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Review

Reconstruction of an average cortical column *in silico*

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ABSTRACT

The characterization of individual neurons by Golgi and Cajal has been the basis of neuroanatomy for a century. A new challenge is to anatomically describe, at cellular resolution, complete local circuits that can drive behavior. In this essay, we review the possibilities to obtain a model cortical column by using *in vitro* and *in vivo* pair recordings, followed by anatomical reconstructions of the projecting and target cells. These pairs establish connection modules that eventually may be useful to synthesize an average cortical column *in silico*. Together with data on sensory evoked neuronal activity measured *in vivo*, this will allow to model the anatomical and functional cellular basis of behavior based on more realistic assumptions than previously attempted.

Contents

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1. Circuits and behavior

One challenge of contemporary neuroscience is the mechanistic understanding of the cellular basis of simple behaviors that involve the mammalian cortex. To achieve this goal, a precise anatomical description of local and long-range circuits is necessary, and the electrical signal patterns in these circuits must be known. Ramón y Cajal pioneered the description of individual neuronal cell types. Nevertheless, a detailed anatomical description of even simple circuits formed by ensembles of neurons is – a century after Ramón y Cajal – still lacking. The present essay aims at illustrating how eventually such circuit-level descriptions might be achieved in the future and how computer based modeling of cortical networks may help to understand simple behaviors. We use the term *in silico* to refer to this approach of using mechanistic numerical models to complement experiments done *in vitro* and *in vivo*.

The basic computational unit of the cortex is the "functional" cortical column (Mountcastle, 1957, 1997). In the rodent somatosensory cortex, an anatomical equivalent of a functional column exists. Here single "barrels" in cortical layer 4 (L4) are defined anatomically and functionally, since the deflection of each facial whisker is represented in a point-to-point fashion by structurally identifiable ensembles of neurons comprising single "whisker columns" or barrels (Woolsey and Van der Loos, 1970; Simons, 1978; Armstrong-James et al., 1992). Remarkably, a simple two-choice behavior like "gap-crossing" (to-cross or not-to-cross) can be driven by sensory input from a single whisker in rodents (Fig. 1A) and thus presumably by the action potentials (APs) emitted from a single column (Hutson and Masterton, 1986; Celikel and Sakmann, 2007). This simple experimental situation therefore offers the possibility to delineate the anatomical basis and some of the (electro)physiological equivalents of "decision making". We suggest that by modeling a column *in silico* (i.e., re-engineer the 3D geometry of all neurons that constitute a cortical column and animate them with a time dependent pattern of pattern of postsynaptic potentials (PSPs) and action potentials (APs) at a resolution of a few milliseconds that corresponds to signals measured in the real column) it will be possible to obtain a mechanistic understanding of the synaptic activity needed to predict, for example, a binary decision.

1.1. *Single column driven behavior*

A first and essential requirement for constructing a column *in silico* is to establish a detailed anatomical wiring diagram of the cortical area that integrates the afferent sensory signals and then generates output APs (Fig. 1B). The output AP pattern from this area could be compared to a memory of previous sensory signals and eventually will generate or more likely trigger a motor action.

The *anatomical* and *functional* constraints, or "budget", of a cortical column can be calculated first. Because of the almost punctate single-whisker-to-single-column projection from the thalamus to the cortex, one can estimate the number of axons projecting into the cortical column (the input lines) and compare it with the number of axons projecting out of a column (the output lines). In addition, estimates of the afferent AP activity into a column and the AP output of cells in a column can be used to establish a *functional* AP budget or a cortical AP input–output relation for a behavior.

Because the cortex is a layered structure containing different types of neurons that project to specific cortical and subcortical areas, the second aim is to more specifically identify input PSP and output AP patterns for the different layers and the different neuronal cell types within a column. This description at cellular

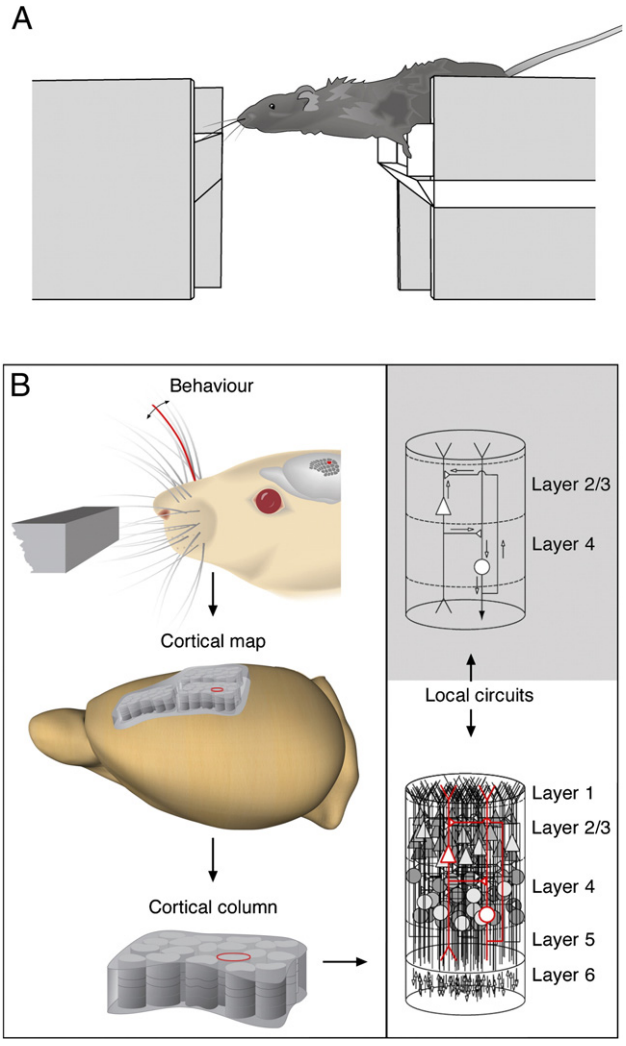

Fig. 1 – Single column driven behavior. (A) Gap-crossing task. (B) Schematic view of local circuits that can drive gap-crossing behavior. Analysis of the local circuitry by combined *in vitro* (grey background) and *in vivo* measurements (white background).

resolution is essential because the layer and cell-type-specific AP output largely define the strength with which other brain areas are activated upon sensory stimulation. By establishing the exact projection targets of the different cell types in a column one may also be able to identify the anatomical substrates and electrical signals of the operationally defined components of decision making such as the "comparator" and the location of the "memory" that is essential for making the comparison.

