PRIZE ESSAY

FINALIST

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Tomasz J. Nowakowski received his B.Sc (Hons.) and his Ph.D. from the University of Edinburgh. He completed his postdoctoral training at the University of California, San Francisco. Dr. Nowakowski is now an assistant professor at the University of California, San Francisco, where his research team seeks to identify the molecular mechanisms underlying cell fate specification and microcircuit formation in the developing cortex.

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NEUROBIOLOGY

Building blocks of the human brain

Area-specific excitatory neuron subtypes emerge before sensory experience

By **Tomasz Jan Nowakowski**

Classical computer software programs perform tasks in series: One algorithm has to be performed, and the output of that algorithm is used as an input into another. However, the most powerful computers in the world take advantage of the fact that complex computational tasks can be broken down into independent components. Distributing the computation over many parallel processes taking place simultaneously rapidly increases the overall speed. This paradigm is very similar to how the human brain performs its complex functions.

Our brains constantly receive information from the outside world; hundreds of thousands of sensory stimuli have to be processed in parallel and associated with each other to support a conscious perception of our environment. Unlike the engineered architecture of the computer circuit board, however, the biological hardware performing the complex operations in the brain is far from understood.

RADIAL GLIA AND CORTICAL DEVELOPMENT

The human cerebral cortex includes billions of neurons organized into six sheetlike layers. More than a century ago, Santiago Ramón y Cajal appreciated the astonishing diversity of cell types in the brain, but even today, it is unclear how the different neuronal cell types are assembled and distributed across the distinct anatomical areas of the cortex to support its diverse functions.

We know that excitatory neurons of the cortex are born from a uniform population of radial glia. During development, radial glia line the fluid-filled ventricles and extend long fibers that connect with the outer pial surface. Newborn neurons migrate along radial glia fibers to the outer layers of the tissue, where they form the cerebral cortex. This radial glia scaffold inspired the longstanding radial unit hypothesis (*1*), which posits that neurons born from the same group of radial glia migrate along the physical scaffold of the radial glia fibers to occupy a nearby position in the cerebral cortex.

The vestige of this developmental process is reflected in a tissue architecture feature called the "minicolumn" (*2*). The concept of an elementary functional unit, with a stereotyped microcircuit and a common input and output architecture, has resonated throughout studies of neuroanatomy, physiology, and developmental biology (*3*–*5*) because it provides a tractable framework for understanding the computational strategy of the human brain. Human cortical development involves a greatly expanded and diversified pool of radial glia compared with that of mice (*6*). I hypothesized that radial glia cell diversity in humans could influence the radial glia scaffold and the composition of neuronal cell types across cortical areas and layers.

MAPPING DEVELOPMENTAL TRAJECTORIES IN THE CORTEX

As a first step toward unraveling the diversity of cell types and developmental processes involved in cortical development, I pioneered the use of single-cell RNA sequencing for classifying diverse cell types mixed together in tissue samples (*7*–*9*). Using these transcriptomic signatures as biomarkers, I could selectively label distinct subtypes of radial glia and study their abundance, morphology, and distribution in the developing human brain (*10*).

Unexpectedly, I found that the radial glial scaffold undergoes a transformation from a physically continuous to a physically discontinuous structure, composed of radial fibers derived from two distinct types of radial glia, midway through human neurogenesis (*7*). This observation suggests that in primates, developmental histories of cells in the same cortical minicolumn may follow more complex trajectories than previously thought, particularly in the upper cortical layers, which are believed to support the higher-order cognitive functions. I synthesized these

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findings into a revised model of human brain development (*7*) that provides a new framework for the study of lineage relationships during cortical minicolumn formation.

Cortical minicolumns are arranged serially throughout the cerebral cortical sheet and are composed of a relatively small number of major neuronal cell classes (*11*, *12*). This modular organization has inspired a uniformity hypothesis (*13*, *14*) that the cortex is composed of elementary units with similar cellular composition and a stereotyped connectivity pattern.

However, differences in cortical cytoarchitecture and gene expression across functional areas have long been appreciated (*15*–*18*). It is currently unclear whether these molecular differences are established by bona fide, area-specific neurodevelopmental programs or by neuronal activity. I reasoned that studying neuronal cells during their generation could reveal the molecular dynamics that regulate the emergence of diverse cell types in the brain and could address questions about cortical minicolumn composition, microcircuit development, and ultimately, brain function.

