Journal of Neurocytology **31**, 387–416 (2002)

Neocortical circuits: Evolutionary aspects and specificity versus non-specificity of synaptic connections. Remarks, main conclusions and general comments and discussion

JAVIER DEFELIPE[1,*], GUY N. ELSTON[2], ICHIRO FUJITA[3], JOAQUÍN FUSTER[4], KIMBERLY H. HARRISON[5], PATRICK R. HOF[6], YASUO KAWAGUCHI[7], KEVAN A.C. MARTIN[8], KATHLEEN S. ROCKLAND[9], ALEX M. THOMSON[10], SAMUEL S.-H. WANG[5], EDWARD L. WHITE[11] and RAFAEL YUSTE[12]

[1]*Instituto Cajal (CSIC), Madrid, Spain;* [2]*Department of Physiology and Pharmacology, Vision, Touch and Hearing Research Centre, School of Biomedical Sciences, The University of Queensland, Brisbane, Australia;* [3]*Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan;* [4]*Neuropsychiatric Institute and Brain Research Institute, UCLA School of Medicine Los Angeles, California, USA;* [5]*Department of Molecular Biology, Princeton University, Princeton, New Jersey, USA;* [6]*Fishberg Research Center for Neurobiology, Kastor Neurobiology of Aging Laboratories, and Advanced Imaging Program, Mount Sinai School of Medicine, New York, USA;* [7]*Division of Cerebral Circuitry, National Institute for Physiological Sciences, Okazaki, Japan;* [8]*Institute of Neuroinformatics, University Zürich and ETH Zürich, Switzerland;* [9]*Laboratory for Cortical Organization and Systematics, RIKEN Brain Science Institute, Wako-shi, Saitama, Japan;* [10]*Department of Pharmacology, The School of Pharmacy, London University, UK;* [11]*Department of Morphology, Faculty of Health Sciences and Zlotowski Center for Neuroscience, Ben-Gurion University of the Negev, Beer Sheva, Israel;* [12]*Department of Biological Sciences, Columbia University, New York, USA*

Received February 6, 2003; accepted February 6, 2003

Introduction

In this article the main conclusions, and some of what the guest editor (Javier DeFelipe) considers the most interesting remarks, have been extracted from each of the individual articles. These commentaries are not necessarily directly derived from the original work of the authors, and may be the result of the collective work of several different laboratories. As indicated in the Preface to this Special Issue, the aim of this summary is to provide the reader with a synopsis of current thoughts about cortical circuitry and function, by transcribing the authors' ideas in their own words. The article presents the remarks and the main conclusions of each of the individual articles, followed by a section dedicated to the general comments and discussion of the issues raised therein. The authors who have participated in this article are listed in alphabetical order.

Alan Peters

Examining neocortical circuits: Some background and facts.

REMARKS AND MAIN CONCLUSIONS

1. The first definitive studies of where afferents to cerebral cortex terminate were made possible by the finding that as they degenerate axon terminals become electron dense. Gold toning of Golgi impregnated neurons allowed the postsynaptic targets of these afferents to be identified by electron microscopy and also allowed the termination sites of axons from a variety of types of cortical neurons to be ascertained. It was shown by a number of investigators that the most common elements postsynaptic to the thalamic afferent are dendritic spines (about 85%), with fewer axon terminals synapsing with dendritic shafts (about 15%) and neuronal perikarya (about 2%). The distribution of thalamic terminals relative to their postsynaptic partners is similar for the thalamic inputs to visual cortex, somatosensory cortex and motor cortex.

2. The most surprising fact to emerge from these degeneration studies of the thalamic input to cerebral cortex was that only some 5 to 10% of the synapses in layer 4 appeared to originate from the thalamic input, and for

* To whom correspondence should be addressed.

the callosal inputs to layer 3, the observed percentages were even lower.

3. Since layers 3 through 5 are the cortical layers that receive most of the input from afferents originating outside the cerebral cortex, 90 to 95% of the axon terminals synapsing on neurons within cerebral cortex must originate from cortical neurons themselves. This means that the axonal plexus of an individual cortical neuron must produce several thousand intracortical synapses.

4. The investigation of neuronal types using gold toned-Golgi impregnated material reinforced the important conclusion derived from earlier studies using unaltered Golgi impregnated material, namely that all axon terminals formed by a single neuron have similar features and form synapses of the same type. It was also shown that the spiny neurons, namely the pyramidal cells and the spiny stellate cells, have axons whose terminals form asymmetric, or type 1 synapses, while the neurons with smooth or sparsely spinous dendrites have axons that form symmetric, or type 2 synapses.

5. One of the surprising findings to emerge from these studies is that with the exception of the chandelier cells, whose axons form synapses almost exclusively with the axon initial segments of pyramidal cells, all other types of cortical neurons lack specificity. They prefer one specific type of postsynaptic element, but they also form synapses with others. As pointed out, the same is true of the thalamic inputs to cerebral cortex. While they terminate principally in layer 4, the thalamic afferents lack specificity in the sense that they synapse with any postsynaptic element capable of forming asymmetric synapses.

6. Soon after the Golgi-gold toning method had been worked out, cortical physiologists began to inject neurons intracellularly with electron dense markers. It became evident that intracellular filling provided a much more complete view of the axons, making it obvious that Golgi impregnation rarely showed the complete axonal plexuses of cortical neurons. There was the added advantage that even myelinated axons, which do not impregnate in Golgi material, could be filled by intracellular markers.

7. While we have a reasonably complete picture of where the axons of extra- and intra-cortical neurons form their synapses, we know very little about the sources of the overall input to any given type of cortical neuron. The neurons that we probably know most about are the spiny stellate cells of layer IV in cat and monkey striate cortex. Analysis of the thalamic input to layer IV of cat visual cortex shows that of the 2500 asymmetric synapses received by each spiny stellate cell, only 5% of the total, some 100 to 125 synapses, are provided by the geniculocortical afferents. Further it seems that the plexus of an individual geniculocortical axon provides only a single synapse to one in four of the layer IV neurons encompassed by its plexus. A somewhat similar situation exists in monkey striate cortex. . . it is calculated that the total thalamic input to each layer IVC neuron originates from the axonal plexuses of 24 different thalamic neurons, so that an individual plexus provides not more than one or two of the thalamic synapses received by each neuron. Yet, despite the paucity and divergence of the thalamic input, it dominates the response properties of neurons in visual cortex.

8. The number of asymmetric synapses the axon of an individual pyramidal cell forms with other cortical neurons, both other pyramidal cells and non-pyramidal neurons, is generally low (from 1 to 8 synapses). In contrast to the generally low number of synapses formed by axons of pyramidal cells with other neurons, individual inhibitory, local circuit, neurons can provide several symmetric synapses to a postsynaptic neuron (from 6 to 15).

9. Since each cortical neuron receives several thousand synapses, it is obvious that there must be convergence of several neurons of the same type on to a postsynaptic neuron to provide its total input. For example, it has been estimated that as many as 10–25 large basket cells would be required to provide all of the inhibitory synapses on the cell body of a layer 3 pyramidal cell in cat visual cortex. At the other extreme is the situation that occurs with the inhibitory chandelier cells, which form strings of boutons making inhibitory synapses with the axon initial segments of pyramidal cells. Although it is not uncommon for the axons of two or three chandelier cells to converge on the same axon initial segment, all of the axo-axonal synapses of a given pyramidal neuron can be formed by a single string of chandelier boutons.

10. The point to emphasize is that each neuron in cerebral cortex gets its intrinsic input from a large number of other cortical neurons. Most of these are spiny neurons, each of which forms a few excitatory, asymmetric synapses, and if one assumes that the total number of synapses on a hypothetical pyramidal cell is of the order of 5,000, and 85% of these synapses are excitatory, then there would be some 4,250 asymmetric synapses. They are mainly located on the dendrites and on the basis of the data cited above, these synapses probably originate from about 1,000 presynaptic excitatory neurons that converge on the postsynaptic cell. Inhibitory, symmetric synapses predominate on the cell body, initial axon segments and proximal dendrites of cortical neurons. They comprise only 15% or so of the total synaptic input, so that in our hypothetical case the neuron would receive some 750 inhibitory boutons from the local circuit neurons. If, on the basis of the available data, it is conservatively assumed that each inhibitory neuron provides an average of 10 synapses to our pyramidal cell, then the inhibitory input to this pyramidal cell would be provided by some 75 neurons. However, at present we have very little specific information about the origins of the overall synaptic input to the various

types cortical neurons. Without that knowledge it is difficult to interpret the specific role that any given type of neuron plays in the circuitry and functioning of cerebral cortex.

GENERAL COMMENTS AND DISCUSSION

Fuster

The evidence that the great majority of axon terminals synapsing on cortical neurons originate from other cortical neurons suggests that, in the cortex, the bulk of the encoding and processing is "autonomous," that is, intracortical and independent of external inputs. This means that, in the cortex (especially association cortex), both the encoding and the processing are operations of cortical networks, which is consistent with the largely "autonomous" character of cognitive functions.

White

Citing degeneration studies, Peters mentions 5–10% as the correct value for the proportion of thalamocortical synapses in sensory cortex, in contrast to the value of 20% reported for mouse barrel cortex (*e.g.*, White, *J. Comp. Neurol* **181**, 627–662, 1978, pages 75 and 78 of White, *Cortical Circuits: Synaptic Organization of the Cerebral Cortex. Structure, Function and Theory*, Birkhäuser, 1989). For lesion-induced degeneration to be used to quantify terminals reliably, it is necessary that all affected terminals degenerate simultaneously. This is clearly not the case in most areas and species examined (*e.g.*, figures in Shanks & Powell, *Brain Res* **218**, 35–47, 1981 show early, middle and late stages of degeneration to occur at 5 days postlesion in monkey). This phenomenon undoubtedly accounts for the peak on postlesion day 3 in the number of degenerating terminals/synapses in the results cited by Peters: In the absence of simultaneous degeneration, some affected terminals are phagocytosed before others have begun to be recognized as degenerating. In essence, the "peak" describes a post-lesion period during which the maximum number of degenerating terminals are recognizable; in the absence of simultaneous degeneration, the peak number observed does not reflect the entire complement of affected terminals; reliable quantification is impossible even at the peak. In contrast, in mouse barrel cortex, terminals that are affected by thalamic lesions degenerate simultaneously such that all affected terminals display the same stage of degeneration at any single post-lesion survival time; there is no peak of degeneration, and on each day during the post-lesion period when degeneration is clearly visible, about 20% of the synapses are made by degenerating thalamocortical axon terminals (White, *J. Comp. Neurol* **181**, 627–662, 1978). Significant in this context are the results of LeVay and Gilbert (*Brain Res* **113**, 1–19, 1976) who, using EM autoradiography to quantify geniculocortical terminals and synapses in cat visual cortex, report the percentage in layer IV of thalamocortical synapses to be about 20%—in stark contrast to the 5–10% value reported in every other study of visual and somatosensory cortex in cat and monkey; the other studies used degeneration. Presumably, the simultaneous degeneration of mouse thalamocortical terminals is related to the relative homogeneity of cell body (and their axon) diameters in mouse "barreloid" thalamus: the terminals degenerate simultaneously and can be quantified reliably in mouse barrels without resorting to EM autoradiography which would seem the method of choice for thalamocortical systems composed of axons of markedly different diameters. In conclusion, 20% seems to be the appropriate value for the percentage of thalamocortical synapses in layer IV of mammalian sensory cortex.

Yuste (point 5)

I would debate the conclusion that "with the exception of the chandelier cells, whose axons form synapses almost exclusively with the axon initial segments of pyramidal cells, all other types of cortical neurons lack specificity. They prefer one specific type of postsynaptic element, but they also form synapses with others." I think this definition of specificity is too narrow, since it is based on contacting a single population of targets. I think we need to agree on a definition of specificity. I would define specificity as the ratio of the number of chosen targets divided by the number of potential targets. It seems to me that, in spite of a hundred years of anatomy, we still don't know the denominator, *i.e.*, how many classes of cortical neurons there are. As Solnick and Sterling concluded in 1984 (*Proc. Natl. Acad. Sci. USA* **81**, 3898–3900), even in layer 4 there could be more than a dozen types of spiny stellate cells. Therefore, we could conclude that a projection lacks specificity if we don't properly identify or distinguish the nature of its targets (numerator) or if we do not know what is the existing population of potential targets (denominator). I am assuming that there are indeed different types of cortical neurons, rather than a continuum of morphological or physiological cell types. This of courses raises the issue of how can one define objectively the existence of a class of cortical neurons. Without agreeing on these basic terms it is difficult to discuss these issues. I propose to arrange an international meeting focused on the nomenclature of classes of neocortical neurons.

Martin

In view of the considerable and cumulative efforts of neuroanatomists over the past 100 years, it comes as a shock to read Alan Peters' view that, 'we know very little about the sources of the overall input to any given type of neuron'. His assessment however, explains the otherwise curious fact that physiologists have been more proactive in devising circuits than anatomists. If

we know so little about the convergence of intrinsic inputs to individual neurons, then it is clearly impossible to devise any comprehensive description, even at a qualitative level, of the circuits of the neocortex. The quantitative descriptions of the total input to spiny stellate and small basket cells in layer 4 of the cat (Ahmed *et al.*, *J. Comp. Neurol* **341**, 39–49, 1994; *J. Comp. Neurol* **379**, 1–13, 1997; Stratford *et al.*, *Nature* **382**, 258–261, 1996; Tarczy-Hornoch *et al.*, *J. Physiol* **508**, 351–363, 1998; *Cerebral Cortex* **9**, 833–843, 1999) are the most complete descriptions we have of the anatomy and physiology. Of course, these descriptions have the disadvantage of being essentially one-dimensional spatially. We would obviously like to know many more details about the biophysical, and synaptic properties, as Ed Callaway points out. However, at this stage even one-dimensional knowledge of the overall pattern of connections for the major cell types within a given cortical area would be a tremendous step forward. Paradoxically, however, our increasing reliance on physiological methods—paired recordings, caged glutamate, or calcium imaging, to explore the anatomy of cortical circuits, makes it less rather than more likely that this significant gap, identified here by Alan Peters, will be filled. But without it, as Alan Peters concludes, 'it is difficult to interpret the specific role that any given type of neuron plays in the circuitry and function of cerebral cortex.' This is a strong challenge to neuroanatomists.

Another curious observation made by Alan Peters also requires stronger consideration: the thalamic input to layer 4 provides less than 10% of the excitatory input to the layer. This means that the thalamic input to any cortical column or 'module', if such there be, constitutes a fraction of one percent of the synapses in the column. Yet, it is widely believed that this thalamic input dominates the response properties of the sensory cortex. That is, the output from an area is largely a function of its patterns of feedforward input. Where does this leave the issue of the cortical computations?

