NEUROSCIENCE

A cookbook for neuronal flavours

Whether cell types in the brain have been conserved during evolution is not clear. A comparison of the molecular recipes that define brain cell types in humans and mice reveals similarities and differences between species. SEE ARTICLE P.61

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To create a new dish, a chef must select ingredients and mix them to achieve different tastes and textures. Similarly, the expression of various combinations of thousands of genes creates and maintains the many diverse 'flavours' of each type of cell in the brain. On page 61, Hodge *et al.*[1] report their analysis of gene expression in single cells from the brain to present a 'cookbook' of molecular recipes for the neuronal cell types in the human cerebral cortex, a part of the brain required for many cognitive processes. By comparing gene-expression profiles of human and mouse neurons, they reveal a striking conservation of molecularly defined cell types during evolution, but also highlight many key species-specific differences in gene expression within conserved cell types.

The classification of neuronal cell types in the brain represents a long-standing goal in neuroscience that goes back to the anatomists of the early twentieth century. In the cerebral cortex alone, billions of neurons are organized into six sheet-like layers and distributed across dozens of anatomically distinct regions. Given the many sources of variation between individual neurons, systematically annotating brain cells under a common taxonomy represents an enormous challenge[2]. Previous studies have gained insight into the molecular landscape of the brain by measuring gene expression in different brain regions[3] and by using bioinformatic analyses to identify molecular signatures of the major cell classes[4]. These approaches have been extended by technologies that measure gene expression in single cells[5], but isolating intact cells from human brain tissue is technically challenging and many cells are lost in the process.

Hodge *et al.* overcame this problem by measuring the levels of different RNA transcript molecules in single nuclei isolated from samples of human brains obtained either post-mortem or during surgery. The authors then carried out a statistical analysis of the transcriptomic data; this grouped individual data points (each representing a single cell) into clusters that corresponded to the types of RNA transcript expressed by the cells. The analysis revealed 75 distinct clusters,

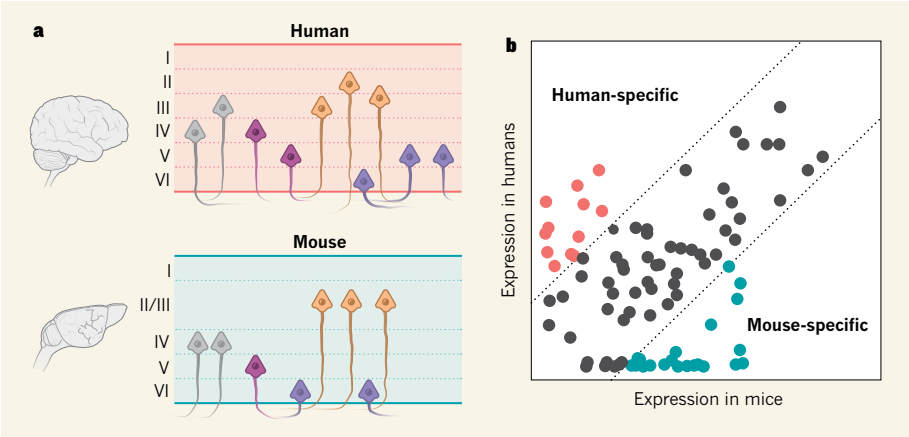

Figure 1 | Shared cell types in human and mouse have divergent properties. Hodge *et al.*[1] measured RNA levels in individual nuclei from cells isolated from the six layers of the human cortex and used these data to classify cells into different types. **a**, Most human cell types (depicted in different colours) were distributed across multiple cortical layers. Comparison with previous single-cell RNA sequencing in the mouse visual cortex[6] revealed that cell types shared by both species (depicted by colours shared with human cells) were more strictly segregated into single layers in the mouse cortex. (The cell distributions illustrated do not correspond to actual data.) **b**, The authors analysed the expression of 14,553 genes in the cell types shared by both species. The scatter plot shows the expression of genes that act as markers of an example cell type. Although most genes were expressed at similar levels in both species (black dots), many genes — including marker genes — were more highly expressed in either humans (red dots) or mice (blue dots).

including 24 types of excitatory neuron (the main signal-generating cells in the brain), 45 types of inhibitory neuron (which suppress neural activity) and 6 non-neuronal cell types. Many of those clusters represent previously defined cell types, but others revealed previously unknown distinctions within broad neuronal classes. Each cell type was given a four-part name on the basis of its general function (excitatory, inhibitory or non-neuronal), its anatomical position, and its expression of genes characteristic of major classes and of specific cell types. This systematic nomenclature integrates multiple modes of information that have historically been used to classify neuronal cell types.

The layer of the cortex in which a neuron resides (also known as its laminar position) has conventionally been considered a fundamental feature of neuronal identity, and so molecular markers of laminar position have previously been used to study cortical organization[6]. Cortical layers are generated sequentially during development, such that each neuron's birthdate predicts its eventual laminar position[7]. To determine how well the laminar position of a cell predicts its type, the authors recorded the layer of origin of each cell and examined whether this correlated with the cell-type taxonomy defined by gene expression.

In some cell types, the expression of certain genes correlated with precise laminar position. However, in striking contrast to recent findings in the mouse[8], Hodge *et al.* found that, in humans, almost all types of excitatory neuron reside in more than one layer (Fig. 1a). This finding was validated using fluorescent labelling of cell-type-specific RNA transcripts in intact tissue samples. It is further supported by a gene-expression survey[9] in the developing human cortex that found that molecular signatures of cell type do not correlate well with birthdate, and that most molecular layer markers are not expressed when new cells are generated during development. Together, these studies challenge long-held assumptions about the discrete laminar organization of neuronal cell types in the cerebral cortex.

