Rethinking Functional Segregation: Gradients of Gene Expression in Area CA1

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An emerging view, based on gene expression patterns, is that discrete cell types occupy different regions in the hippocampus. In this issue of *Neuron*, Cembrowski et al. (2016) challenge this concept by identifying gradients of gene expression that suggest a molecular continuum of excitatory neurons and provide insights for organizational motifs at hippocampal poles.

A fundamental feature of brain organization is the compartmentalization of functions into discrete anatomical regions. In the hippocampus, for example, different functions have often been ascribed to the dorsal versus ventral regions: the rodent dorsal hippocampus (corresponding to the posterior hippocampus in primates) is generally associated with cognitive functions like memory and spatial navigation while the ventral region (corresponding to the anterior hippocampus in primates) is primarily associated with stress and emotional behaviors (Fanselow and Dong, 2010; Strange et al., 2014). Whether these functional differences arise exclusively from the distinct inputs and outputs (Risold and Swanson, 1996) or intrinsic differences in cell types and circuits is an important question.

The four basic elements of any neural circuit are excitatory neurons, inhibitory neurons, modulatory inputs, and glial cells. The degree to which molecular heterogeneity within a cell type can influence circuit function is currently an open and exciting area of research. While interneurons have readily distinguished themselves by distinct morphologies, arborization and projection patterns, and peptide signatures (Klausberger and Somogyi, 2008), excitatory neurons have yet to reveal a similar diversity. The absence of a simple segregation by peptide expression or other molecular markers, for example, has led many to conclude that the differences between principal excitatory cells might be relatively small. This idea has been countered, in part, by several recent investigations of regionalized gene expression patterns in excitatory cells of the hippocampus. For example, using data from the Allen Brain Atlas, Thompson et al. (2008) identified up to nine different molecularly discrete regions in hippocampal area CA3 and Dong et al. (2009) proposed three different regions in area CA1. In contrast, a very recent study that used single-cell RNA-seq reached the (rather disappointing) conclusion that there are only ~2 types of pyramidal neurons (in the neocortex and hippocampus) (Zeisel et al., 2015).

To examine neuronal cell diversity and gene expression along the three CA1 axes (dorsal-ventral, proximal-distal, and superficial-deep), Cembrowski et al. (2016) used next-generation RNA sequencing, in situ hybridization, immunohistochemistry, and electrophysiology. The authors sampled cells at different positions in the hippocampus by microdissection and manual sorting of GFP-expressing pyramidal neurons and then conducted RNA sequencing to identify and quantify transcripts in each region. In contrast to previous reports, Cembrowski et al. discovered a surprising molecular heterogeneity among principal cells. Using a statistically weighted enrichment criterion, 33, 71, and 265 transcripts were significantly differentially expressed in the proximal-distal, superficial-deep, and dorsal-ventral axes, respectively. Surprisingly, the transcript diversity present within area CA1 is greater than that observed between area CA1 and its presynaptic partner, area CA3. The genes that were differentially expressed along the dorsal-ventral axis of area CA1 include those involved in the regulation of transcription, Ca^{2+} signaling, the cytoskeleton, axon guidance, and synaptic transmission. The authors validated the differential expression of some transcripts with fluorescence in situ hybridization and confirmed the corresponding differential protein expression with immunohistochemistry. They also conducted electrophysiological recordings from individual neurons that indicated axial differences in input resistance, resting membrane potential, and spike threshold (Dougherty et al., 2012), consistent with the differences in ion channel expression observed. Others have noted differences in synaptic plasticity, for example, long-term potentiation, between the two poles (Papatheodoropoulos and Kostopoulos, 2000).

