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Cortical Microcircuits: Diverse or Canonical?

Meeting Report

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All regional anatomical explorations implicate this postulate: a common functional identity [is determined by] the same type of structure and connections, whatever the mammal examined.

—Cajal, 1922

In spite of the very persistent labor of so many investigators and despite analytical methods newly introduced into science, the problems awaiting solution, with regard to the structure and function of the cerebral cortex, are as numerous as they are transcendental.

—Cajal, 1892

A century and a decade after Cajal wrote these words, many of the enduring questions of cortical neurobiology that he helped identify remain unanswered. One such question is the degree to which computations in different cortical regions of different species can be encapsulated in a single canonical microcircuit: a kind of basic wiring diagram which, although embellished, remains fundamentally unaltered from mouse to man and across all cortical regions.

Hoping to answer or at least to argue about this question, 48 anatomists, physiologists, theorists, and molecular biologists working on the mammalian cerebral cortex met this past June in Madrid, the city in which Cajal worked for much of his professional life and was buried and which still contains an Institute named for him, for a workshop on the structure of the cortical microcircuit. The 23 speakers ushered participants through a dense thicket of data obtained with a dizzying array of classical and cutting-edge techniques. In an effort to keep sight of the forest amongst so many beautiful trees, I have tried to organize this report around three themes that ran through many of the presentations.

Circuitry: Precision versus Randomness

Clarification of the mode of connection between the innumerable endogenous and exogenous [elements], terminal and collateral branches arising from the thalamic, callosal, and association fibers at present constitutes an insuperable problem. In it many generations of future neurologists will put their sagacity and their patience to the test.

—Cajal, 1933

How precise are the connections between one cortical neuron and another? Does pyramidal neuron number 62,484 know that it should connect to pyramidal neuron number 118,651? Or do they connect only if they encounter each other by chance? Kevan Martin argued for randomness. His talk was scheduled to open the meeting, a fitting choice because his 1989 paper with Rodney Douglas (Douglas et al., 1989) not only introduced the term *canonical microcircuit* into the cortical literature (see Figure 1) but was also the first to explicitly model this circuit on a computer. Unfortunately, owing to a random computer problem (or perhaps to a precisely orchestrated conspiracy, we will never know), the talk was postponed and wound up close to the end of the meeting. Martin presented anatomical analyses of cortical axons in cat visual cortex. There was clear cell-type specificity in the distributions of distances between synaptic boutons, the thickness of axons and boutons, the size and nature of the postsynaptic targets, and the ratio of terminal to en passant boutons. However, Martin emphasized the essential randomness of the distribution of distances separating boutons within each class of axons. He concluded, as had Braitenberg and colleagues from an analysis of rodent cortical axons (Braitenberg and Schüz, 1998), that connections may be specific with respect to cell classes but are likely to be highly random with respect to individual neurons within a class.

Arguing eloquently on the side of precision, Rafael Yuste presented data suggesting that not only are the cell classes targeted by cortical axons highly specific, but that at least in some cases even the relative position of the target neurons are precisely specified (see Figure 2). This is the kind of precision found in many invertebrate ganglia but which runs counter to popular intuition about cortical circuits. Yuste and colleagues used a novel optical probing technique to identify postsynaptic targets of thick-tufted layer 5 neurons in mouse visual cortex. A spot of laser light was used to repeatedly uncage glutamate near the presynaptic neurons, causing them to fire. The firing of postsynaptic targets was then observed using calcium imaging after bulk loading of many neurons within the slice with the calcium indicator fura AM. The method identified a specific class of dangling pyramidal neurons with basal dendrites extending into layer 6. Surprisingly, the tangential position defined by the angle between the somas of the presynaptic and follower neurons was nonrandom. In addition, these connections were mediated by multiple synapses from a single axon that tended to occur at roughly the same distance along different dendrites of the follower neuron. Yuste likened the precision of this structure to that of a crystal, although he admitted that this static view must be amended to encompass the dynamics of cortical activity (see below).

The theme of specificity of connections was echoed in many of the talks. Alex Thomson described paired recordings from cat visual cortex which revealed that interlaminar connectivity is often not reciprocal: layer 4B spiny stellates and pyramidal neurons project to layer 2/3 pyramids but do not receive excitation from them,

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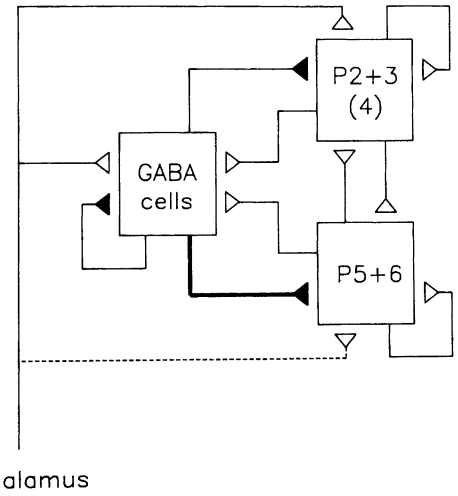

