Strength in numbers from integrated single-cell neuroscience

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Combining epigenomics and transcriptomics data from human brain samples reveals regulatory mechanisms that underlie cell type identity.

Single-cell genomics is poised to transform our understanding of the molecular diversity in complex biological systems[1], but substantial technical and analytical challenges remain when processing human tissue. In this issue, Lake *et al.*[2] develop two highly scalable sequencing technologies alongside innovative integrated analytical tools, and apply them to molecularly define the transcriptional and epigenetic states of single cells in the human brain (**Fig. 1**). In particular, they discover dozens of transcriptomically defined cell types, alongside enhancers and genomic elements that regulate this diversity in healthy and diseased states. Their work highlights the potential for integrated single-cell analysis to yield new insights in human neuroscience, and demonstrates the power of new experimental strategies that may be relevant for the study of other human tissues.

The human brain represents a unique challenge for molecular single-cell profiling, as protocols for single-cell RNA sequencing (scRNA-seq) require fresh biopsies and cleanly dissociated cellular suspensions. Archived post-mortem neuronal tissue is compatible with neither of these requirements, but can easily generate suspensions of single nuclei. The same group was previously among the first to demonstrate that single-nucleus RNAsequencing could identify human neuronal subtypes[3], but required the use of low-throughput technologies. To dramatically increase scale, the authors begin this work by demonstrating how massively parallel, droplet-based scRNA-seq technologies[4] can be extended to profile single-nuclei transcriptome sequencing scNuc-seq by optimizing the lysis procedure through mechanical shearing. This enabled them to profile the transcriptomes of 36,166 single nuclei, representing an order of magnitude increase in data compared with their previous efforts[3], at a small fraction of the cost. The scNuc-seq approach is complementary to the recently published massively parallel The sNuc-seq approach is complementary to the recently published DroNc-seq method[5], which accomplishes similar goals with a modified microfluidic chip design, suggesting that ideas from both technologies could be synergistically combined.

Alongside their transcriptomic data, the authors introduce a new strategy to profile chromatin accessibility in single cells, by extending their previously developed transposome-hypersensitive site sequencing (THS-seq) assay[6] to utilize combinatorial indexing. The method is conceptually similar to the recently published method of single-cell assay for transposase-accessible chromatin using sequencing (ATAC-seq)[7], but with a modified amplification strategy, alongside enzymes and reagents that are fully available. Lake *et al.*[2] apply single-cell THS-seq (scTHS-seq) to measure chromatin accessibility profiles of 32,869 single nuclei from the visual and frontal cortex, and the cerebellum. Although the transcriptomic and epigenetic profiling strategies were applied to separate groups of cells, the output of these two approaches represent a breathtaking data set of molecular diversity in the human brain, with exciting potential to identify both subtle and rare cellular states.

Toward this goal, the authors first proceeded to cluster the two data sets individually. They identified 35 distinct clusters of neuronal and non-neuronal cells based on RNA measurements, representing both broad and fine-grained differences in cell type, anatomical structures, and cortical layers. Importantly, these clusters were consistent with previous efforts to define human neuronal cell states[2], yet with substantially higher resolution, in particular for inhibitory (11 clusters) and excitatory (14 clusters) cells. These clusters, and their associated transcriptomic markers, represent a rich community resource, and will enable the definition of exciting candidates for spatial or functional profiling.

Applying a similar strategy to identify epigenetically defined clusters, the authors were able to discriminate broad differences

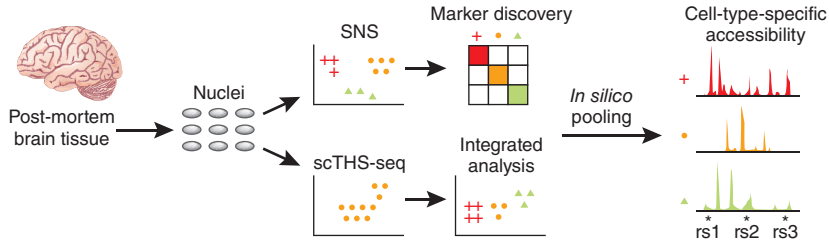

Figure 1 Schematic of integrated single cell analysis of the human brain. Nuclear suspensions from preserved human neuronal tissue are sent for single cell transcriptomic (scNuc-seq) and epigenetic (scTHP-seq) profiling. Unsupervised clustering of transcriptomic data is used to guide the classification of single epigenetic profiles, which are then pooled together *in silico* to create cell type-specific maps of open chromatin for downstream analysis.