2. Reconstruction of an average anatomical network

The stream of excitation and inhibition sweeping through the different layers of a column following a sensory stimulus may be understood mechanistically only when estimates exist of the total number of neurons in a column. One also needs to know the anatomical and functional connectivity between the cells. Ideally the complete network of a single column would be reconstructed by serial electron microscopy (EM) but this is, as yet, not possible. Therefore one may rely on a statistical approach for the reconstruction of an "average" anatomical column. Here stereotyped functional connections between different neuronal cell types in the patch of cortex are reconstructed anatomically (e.g., Markram et al., 1997; Feldmeyer et al., 2002). The aim of this approach is to estimate the mean values and variability of morphological parameters for individual neurons as well as anatomical and physiological parameters for connections between cells. One could, in principle, reconstruct an average columnar network by measuring a set of typical connections between different projection and target neurons (Fig. 2). To do so, two assumptions have to be made: (1) For any given neuronal cell type in the column, its synaptic input and output properties are homogenous. (2) Major properties of the wiring diagram can be reproduced by pair-wise measurements of connectivity alone and do not require higher order statistics (e.g., recordings from triplets of neurons).

Using the "pair reconstruction" approach a set of all existing pair modules is generated. Subsequent "cloning" of these modular connections can be used to synthesize an average column *in silico*. The connections of the reconstructed columns have, possibly on average, the same statistical values as the connections in the real column. In the best case, the column *in silico* reproduces the average properties of the *real* columnar network. Once the synthesis of an average column *in silico* has been achieved, it is possible to model cortical phenomena observed experimentally, such as the spatial and temporal variability of responses to sensory stimuli (Kerr et al., unpublished observations) and their plasticity, attention-related changes in responses, learning of sensorimotor tasks and eventually sensory-guided decision making.

2.1. *Anatomical and functional connectivity*

For the purpose of reconstructing the average 3D pattern of subthreshold PSPs and suprathreshold APs appearing and disappearing following a stimulus, we need to know: (1) the number of different neuronal cell types in each layer, (2) the anatomical connectivity between projection and target cells

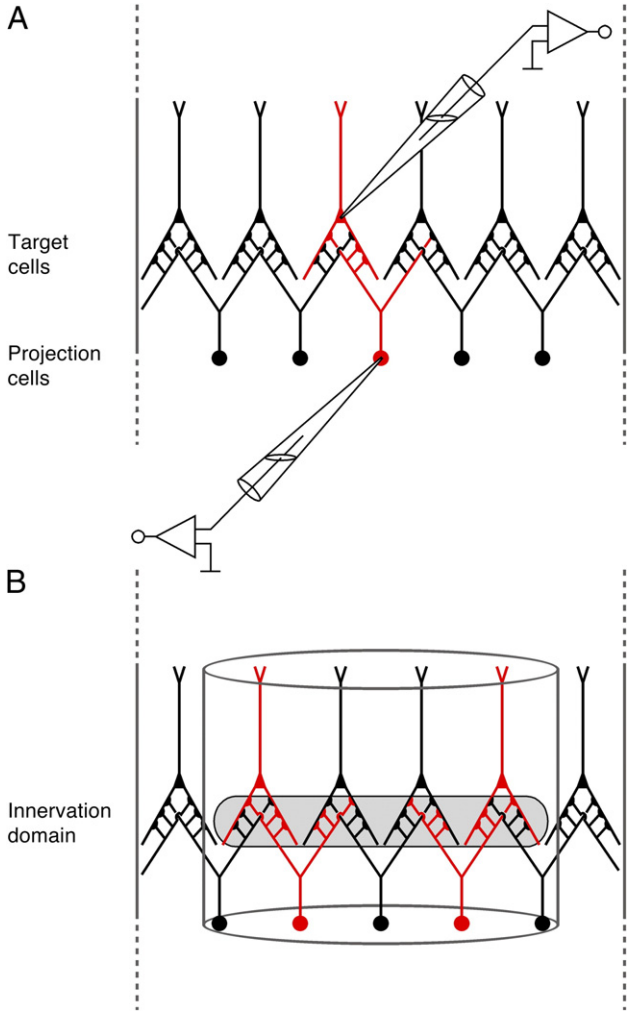

Fig. 2 – Reconstruction of an average network. (A) Reconstructions in a network from pair recordings and reconstructions of projection and target neuron. (B) The innervation domain (gray) of axon arbors of projection neurons with dendritic arbors of target cells determines the average axonal length of a cell innervating a given target cell.

(Fig. 2A), (3) the fraction of neurons in each layer that receive a PSP following a sensory stimulus (functional connectivity) and (4) the fraction of neurons in each layer that emit one or several APs upon a sensory stimulus.

The anatomical connectivity of a network is constant on the time scale of a sensory stimulus. The numbers describing the anatomical connectivity can be derived experimentally from *in vitro* measurements by pair-reconstructions of the projection and the target neuron, respectively (Fig. 2A). Anatomical connectivity can be characterized as the overlap zone between the axon arbors of the projection neuron and the dendritic arbors of their target neurons. This area of overlap is designated as the "innervation domain" (Fig. 2B). From the average axonal length of the projection cells and the average number of boutons per axonal length, the total average number of boutons is estimated. In combination with the average number of synaptic contacts made by a connected pair, the maximal

number of possible synaptic connections that a single projection cell can make with a target cell population is derived (*divergence*).

Taking into account the number of target neurons, the number of projection neurons contacting a single target neuron can also be calculated (*convergence*). Thus, although only pairs of neurons are analyzed, the constraint imposed by the number of pre- and postsynaptic neurons allows the prediction of neuronal convergence and divergence in the wiring diagram of a cortical column.

The number of stimulus-evoked APs in the target neuron population compared to the projection neuron population defines the functional connectivity of the network. Functional connectivity can also be characterized by a "connectivity ratio", which is the number of projection neurons that generate an AP within a given time interval during sensory physiology experiments. The properties that are relevant for describing the functional connectivity can be estimated by combining the anatomical connectivity with *in vivo* PSP and AP measurements from anatomically identified types of neurons.

The exact pattern of APs and PSPs in a column at any point in time then depends critically on the size and time course of the input to the network. In analogy to the kinetics of a multi-step chemical reaction which is driven by an input function (e.g., by a pulse-shaped increase and decrease in the concentration of a ligand), the columnar network is driven by the input pattern of APs that is generated in the thalamus by a sensory stimulus. This pattern impinges then onto the different layers of the cortex and generates a layer and cell-type-specific output AP pattern.