Figure 1. The Canonical Cortical Microcircuit, circa 1989

Three functional classes of cortical neurons are distinguished: superficial pyramidal cells (P2+3; including also layer 4 spiny stellates), deep layer pyramidal neurons (P5+6), and GABAergic neurons. Figure supplied by Kevan Martin.

even though the apical dendrites of the layer 4 pyramidals pass through layer 2/3 near synapses made by layer 2/3 axons with other targets; similarly, layer 3 pyramids connect to layer 5 but not vice versa. The rules differ for inhibitory connections and even among specific subtypes of inhibitory connections.

Ed Callaway described photostimulation experiments demonstrating cell-type specific connectivity. The approach taken was complementary to that taken by Yuste's group. Instead of photostimulating a single presynaptic target and probing for many candidate postsynaptic targets, whole-cell recording was used to monitor a single postsynaptic neuron while probing many candidate presynaptic neurons. The approach sacrifices the temporal and spatial precision obtained from paired recording experiments in which only a single presynaptic neuron is activated but reveals a much more complete picture of the laminar and tangential distribution of presynaptic partners. Using this technique, Dantzker and Callaway (2000) found that different morphological classes of rat visual cortical neurons within layer 2/3 have identifiable patterns of input. Pyramidal neurons and one type of inhibitory neuron, the fast-spiking basket cell, received strong feed-forward input from layer 4B. A second physiological class of GABAergic neurons, those exhibiting spike frequency adaptation, received most of their input from layers 5 and 6 or from lateral connections within layer 2/3.

Cortical GABAergic interneurons are also coupled by electrical synapses. The functional role of these gap junctions was discussed in three separate presentations by Shaul Hestrin, Hannah Monyer, and Peter Jonas (see below). Importantly, coupling is cell-type specific; it is frequent between neurons of the same physiological class but is absent between neurons of different classes.

The question of specificity of connections can also be framed at a more macroscopic and functional level. For example, how specific are intracortical connections

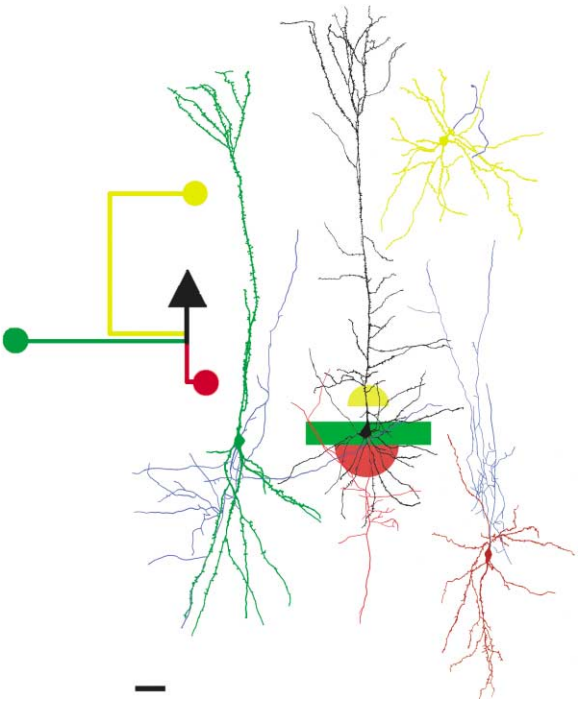

Figure 2. Specificity of Layer V Subcircuits

A layer V corticotectal pyramidal "trigger" neuron (soma and dendrites in black; axon in red) surrounded by examples of three stereotyped classes of postsynaptic "followers" (axons in blue) identified by optical probing: large triangular interneuron (yellow), fusiform interneuron (red), and "dangling" pyramidal neuron (green). Somata of neurons from each class were found in stereotyped regions surrounding the trigger neurons. These regions are depicted as color-coded shapes surrounding the corticotectal soma. Each shape contained all somata of the corresponding follower class, with the exception of a single pyramidal follower, which was offset medially from the trigger due to a distortion in the cortical plane within this slice. Scale bar, 50 μm. These results indicate that a circuit diagram (upper left) specifying neuronal classes (indicated by color) and soma positions (circles) will emerge from extensive studies of the cortical microcircuit. Figure supplied by James Kozloski and Rafael Yuste.

with respect to physiological properties like orientation and direction selectivity? Charles Gilbert, who with Torsten Wiesel and their colleagues did pioneering work on this question in cat and monkey visual cortex, described recent experiments revealing that the orientation specificity of connections may vary with distance. Local connections (within 750 μ of the injection site) tended to occur more randomly with respect to orientation, whereas more distant connections were restricted to orientation domains matching that of the injected region. These long-range connections link cells over separations within the visual field that match the maximum separations over which perceptual interactions between oriented stimuli can occur. Interestingly, feedback projections from V2 exhibited less periodicity and were not correlated with orientation. This last point could imply that feedback connections from higher cortical regions may be organized more diffusely than local connections, consistent with a more contextual role.

