NEOCORTEX

RODNEY DOUGLAS, HENRY MARKRAM,
AND KEVAN MARTIN

NEOCORTEX

RODNEY DOUGLAS, HENRY MARKRAM,
AND KEVAN MARTIN

Many of the brain regions discussed in this volume are examples of cortical ("bark-like") structures. Taking its place alongside the *archicortex* (hippocampus) and *paleo-cortex* (olfactory bulb and olfactory cortex) is the *neocortex*, which is the most recent arrival in evolutionary history and arguably the most impressive example of the genre. It has certainly impressed paleontologists, whose research on the fossil record of hominids has demonstrated that the size of the hominid brain has trebled over the past 3 million years. Endocasts of the fossil hominid skulls indicate that this increase in size is largely due to the expansion of the neocortex and its connections. The massive and rapid changes in the size of the neocortex are paralleled in the phylogenetic differences we see in contemporary mammalian brains (Fig. 12.1). Of land mammals, the primates have the largest brains in proportion to their body weight. However, the human brain is three times as large as might be expected for a primate of equivalent weight (Passingham, 1982). Furthermore, the human brain is not simply a scaled-up version of our closest primate relatives, i.e., the chimpanzee. The greatest expansion is in the cortical structures, particularly the cerebellum and neocortex. Within the neocortex itself, the expansion is uneven. In comparison with nonhuman primates of equivalent body weight, the association and premotor areas have expanded relative to the sensory areas. When added together, the neocortex and its connections form a massive 80% by volume of the human brain (Passingham, 1982).

In all mammals, the neocortex consists of a sheet of cells, about 2 mm thick. Conventionally it is divided into six layers, but in many regions more than six laminae are in evidence (Fig. 12.2). Each cubic millimeter of cortex contains approximately 50,000 neurons. The study of the laminar organization of these cells in the neocortex began in the early part of the 20th century and became known as *cytoarchitectonics*. In conjunction with studies of the organization of myelinated fibers, called *myeloarchitectonics*, cytoarchitectonics was applied by Campbell in England and by Vogt and Brodmann in Germany, to divide the neocortex into about 20 different regions. Although many more areas have since been identified, there are three major cytoarchitectural divisions of the neocortex. The *koniocortex*, or granular cortex, of the sensory areas contains small densely packed neurons in the middle layers. These small neurons are largely absent in the *agranular* cortex of the motor and premotor cortical areas.
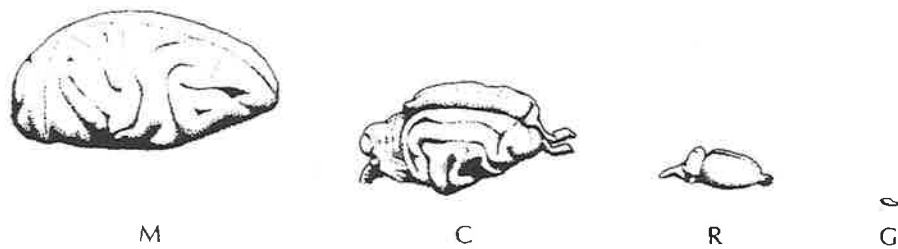
Fig. 12.1. Brains of modern vertebrates: goldfish (G), rat (R), cat (C), and Old World monkey (M). Scaled to body weight, the neocortex and its connections form an increasingly greater proportion of the brain volume. The neocortex in monkey completely envelops all other brain structures.
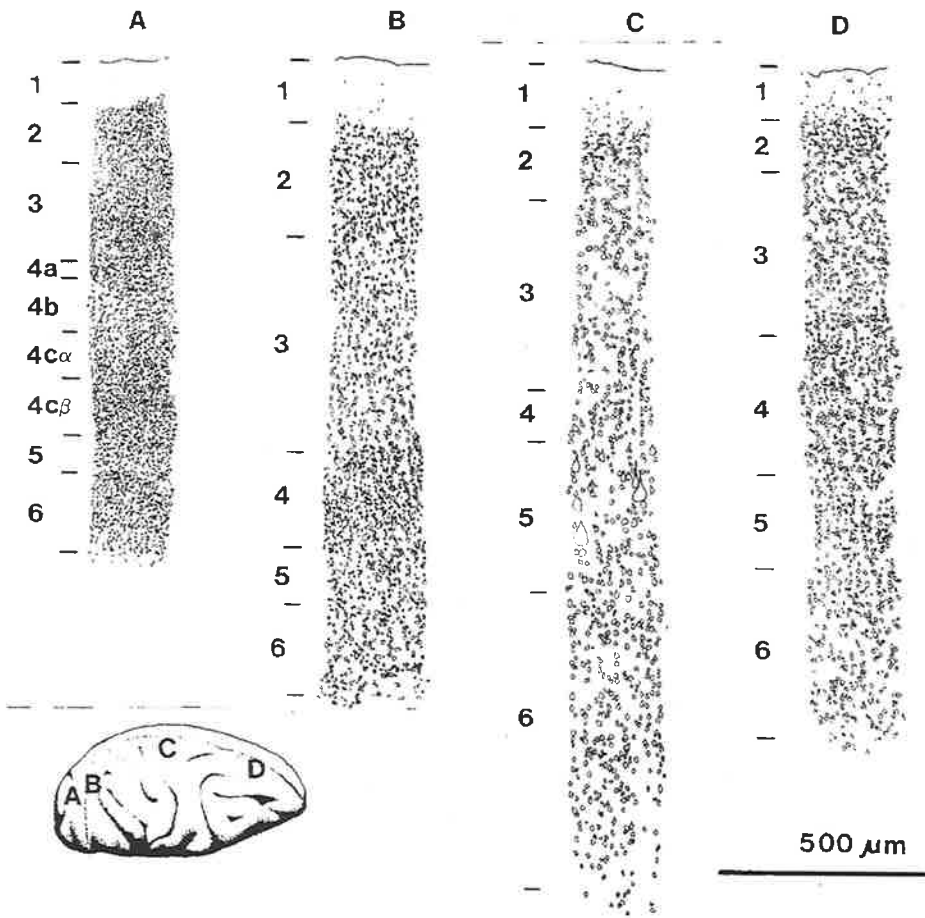