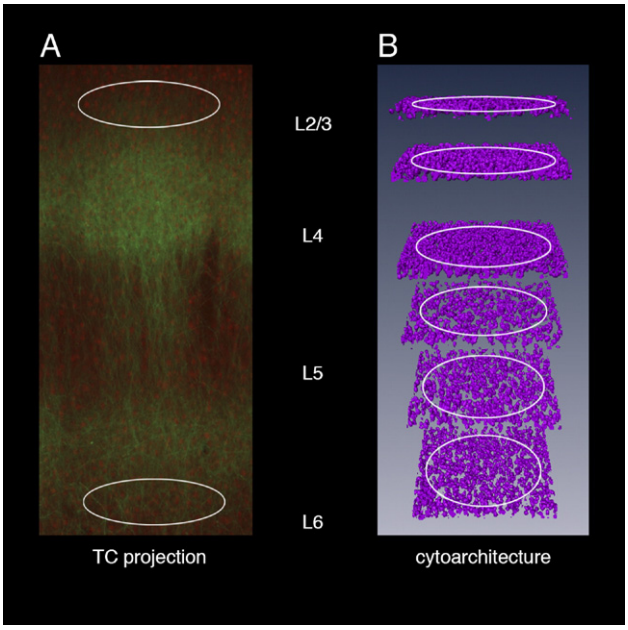

Fig. 3 – Dimension of a column. (A) Dimensions of VPM projections (green) into different cortical layers of a column. Somata of cortical neurons are shown in red. From Wimmer et al. (in preparation). (B) Cytoarchitecture of a column. 3D reconstructions of neuron cell bodies in different layers of the cortex. Optical sections of cell bodies in a column are shown in magenta. Cell bodies were stained with NeuN antibody.

3. Dimensions of a column and of its layers and neuronal types

To quantify the electrical representation of a sensory stimulus in a column, one has to estimate the number of neurons that, on average, constitute a column and determine their distribution in the different layers of a column. For this purpose, the approximate tangential boundaries of a column must be delineated and a cell density profile in the vertical direction of a column must be established. Fig. 3A illustrates the (virtual) vertical and tangential boundaries of a thalamocortical (TC) "innervation-column" in rat somatosensory cortex. Borders are given by the dimensions of the ensemble of TC axon arbors arising from a single barreloid in the ventroposterior medial part of the thalamus (VPM). In the dorsomedial part of the somatosensory cortex of a 4 week old rat, an innervation column has a height of slightly less than 2 mm and a cross-sectional area of about 120,000 μm^2. The definition of the neuronal cell layers of a column relies upon differences in cell density as well as on the shape of cell somata and their dendrites as a function of the distance from the cortical surface. Fig. 3B illustrates the quantification of cell numbers located within the boundaries of a TC innervation column. In L2/3 and L4 of a column, the cell density is approximately constant. About 3000 L4 spiny neurons project to about 3000 L2/3 pyramidal cells, e.g., in the D2-whisker column. In L5, neuron density drops to about one half of that in L4, and in L6 it increases again (V. Wimmer and B.S., unpublished data). In addition, the distribution of cell bodies located in the different layers has to be complemented by determining the geometry and the density of their dendritic and axonal arbors. This can be done for instance by the pair recording and reconstruction approach.

4. Anatomical connectivity

To illustrate the "pair-reconstruction" approach that establishes connection modules between neuronal cell types, we have chosen the interlaminar excitatory connection between L4 and L2/3 (Fig. 4A). Here both anatomical and functional data are available. The predominant cell types are known to be spiny neurons in L4 and pyramidal neurons in L2/3. The anatomical and functional estimates enable one to reconstruct the "average" anatomical and functional connectivity between L4 neurons and L2/3 neurons and eventually simulate the signal flow in an *in silico* model of this module.

The anatomical connectivity or the number of L4 axons converging on L2/3 dendrites is derived from the mean axonal length within the 2D projection of the innervation domain (Fig. 4A, lower panel, yellow area bounded by an 80% contour line) and the number of boutons in the innervation domain of an average axonal arbor of a single projection neuron (Fig. 4A, upper panel inset). In this module, it is estimated that there are about 2000 boutons per presynaptic neuron that mediate interlaminar signaling (Lübke et al., 2003). Anatomical as well as functional analysis suggests that in L4–L2/3 connections, on average, 4.8 synaptic contacts are established per connected cell pair (Silver et al., 2003). Dividing the mean number of boutons of a single cell axonal arbor within the L4–L2/3 innervation domain by the mean number of synaptic contacts in an L4–L2/3 neuronal connection