Questioning this anatomical observation, Jenny Lund reported that in using biotinylated dextran amine as an

orthograde tracer or cholera toxin B as a combined orthograde/retrograde tracer (Angelucci et al., 2002a, 2002b), much more periodic labeling of feedback connections had been observed, consistent with a high degree of functional specificity (e.g., orientation specificity). She, like Gilbert, attempted to directly relate patterns of local cortical connections to contextual effects in the visual cortex. Lund focused, however, on the observation that these contextual effects are contrast dependent. At high contrasts, surrounding stimuli have a primarily inhibitory effect, and the central receptive field is small. At low contrasts, surrounding stimuli are primarily excitatory, in effect causing the RF to grow in size. The smaller RF observed at high contrast matches the spread of feed-forward inputs, whereas the larger RF observed at low contrast matches the spread of horizontal connections (Levitt and Lund, 2002; Angelucci et al., 2002b). Long-range horizontal connections and feedback axons can provide monosynaptic input to other pyramidal neurons and, by activating local basket cells or other classes of inhibitory interneurons, also provide disynaptic inhibition. Contrast may alter the balance between these sources of excitation and inhibition. This view emphasizes the importance of local inhibitory circuits, particularly those involving basket cells, in regulating the sign and degree of interaction between neighboring cortical columns (Lund et al., 2002).

The relative roles played by inhibition and excitation in generating cortical orientation selectivity have been intensively debated in recent years (for reviews, see Sompolinsky and Shapley, 1997; Ferster and Miller, 2000). Yves Frégnac described recent experiments that suggest that the inhibitory circuits that generate orientation selectivity in the primary visual cortex of the cat may be far more diverse than previously thought. Prior intracellular studies have typically observed iso-oriented excitation and inhibition: excitation and inhibition that are strongest at the cell's preferred orientation and weakest at the nonpreferred orientation. Frégnac and colleagues assessed the relative tuning of excitatory and inhibitory synaptic input and found a diversity of relative tuning. Some cells had the previously described iso-oriented tuning, whereas other cells exhibited iso-oriented excitation but had inhibition that appeared strongest at the orthogonal or "cross" orientation. Several prior intracellular studies have focused on layer 4 simple cells, because it is these cells that can best be viewed as the input neurons of the cortex. In the more recent experiments, cells were recorded throughout the depth of the cortex, and the cell type and laminar position were not known. This suggests the possibility that circuits generating orientation selectivity may differ depending on the location of the target cell in the orientation/direction map (see Figure 3) and/or from layer to layer, a conclusion also reached in a recent paper by Luis Martinez, also in attendance, and colleagues (Martinez et al., 2002).

Taxonomy: Lumpers versus Splitters

…with regard to structural concepts of the cerebral cortex, there are two trends: the ***unitary*** *supported by many who defend the structural unity of the gyri and explain the functional diversity [of the cortex] by the different peripheral connections of the cerebral cells; and the* ***particularist*** *(dualist or pluralist) which claims that the special function of each cortical area implicates both a structural specialization and a diversity of connections of the cortical cells.*

—Cajal, 1899

Heterogeneity at the functional level and a high degree of specificity at the microcircuit level raises the question of just how many classes of cortical neurons exist. Systems neuroscientists recording extracellularly from cortical neurons in intact animals have typically distinguished only a few classes of cortical neurons within a particular region, and such distinctions have been made largely on the basis of differences in receptive field properties: for example, simple cells versus complex cells in cat primary visual cortex or pattern motion- versus component motion-sensitive cells in macaque MT. Anatomists and cellular neurophysiologists have arrived at different sets of categories on the basis of morphology, firing properties, and the expression of various markers such as peptides and calcium binding proteins. As increasing numbers of anatomical markers and physiological properties are studied, the potential number of cell classes has exploded. The problem is especially intense for the GABAergic interneurons. The taxonomy of these neurons has been the subject of recent scrutiny in the neocortex (Cauli et al., 2000) and hippocampus (Parra et al., 1998).

Yasuo Kawaguchi, who in earlier work systematically described several of the physiological classes of cortical interneurons now recognized, described very detailed and comprehensive studies in which he asked: Do classes of interneurons defined on morphological grounds match up with those defined on physiological and immunocytochemical grounds? The morphological parameters were subjected to a principal component analysis (PCA), which revealed clusters which largely matched the groupings of cells identified by their firing pattern. These parameters were also well correlated with the pattern of expression of calcium binding proteins and peptides, although the match-up was far from perfect. Several attendees wondered if additional clusters (cell types) would be revealed if PCA was performed on all parameters, and not simply the morphological parameters, or if other nonlinear analysis methods would provide greater separation of cell types.

