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The anatomical basis of thalamic activation of neuronal networks in cortical columns of rat primary somatosensory cortex

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The information on sensory input is relayed to the cortex by thalamic neurons. Individual cortical neurons in whisker columns react to peripheral stimulation in a type-specific manner. To obtain a quantitative understanding of thalamocortical activation, however, precise knowledge of the anatomy at subcellular level is required.
The aim of this thesis was to quantify the anatomical constraints of thalamic input to cortex that determine sensory-evoked responses and subsequent cortical processing. A fundamental part of any model of thalamic column activation is the postsynaptic population, that is, neurons in columns that are targeted by synaptic boutons coming from thalamic axons.
In a first step, this population was quantified at a cellular level. At a precision that was not available by then, the number and distribution of neurons in all layers of a column were measured directly by counting all neurons in entire columns. A near-exhaustive count including 89,834 somata in a 1.15 mm^3 volume of cortex was made. In contrast, previous estimates of the number of neurons in a column had to rely on artifact-sensitive neuron density measurements and independently determined volume measurements. The direct measurement of the number of all neurons in one complete column was entirely lacking.
In a second step, the anatomical determinants of thalamocortical activation were quantified at a subcellular level. Dendritic morphology of 82 neurons was measured and, combined with the above cell counts, used to estimate the total dendritic density in a cortical column per cell type at a subcellular level. By further combining with measurements of thalamic bouton density, the number and distribution of thalamic boutons potentially contacting the dendritic trees of different neuron types in a column could be quantified.
The main findings are: (1) A single column contains, on average, 19,109±444 neurons (17,560±399 for a standard-size projection column), of which 63±10 are in cytoarchitectonic L1; 2,039±524 are in L2; 3,735±905 are in L3; 4,447±439 are in L4; 1,737±251 are in L5A; 2,235±99 are in L5B; 3,786±168 are in L6A; and 1066±170 are in L6B. (2) Estimates of the action potential output of a cortical column upon sensory stimulation (4441 within 100ms post stimulus) confirm previous reports, suggesting that the ensembles of spiny L4 and thick-tufted pyramidal neurons emit the major fraction of APs of a column. (3) all types of excitatory neurons potentially receive substantial TC input (90-580 boutons per neuron); (4) pyramidal neurons in L3-6 receive dual TC input from both VPM and POm that is potentially

of equal magnitude for thick-tufted L5 pyramidal neurons (approximately 300 boutons each from VPM and POm); (5) L3, L4, and L5 pyramidal neurons have multiple (2-4) subcellular TC innervation domains that match the dendritic compartments of pyramidal cells; (6) a subtype of thick-tufted L5 pyramidal neurons has an additional VPM innervation domain in L4. The multiple subcellular TC innervation domains of L5 pyramidal neurons may partly explain their specific AP patterns observed in vivo.

To achieve these results, both new experimental procedures and analysis tools needed to be developed: (1) a pipeline of tissue processing, immunohistochemistry, and large volume confocal imaging that was designed to label and image all neurons in consecutive cortical slices (2) reliable, unbiased and reproducible algorithms for manual neuron detection (3) analysis software that allowed the alignment of neuron positions obtained from consecutive slices, the relation of neuron positions to barrel-column outlines, and an automated analysis of neuron distribution (4) analysis software that allowed an automated quantification of single and average dendritic length density distributions of neurons from manual reconstructions, the convolution of neuron density and dendritic density distributions, and the calculation of bouton dendrite overlap.

The substantial potential thalamic innervation of all excitatory neuron types in a cortical column quantified in this thesis constitutes an anatomical basis for the initial, near-simultaneous representation of a sensory stimulus in different neuron types, and thus contradicts models of serial signal flow that originates in L4 and L6 and then spreads to supra- and infragranular layers. Together with the data on the number and distribution of neurons, these results will provide the reference frame for a mechanistic model of the synaptic substrates underlying the representation and the processing of sensory input in a cortical column.