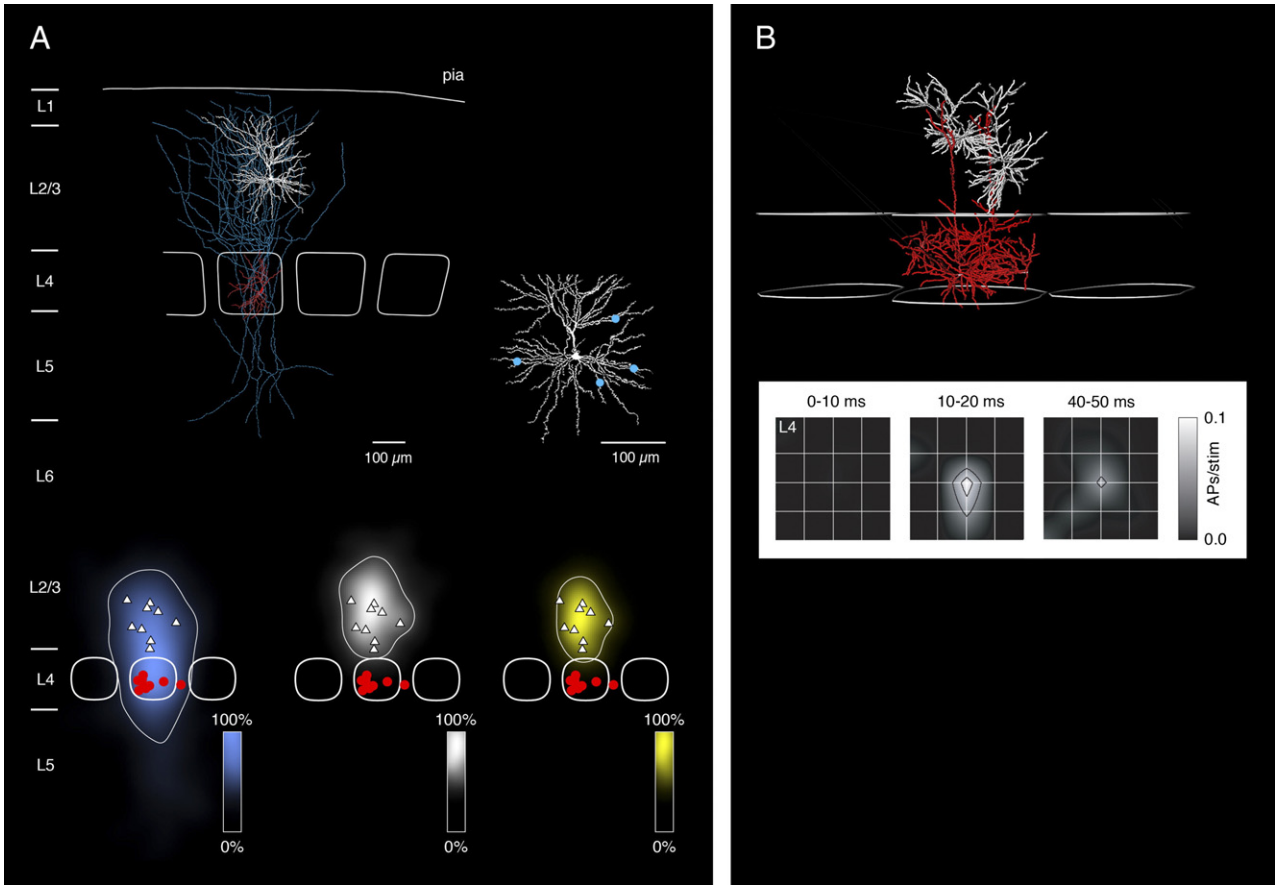

Fig. 4 – Anatomical and functional connectivity. (A, upper panel) L4–L2/3 pair connection. L4 neuron soma and dendrites are shown in red, L4 axons in blue. L2/3 cell dendrites in white. Blow up of L2/3 basal dendrites with synapses indicated in blue are shown in the inset. (A, lower panel) Innervation domain. L4 axon domain in blue, L2/3 dendrite domain in white. L4–L2/3 innervation domain between L4 and L2/3 cells in yellow (from Lübke et al., 2003). (B) Reconstruction of neurons in 3D. Illustrated are dendrite reconstruction of L4 (red) and L2/3 neurons (white). White horizontal structures represent upper and lower borders of three adjacent barrels. Reconstructions made from biocytin filled cells, loaded during *in vivo* recordings. Lower panel shows schematically the size of AP-RFs of L4 neurons as measured by deflection of different whiskers surrounding the principal whisker. Note RF restriction to the principal whisker (from de Kock et al., 2007).

yields an estimate of the anatomical *divergence* of 300/1 (corrected for the assumption of approximately 20% of the boutons innervating interneurons in L2/3). This means that each L4 neuron innervates on average 300 L2/3 pyramidal neurons. In addition, given an estimate of the number of neurons in L4 and L2/3, the *convergence* can also be calculated. If there are approximately 3000 excitatory neurons in L4 that each have about 2000 boutons in the innervation domain, this amounts to approximately 6 million projection boutons in the L4-to-L2/3 innervation domain in a D2 whisker column. If again about 80% of the boutons innervate the estimated 3000 L2/3 pyramidal neurons, we arrive at an estimate of the *convergence* of 6,000,000*0.8/3000/4.8≈300. This means that each pyramidal cell in L2/3 is, on average, innervated by about 300 projection neurons located in L4. For this calculation, we assumed homogeneous populations of L4 neurons and of L2/3 pyramidal neurons. Then, *divergence* and *convergence* are approximately the same in this L4-to-L2/3 connection, because the number of neurons in L4 and in L2/3 of a cortical column is approximately the same (Lübke et al., 2003).

The shape of the boundary of the axonal projection domain (Fig. 4A, lower panel, blue area) indicates that L4 axons are largely "column-restricted", extending mostly into L2/3 and less densely into L5A. The L4 axonal architecture contributes to the column restriction of excitation as seen, e.g., in the receptive field (RF) architecture of L4 and L2/3 cells which is described in the next section.

5. Functional connectivity

In a simplified view, the "rigid" and stereotyped anatomical network is transiently populated by PSPs and APs after a sensory stimulus. On the time scale of a sensory stimulus, the anatomical connectivity of a network is constant. In contrast, the functional connectivity (number of co-active inputs to a single target neuron) of the same network is changing rapidly with time depending on the stream of APs into the input layers of the network. The AP and PSP patterns can be measured in the real

network by *in vivo* experiments from a small number of neurons (Kerr et al., unpublished observations) and then is extrapolated to the actual number of neurons in a column to arrive at average values of PSPs and APs in the column. To relate the *in vivo* measured PSPs and APs (Brecht and Sakmann, 2002; Brecht et al., 2003; Bruno and Sakmann, 2006; de Kock et al., 2007) to the properties of pair modules that were measured *in vitro*, we reconstructed the cells recorded *in vivo* and implemented them in 3D with reference to the barrel coordinates and pia distances (Fig. 4B). These reconstructed neurons can then be classified using similar criteria applied to the neuron reconstructions made from *in vitro* pair experiments. Excitatory neuronal cell types reconstructed from *in vivo* and *in vitro* experiments were found to be comparable. It is clear, however, that the axonal arbors reconstructed from *in vitro* experiments using acute cortical slices are missing large portions when compared to those measured *in vivo*. These deficits can prevent an unequivocal cell identification, especially of non-local projection neurons, based on axonal arbor geometry.

As an example to illustrate the estimation of the functional connectivity from the combined results of *in vitro* and *in vivo* experiments, we focus on the L4-to-L2/3 connection module in the somatosensory cortex. From *in vitro* experiments, we conclude that the average anatomical connectivity in this pathway is about 300/1 and the innervation domain is restricted to the width of the upper third of a column. *In vivo* experiments, recording from L4 and L2/3 neurons of the same type (Fig. 4B), indicate that following a principal whisker (PW) deflection about 10% of all L4 neurons generate an AP on average in the time interval of 10–20 ms (Fig. 4B). This means that out of the 300 projection neurons in L4 that are connected (on average) to a single target pyramidal cell in L2/3, only 30 of them will generate, on average, an AP in response to a whisker deflection. Thus a compound excitatory postsynaptic potential (EPSP) in L2/3 pyramidal cells would be generated by the superposition of ~30 unitary EPSPs.