A similar case can be made for the power of the 'feedback' or 'top-down' connections, which have been extensively studied by Jenny Lund and Kathy Rockland, and which are often endowed by physiologists and psychophysicists with rather extraordinary efficacy. That extrinsic sources of excitation seem to be so much more powerful in determining the output of the cortical circuit than all the variety of intrinsic neurons, with their plethora of biophysical and neurochemical nuances, is an issue that lacks coherent explanation. Are we really to believe that all the spatial and temporal properties of cortical neurons are determined by the pattern of the thalamic input to the column and that the dynamics of the circuit are modulated only by the 'top-down' connections? If we do believe this, then the fine details of difference in structure should not preoccupy neuroanatomists for a moment longer. We should instead ponder why, *e.g.* in the discussion of specificity, that the all important thalamic input appears quite promiscuous in its connections- spine, dendritic shaft, soma, apical or basal dendrite, smooth or spiny neurons, any target is possible, and the bouton distributions of the thalamic afferents show no obvious selectivity, beyond the retinotopy and left and right eye segregation. Yet the most selective receptive field properties seem to emerge from this thalamic feedforward connections. If this is indeed true, then can we really say anything about the anatomical specificity underlying the functional architecture of the cortex on the basis of fine anatomy. We seem to believe we can, but we need to face the hard fact that anatomy has not one example of 'solved' circuit for any of the well-studied properties of sensory cortex. Simply adding to the list of known connections, at ever increasing detail, will not solve the basic issue of what the microcircuits are that underly the physiological expression seen in cortical circuits *in vivo*.

Rockland's response to Martin's comment on Alan Peters. I agree with Kevan Martin that Alan's statement about persisting lack of basic data is shocking; but Anderson *et al.* have also made a very similar comment ("only fragments of the cortical circuit have been assembled"). These statements are in part a salutory (sobering?) reminder of the early state of the field, but can more positively be seen as a challenge for new synthesis and concepts, *even as* we continue the work toward better data.

Edward L. White

Specificity of cortical synaptic connectivity; emphasis on perspectives gained from quantitative electron microscopy.

REMARKS AND MAIN CONCLUSIONS

1. Initial findings indicated that any neuronal type having a dendrite within a particular layer would receive input from pathways terminating within that layer, and additionally, that a high degree of interconnectivity obtains between different morphological types of cortical neurons. The plethora of cortical synaptic connections and the inability for the most part to identify consistently in a single preparation the cell types of pre- and postsynaptic elements, rendered cortical synaptic connectivity nearly undecipherable and helped promote the impression that cortical wiring was unorganized or "random". The basic idea was that everything was connected to everything with no particular order, a line of thought leaving little room for specificity. The discovery of cellular recognition proteins and the development of models of synaptic specificity that rely on the activities of these molecules lay in the future.

2. Stereotypic patterns of synapses are a general feature of the cerebral cortex. Perhaps the most basic

expression of this are the contrasting patterns of synapses on the surfaces of spiny vs. non-spiny neurons, anatomical features of consequence for the physiology of these neuronal types. Briefly, the dendritic shafts and cell bodies of neurons whose dendrites have many spines, such as pyramidal and spiny stellate cells, are postsynaptic nearly exclusively at symmetrical, presumed inhibitory synapses; the spines of these neurons are targeted preferentially by asymmetrical, presumed excitatory synapses. In contrast, all types of non-spiny neurons display both asymmetrical and symmetrical synapses on all parts of their cell bodies and dendritic shafts.

3. The high degree of order discerned in many aspects of cortical organization is mirrored by an equally ordered arrangement of synaptic connections involving specific types of neurons, *i.e.*, specificity is a basic feature of cortical organization.

4. Parenthetically, the author would like to state that although much evidence for the specificity of cortical synaptic connections has been obtained by quantitative electron microscopy, there is precious little regarding qualitative aspects of synaptic connectivity in the cerebral cortex that escaped the observations of the Golgi masters such as Ramón y Cajal and Lorente de Nó despite their inability to visualize synapses with the tools and approaches at their disposal.

5. The observation that thalamocortical synapses may be spaced regularly along dendrites is consistent with the view that cortical circuitry is highly ordered. Additional support for this thesis has been inferred also from comparisons of the spatial distributions of other synaptic types along reconstructed dendrites.

6. The distribution of thalamocortical synapses onto neurons identified by their morphology and/or by the targets of their distant axonal projections has been studied extensively in layer IV of the large barrel region of mouse primary somatosensory cortex. The results of this work demonstrate that the proportions and distribution of thalamocortical synapses is characteristically different for different neuronal types. Similar observations were made for the synaptic connectivity of callosal afferents in mouse motor cortex. These indications of specificity in synaptic circuitry, contrast sharply with the nonselectivity, often (and erroneously) equated with randomness, with which thalamocortical axon terminals synapse with every type of dendrite in layer IV of the primary visual and somatosensory areas of the cortex in different species. The "randomness" of cortical synaptic connectivity is more apparent than real; the *specific* nature of these synaptic connections is evident only when accurate methods of quantification are employed.

7. It has been shown in a variety of cortical areas and species, that different extrinsic and intrinsic axonal pathways form specific proportions of their synapses with specific postsynaptic elements, *i.e.*, spines vs. dendritic shafts. Given the general association of excitatory, axo*spinous* synapses with excitatory neurons (*e.g.*, pyramidal and spiny stellate) and excitatory, axo*dendritic* synapes with inhibitory cells, these observations indicate that axonal pathways are highly selective with respect to the proportions of the different types of neurons they target.

8. The wealth of evidence to support specificity of synaptic connectivity should not be construed as sufficient grounds for a complete rejection of "randomization in cortical connectivity", if for no other reason than that biological systems are not so rigidly programmed as to exclude the occurrence of mistakes or sloppiness in normal development and operation.

9. Ample evidence exists to view the cerebral cortex as a highly ordered system of neurons massively interconnected in a specific fashion. Dynamic properties of the cortical system could be achieved by the selective transitory or permanent reinforcement of specific "hard-wired" synaptic pathways or by the destruction of old synapses and/or the formation of new ones. The formation of specific synaptic connections is not inconsistent with the fluidity of brain structure implied by the formation of new synapses in the adult. In any event, there is no reason to conclude that plasticity of wiring and the immutability of it cannot co-exist and both could involve specific synaptic connectivity.

10. Specificity, by limiting possibilities for interneuronal communication, can impose an order on synaptic interactions even as dynamic selection or synaptic remodeling processes ensure the constant formation and dissolution of individual circuits. Collectively, these operations make maximal use of the richness of cortical synaptic connections to provide the system with the highest degree of flexibility, irrespective of the degree of hard-wiring, mutability, randomness or specificity that obtains for cortical synapses at any particular time.

GENERAL COMMENTS AND DISCUSSION

Fuster

Functional specificity most probably derives from connectional specificity, not from the features of morphologically defined "specific" neurons or synapses.

DeFelipe

I would like to ask White what is his concept of "specific connections" and to comment on his idea about species differences in the thalmocortical circuits.

White's response to DeFelipe. In this paper, "specific" is used to describe synaptic connections between *"specific neurons or neuronal types*." Mentioning two possibilities for specificity is, in part, a purposeful effort to be comprehensive, but it is also a cautious comment related to the limitations of combining the EM study of

individual, labeled neurons with lesion induced degeneration for labeling afferents. These limitations include the labeling only of populations of *presynaptic* elements due to the inability to trace individual, degenerating axons. That said, the results from EM studies of synaptic organization in barrel cortex, and the "general rules" (see text) used to describe them, are entirely consistent with the suggestion of Anderson *et al.* (this issue) that cortical specificity is first and foremost a specificity that obtains between *populations* of neurons, *i.e.*, neuronal types. Methodological limitations prevent interpreting these results in connection with "specificity at a finer grain" (Anderson *et al.*), *i.e.*, that between individual neurons.

In response to the second part of your question concerning species differences of thalamocortical circuits, I refer first to your own work (this issue) in which you make it clear that different areas of cortex are at the same time similar and different. A considerable amount of quantitative data on the proportion of synapses formed by thalamocortical afferents are available for mouse barrel cortex; both lesion induced degeneration (LID, see *e.g.*, White, 1989, *Cortical Circuits: Synaptic Organization of the Cerebral Cortex. Structure, Function and Theory*, Birkhäuser, and the papers cited in this issue) and the anterograde transport of PHA-L (Keller *et al.*, *Brain Research* **343**, 159–165, 1985) consistently show thalamocortical afferents to form about 20% of the synapses in barrel hollows. This value is consistent with the results of LeVay and Gilbert's (*Brain Res* **113**, 1–19, 1976) EM autoradiographic study of cat primary visual cortex. Lower values of 5–10% have been reported by others for the same area of cat visual cortex; thus the difference in values is not a species difference but rather a difference related to the conditions under which LID can be used to obtain reliable data and when it cannot. A comment on this point appears at the end of Peters' paper in this issue. For me, roughly 20% is an acceptable value for primary sensory areas, and I view this value as a basic characteristic of the tissue, similar to the "standard" 80 to 90% value observed in layer IV of the primary sensory areas for proportion of thalamocortical synapses formed with spines vs. with dendritic shafts and cell bodies. That certain, "standard" values occur in different cortical areas and in different species is not astounding; comparative studies of mammalian limbs, for example, evidence great similarities in the types and arrangements of bones; evolution and/or creation are conservative; indeed, why reinvent the wheel. That there are differences in synaptic organization that undoubtedly reflect differences in function is also true. I am neither sufficiently knowledgeable nor intelligent to explain the functional bases for the commonality of certain features of cortical organization, nor for the differences in other features. The identification of common features of cortical organization helps us identify what is *not* common, and that knowledge will afford us the possibility of relating the differences to differences in function. Pertinent is R. Tarfon's comment: "It is not for you to complete the work, but neither are you free to desist from it" (*Ethics of the Fathers*, chap. 2/21, *circa* 100 C.E.).

Yuste

To complement White's argument, I would propose the following idea: there are two types of excitatory cortical connections, strong and weak. Strong connections would be specific whereas weak connections would be unspecific or random. Strong connections could be implemented by either multiple synaptic contacts on the same target cell, biophysically stronger synapses or perhaps postsynaptic factors such as the ability of an input to trigger local dendritic spikes and thus cause the target to spike. They would become dominant in quantitative reconstructions of specific pathways, such as White's, and perhaps be selected for with techniques that focus on the functionally strongest connections, such as the optical probing that used in Kozloski's study. These strong connections would be responsible for the receptive field properties that also show tremendous specificity, as discussed by White. Perhaps an even more dramatic example of receptive field specificity is the agreement in the detailed characteristics of receptive fields stemming from each eye in the upper layer binocular neurons in V1, as pointed out by Hubel in his book and, I believe, first by Barlow. The entire retina/geniculate/layer4 connection matrix arriving to each one of these binocular neurons must be functionally identical (and matched) for the two pathways.

On the other hand, weak connections could be implemented by the documented instances of single synaptic contacts between excitatory neurons. They will also show up, and perhaps dominate in numbers, in all studies that are not selecting the population of neurons or axons to be reconstructed. This could explain some discrepancies in the literature. These weak connections would not alter the receptive field structure, but serve to modulate the function of the networks of neurons dominated by strong connections. They could be unusually plastic and endow the rigid network of strong connections with certain potential for functional rewiring. Strong connections would be genetically determined, since nature would ensure that the brain of the animal "works" from the get go. Weak connections could be modified by activity dependent rules. You can to some extend equate weak/strong with a more general version of the driver/modulators idea of Koch/Crick/Sherman.

Could White, or whoever is interested, comment from his own experience on what % of spines are devoid of synaptic terminals and conversely, what % of terminals did not terminate on a spine or a dendrite?

White's response to Yuste. Spines without synapses: Studies of spiny stellate cells in layer IV of mouse barrel cortex show that upwards of 10% of their spines do not form synapses (White & Rock, *J. Neurocytol* **9**, 615–636, 1980; Benshalom & White, *J. Comp. Neurol* **253**, 303–314, 1986). It cannot be excluded that some proportion of the synapse-less spines form synapses but that the synapses are not able to be visualized due to an unfavorable plane of section. I know of no similar data on the apical and basal dendrites of pyramidal neurons in barrels or elsewhere.

Terminals that don't make synapses: As with so many things having to do with electron microscopic studies of the brain, this is a very good question with no good answer. To identify a synapse-less terminal, one must first identify the terminal. Let us examine what has been considered by microscopists to be a terminal: A sphere at the end of a short preterminal axon is certainly an axon terminal, but what of axonal varicosities or cylindrical portions of axons that form synapses? In single and even in serial thin sections through labeled axons, a synapse-forming axonal varicosity is likely to be referred to as a terminal, especially by English speakers who in their shortening of bouton terminal to terminal often have included erroneously within this designation boutons en passant. Some types of axons, such as thalamocortical afferents form most of their synapses at varicosities, but I suspect that varicosities that don't form synapses would be considered as simply part of the axon instead of being regarded as a synapse-less terminal. In other words, the lack of an en passant synapse is not a clearly identifiable structure. The situation with unlabeled axons is even more difficult: the literature is full of examples from thin sections of unlabeled, presynaptic profiles that are identified as "terminals", in fact, only because they are presynaptic—according to this criterion, profiles that are not presynaptic would not be identified as terminals, hence, a synapse-less terminal would escape detection.

Thomson

Ultra-structural analysis of connectivity patterns has provided us with some of the most fundamental ground rules from which we have built hypothetical circuits. Some combinations of pre- and post-synaptic elements rarely if ever occur, while others are common. Quantitative studies can define neuronal populations that are the preferred targets of a given pathway and those less densely innervated.

Jennifer Lund

Specificity and non-specificity of synaptic connections within mammalian visual cortex.

REMARKS AND MAIN CONCLUSIONS

1. The majority of cortical neuron classes are of ancient lineage-perhaps as old as the mammalian cerebral cortex itself. This strongly suggests that many of the cortical neuron classes are genetically determined and have highly conserved form in terms of evolution.

2. While there are clearly differences in morphology between local circuit, GABAergic neurons-especially in their axon morphologies, it is more difficult to decide if there are genetic differences between the excitatory, spine bearing neurons. They differ from local circuit neurons in transmitter and morphology but between each other, distinguishing features are less certain.

3. There is an impression that lateral excitatory connections in each layer are more plastic in their earlier stages of development than interlaminar connections.

4. There is every reason to believe that the neurons of every layer are organised with similar complexity in terms of interlaminar excitatory neuron circuitry. Similarly, the array of specific interneuron circuits that is apparent in and between single layers of area V1 is another very strong indicator of highly specific circuitry that is almost certainly genetically determined.

5. Available information on synaptic targets of specific interneuron populations suggests that their targets are also rigidly determined in terms of the nature of the postsynaptic neuron and the region on that neuron where synapses are established.

6. The majority of interneurons are not obligatory companions of every pyramidal neuron but that they most likely play very specific roles in the particular stage of functional processing that occurs in their home layer; they also appear to have predetermined roles that they play in other layers since each interneuron usually has specific interlaminar projections as well as intralaminar axon processes. Since each layer in cortex appears to have different functions, even morphologically similar varieties of interneuron are quite likely to play very different roles, depending on the layer in which their dendrites and local axon processes lie and those layers they also innervate.

7. One important difference between excitatory and inhibitory neurons is that most commonly only the excitatory neurons have very long lateral projections.

8. One difference between interlaminar and intralaminar excitatory projections is that generally intralaminar projections are reciprocal, whereas interlaminar projections are not always reciprocated.

9. Each layer has its own unique circuits for its constituent excitatory and inhibitory neurons that are likely to be largely genetically determined. These circuits may, however, coexist with more labile, functionally determined patterns of connections.

10. Even though the postsynaptic targets of the axons of each variety of cortical neuron may be genetically determined as to the class of cell and the region

of that cell's surface it must contact, the actual numerical weight of synapses contributed in each case may be open to considerable variation, depending on the specific circuit involved, the balance of inputs on each cell and the vagaries of development and environment within the brains of individual animals.

