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Previews

The Interneuron Class Struggle

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In this issue of *Cell*, Gouwens et al. establish the state of the art for defining inhibitory cell types in the mouse neocortex. By combining morphological, electrophysiological, and transcriptomic features to classify interneurons in the mouse visual cortex, this work provides a roadmap for understanding the diversity of cell types and their functional role in cortical computations.

It has long been recognized that the brain is made up of distinct cell types. Their beauty was first put on display by the work of Ramon y Cajal, who showed that among cells of the cerebral cortex there are a diverse array of "short axon cells," now known as cortical GABAergic inhibitory neurons (INs), or interneurons (Ramón y Cajal, 1899). Cortical interneurons are often categorized by subtle differences in gene expression, physiology, morphology, and connectivity, among other attributes, making their classification extremely challenging. Thus, many years after Ramon y Cajal, we still struggle to understand and categorize the diversity of inhibitory neurons in the cortex; however, in this issue of *Cell*, Gouwens et al. (2020) represents a major step forward in this effort.

To understand the function of cortical circuits, it is necessary to catalog their cellular diversity. Examining strictly defined cell types has yielded deep mechanistic understanding of systems such as the *C. elegans*, crustacean stomatogastric ganglion, and the vertebrate retina (Zeng and Sanes, 2017). A major advantage that these biological systems have relative to the murine cortical system is a broad consensus about the classification of cell types, making it possible for researchers to compare their independent findings. Similarly, there has been a long-standing effort to catalog murine cortical neurons by using various cellular features (Ascoli et al., 2008); however, such efforts have not yet yielded a consensus on the diversity and nomenclature of cortical INs. Over the last decade, the advent of transgenic Cre-expressing mouse lines has made it possible to target specific IN classes based on their marker expression (e.g., somatostatin, SST; parvalbumin, PV; vasoactive intestinal peptide, VIP). This allowed researchers to investigate the role of each IN class in cortical function. Such tools have been extremely useful; however, because each one of the Cre lines encompasses a variety of IN types, it has been difficult at times to achieve consensus on the function of an IN class (Fishell and Rudy, 2011). Recently, single-cell RNA deep sequencing has provided a major breakthrough in the quest for IN types (Poulin et al., 2016; Tasic et al., 2016). Transcriptomics has shown us that INs group in clusters that constitute cardinal IN classes that often correspond with cell types previously defined by molecular markers and morphological or physiological criteria (Fishell and Rudy, 2011). Furthermore, each one of those IN clusters can be subdivided into IN subtypes by using combinatorial gene expression. Despite the major contributions of transcriptomics toward cellular classification, this method alone has not established absolute boundaries between cell types and does not allow us to establish equivalences between gene expression and morphology or physiology, making it often difficult to map transcriptomically defined cell types onto historical research on distinct IN cell types.

The work by Gouwens et al. makes a remarkable contribution toward cataloging the variety of inhibitory cell types of the visual cortex and provides a "ground truth" of the transcriptomic cell types. We finally have a solid framework for IN classification for future studies. By using the Patch-seq technique in a highly standardized setting, the authors have created a database of 4,270 INs with information on the electrical properties and transcriptomics of these cells, with 517 of these cells having morphological data (Figure 1A). These data allow for unprecedented global understanding of the landscape of inhibitory cell types in the neocortex by using distinct modalities never before combined at this scale. With this data, the authors were able to draw boundaries among IN types by clustering sampled cells based on similarities in gene expression, morphology, and electrophysiology. Powerfully, they were able to relate the Patch-seq data back to previously defined transcriptomic cell types (t-types) and to compare the boundaries defining cell types across modalities. The result is a consensus morpho-electric-transcriptomic typing (met-type) defining 13 met-types within the SST-expressing cells, five met-types within the PV-expressing cells, two within the Lamp5 cells, three within the Sncg cells, and five within the VIP cells.