6. Classes of neuron pair modules in a column

Which pair modules must be analyzed to be able to reconstruct those connections that are most relevant for establishing an average column? A simple way to approach this problem is to first identify experimentally the most frequently occurring types of neurons, then to determine *in vivo* the most active types and measure *in vitro* pair connections between these types of neurons. Pair recording experiments are guided by anatomical evidence of the overlap of axon arbors of projection cell types with the dendritic arbor of target cell types (Binzegger et al., 2004). The different connection pairs within a column can be subdivided into modules of vertical connections between cells in two layers and into modules of horizontal connections between cells located in the same layer. The description of different types of pair connections given below follows the likely activation pattern of cortical neurons when AP activity, evoked by a sensory stimulus, arrives from the thalamus and then activates cortical layers.

Firstly, all layers are excited by direct TC input. Anatomical and functional data suggest that in all cortical layers cells are innervated mono-synaptically by axonal projections from VPM (Fig. 5A) or POm (posterior medial nucleus of the thalamus) (Chmielowska et al., 1989; Lu and Lin, 1993; de Kock et al., 2007). *Secondly*, within the cortex, the densest projections are by the vertically oriented axon arbors between different layers. Here the L4 neurons act as a hub for additional excitation of supra- and

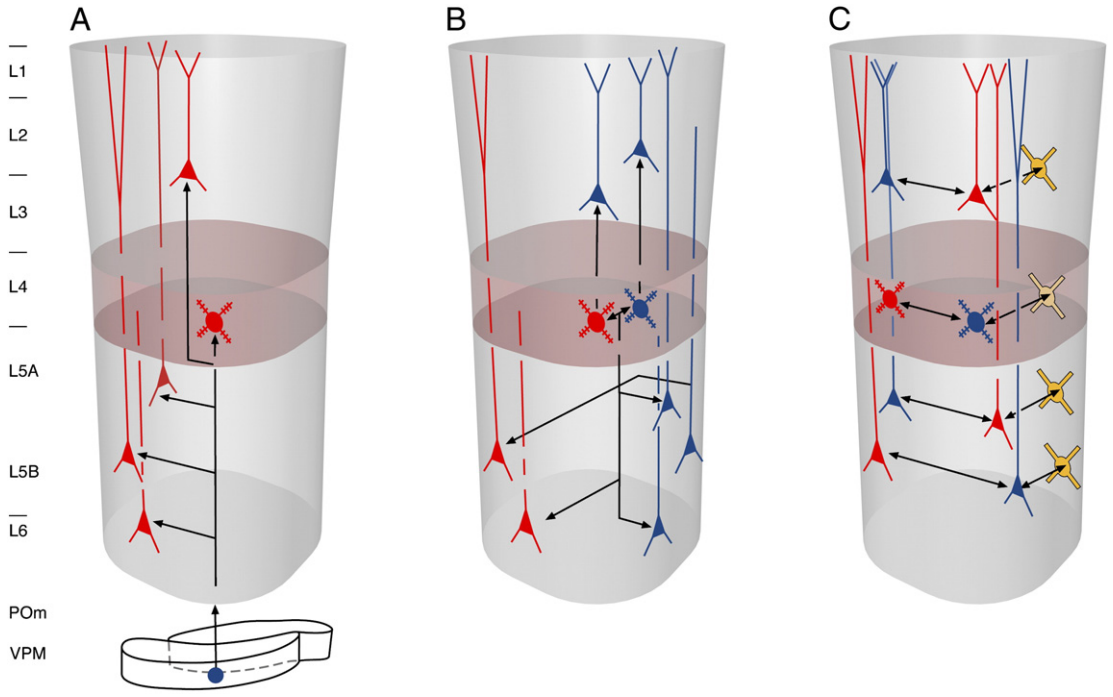

Fig. 5 – Neuron pair modules. (A) Schematic illustration of excitation of neurons in a cortical column by VPM axon APs. (B) L4 activation and interlaminar spread of excitation from L4. (C) Intralaminar excitation and inhibition in each cortical layer.

infragranular layers (Fig. 5B). *Thirdly*, within layers, both excitatory and inhibitory connections exist which are in many cases reciprocal (Fig. 5C) (e.g., Markram et al., 1997; Reyes et al., 1998; Reyes and Sakmann, 1999). *Fourthly*, inter-laminar combinations of excitatory–inhibitory connections exist that are part of a "triple neuron module" (not shown). Here inter-layer *excitation* of inhibitory interneurons located in the target layer is complemented by an intra-layer inhibitory projection with excitatory neurons as targets. Effectively, an excitatory target neuron in this layer (L2/3) receives two inputs from a projection neuron, one *direct* excitatory input and a second *indirect* inhibitory input. Possibly these triple modules shape the AP pattern via a sharply timed sequence of EPSPs followed by inhibitory postsynaptic potential (IPSP) and prevent over-excitation between layers. In addition, inhibitory connections may serve to synchronize excitatory input (e.g., Mishra et al., 2006; for a review see Ritz and Sejnowski, 1997).

7. Cell-type-specific population AP patterns emitted by a column

From the anatomical convergence of the L4-to-L2/3 connection, one can estimate that nearly all neurons in L2/3 will be activated by L4 at the subthreshold EPSP level. However, only a small percentage of cells will generate and emit APs ("sparse" AP population coding). Making the simplifying assumption that the response properties are homogeneous across the relatively small sample of recorded cells (de Kock et al., 2007), one can derive from the value of APs/stimulus/cell the number of active neurons in a layer. Assuming, for example, that spiny neurons in L4 respond with ~0.1 APs/stimulus (Brecht and Sakmann, 2002), one can estimate that ~10% of all L4 neurons are active. This means that during a time interval of 100 ms, only ~300 cells in L4 are active. Using a different recording method, a higher estimate of ~0.4 APs/stimulus/cell was reported recently (de Kock et al., 2007) that would increase the number of active cells in L4 to ~1200. Finally the response magnitude depends on the duration of the integration interval assumed for measuring the response. In the above example, we used a 100 ms window. The frequency of whisking is about 10 Hz, suggesting that activity during a 100 ms time window is also behaviorally relevant, meaning that it is comparable to the integration of sensory input that drives decision making.