Fig. 12.2. The laminar organization of neurons in different cortical areas of the macaque monkey cortex (inset). **A:** Area 17 (striate visual cortex). **B:** Area 18 (extrastriate cortex). **C:** Area 4 (motor cortex). **D:** Area 9 (frontal cortex). A basic six-layer structure can be identified in all areas. The pia covers layer 1; the white matter is below layer 6. Note the marked difference in cell size and density among the different areas, but additional layers are apparent in some areas (e.g., area 17). The giant neurons in layer 5 of area 4 are the Betz cells. (Celloidin-embedded brain, cut in 40-μm-thick parasagittal sections, stained for Nissl substance, uncorrected for shrinkage.)

The third type of cortex has varying populations of granule cells and is called *eulaminate*, or *homotypical*, cortex. It includes much of "association cortex," which is a convenient description for cortex whose function has yet to be discovered (Fig. 12.2). Within each of these areas are many subdivisions, both functional and anatomical. Some are clearly delimited by their cytoarchitectonic structure, as in the case of area 17, the primary visual cortex, or by myeloarchitectonics, as in the case of the middle temporal visual area (MT). Other areas, such as area 18 in the monkey, can only be subdivided by more elaborate immunochemical, histological, or physiological methods.

In a planar view, the map of these architectonically defined areas looks like a patchwork quilt. The functional properties and subdivisions of these have been mapped most extensively in the monkey cortical areas concerned with vision. The exponential growth of functional imaging studies in humans means that increasingly more is becoming known about the equivalent subdivisions of the human brain. In addition to the basic sensory and motor functions, the cortex appears to be particularly involved in high-level functions, such as speech production and comprehension. Indeed, the concept of cortical localization of function derives from studies in the early 1900s that correlated damage of specific areas of human cortex with specific deficits in speed production. Similar modern case studies of aphasias have become celebrated in the popular culture of books, television, and films. With the advent of functional imaging studies with positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and electroencephalography (EEG), there has been a rapid increase in our knowledge of the functional and anatomical map of human cortex. These techniques do not attempt to identify the mechanisms or neuronal circuits responsible for these functions. Thus, the challenge is to discover what is actually happening when different regions of the cortex are activated under different sensory or behavioral tasks. Fundamental to this central endeavor is an understanding of the structure and function of the microcircuits of the neocortex and their components.

EMBRYONIC DEVELOPMENT

