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Specificity and randomness in the visual cortex

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Summary

Research on the functional anatomy of visual cortical circuit has recently zoomed in from the macroscopic level to the microscopic. High-resolution functional imaging has revealed that the functional architecture of orientation maps in higher mammals is built with single-cell precision. In contrast, orientation selectivity in rodents is dispersed on visual cortex in a salt-and-pepper fashion, despite highly tuned visual responses. Recent studies of synaptic physiology indicate that there are disjoint subnetworks of interconnected cells in the rodent visual cortex. These intermingled subnetworks, described *in vitro*, may relate to the intermingled ensembles of cells tuned to different orientations, described *in vivo*. This hypothesis may soon be tested with new anatomic techniques that promise to reveal detailed wiring diagrams in cortical circuits.

Introduction

Over the past 50 years, the visual cortex has served as a model system for the study of cerebral cortical circuits. Several themes have dominated this literature: hierarchical vs. recurrent processing, specific vs. random synaptic connectivity, and functional architecture vs. intermingling of response types. A classical view of visual cortical processing [1] has concentrated on one side of each of these dichotomies: that the visual cortex is best understood as a hierarchical system, whose receptive fields are created through specific connections, within a framework of crystalline functional architecture. At various times, however, each of these views has been called into question.

Orientation selectivity and hierarchy

The hierarchical model of visual processing was proposed at the beginning of modern studies of the visual cortex, when it was proposed that selectivity for stimulus orientation emerges from the specific connections from thalamus to cortical simple cells [2]. In the cat visual cortex, there is evidence that multiple thalamic afferents, each of which is substantially unoriented, add together to produce a strongly oriented afferent input to simple cells in layer 4 [3,4]. This evidence comes from cross-correlation studies [5] as well as studies of orientation selectivity of thalamo-recipient cells when the intracortical circuit has been silenced [6–8].

While an increasing body of evidence has shown that thalamo-cortical connections are related to the establishment of orientation selectivity, the role of intracortical connections has been

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much more difficult to study. Cross-correlation techniques have shown that simple cells in layer 4 connect to iso-orientation complex cells [9], but the detailed logic of these connections is not clear. There is new evidence that simple cells predominate in layer 4 and complex cells in other cortical layers [10], but the relative importance of feedforward, recurrent and feedback connections in the cortical circuit is still vigorously debated. Without newer tools to study the relationship between intracortical synaptic connections and visual response properties, however, it will be difficult to resolve these issues.

While there is strong physiological evidence for the role of thalamo-cortical input in orientation selectivity, a more direct anatomical proof has been elusive. In some species, however, the main thalamo-recipient cells in layer 4 are not orientation selective, but cells in the next stage - layer 2/3 are orientation selective. Their orientation selectivity must develop through intracortical connections from layer 4 to layer 2/3. In an elegant recent study of one such species, the tree shrew, an elongated topographic organization of connections from layer 4 to layer 2/3 correlated well with the emergence of orientation specificity [11].

Coarse and fine-grained specificity of intracortical connections

Since the Hubel and Wiesel's model was first put forth, the specificity of the anatomical connections has been studied at increasingly fine levels of detail: from areal maps, to functional or laminar architecture, fine-scale geometry, and finally individual synaptic connections. At the coarsest levels are cortical regions, which often have large-scale maps, such as the retinotopy of the visual cortex. At the next level is functional architecture, in which different features of a cortical computation can be segregated at a scale of tens to hundreds of microns [1]. The afferent connections to cortex often respects these functional boundaries, such as in the ocular dominance columns in the input layers of primate visual cortex [12], or in the cortico-cortical projections between different functional compartments [13,14]. Many studies have also demonstrated specificity in intracortical connections, either between cortical layers [15, 16] or in long-range connections within layers [17,18].

The next finer scale has been termed the *geometrical* level: at which the close proximity of axons and dendrites are considered on the scale of ~0.5–2 μm [19,20*,21]. At this level and the next—that of actual synaptic connections—the debate about specificity has been couched in terms of *Peters' rule* [19,22,23], that is, axons make connections randomly in direct proportion to the occurrence of all synaptic targets in the adjacent neuropil, with no local specificity.