The apparent diversity increases further as additional cellular properties are considered. Aniruddha Gupta, from Henry Markram's lab, described an extensive paired recording study that revealed an astounding diversity of interneuron types. Eight different classes of neuronal firing properties were distinguished based on the presence or absence of several features in the response to current injection. Interneurons were also classified into one of five morphological types and further classified on the basis of short-term plasticity properties of their synaptic output into one of three synaptic classes. Although multiple synapse types could occur for individual classes defined on the basis of morphology or firing properties alone, when classes were defined anatomically and physiologically, only a single type

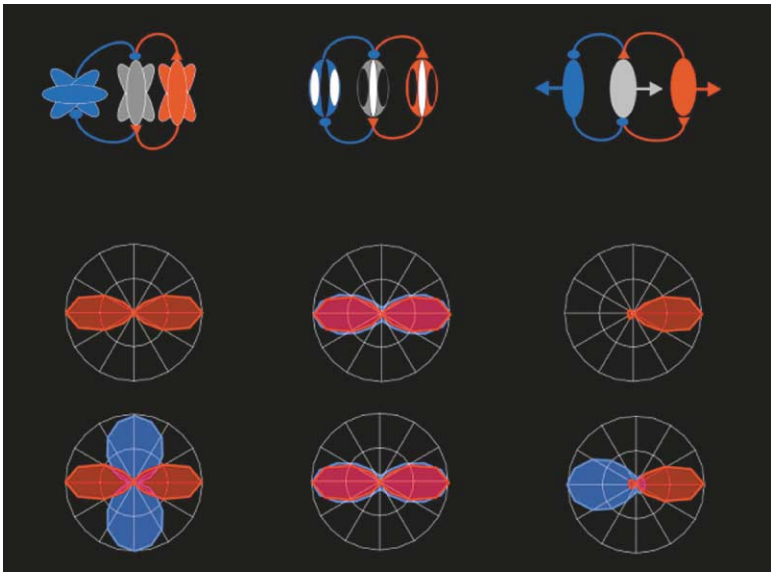

Figure 3. A Diversity of Inhibitory Connections May Underlie Orientation Selectivity in Cat Visual Cortex

The top row shows the predicted connectivity with excitatory (red) and inhibitory (blue) connections between cells with similar or dissimilar receptive field properties. In column 1, excitation and inhibition are tuned to orthogonal orientations. In column 2, excitation and inhibition are tuned to the same orientation but occur at different phases of the responses. In column 3, excitation and inhibition are tuned to the same orientation but to opposing directions of motion. Predicted input tuning for cells in the center of an iso-orientation/direction domain or at singularity points of the orientation/direction maps is shown in the middle and bottom rows, respectively. Figure supplied by Yves Fregnac.

of output synapse was observed. Given the heterogeneity, many of the classes contained small numbers of members (two of the classes contained only a single member). In a recent review, Silberberg et al. (2002) suggests that, given the fact that each cell type is present in differing proportions in each layer, a minimum "canonical" circuit may require on the order of 400,000 to 800,000 neurons. For comparison, this is within a factor of two of the number of neurons contained within the entire primary visual area in one hemisphere of the rat neocortex (Peters and Payne, 1993). If cortical circuits can only be understood in such large chunks, the idea of a canonical microcircuit is lost.

A similar diversity of interneuronal morphology, firing properties, and marker expression has been documented in the hippocampus, causing some workers to despair of any simple classification scheme that links physiology and anatomy (e.g., see Parra et al., 1998). The Gupta et al. study mentioned previously raises hope that diverse classes of neurons defined on morphological or molecular grounds may coalesce into functionally related groups defined on the basis of their output synapses. Rosa Cossart presented intriguing results suggesting that the kinetics of input synaptic currents may provide a similar organizing principle in the hippocampus. Cossart and colleagues found that cells that have overlapping axonal arborizations within the hippocampus receive IPSCs and EPSCs with a particular "kinetic signature."

Although most of the taxonomy issues centered on inhibitory neurons, there are reasons to think that similar issues arise for excitatory neurons. Ed Callaway made the point that spiny stellate cells and small pyramid neurons within layer 4 of macaque visual cortex make different connections, suggesting that these two similar cell types, which have often been lumped together, may serve very different functional roles.