The cerebral cortex develops in the walls of the telencephalic vesicles of the reptilian and mammalian forebrain (Fig. 12.3). It develops from the cortical plate that is itself embedded in the primordial cortical preplate. The preplate "pioneer" neurons regulate neuronal migration of the cortical plate neurons, and form the first axonal connections. As the cortical plate expands, it splits the preplate into a superficial layer or marginal zone, and a subplate that lies beneath the cortical plate at its boundary with the white matter. Eventually, the cortical plate differentiates into cortical layers II–VI. The marginal zone becomes layer I, and the subplate transforms into layer VIb, or vanishes (see Supér and Uylings, 2001).

Most postmitotic neurons are generated by the neuroblasts of the ventricular zone (VZ) beneath the region of the cortical plate they will finally occupy. However, some fraction of the GABAergic neurons are generated in the subventricular zone (SVZ), or in the ganglionic eminence, which lies some distance from their final location in the cortical plate.

The ventricular cells have two modes of division. Symmetrical division gives rise to two daughter cells that both maintain their proliferative properties, and so increases the
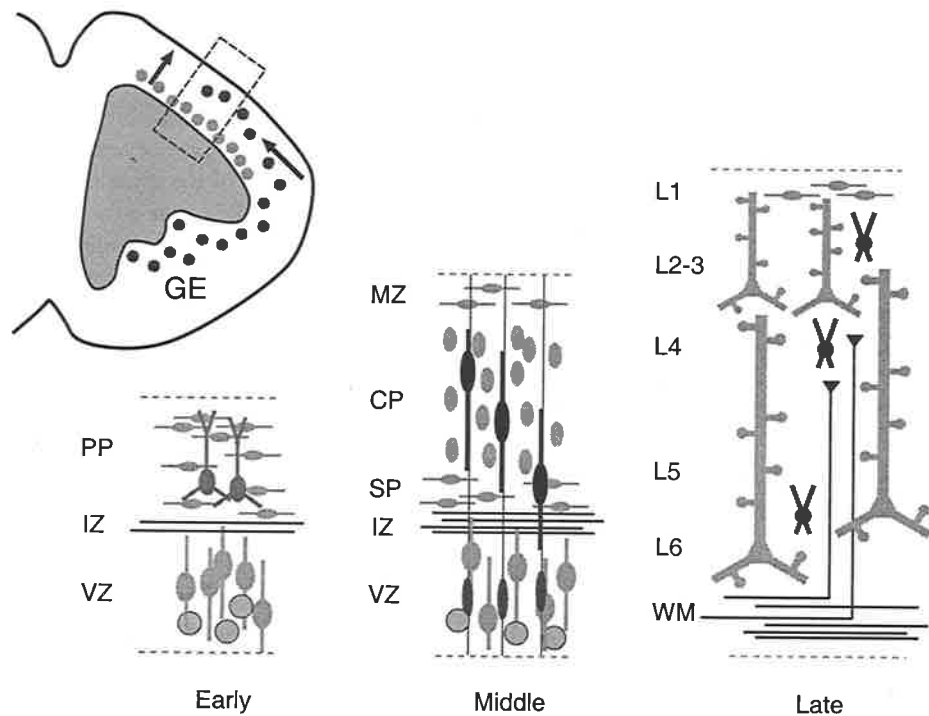
Fig. 12.3. The embryonic cerebral cortex develops in the wall of the bilateral telenephalic vesicles (one vesicle, with shaded ventricle, is shown in the schematic anatomical coronal cross section at top left). The construction sequence of increasing cortical organization in a typical region of the telencephalic wall (dashed rectangle) is shown for the early, middle, and late phases of embryonic development. All the spiny neurons and some smooth neurons arise (radial arrow in anatomical cross section) from the neuroblasts of the ventricular zone (VZ, light filled circles in cross section, cross-hatched circles in diagrams below). The majority of GABAergic neurons arise in the VZ of the ganglionic eminences (GE) and then migrate tangentially (tangential arrow in anatomical cross section) along the telencephalic wall at about the level of the intermediate zone (IZ), before taking up position in the cortical plate (CP) where they complete their differentiation (dark vertical bipolar cells in "Late" phase). The preplate (PP, "Early" phase) is the earliest structure generated by the neuroblasts of the ventricular zone. Postmitotic neurons migrate vertically (black vertically elongated cells in "Middle" phase) into the PP guided by a scaffolding of radial glial cells (dark vertical lines with elongated somata in "Middle" phase), which extend from the ventricular wall to the outer surface of the telencephalon. These neurons form the cortical plate (CP) within the preplate, so splitting the preplate into a superficial marginal zone (MZ) and a deep subplate (SP). The cortical plate neurons are assembled in inside-out order. During this phase afferent axons advance tangentially in the intermediate zone, before turning vertically to make contact with their targets in the CP. Cortical plate neurons, derived from radial migration from VZ and tangential migration from GE, differentiate into the spiny and smooth neurons that compose layers 2–6 of neocortex ("Late" phase. [From Parnavelas, 2000.]