This work also highlights and expands upon the outstanding past research defining IN types through electrophysiological, morphological, and histological properties. In contrast with the limitations provided by using transcriptomics alone, this work allows mapping of historical data to a common naming scheme. The authors point out several instances of previously defined cell types mapping to their consensus met-type approach: e.g., the so-called L1 canopy cells map to Lamp5-MET-1 (Schuman et al., 2019); the layer 2/3 and 5/6 fanning-out Martinotti cells map to the SST-MET-3 and 4, respectively (Muñoz et al., 2017); and the translaminar fast-spiking cells map to Pvalb-MET-4 (Bortone et al., 2014; Frandolig et al., 2019). They also reveal new signatures of cortical inhibitory cell types

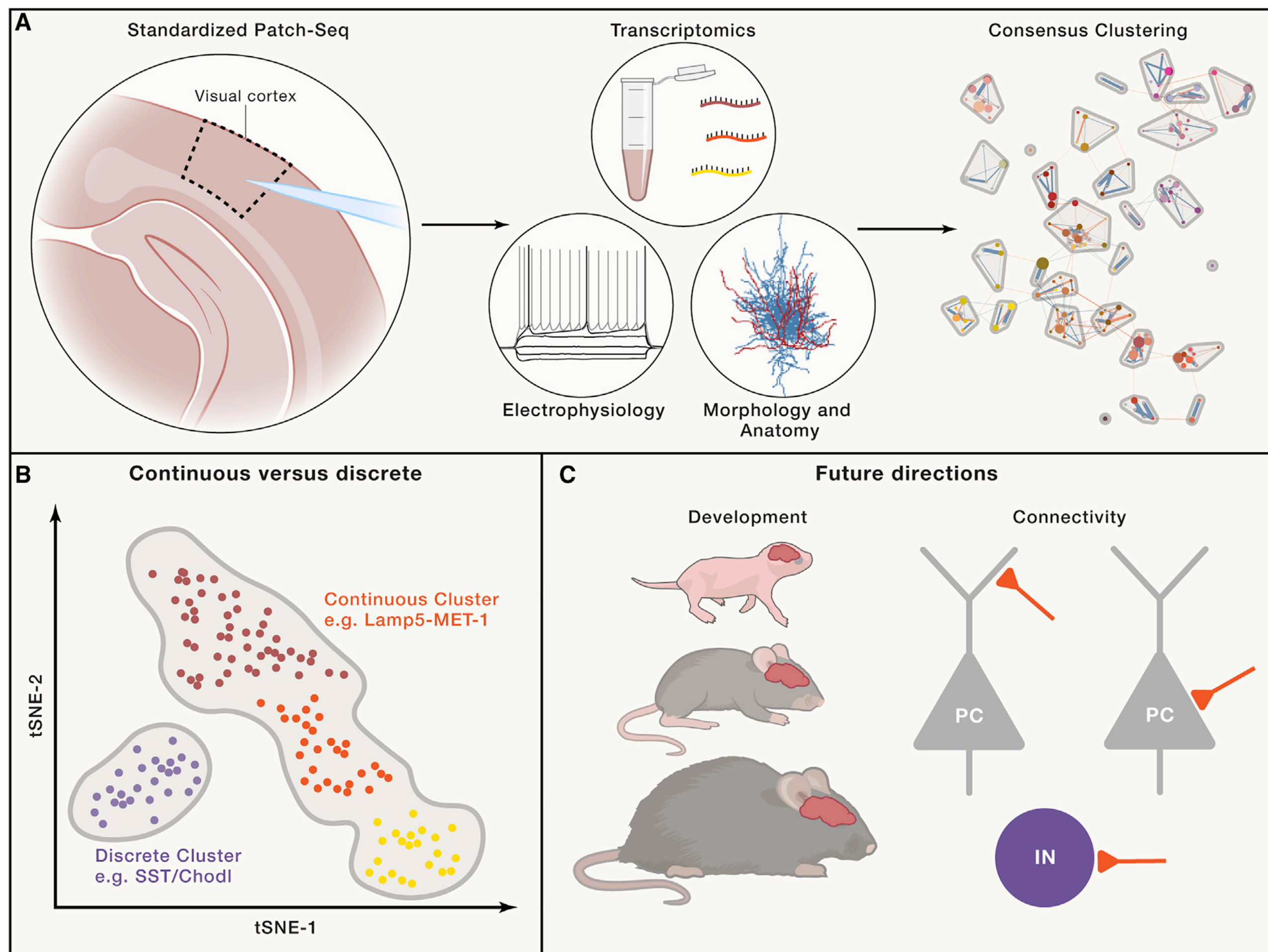

Figure 1. Consensus Cell Type Definitions Combining Data Modalities with Future Directions

(A) Schematic of the standardized Patch-seq pipeline combining transcriptomic, morphological, and electrophysiological data to generate consensus cell types definitions.

