paused for about half the time, which is not a useful generalization. Our definitions, like our tools, need sharpening if they are to sustain claims about unusual climate events. ■

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HUMAN DEVELOPMENT

Advances in mini-brain technology

Two studies integrate cutting-edge techniques to grow and analyse 3D cultured tissues that resemble human brain structures, enabling examination of how brain regions interact and neurons mature. SEE ARTICLES P.48 & P.54

J. GRAY CAMP & BARBARA TREUTLEIN

Over the past few years, the production of human-brain-like tissue from stem cells in 3D cultures[1,2] has allowed sophisticated analyses of how the brain develops, how its development has changed during evolution and how it is affected by disease[3–5]. But it has remained unclear precisely what cell types arise in these brain 'organoids', how much individual organoids vary, and whether mature neuronal networks can form and function in organoids. Using a combination of sophisticated techniques, two papers[6,7] in this issue describe key steps towards addressing these questions.

There are two general strategies for growing human brain organoids. In the first strategy, pluripotent stem cells (PSCs, which can give rise to any cell type in the body) are guided to form a layer of stem cells called a neuroepithelium that can make neurons. The neuroepithelium is left to develop alone, and this can result in the generation of multiple brain regions (Fig. 1a). This self-patterning strategy offers the potential to understand how brain regions self-organize and interact. However, there are often substantial differences between individual organoids, and between batches grown separately.

The alternative strategy is to use signalling molecules to control patterning of the neuroepithelium so that a defined region forms — the forebrain or hypothalamus, for instance (Fig. 1b). This technique might increase reproducibility, but researchers have yet to define all of the signals that create each subregion of the brain. Moreover, it is unclear whether certain regions can form in the absence of adjacent structures.

Quadrato *et al.*[6] (page 48) set out to examine the composition and functionality of maturing brain organoids in detail. The authors modified a self-patterning protocol[3] to reduce cell death, which can occur towards the centre of organoids owing to a lack of oxygen. Organoids grown using this modified method progressively matured for more than nine months, making it possible to study how neurons develop over a time equivalent to that of human gestation.

The researchers analysed the gene-expression profiles (the transcriptomes) of more than 80,000 cells from 3- or 6-month-old organoids — the most comprehensive single-cell analysis of organoid composition performed so far. These data revealed diverse cell populations from different brain regions. Focusing on retinal cell types, the investigators demonstrated that their organoids contained almost every cell type found in this tissue *in vivo*. They also found evidence that neurons mature over time, beginning to express genes that mediate synaptic connections with other neurons.

How mature do these neurons become? Electron microscopy on serial slices of tissue revealed that an 8-month-old organoid contained a density of synapses approximately

50 Years Ago

The evolutionary origin of mitochondria, chloroplasts and kinetoplasts has recently been the subject of some intriguing speculation; several workers have suggested that these organelles have had an exogenous origin, perhaps evolving from symbiotic bacteria. These ideas stem from genetic evidence for the existence of extrachromosomal genes and the discovery that mitochondria and chloroplasts contain DNA and ribosomes and are capable of synthesizing proteins *in vitro* … Although it is unlikely we shall ever be able to prove or disprove the hypothesis of the exogenous origin of these organelles, the fact that chloroplast and probably mitochondrial ribosomes differ from cytoplasmic ribosomes suggests that cells contain two independent protein synthesizing systems perhaps subject to different control mechanisms.
From *Nature* 6 May 1967

100 Years Ago

It is usually stated that the carat weight of jewellers and diamond merchants is derived from the hard seeds of the locust tree, *Ceratonia siliqua*, which were anciently used as weights. Having had occasion to obtain some of the beans, I weighed several of the seeds to see what sort of error would be incurred if they were used as weights … It would appear … that the carat weight could be recovered with some approach to accuracy by weighing a number of seeds of the locust bean. It is also evident that the use of such seeds as weights must have given opportunities for fraudulent dealing in the precious commodities gauged by means of them, since deviations of from 30 to 40 per cent. from the average may occur.
From *Nature* 3 May 1917