pool of neuroblasts. Asymmetrical division gives rise to one proliferative cell (so conserving the pool of neuroblasts) and one postmitotic cell. The postmitotic cells migrate radially upward through the subplate, into the neocortical plate (Rakic, 1971). The migration is an inside-out stacking process, whereby the earliest migrators settle in the deepest layers and subsequent migrators pass through the previously settled layers and

finally come to rest in the outer layers. This radial migration of cortical neurons is supported by a transient scaffolding of radial glial cells; it is controlled by preplate pioneer cells, such as the Cajal-Retzius cells (Ogawa et al., 1995). Radial migration has two phases. Initially, the entire neuron migrates. Later, migration is dominated by translocation of the nucleus within the radially extended cell to the layer location where the soma will finally be established.

Afferents growing into the mammalian neocortex advance tangentially beneath the cortical plate in the subplate and intermediate zone. Then they turn radially and ascend vertically through the subplate to reach their target neurons within the plate. The subplate plays a major role in the organization of the afferent connections with the newly settled cortical neurons. Its thickness is proportional to the complexity of the mammal's behavior. It is always very thin in rodents, but in humans it transiently attains a thickness nearly six times that of the cortical plate it serves (Supér and Uylings, 2001).

This uniform two-dimensional radial construction mechanism could provide a simple explanation for the more than 1000-fold increase in the cortical area without a comparable increase in its thickness. A simple mutation of a regulatory gene (or genes) that control the rate and duration and the mode (symmetrical/asymmetrical) of cell division in the proliferative zone, coupled with constraints in the radial distribution of migrating neurons, could create an expanded cortical plate with enhanced capacity for establishing new patterns of connectivity that are validated through natural selection (Rakic, 1995). This simple picture has been complicated by the discovery that significant numbers (at least 75% in mice) of the GABAergic neurons migrate along distinct pathways from the ganglionic eminence, probably along corticofugal fibres (Parnavelas, 2000). They travel close to the VZ and then turn to migrate radially. They appear first in the marginal zone, some in the form of Cajal-Retzius cells, which are thought to influence the migration of pyramidal neurons generated in the VZ. Later, they appear in the intermediate zone and cortical plate. The second population of GABAergic neurons, generated in the SVZ, form themselves into chains, which they use as a scaffold for their own radial migration. The GABAergic neurons exhibit a great morphological diversity, which may also be due to their exposure to different differentiating factors in the course of their various migrations from different sources. One of the major fascinations of cortical anatomists has been to discover how the different pieces of the cortical jigsaw fit together to form functional circuits of such evident sophistication.

