Curr. Opin. Neurobiol.* **18,** 633–641 (2008).
7. Euler, T., Detwiler, P. B. & Denk, W. *Nature* **418,** 845–852 (2002).
8. Douglas, R. J. & Martin, K. A. C. *Annu. Rev.*

50 Years Ago

The Mango. By Dr. Lal Behari Singh — It is pleasing, as it is unexpected, to find a new series of books on world crops starting off not with such solid fare as wheat or potatoes or cabbage but with something as exotic and appetizing as the mango. This "choicest fruit of Hindoosthan" has spread far beyond the bounds of India and has become one of the most cherished fruits of tropical lands. It is still found in greatest variety and excellence in India, and this book comes, fitly enough, from the pen of a distinguished Indian horticulturist. This book may be counted on to commend itself as the most complete study of the mango so far published … While the botanical chapters refer mainly to the common mango, *M. indica*, the author directs attention to fifteen other species of *Mangifera* with edible fruits. Evidently there is great wealth of related material here, of possible value for future breeding … The section on utilization is necessarily brief, since the best way to use a mango is still to eat it as promptly and as dexterously as possible, but some mango recipes are also given … This book deserves to be welcomed and to be gratefully added to the small, but growing, collection of hand-books on the tropical crops.
From *Nature* 11 March 1961

100 Years Ago

Can any correspondent of *Nature* recall a case of a cat playing with a shadow? I know of a cat — a blue Persian — which appears to wait until the morning sun throws the shadow of a cage-bird on the wall of a room, and then seems to play at catching the shadow of the bird as it moves about.
From *Nature* 9 March 1911

Neurosci. **27,** 419–451 (2004).
9. Sohya, K., Kameyama, K., Yanagawa, Y., Obata, K. & Tsumoto, T. *J. Neurosci.* **27,** 2145–2149 (2007).
10. Niell, C. M. & Stryker, M. P. *J. Neurosci.* **28,** 7520–7536 (2008).
11. Kerlin, A. M., Andermann, M. L., Berezovskii, V. K. & Reid, R. C. *Neuron* **67,** 858–871 (2010).
12. Runyan, C. A. *et al. Neuron* **67,** 847–857 (2010).
13. Douglas, R. J., Koch, C., Mahowald, M., Martin, K. A. & Suarez, H. H. *Science* **269,** 981–985 (1995).
14. Somers, D. C., Nelson, S. B. & Sur, M. *J. Neurosci.* **15,** 5448 (1995).
15. Ben-Yishai, R., Bar-Or, R. L. & Sompolinsky, H. *Proc. Natl Acad. Sci. USA* **92,** 3844–3848 (1995).
16. Jain, V., Seung, H. S. & Turaga, S. C. *Curr. Opin. Neurobiol.* **20,** 653–666 (2010).
17. Seung, H. S. *Neuron* **62,** 17–29 (2009).
18. Fukushima, K. *Biol. Cybernet.* **36,** 193–202 (1980).
19. Mesulam, M. *Ann. Neurol.* **57,** 5–7 (2005).

MATERIALS SCIENCE

Bubble wrap of cell-like aggregates

Using a microfluidic device, tiny polymeric capsules have been made in which different compounds can be isolated in separate, membrane-bound compartments — a prerequisite for the development of artificial cell aggregates.

TAKAMASA HARADA & DENNIS E. DISCHER

Adding milk to coffee or tea leads to a homogeneous (and tasty) mixture, but how could one keep such fluids apart when they are combined? Aqueous fluids tend to flow and mix together even on the micrometre scale of biological cells, and so keeping such fluids apart requires a robust partition. Cells in tissues achieve this by using flexible membranes not only to define and delimit each cell, but also to compartmentalize their nuclei and other organelles. Reporting in *Angewandte Chemie*, Shum *et al.*[1] describe similar hierarchical assemblies of synthetic cell-like structures, which use polymeric surfactants as the building blocks of membranes instead of natural lipids and proteins. These robust, deformable microcapsules adhere tightly to each other while confining distinct aqueous fluids, in the same way that cells cluster together in tissues while enclosing separate portions of cytoplasm.

To achieve this feat, Shum *et al.* used a microfluidic device to make water-in-oil-in-water (W/O/W) double emulsions as templates for the polymeric assemblies (Fig. 1). Such double emulsions consist of a water droplet (or droplets) surrounded by an oil phase, which is itself suspended in a second aqueous solution. The authors precisely controlled the initial size of their emulsions by changing the volume fraction of each phase being passed through the glass capillaries of the microfluidic device, and/or by changing the capillary diameter and flow rate. Importantly, the number of water droplets incorporated into the double emulsions could also be varied by controlling the flow rate of the phases.