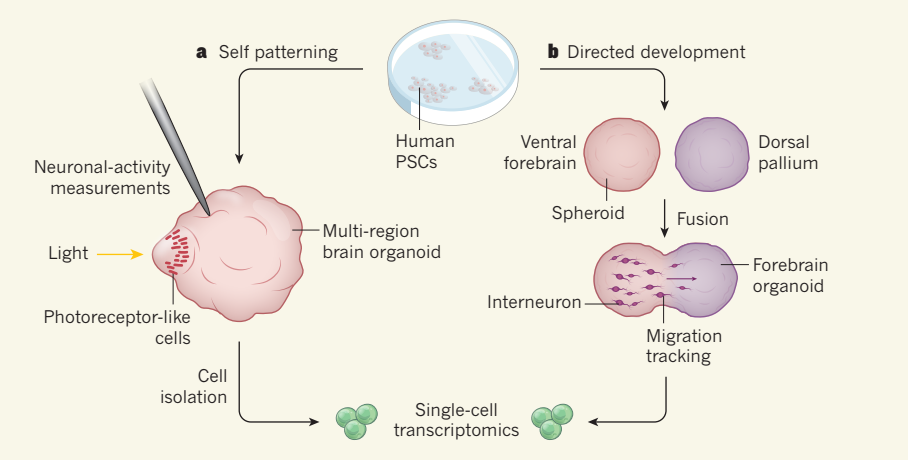

Figure 1 | Two routes to a mini-brain. Structures called organoids that resemble regions of the embryonic human brain can be grown *in vitro* from human pluripotent stem cells (PSCs), which can give rise to every cell type in the body. **a**, Quadrato *et al.*[6] induced PSCs to form a cell layer that self-patterned into organoids composed of multiple brain regions. Measurements of neuronal activity revealed that the organoids contain cells that respond to light in a similar way to photoreceptor cells of the retina, demonstrating that neurons can mature in organoids. The authors isolated single cells and analysed the gene-expression profiles (transcriptomes) to determine the range of cell types in the organoids. **b**, Birey *et al.*[7] used signalling molecules to direct the development of 3D structures called spheroids that resemble two regions of the forebrain (the ventral forebrain and dorsal pallium). Spheroid fusion led to the formation of forebrain-like organoids. Imaging revealed that neurons called interneurons migrated from the ventral to the dorsal region, providing information on brain-region interactions. These authors also performed single-cell transcriptomics.

equivalent to that in the cortex of an embryonic human brain. Individual neuronal projections (dendrites) often made multiple synapses, suggesting that complex networks form by this stage. Quadrato *et al.* found that these synapses could fire, and periods of coordinated firing indicated that organoids contained active neuronal networks.

Finally, the authors showed that a subpopulation of neurons had lowered firing rates after exposure to light. Coupled with transcriptomic data suggesting that organoids contained light-sensing retinal neurons called photoreceptor cells, these data indicate that organoids could be used to investigate how neuronal networks are modulated by physiological stimuli.

It is of note that Quadrato and colleagues found extensive differences in cell composition between organoid batches. This highlights the need to control organoid engineering so as to improve reproducibility when studying disease mechanisms, for example. In the second study, Birey *et al.*[7] (page 54) take a leap in this direction.

The authors used a controlled-patterning protocol to generate 3D structures, which they called spheroids, that resemble one of two forebrain regions: the ventral forebrain, which produces inhibitory neurons called interneurons; and the dorsal pallium, which contains excitatory neurons. Single-cell transcriptomics showed that the spheroid cells were remarkably similar to those from corresponding regions of the human fetal brain.

Next, Birey and colleagues placed spheroids of different types next to each other, and allowed them to fuse over a few days to form forebrain-like organoids. Interneurons from the ventral spheroids migrated into the dorsal regions — similar to the migration route taken by these interneurons *in vivo*. After migration, the interneurons matured. The authors detected stronger neuronal firing in fused organoids after interneuron migration than before.

The researchers used live-cell imaging to track individual interneurons, comparing their behaviour with that of equivalent cells in the human and mouse brain. Certain human-specific aspects of migration were accurately replicated in the fused organoids, presenting exciting possibilities for studying the genes that mediate migration. Indeed, Birey *et al.* next used their system to study the neurodevelopmental disorder Timothy syndrome, which is associated with mutations in a protein that regulates interneuron migration[8].

The authors produced fused forebrain organoids from PSCs that had been generated by inducing mature differentiated cells from patients with Timothy syndrome to become pluripotent. The patient-derived interneurons migrated less efficiently than controls. This is probably due to a defect in the interneurons themselves, rather than in the dorsal environment, because migration remained defective when the researchers fused patient-derived ventral spheroids to wild-type dorsal spheroids. Birey *et al.* have therefore succeeded in generating a controlled 3D system that has both excitatory and inhibitory neuronal activity, and that can effectively model an interneuron migratory disorder.

Both studies reveal the power of high-throughput, single-cell transcriptomics to quantify cell-type diversity in brain organoids. These methods will be valuable for studying how cell composition and gene-expression networks are dysregulated during disease. Nevertheless, it is unclear how transcriptomes will vary between organoid batches, between different stem-cell lines generated from the same patient, and between patients with the same disease. This is particularly important when comparing patient and control organoids, and should be addressed in detail.

It was known[4] that organoid neurons could create synapses. However, serial electron microscopy is a new and elegant approach to studying detailed synaptic structures in organoids, and, it is hoped, to studying the complete map of neuronal networks. It remains to be seen how neurons 'decide' to connect with one another in these complex and heterogeneous tissues, and whether the connections mirror those in the developing human brain.

Much work shows that brain tissue engineered from induced PSCs can accurately model neuronal-progenitor behaviour[9]. The new studies showcase how brain-organoid technology can be improved to study the interactions between brain regions and the way in which mature neurons might function in a coordinated network. Time will tell just how much information these mini-brains can provide. ■

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CORRECTION

The News & Views 'Biochemistry: Origin of a key player in methane biosynthesis' (*Nature* **543,** 49–50; 2017) by Tadhg P. Begley indicated that a paper by Moore *et al.* (*Nature* **543,** 78–82; 2017) solved an outstanding problem — the biosynthesis of coenzyme F_{430}. However, a paper (K. Zheng *et al. Science* **354,** 339–342; 2016) that solved this problem was published shortly before the work by Moore and colleagues. A corrigendum for the paper by Moore *et al.* can be found on page 116.