From these data, Cembrowski make the bold proposition that the molecular description of hippocampal neuron cell types is best characterized by gradients of multiple gene expression patterns that, for the most part, span the dorsal-ventral axis. This is in contrast to what was the emerging view, that there are discrete cell types; this idea is represented most recently and dramatically by Zeisel et al. (2015). Using single-cell sequencing, Zeisel and colleagues identified two classes of CA1 pyramidal cells in the hippocampus (but also noted dorsal-ventral patterned genes). Cembrowski et al. considered this possibility—that there exist just two cell types, each one concentrated at the dorsal or ventral pole of the hippocampus. They assert

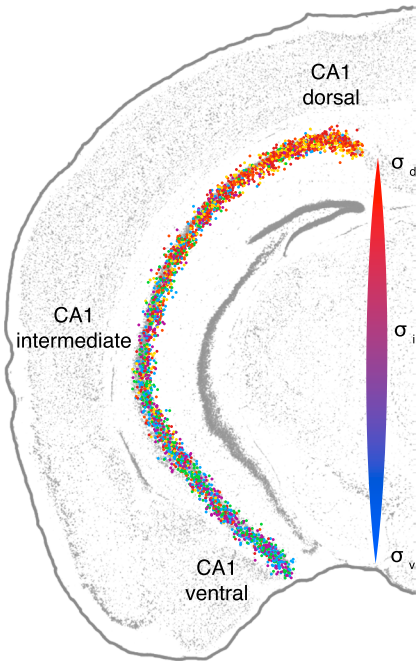

Figure 1. Continuous Gradients of Gene Expression along the Dorsal-Ventral Axis of Hippocampal Area CA1

Cross-section of a rodent brain revealing the longitudinal axis of CA1 region. The colored puncta represent pole-specific marker genes in either dorsal (warm colors) or ventral (cold colors) CA1 regions. The color bar depicts the gradual mix of those genes' expression along the axis, whereas the width of the color bar correlates with the observed variance of gene expression. Pole-specific marker genes tend to have higher expression variance in the intermediate section relative to the tips ($\sigma_{d,i,v}$). The cross-section image is from the Allen Brain Atlas (Lein et al., 2007).

that if this model is valid, these two cell types should gradually mix toward the middle of the dorsal-ventral axis. To examine this directly, Cembrowski et al. conducted RNA sequencing of the pyramidal neuron population that occupies the intermediate region of CA1. If the two-cell model is correct, then the abundance of dorsal and ventral genes should sum to 1, reflecting the ratio of the two cell types. In addition, the decay of gene expression from each of the poles should be equivalent for all genes. The data do not support this view. The gene expression profiles, while graded from one pole to the other, show unique profiles of decay, suggesting that there are many different cell types. Supporting the idea of multiple cell types, Cembrowski et al. also discovered that within the ventral pole, the gene expression patterns of clusters of cells with similar projections (e.g., to the nucleus accumbens or the amygdala, identified by retrograde labeling) are distinct from one another. Taken together, the data and simulations indicate a differentially graded monotonically decreasing expression pattern of distinct genes that originate from either the dorsal or ventral pole (see Figure 1).

One open question is how these continuous gradients of gene expression arise. The CA1 pyramidal neurons of the dorsal and ventral hippocampus are born at the same time, but the dorsal hippocampal neurons project to earlier-born cells in target structures such as the lateral septum, whereas ventral hippocampal neurons project to later-born cells in the same structure (Bayer, 1980). As such, target-derived factors or diffusible molecules with different concentrations at the hippocampal poles could influence transcription factor profiles and gene expression patterns.

The challenge now is to understand how the CA1 neuron populations represented by these gradients carry out the functions that have been ascribed to different regions of the hippocampus. Although inputs and outputs to the different hippocampal poles are often described as "discrete," these anatomical boundaries are rarely sharp. In addition, the trisynaptic circuit, comprising the synapses between the dentate gyrus, area CA3, and area CA1, is represented along the entire dorsal-ventral axis and has been proposed to perform specific computations. Along with the differing inputs and outputs that are observed along the dorsal-ventral axis, we must now consider the graded molecular profiles of the CA1 neurons that are an integral component of the circuit. Although neural circuits can perform similar computations with diverse molecular solutions (Prinz et al., 2004), it is clear that the molecular profile and physiology of the neurons at the dorsal and ventral poles is different—and this will certainly contribute to some of the important behavioral differences that have been described.

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