Horizontal integration in the neocortex

Charles D. Gilbert

From the classical work of Golgi anatomists and the discovery of columnar functional architecture, the cortex came to be thought of as a structure containing independent modular units (the columns), with most of the internal circuitry involving vertical connections within the modules (across cortical layers) and little horizontal communication between modules. However, the development of new techniques for tracing neuronal pathways has revealed the existence of extensive communication between distant cortical modules within each cortical area and considerable convergence in the projection from one cortical area to another. These discoveries have importance in understanding the way the cortex analyses the sensory environment, and are likely to be general features for all the operations the cortex performs.

The visual cortex of the cat and monkey is a good model for understanding the implications of the horizontal cortical connections. It is divided into many distinct areas, each of which is mapped retinotopically (for review see Van Essen and Maunsell). The retinotopic order is laid onto the cortex by the thalamic input, and is most clearly observed in layer 4, where the thalamic afferents terminate. However, as will be described below, the retinotopic order tends to degenerate somewhat outside of layer 4, and the receptive fields of some of the cells get substantially larger. This is likely to be due to the intrinsic connectivity of the cortex.

In the striate cortex there is a systematic relationship between cortical distance and distance travelled within the visual field. Considerably more cortical territory is devoted to a degree of visual space in the foveal representation than in the periphery, and receptive field size and scatter in receptive field position are much smaller in the fovea than in the periphery. Wherever one records in the striate cortex, moving a constant distance (about 1.5 mm) within the cortex corresponds to moving to a new part of the visual field, so that the receptive fields of cells separated by this distance do not overlap. This distance corresponds to two 'hypercolumns'. A hypercolumn is defined as a full cycle of orientation or ocular dominance columns, and in the primate is about 700 to 800 μm wide. The fact that one can traverse a full hypercolumn and remain in the same part of the visual field allows each point of the visual world to be analysed for all values of each parameter (all orientations and ocular dominances, for example). The retinotopic map of the cortex is therefore not so much a point-to-point representation as a point-to-patch representation.

Because of the retinotopic order of the striate cortex, it is somewhat surprising that cortical points are connected over very long distances, with individual cells projecting for more than 4 mm tangentially to the cortical surface. This extent of projection would presumably connect areas dealing with non-overlapping parts of the visual field. Studying the nature and functional implications of the long range horizontal cortical connections is likely to provide insights into the nature of cortical analysis of sensory information as well as the higher functions the cortex performs.

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Fig. 1. 3-Dimensional computer reconstruction of axon belonging to a layer 3 pyramidal cell. (a) and (b) represent two views rotated at 90° about the vertical axis. The axon extends from the cortical surface at the top of the reconstruction to the white matter at the bottom. The axon innervates two layers, with one set of collaterals in layer 2-3 and the other in layer 5. These two portions of the axon are indicated by the dotted line dividing them. In (b) the axon can be seen to extend for several millimeters, and the axon collaterals are grouped into distinct clusters. The clusters of collaterals in layer 5 lie directly below the clusters in layer 2-3, suggesting that the clustering pattern might be related to the cortical columns. Scale bar = 100 μm. Reprinted from Gilbert and Wiesel.
Intrinsic cortical connections

The existence of the long range horizontal connections was first described from lesion and degeneration studies, was later shown with intracellular horseradish peroxidase (HRP) injections into cortical pyramidal neurons and was also seen in the distribution of label after focal extracellular injections of HRP (Refs 7–9). This pattern of connections, originally shown in V1, has since been shown in several areas of prefrontal cortex (V2, V4 (Ref. 10) and MT (Ref. 11). It therefore is likely to represent a general feature of intrinsic connectivity in the cortex.