Recently, several fine-scale geometrical analyses have been performed for cortical circuits. For pyramidal neurons in somatosensory cortex, at least one direct apposition of axons and dendrites (regardless of the existence of a synapse) was observed for every pair of pyramidal neurons sharing the same cortical column within 300 μm [20*,21]. This supports Peters' rule for the geometrical contact between axons and dendrites. But this level can only reveal potential connectivity: actual synaptic boutons were found in only a fraction of these potential connectivity. The number of synaptic boutons was correlated to the synaptic responses of pairs of neurons [20*].

A version of Peters' rule was tested directly by comparing geometrical and functional connectivity [24*] in barrel cortex. Functional connectivity could not be predicted solely from the overlap of dendrites and axonal arbors, but much of the variations could be explained when other factors were considered. The probability of finding synaptic connections depended on the class and laminar location of each neuron. Further, synaptic connectivity also depended on a neuron's location in the cortical map, *i.e.* whether it was in a barrel or a septum.

Physiological studies of synaptic connectivity have also shown that neurons are not connected randomly to potential targets. Dual intracellular recording studies in slices have shown that neurons are synaptically connected to only a small fraction of their neighbors, and such synaptic connections are specific to cell types [25–29]. Even within a single cell type, pyramidal cells, a recent statistical analysis of simultaneous recordings has shown that connections are far more clustered than in a random network [30].

Two recent studies of rodent visual cortex have revealed the existence of spatially overlapping but distinct subnetworks of cells. In one study of layer 2/3 pyramidal neurons [31*], lateral connections between cells were frequently found when they received common inputs from layer 4 (Figure 1c). When they did not receive common inputs, even adjacent neurons were rarely connected to each other. The existence of subnetworks was reinforced with a subsequent study of two classes of layer 2/3 inhibitory interneurons: fast-spiking and adapting [32]. Pairs of pyramidal cells and fast-spiking (FS) interneurons are likely to be reciprocally connected only when they share common input. Fast-spiking cells are thus specifically connected with pyramidal cell subnetworks. In contrast, connections from adapting interneurons were not specific. The functional role of these cortical subnetworks is not immediately apparent, but new imaging techniques hold the promise of relating network connectivity and function.

Cellular imaging *in vivo*: the fine scale architecture of visual processing

Early extracellular recordings of single cells in visual cortex have shown that neighboring neurons often exhibit similar response properties, such as ocular dominance and orientation selectivity. Functional groups are arranged in columns, as demonstrated by penetrations made normal to the cortical surface. Tangential penetrations, on the other hand, show regular variations and predictable shifts, suggestive of systematic maps [2]. The orderly progression of receptive-field types across the cortical surface along with the similarity within columns is known as the functional architecture of visual cortex. Optical imaging revolutionized the study of functional architecture by showing the overall geometry of functional maps [33,34]. The spatial resolution of conventional optical imaging (> 100μm) is well matched to most features of cortical maps, but it is inadequate to examine the fine-scale features, such as orientation pinwheels and direction fractures [35].

In vivo two-photon calcium imaging of population of neurons is a new technique [36] for single-cell resolution functional imaging. *In vivo* calcium imaging in the cerebral cortex had only been achieved by intracellular labeling of single neurons [37], until a method for simultaneously loading many neurons with a cell-permeant (AM-ester) form of calcium indicators was developed recently [36]. Using this method, it has become possible to map the response selectivity of all neurons in a local cortical circuit (300–600 μm in diameter) at single cell resolution [38**]. This technique is unique in its ability to determine not only the physiological response of hundreds of cells simultaneously but also their precise location in the cortical circuit.

Sharp functional borders

Sharp transitions across functional borders have been observed from the outset of the discovery of functional columns by Mountcastle and colleagues [39]. More extensive studies revealed honeycomb-like structures of discrete functional columns in the somatosensory cortex [40]. In the visual cortex, preferred orientation changes smoothly, except for occasional abrupt discontinuities in the sequence [41].

Two-photon calcium imaging has recently demonstrated the extraordinary precision of the functional architecture of orientation and direction selectivity in cat visual cortex [38**,42]. In area 18 of cat visual cortex, iso-orientation domains were known to contain subregions with

opposite preferred directions [43]. Two-photon calcium imaging demonstrated that neurons with opposite preferences for stimulus direction were segregated by remarkably precise "direction fractures", with columnar borders one to two cells wide [38**].

