The organization of inhibitory connectivity across cell types in an electron microscopy reconstruction of a mouse cortical column

AA.12

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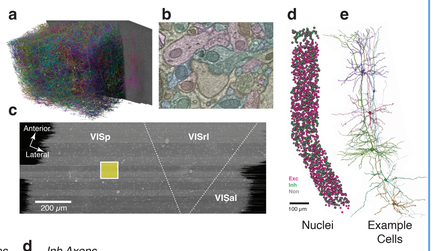
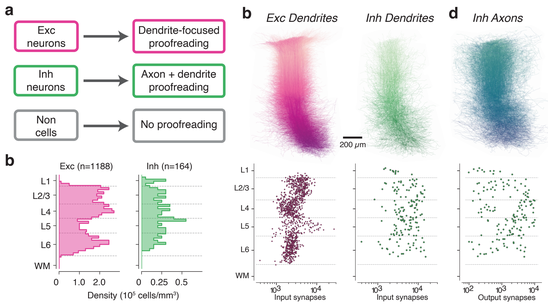
Introduction

The tremendous diversity of intermingled cell types is one of the hallmarks of mammalian cortex. Recent studies have surveyed the transcriptomic, molecular, morphological, and electrophysiological characteristics of neurons within and across regions, sometimes with multiple modalities per neuron using techniques such as Patch-seq. Perhaps the most important differences relate to how cells communicate with one another, but it remains difficult to comprehensively map the rules underlying which neurons connect to which and how strongly. **Here, we use a millimeter-scale electron microscopy volume of mouse visual cortex to densely reconstruct the anatomy of neurons across more than 1300 cells in all layers, produce a data-driven analysis of anatomical cell types, and map the precise patterns of synaptic connectivity of inhibitory neurons.** Collectively, these results find pervasive specificity in early sensory cortex and and new principles of inhibitory connectivity.

1. Dense proofread EM reconstructions across VISp

Figure 1.1 — As part of the broader MICrONs project, we generated an EM volume from visual spanning all layers, more than 1 mm wide and 500 μm deep (**a**) with resolution sufficient to capture ultrastructural details (**b**). Segmentation and synapse detection was run across the whole volume. We focused on a 100 x 100 μm column in VISp (**c**) containing more than 1300 neuronal nuclei (**d**). The column continuously followed neuronal arbors across the full depth of cortex (e).

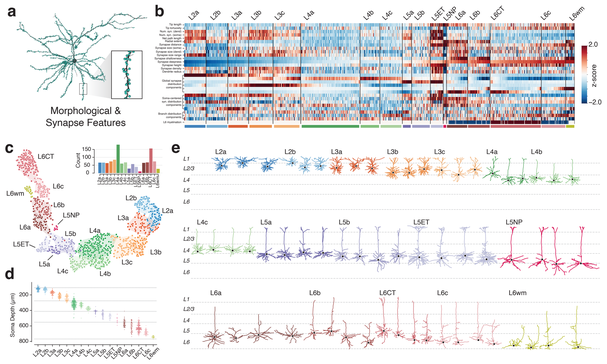


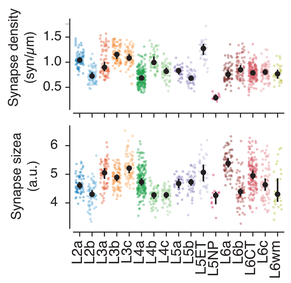


We extensively proofread excitatory neuron dendrites and inhibitory neuron axons and dendrites (**a**). Semi-automated cell typing found an expected laminar pattern of cell densities (**b**). Reconstructions of individual cell dendrites had thousands of synaptic inputs (**c**), and axons spanned from hundreds to 10,000 synaptic outputs per cell (**d**).

2. Anatomical cell typing of excitatory neurons

Using set of 25 robust morphological feature (e.g. net path length), synapse features (e.g. synapse density), and combinations thereof (e.g. synapse distribution vs depth), we generated a landscape of the dendritic diversity of excitatory neurons (**a–c**). A consensus clustering on this landscape identified 17 anatomical types (***a-types***) across all layers, many intermingled across the same laminar domains (**d, e**).

