Increased Somatostatin-axon density in Layer 1 does not predict input density

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Introduction

Why study Layer 1 and Somatostatin-cells (SST)?
• Layer 1 (L1) is a hub of activity. Lots of exchange of information occurs here1
• SST-expressing inhibitory neurons synapse onto and control the activity of the main excitatory neurons of the cortex (pyramidal neurons)2
• SST neurons have high axon density in L1, suggesting they synapse onto the apical dendritic tuft of pyramidal neurons2
• SST axons in L1 disappear during learning, suggesting their connections are plastic
• Our method of analysis would be a way to quantitatively determine changes during learning

Questions:
• Which part of the pyramidal neurons do SST-cells preferentially innervate? The dendritic shaft or the dendritic spines?
• Does the location of the dendrite influence the density of SST input?

Methods

We used the image analysis software, Imaris, to reconstruct presynaptic SST neurites and the synapses (puncta) and spiny dendrites associated with a pyramidal neuron, from a fluorescence image. We then collected the points of contact between the puncta and SST at different distances away from the dendritic shaft (0.1, 0.5, 1.0, 2.0, and 3.0 µm). The dendrites were then labeled with their location in the cortex (upper-L1, lower-L1, and L2).

Labeling of presynaptic-SST Neurons

A13 mice contain the gene for enhanced yellow fluorescent protein (EYFP) but cannot express it without Cre recombinase (Cre)

SST-IRE6-Cre mice

Labeling of Synapses

Cre-independent FAPost and dTom expression → Cells infected by the AAV virus will express FAPost and dTom. FAPost will localize to synapses and fluoresce.

Methods (cont.)

- dTomato fill of neurons infected by AAV
- Reconstruction of a L2 Pyramidal neuron with spots representing assigned and unassigned puncta

Distance Threshold Test

Puncta with a point of contact were SST-assigned.

SST-puncta density = # points of contact
dendrite length

How does SST-assigned puncta density change as the distance from the dendritic shaft increases?

Results

SST-input density increases as the distance from the shaft increases

The overlap between the green and blue fluorescence is a point of contact

As the distance threshold is increased, the SST-assigned puncta density also increases, suggesting that SST-input density is greatest furthest away from the dendritic shaft (on spines).

Overall SST-input density is higher in layer 1 than it is in layer 2

SST-input density is higher in L1 than in Layer 2 at all distance thresholds.

The change in SST-puncta density with distance slightly more in L1.

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