Javier DeFelipe emphasized the evolutionary diversity of cortical structure. He first presented histological sections from the cortices of platypus, giraffe, and many other species, demonstrating that a single cortical region can vary widely in cell density and other cytoarchitectonic features. He went on to describe in more detail a particular subtype of GABAergic interneuron, the double bouquet cells. These neurons, which can be visualized with antibodies to calbindin, are organized in a repeating columnar fashion in human and monkey cortex (see Figure 4), but this structure is absent in rodent cortex. DeFelipe summarized the tension between diversity and commonality, nicely noting it as follows:

although most of us…would agree that pyramidal cells and their input-output connections form the skeleton of the basic microcircuit [and] that the basic pattern of synaptic connectivity is generally conserved in all cortical areas and species,…significant differences have

Figure 4. Photomicrograph of a Human Temporal Neocortex Preparation, Immunostained for Calbindin, Illustrating How Numerous Double Bouquet Cell Axons Are Distributed in the Human Cortex

Double bouquet cells generate a widespread inhibitory microcolumnar system in humans and monkeys, whereas in other species, such as rats, this microcolumnar organization is apparently absent. Scale bar, 200 μm. Figure supplied by Javier DeFelipe.

been observed amongst the neuronal elements that make up the basic microcircuits in different cortical areas of the same or different species…Assuming that the functional signatures of neurons in different cortical areas are in part determined, by their microanatomy and by the intraareal circuitry, differences in microcircuitry are likely to be instrumental in determining neuronal function throughout the cortex. Therefore, it is of great interest to discern what are the basic or fundamental bricks of cortical microcircuits that are common to all cortical areas and species, and what are the specific variations in a given cortical area and species.

By the time Kevan Martin got his computer working on the final day of the meeting, many of us were feeling a bit exhausted by diversity. For some, it was perhaps more the diversity of late-night eating and drinking establishments, but even those who made sure they slept at least twice as much as the average Madridian were clearly affected by the bewildering variety of cell types and circuit permutations. Martin and several other attendees asked the following: Are all of these differences functionally important? Do they represent fundamentally different types, like cars and buses, or at least Volkswagens and Fords, or are different molecular markers more like different colors of paint? "Call me Henry Ford," Martin mused later, "but I think the wheels are probably more important than the color of the car."

Perhaps the question of which differences are substantive and which are window dressing requires a functional genomics of the neocortex. Ultimately, what distinguishes cells from each other are the proteins they express. By comparing genome-wide expression patterns across anatomically and functionally defined cell types in different species and regions and under different conditions, clusters of commonality may emerge. Hopefully, this will permit an objective distance metric to be developed, a way to quantify the intuition that a thick-tufted layer 5 pyramidal neuron in mouse visual cortex has more in common with the homologous neuron in human cortex than with the basket cell sitting next to it. Separating the "wheels" from the "paint" then requires the further step of manipulating gene expression to see which expression differences are causal and which are epiphenomenal.

Henry Markram described some ambitious first steps toward matching up anatomical, physiological, and gene expression properties of cortical neurons. Single-cell multiplex PCR was used to assay the presence or absence of dozens of channel subunits and other neuronal proteins. The resulting "G-code," as he referred to it, was then compared with an array of morphological features (the "M-code") and electrophysiological features (the "E-code") measured from the same individual neurons. Associations between these properties occurred at ten times the rate predicted by chance, but it was difficult to evaluate from the short presentation the degree to which the molecular basis of particular neuronal traits could be identified or to which repeatable neuronal types could be quantitatively identified. Although it is clearly early days, the prospect of being able to link properties of individual cell types to their gene expression is exciting, and this approach or one like it is likely to be a fruitful one.

Dynamics: Putting the Pieces Back Together

Ultimately, studying cortical microcircuit structure is valuable only in what it can tell us about cortical function. In many respects, relating structure and function of the cortical microcircuit is an enterprise that requires linking multiple levels of description. How do perceptual, motor, and cognitive abilities arise out of complex patterns of neuronal activity across the cortex (linking the cognitive and systems levels), and how in turn do those patterns of activity arise out of the physiological properties of individual cortical neurons and their connections (linking the systems and cellular levels)? Making these links is enormously difficult, not only because of the lack of widespread agreement on how to classify the cortical neurons that comprise the circuit or on precisely how they are wired up, but also because of the complex and highly nonlinear dynamics of the component cells and synapses. The problem is compounded by the fact that cellular and synaptic dynamics not only influence activity; they are themselves plastic in an activity-dependent manner.

Despite the difficulty of this problem, there were some hints that progress is being made.

At short spatial and temporal scales, circuit dynamics are governed primarily by the intrinsic firing properties of cortical neurons and by short-term plasticity of their synapses. Alex Thomson, a pioneer in the study of short-term plasticity at neocortical synapses, noted the complexity of these phenomena. An apparently unitary physiological property like short-term synaptic depression can, in fact, reflect the operation of multiple underlying biophysical mechanisms. Despite this biophysical diversity, however, recent studies from several attendees (Tsodyks and Markram, 1997; Varela et al., 1997) and others (Dittman et al., 2000) have shown that essential features of short-term synaptic dynamics can be captured in simple computational models that can then be used to generate hypotheses about functional implications at the circuit level. Thomson suggested that the kinetics of short-term facilitation and depression may be significantly more complex than those captured in these models. Additional rounds of matching theory and experiments may be required.