While the horizontal connections are extensive, they appear to be selective in the regions that they innervate. This is apparent in the distribution of the axon collaterals of the projecting cells, which are distributed in distinct clusters4–7. The extensive clustered projections are common, yet they constitute a component of cortical processing that had not been seen with the Golgi technique. The distance between clusters approximates the width of a hypercolumn, suggesting a possible relationship between the horizontal connections and the columnar systems in cortex. Another feature of the axon collaterals of these cells, apparent in 3-dimensional reconstructions made from intracellular HRP injections, is that they do not project equally along all cortical axes. Instead, they tend to have long and narrow axonal fields. The axon of one such cell is shown in Fig. 1. This axon shows the clustering pattern, extends for about 4 mm along the anteroposterior cortical axis, and is much narrower along the orthogonal dimension. The factors that determine the axis of orientation of a cell’s axonal field are not known. On the other hand, there is preliminary evidence linking the phenomenon of clustering with cortical columns, as will be described below. The distribution of axon collaterals seen after intracellular HRP injections has also been demonstrated by making focal extracellular HRP injections. In these experiments, originally done by Rockland and Lund, the HRP is transported away from the injection site and forms a pattern of discrete periodic patches.8–9. Based on this labeling pattern, Mitchison and Crick developed a model showing how the pattern could reflect orientation-column-specific projections from the injection site. Their model proposed oriented axonal fields innervating columns of similar orientation preference as the parent cells.

The pattern of label seen after extracellular HRP injection has also been related to the distribution of the enzyme cytochrome oxidase. This enzyme, a participant in the energy metabolism of cells, is distributed nonuniformly in the cortex. In tangential sections of the primary visual cortex (V1), particularly in the superficial layers, one can see patches of high cytochrome oxidase activity, and these patches are distributed in a series of regularly spaced rows and columns. This pattern is correlated with the functional architecture of cortex: the patches are centered on the ocular dominance columns. Also, cells located inside the patches have receptive fields that are unoriented and selective for color, while cells outside the patches are orientation specific. The similarity in the distributions of endogenous cytochrome oxidase and HRP after extracellular injections is suggestive of a relationship between the two patterns. While Rockland and Lund found no systematic relationship between the two patterns, Livingston and Hubel subsequently found that when the HRP injections were restricted to the blobs, the label was transported preferentially to nearby blobs, skipping over the intervening non-blob areas. Conversely, injections in non-blob areas resulted in a labeling pattern that avoided blobs, with label found preferentially in the surrounding non-blob areas.

Recent physiological experiments provide evidence for even greater specificity in the innervation pattern of the horizontal clustered connections. These studies show, in support of the model of Mitchison and Crick, that the connections are orientation specific. Using cross-correlation analysis, Ts’o et al.15 found that within a given layer, cell pairs at varying horizontal separations, up to 2 mm, showed correlated firing. These correlations probably reflected both connections between the cells being studied and common inputs to the cells from intrinsic cortical sources. In either event, they provided a possible physiological demonstration of the long range horizontal intrinsic connections. An important finding in this study was that cell pairs with positive correlations showed a consistent relationship in their receptive field properties. These pairs had the same orientation specificity, preference for the direction of stimulus movement, and ocular dominance. Thus it appears that the horizontal connections relate cells in different columns with the same specificities, separated by considerable distances. This idea is represented schematically in Fig. 2. The receptive fields of correlated cells separated by these distances were only partially overlapping, consistent with the extent of the horizontal connections seen anatomically. Finally, the correlations, particularly the long distance ones, were excitatory in nature. There are other studies, however, that seem to contradict these findings. By comparing maps in area 18 of the orientation columns determined physiologically with the clustered distribution of label after focal extracellular HRP injections, Matsubara and Cynader16 found that the labeled columns had orientations, opposite to the orientation of an injection site, suggesting that these connections were inhibitory. The discrepancy between this and the cross-
correlation study may be ascribed to methodological difficulties, or may represent a different set of horizontal connections to those observed with the cross-correlation technique. An independent method for determining whether these connections are excitatory or inhibitory is to examine the morphology of the synapses formed by the projecting cells and to determine the identity of their postsynaptic cells. The degeneration studies showed that the terminals degenerating far from the site of the lesion formed type I synapses, which are thought to be excitatory, and most of the contacts are with spines. Spiny cells are themselves thought to represent sources of excitation in the cortex. The EM findings would therefore be consistent with the results of the cross-correlation studies and an excitatory model of the long range horizontal connections.

Cortico-cortical connections

An individual cortical cell can integrate input from large regions of cortex not only through the long range intrinsic cortical connections but also through its input from other cortical areas. The visual cortex is divided into a number of areas, each with a retinotopic map of varying precision. One theory of the functional role of multiple areas serving a single sensory modality is that each specializes in analysing particular properties within that modality. In the visual cortex, different areas have been shown to specialize in color and movement. The diversity of functional properties seen for cortical cells is likely to arise from the intrinsic and cortico-cortical connections. While different cortical areas receive input from different thalamic nuclei, the receptive field properties of thalamic cells are not complex enough to generate the functional differences seen in the various cortical areas.