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across cell types (e.g., separating excitatory from inhibitory neurons), but were unable to robustly resolve fine-scale differences based on unsupervised analysis. This is likely due to the reduced dynamic range of chromatin assays compared with transcriptomics, which both leads to substantial levels of sparsity in the sequencing data sets and represents a key challenge for the analysis of single-cell epigenetic data. In principle, cells with similar molecular profiles can be blended together *in silico* to increase the effective depth of profiling. The authors reasoned that although the scTHS-seq data may not lead to the discovery of new cell types, it could be powerfully applied to resolve differences in chromatin accessibility between previously identified classes.

The authors therefore designed a clever and powerful approach to integrate their epigenetic and transcriptomic data sets. Their key insight was to use a supervised classification analysis to individually assign data points from their chromatin-accessibility data set to their transcriptomically defined clusters. This strategy effectively transfers the knowledge learned from the RNA-based clustering onto the scTHS-seq data, yielding a coherent set of cell states for integrated analysis. To accomplish this, the authors first used gradient-boosted machines to build a model relating the expression of transcriptomic markers to the accessibility of genomic sites, for clusters that could be detected independently in both transcriptomic and epigenetic datasets. They then applied this model to classify the remaining THP-seq accessibility profiles based on their transcriptomic clusters. By pooling cells with the same classification together, the authors could reconstruct chromatin accessibility profiles of fine-grained cell types, and explore the regulatory processes that drive the specification and maintenance of cellular diversity.

Changes in chromatin accessibility are associated with the differential binding of transcription factors (TF), and the activation of downstream targets. The authors therefore searched for TF binding motifs that were statistically associated with each cell type, revealing both well-known relationships (e.g., regulatory activity of TFAP2A in GABAergic populations), but also identifying TFs with different activities across cortical layers and subcortical regions. Focusing on the differentiation of oligodendrocyte precursors, a process that underlies myelin regeneration in adults, they identified striking changes in chromatin accessibility during differentiation, suggesting a mutually antagonistic regulatory network involving SOX9 and TCF4. These initial findings raise exciting possibilities for future work, enabling single-cell analysis to resolve not just a taxonomy of cell types, but also a detailed understanding of their genetic and epigenetic regulation.

The authors conclude with an intriguing analysis, searching for the potential enrichment of genetic variants associated with neurodegenerative diseases in the open chromatin regions for each cell type. Strong enrichments could indicate a potentially pathogenic role for specific populations, generating hypotheses for downstream functional analysis. Interestingly, the authors inferred associations between microglia and Alzheimer's disease, and particular subpopulations of excitatory cells and Parkinson's disease. Though these analyses are at an early stage, they again suggest that although RNA measurements will help define cell types, integrated analyses with other modalities can yield new insights into the potential associations between specific populations and human physiology.

Large-scale international collaborations, including the Human Brain Project[8] and Human Cell Atlas[9], aim to transform our understanding of the human brain, and will rely heavily on single-cell technologies to define human cell states. Given the rapid pace of recent developments, new technologies are likely to continue to appear, potentially with dramatic increases in sensitivity and profiling depth, along with the potential to simultaneously measure transcriptomic and epigenetic states in the same cell. The work of Lake *et al.*[2] takes important steps toward this goal, demonstrating not only the feasibility of profiling challenging tissues, but also the value of highly scalable and integrated analysis.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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Viruses leave their stamp on single cells

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A method for 'stamping' viruses achieves efficient transduction of single target cells in cultured tissues and in the mouse brain.

Genetic manipulation of single cells or defined cell populations has become an indispensable technique in the quest to understand many biological processes. In neuroscience, an ever-expanding arsenal of targeting technologies is used to read out or interfere with the function of defined populations of neurons and to trace their connectivity. A key goal now is the development of improved tools for genetic modification of single cells, including in the brain of living animals. In this issue, Schubert *et al.*[1] present a simple, versatile, and efficient method for viral transfection of single cells in cell culture, tissues, and the brains of live mice. The approach relies on mechanical contact of the cell with a micropipette or magnetic bead decorated with the virus. This makes the reliable and selective infection of single cells in culture or superficial tissues child's play, and opens up new avenues for targeted *in vivo* infections as well.

In recent years, several new techniques have been introduced to transfect individual neurons, often in combination with a device to record their functional properties (**Fig. 1**). Plasmids carrying diverse genes, such as specialized receptors and fluorescent proteins, can be delivered via whole-cell recording[2], single-cell electroporation[3], or electrophoresis[4]. Other methods to target gene expression to relevant neurons include genetic tricks such as mosaic analysis with double markers[5] and activity-dependent expression of target genes[6]. However, these latter approaches often lack single-cell sensitivity or make it tricky to

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