(B) Schematic highlighting the difficulties in dividing cell types that could exist in functional continua.

(C) Future directions suggesting that including developmental and detailed connectivity information will likely improve the multimodal cell type classification schema.

such as clear soma depth profiles and axonal laminar innervation patterns. SST MET-types, for instance, exhibit a cell-type-specific preference for different cortical layers. By connecting data across modalities, the authors also observe a greater diversity of inhibitory neuron types than many previous studies. Mapping cell types across modalities and achieving consensus about IN types across labs is critical in order to comprehend the functional role of each IN type in cortical computations; however, the success of systematic annotation is still highly dependent on the cell type in question. For instance, it should be simple to map cell types across labs that are morphologically and transcriptomically unique and cluster tightly in these spaces (e.g., SST/Chodl and Chandelier cells; Figure 1B). Such a task might prove difficult for IN classes that appear to exist on a genetic/functional continuum in which the functionally relevant boundaries are more difficult to define (e.g., Lamp5-MET-1 and VIP cells; Figure 1B).

The journey toward achieving a universal classification system for cortical INs is still ongoing. Although the work by Gouwens et al. considerably moves the needle of cortical interneuron classification, future work will likely benefit from examining additional parameters such as connectivity, gene expression during development, and *in vivo* activity (Figure 1C). Given the systematic and multidimensional approach toward classification of cell types practiced at the Allen Institute for Brain Science, we might have these answers in the near future. New developments, like the details of cellular and subcellular connectivity that electron microscopy will bring to the cell type classification system, are especially exciting. Finally, we would like to end with a reflection on what this work means for future research in the IN field. It is clear now that functionally relevant and distinct cell types require multiple parameters to define. However, it is still common practice and will likely remain common

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practice to use single Cre lines to define a cell type due to experimental limitations. The work by Gouwens et al. shows the need for care when interpreting data acquired with these genetic methods alone. Overall, this work is a great leap forward in our ability to define functionally relevant cell types in an accessible way, guiding comparisons and reproducibility in future work.

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Some Like It Sweet: Dendritic Cells Add Sugar to Their T(ea)

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In this issue of *Cell*, Liu et al. present FucoID, a glycosyltransferase-mediated tagging platform, to biochemically label and capture antigen-specific T cells. With this technology, the authors isolate and characterize tumor-specific CD8+ and CD4+ T cells in murine tumor models. FucoID shows promise as a tool to enhance the understanding of anti-tumor immune responses.

Tumor-infiltrating CD8$^+$ and CD4$^+$ T cells (TILs) play a fundamental role in the recognition and elimination of cancer and/or in the suppression of protective anti-tumor immunity (Dunn et al., 2002). Integral to their ability to elicit an immune response is their recognition of short peptides presented on major histocompatibility (MHC) molecules through their cell-surface T cell receptor (TCR). Over the past decade, we have begun to appreciate both the heterogeneity and antigen-specificities of T cell responses in the tumor. Cell-surface markers such as PD-1 and CD137 (4-1BB), or peptide-MHC (pMHC) multimers have been used to enrich tumor-reactive T cells and enable subsequent phenotypic and functional characterization (Gros et al., 2014; Newell et al., 2013; Wolfl et al., 2007). However, in addition to aberrantly expressed self-proteins and mutation-harboring neoantigens, activation markers also often isolate virally reactive, bystander CD8$^+$ T cells (Scheper et al., 2019; Simoni et al., 2018). Moreover, pMHC multimers rely on our computational ability to provide a pre-filtered set of candidate tumor antigens for study, and, as such, *in silico* approaches still cannot accurately predict stimulatory T cell epitopes. Thus, there is a need in the field for a strategy to pull out true tumor-reactive T cells (for both CD8$^+$ and CD4$^+$ subsets) from bystander cells that are unlikely to be beneficial for anti-tumor immunity.

In this issue of *Cell*, Liu et al. (2020) present FucoID—a glycosyltransferase-mediated tagging platform that provides an unbiased approach to study tumor-specific CD8$^+$ and CD4$^+$ T cells. FucoID relies on a chemoenzymatic labeling reaction whereby *H. pylori* fucosyltransferase (FT)-labeled dendritic cells (DC-FTs) transfer functionalized GDP-fucose-biotin (GDP-Fuc-Biotin) substrates onto