11. Long lateral intralaminar connections are made by many of the pyramidal neurons and some populations of spiny stellate neurons in several layers of area V1. This is a common feature across many if not all cortical areas of the macaque and many other species. The lateral connections from pyramidal neurons lying within any small point in layers 2/3 establish regularly spaced columns of axon terminals in an extended field in the same layers around the neurons of origin.

12. The lateral connections of the pyramidal neurons target both excitatory and inhibitory neurons within each column of terminals and the question is how specific is their choice of location for the column of terminals? Since the column system appears to be a continuum of overlap of connections across cortex it would seem unlikely to be genetically defined as to specific loci. Since the column diameters are the same size as dendritic fields of single neurons, one may ask if the terminals are targeting the dendritic fields of just a single column of neurons with somata at the column center? Or are the axons terminating on any dendrites that enter that column space, irregardless of where their somata lie- for instance even the dendrites of cells whose somata lie well outside the column but who have partial dendritic intrusion into the column space? The latter pattern is thought to be the most likely, such that the axons terminating in the column can select any portions of dendritic fields that lie within the column boundary.

13. Lateral connection architectures (patterning and extent of these connections) [in different species] seem to depend more on cortical size, afferent scaling factors, and functional maps for their patterns, rather than being linked directly to genetically predetermined loci.

GENERAL COMMENTS AND DISCUSSION

Fuster

The local nature of most inhibitory connections has the obvious functional implication that in the cortex, as in the periphery, lateral or recurrent inhibition facilitates contrast, a local process that serves a number of important cognitive functions (*e.g.*, attention, discrimination).

White (point 12)

The thesis is put forth that inputs to columns terminate on dendrites irrespective of the location of the parent cell body of the postsynaptic neuron. This notion is consistent with observations on the synaptic connectivity of thalamocortical afferents to mouse barrel hollows.

John Anderson, Tom Binzegger, Rodney Douglas, Kevan Martin

Chance or design? Some specic considerations concerning synaptic boutons in cat visual cortex.

REMARKS AND MAIN CONCLUSIONS

1. The complexity of the cortical circuits led early investigators to see only tangled thickets, a random mesh of connections in which no specic cortical circuits were to be found. Theories were developed based on the assumption that the patterns of connections of individual neurons were irrelevant and that the mass action of an aggregate of neurons was the key to understanding cortical function.

2. Modern studies have largely overturned this view in favour of highly specied and stereotyped connections between cortical neurons that are repeated over and over to generate a crystal-like cortical architecture. In the most recent models, the circuit has evolved such precise wiring that the specicity extends beyond simply connections between diferent types of neurons to the actual position of the connection on the postsynaptic neuron. On this view, the surface of each neuron is an intricate mosaic of specic synaptic connections made with select presynaptic partners. The hope is that when all these synapses are mapped, and their stereotyped and specific 'weights' known, then the role of the different neurons in the circuit will be plain.

3. The debate over the precise form of the circuits has yet to begin. Even in very intensively studied circuits of the rat barrel cortex, or cortical areas 3 and 17 of monkey, only fragments of the cortical circuit have been assembled. The discussion of the specicity of connections is pursued at quite different levels. The oftcited epitome of specicity is the chandelier cell, which forms its synapses exclusively on the initial segment of pyramidal cells. However, at the next level the question remains unanswered as to whether the chandelier cell specically 'selects' particular pyramidal cells or simply forms multiple synapses with the axon initial segment of any pyramidal cell it comes across.

4. The axo-axonic cell is also at one extreme in selecting a single (albeit broad) class of neurons—the pyramidal cells. All axons have preferred targets, but in general they form synapses with many different types of neurons and multiple sites: with spines, dendritic shafts and somata.

5. Analyses of fragments of Golgi-stained axons in the rodent neocortex have given the rather surprising result that the placement of boutons along individual axon collaterals is random, *i.e.*, the interbouton intervals approximate an exponential distribution (Braitenberg & Schüz, 1981). The authors interpret this as indicating that the cortical wiring is 'essentially random'.

6. To understand the rules by which axons lay down their synaptic boutons we analyzed the linear bouton

distributions in neurons (spiny and smooth cells types) and thalamic axons, which were labelled intracellularly with horseradish peroxidase in cat area 17. Cells of similar type tend to have similar bouton density and interbouton interval distributions. Median interbouton interval on distal collaterals (*i.e.*, first and second order segments) covered a range between 3 μm and 11 μm, which presumably reflects some rule governing the connection of the different cell types to their targets. Spiny cells and thalamic afferents tend to have larger median interbouton interval than smooth cells. A basket cell axon in layer 4 forms a bouton about every 5 μm, whereas for the spiny stellate cells it is only about every 8 μm.

7. En passant boutons form beads along the trajectory of the axons, whereas the terminaux boutons are located on spine-like process that project a few microns from the axis of the axon. Neurons form mainly en passant boutons in making their synapses. The exception is the type of layer 6 pyramidal cell [cat visual cortex] that projects to layer 4, which have a high proportion of terminaux boutons.

8. An active role of the postsynaptic targets themselves are rarely considered in discussions of bouton formation. However, it is clear that the targets could themselves contribute to forming specific connections. We now know that dendritic spines can be generated de novo and thus they could provide a means whereby the dendrites themselves can be active in capturing specific passing axons.

9. If individual basket cells have to make their multiple synapses with a spatially restricted domain of their target neurons (*e.g.*, the soma and proximal dendrites), this may require either more branches or the interbouton interval to be as small as possible. Among the spiny cells, the layer 6 pyramidal cells also have small interbouton interval. However they do not form multiple synapses with their targets so the close proximity of their boutons does not re multiple inputs to single cells, but may re the density of suitable targets in the neuropil. These observations remind one that we know very little about the environment within which axons are distributing their boutons.

10. The analysis of these axons [spiny, smooth and thalamic axons] shows the rich variety of ways in which the different cell types distribute their synaptic boutons along the axons. In seeking for the underlying rules one central consideration has been the manner in which the boutons distribute themselves along the axon. The strong claim made by Braitenberg and Schüz (1991) is that the concept of a 'terminal arbor' is meaningless because cortical pyramidal cells distribute their synaptic boutons diffusely over the whole tree. They supposed that the location of synapses was decided by the surrounding dendrites in the neuropil, which offer the axons postsynaptic sites. They argue that because the network of dendrites is 'to all intents and purposes 'random' the wiring of the cortex is also essentially random.

11. Our data and analysis leads us to a rather different interpretation. Different cell types have characteristically different bouton distributions. The axons do not have a diffuse distribution of boutons over the whole tree, but instead have specific laminar targets and form clustered projections within those target lamina.

12. In the case of random wiring, a given axon traverses the neuropil and probabilistically forms synapses with any dendritic tree it encounters in the neuropil. If the location of the target neuron is distributed by a Poisson process, the interbouton intervals will be exponentially distributed. In the converse case, where the axon forms its synapses selectively with a specific class of cells, the same exponential distribution of interbouton intervals will be achieved if the class of target neurons is distributed by a Poisson process.

13. It is not unreasonable to suppose that different classes of cortical neurons are distributed distributed by a Poisson process. It is as if the different classes of cells lie suspended in a sea of neuropil, each class forming different densities of Poisson distributions at different depths.

14. Our hypothetical example shows that if the rule required any neuron to connect to specific individual neurons, rather than any member it encountered of a specic class, then the axon could not take a straight trajectory, but would have to twist and turn to make connections with specic neurons even if the neurons were organized in a geometric grid. Axons of cortical neurons are not highly contorted in general, which is consistent with our proposal that the specicity lies in connections between classes of neurons rather than between specic individual neurons. By allowing the axons to take relatively straight trajectories, such a rule of connectivity greatly contributes to optimizing the length of axon required to make a given set of connections.

15. The relatively short median interbouton intervals we observe implies that if an axon forms its synapses with only one class of neurons, then the members of that class must occur at relatively high density. The experimental evidence, however, indicates that most neurons form synapses with multiple classes of neurons. If these classes are all distributed by a Poisson process, then the resultant bouton distribution would still be exponentially distributed. The number of synapses made by one neuron on another will depend on the density of the axonal boutons and the target cells.

16. The simple rule of specificity for classes of neurons rather than particular neurons would not exclude specificity at a finer grain. For example a rule could be that the synapses be formed with spines rather than dendritic shafts. The hypothesis also allows for a coarser level of specificity, as is evident in the vertical interlaminar connections or the horizontal eye-specic clusters of thalamic afferent boutons that create the

ocular dominance columns, for example. Thus, quite simple rules of connectivity could generate the circuits that display the exquisite functionality seen with the microelectrode and imaging.

GENERAL COMMENTS AND DISCUSSION

Fuster

"Random", "order", and "specificity" are difficult to ascertain on morphological grounds alone. The "randomness" of Braitenberg and Schütz may be as defensible as the observation of systematic distribution of synaptic terminals. In functional terms, what appears at first as "randomness" may develop into order and "specificity" without obvious morphological change. The morphological evidence of synapses is not evidence of their functional viability.

White

A pertinent comment relating to the consistency between the results of quantitative data on synapses in mouse barrel cortex and the thesis of Anderson *et al.*, that cortical specificity is primarily one between "*classes* of neurons rather than particular neurons" appears in the comments section at the end of White's paper in his response to DeFelipe.

Kawaguchi

Thomson and Morris (in this issue) suggest that axons from interneurons may actively seek their targets while pyramidal cells are more passive. Anderson *et al.* revealed bouton intervals show gamma distribution in most cells including both spiny and aspiny ones, although mean intervals are different among cell types. This suggests that formation rule of axonal boutons may be similar between pyramidal cells and interneurons. Spatial distribution of specific classes of pyramidal or non-pyramidal cells may give important hints for understanding the cortical wiring.

Rockland

Anderson *et al.*, and also Thompson and Morris, make the observation that axons take relatively straight trajectories, and suggest this as evidence that specificity lies in connections between classes of neurons, rather than between specific neurons. In the latter case, that is, one might expect a more convoluted trajectory. The actual terminal arbors, however ,are locally convoluted; and it would seem that their configuration can be considered compatible with either interpretation.

Thomson

The analyses presented here provide a framework within which questions of random versus specific are addressed. For example, are individual targets belonging to a single class of cortical neurones selected by a given axon or, does the axon innervate all possible targets belonging to a specific class that it encounters as it traverses the neuropil, with the same probability. This alternative need not exclude selective innervation of one cell class over another, or of one type of postsynaptic subcellular compartment over another, provided these elements are distributed by a Poisson process. Rather, it poses the possibility that within these constraints there may be no further selection of individual cells. The lack of convolution in the axonal trajectories at least of many of the excitatory cells indeed suggests that the axon itself may not seek out specific targets within these subpopulations, but does not exclude the possibility that spiny targets can 'reach out' to selected inputs.

DeFelipe (point 3)

I personally think that the chandelier cells select particular pyramidal cells. In support of this position, there are two important pieces of evidence. First, it appears that not all pyramidal cells are innervated by chandelier cells. For example, it is unlikely that corticothalamic projecting pyramidal cells in layer VI of the cat visual cortex are major postsynaptic targets of chandelier cells. These cells have few synapses on the initial segment, some of them receiving only 1 or 2 axo-axonic synapses (Fariñas & DeFelipe, *J. Comp. Neurol* **304**, 70–77, 1991). Second, a given chandelier cell does not form synapses with the initial axon segment of any pyramidal cell it comes across since the number of neurons within the axonal plexus of a chandelier cell is relatively high when compared to the number of chandelier cell axon terminals. To demonstrate this, we identified all the pyramidal cells in a series of semi-thin sections through the central, dense region of Golgi-impregnated chandelier cell axonal plexuses in layers II and III of area 4 of the monkey. From this study, it was clear that only a small proportion of the pyramidal cells (up to 20%) were innervated by impregnated chandeleir cells (DeFelipe *et al.*, *J. Comp. Neurol* **231**, 364–384, 1985).

Edward Callaway

Cell type specificity of local cortical connections.

REMARKS AND MAIN CONCLUSIONS

1. The function of the cerebral cortex is dependent on the precise organization of the circuits formed by its component neurons. The connections between neurons are not random, but are specific at multiple levels of organization.

2. Typically, structural differences are highly predictive of functional diversity. But, often functional diversity is not apparent from anatomy alone. For example, inhibitory neurons with similar anatomical features

can differ functionally as a result of differing intrinsic membrane properties or differences in neuropeptide expression. Thus, identification of the full range of neuron types requires both functional and structural studies.

3. In order to understand how each neuron type contributes to the neural mechanisms that mediate perception and behavior, each of several properties must be understood. These properties include: (1) Synaptic Integration. How are the chemicals released at thousands of inhibitory, excitatory, and modulatory connections which are distributed over complex dendritic geometries converted into temporal patterns of action potentials propagating through the axonal arbor? (2) Synaptic Output. How are patterns of action potential firing in axonal arbors converted into release of chemicals at synaptic contacts? (3) Connectivity. What other neurons in the network make connections onto or receive connections from each cell and what are the properties of these connections with respect to synaptic integration and synaptic output?

4. Intracellular recordings from neuron pairs allows identification of the morphology of both pre- and postsynaptic cells and tight temporal control of action potential initiation in the presynaptic cell. But since only a few neurons can be sampled at a time, only a minute subset of possible connections can be tested. Photostimulation by local uncaging of glutamate, combined with intracellular recording allows high spatial resolution mapping of the sources of functional input to single neurons.

5. . . . when axons arborize in a particular cortical layer they do not necessarily connect to all cell types in the layer according to the relative densities of their dendritic arbors. Instead axons connect preferentially to some cell types while avoiding others.

6. While inhibitory neurons are highly diverse with respect to multiple anatomical, functional, and neurochemical features, excitatory neurons are relatively uniform. Although some excitatory pyramidal cells have distinct intrinsic membrane properties that give rise to a "bursting" phenotype, the majority of excitatory neurons are regular spiking.

7. Photostimulation studies show that there are consistent differences in the sources of local input to different types of excitatory neurons. There were also systematic differences in the laminar sources of excitatory input as well as the extent of lateral input from adjacent cortical columns.

8. In addition to specificity of input to distinct cell types, individual neurons with apparently uniform morphological features can receive diverse laminar patterns of synaptic input. It is unlikely that this diversity results from random connectivity. Instead there appear to be selective connections onto pyramidal neurons which are likely to be functionally diverse despite apparent anatomical uniformity.

9. Each cortical layer is composed of many neuron types whose axonal and dendritic arbors are intertwined. In addition, each layer is targeted by axonal arbors from a diverse set of cortical and subcortical neurons. The patterns of connectivity between these afferent axons and the neurons in the recipient cortical layer can not be predicted based on their spatial overlap. Instead functional connectivity is highly precise. Afferent axons can selectively target some neurons while avoiding others giving rise input patterns that are often similar for neurons of the same type but differ between cell types.

GENERAL COMMENTS AND DISCUSSION

Fuster

"Random connectivity" seems to be an inference that derives from ignorance.

Thomson

Although the studies outlined in this review do not allow precise identification of the subclass(es) of presynaptic neurone(s) activated by the caged glutamate, they do accurately identify the postsynaptic targetand the layer/sub-layer(s), activation of which provides the most powerful inputs to that cell. Paired intracellular recordings more accurately identify the presynaptic neurone(s), but yield population data only very slowly. However, in the relatively small selection of interlaminar connections that have been studied with both techniques to date, there is good agreement in the high degree of selectivity with which these connections are formed. In brief, that while intralaminar connections may be relatively non-selective in the classes of neurones involved, each type of interlaminar connection selects specific classes of postsynaptic targets, targets that can vary with both the laminar origin of the axon and its layer of termination.