7.1. *Estimates of APs emitted by a column*

The behavior in the gap-crossing task is controlled by the asynchronous AP output pattern generated within a column in response to an almost synchronous thalamocortical AP input to the column. A budget of the number of APs exciting a column and of APs emitted by a column can be established by integrating the number of APs emitted from the thalamus and the integrated number of APs emitted from the different layers of a column (de Kock et al., 2007). The estimated number of "input" axons into a column is in the order of 200 TC axons whereas the number of output axons, considering only those of pyramidal neurons in L2/3, L5A and L5B, is about 6000 axons, suggesting a high input–output divergence. The AP activity in the ensemble of input and output axons, respectively, can also be roughly estimated. The number of input APs occurring within a time interval of ~100 ms is about 60 APs (de Kock et al., 2007). A rough estimate of output APs is in the order of about 1800 APs. Thus, as a first-order approximation, a column acts as an amplifier for APs, however, with a relatively low amplification factor (the barreloid consists of ~200 cells and the cortical column of ~12,000 cells). More important is the fact that the patterns of APs emitted by a column are asynchronous in their time structure and in addition the AP output is split into different projection pathways.

To understand the transformation of input to output AP patterns in the future, the neuronal cell-type-specific output from each layer, as determined in individual experiments for single neurons, must be extrapolated to the ensemble of neurons of a particular type in each layer (Fig. 6). This goal requires a detailed measurement of cell density profiles in a column and estimates of the number of excitatory versus inhibitory neuronal cell types in each layer. In addition subtypes of inhibitory neurons are present in each layer that contribute about 10–20% to the total cell number. Finally when examining the layer-specific AP output, one has to take into account cellular mechanisms that are determinants of AP outputs such as coincidence of synaptic inputs from different layers (Larkum et al., 1999a,b).

7.2. *Long-range target cells of a column's output*

The target areas of the long-range columnar output projections are specific for each layer of a column (Alloway et al., 1999; Jenkinson and Glickstein, 2000; Hoover et al., 2003; Hoffer et al., 2005). A clear separation exists between axon projection targets of the L2/3 pyramidal cells on the one hand and pyramidal neurons of the infragranular layers L5A and L5B on the other. Long-range axonal projections of target L2/3 pyramidal neurons in the secondary somatosensory cortex (S2) and in the vibrissal motor cortex (M1) are illustrated schematically in Fig. 7A. Here a topographical relation exists between a column in S1 and a column of the same whisker in M1 (Izraeli and Porter, 1995). In addition, projections to the insular cortex and to S1 of the contralateral hemisphere exist (Fig. 7B). The delayed and variable AP patterns of L2/3 pyramidal cells, when compared to the brisk and less variable response of L5B (de Kock et al., 2007), could indicate, in combination with the projection pattern of L2/3 pyramidal cells, that the AP output from L2/3 modulates whisking, possibly via a direct projection from primary sensory cortex S1 to primary motor cortex M1 and from there to the facial nucleus (Grinevich et al., 2005). The contribution of the L2/3 output to triggering gap-crossing is not very clear at present. It has been shown that an intact L2/3 is required during the learning phase of gap-crossing but not when the task has been learned (Hutson and Masterton, 1986).

The long-range projections of L5A pyramidal cells target predominantly the striatum, a motor control area (Alloway et al., 1999; Hoffer and Alloway, 2001). Here the output from a single column projects to distributed but clustered targets in the striatum. The low AP activity of L5A pyramidal cells in the anaesthetized animal makes it difficult to assign a clear function of this cell class in gap-crossing, at least based on single whisker deflections. Clearly L5A activity is expected to be higher in the awake behaving animal as POm afferents innervate these cells dually and POm activity could be increased in the awake state

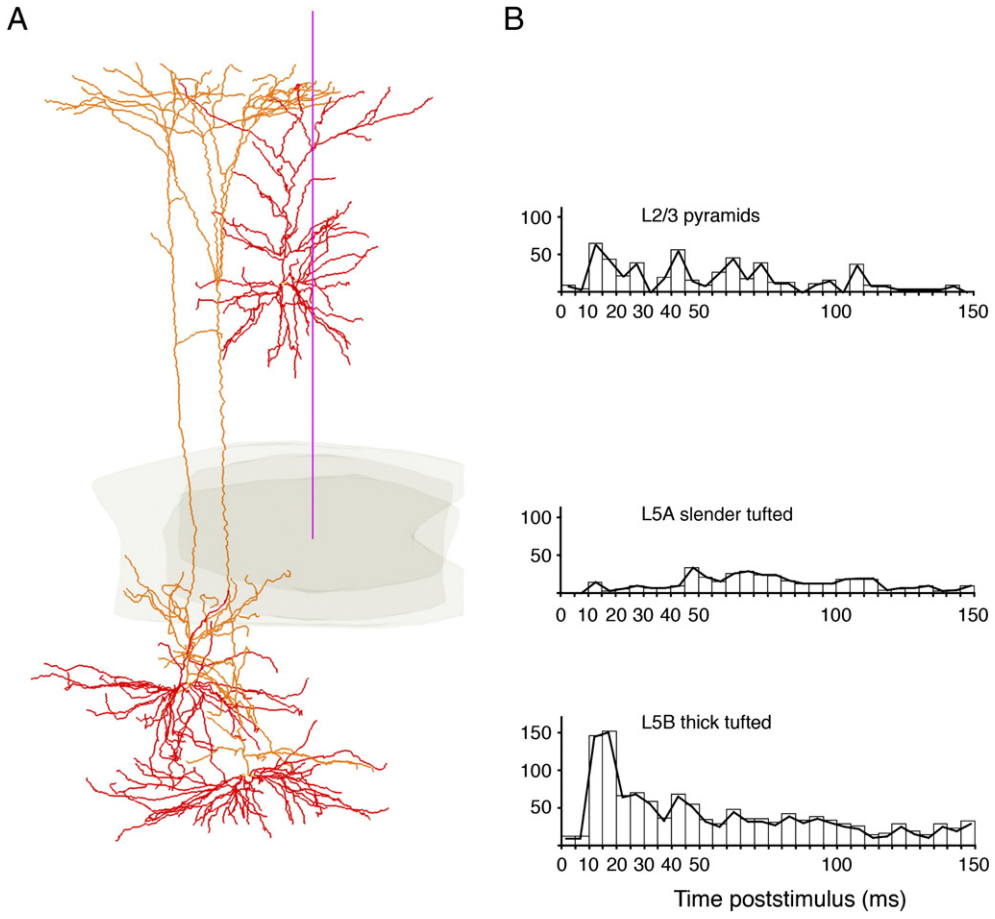

Fig. 6 – Cell-type-specific AP output from a column. (A) 3D reconstruction of main columnar output neurons in L2/3, L5A and L5B. These are L2/3 pyramids, slender tufted and thick tufted pyramidal cells, respectively. Barrel surfaces are indicated in light gray. (B) Post-stimulus time histogram of AP output from 3 classes of output pyramids showing time dispersed (asynchronous) AP output from different neuronal cell types. Ordinate in each histogram represents total number of APs per bin (from de Kock et al., 2007).