In addition to understanding the integrative properties of individual synaptic connections, it is crucial to rigorously understand the rules by which multiple synaptic inputs are combined. An enduring debate concerning these rules centers on the degree to which independent inputs are summed linearly. Gabor Tamas described some particularly elegant triple-recording studies that quite directly address this question. Tamas and colleagues studied convergent input from two presynaptic neurons onto one or more postsynaptic targets and then used light and electron microscopy to identify the location of the synapses. Interactions tended to be remarkably linear. Modest sublinearity was detected only for inputs located quite close to one another on the same dendritic branch. Combining triple recording, pharmacology, and electron microscopy is a technical tour de force, rightfully earning Tamas' student Szabadics a

prize for the best poster presentation at the meeting. The findings fit nicely with models of cortical circuits as linear filters of their synaptic input (Ferster, 1994) but would seem not to agree with recent observations of robust shunting inhibition during sensory stimulation (Borg-Graham et al., 1998; Hirsch et al., 1998). Apart from methodological issues, this may, however, highlight the huge gap that exists between understanding the interactions between three synaptically connected neurons in a slice and understanding the dynamics of activity in a fully connected, spontaneously active intact cortex. Linearity in one regime may not preclude significant nonlinearity at the other end of the activity spectrum. It may be necessary to use a combination of modeling and novel physiological approaches to help fill this gap.

One such novel approach is that described by Gilad Silberberg, a student in Henry Markram's laboratory. Silberberg made multiple whole-cell recordings from pyramidal and nonpyramidal neurons. Rather than studying connections between these neurons in the silent slice, he studied correlations in subthreshold activity after elevating network activity by changing the ionic constituents of the bathing medium to find that the shape of the cross-correlogram was related reproducibly to the cell classes studied. A similar "active slice" preparation was developed by Maria Sanchez-Vives (also in attendance) and David McCormick (Sanchez-Vives and McCormick, 2000) and offers a promising intermediate level of circuit complexity between traditional in vivo and in vitro approaches.

Similar to the slow oscillations present in "active" slices, spontaneous fluctuations in membrane potential reflecting transitions between an UP state and a DOWN state are a prominent feature of in vivo intracellular recordings that were first characterized by the laboratories of Steriade (Steriade et al., 1993) and Wilson (Cowan and Wilson, 1994). Carl Petersen described experiments performed in Bert Sakmann's lab using whole-cell recording and voltage-sensitive dye imaging to study the interaction between these spontaneous transitions and sensory responses. Responses during the *up* state were small, brief, and confined to a small cortical region relative to the *down* state responses. This is somewhat surprising, because neurons in the down state are relatively hyperpolarized, and this might be expected to diminish propagation of responses. On the other hand, the intense synaptic activity that accompanies the *up* state may lead to synaptic depression, increased inhibition, or reduced input conductance, all of which could contribute to the observed reduction in responsiveness. In some cases, spontaneous events appeared to replay recently evoked sensory activity. This study and prior observations in primate visual cortex (Tsodyks et al., 1999) suggest that sensory responses and spontaneous activity engage the same cortical circuits and that interactions between the two account for much of the oft-noted variability of sensory responses.

A particularly rich and detailed picture of the relationship between synaptic properties and circuit dynamics emerged from three thematically related presentations on electrical synapses between interneurons. The existence of these electrical synapses, once thought to be rare in the adult, was discovered simultaneously in the laboratories of Shaul Hestrin (Galarreta and Hestrin, 1999) and Barry Connors (Gibson et al., 1999). In these initial recordings, slices were obtained from juvenile animals, and skeptics have continued to wonder whether these gap junctions, known to be prominent in the developing cortex, persist into adulthood. Using mice generated in Hannah Monyer's lab, in which specific populations of interneurons selectively express GFP under the parvalbumin promoter, Galarreta and Hestrin have now shown that electrical synapses between interneurons are indeed prominent in the adult.

What is the functional significance of these electrical synapses? Hestrin described how the combination of rapid excitation through electrical synapses and slightly delayed inhibition through chemical synapses made networks of interneurons function as synchrony detectors. Such synchronization is known to occur at several time scales, including those of the θ ($\sim$5–10 Hz) and γ ($\sim$40–80 Hz) rhythms and the much faster so-called 600 Hz ripple (Jones et al., 2000). These oscillations can be observed in vivo and in slices under suitable conditions.