Alex Thomson, Oliver Morris

Selectivity in the inter-laminar connections made by neocortical neurones.

REMARKS AND MAIN CONCLUSIONS

1. What we have observed suggests that there is indeed exquisite selectivity in the intracortical connections made by cortical neurones. This is in addition to the selectivity 'imposed' on the circuit by the arborization patterns of their axons. Clearly, an axon cannot contact a target that is not within its terminal field and the arborization patterns of many cortical neurones are highly organised and often layer specific. Even within these restricted arbors, however, there is often selection of the targets innervated. Moreover, these selections can also be layer specific, a given axon

preferentially targeting spiny excitatory cells in one layer and aspiny inhibitory interneurones in another.

2. In neocortex there exists a vast range of interneuronal morphologies, immunocytochemical profiles and target preferences. Their distribution over 6 layers results in a huge array of axonal arbor patterns even amongst cells with, for example, similar subcellular compartment target preferences and immunocytochemical profiles. The task before us, to determine the relative probabilities of each intra- and inter-laminar connection involving each class of postsynaptic interneuronal target type, is therefore enormous and we have as yet only scratched the surface.

3. Does the axon choose its target, or the target cell choose the axon? This is clearly an immensely important question in developmental neurobiology, but one that is beyond the scope of this article. The shapes of mature axons and dendrites may, however, give some clues. With the exception of layer 6 axons ascending to layer 4, which have complex side-spine arrays with small boutons (in cat and primate visual cortex), the axons of most pyramidal cells follow near linear trajectories forming *en passant* boutons and do not give the impression of having had to become convoluted to 'find' their appropriate targets. In contrast, the axonal arbors of many interneurones are extremely complex, with multiple side branches and often tortuous paths.

4. From axonal morphology, one might predict that many interneuronal axons actively seek their targets while pyramidal axons are more passive.

5. Unlike their axons, the paths taken by the dendrites and dendritic spines of pyramidal cells are often complex. Although the general pattern of the dendritic arbor of a particular class of pyramidal cell may be stereotypic, in fine detail the dendrites are far from a straight and constant diameter cylinder. Moreover, spines with very different lengths can emerge from dendritic shafts and at a range of angles. This complexity suggests that these spines seek their excitatory inputs, rather than *vice versa*, a suggestion that is strengthened by observations of spine plasticity *in vitro* and *in vivo*. Interestingly, although most interneuronal dendrites lack spines in the adult, they are medium to densely spiny in immature cortex. It therefore seems possible that during development and possibly later, neurones actively seek their most appropriate excitatory inputs, while inhibitory fibres seek their targets. The targets of inhibitory interneurones may be more fixed in space because they are typically less plastic elements—somata, axon initial segments, dendritic shafts and spine necks—rather than spine heads.

GENERAL COMMENTS AND DISCUSSION

Fuster

In terms of cortical self-organization, whether an axon "chooses" its target or vice versa is a nonsensical question. They choose each other; growth and modulation by usage (within certain critical periods) are the most relevant factors in that mutual choice.

Fujita

Given the striking specificity of projections between layers or between particular component neurons in respective layers described in this and other articles of this issue, little is known on the laminar distribution of neurons with different response properties for a cortical area with the notable exception of the primary visual cortex. The lack of this information may be mainly due to technical difficulty to determine the recording site with respect to layers, especially in experiments using monkeys. Another reason may be that, as shown in this paper, projection patterns are variable among different types of neurons in each layer, *e.g.*, projections from layer 3 pyramidal neurons to large, but not to small, pyramidal neurons in layer 5. Tabulation of response characteristics according to the laminar location of soma may not reveal the fine layer arrangement of neurons with different properties. Here is a largely unexplored research area, which remains a challenge to neurophysiologists.

Kathleen Rockland

Non-uniformity of extrinsic connections and columnar organization.

REMARKS AND MAIN CONCLUSIONS

1. Extrinsic [interareal] cortical connections are an important component of the cortical microcircuitry; but, in contrast with interneurons, thalamocortical, and intrinsic horizontal connections, much less information is available. In particular, for these connections it is very difficult to completely characterize and correlate identified parent neurons, identified postsynaptic groups, and functional architecture of both source and target areas.

2. What is known about extrinsic connections, however, seems to clearly indicate a high degree of diversity. Projection neurons, although predominantly or exclusively (in primates) of the pyramidal category have been subdivided as early as the 1970's on the basis of axon collateral and dendritic arborization, and are not identical, even within a single projectional system. Systematic subdivisions by other criteria such as receptor profiles, firing pattern, and even spatial position are likely in the near future.

3. On the side of afferent terminations, the connectional environment is highly variable for different cortical areas. The density and mix of callosal, thalamocortical, and cortical connections may be supposed to be area-specific; and multivariate analyses of receptor binding and connectivity data have in fact been used to produce area-specific "fingerprints". Even within areas,

the microcircuitry is unlikely to be "uniform"; for example, most areas have zones of both callosal and acallosal connectivity.

4. What are the projection patterns of neighboring neurons? Do adjacent projection neurons, in the same "column," have recognizably similar, "stereotyped" projections? Results from intracellular injections in pairs of adjacent projection neurons in rats suggest that single cells in target regions are unlikely to receive input from both of the injected neuron pairs, and that any modular grouping of cells would have to be irregular in nature. An alternative interpretation, still interesting, is that not all "adjacent" neurons, although physically nearby and having intermingled dendritic and axonal arbors, are identical. They might instead represent closely related subtypes, and the variation in projection patterns may correlate with the finer subtypes. Neither of these features—selectivity of target areas, and non-overlap of arbors--are consistent with a strict uniformity of connections. This is not to suggest that they imply "randomness." Some other organizational rule might be in effect.

5. The same phenomenon, of partially overlapped convergence of terminations, is sometimes seen in small foci of three to four axons labeled by large extracellular injections of anterograde tracers, although the exact relationship of the parent neurons cannot be specified by this method.

6. Projections to multiple areas, via collaterals, have also been demonstrated in other experiments, such as double retrograde tracer injections or single axon analysis. For example, in marmoset, the large infragranular Meynert cells, which are commonly considered as a homogeneous population, project to extrastriate areas MT and 19DM, and the superior colliculus in all possible combinations.

7. Axons with multiple arbors. Cortical axons commonly have multiple arbors even within one target area. The arbors, however, are not uniform, in that one arbor, larger and with more terminations, can be distinguished as a "principal" arbor associated with smaller, auxiliary arbors.

8. In higher cortical areas, connections frequently are shown as terminating in a columnar pattern from pia to white matter. There are some reports that column size, at least of intrinsic terminations, increases in early visual and higher order association areas. The increased size, however, properly speaking refers to convergence factors of multiple axons and less information is available for individual arbor size.

9. An important question for continued investigation is the manner in which different connectional systems converge and interact in different cortical areas. In contrast with structures like the hippocampus and cerebellum, very little information concerning the modes of termination and interaction of the main afferent systems is available for cortical areas, largely because of the difficulty of electron microscope investigation of identified terminals and postsynaptic neurons together. At the light microscopic level, early investigations with double anterograde tracers have shown interdigitation of contralateral and ipsilateral columnar projections in frontal association cortex of primates. More extensive mapping, however, reveals a complicated spatial distribution. Of fifteen cortical regions receiving common input from frontal and parietal injection sites, some regions showed an arrangement of interdigitating columns, but others exhibited a laminar complementarity.

10. The issue of non-stereotyped projections at the columnar level is further considered by analysis of V1 axons terminating in primate area MT/V5 (an early visual area), and of an axon from temporal cortex terminating in area 7b (a higher cortical area). Both these axons have multiple non-uniform arbors. The implication is that each arbor recruits different numbers and possibly different combinations of postsynaptic elements. While more data are needed concerning convergence of connectional systems, and the actual identity and numbers of postsynaptic targets, the distributed spatial and laminar patterns do not evoke a repetitive uniformity, but rather a columnar substructure and the combinatoric possibilities of the 3-dimensional cortical organization.

GENERAL COMMENTS AND DISCUSSION

Fuster

Axons with multiple arbors, one arbor synapsing to multiple cells. The functional implications of these facts are clear and wide-ranging. For one thing, they support both the convergence and the divergence of connections in the cortex, both so important for the formation of cognitive networks and for the encoding of information in those networks. A cell or group of cells (assembly, module, etc.) can be part of many networks, and thus of many items of memory or knowledge.

Wang, Hof and Harrison

Rockland describes progress in working out specific circuitry leading to area MT, which is thought to play an important role in the processing of moving visual stimuli. One exceptional specialization is the projection from V1 to MT, which is rich in very large axons (up to 3 microns in diameter) with short conduction times ($\sim$1.3 msec; Movshon & Newsome, *J. Neurosci* **16**, 7733–7741, 1996). These large axons likely project from Meynert cells in V1, which like other large, neurofilament-rich projecting cells are particularly vulnerable to degeneration in Alzheimer's disease (Morrison & Hof, *Science* **278**, 412–419, 1997)—a possible basis for deficits in motion processing associated with the disease (Hof & Morrison, *J. Comp. Neurol.* **301**, 55–64, 1990; Hof *et al.*, *Vision Research* **37**, 3609–3625, 1997).

The high conduction speed of these axons provides, in addition to speed, a previously unremarked advantage for motion detection: reduced variability in conduction time. Recent firing history can increase conduction velocity by over 20% (Swadlow, 2000, in *Time and the Brain*, edited by Miller, R.). For narrow axons, this increase in conduction velocity would decrease by milliseconds the time taken for an action potential to go from V1 to MT. This variability of conduction times within an individual axon would degrade precise timing differences in spikes between different axons. Conversely, for large-diameter axons, there would be less timing jitter in the timing of within-axon and between-axon spikes. The prevalence of wide axons in the V1-MT projection suggests to us that relative spike timing might contribute to some aspects of motion processing in MT.

Zoltán Kisvárday, Alex Ferecskó, Krisztina Kovács, Péter Buzás, Julian Budd and Ulf Eysel

One axon—multiple functions: specificity of lateral inhibitory connections by large basket cells.

REMARKS AND MAIN CONCLUSIONS

1. Most visual cortical models consider inhibitory interactions as rather non-specific, for example deriving equally from cells of all orientation preferences. This "nonspecificity concept" of GABAergic inhibition stems from two main sources. First, anatomical studies showed that inhibitory projections are rather diffuse and collectively display low specificity to stimulus parameters such as orientation. Second, electrophysiological studies revealed that suppression is broadly tuned to a number of stimulus attributes. These observations raise the bold question why do we have so many inhibitory cell types if they are used for "general inhibition"? At this point it is important to emphasise that the above anatomical and electrophysiological observations reflect, invariably, population data that could not tackle the issue of specificity at the individual neuron level. Our working hypothesis is that inhibition is provided by specific connections at the single cell level and that "general inhibition" detected by various techniques is in turn, the composite effect of a large population of specific inhibitory connections.

2. GABAergic large basket cells represent the most thoroughly studied inhibitory cell type in the cat visual cortex. They are known to provide lateral projections up to 2 millimetres from their somata providing fast $GABA_A$, possibly shunting inhibition on the perisomatic region of the target cells.

3. Since large basket cells represent the sole inhibitory cell type that can project up to 2 mm laterally from the soma, any functional consideration utilising lateral inhibition must take them into account. The most relevant propositions in this regard concern orientation and direction selectivity which are likely to be under the influence of lateral inhibition.

4. Recent evidence obtained for six layer 3 large basket cells in the cat visual cortex indicates that a single large basket cell contacts 960 (average) neurons on the soma and proximal dendrites, each of which received at least 1 (average = 4) perisomatic contact from the basket axon. Obviously, the target neurons represent only a subset of all neurons in the cortical cylinder of the axon. We estimated that in layer 2/3 where the bulk of the boutons (87%) of a basket cell was found only 3 % of all neuronal somata were contacted. This raises the question whether these target cells are selected randomly by the basket cell or according to functional features favoured by the basket cell.

5. We assume that functional preferences should be reflected by the density of connections and, in the present case, be captured in the anatomical distribution of target cells receiving somatic contacts from the basket cells. In other words, higher density of such target neurons would indicate regions of strong preference and low density would indicate weak preference.

6. The distribution of labelled basket cell axons [after iontophoretic injections of biotinylated dextraneamine] and that of the target cells were three-dimensionally reconstructed and compared quantitatively to orientation, direction and ocular dominance maps obtained with the intrinsic signal optical imaging technique. It was found that although the functional distributions (orientation, direction and ocular dominance) for the entire cell were multi-modal and broadly tuned, individual main branches of the same cell displayed highly specific topography. Furthermore, 2-dimensional probability density estimates of the target cell distributions revealed clear clustering which may be important for local subfield antagonism. These findings provide support to the idea that the same basket cell mediates several specific receptive field operations depending on the location of the target somata in the functional maps.

7. Our findings are clearly at variance with the concept of nonspecific lateral inhibition. We demonstrate here that the main axonal branches of individual large basket cells establish highly specific topography to the representations of stimulus orientation, direction and ocular dominance. Further findings led us to propose that that the main axon branches represent functionally distinct units that can mediate specific receptive fields interactions.

GENERAL COMMENTS AND DISCUSSION

Fuster (point 4)

"Randomness" is in the eye—and the tool—of the beholder.

Kawaguchi

The approach combining the functional cortical map and morphological analysis of axonal arborizations of a single cell is promising for revealing a functional role of each cortical cell type for generating receptive fields in the visual cortical microcircuits. The large basket cells studied here may be further heterogeneous. From Figure 4, some basket cells may make somatic inhibition only locally, and others make inhibition on pyramidal cells in multiple columnar structures. The visual response characteristics of each basket cell may show functional differentiation among basket cells.

Thomson

When you reconstruct the axons of one of these large baskets filled *in vitro*, you are struck by the apparent determination with which their thick myelinated horizontal branches travel to far flung places before making a tight terminal arbor; trajectories quite unlike those of many spiny excitatory cell axons. They seem to know precisely where they are going and to ignore many target regions en route. With in vitro fills, however, you have no clear idea where this might be in functional space. The combination of *in vivo* imaging and neuronal reconstruction described in this paper provides an invaluable link between functional mapping and structure that we hope will be extended to many different classes of neurones.

David Lewis, Darlene Melchitzky, Guillermo Gonzalez- Burgos

Specificity in the Functional Architecture of Primate Prefrontal Cortex.

REMARKS AND MAIN CONCLUSIONS

1. The primate prefrontal cortex subserves a number of cognitive processes, including those that require working memory, which involves the maintenance of information "in mind," in the absence of sensory cues, in order to guide behavior. The cellular basis for keeping such information "on line" during working memory has been proposed to be the sustained firing of specific populations of prefrontal cortex pyramidal neurons.

2. Although not restricted to this location, activity during the delay period of working memory tasks is prominent in prefrontal cortex layer 3. Interestingly, the anatomical features of layer 3 pyramidal neurons suggest that they play a central role in the flow of information both between and within cortical regions. The axons of these excitatory neurons furnish three major types of projections: (1) principal axons that pass through the white matter and terminate in other cortical regions (associational projections), (2) long-range axon collaterals that travel parallel to the pial surface through the gray matter for up to 3–4 mm (long-range intrinsic projections), and (3) local axon collaterals that arborize within 300 μm of the cell body (local projections).

