As one might expect of a mechanism of this novelty and breadth of scope, many questions remain unanswered. In a broad survey of primary cancer samples, to what extent are CD95 and H1 histone isoforms overexpressed compared to non-malignant cells? What exactly is the mechanism by which extra-nuclear H1 histones induce cell death? What drugs might be added to a PPE-based therapy to improve *in vivo* efficacy? Will resistance rapidly develop via selection of reduced CD95 or H1 histone expression? What antigens are provoked by ELANE or PPE treatment that sustain the abscopal effect?

Mechanisms as striking, novel, and unexpected as those reported in this paper certainly deserve considerable scrutiny in further experiments that will no doubt now be performed by the authors and others. We can all look forward to the fleshing out of the many aspects of cancer biology revealed in this report. Let us hope that the discoveries reported here turn out to be as exploitable as they are astonishing.

DECLARATION OF INTERESTS

A.L. is an equity-holding member of the scientific advisory boards of Zentalis Pharmaceuticals, Flash Therapeutics, and Dialectic Therapeutics.

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Seq-ing out cell types across the isocortex and hippocampal formation

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A central quest in neuroscience is to gain a holistic understanding of all cell types in the brain. In this issue of *Cell*, Yao et al. establish a molecular architectural view of cell types across the entire adult mouse isocortex and hippocampal formation and reveal surprising similarities of cell types in these two brain regions.

Equipped to perform various functions, our brain consists of many different cell types in a complex organization. Classic studies by Santiago Ramón y Cajal using the Golgi staining technique provided the first systematic classification of neural cell types in the brain based on morphology and location (Ramón y Cajal, 1913). Over the past century, researchers have further classified neural cell types based on their electrical properties and immunohistological markers. Within the past decade, advances in single-cell RNA sequencing (scRNA-seq) technologies have provided a scalable platform to better define different cell types based on whole transcriptomes, instead of a few marker genes (Macosko et al., 2015). Recent studies have started systematic characterization of cell types in different regions of many tissues both during development and in the adult (Chen et al., 2018). In this issue of *Cell*, Yao and colleagues provide an unprecedented molecular architectural blueprint of all cell types across the adult mouse isocortex (CTX, also called neocortex) and hippocampal formation (HPF) and suggest novel concepts about the development, evolution, connectivity, and