Another singularity in the cortical map is an orientation pinwheel center [33,34]. Conventional optical imaging first demonstrated these pinwheels, but the technique lacked the spatial resolution to determine the response properties and arrangement of cells near pinwheel centers. Electrophysiological recordings later demonstrated sharply selective neurons near pinwheel centers [44,45], but it remained unclear whether they were arranged randomly or in an orderly fashion. Two-photon calcium imaging revealed that pinwheel centers are highly ordered: neurons selective to different orientations are clearly segregated even in the very center of pinwheels [42] (Figure 1a). Thus, pinwheel centers truly represent singularities in the cortical map.

The finding of sharp discontinuities in cortical maps raises more questions than answers. The discontinuities are more precise than the spatial scale of the dendritic trees of cortical neurons, so one can ask: what are the mechanisms underlying this precision? Several possibilities are (1) that selective connections between individual neurons with similar tuning dictate the receptive-field properties on either side of the border, (2) that smaller anatomical features, such as bundles of apical dendrites [46,47] or inputs to proximal dendrites, are functionally important, or (3) nonlinear input–output transformation, such as the threshold for spike generation, could explain the sharp tuning of neurons around functional discontinuities [45, 48]. Single-cell calcium imaging combined with other techniques might distinguish these different possibilities. For example, relation between anatomy and physiology could be obtained by labeling cells to reveal their transmitters or other molecular markers, their projection patterns, or their detailed dendritic and axonal morphology.

How do functional features such as directional fractures develop?

Orientation maps exist at the time of eye opening [49], but direction maps appear several days after eye opening, and do not develop in animals deprived of visual experience [50]. The emergence of the directional map is experience-dependent and selective to experience. In a recent study, visually naïve ferrets were exposed to bars of a single orientation moving in two opposite directions, and direction selective patches were induced very rapidly, after 12–18 hrs of visual experience (Li et al., abstract in Soc Neurosci Abstr 2006, 619.6). It would be very interesting to observe the rapid plasticity of direction fractures at a single-cell level.

Response selectivity without a functional map

We saw that orientation maps in higher mammals are crystalline and organized at the level of single cell, but what is the significance of these maps? Hubel and Wiesel [51] proposed that:

> It seems reasonable to suppose that the closer cells are in a nervous structure the better their chances will be of having interconnexions or of sharing connexions: there is at least a certain economy in having cells that share connexions close to one another.

But the functional architecture of the cortex is not always necessary to obtain highly selective response [38**,52*,53] (reviewed in [54*]). In visual cortex of mice [55–57] and rats [58–60], no evidence has been found for orientation maps, although neurons are sharply tuned to orientation. Two-photon calcium imaging confirmed that, in the rat, a mixed salt-and-pepper organization can still yield strong stimulus selectivity [38**] (Figure 1b). Similar salt-and-pepper organization was found in mouse visual cortex [61,62*] (Mrsic-Flogel et al., abstract in Soc Neurosci Abstr 2005, 742.6).

The mixed, salt-and-pepper arrangement of preferred orientation in the rodent [38**] argues for specific connectivity between neurons. Orientation tuning in any given neuron cannot be achieved by simply 'going along for the ride' within a neighborhood of similarly tuned cells. Strong orientation tuning in the rodent is likely instead the result of specific mechanisms, such as selective connections amongst cells with similar response properties, which may form the subnetworks of neurons [31*].

But why do smooth orientation maps not exist in the visual cortex of rodents? It could be because they have too small area of visual cortex to have maps, or because they do not have good visual acuity. However, the gray squirrel, a rodent with a relatively large visual cortex (larger than tree shrew; comparable with ferret) and good visual acuity (comparable with tree shrew), does not have orientation maps [52*,63].

This may be explained by differences in intracortical circuits and wiring length minimization [64]. As might be expected from the above quotation from Hubel and Wiesel [51], the existence of functional architecture in cat visual cortex may be explained by wiring length minimization. But recent theoretical studies have suggested that the absence of functional architecture in rodent visual cortex could be more efficient for a specific class of functional wiring diagrams of a cortical circuit [64]. We currently do not know the difference in the wiring diagrams of rodent and cat visual cortex. Such local connectivity can be studied only when microscopic functional imaging is combined with higher-resolution anatomical techniques that elucidate neuronal morphology and even individual synaptic connections.