3. The associational projections through the white matter to other ipsilateral prefontal cortex regions form clusters of axon terminals that span layers 1–6, whereas the long-range intrinsic projections give rise to discrete clusters of terminals restricted to layers 1–3. In addition, the neurons that contribute axon collaterals to this circuitry are also arranged in a stripe-like fashion. Ultrastructural studies revealed that pyramidal neurons projecting to a given stripe receive synaptic input from pyramidal neurons located within the targeted stripe.

4. Consistent with the anatomical observations, electrophysiological studies in an *in vitro* slice preparation of monkey prefrontal cortex demonstrated that the horizontally-oriented, long-range intrinsic axon collaterals provide monosynaptic excitatory inputs to layer 3 pyramidal neurons, and suggested that most pyramidal neurons in layer 3 are targets of these long-range intrinsic projections. It seems likely that a minority (perhaps up to one-fourth) of layer 3 pyramidal neurons do not participate in such long-distance interactions. Interestingly, in monkey visual cortex, about 25% of layer 2/3 pyramidal neurons give rise only to local connections.

5. By analogy to the link between iso-orientation columns in the visual cortex, the long-range intrinsic connections in the prefrontal cortex have been proposed to link clusters of cells sharing memory fields.

6. By spanning several mm parallel to the cortical surface, the intrinsic connections are ideally suited to mediate the horizontal communication of information that may be carried by input pathways arriving to the prefrontal cortex at physically distant points, without converging on single neurons or closely neighboring groups of cells.

7. It is suggested that specific groups of pyramidal neurons, clustered as stripes that are reciprocally interconnected by long-range intrinsic axon collaterals, form a functional module that may serve to recruit and/or coordinate the activity of specific, spatially-segregated populations of prefrontal cortex pyramidal cells. Furthermore, the neurons within the interconnected stripes appear to be preferentially involved with that particular module. However, the presence of some overlap in the distribution of stripes labeled from adjacent injection sites suggests that at least some pyramidal neurons participate in more than one module, and thus that the number of modules is not determined solely by the surface area of the prefrontal cortex.

8. Specificity is also evident in the synaptic targets of the three types of axonal projections furnished by prefrontal cortex layer 3 pyramidal neurons. Both the associational (92%) and long-range intrinsic (96%) axon terminals preferentially target dendritic spines, with the remainder of both types of terminals forming Gray's Type I synapses onto dendritic shafts. In addition,

because the associational projections arborize in all cortical layers, whereas the long-range intrinsic projections only terminate in layers 1–3, the associational projections are likely to target a broader range of pyramidal neuron types, including the subcortically projecting cells located primarily in layers 5–6. In contrast to these associational and long-range intrinsic axon terminals, the targets of the local axon collaterals of layer 3 pyramidal terminals are equally divided between dendritic shafts and spines.

9. The majority, if not all, of the dendritic shafts targeted by the local axon collaterals of layer 3 pyramidal cells arise from GABA neurons. Thus, in contrast to the associational and long-range intrinsic projections, which appear to be specialized for feed-forward excitation, a substantial proportion of the local axon collaterals of layer 3 pyramidal neurons provide input to inhibitory elements.

10. The finding that local excitatory connections of layer 3 pyramidal cells are preferentially directed to the parvalbumin- and not the calretinin-containing class of GABA neurons provides insight into the possible functional role of these connections. The connectivity of parvalbumin-containing neurons enables them to serve a critical role in feedback inhibition. PV-positive neurons (which are specialized to provide potent inhibitory control over pyramidal cell output) extensively sample the excitatory output of neighboring pyramidal neurons whose associational and long-range intrinsic axonal projections appear to contribute to the recruitment of a broadly distributed network of cortical pyramidal cells. Thus, the resulting activation of parvalbumin-positive neurons would serve to constrain the propagation of pyramidal cell excitation both locally and at a distance.

GENERAL COMMENTS AND DISCUSSION

Fuster

To the anatomist, the "modules" of association cortex appear to be extremely elusive—to wit the overlap of labeled stripes that the authors mention. To the physiologist, they are even more elusive. Physiologically, the idea of modules in cortex of association cortex (*e.g.*, prefrontal) seems almost a contradiction in the terms. For the physiology of this cortex cannot be understood in terms of columns and feature detectors (as in sensory cortex), but rather in terms of the cortico-cortical associations between neurons in non-contiguous domains that make up the cognitive networks.

Yasuo Kawaguchi, Satoru Kondo

Parvalbumin, somatostatin and cholecystokinin as chemical markers for specific GABAergic interneuron types in the rat frontal cortex.

REMARKS AND MAIN CONCLUSIONS

1. Before models of cortical circuitry can be generated, it is necessary to define functional classes of cortical cells. Limited cell types would be preferable to construct modeled circuits, but cortical cell types and interconnections appear highly diverse.

2. Nonpyramidal cells take various forms of dendritic and axonal arborization, which have been used for their morphological classification. Surface domains of cortical cells, including somata, axons, dendritic shafts and spines, are innervated by GABAergic terminals, most of which are derived from cortical nonpyramidal cells. The variability in the spatial distribution of axon collaterals and in their postsynaptic targets suggests diverse roles of GABAergic nonpyramidal cells in cortical function.

3. Although Golgi or intracellular staining have revealed morphological diversity in nonpyramidal cells, chemical and physiological properties do allow morphologically diverse nonpyramidal cells to be assigned into several groups. From our initial observations, we proposed that only a few subtypes constituted the cortical GABAergic population and have conducted a search for other cell types concentrating on firing patterns and immunoreactivity for calcium-binding proteins and peptides in layers II/III and V of the frontal cortex.

4. Recent physiological and pharmacological studies to define functional subsets of cortical interneurons have yielded findings contradictory to the simple classification scheme. Cortical inhibitory interneurons can be grouped into dozens of functionally related classes according to the discharge pattern, anatomy and short-term dynamics of synaptic transmission. Single-cell multiplex PCR of nonpyramidal cells showed highly diverse expression patterns of biochemical markers. Therefore, it has become assumed that cortical GABAergic interneurons cannot be simply classified and may be composed of a large number of classes.

5. We have divided GABA cells in the rat frontal cortex into 3 groups, based on their firing characteristics: fast-spiking cells, late-spiking cells, and non-fast-spiking cells. Expression of calcium-binding proteins and peptides could be shown in separate groups of GABA cells in layers II/III and V of the frontal cortex: (1) parvalbumin cells, (2) somatostatin cells, (3) calretinin and/or vasoactive intestinal polypeptide (VIP) cells [partially positive for cholecystokinin (CCK)] and (4) large CCK cells (almost negative for VIP/calretinin). Combining the physiological and chemical properties of morphologically diverse nonpyramidal cells allows division into several groups, including fast-spiking basket cells containing parvalbumin, non-fast-spiking somatostatin Martinotti cells with ascending axonal arbors, and non-fast-spiking

large basket cells positive for CCK. These subtypes show characteristic spatial distributions of axon collaterals and the innervation tendency of postsynaptic elements.

6. The differences in firing patterns, axonal morphologies, synaptic connections and expressed substances suggest these GABA cell subtypes are differentially involved in cortical function. However, another classification employing other properties would be expected to yield categorization of cortical GABA cells differing from the above. For example, expression patterns of 21 glutamate receptor subunits cluster cortical GABA cells differently. Incorporation of more firing subtypes and short-term plasticity patterns creates another type of classification.

7. Cortical GABA cells are mutually connected not only by inhibitory synapses but also by electrical coupling. This latter occurs frequently in the same class: between parvalbumin fast-spiking cells, or between somatostatin cells, but not between fast-spiking and somatostatin cells.

8. In the cortex, inhibitory as well as excitatory circuits generate synchronized periodical activity. Cholinergic inputs from the basal forebrain exert profound effects on cortical activities such as rhythmic synchronization. The excitability of cortical GABAergic cell subtypes is differentially regulated by acetylcholine.

9. On the assumption that there are several basic neuronal types in cortical interneurons, we have classified them according to the firing pattern in response to depolarizing current pulses and the expression pattern of calcium binding proteins and peptides. We have no proof that these groupings have functional meaning, but each group classified in this simple way shows a characteristic spatial distribution of axon collaterals and innervation tendency on postsynaptic elements.

10. During synchronized activity induced by cortical excitatory or inhibitory circuits, firing patterns also differ among the subtypes. Our tentative classification is also supported by the following: the same subtype of cells appears selectively connected by electrical couplings. We hope that this tentative morphological, physiological and chemical catalogue of cortical cells may have relevance to the functional classes revealed *in vivo* experiments.

11. Diseases due to cortical dysfunctions such as schizophrenia seem to be associated with alteration in GABAergic inhibition in the frontal cortex. Frontal cortical GABA cells may have unique physiological and connectional characteristics, and influence pyramidal cells differently from those in other cortical areas. To clarify the functional architecture in the frontal cortex, it will be important to reveal the connectional characteristics of GABA cell subtypes and their relation to those in other cortical regions.

GENERAL COMMENTS AND DISCUSSION

Fuster

If, as it has been said, GABA is the most abundant neurotransmitter in the cortex of association (*e.g.*, prefrontal), why aren't inhibitory neurons and synapses more common there than they appear to be?

Thomson

These authors tackle a question that has been hotly debated for more than a decade. Is there a finite number of quite distinct cortical interneuronal phenotypes, or do these cells form a continuum with overlapping properties ? [The importance of this question lies in our need to correlate information from a wide range of experimental approaches if we are to understand cortical circuitry. Is the large basket-type cell described in an *in vivo* study of responses to a sensory input synonymous with a large basket cell described in an ultra-structural study detailing the cell types receiving thalamo-cortical input, or one in which the local circuit inputs to a large fast spiking cell are investigated.] The answer probably lies between the two extremes, that within multidimensional parameter space there are properties that cluster, resulting in large populations of cells sharing the same five or ten properties, but that some cells fall outside these clusters. Whether these outliers are aberrations, albeit successful ones, resulting from their being born in the 'wrong place', or at the 'wrong time', or whether the expression of each property is essentially independent, the observed correlations resulting from multiple external cues, remains to be determined.

Kimberly Harrison, Patrick Hof, Samuel Wang

Scaling laws in the mammalian neocortex: does form provide clues to function?

REMARKS AND MAIN CONCLUSIONS

1. The neocortex has features common to all mammals. While the neocortex can occupy anywhere from 25 to 80 percent of the brain and range in mass from about 14 mg in shrews to over 9 kg in whales, the following design elements are always observed. On the outside is a sheet of gray matter 0.4 to 4 mm thick, consisting mainly of neuronal cell bodies (chiefly pyramidal neurons) and synaptic terminations arranged in visible cell and fiber layers. Interior to the gray matter is a core of white matter composed of myelinated and unmyelinated axons. Each neuron makes synaptic connections with thousands of other neurons, mostly elsewhere within the neocortex, thus creating a structure with strong internal connectivity.

2. In large brains, the most conspicuous variations occur in three quantities: the number and variety of functional areas, the folded surface area, and the ratio

of white matter to gray matter volume. These quantities increase with brain size. Most apparent is the increasingly folded appearance of the neocortical surface in larger animals. For example, in the human neocortex, total folded surface area exceeds exposed surface area by a factor of three. Accompanying this increased folding is an increase in the thickness of the gray matter sheet. Furthermore, the white matter increasingly dominates neocortex, comprising less than 10% of the neocortex in shrews and galagos but over 40% in man and whales.

3. Analysis of relationships among anatomical parameters leads to the identification of a number of sets of interrelated neocortical parameters. They include (a) gray matter volume, gray matter thickness, and surface area; (b) gray matter volume, axon length, axon caliber, and neuron density; (c) white matter volume, axon length, axon caliber, and neuron density; and (d) gray matter volume, neuron density, synapse density in gray matter, and number of synapses per pyramidal neuron. Of these parameters, only the macroscopic ones (gray matter volume, gray matter thickness, neocortical surface area, and white matter volume) are well measured. In contrast, ultrastructural measurements are less common, involve a narrower range of species, and are often compromised by poor stereological technique. Reconciliation of macroscopic neocortical structure in terms of components is thus currently limited by the availability of accurate microscopic measurements.

4. Axons are the principal component of white matter and as such are the principal determinants of its volume. This suggests that the runaway in white matter volume relative to gray matter may be accounted for by disproportionate increases in the total number of neurons giving rise to axons, their average length, and/or the average axon cross-sectional area. Because the neuron density actually decreases rather steeply with brain size, calculations suggest that the only way to account for white matter runaway is for axon cross-sectional area to increase with brain diameter.

5. Why would axons scale up with increasing brain size? One possibility is that scaling of axon size may optimize conduction of action potentials over long distances. Conduction time is a limiting factor in cortical processing, and propagation of action potentials through the white matter may be a major component of overall neocortical processing time. Cross-brain conduction times, which can exceed 100 ms, may dominate processing time since other neural events take much less time, such as action potentials (1 ms) and synaptic transmission delays (0.3 ms). However, cross-brain delays are variable because brains vary in diameter and because conduction properties of axons depend on their diameter and type.

6. Axon conduction velocity is faster in wider axons due to a relative reduction in axial vs. leak resistance. Another major speed-enhancing innovation in vertebrate nervous systems is the wrapping of axons in a fatty myelin sheath. Myelination increases the conduction velocity of action potentials by decreasing membrane capacitance and increasing leak resistance. Wider brains may require faster impulse conduction to maintain low cross-brain conduction times. This suggests that myelination would be more prevalent in large-brained mammals. We have confirmed this experimentally, and our results indicate that myelination occurs principally for axons wider than 0.6–0.8 μm.

7. Another notable scaling phenomenon in white matter axons is a subpopulation of large, myelinated fibers that scale dramatically with brain size. For example, the widest axons are approximately 2 μm in diameter in mice and 8 μm in macaque, which is proportional to the increase in brain diameter in these species from 2 cm to 8 cm. Since in this type of fiber conduction velocity is proportional to axon diameter, the largest axons may therefore hold cross-brain conduction time relatively constant across species.

8. However, the benefits of building larger axons come with several metabolic costs. Wider axons require more metabolic energy to construct and maintain, as does the addition of myelin. These adaptations also increase mean conduction distance by increasing the total white matter volume. Moreover, axonal capacitance must be discharged and recharged during an action potential. Thus wider axons tend to consume more energy (though myelination drastically reduces the energetic cost of an action potential). These trade-offs may place white matter under selection pressure to balance the benefits of fast conduction velocity against the costs of building and operating wider axons.

9. The neocortical surface area to brain volume observes a power law relationship that accurately predicts surface area in most species within 15 percent. Deviations from this relationship can be categorized relative to the overall trend as either less folded than expected (lissencephalic) or more folded than expected (gyrencephalic). Animals such as the manatee, beaver, and platypus are lissencephalic, while the spiny anteater is gyrencephalic. A variation on this theme is cetaceans, which have folds that are more numerous than expected but also are decreased in diameter and depth (polymicrogyria). In some cases the thickness of the gray matter sheet is also aberrant: in manatees the gray matter is unusually thick (approx. 4 mm), while in cetaceans the cortical sheet is unusually thin.

10. If white matter is composed of closely packed axons, surface area is expected to be proportional to both the number and the mean cross-sectional area of white matter-projecting axons. This model makes quantitative predictions about how these cellular parameters are related to macroscopic folding. One possibility is

that lissencephaly may be caused by a shortage in the number of neurons or white matter-projecting axons. Alternatively, axons in lissencephalic animals may be unusually narrow relative to the general scaling trend. In some cases, such as the beaver, natural lissencephaly is accompanied by the presence of an exceptionally thick gray matter layer compared with similarly sized mammalian brains. It could be that the optimization principles operating in other mammals do not apply to lissencephalic animals, or that some biophysical or other functional adaptation leads to both the appearance of surface smoothness and thicker gray matter. In either case the coincidence of several unusual macroscopic parameters warrants further scrutiny on a microscopic level.