EXCITATORY NEURONS AND CONNECTIVITY

As a corollary to the radial unit hypothesis, I postulated that cortical areas would emerge from a primordial "protomap" encoded by radial glia. In this model, a mosaic of prespecified cortical area information would be projected onto the nascent cerebral cortex via lineage relationships between radial glia and neurons.

As part of my laboratory's effort to characterize the diversity of cell types in the developing brain by using single-cell RNA sequencing, we profiled cells from frontal and occipital lobes of the cortex at stages of neurogenesis, before sensory input shapes neuronal activity in the cortex. Whereas most cell types shared the same transcriptional signatures in prefrontal and visual areas, excitatory neurons segregated into molecularly distinct groups according to the area where they were generated (*19*). Many of the genes that distinguish excitatory neurons found in different cortical areas included factors previously shown to determine patterns of neuronal connectivity.

Although future experiments are needed to compare cortical microcircuit architecture in specific cortical areas, our findings suggest that in the developing human cortex, topographically distinct minicolumns consist of different classes of excitatory neurons. Importantly, similar area-specific neuronal signatures have been reported in the adult mouse brain (*20*), indicating that the topographic variation that we described reflects differences in neuronal identities, rather than transient developmental variation (*21*).

A COMPLEX SYSTEM EMERGES

Together, our studies show that the human radial glia scaffold transforms during development, which may increase the dispersion of upper-layer cortical neurons. In addition, even before sensory experience, cortical neurons are functionally distinct across cortical areas in addition to layers. These findings support a model of serial homology, in which topographic hierarchy of neurodevelopmental programs orchestrates differences in excitatory neurons across individual cortical areas and may contribute to interareal differences in microcircuit connectivity patterns to support higher-order information processing (*22*–*24*). This model could have important implications for the design of improved deep learning algorithms that seek to faithfully recapitulate computational processes in the brain (*25*, *26*) and has the potential to reveal developmental processes underpinning human cognition. ■

REFERENCES

1. P. Rakic, *Postgrad. Med. J.* **54**, 25 (1978).
2. N. R. De Lorente, *Physiology of the Nervous System*, 288-330 (1949).
3. D. H. Hubel, T. N. Wiesel, *J. Physiol.* **160**, 106 (1962).
4. P. Rakic, *Science* **241**, 170 (1988).
5. J. DeFelipe, S. H. Hendry, T. Hashikawa, M. Molinari, E. G. Jones, *Neuroscience* **37**, 655 (1990).
6. D. V. Hansen, J. H. Lui, P. R. Parker, A. R. Kriegstein, *Nature* **464**, 554 (2010).
7. T. J. Nowakowski, A. A. Pollen, C. Sandoval-Espinosa, A. R. Kriegstein, *Neuron* **91**, 1219 (2016).
8. A. A. Pollen *et al.*, *Cell* **163**, 55 (2015).
9. A. A. Pollen *et al.*, *Nat. Biotechnol.* **32**, 1053 (2014).
10. Z. Molnár *et al.*, *Eur. J. Neurosci.* **23**, 921 (2006).
11. A. Zeisel *et al.*, *Science* **347**, 1138 (2015).
12. B. Tasic *et al.*, *Nat. Neurosci.* **19**, 335 (2016).
13. O. D. Creutzfeldt, *Naturwissenschaften* **64**, 507 (1977).
14. V. B. Mountcastle, *J. R. Soc. Med.* **71**, 14 (1978).
15. G. N. Elston, *Cereb. Cortex* **13**, 1124 (2003).
16. S. A. Bayer, J. Altman, *Neuroscience* **45**, 391 (1991).
17. D. D. O'Leary, *Trends Neurosci.* **12**, 400 (1989).
18. M. B. Johnson *et al.*, *Neuron* **62**, 494 (2009).
19. T. J. Nowakowski *et al.*, *Science* **358**, 1318 (2017).
20. B. Tasic *et al.*, *bioRxiv* 229542 [Preprint]. 6 December 2017. https://doi.org/10.1101/229542.
21. H. Li *et al.*, *Cell* **171**, 1206 (2017).
22. N. T. Markov *et al.*, *Science* **342**, 1238406 (2013).
23. B. B. Averbeck, A. Battaglia-Mayer, C. Guglielmo, R. Caminiti, *J. Neurophysiol.* **102**, 1911 (2009).
24. K. D. Harris, G. M. Shepherd, *Nat. Neurosci.* **18**, 170 (2015).
25. G. J. Rinkus, *Front. Neuroanat.* **4**, 17 (2010).
26. Y. LeCun, Y. Bengio, G. Hinton, *Nature* **521**, 436 (2015).

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