To address the question of how these oscillations depend on electrical connections between interneurons, two groups have now knocked out the sole connexin subtype (connexin36) present in the cortex (Hormuzdi et al., 2001; Deans et al., 2001). Hannah Monyer, senior author of the Hormuzdi et al. study, presented these results. As expected, electrical coupling was virtually absent among hippocampal interneurons, and similar results were obtained in the neocortex (Deans et al., 2001). Although θ and γ oscillations remained in pharmacologically activated slices from the knockout animals, the γ oscillations were less robust and less widely synchronized. Interestingly, the very fast oscillations (200–600 Hz) were unaffected. It remains possible that other non-connexin proteins may contribute to gap junctions underlying these rhythms within the cortex and hippocampus.

Monyer's lab also showed that long-range synchrony of the γ range oscillations depends on the kinetics of excitatory inputs to interneurons by creating mice in which GluRB receptor expression is elevated in interneurons, thus slowing the kinetics of their AMPA currents (Fuchs et al., 2001).

Peter Jonas rounded out the set of three talks on interneurons and oscillations by describing studies in which a detailed Hodgkin and Huxley model of the dentate gyrus basket cell network was constrained with data obtained from paired recordings of basket cell connections. Unitary IPSCs were remarkably large and fast. A prior simulation study (Wang and Buzsaki, 1996) had demonstrated the importance of IPSC kinetics in determining the oscillation frequency in this circuit. Jonas and colleagues implemented a similar model but used the measured conductance amplitudes and kinetics and found that this allowed the network to oscillate at higher frequencies and to be more robust in the face of heterogeneity in the excitatory synaptic inputs.

The combined molecular, synaptic, and computational approach outlined in the three talks on interneuron synchrony is especially exciting in light of the ongoing debate about the functional importance of γ range oscillations for sensory coding (see Roskies, 1999). Now that interneuronal circuits are understood in sufficient detail to enable accurate simulation and the molecular under-

pinnings are understood in sufficient detail to permit direct manipulation, functional hypotheses can be rigorously tested…provided, of course, one can figure out how to study the binding problem in a mouse.

Oscillations aside, specificity in the dynamics of synaptic connections and in the pattern of those connections may give rise to reproducible patterns of network activity. Rafael Yuste, in the second part of his presentation, described recent findings from his laboratory on recurring spatiotemporal patterns of spontaneous activity in slices from mouse visual cortex (Mao et al., 2001). These highly nonrandom activity patterns are reminiscent of the concept of "synfire chains" proposed by Moshe Abeles (1991). This similarity was not lost on Abeles, who devoted a substantial portion of his talk to general comments on the importance of choosing the correct temporal and special scale at which to study neural coding in the cortex. Paraphrasing Valentino Braitenberg, he noted "You don't read a newspaper with a microscope." Activity and circuitry that appears random at one scale may be highly deterministic and reproducible at another scale. If, as Abeles suggests, these patterns involve chains of interconnected neurons, they will only be visible when recording from multiple neurons simultaneously.

How might small networks of neurons be wired up so as to repeatedly produce particular spatiotemporal patterns of firing? One attractive candidate is spike timing-dependent plasticity (STDP), a form of plasticity in which the strength and magnitude of plasticity depend on the relative timing of pre- and postsynaptic firing (Markram et al., 1997; Bell et al., 1997; Abbott and Nelson, 2000). In my own presentation, I described recent experiments carried out by Per Jesper Sjöström in my lab aimed at uncovering the mechanism of spike timing-dependent long-term depression (tLTD) in rat visual cortex. Using paired recording and pharmacological manipulations, we found that induction of tLTD requires release of endogenous cannabinoids from the postsynaptic neuron, which appear to act retrogradely on the presynaptic terminal. Intriguingly, agonist-induced LTD is still NMDA dependent, and these NMDA receptors appear to be located on the presynaptic terminal. The temporal overlap between activation of presynaptic CB1 and NMDA receptors appears to control the temporal window over which pre- and postsynaptic firing can induce LTD, since manipulations that alter the availability of cannabinoids at the presynaptic terminal alter the temporal window.

Cellular and synaptic properties change as a result of the actions of neuromodulators. Pat Goldman-Rakic reviewed her findings on the dopaminergic modulation of working memory in the prefrontal cortex of the macaque. Neurons in this region continue firing after the offset of stimulus, marking a location that the animal must remember to receive a reward. This persistent activity is diminished after application of dopamine (DA) agonists. Goldman-Rakic also described slice studies of modulatory effects of dopamine on firing properties and synapses of pyramidal neurons in ferret prefrontal cortex. Using paired recording, Wen-Jun Gao (Gao et al., 2001) showed that DA acts presynaptically via D1 receptors to inhibit release at synapses between layer 5 pyramidal neurons. These effects are likely to contribute to the inhibitory effects of DA on prefrontal firing observed in vivo in the primate. Because DA can have multiple effects on multiple cellular and synaptic elements of the circuit, however, rigorously linking particular biophysical mechanisms to systems level properties can be difficult. One approach that may help this effort is computer simulations.