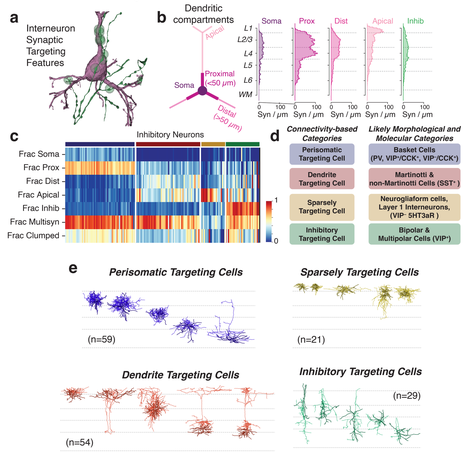




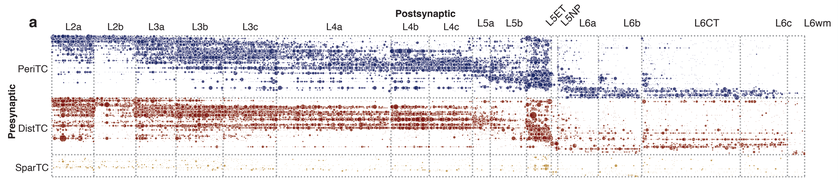
Synapse properties differ across a-types. For example, layer 2/3 excitatory cells differed in synapse density and synapse size, separating layer 2/3 cells into 2 layer 2 a-types and 3 layer 3 a-types. Layer 5 subtypes corresponding to different projection targets cells were previously shown to vary in morphological properties, and also do so in synaptic properties. In particular, layer 5 near projecting (NP) cells have very low synapse density and small synapses.

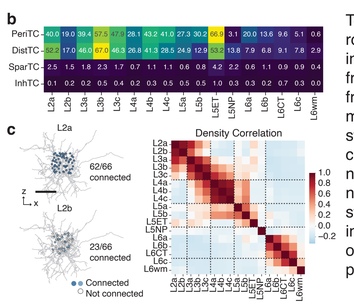
3. Anatomical cell typing of inhibitory neurons

To generate structural analogues of classical cell types often defined by molecular properties (e.g. SST or VIP neurons), we measured how each of the 164 interneurons distributes synapses across target neurons (**a**). We split excitatory neurons into four somatic and dendritic compartments and treated inhibitory neurons as a de facto fifth compartment (**b**). Together with a measure of frequency of multisynaptic connections and a measure of spatial clustering of multisynaptic connections, we found four classes of inhibitory neurons based purely on anatomical features that correspond approximately to classical or molecular categories (**c–e**).



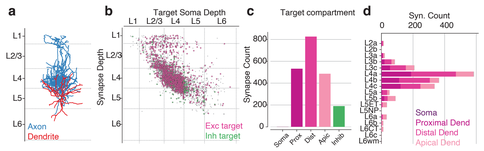
4. Inhibitory connectivity in a cortical column





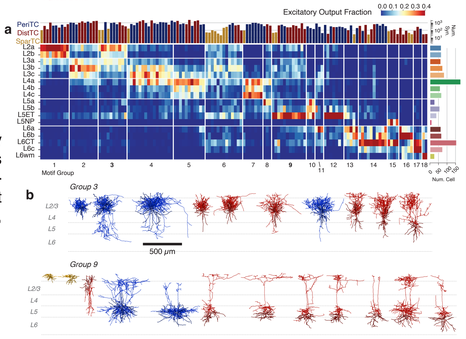
To test if the excitatory a-types we found actually have a different role in the cortical circuit, we looked at the connectivity of inhibitory interneurons across a-types. The data contain 68,527 synapses from inhibitory neurons onto excitatory cells (**a**). An average cell from nearly all a-types receieved numerous synapses from perisomatic targeting cells and distal dendrite targeting cells (**b**). To understand the organization of how cells distribute their inhibition across cell types, we measured the density of connections from each interneuron onto each target a-type (**c**). High values indicate many interneurons co-target (and avoid) the same a-types. The correlation structure of these densities revealed that input into layer 2 is largely independent from layer 3, that each layer 5 subtype is only weakly overlapping with one another, and that inhibition onto layer 6 is independent from all other layers.