(Trageser et al., 2006; Yu et al., 2006). The long-range projections of L5B are densest to the tectum and to the motor nuclei in the spinal cord (Jenkinson and Glickstein, 2000; Leergaard et al., 2006). Eventually, they activate the cerebellum in a distributed fashion (Sharp and Gonzalez, 1985), which exhibits whisker-evoked responses (Chadderton et al., 2004). The corticopontine pathway that is mediating this projection could thus contribute to coordination of limb movements before and during a motor action like a gap-cross jump.

Clearly a major challenge in the future will be to exactly delineate the anatomical connectivity of different cell types in a cortical layer with long-range projections to these subcortical targets and in addition characterize their synaptic transmission using the AP patterns that are characteristic for each type of pyramidal cell.

8. Conclusions

8.1. *Neuron-specific stimulus representation in the somatosensory cortex*

Decision making involves, conceptually, 3 steps—processing of sensory signals, generation of a decision signal and execution of a motor action. The fact that the first step can rely on a single cortical column seems to make it feasible to completely understand the processed sensory signals provided one can reconstruct the cellular anatomy of a cortical column. One first conclusion from combined anatomical and physiological analysis is that the representation of a whisker deflection is highly specific for the individual cortical layers, and in addition specific for individual cell types within a layer. Both the subthreshold (PSP) and suprathreshold (AP) representations are dynamic. Receptive field (RF) size increases and collapses within tens of milliseconds in a layer-specific way, one major difference being the higher reliability of PSP responses. Thus an anatomical column is far from being a functional unit consisting of cells with similar functional properties. The differences in layer and cell type representation, both at the PSP and AP level, may thus be related tentatively to the determinants of simple behaviors. The columnar output that triggers a decision to jump across the gap or not depends on the APs emitted from L2/3, L5A and L5B following a single or a few repetitive whisker deflections. The targets of their long-range projections are located in very different brain regions and layers. On the short time scale of 0.4–2 s during which a decision is made (Celikel and Sakmann, 2007), it seems likely that the strong

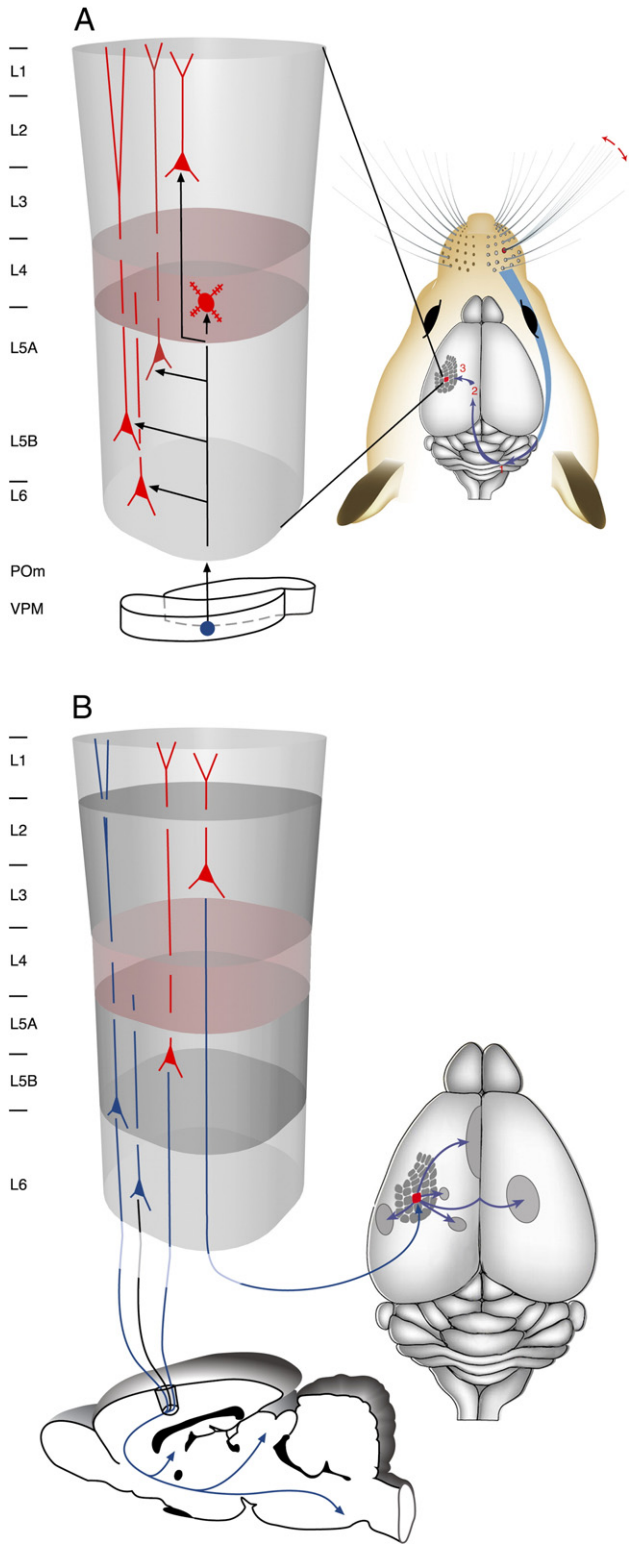

Fig. 7 – Long-range target cells of pyramids driving decision making behavior. (A) Schematic view of TC input projections into a column from VPM. Right: Location of column in somatosensory cortex. (B) Schematic view of long range output projections from a column. Pyramids in L2/3 project to S2 and other cortical areas as indicated. Output from "slender tufted" pyramidal cells in L5A to striatum. Output from "thick tufted" pyramidal cells in L5B to thalamic nuclei, tectum and pons.

and stimulus locked AP output from thick tufted pyramidal cells in L5 is the most important signal stream. The less precisely time locked and less synchronous output of pyramidal cells in L2/3 and slender tufted pyramidal cells in L5 might contribute to the learning of decision making and imprint those local circuits that represent the putative comparator. If this was the case, then the triggering of a decision based on integrated sensory signals is caused by only a few hundreds of APs conveyed from the infragranular layers, presumably to hypothetical "comparator" circuits and eventually to motor cortical areas that control the animals limbs.