How cortico-cortical connections contribute to the functional properties of cortical cells is not yet known. These connections are relevant to the present context because of intriguing similarities in the pattern of connections between different cortical areas and the distribution of horizontal cortical connections within an individual cortical area. Cortico-cortical connections may therefore have an important bearing on the issue of horizontal integration in cortex. For example, there is considerable convergence in these connections. Consider, for example, the projection from area 17 (the primary visual cortex, V1) to the extrastrate area 18 (V2). A small injection of HRP in area 18 will label, by retrograde transport, the cells in area 17 that project to the injection site. An example of one such experiment is shown in Fig. 3. The labeled cells cover a large region, 5 or 6 mm across. Retinotopically, it appears that a much larger area of labeled cells may be labeled in area 17 than is covered by the injection site in area 18, indicating a considerable convergence in the projection. While the degree of convergence has not yet been quantified in terms of visual field area, it may be greater than one would expect from the increase in receptive field size and scatter that one sees in going from striate to prestriate cortex.

A second similarity between the cortico-cortical and intrinsic connections is that within the large area of labeled cells, the cells are distributed in clusters. Also, the terminals of cells projecting from one area to another are distributed in clusters. This projection pattern is a common feature in many regions of cortex, including visual, auditory, somatosensory and frontal. In some of these areas the clustering pattern has been shown for callosal as well as ipsilateral cortico-cortical projections. Another important feature of these projections is that they are specific, in that distinct populations of cells in V1 are responsible for projections to V2 and to V3 (Refs 20 and 30).

The functional correlate of this projection pattern has been studied in a few instances. In auditory cortex the callosal cortical projection bands appear to be aligned with the binaural summation response columns, and the ipsilateral projection bands are associated with contralateral dominant suppression responses. The function of these columnar systems in the auditory system is probably the localization of sound sources in space. In the visual cortex, no such correlation has yet been seen with either orientation or ocular dominance columns. In the monkey visual cortex, however, a relationship has been demonstrated between projection bands and the pattern of staining for the enzyme cytochrome oxidase. As described above, in the superficial layers of V1 cytochrome oxidase is distributed in a pattern of rows of 'blobs'. In other areas of cortex the cytochrome oxidase staining pattern is different; in V2, it appears as alternating thick and thin dark stripes separated by thin light 'interstripes'. Livingstone and Hubel found that the cytochrome oxidase dense patches in V1 project to the thin bands in V2. Whereas the functional specificity of cells in V1 cytochrome blobs has been determined, the physiological correlate of the staining pattern in V2 is not known. Once characterized, it should provide insight into the functional role of the clustered pattern of the projection between V1 and V2.

The specialization of projection target for cells participating in cortico-cortical connections has also been observed in other cortical areas. Bands of cells projecting from V2 to V5 (also

Fig. 3. Labeled cells in cat area 17 after an injection of horseradish peroxidase (HRP) in area 19. The HRP is taken up by nerve terminals in area 19, and transported retrogradely to the somata of cells in area 17 that project to the injection site. The labeled cells are located in the superficial cortical layers, cover an area 5 to 6 mm across, and are distributed in discrete clusters. In this view the cortical surface is at the top, and the lower dark band of diffuse label is in layer 5. The distance between the centers of the two prominent cell clusters is approximately 800 μm. Reprinted from Gilbert and Wiesel.

Fig. 4. A computer aided reconstruction of the distribution of cells projecting to V5 (dots) and of regions staining densely for cytochrome oxidase (lines) in area V2 of macaque visual cortex. The view is presented as if one is looking down on the surface of the cortex. Each row of lines represents a single section. The output to V5 arises from alternate cytochrome oxidase dense bands, seemingly the thicker ones. Similar experiments, not illustrated, demonstrate that cells in the thin bands project to a different visual cortical area. V4. Scale bar is 1 mm. From Shipp's and Zeki's, unpublished results.

called MT; see below) alternate with bands of cells projecting from V2 to V4, and the patterns of these bands correspond to the pattern of cytochrome oxidase staining in V2, with cells in the thin bands projecting to V4 and cells in the thick bands projecting to V5.32,33. Fig. 4 illustrates the relationships between the cytochrome oxidase pattern and the cells projecting to V5. The tendency for interdigitating cortical projections had been observed previously in the callosal and association projections to auditory38 and frontal cortices.48