5. Diverse inhibitory motif groups with similar output distributions

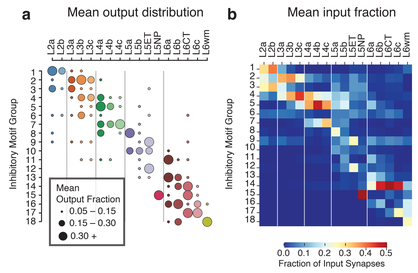


For each inhibitory neuron (a), we have the exact distribution of synaptic outputs across space (b), compartments (c), and a-types (d).

We computed the fraction of outputs each inhibitory cell makes onto target a-types (a). Clustering this output distribution vector reveals "motif groups" of-cells with similar output connectivity, but different morphology and even compartment targeting (b),

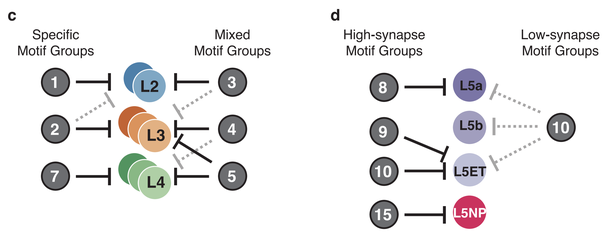


6. Motif groups organize inhibition in upper and lower layers

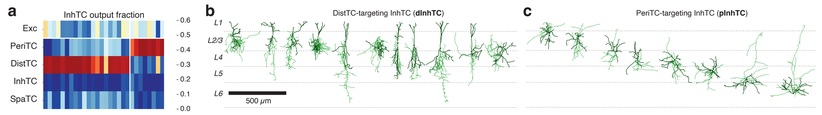


Each motif group has a characteristic output pattern across target types. Motif groups distribute their output across 1–3 dominant a-types (**a**). Many but not all motif groups are responsible for a significant fraction of the mapped inhibitory input onto target cells (**b**).

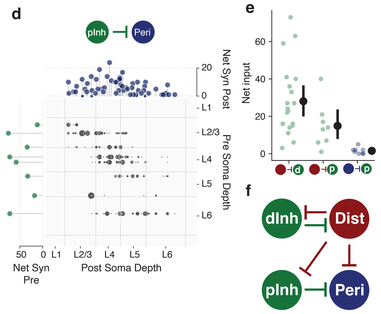
In layers 2–4, each a-type recieves inhibition from two or three motif groups that have distinct but overlapping combinations of targets (**c**). Some are specific within a layer (counting layer 2 and 3 as distinct), while others cross layer boundaries. In contrast, in layer 5, the most synapse-rich motif groups were specific to single a-types (**d**).



7. Inhibition of inhibition reveals a new class of disinhibitory specialist that targets basket cells



The common view of inhibitory cell types is that VIP+ cells are disinhibitory specialists that preferentially target SST+ cells. We were thus surprised to find two classes of disinhibitory specialists in the column data: One that targets distal dendrite-targeting cells (consistent with the common view) and a second that targets perisomatic targeting cells (***pInhTC***) (**a**). The first type are concentrated in layers 2–4 and have bipolar dendrites (**b**), while the second are across all layers and have multipolar dendrites (**c**). The targets of pInhTC are found across layers (**d**). However, similar to other InhTCs pInhTCs receve input from distal dendrite targeting cells but not perisomatic cells (**e, f**).



Conclusion: key observations and principles

In addition to creating a rich anatomical resource for neuronal anatomy and single cell connectivity, our data reveal several organizing principles for cortical connectivity:

- Excitatory cell types can differ not only in morphology, but synaptic properties like synapse density and synapse size.
- Inhibitory neurons specifically target (or avoid) intermingled a-types, suggesting the cortical circuit reflects the anatomical differences measured here.
- Inhibitory cell types collect into "motif groups", collections of cells that distribute their output onto multiple compartments of the same target a-types. This suggests parallel pathways to inhibit the same excitatory populations, perhaps under different network conditions or behavioral states.
- Excitatory neurons in layer 2 and layer 3 are inhibited by distinct, partially overlapping, populations of cells, allowing potentially independent control of subnetworks.
- Each layer 5 projection class (IT, ET, and NP) has distinct populations of interneurons that could control subnetworks or gate output with extraordinary precision.
- A novel class of disinhibitory specialist interneurons can modulate perisomatic inhibition across layers.

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