Eventually, to delineate the AP pattern emitted by the different layers that triggers a decision, one will have to record from ensembles of anatomically identified cells in the same column during the behavioral tasks.

8.2. *Synthesis of an average functional column*

Independent of the issues concerning various anatomical and electrical substrates triggering decision making, it is essential to derive numbers that quantify how the columnar output AP pattern is generated from the thalamocortical input. This means that one has to find out how the synchronous input AP activity is amplified and desynchronized in a layer-specific or cell-type-specific way. The *in vitro* pair recording and reconstruction approach outlined above has given anatomical clues as to the determinants of column restricted initial excitation and the subsequent spread into neighboring cortical areas (Egger et al., 1999; Feldmeyer et al., 1999, 2002, 2005, 2006; Lübke et al., 2003; Silver et al., 2003). Functionally all connections examined in pair recordings are weak, with small EPSPs in the order of a millivolt or less but reliable (e.g., Silver et al., 2003). On the other hand, the estimated anatomical convergence within and between layers is high (>50). These findings suggest that the size of cortical AP responses observed *in vivo* is strongly dependent on the input synchrony of weak but reliable individual inputs. Possibly a high convergence of reliable, weak individual inputs on target cells is one principle by which ensembles of cortical cells are selectively activated.

8.3. *Plasticity of behavior and its relation to changes of the anatomical or functional connectivity*

For the gap-cross behavior, it seems likely that a detailed understanding of the anatomy and the functional determinants of the VPM-to-L5B thick-tufted pyramidal neuron connections is relevant for linking this behavior to a pattern of AP activity. However, solving of behavioral tasks includes a learning phase and presumably this involves a change in the AP pattern that sweeps through the columnar network and is generated by a sensory stimulus. Alternatively a change in the effectiveness of the AP output pattern in the target neurons may underlie learning. In the case of sensory learning, it raises the question as to what are the differences in PSP and AP patterns in a column. In which layer and in which cell types are they altered between the two behaviors? Further questions are whether changes in AP pattern are generated by anatomical changes in connectivity or do they involve only functional changes in synaptic effectiveness?

9. Outlook

9.1. *Simulation of signal flow in a cortical column in silico*

Once a detailed wiring diagram including synaptic weights for the different connections is available, it will have to be made "live" by simulations using measured population AP patterns. Simulating the electrical signal spread in a column allows calculating, e.g., the predicted AP output of a given neuronal cell type in a given layer. This AP output can be experimentally determined. The simulation itself relies on many parameters of the column model (synaptic parameters, passive and active membrane properties, morphologies, and connectivity assumptions). Thus, by simulations, the parameters of the mechanistic model of a cortical column can be transformed into experimentally accessible parameters (e.g., AP output) for validation.

The APs, for example that are generated in L4 in response to a whisker deflection are, most likely, not perfectly synchronous. Because of the distribution of first AP latencies in L4 (de Kock et al., 2007), one expects that the amplitude of the compound EPSP in L2/3 is smaller than the value calculated on the basis of the functional connectivity that assumes perfectly synchronous activation of all presynaptic neurons. Experimentally, the evoked EPSPs are, on average smaller (~10 mV; Brecht et al., 2003) than the calculated value of >30 mV. In fact the PSPs in L2/3 pyramidal neurons fluctuate in amplitude between trials and the time of occurrence of APs is also fluctuating widely.

The fact that the functional connectivity between two layers in the cortex depends strongly on the population synchrony of the AP inputs from the projection layer means that a realistic description of average pair modules and columnar networks must take into account measured population synchrony. Population synchrony with sparsely firing neurons, however, means coincidence of single APs in time windows of 1–10 ms. Population synchrony could be "simulated" by modeling the target cells' PSP–AP responses by using the *in vitro* measured properties of PSPs in conjunction with the passive and active electrical properties of target cells. Using the distribution of *in vivo* measured AP probabilities of the projection cell layer (L4), the L2/3 cell activation was simulated based on realistic assumptions (Sarid et al., unpublished observations). Such detailed simulations of modules reveal that the AP response in the projection cell population is indeed fluctuating between different stimulations (trial-to-trial variability; Sarid et al., unpublished observations). Here the fluctuations are due to both population synchrony and intrinsic membrane properties. Thus the strength of population synchrony of APs in ensembles of projecting cells (L4 in the above example) is a further determinant of the functional connectivity as it has been demonstrated for TC activation (Bruno and Sakmann, 2006).

At present the effect of population synchrony on functional connectivity can only be estimated by simulations or by simultaneous recordings from small cell groups because of the lack of methods to measure the AP activity in large (>100 cells) ensembles of morphologically identified projection cells in different layers. However, in supragranular cortical layers like L2/3 the number of active neurons and their stimulus-evoked synchrony can be measured directly *in vivo* using new optical methods (Kerr et al., unpublished observations). These AP measurements as well as measurements with voltage sensitive dyes that report an estimate of PSP activity are providing a tool for the "validation" of modeling *in silico*.

9.2. *Large-scale anatomy at high resolution*

The single whisker-guided gap-cross behavior seems simple enough to eventually obtain a time resolved description of the AP input into the cortex and of the AP output from the cortex that drives this behavior. The challenge is to rationalize the transformation of an almost synchronous input AP pattern of thalamocortical afferents into the time dispersed and spatially distributed output AP pattern. The essential requirement for this goal and similar attempts in other areas of the cortex to understand the basis of behavior is a detailed anatomical description of ensembles of connected cells. Electrical recordings from single or multiple neurons without anatomical identification of recorded cells are of limited value for identifying behaviorally relevant cortical circuits. We have outlined the value of making recordings for anatomically identified neurons combined with the pair reconstruction approach to construct an entire "average column" *in silico*. It may reproduce salient properties of a real cortical column. Eventually this effort will have to be complemented by a complete anatomical reconstruction of an entire columnar network by serial EM (Denk and Horstmann, 2004; Briggman and Denk, 2006) and its representation as a real column *in silico*. This latter approach will also allow the determination of higher-order connectivity patterns among ensembles of neurons and the identification of specific anatomical wiring patterns that are lost in the average column.

Finally anatomical details of long-range connections, in the case of a whisker column, the axonal projections to the other cortical areas, the striatum, tectum and pons will have to be described for identified neuronal cell types that were reconstructed following pair recordings.

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