The considerable convergence and divergence in the cortico–cortical connections could serve to endow areas with the ability to analyse particular properties independently of position within space. In extrastriate cortex the receptive fields tend to be larger than in striate cortex, and the visual field maps less precise. This would allow spatial resolution to be degraded in favor of greater specificity for other visual properties such as color or movement. It also makes it possible to generalize these properties over a large area, which would be advantageous for maintaining economy of cortical area and for position-independent object recognition. Such a mechanism was suggested by Hubel and Wiesel35 in discussing the difference between simple and complex receptive fields. ‘Complex cells’ are far less critical in their requirements as regards stimulus placement. Their responsiveness to the abstraction which we call orientation is thus generalized over a considerable retinal area.

Another advantage of the convergence in cortico–cortical connections, as with the intrinsic cortical connections, is to allow cells to analyse properties within the context of the surrounding environment. We will develop this idea further in the next section on the possible functional uses of the long range intrinsic and cortico–cortical projections.

Functional significance

Perhaps the first studies relevant to the functional role of horizontal cortical connections were performed by Sperry and Miner36, who inserted mica plates in the cortex and tested the animal’s capability to distinguish simple geometric figures. They interpreted their results to indicate that any deficits were related to local tissue damage, instead of global interactions within the cortex such as ‘electrical fields’. Doing similar experiments, Berkley and Bush37 made slices spaced 1 mm apart in the cortex. The most significant effect observed was a reduction in contour orientation acuity. Psychophysical and physiological studies provided evidence suggesting that orientation sensitivity in the cortex is due at least in part to inhibitory horizontal interactions between neighboring columns. Thus cells with vertical orientation preference in one column might sharpen the orientation tuning of horizontally oriented cells in the appropriate columns nearby. Sillito38,39 has made the most direct demonstration of this by applying antagonists to the inhibitory neurotransmitter GABA to cortical cells. Using one antagonist, N-methyl bicuculline, he found a marked reduction, and in some instances total elimination, of orientation specificity among visual cortical cells. The results of Matsubara and Cynader16, mentioned above, could represent the anatomical substrate of these horizontal inhibitory interactions, though the observations of Sillito do not require interactions over more than a single hypercolumn. The horizontal connections, as mentioned above, span many hypercolumns, and may allow communication between cells whose receptive fields are spatially separate, which does not seem entirely appropriate for generating orientation specificity.

There are other features of the receptive fields of visual cortical neurons that could be produced by the long range intrinsic connections. Some cells have very long receptive fields, integrating input from a large portion of the visual field, and thus requiring some form of horizontal interaction.9 In discussing the role of cortical connections in receptive field structure, one has to take other components of a cell’s receptive field into account. Among visual cortical cells, beyond the portion of the visual field where a stimulus can cause excitation, there are flanking inhibitory regions that produce the properties of end-inhibition (selectivity for edges or lines of restricted length), side-band inhibition, and selectivity for position in depth. Taken together, these flanking inhibitory regions make receptive fields much larger than the excitatory portion alone, and one could imagine that horizontal connections could generate such properties. A cell’s firing can be further influenced by visual stimulation beyond even the excitatory and inhibitory subfields of the classic receptive field, though the more peripheral influences cannot be observed in the absence of stimulation of the more central portions of the receptive field (for a review of this subject, see Allman et al.40).
leads to something of a semantic difficulty in defining receptive field: at times the receptive field is referred to as the area where a stimulus can elicit a discharge, at other times it is meant to include the inhibitory flanks, and in its most general use might also include the peripheral regions providing subthreshold facilitatory influences. For the ease of the present discussion the term receptive field is meant to include only the excitatory center and inhibitory flanks.

The more peripheral influences were originally observed in the retina by Mollwain, who found that the response of retinal ganglion cells could be facilitated by stimulating retinal points distant from the cells' receptive fields. In the lateral geniculate nucleus, Fisher and Kruger found a similar influence on firing by presenting large grating patterns outside the cells' receptive fields. These influences can cover an extensive portion of the visual field, much larger than the receptive field area of individual cells.