11. A functional rationale for cellular scaling relationships should ultimately be able to explain differences not only among species, but also among different cell types. During brain evolution the neocortex has undergone expansion, addition of areas, and the elaboration of novel neuronal subtypes leading to great diversity in sensorimotor and cognitive specializations. In particular, primates possess three unusually large neuron types of the neocortex: Betz, Meynert, and spindle cells. These cell types are exceptionally large in big-brained primates, and may give rise to axons that are disproportionately large and have faster conduction velocity. This would be consistent with the possibility that certain neo In addition to these natural exceptions, other useful tools for investigating neocortical form are induced malformations. Such exceptions are informative because they usually generate suboptimal neocortical layouts by perturbing developmental mechanisms, thereby illuminating the link between development and evolutionary optimization.

12. Other useful tools for investigating neocortical form are induced malformations. Such experiments are informative because they usually generate suboptimal neocortical layouts by perturbing developmental mechanisms, thereby illuminating the link between development and evolutionary optimization. For example, removal of frontal lobes leads to the formation of convolutions in visual cortex. This result is consistent with the interpretation that when long-distance projections are removed, local connections generate much of the remaining force. A more extreme version of this manipulation is global chemical ablation of projection neurons of layer III, a major source of long-distance corticocortical axons; this manipulation leads to polymicrogyria. Eye enucleation, which leads to reduction in the size of the lateral geniculate nucleus, induces folds in primary visual cortex. In this case a key event may be reduction in the amount of input from the lateral geniculate nucleus, and a concomitant reduction in force or synaptic volume due to the axons of that pathway.

GENERAL COMMENTS AND DISCUSSION

Fuster

Very interesting observations on myelin and on the surrogate development of cortical areas. Regarding the latter, has anyone studied the functional consequences—in terms of vision—of the extraordinary development of convolutions in the striate cortex after removal of frontal lobes?

Kawaguchi

To construct the structural model of cortex quantitatively, we do not have the quantitative parameters at the electron microscopic level, such as the synaptic density of axons from each neuron type. Stereological assessment of number of specific synapses per unit volume will be necessary.

Javier DeFelipe, Lidia Alonso-Nanclares, Jon Arellano

Microstructure of the neocortex: Comparative aspects.

REMARKS AND MAIN CONCLUSIONS

1. The neocortex of all species contains a set of elements similar to that of any other part of the brain. That is, two major types of neurons (projection cells and interneurons), glia (astrocytes, oligodendrocytes and microglia), nerve fibers (extrinsic and intrinsic) and blood vessels. Furthermore, the physiological properties, neurotransmitters and neuroactive peptides, receptors and ion channels, and other compounds generally expressed by cortical neurons are not unique to the neocortex, but are found throughout the brain. Thus, one of the fundamental questions in neuroscience is, what neural substrates make a human being human? In other words, what is special about the neocortex of humans and how does it differ from that of other species?

2. In general, despite of the large number of different elements that contribute to neocortical circuits, it is thought that neocortical neurons are organized into multiple, small repeating microcircuits, based around pyramidal cells and their input-output connections. These inputs originate from extrinsic afferent systems, excitatory glutamatergic spiny cells (which include other pyramidal cells and spiny stellate cells), and inhibitory GABAergic interneurons. These microanatomical characteristics have been found in all cortical areas and species examined so far and, therefore, they can be considered as fundamental aspects of cortical organization.

3. It is clear that cortical areas show important differences within the same and different species. The contention is that, since the times of Cajal, some researchers have emphasized the similarities whereas others the differences. For the former group of researchers the

histological differences are essentially fortuitous, the functional differences of the various cortical areas are a consequence of the differential connectivity of their afferent and efferent fiber systems. For the second group, the morphological differences between cortical areas are fundamental as are the differences in connectivity.

4. In 1980, a highly influential paper by Rockel, Hiorns and Powell emphasized the basic uniformity of the cortex. They conclude "in mammalian evolution the area of neocortex increases in larger brains but the number of and proportions of neuronal types through the depth remains constant, except in area 17 of primates. From these and other findings it is suggested that the intrinsic structure of the neocortex is basically more uniform than has been thought and that differences in cytoarchitecture and function reflect differences in connections." However, using other more appropriate quantitative methods (including the disector method), it has not been possible to confirm these conclusions in the rat and cat, nor that each cortical unit contains a similar number of neurons in a variety of other species. Thus, the notion of a basic uniformity in the neocortex, with respect to the density and types of neurons per column is not valid for all species. Therefore, these features are not an essential or general feature of the neocortex.

5. A number of studies support the theory of evolutionary diversity with respect to the morphology and neurochemical characteristics of both pyramidal cells and interneurons. With regards interneurons, a remarkable case of interspecies variation is that of the so-called double bouquet cell, the source of a large number of inhibitory synapses on dendrites within a very narrow column of cortical tissue. These cells seem to constitute a key element of the minicolumnar organization of the cortex. However, while the morphology and distribution of double bouquet cells is similar in the human and macaque neocortex, they are modified or less numerous in the neocortex of other species (*e.g.*, the cat), and may even be absent (*e.g.*, in the mouse and rat). Thus, differences in the morphology, number or distribution of double bouquet cells may represent fundamental differences in cortical microorganization between primates and other species.

6. Immunocytochemical analyses have demonstrated important differences in the proportions of interneurons between species. For example, in the rat GABAergic cells form 15% of the population in all cortical areas whereas in the primate, they reach 20% in the visual cortex and upto 25% in other cortical areas. The higher proportion of GABA neurons in the primate cortex raises three intriguing but not mutually exclusive possibilities. Firstly, there could be an increase in the number of all types of interneurons already present in non-primate mammals. Alternatively, an increase in the number of certain types of interneurons may have occurred. Thirdly, new types of interneurons may have been added during evolution in the primate cortex.

7. Differences or similarities in the density of synapses have been reported in various cortical areas and species. A pioneer in comparative ultrastructural studies of the neocortex, Cragg observed that there was relatively little variation in synaptic density between areas that were so cytoarchitectonically and functionally different as the motor and visual cortex of the mouse and monkey. From their own data, and that of Cragg, a number of authors argued that the numerical density of synapses was relatively constant throughout the cortical layers, as well between different cortical areas and different species.

8. The uniformity in synaptic density led to some authors to propose that it probably reflects the optimal number of synapses, and that it may due to some limiting metabolic or structural factor. However, the methods used by the different authors to estimate synapse number are rather different, including whether or not stereological methods were used. In addition, the cortical layers have not been examined systematically and most comparisons are only qualitative and not based on statistical analysis. Furthermore, there is no general consensus for counting synapses and, therefore, it is often difficult to compare data obtained in different laboratories. Here we have reviewed some of these issues and compared them with data we have obtained regarding the differences in the cortical circuits of distinct species and cortical areas. We have studied the morphology and density of synapses, in relation to their depth in the cortex (from layer I to layer VI), in the adult mouse, rat and human. We applied the same method to quantify synapses in the thin neuropil of the visual cortex and the somatosensory cortex of the mouse (area 17 and barrel cortex, respectively), the rat hindlimb area, and the anterolateral temporal cortex (T2–T3) of the human. Furthermore, the neuronal density was also calculated in this material to compare and estimate the number of synapses per neuron in each cortical layer and species.

9. Since the thickness and neuronal density in different layers and species may differ, comparing the density of synapses between species alone is difficult to interpret in terms of connectivity. Therefore, it is common to divide the synaptic density by the corresponding neuronal densities in the same brains to estimate the average number of synapses on each neuron. Using this approach, several authors found an inverse relationship between neuronal density and the number of synapses per neuron and suggested that this inverse relationship was due to a limiting factor such that neurons receiving more synapses would have a more complex dendritic arborization, increasing the distance between their cell bodies. In contrast, neurons

receiving fewer synapses would have a less complex dendritic arbor, allowing them to be more densely packed.

10. We found here that there are dramatic differences in the synaptology of the neuropil between the human, rat and mouse. There were significant differences in the density of synapses between the three species. Regarding, the proportion, length and density of asymmetrical and symmetrical synapses, and the ratios of asymmetrical and symmetrical synapses per neuron, there were also notable laminar specific differences between human, rat and mouse, which did not necessarily affect the same layers. Furthermore, the density of synapses was not inversely correlated with the density of neurons in the three species. Thus, certain general features of the neocortical synaptology are applicable to the human, rat and mouse, but we also detect significant differences and this means that the pattern of synaptic organization is characteristic of each cortical area and species.

11. The main sources of asymmetrical synapses are the corticortical and thalamocortical axons, and the local axon collaterals of pyramidal cells and spiny stellate cells, which are known to be excitatory. In contrast, the main sources of symmetrical synapses are the inhibitory GABAergic interneurons. Furthermore, synaptic size plays an important role in the functional properties of synapses. For example, larger synapses seem to contain a greater number of postsynaptic receptor and are associated with a greater number of docked synaptic vesicles. We have shown that in each species, the cortical neuropil has its own characteristic layer-specific synaptology. Thus, differences in the density, proportion and size of excitatory and inhibitory synapses among cortical areas or species probably reflects the functional differences of cortical the circuits involved.

12. There is approximately a 10% increase in the proportion of GABA interneurons in primates when compared to rats. This must be considered in conjunction with the fact that certain subtypes of interneurons are lacking or greatly modified in some species. If the species differences in the number of synapses per neuron and, therefore, in the synaptic weights, are also taken into account, then this might serve to emphasize the variability in the design of microcircuits between cortical areas and species.

13. The laminar specific similarities between the human, rat and mouse and other species with respect to the percentage, length and density of asymmetrical and symmetrical synapses, and in the number of synapses per neuron, might be considered as basic bricks of cortical organization. In contrast, the differences probably indicate evolutionary adaptations of excitatory and inhibitory circuits to particular functions.

GENERAL COMMENTS AND DISCUSSION

Fuster

A unique application of a uniform and meticulous morphometry of synapses to a comparative (across-species) study. I would venture that the relatively large increase of GABA interneurons in the primate has to do, among other things, with the relatively greater importance of inhibition and selectivity in perceptual and executive cognitive functions.

Rockland (point 4)

The 1980 paper by Rockel *et al.* has indeed been highly influential, although the result was already questioned in 1989 by Beaulieu and Colonnier. It is important to emphasize, as do DeFelipe *et al.*, that the original result needs to be regarded with caution, and in fact does not seem valid in its original formulation.

Wang, Hof, and Harrison

DeFelipe *et al.* quite accurately note that the number of neurons per discrete vertical cylinder of neocortical gray matter is unlikely to be constant. However, columns tend to be wider in larger-brained mammals (see our review, this volume). A constant quantity may instead be the number of neurons per column. It would be of great interest to probe this both within and among species (especially in unusual cases; see Catania review, this volume). The issue of whether neocortex is always parcellated into columns is unsettled. But just as repeating structures in crystals have a characteristic unit diameter, so too the neocortex may have motifs with a characteristic lateral length scale, with columns representing a special case. In this case the true invariant quantity may be the number of neurons per "repeating unit column." This quantity is also of interest in terms of developmental mechanisms (Rakic, *TINS* **18**, 383–388, 1995).

Guy Elston

Cortical heterogeneity: implications for visual processing and polysensory integration.

REMARKS AND MAIN CONCLUSIONS

1. Pyramidal cells constitute the largest group of cortical neurones, and form the majority of interareal connections. Arguably, they are the principal neurones of the cerebral cortex, generating nearly all facilitation (excitation) of cortical origin. They are characterised by many types, but are distinguished by their prominent apical dendrite and basal dendritic arbor.

2. Pyramidal cell morphology has been shown to vary in a systematic fashion such that cells in visual association areas are larger and more spinous than those in the primary visual area. Various aspects of these

structural differences appear to be important in influencing neuronal function.

3. As a relative measure of the number of excitatory inputs received by a given pyramidal neurone, one can count the number of dendritic spines, each of which receives at least one asymmetrical synapse, the presynaptic terminals of which have been shown to contain the excitatory neurotransmitter glutamate. Thus, more spinous pyramidal cells such as those in cytoarchitectonic areas TEO, TE and the superior temporal polysensory area (STP) are likely to receive more excitatory inputs than less spinous cells such as those in V1.

4. The majority of inhibitory inputs to cortical pyramidal cells are located on the dendrites and dendritic spines; thus, differences in the arbor structure of pyramidal cells may result in the integration of different numbers of inhibitory inputs within their arbors... pyramidal cells with larger dendritic arbors may be able to integrate from a greater number, and diversity, of interneurones than those with smaller arbors.

5. Cortical neurones characterised by a smaller dendritic arbor may integrate inputs over a smaller region of cortex than larger cells. In topographically organised visual cortex, this translates to a smaller portion of the sensory map being sampled.

6. The size of the intrinsic axonal patches that arise from supragranular pyramidal cells is also correlated with the size of their dendritic arbors.

7. It has been suggested that a correlation in the geometrical relationship of axon modules (*e.g.*, intrinsic patches, cortico-cortical arborisation, or thalamocortical afferents) and dendritic arbors determine the sampling ratios of neurones. According to this theory, the correlation between intrinsic patches and the basal dendritic arbors of pyramidal cells in supragranular cortex would result in maximal sampling diversity. Furthermore, the correlation between interareal axon arborisation and the dendritic arbors of pyramidal cells further suggests that the geometrical relationship between inputs and pyramidal cells is functionally significant.

8. However, while columnar axonal arborisations may be a feature of cortical organisation, not all projections form such arborisations. In many cases, interareal projections form more diffuse arborisation which may, or may not, extend throughout the cortical depth. Thus, any particular region in cortex may receive projections from different sources, which are characterised by different arborisation patterns. Consequently, cells throughout the cortical layers may sample inputs with different transverse and tangential geometrical relationships. Thus, in some cortical areas such as V1 and V2 it may be advantages to maintain distinction between inputs within modules, whereas in other areas, such as those in inferotemporal cortex, different sampling strategies may subserve different functional requirements.

9. Various converging lines of research suggest that visual processing is more complex than the proposed serial hierarchical schemes. For example, inactivation of V1 doesn't result in complete blindness, the response latencies of neurones in different cortical areas and the pattern of their corticocortical projections don't necessarily comply with the proposed hierarchical schemes, and imaging studies reveal that large ensembles of neurones in different hierarchical levels may be activated during a particular task. An alternative, and more flexible, theory is that cortical processing occurs within distributed systems.

10. Simultaneous activation of cortical areas during a particular task not only depends on the patterns of connectivity between cortical areas, but also the degree of connectivity: the degree of connectivity being conferred by both the number of axon projections/boutons and the number of inputs that can be received by target neurones.

11. It is not unreasonable to conclude that regional specialisation in pyramidal cell structure within distributed system potentially leads to a richness of diversity of, and functional cohesiveness in, cortical function not attainable in cortex composed of the same basic repeated circuit. How regional specializations in pyramidal cell (and circuit) structure act in concert with subcortical and interhemispheric connections, as well as top-down modulation, in visual processing remain challenges for future studies.

