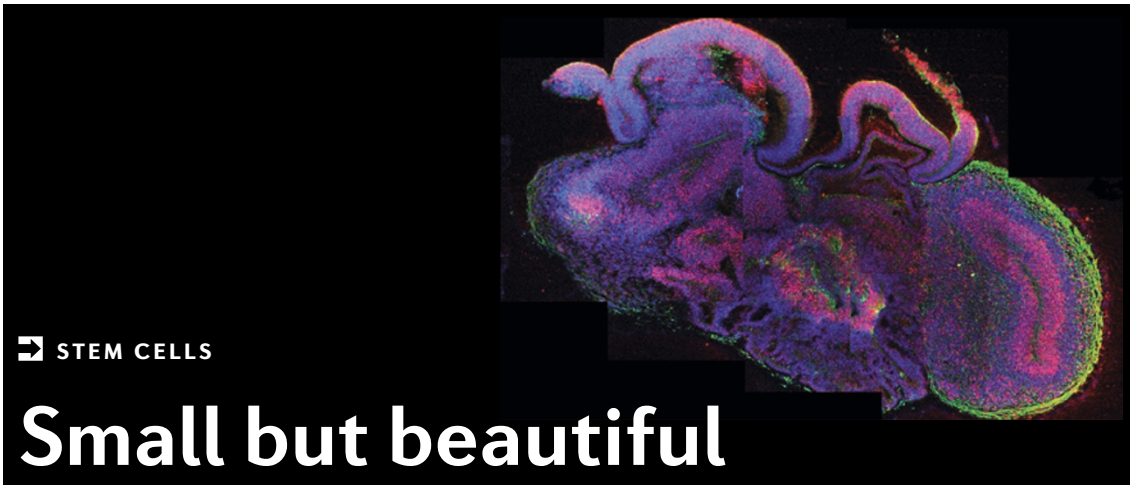
Nature Reviews Neuroscience | AOP, published online 12 September 2013; doi:10.1038/nrn3600



A cross-section of an entire organoid showing development of different brain regions. All cells are in blue, neural stem cells in red and neurons in green. Image courtesy of M. A. Lancaster, Institute of Molecular Biotechnology of the Austrian Academy of Science, Vienna, Austria.

STEM CELLS

Small but beautiful

Although animal studies have yielded many insights into brain development, some aspects of cortical development are unique to the human brain. In a study published in *Nature*, Lancaster *et al.* now show that neuroectoderm derived from human pluripotent stem cells can self-organize *in vitro* into a three-dimensional brain-like structure, providing a potential new tool to model normal and abnormal human brain development.

The authors used human embryonic stem (hES) cells and induced pluripotent stem (iPS) cells derived from fibroblasts to generate neuroectoderm tissue. They embedded pieces of neuroectoderm tissue inside Matrigel droplets that provided a scaffold for three-dimensional expansion and were kept in a spinning bioreactor to promote nutrient uptake. After 15–20 days in culture, the neuroectoderm samples had developed into brain-like tissues that the authors termed 'cerebral organoids'. The organoids reached their maximum size (~4 mm diameter) after 2 months.

Regardless of whether they were grown from hES cells or from iPS cells, the organoids displayed several features of the developing human brain. For example, immunohistochemistry performed on 35 of the 100s of organoids that had developed revealed, on day 16, distinct regions expressing specific markers for the forebrain, midbrain and hindbrain. Many forebrain-like regions expressed a marker of dorsal cortex, with subregions expressing markers of prefrontal cortex, the frontal lobe or the occipital lobe. In addition, there were regions that stained positive for markers of the hippocampus or the ventral forebrain, structures resembling the choroid plexus and, in 4 of the 35 organoids, immature retinal tissue.

Further analysis of the dorsal cortex area using markers for radial glial cells, intermediate progenitors and neurons showed that the organoids had a progenitor zone organization typical of developing mammalian brains. Live-imaging experiments showed that the morphology of radial glial cells changed over time, mimicking the changes seen during development. Moreover, these cells showed interkinetic nuclear migration, which is typical for cortical progenitor cells, and a pattern of mitotic spindle organization similar to that recently described in human cortical development.

A unique feature of the human progenitor zone is the existence of an outer subventricular zone (containing outer radial glial cells and intermediate progenitor cells) that is separated from the inner subventricular zone by an inner fibre layer. The authors showed, again using immunohistochemistry, that this aspect of human cortical development was recapitulated in the organoids.

Calcium dye imaging of organoid neurons revealed spontaneous oscillations in calcium concentration that became more frequent after application of glutamate. Addition of tetrodotoxin (which inhibits action potentials) reduced the amplitude of the calcium oscillations. These findings suggest glutamate receptor expression and the presence of functional neurons in organoids.

Although the organoids recapitulated several aspects of human brain development, their overall morphology was not consistent, and they did not exhibit the distinct six cortical layers (although a separation between a deep and a superficial cortical layer was apparent on day 30). Size was also severely restricted, and cell death occurred in the centre of the organoids, probably owing to insufficient nutrient supply. Despite these limitations, organoids can provide a useful model for normal and abnormal human cortical development. To show this proof of principle, the authors grew cerebral organoids from iPS cells derived from skin cells of a patient with microcephaly caused by a specific genetic mutation. They found that compared with controls, these organoids were smaller, had a lower number of progenitor cells and more neurons (suggestive of premature differentiation), and abnormal spindle orientation.

The unique properties of this mini-model of the human brain offer an advance on currently available techniques that is likely to enable the study of some human-specific aspects of brain development and disease that are difficult to model in animals.

Leonie Welberg

ORIGINAL RESEARCH PAPER Lancaster, M. A. *et al.* Cerebral organoids model human brain development and microcephaly. *Nature* http://dx.doi.org/10.1038/nature12517 (2013)

> “These findings suggest glutamate receptor expression and the presence of functional neurons in organoids”