In the cortex most receptive field analysis has focused on the central excitatory and flanking inhibitory regions. It is possible that the horizontal connections can be responsible for generating either larger excitatory centers or inhibitory flanks. Some of the inhibitory flanks endow cells with the property of end-inhibition, where the cells can only be activated by bars or edges of a restricted length. These flanks have the same orientation specificity as the excitatory regions. Other inhibitory regions, known as side-bands, may be involved in the mechanism of stereopsis or the depth-sensitivity of visual cortical receptive fields. Both of the flanks described above are equivalent in area to the excitatory cores of the receptive fields. There may also be a much more extensive influences seen in the retina and lateral geniculate nucleus, and several investigators have found both inhibitory and excitatory peripheral influences beyond the standard receptive field. Insights into the functional role of horizontal connections may require more sophisticated visual stimulation techniques. Poggio et al. have found that many cells in V1 are sensitive to the disparities observable in random dot stereograms, requiring a global calculation of disparity over a large area, averaged over many discrete points. While it is not clear to what extent this phenomenon requires a global calculation rather than a sensitivity to distinct pattern present within the stereograms, the horizontal interactions in cortex could conceivably be responsible for generating these effects.

Peripheral receptive field effects have been observed in visual cortical areas beyond V1. In V5 (also referred to as STS) of rhesus monkeys, an area specializing in stimulus movement, the cells show preference for the direction of movement. An homologous area in owl monkeys, called MT, has been shown to show peripheral field effects related to the movement specificity of the area. When the 'background' outside the receptive field is moved in the direction opposite to the optimum direction of the receptive field itself, the cell's response can be greatly facilitated. The area over which this effect can be seen is up to 10 times the diameter of the receptive field proper. The surround effect for one cell in MT is illustrated in Fig. 5. The facilitatory effect is seen only for cells in MT, not for directional cells in V1 or elsewhere. The physiological findings correlate well with the extent of the horizontal connections found in MT (Ref. 11). Some cells in MT have another property, distinct from cells in V1, that is important for the analysis of motion. A moving object may contain many edges of different orientation. If one views a single edge through an aperture, so that none of the other edges are visible, the edge always appears to move in a direction perpendicular to its orientation, regardless of the actual direction of movement of the entire object. In some way we integrate the movement of the various edges of an object to perceive the actual direction of movement of the object as a whole. Cells in V1 act as apertures, but some cells in MT respond to the movement of the whole object, rather than to the apparent movement of individual edges viewed in isolation. This property would require individual cells to integrate information coming from different parts of an object, and consequently from widely separated parts of the visual world. One can speculate that the horizontal connections might play a role in this kind of integration.
Another possible role for long range horizontal connections can be seen in V4, an area that has a concentration of cells showing specificity for the color of the stimulus. In this area cells have the property of 'color constancy', similar to the phenomenon demonstrated in human psychophysical experiments by Land. The experiments by Land showed that to a human observer an object will appear to have the same color regardless of the color balance of the incident light. Thus the observed color does not change even though at different times the object may be reflecting different wavelengths. Similarly, a cell in V4 will respond well to stimuli that appear identical to the experimenter but actually contain different distributions of wavelengths. This property also is dependent on the colors of objects located outside the cell's receptive field. As with the surround facilitation in MT, the effect is seen only for cells in V4, and not, for example, in V1.

For both V4 and MT there are obvious benefits to the surround effects: color constancy is necessary to permit object identification independent of the greatly varying lighting conditions occurring during the day. The surround movement effect in MT may enable the observer to detect differential motion of object and background and to use motion as a cue for depth.

Conclusion
The horizontal cortical connections and the peripheral field influences may play an important role in endowing each cortical area with the function that is specific to that area. Though the pattern of cortical connections seems to be stereotyped, with the basic features repeated from one cortical area to the next, each cortical area seems capable of developing a range of specialized properties. The singular properties that characterize each cortical area may be derived at least in part from the relationship between the horizontal cortical connections with the area's columnar structure. Another factor in determining cortical specificity is the selectivity in cortico-cortical connections, where distinct populations of cells in a given cortical area are responsible for projections to other cortical areas. Understanding the properties that a given area arranges in columns and the way in which these columns are interconnected within and between different cortical areas can serve as a guide in exploring the more sophisticated properties of cortical cells.

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Charles D. Gilbert is at the Rockefeller University, 1230 York Avenue, New York, NY 10021-6390, USA.