Xiao Jing Wang has been at the forefront of efforts to model persistent activity in the prefrontal cortex (see Wang, 2001, for review). He described recent modeling work aimed at understanding how multiple classes of GABAergic interneurons contribute to persistent activity in the prefrontal cortex. Physiological recordings from Goldman-Rakic's lab and others have revealed that persistent activity is long lasting, stimulus specific, and resistant to the presence of distracting stimuli. Wang's simulations suggest that different classes of interneurons, by virtue of their different patterns of projection, may play distinct roles in generating these features. Stimulus specificity arises from the combination of widespread inhibition mediated by parvalbumin-containing interneurons and localized disinhibition of pyramidal cells via projections from calretinin-containing interneurons to calbindin-containing interneurons. The calbindin-containing interneurons permit persistent activity to be resistant to distracting stimuli by inhibiting dendrites of pyramidal cells not engaged in encoding the stored stimulus.

Coda: Back to the Future

Several attendees came away feeling that diversity rather than generality had ruled the day. Pat Goldman-Rakic remarked that "the meeting displayed the emergence and definition of a new field, a kind of neo-anatomy," but cautioned that although this was "giving new power [and] a new respectability for anatomical investigation…there is a danger of cataloguing diversity for its own sake." Similarly, Kevan Martin remarked "perhaps we have just moved into the neoclassical era of cortical microstructure—repeating the golden age of Cajal and Lorente with new techniques."

Shaul Hestrin noted the parallel to the situation in the ion channel field before sequence information: "The meeting made it very clear to us that we do not have physiological and morphological 'hard data' at hand to agree on a useful scheme of cell types. By comparison, naming ionic channels became possible when unambiguous sequence data was obtained, and we are not there yet in the study of cortical neurons."

Nevertheless, I remain convinced that a new synthesis of the cellular and systems neurobiology of the cortex is at hand. With more sophisticated analyses such as the ability to classify and identify cells on the basis of the whole transcriptome, rather than one or few markers, more rapid progress will be made on the difficult task of mapping functional classes like "simple cell" or "memory cell" onto cell types defined at the cellular level. More detailed and sophisticated computer simulations will allow theorists to develop specific hypotheses about the network consequences of cellular and synaptic properties, and the ability to manipulate candidate genes known to be crucial for those cellular and synaptic properties will allow physiologists and molecular biologists to test those hypotheses.

Will the end of this process be an understanding that emphasizes commonality or diversity? Both, of course. Ion channel sequence data have not only revealed their incredible diversity but also their fundamental relatedness and, when combined with structure/function studies, have revealed principles governing the ways in which particular motifs and domains function across channels. Similarly, as noted by Kevan Martin, the study of the vertebrate retina has revealed "complexity of structure [that] can be reduced to some basic essential functions common across…all species despite huge variations in structure at all magnifications." My own admittedly optimistic hunch is that the current neoclassical flowering of interest in the diversity of cortical microstructure will move seamlessly into a renewed and more sophisticated attempt to unravel the common ways in which these circuits function.

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The canonical microcircuit is the basic model of these layer-to-layer connections, and it is innately related to the concept of the cortical columns. Accordingly, an inherent bias would be expected in our chosen articles towards both the cortical column and the canonical microcircuit. Nevertheless, our analysis demonstrates a mixed approach towards both concepts. The relation of cortical columns to connectivity modelling has been put in doubt, with some commentaries going as far as writing â€˜obituariesâ€™ for the column and calling it â€˜a structure without functionâ€™ (Feldmeyer 2012). This putative "canonical" cortical microcircuit contains 400,000 neurons across six layers with a particular connectivity pattern between the excitatory pyramidal and inhibitory interneurons. Connecting the Brain to Itself through an Emulation. Article.Â There has been considerable debate on whether cortical microcircuits are diverse or canonical [Buxhoeveden & Casanova, 2002;Nelson, 2002] but we argue that the differences are variations of the same underlying cortical algorithm, rather than entirely different algorithms. This is because most cortical areas seem to have the capability of processing any type of information. ... In the primate prefrontal cortex, the â€œcanonicalâ€ microcircuit was shown to be subject to modifications from the striate circuit (Heinzle et al., 2007; Godlove et al., 2014). More generally, abundant data is available on variants of intrinsic connectivity in cortical regions such as prefrontal cortex (Melchitzky et al., 2001), somatosensory cortex (LÃ¼bke and Feldmeyer, 2007; Petersen, 2007; Lefort et al., 2009; Feldmeyer et al., 2013) or auditory cortex (Barbour and Callaway, 2008; Oviedo et al., 2010; Watkins et al., 2014).Â To probe the local microcircuitry, diverse experimental methods with different degrees of sensitivity and reliability have been used.Â Canonical microcircuits for predictive coding. Neuron 76, 695â€“711. doi: 10.1016/j.neuron.2012.10.038.