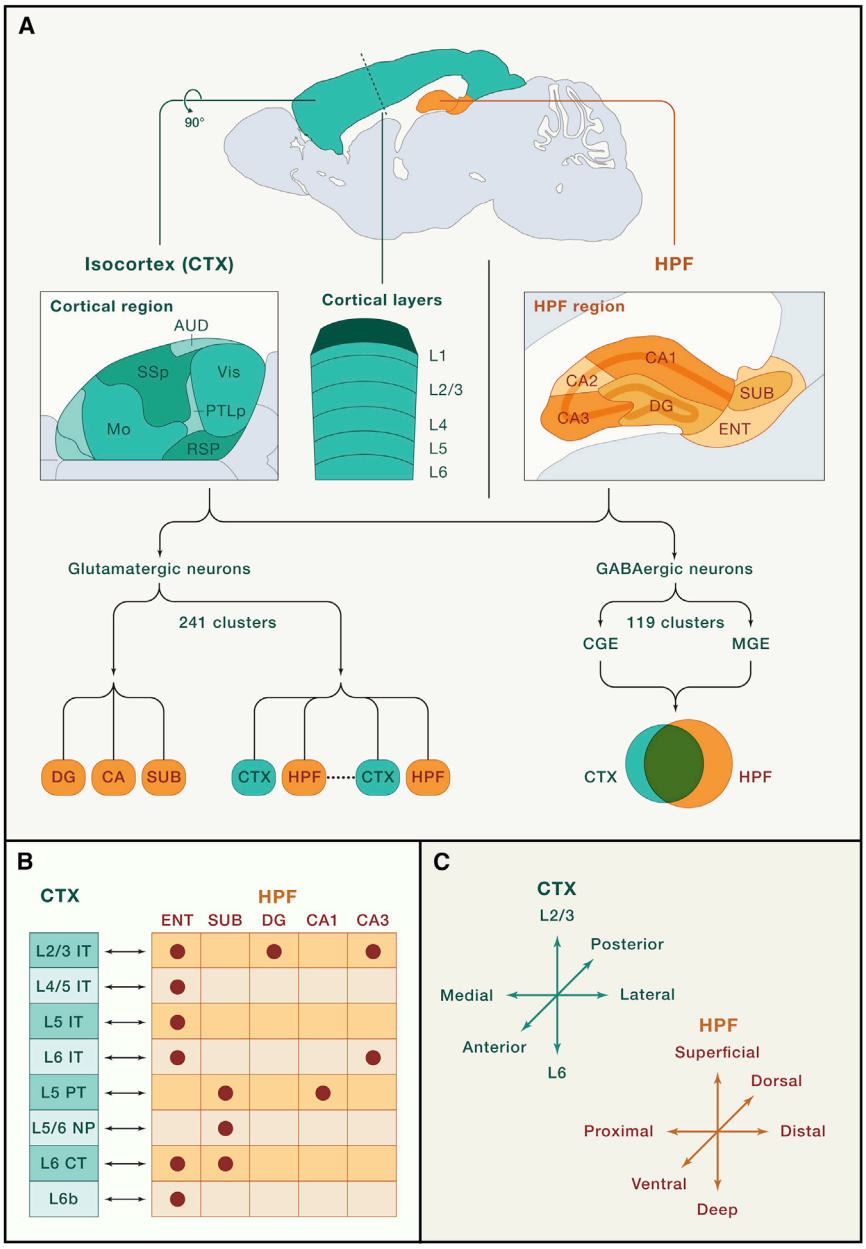

Figure 1. A molecular architecture view of different cell types across the entire isocortex and hippocampal formation in the adult mouse brain

(A) Schematic view of brain regions subjected to scRNA-seq and classification of neuronal subtypes. The current study covers the entire isocortex (CTX, including different cortical areas and different cortical layers, such as auditory cortex [AUD], motor cortex [MO], primary somatosensory area [SSp], posterior parietal association area [PTLp], retrosplenial area [RSP], and visual cortex [Vis]) and the hippocampal formation (HFP, including multiple subregions, such as cornu ammonis [CA] 1, CA2, and CA3; dentate gyrus [DG]; subiculum [SUB]; and entorhinal cortex [ENT]). The neuronal clusters can be divided into two large groups (glutamatergic neurons, 241 clusters; and GABAergic neurons, 119 clusters). The glutamatergic neurons can be further classified into hippocampal neurons of different subregions (CA, DG, and SUB) and neurons of other HPF regions mixed with CTX subgroups. The GABAergic neurons can be classified into two large groups developmentally derived from caudal ganglion eminence (CGE) or medium ganglionic eminence (MGE). Most GABAergic subtypes are shared in CTX and HFP.

(B) Parallel correlation of transcriptome and anatomical organizations of cell types in CTX and HPF. Different neuronal clusters in different cortical layers have corresponding cell types in HFP subregions, including ENT, SUB, CA, and DG.

(C) Glutamatergic neurons in CTX exhibit a gradual vertical transition of all neuronal clusters along the cortical depth (from L2/3 to L6) and a continuous and graded distribution along anterior-posterior and medial-lateral dimensions, whereas those in HPF exhibit gradual changes in gene expression along superficial-deep, proximal-distal, and dorsal-ventral dimensions.

(legend continued on next page)

function of these two brain regions (Yao et al., 2021).

While previous scRNA-seq studies have profiled cell types within several individual regions of CTX and HPF (Ecker et al., 2017), Yao et al. (2021) covered all these two regions of the adult mouse (Figure 1A) with over 1.3 million cells. They combined dissection of different CTX and HPF areas and fluorescence-activated cell sorting of many transgenic reporter mouse lines to enrich for different neuronal subtypes and used two scRNA-seq platforms, with 10x Genomics to cover a large number of cells and SMART-seq to generate more in-depth transcript coverage. All data were integrated together, resulting in 388 consensus clusters, including many newly identified cell types. They further constructed a taxonomy with different granularity by hierarchical clustering based on differentially expressed genes. While multiple subclasses of non-neuronal subtypes were identified, the current study focused exclusively on GABAergic and glutamatergic neuronal types. Taking advantage of the existing Allen Brain Atlas RNA *in situ* hybridization database (Lein et al., 2007), they were able to reconstruct the brain by correlating molecularly identified cell types with anatomical distribution patterns.