New techniques for mapping connections in cortical microcircuits

While the new results in two-photon functional imaging raise many questions, many of these questions might be addressed by new anatomical and physiological techniques for analyzing neural circuits. At one extreme, automated methods in serial-section electron microscopy [65] have renewed hope in some day making large-scale wiring diagrams of cortical circuits. In the shorter term, the ability to trace subnetworks of neurons with viruses is being constantly refined [66–68]. Most notably, a newly modified rabies virus has been demonstrated to label specifically all of the neurons that provide monosynaptic input to a single target neuron [69**]. Combining single-cell resolution functional mapping with these anatomical techniques will give increasingly complete functional and anatomical picture of cortical circuitry.

The evolution of genetic tools in mice has fostered an unprecedented opportunity to study neurons in living animals. The ability to stably label specific subsets of neurons with genetically encoded reporters allows the dissection of cell type-specific function in the dauntingly complex cortical circuit. Several groups have generated mouse lines with subsets of neurons labeled with fluorescent proteins using various promoters [70–74]. By combining power of two-photon-calcium imaging and the cell type-specific labeling afforded by mouse genetics, the complexity of the cortical network can be studied by examining cell type-specific function in intact cortical circuits [65*]. This approach can lead to a more complete understanding of sensory physiology of interneurons and their role in cortical information processing.

Finally, there is an increasingly rich arsenal of techniques for influencing the activity of cortical circuit with cellular resolution [75,76,77*]. Cell-type specific suppression [78] may reveal the function of each cell-type in cortical processing. Optical control of neural activity with genetically introduced light-gated channels, such as channelrhodopsin-2 and halorhodopsin [79**,80*,81*] is opening up a way to manipulate neural activity at millisecond resolution. These techniques in combination with calcium imaging should soon allow us to turn on and off individual neurons while observing circuit activity in response to sensory stimulation.

Conclusion

There have been considerable debates on the specificity and randomness of the connections in the cerebral cortex. Recently, cellular-resolution imaging of cortical circuits *in vivo* has started to reveal the functional micro-organization of visual cortex. Results in rodent visual cortex, which displays highly selective visual responses without functional architecture, are consistent with specific connections among subnetworks of cells. Conversely, an increasing number of *in vitro* studies have revealed specific connections and subnetworks, but do not reveal their function. Over the coming years, the relationship between functional micro-organization and fine-scale specific connections should be revealed by combination of functional imaging and new anatomical techniques.

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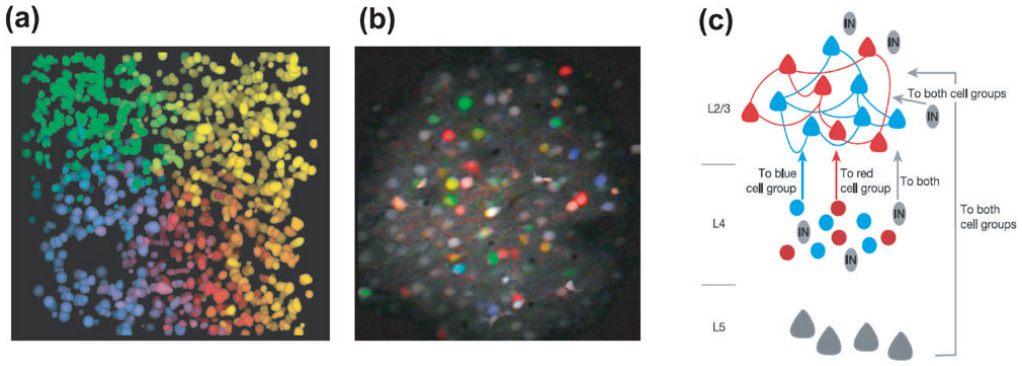

Figure 1.

Single-cell resolution orientation maps from **(a)** a pinwheel in cat visual cortex [42], and **(b)** rat visual cortex [38**]. Cells are colored according to their preferred orientation. In **(a)**, ~1,000 cells from nine different depths are overlaid. Cells are arranged up to the very center of the pinwheel. In **(b)**, cells in one depth are displayed. Even neighboring cells are tuned to different orientations. **(c)** In rat visual cortex, relatively independent subnetworks are embedded in larger-scale functional architecture [31*]. Excitatory connections from layer 4 to layer 2/3 and within layer 2/3 define subnetworks of selectively interconnected neurons (red or blue). The excitation from layer 5 (gray triangles) and inhibition from layers 2/3 and 4 adaptive interneurons (IN, gray ovals) does not respect the subnetworks.