In addition to providing insights into cell type and organization, single-cell analyses enable insights into the evolutionary conservation of cell types. Hodge *et al.* analysed

the pooled single-cell gene-expression data sets from human and mouse cerebral cortex and again used a clustering analysis to identify matching cell types. Using this method, the authors determined that almost all of the 75 cell types identified in the human cortex showed correspondence (homology) with cell types described in the mouse. They also found, however, that the diversity of neurons in cortical layers I–IV, which are thought to be responsible for many aspects of cognition specific to primates[10], is greater in human cortex than in mouse cortex. Moreover, the authors noted that, within homologous cell types, the expression of certain genes was highly variable between species, including genes used to define these cell types in either species' data set (Fig. 1b). Microglia, the immune cells of the brain, showed the greatest divergence in gene expression between humans and mice, suggesting that the role of microglia in neuroimmune disorders might differ considerably between species.

One shortcoming of Hodge and colleagues' study is the use of data that were obtained by different profiling methods (the authors profiled single nuclei from the human samples, whereas whole cells were profiled in the mouse analysis) and from different cortical areas (temporal lobe in the human and visual cortex in the mouse). Nevertheless, the analysis demonstrates a new approach that could one day enable a thorough comparison of cell-type homology across many species[11]. Notably, genes that encode certain receptors for neurotransmitter molecules were among the genes that showed the greatest divergence in expression between human and mouse, suggesting that the authors' observations could have implications for attempts to model the function of synaptic connections between neurons, and their disorders.

Hodge and colleagues' study advances our understanding of the cellular composition of the cerebral cortex, and reveals previously unrecognized distinctions between the organization of this brain region in humans and mice. The authors' comparison of conserved cell types in humans and mice calls for further investigation of how cell types have been conserved during evolution and the importance of species-specific changes to the recipe for the brain. ■

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ORGANIC CHEMISTRY

Fluorine and amide groups together at last

A practical method has been devised to synthesize compounds that have previously been almost impossible to make. The combination of fluorine atoms and amide groups in the compounds might be useful in pharmaceuticals. SEE LETTER P.102

JONATHAN CLAYDEN

In medicinal chemistry, the chemical group known as the amide is king. Amides consist of a nitrogen atom linked to a carbonyl group (C=O), and featured in all but four of the top 40 bestselling drugs in 2018 (see go.nature.com/30f709w). On page 102, Scattolin *et al.*[1] expand the range of amides available for drug discovery by showing how to synthesize a previously inaccessible group of compounds known as *N*-trifluoromethylamides, in which the amide is closely associated with three fluorine atoms.

Amides are prevalent in medicinally important compounds not only because they are particularly stable, but also because they are polar (they contain regions of high positive and negative electrical charge density), which allows amide-containing drugs to interact with biological receptors and enzymes. In these respects, the use of amides in drugs follows nature's example — amide groups provide the links between amino-acid residues in proteins. But drugs don't just have to interact with biological targets; they must also resist rapid metabolic degradation in the complex environment of the human body.

One well-established way to protect molecules from such degradation involves the use of fluorine atoms. Like amides, carbon–fluorine (C–F) bonds are polar and unreactive. But, unlike amides, they are almost entirely alien to biology — which means that metabolic enzymes struggle to degrade them rapidly. This makes the incorporation of fluorine atoms an effective way to increase the metabolic stability, as well as other desirable properties, of drug compounds[2].

Combining amide groups with fluorine atoms can be a particularly fruitful strategy for drug development — indeed, the best-selling drug of all time, cholesterol-reducing atorvastatin, contains both an amide and a fluorine substituent, albeit in different parts of the molecule. But the synthesis of molecules in which amides and fluorine atoms are closely associated is far from straightforward. Attempts to assemble fluorinated amides typically require reaction conditions that can cause the degradation of sensitive chemical groups in the target molecule.

Scattolin *et al.* describe a practical solution for the synthesis of an otherwise almost inaccessible family of fluorinated amides that contain an *N*-trifluoromethyl group

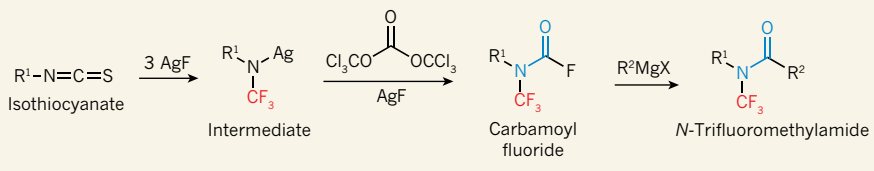

Figure 1 | The synthesis of *N*-trifluoromethylamide compounds. *N*-trifluoromethylamides contain a trifluoromethyl group (red) attached to an amide group (blue), and have been almost impossible to make. R^1 and R^2 represent any chemical group. Scattolin *et al.*[1] report a practical synthesis of these compounds, which are of interest for drug discovery. The authors treated an isothiocyanate with silver fluoride (AgF), producing an intermediate compound in which a trifluoromethyl group is attached to a nitrogen atom. This intermediate reacts with bis(trichloromethyl) carbonate ($CO(OCCl_3)_2$) in the presence of silver fluoride to make a carbamoyl fluoride. Treating this compound with magnesium-containing reagents known as Grignard reagents (R^2MgX, where X is a halogen atom) produces *N*-trifluoromethylamides. Side products of the reactions are not shown.