The GABAergic neuronal class is divided into two large classes that correlate with their distinct developmental origins, and each large class can be further divided into multiple subclasses and then more subtypes with a total of 119 types/clusters (Figure 1A). Most GABAergic neuronal types are present in all CTX areas and HFP regions, but a small number of them are specific to CTX or HPF.

The glutamatergic neuronal class is much more complex than the GABAergic neuronal class with a total of 241 types/clusters (Figure 1A). Across all CTX areas, most glutamatergic neuron types are shared among multiple areas, while a few are specific to one or two areas. Evolutionarily, it is generally believed that HPF together with the

olfactory cortex are older structures with three to five neuronal layers, whereas CTX emerged later and expanded significantly in mammals with six neuronal layers (Rakic, 2009). Interestingly, the comparison of glutamatergic neuronal types between HFP and CTX uncovered parallels between the transcriptomic and anatomical organization of cell types in both regions (Figure 1B). These corresponding cell types not only exhibit similarities in overall gene expression but also in a large set of canonical CTX layer-specific cell type markers. For example, the HFP entorhinal (ENT) groups has 10 subtypes organized in a layer-specific manner (in the order of L2, L2/3, L3, L5), corresponding to CTX L2/3-L6 types. Based on the observed homologous relationship, the authors suggest a novel model that both CTX and HPF in mammals may evolve from the simpler three-layered cortex in reptiles into two parallel six-layered organizations, with CTX going through an accelerated evolution resulting in a multiplication of areas. These results also raise the possibility that axonal projection patterns may follow the same rule for corresponding neurons in CTX and HPF, resulting in similar basic circuit organization and function.

In the HPF, glutamatergic neurons exhibit gradual gene expression changes along superficial-deep, proximal-distal, and dorsal-ventral dimensions (Figure 1C). For example, in cornu ammonis (CA)1 and CA3 subfields of the hippocampus (Figure 1A), key genes contributing to the first principal component correspond to a dorsal-ventral gradient, whereas those contributing to the second principal component correspond to a superficial-deep radial axis. In the CTX, there is a gradual vertical transition of all neuronal clusters along the cortical depth (Figure 1C). Across CTX areas, glutamatergic neuronal types are distributed in a continuous and graded manner, often along anterior-posterior and medial-lateral dimensions. Due to this holistic picture of the whole CTX, the current study corrects the previous assumption of area-specific glutamatergic neuron types based on studies of individual CTX regions without the knowledge of a continuous gradient (Tasic et al., 2016).

The comprehensive study by Yao et al. (2021) provides a molecular blueprint of different neuronal types and their distribution in the entire CTX and HPF. It raises a number of questions. For example, how are diverse cell types generated and organized during brain development, especially those unique to specific regions? What are the functions of different cell types, especially those newly identified ones? The current study also has limitations. The characterization of non-neuronal cell types is rather limited with around only 17,000 cells. It will be interesting in the future to investigate various subtypes of glia cells and neural progenitors that support continuous cell genesis in the CTX and HPF (Ming and Song, 2011). A mixture of male and female mice was used in the current study, but sexually dimorphic gene expression of different cell types was not examined. Use of mice from intercrossing of strains with different genetic backgrounds, instead of pure C57BL/6J, would also provide the opportunity to investigate differential paternal and maternal allelic gene expression in different cell types.

Aligned with the goal of the BRAIN Initiative for Cell Census (Ecker et al., 2017), such a large-scale and comprehensive analysis by Yao et al. (2021) provides an extremely useful resource for the neuroscience community and will have a significant impact for years to come. Similar comprehensive studies are needed to map other brain regions and different stages of brain development. In addition, features other than transcriptomes at the single-cell level, such as epigenomes (DNA methylation, ATAC-seq, histone marks, HiC), epitranscriptomes (m^6A and other mRNA modifications) (Vissers et al., 2020), and proteomes, may help to provide a more complete understanding of different cell types. Given the large differences in the number, type, and properties of cells in the mouse and human brains, it will be a major step forward to understand evolutionary patterns and organizational principles by extending such comprehensive analyses to humans.

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