GENERAL COMMENTS AND DISCUSSION

Fuster

Here are morphological differences between cortical areas that make considerable sense in functional terms. Small dendritic arbors make sense in primary sensory cortex, where the mapping of the external environment is based on spatial topology and relies heavily on modular organization. Spinous pyramidal cells with long axons make sense in association cortex (*e.g.*, TE, STP), where long-distance association is the rule.

Fujita

Comparison of neuronal morphology among different cortical areas provides a new dimension where the relation of morphology and physiology is addressed to obtain clues for a basic understanding of the workings of the cerebral cortex. Given the marked and systematic difference in the dendritic and axonal morphology of pyramidal neurons, similar analysis should be applied to each subtype of GABAergic interneurons to address whether any regional difference exists. Not only the density and the proportion of subtypes of GABAergic neurons, but the size or the complexity of dendritic arbors of each subtype may differ among different cortical regions. Studies of prenatal and postnatal development

of pyramidal neurons also tell us about how their area-specific morphology comes about, and whether the correlation between the size of the intrinsic horizontal axonal patches and the dendritic arbors reported in adult animals holds for developmental changes.

Ruth Benavides-Piccione, Inmaculada Ballesteros-Yáñez, Javier DeFelipe, Rafael Yuste

Cortical area and species differences in dendritic spine morphology.

REMARKS AND MAIN CONCLUSIONS

1. Dendritic spines receive most excitatory inputs in the neocortex and are morphologically very diverse. Recent studies have demonstrated that spines compartmentalise calcium, are constantly moving and changing shape and that spine formation, plasticity and maintenance depend on synaptic activity and can be modulated by sensory experience. In spite of these recent results, the function of dendritic spines is still somewhat mysterious. Because excitatory inputs can be made on dendritic shafts, spines must be serving a specific function, which could range from implementing learning rules to minimising axonal wire.

2. An important aspect of the dendritic spines is the enormous diversity in their morphologies, something which was already noted by Cajal and which could be important to understand their function. Indeed, there appears to be a clear relationship between the morphology and function of the spine, particularly with relation to the size of the spine head and the length of the neck. For example, the volume of the spine-head is directly proportional to the size of the postsynaptic density, the number of postsynaptic receptors, to the presynaptic number of docked synaptic vesicles and the ready releasable pool of neurotransmitter. Also, spines with longer necks show longer time constants of calcium compartmentalisation than spines with shorter necks. Therefore, the morpholoy of dendritic spines has a direct functional relevance since it reveals key characteristics of synaptic inputs and their biochemical compartmentalisation.

3. In order to explore whether systemic differences in spine morphologies or densities exist among species or among cortical areas, we reconstructed and measured spines from human temporal cortex and mouse temporal and occipital cortex. Spines were labelled using intracellular Lucifer Yellow injections in fixed material and immunocytochemistry.

4. Our first finding is the existence of large differences in spine densities between human and mouse cortex. Differences in spine densities across species have been reported before and our results confirm and extend these findings. It is commonly assumed that every spine has an excitatory synapse, although to our knowledge, this has not been demonstrated unambiguously. Nevertheless, the large differences in spine densities that we report between mouse and human cells, together with the larger dendritic length of human cells ($\sim$70% larger average dendritic length in human vs. mouse basal dendrites) implies that human pyramidal neurons can integrate a substantially higher number of inputs that their mouse counterparts.

5. In addition, we also encountered major differences in spine head areas in different cortical regions, whereby spines from human temporal cortex are larger than those from mouse temporal cortex, which are themselves larger from those in mouse occipital cortical neurons. These differences in maximal cross sectional area must translate into even larger differences in volume and these volume differences linearly translate into differences in a host of physiological parameters.

6. Assuming as an approximation that spine heads are spherical, we can estimate that human temporal spines, which are 60% larger in area than mouse temporal spines, should have on average close to 100% larger volume. This could correlate into a doubling of the number of postsynaptic receptors, double the number of docked vesicles and of ready releasable pool in the presynaptic terminals. Therefore the functional impact of human spines, and the current that they inject into the dendrites, must be much larger than those from mouse neurons. A similar estimate, comparing mouse temporal and occipital spines, would suggest that mouse temporal spines, with 20% larger area than occipital spines, could have $\sim$30% larger volumes and similarly larger number of receptors and docked vesicles.

7. It is important that there are significant differences between cortical areas in spine size, because this implies that the average synaptic current is modulated according to cortical region.

8. We also observe differences in spine neck length between human and mouse samples, whereby human spines have $\sim$30% longer necks than mouse temporal or occipital ones. Interestingly, in all three populations, the bimodal distribution of spine neck lengths indicates the existence of at least two populations of spines: one with no necks, and another with necks. Given the relation between spine neck length and biochemical compartmentalization, we hypothesize that, rather than a continuum of spine with respect to their neck lengths, there are two distinct populations of spines, ones which are biochemically isolated from the dendrite and another one which are not. In addition, humans spines appear to be on average more biochemically isolated that mouse ones.

9. We have searched for co-regulation of spine density, head size and neck length and have failed to encounter significant correlations among these three parameters. The lack of correlation between spine head area and neck length implies that they are regulated independently.

10. It is conceivable that the neck length reflects the consequences of a developmental process, by which the spine grows to different length according to the input it wants to contact. Meanwhile the spine head size could be determined by the nature of that input and the life history and previous use of that synapse.

11. We would argue that spines have a specific function, one that is likely to be of central importance in the cortical circuit. Whatever this specific function is, it appears to be carried out more effectively in human cortex than in mouse cortex. If spines are providing the circuit with implementations of local learning rules, humans could have a richer and more flexible circuit with more opportunities to regulate inputs. Even a cursory comparison between human and mouse spines underscores this point: human spines are enormous and have large necks and occur in great densities.

12. It is therefore fair to argue that human pyramidal neurons are more "spiny" and is tempting to speculate that mental differences between humans and other mammals could be attributed to the increased number of spines. Furthermore, our data provides evidence for there are substantial morphological differences at the spine level, differences that might underlie cognitive differences.

GENERAL COMMENTS AND DISCUSSION

Fuster

Assuming that large spines facilitate the temporal and spatial summation of EPSP's, their prevalence in the human brain would seem to indicate that this brain is well equipped to encode information that integrates many inputs and sources across space and across time.

Elston

This is an exciting finding in which Benavides-Piccione and colleagues demonstrate regional differences in spine morphology between pyramidal cells in the occipital and temporal lobes of the mouse. Because spines were sampled from two cortical regions of the same hemisphere the finding is not subject to possible interindividual error or functional state of the animal. Instead, the data are a robust demonstration of regional differences in spine structure at an instance in time. These data are compared with those obtained from human temporal lobe, which are shown to be measurably different. In this study the authors reveal regional/species specialisations in cortical circuitry at a new level, to the resolution of the dendritic spine. Studies designed to address the functional implications of these morphological specialisations are likely to yield important findings.

Wang, Hof and Harrison

The physical properties of neurons play a critical role in how the mammalian neocortex processes information (see our review, this volume), and biophysical analysis may be of particular help in understanding comparative ultrastructural measurements. Benavides-Piccione *et al.* report new measurements demonstrating that basal dendritic spines of human layer 3 pyramidal neurons have larger areas and longer necks than their mouse counterparts. They suggest that larger spine areas may imply more glutamate receptors. We suggest that this difference may be a general scale-up property of all large brains, not just human ones. Since larger brains have a thicker gray matter layer, pyramidal neurons have longer dendrites on average and may attenuate synaptic potentials more as they are transmitted from spine to soma. More receptors would then be necessary to compensate. Indeed, in hippocampal pyramidal neurons, more distant synapses may have more glutamate receptors (Andrasfalvy & Magee, *Journal of Neuroscience* **21**, 9151–9159, 2001). Benavides-Piccione *et al.* argue that in spines with necks, necks length may influence compartmentalization of biochemical signals. However, in the case of calcium this is unlikely because in the absence of indicator dye, diffusion through spine necks makes very little contribution to calcium clearance (Sabatini *et al.*, *Neuron* **33**, 439–452, 2002). Differences in spine neck length may have other consequences such as altering the number of possible presynaptic partners (Stepanyants *et al.*, *Neuron* **34**, 275–288, 2002).

Yuste's response to Wang, Hof and Harrison

We disagree with Wang's and colleagues interpretation of Sabatini's data. The role of calcium pumps, which we ourselves first discovered (Majewska *et al.*, *J. Neuroscience* **20**, 1722–1734, 2000) appears to vary from spine to spine (see Holthoff *et al.*, *Neuron* **33**, 425–437, 2002), to the point that we suspect that some spines could even lack functional pumps. Also, I am sure everyone would agree that diffusion is likely to dominate calcium kinetics in stubby spines without necks, which constitute a substantial percentage of the spines we have reconstructed (see our Fig. 2C), and are also generally underestimated due to their small sizes. We have provided a more detailed rebuttal to Sabatini's criticisms in Holthoff *et al.* (*Trends Neurosci* **25**, 433–435, 2002). In addition, Wang and colleagues should consider that calcium is probably just one out of many biochemical pathways that are likely to play important roles in spine function. Therefore the length of the spine neck many not just influence calcium compartmentalization but regulate spine biochemical compartmentalization in general. This is the argument we are specifically making in our discussion.

DeFelipe's response to Wang, Hof and Harrison

Regarding the argument of Wang and colleagues that "since larger brains have a thicker gray matter layer,

pyramidal neurons have longer dendrites," in a previous study (Elston *et al.*, *J. Neurosci* **21**, RC163, 1–5, 2001) we have shown that the size of the basal dendritic arbor of pyramidal cells does not necessarily correlate with brain size. As pointed out by Elston and colleagues, pyramidal cells in different cortical regions/species are not merely scaled versions of the same cell type, but are structurally different (Elston & Jelinek, *Fractals* **9**, 297–303, 2001; Jelinek & Elston, *Fractals* **9**, 287–295, 2001).

Kenneth Catania

Barrels, stripes, and fingerprints in the brain—Implications for theories of cortical organization.

REMARKS AND MAIN CONCLUSIONS

1. The columnar hypothesis suggests that the fundamental unit of cortical organization is a cylindrical column (a macro column) of interconnected neurons, running from layers II through VI, and having a diameter of approximately 300 to 600 μm.

2. For cortex to have a columnar organization, it is not simply that neurons in one layer are functionally related to those in more superficial and deeper layers, but rather there must be a definable horizontal component to these functional groupings as well. Perhaps the best way to envision this configuration is to imagine the consequences of compressing the 6 cortical layers into a two-dimensional sheet. A cortex organized as cylindrical columns then becomes a series of circular units, much like the barrels visible in sections of mouse layer IV cortex.

3. Barrels are a relatively common structure among rodents, and are also present in rabbits and at least some marsupials. Cortical barrels reflecting the representation of the whiskers have also been identified in Insectivores. In each species the pattern of barrels is dictated by the pattern of whiskers on the face and, significantly, the barrels are harder to identify in a given rodent suborder as brain size increases.

4. The clarity of structures in layer IV cortex was further improved in studies where the cortex was dissected free from subcortical matter and precisely flattened between glass slides. This approach, along with the development of new methods for processing brain sections histologically, particularly the cytochrome oxidase procedure, revealed barrels with great clarity and it became clear that barrel-like structures where present in areas of somatosensory cortex representing a number of different body parts. For example the representation of the forelimb in rats is characterized by a number of cytochrome oxidase-dense ovals that have a form similar to the barrels representing whiskers. These findings, along with the common occurrence of barrels representing whiskers in nissl stained tissue, strengthened the impression of cortex as being composed of fundamentally circular units in the form of the traditional cortical column.

5. Cortical modules reflecting the distribution of mechanoreceptors are not restricted to barrels, nor are they restricted to primary somatosensory cortex. However, much of the data regarding modular representations of sensory surfaces, sometimes called "cortical isomorphs," has been found in relatively small mammals. These cortical specializations correspond to specialized sensory surfaces such as rodent whiskers and the unusual hand of the eastern mole, or stranger structures such as star appendages and the platypus bill. The impression from these studies, reinforced by comparative studies of the rodent barrel system, is that reflections of the distributions of mechanoreceptors in cortical structures are restricted to unusual small mammals. However recent findings in the somatosensory cortex of large-brained primates are beginning to dispel this impression. In particular, the discovery of a series of cortical subdivisions representing the digits and palm in primate area 3b suggests that similar developmental mechanisms operate to shape cortical modules in mammalian species ranging from rodents to primates.

6. In three primate species, flattened sections of cortex processed for myelin were found to contain visible, myelin dense cortical modules in area 3B, faithfully reflecting the distribution of mechanoreceptors from the hand. In essence, the cortical representation of the primate hand, like the representation of rodent whiskers and the mole's star, is visibly reflected in flattened sections of cortex processed for myelin. These findings are surprising in light of numerous studies of cortical plasticity that indicate the primate hand representation is a flexible entity capable of relatively rapid reorganization. Further exploration of area 3B at the rostro-lateral extreme of cortex also revealed a series of myelin rich ovals that represent the teeth, lips, and tongue in a pattern consistent across individuals.

7. These findings in primates indicate that both small and large brained mammals share common developmental mechanisms that segregate sensory maps in similar ways. More generally, results from the diverse somatosensory systems described above- in contrast to inferences drawn from barrel cortex alone—suggest that cortical modules of neuronal circuitry are not constrained to form circular columns. Rather, modules in the somatosensory system tend to reflect the distribution of mechanoreceptors in the sensory periphery. These findings further suggest a ubiquitous instructional role for the sensory surface in guiding the formation of the details of central representational maps.

8. Most current investigators would probably agree that cortical and subcortical sensory maps are formed by a combination of intrinsic (genetic) and extrinsic (epigenetic) influences, although the relative role of each remains the subject of intensive investigation.

Recent evident suggests a number of regulatory genes specify the overall location of sensory areas (*e.g.*, V1, S1, A1) in the cortical sheet by forming complementary gradients of gene expression during embryonic development. However, there is also clear evidence that information from the periphery is required for the formation of accurate representations of the details of sensory surfaces, and these details likely include the shape and form of cortical modules.

9. Information from the peripheral sensory sheet may be conveyed to the cortex by activity patterns or possibly by transported chemical signals. The septa that form borders between the modules described here for the somatosensory system generally correspond to discontinuities in the peripheral sensory sheet. These discontinuities cause sudden transitions in the degree of correlated activity arising from the sensory periphery, providing a potential substrate for Hebbian mechanisms to produce cortical modules that process information from islands of coactivated primary afferents during development. Alternatively, or in addition to information conveyed by activity patterns, transported chemical cues might communicate the locations of groups of co-activated primary afferents. However there is considerable evidence for the importance of activity patterns in shaping cortical modules, particularly in the visual system of primates and similar mechanism likely play an important role in the somatosensory system as well.

10. The importance of information from the periphery in shaping cortical modules raises a number of interesting issues. For example, why do some species exhibit cortical subdivisions while others with similar sensory organs do not? Why are barrels the most prominent and common reflection of the sensory periphery in the brain? Do modules enhance cortical processing, or are they simply a by-product of competitive neuronal interactions inherent to mammalian brain development?

11. Given the relatively widespread occurrence of cortical modules in the somatosensory system, an obvious question is what function they perform and how they might enhance cortical processing. One possibility is that they reflect the need to group neurons dealing with similar areas of sensory space together while excluding connections from inappropriate topographic regions. This kind of organization might allow for maximum efficiency in connecting multiple brain areas into processing networks that require homotopic and often reciprocal interconnections. Once modules originate in a given lineage, they might be modified to perform new and more complex functions.