The real key to their success, however, was in the choice of ingredients for the oil phase. The authors used two organic solvents mixed with a block copolymer (a polymer in which two or more chemically distinct polymer chains are connected together). The solubility of the copolymer was greater in one of the solvents (chloroform) than in the other (hexane). Because the copolymer was amphiphilic — consisting of a hydrophobic chain covalently linked to a hydrophilic chain — it became concentrated at the oil–water interfaces in the W/O/W double emulsions.

Once the double emulsions had formed, the volatile chloroform started to evaporate. Shum *et al.*[1] observed that, as the chloroform left the system, the inner water droplets became coated with a dense monolayer (leaflet) of copolymer. Because the solubility of the copolymer in the remaining hexane was lower than in the original chloroform–hexane mixture, the water droplets started to stick together. The droplets also adhered to the interface of the oil phase with the surrounding water, where another leaflet of copolymer had formed.

The authors then eliminated the remaining hexane either by evaporation or by using shear in the microfluidic flow of their system. The end result was a highly cohesive assembly of polymer vesicles (polymersomes), wrapped together in a shared outer copolymer leaflet. The orientations of the inner droplets to each other and the contact angles of the interfaces between the droplets determined the eventual shapes of the polymersomes in the aggregates. This meant that the authors could make polymersomes of different shapes, but with the same number of compartments.

Shum and colleagues' ensembles resemble aggregates of soap bubbles and also adherent cells such as those seen in the eye[2]. The comparison with adherent cells is telling, because the formation of structured subsets of cells in tissues is a complex process that is also controlled to some extent by the physics of adhesion. Networks of biochemical reactions direct cell crawling and other key processes, but developmental biologists have also noted that simple mixtures of cells sort themselves in a manner reminiscent of the behaviour of immiscible liquids in emulsions. It has been thought for decades that cell sorting *in vivo* might occur because cells preferentially adopt configurations that minimize the surface and bulk mechanical energies of cell clusters. Evidence of this has come most recently from *in vitro* studies[3] in which the adhesion strengths of cells were altered in systems containing small numbers of different cell types.

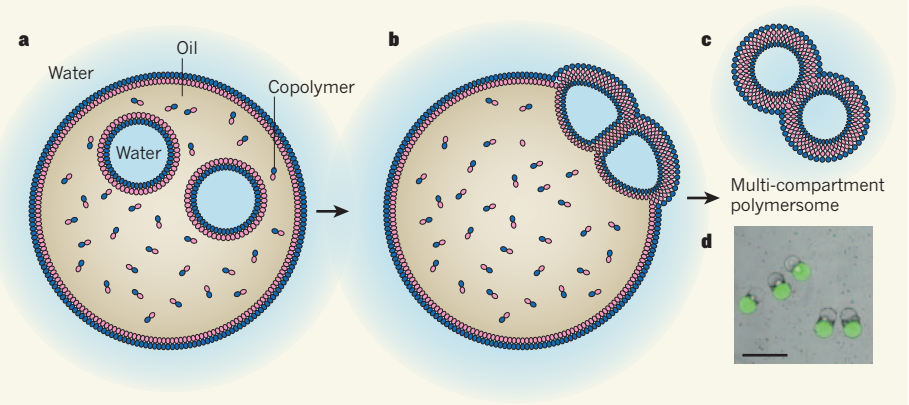

Figure 1 | The synthesis of polymeric cell-like aggregates. Shum *et al.*[1] have used a microfluidic device to prepare multi-compartment polymer vesicles. **a**, The authors began by making water-in-oil-in-water (W/O/W) double emulsions — water droplets suspended in an oil phase, which is itself suspended in water. The oil phase consisted of two solvents and a copolymer, which concentrates at the interfaces of the emulsions as monolayers. **b**, As the more volatile solvent in the oil phase evaporates, the monolayers become adhesive and stick to each other. **c**, The authors then removed the remaining solvent from the oil phase, generating multi-compartment vesicles called polymersomes, whose membranes consist of bilayers of copolymer molecules. (Graphics in **a**– **c** adapted from Scheme 1 of ref. 1.) **d**, This picture overlays optical and fluorescent images of polymersomes. One compartment contains a fluorescent solute, with the other containing a non-fluorescent solute. No cross-contamination occurs. Scale bar, 200 μm. (Image from ref. 1.)