12. In contrast to such functional considerations, it has also been argued that the inconsistent expression of patent modules across species and systems indicates that they are not particularly important for sensory processing. Instead they might simply be the result of general developmental mechanisms that group neurons and their connections together based on coordinated firing patterns and competitive interactions, a process the produces useful circuits throughout the nervous system and sometimes results in visible modules as a by-product. The different groupings of interconnected neurons observed in the somatosensory system, such as barrels, stripes, and finger specific subdivisions, may be part of a continuum of anatomical specialization with the patently visible examples reflecting more general organizational trends that are simply difficult to detect in many cortical areas. It has also been argued that ocular dominance columns are unlikely to play an important role in visual processing because they are capriciously expressed across species and across individuals within a given species, despite the lack of any obviously different visual abilities.

13. A somewhat different approach might be to look for evidence of developmental mechanisms that promote module formation but have no other obvious purpose. One possible conclusion from these observations is that the segregation of sensory organs has no important function in the sensory periphery, but rather functions to induce subdivisions in somatosensory cortex that form a framework for the organization of cortical circuitry.

14. In addition to affecting how cortical modules form in cortex, developmental events centered at the sensory periphery may also affect the sizes of cortical representational areas. For example both the somatosensory fovea in the star-nosed mole and the visual fovea in primates develop earliest and both of these regions are represented by disproportionately large areas of cortical representational space. By developing earliest these regions may get a head start in a competition for cortical space. These observations raise the intriguing possibility that evolution makes use of developmental mechanisms centered at the peripheral sensory surface to shape more efficient organization of the central nervous system through cascades of inductive events.

GENERAL COMMENTS AND DISCUSSION

Fuster

The modular mapping of the periphery in sensory cortex is phylogenetically determined and ontogenetically secured. I do not think it is a by-product of function. If Hebbian mechanisms have intervened there, they must have done it in evolutionary temporal scales.

Elston

Catania's thesis that "cortex is not constrained to form circular units in the form of traditional cortical column" is likely to be controversial. Here Catania presents a persuasive discussion based on data from studies of the somatosensory representation of the bill of the platypus and star nosed mole that "columns" are not a

necessary organizational principle in mammalian cortex. Instead, he concludes that specialized cortical organization sometimes seen in primary sensory areas in mammalian cortex reflect the sensory periphery. In this review Catania provides us with a sober reminder that, even within primary sensory areas, there is great diversity in mammalian cortical organization.

Ichiro Fujita

The inferior temporal cortex: Architecture, computation, and representation.

REMARKS AND MAIN CONCLUSIONS

1. Individual neurons in cytoarchitectonic area TE of the inferior temporal cortex and in the superior temporal sulcus (STS) respond preferentially to particular shapes, textures or patterns, color, shapes combined with color or texture, or complex object images such as faces or objects that are used for learning or familiarization tasks. A substantial population of neurons in the V2, V4, and TEO areas respond better to shapes such as crosses and T-shapes, and to polar or hyperbolic gratings, than to bars, edges, or linear gratings.The stimuli necessary for strong activation of neurons in these areas is, however, generally simpler than those that excite TE neurons. Object information carried by single neurons is thus transformed gradually into complex forms in successive areas. Except for neurons selective for faces or learned objects, however, TE neurons respond to stimuli of intermediate complexity, which are simpler than those from ordinary objects.

2. Neurons responding to similar stimuli or those with correlated stimulus selectivity cluster locally within the TE. These clusters are columnar in shape; neurons with shared stimulus preferences are arrayed vertically across the cortical layers, but are localized within a range of 0.4–0.5 mm across the cortical surface.

3. Neurons in a TE column are not identical, though similar or correlated, to each other in their stimulus selectivity.

4. The columnar organization of the TE suggests that when a monkey sees an object, a particular group of columns will be activated by the image, because an ordinary object is rich in features, different features of an object will activate different columns, and each feature may activate multiple columns.

5. We previously proposed a hypothesis ("visual alphabet hypothesis") about how the TE columns represent objects. In this scheme, we hypothesize that individual columns encode particular object features to which ouput neurons in the respective columns respond. Since different objects contain different combinations of features, which activate different subsets of columns, the combination of active columns, in principle, can specify the whole object. As the 26 alphabets in English produce a million of words, 1300–2000 columns in the TE can represent an enormous variety of objects by virtue of combination of intermediately complex features. Unlike phonetic symbols such as the alphabets, however, the features represented in columns are likely to be constrained by the physical laws or the statistics in the natural scenes. It is noteworthy that we encounter columns of neurons responding to T- or L-shapes or those responding to gradual changes of luminosity across animals.

6. The visual alphabet hypothesis thus postulates that a large number of TE neurons is engaged in representation of an object, but this is not a population coding in the sense of DeCharms and Zador. They distinguish a population coding in which information is explicit only in the relation among activities of multiple neurons and not encoded by individual neurons (as a symphony played by an orchestra) from a coding scheme where information is explicit in activity of individual members of an ensemble (as a voter's opinion in an election). In an orchestra, different instruments play together and create a synergy effect that any of the instruments alone cannot produce. In an election, the result of the election depends on the entire voters, but each voter has his/her own opinion. A combinatorial coding such as the visual alphabet hypothesis in its simplest form is similar to the latter coding scheme in the sense that each column (voter) represents a particular feature (opinion). Given the diversity of selectivity across neurons in a column, however, activities in each column can signal more than "yes" or "no" regarding the presence of a particular feature in an object, and therefore, activities across neurons within a column can form a population code.

7. The TE and other cortices share some of the basic patterns of the intrinsic fiber connection. For example, extensive vertical interlaminar connections exist; cells in layer 3 project heavily to layer 5, and cells in layers 4–6 ascend to layer 3. Another feature common to the TE and other cortices is horizontal axons originating from pyramidal cells and running parallel to the pia mater. Horizontally running axons can be observed in all layers. Those in layers 2 and 3 run for the longest length (4–8 mm), and produce distinct plexuses of terminal arborization (horizontal axonal "patches") at intervals.

8. The topographic features of horizontal axon patches markedly differ between V1 and TE. Patches of axonal arbors in the TE are larger, more widely spaced, and more irregularly distributed than those in V1. When we label horizontal axons by injecting an anterograde neuronal tracer, the labeling intensity of patches gradually declines with increasing injection-to-patch distance in V1, but this tendency is less apparent in the TE. This suggests that horizontal axons in V1 link nearby cortical sites more strongly than distant sites, but the connection by horizontal axons in TE

depends less on the distance between the two sites. The findings, taken together, suggest that the TE is an example of patchy brain map, whereas V1 represents a continuous brain map.

9. The size, center-to-center spacing, and spread of horizontal axon patches in areas V2 and V4 are intermediate between V1 and the TE, and thus there is a gradual change in these parameters along the occipitotemporal pathway.

10. Intrinsic horizontal axons enable distant sites within a cortical area to interact with each other. Horizontal axons in the TE are not for interactions between neurons responding to different parts of the visual field as proposed for V1, considering that TE neurons have largely overlapping receptive fields with each other. It is likely that the connections are related to the stimulus selectivity of columns. It is an open question whether horizontal axons connect columns responding to similar stimuli, different stimuli, or stimuli that occur together more often than the others due to the high correlational structure of the visual world.

GENERAL COMMENTS AND DISCUSSION

Fuster

The "visual alphabet hypothesis" of TE columns constitutes an interesting application of connectionist principles to unimodal association cortex. What is even more interesting is that those principles may continue to operate in higher cortices, there to form perceptual cross-modal classes, classes of classes, classes of classes of classes, and so on toward greater and greater abstraction.

Joaquín Fuster

Frontal lobe and cognitive development.

REMARKS AND MAIN CONCLUSIONS

1. The prefrontal cortex is the cortex of association of the frontal lobe. In the primate, human or nonhuman, the prefrontal cortex has three major anatomical aspects or regions: lateral, medial, and ventral or orbital. Each prefrontal region is subdivided into areas of varying cytoarchitecture, providing the grounds for a number of cytoatchitectonic maps. With few exceptions, such as that of area 8, which is largely devoted to the control of gaze and eye movements, it is not possible to ascribe a specific physiological function to any prefrontal area. However, it seems obvious that the prefrontal cortex is functionally heterogeneous.

2. Whereas it cannot be functionally parceled out with regard to its cytoarchitecture, there is substantial evidence that, as a whole, the prefrontal cortex performs a critical role in the organization of behavioral, linguistic, and cognitive actions. The psychological and physiological analysis of this role in the three action domains yields a topographic distribution of cognitive functions conforming to the following outline. All three prefrontal regions are involved in one or another aspect of attention. In addition, the medial and anterior cingulate region are involved in drive and motivation, the lateral region in working memory and set, and the orbital region (to some extent also the medial region) in the inhibitory control of impulses and interference.

3. The prefrontal cortex, like the rest of the neocortex or *neopallium*, evolves in the dorsal telencephalon between two older structures, the laterally situated olfactory (piriform) pallium and the medially situated hippocampal pallium. The precise evolutionary process that gives rise to the neocortex is unresolved. There are two major lines of thinking in this respect: One, that the neocortex develops as an expansion of those ancient structures; the other, that it develops from a ridge of cells along the dorsal wall of the ventricle. In any case, it is generally accepted that, with evolution, the neocortex as a whole increases in size and volume in proportion to body dimensions. The growth of the neocortex in evolution can be characterized as a veritable phylogenetic "explosion".

4. The most rostral aspect of the developing neopallium in primitive species constitutes what is to become the prefrontal cortex. Whereas the homology of the neocortex as a whole in the various mammalian species is undisputed, the homology of individual neocortical areas, prefrontal areas in particular, is a matter of some controversy. Nonetheless, the evidence from comparative studies of existing species and from the examination of the endocasts of specimens of extinct species leads to the conclusion that, in the course of evolution, the prefrontal cortex grows disproportionately more than other cortical regions.

5. Arguably, the disproportionate evolutionary growth of the prefrontal cortex parallels that of the associative cortex of temporal and parietal regions. It is a legitimate inference, in any event, that the evolutionary expansion of the cortex of association, both posterior and prefrontal, is closely related to the evolution of cognitive functions.

6. Judging from the evolutionary development of surface morphology (*i.e.*, sulci and gyri), as well as of the components of its thalamic nucleus (mediodorsal) and their cortical projections, the various portions of the prefrontal cortex do not appear to evolve equally at the same time. Rather, by those criteria, the lateral prefrontal region clearly evolves later and farther than the other prefrontal regions. This is in obvious agreement with the late and extraordinary development of higher integrative cognitive functions (*e.g.*, language) in higher species, especially the human. These functions, as we see below, are largely dependent on the lateral prefrontal cortex.

7. In accord with the principle that ontogeny recapitulates phylogeny, the prefrontal cortex is one of the cortical areas to develop most and last in the course of individual development.

8. Since the early studies by Flechsig, it has been known that the myelination of the various cortical areas follows a certain order. Although the precise order proposed by Flechsig has been disputed on technical grounds, it seems well established that the primary sensory and motor areas myelinate before the areas of association, the latter including the prefrontal cortex. It has reasonably been argued, on the basis of neuropsychological and linguistic data, that the cognitive development of the child is closely dependent on the development of cortical myelin. Until the publication of recent neuroimaging studies, however, it had not been surmised that in the human the myelinization of higher areas of association, notably the prefrontal cortex, was not complete until the third decade of life.

9. Myelin enhances the speed of axonal conduction, and thus it can be assumed to facilitate the processing in cortical networks. Myelination, however, is only one of the indices of cortical maturation. Others, less readily measurable, include the prolongation of axons and the arborization of dendrites. Perinatally, as in later life, the development of both the axons and dendrites of frontal areas seems to lag chronologically behind that of other cortical areas. Given the role of prefrontal networks in cognitive functions, it is reasonable to infer that the development of those networks underlies the development of highly integrative cognitive functions, such as language, that continue to develop well into adulthood.

10. The cortex of the frontal lobe is exceptionally well connected with other brain structures, both cortical and subcortical. In particular the prefrontal cortex, as studies in the monkey demonstrate, is arguably the best connected of all cortical structures.

11. The precise functional role of the connections of the prefrontal cortex is not entirely known, but can be inferred from the functional role of the structures with which it is connected. In general terms, the prefrontal-limbic connections are involved in the control of emotional behavior, whereas the prefrontal-striatal connections are involved in the coordination of motor behavior. Of special importance for the cognitive aspects of all forms of behavior are the reciprocal connections of the lateral prefrontal cortex with the hippocampus and with the posterior association cortices. There are well-demonstrated reciprocal connections between the hippocampus and the prefrontal cortex, especially its lateral region, although their exact path has not been completely clarified. Given the proven, though still obscure, role of the hippocampus in the acquisition of memory, it appears very likely that those connections participate in the formation of networks of motor or executive memory in the prefrontal cortex.

12. The principal and also most general function of the prefrontal cortex is the temporal organization of actions toward biological or cognitive goals. The prefrontal cortex—its lateral region in particular—specializes in the temporal structuring of new and complex goal-directed series of actions, whether in the form of behavior, speech, or reasoning. It is the novelty and complexity of those actions that qualify the prefrontal cortex as the so-called "organ of creativity." Further, the participation of the prefrontal cortex in the choice between alternatives, in decision making, and in executing temporally structured action are the reasons that this cortex has also been considered the "central executive."

13. In order to perform its integrative role, the prefrontal cortex must be accessible, or have access, to all the items of sensory, motor, and mnemonic information that form the structure of behavior at hand. One way to understand that accessibility in physiological terms is to construe the neuronal populations of the prefrontal cortex as cellular constituents of widely distributed cortical networks representing the structure of behavior and the associations between its constituent items. This would imply that the execution of temporally structured behavior is the result of the activation of that executive network and the timely activation of its constituent neuronal components.

14. Temporal integration is in turn served by at least three cognitive functions of somewhat different prefrontal topography: working memory, preparatory set, and inhibitory control. These functions engage the prefrontal cortex in interactive cooperation with other neocortical regions.

15. The development of language epitomizes the development of temporal integrative cognitive functions and their underlying neural substrate, notably the lateral prefrontal cortex and other late-developing cortical regions.

GENERAL COMMENTS AND DISCUSSION

Elston

This is a scholarly and succinct review in which Fuster highlights multiple converging anatomical and functional data sampled from the developing and mature brain of various species to address the role of prefrontal cortex in cognitive functions. Fuster defines what he considers some basic cognitive functions in prefrontal cortex; including temporal integration, working memory, preparatory set and inhibitory control, and discusses their topography. His seminal findings on the "neuronal correlates of working memory" (Fuster & Alexander, *Science* **173**, 652–654, 1971; Fuster *et al.*, *Exp Neurol* **77**, 679–694, 1982) are discussed and placed in the context of cognitive functions. Recent findings on the structural complexity of pyramidal cells in prefrontal cortex (Elston *et al.*, *J. Neurosci* **21**, RC163, 1–5, 2001)

shed new light on specialisations in PFC circuit structure in different primate species, which may influence the cognitive functions outlined here.

Wang, Hof and Harrison

As reviewed by Fuster, the developmentally late myelination that occurs in human prefrontal cortex appears to accompany cognitive maturation. This suggests a link between myelination, fast conduction, and function during human adolescence. Because of the tremendous decrease in capacitance that results from myelination, it is even possible that before maturation these axons do not conduct at all. In addition to being consistent with the opinions of many parents about adolescents, this speculation can be tested experimentally.