NEURONAL ELEMENTS

Nearly 100 years ago, Ramon y Cajal outlined the basic approach to studying the elementary organization of cortical connectivity (Cajal, 1911). The method is to reveal the complete structure of neurons, including their axons, and then piece together these components in a jigsaw puzzle fashion to produce circuits. He, and many since, studied the morphology and circuitry of the neocortex with the silver impregnation technique discovered by Golgi. Although this technique has been superseded by much more sophisticated modern techniques, the basic classes of neurons revealed by the Golgi technique used by Ramon y Cajal have remained largely unaltered. All three cytoarchitectural divisions of the neocortex contain the same two basic types of neurons: those whose dendrites bear spines (the stellate and pyramidal neurons, e.g., see Fig. 12.5) and those whose dendrites are smooth (smooth cells, e.g., see Fig. 12.7). Occa-

sionally, "sparsely" spiny cells have been described, but these neurons form a very small subclass of cortical neurons.

The proximal shafts of the dendrites of the spiny cell types are nearly devoid of spines. The spine density varies considerably between different types of neurons. At one extreme is the "sparsely spiny" neuron, which may bear fewer than 100 spines over the entire dendritic tree. These neurons form a small subclass of the inhibitory neuron population. At another extreme are neurons such as the Betz cell, a large pyramidal neuron that is found in the motor cortex (area 4) and bears about 10,000 spines. Each spine forms a Type 1 (see later and Chap. 1) synapse with a presynaptic bouton. Thus, simply counting spines gives a lower limit on the number of Type 1 synapses. However, because not all type 1 synapses are formed on spines, the degree of underestimation can only be determined by quantitative electron microscopy of the dendrites of identified neurons. Due to this methodological bottleneck, accurate estimates of the number and positions of synapses onto particular neuronal types are, unfortunately, extremely rare.

Modern electron microscopic and immunochemical techniques have been used to determine the proportion of the different types in the different regions of cortex. These studies have shown that although the different types may be differentially distributed between laminae within a single cortical area, the overall proportions of a given neuronal type remain approximately constant between different areas. The pyramidal neurons form about 70% of the neurons (Sloper et al., 1979; Powell, 1981) and the smooth cells form about 20% of the neurons (Gabbott and Somogyi, 1986) in all cortical areas. These morphological differences in the dendritic structure are only one of many differences between these two basic types. For example, the spiny neurons are excitatory, whereas the smooth neurons are inhibitory. Spiny neurons use quite different neurotransmitters from smooth neurons; their respective synapses are associated with a quite different set of receptors, and this is reflected in the morphology of the synapses.

SPINY NEURONS

Spiny neurons are called such because their dendrites bear small processes called *spines*. Spines are usually club-shaped, with a head of about 1 μm diameter and a shaft or "neck" of about 0.1 μm diameter (see electron micrograph in Fig. 1.7). The length of the neck varies greatly, from virtually nothing as in "stubby" spines, in which the head attaches directly to the dendritic shaft, to necks that are several micrometers long (Jones and Powell, 1969). At the high magnifications achieved with the electron microscope, spines can be distinguished from other dendritic elements in the neuropil by the presence of a characteristic "spine apparatus," composed of calcium-binding proteins, and this has been an important marker for spines in quantitative electron microscopic studies (cf., for example, Chap. 7, Fig. 7.5A).

Spiny neurons are usually defined according to the lamina in which their soma is located. However, many types can be distinguished on the basis of their dendritic morphology. The clearest distinction is that some spiny neurons have an apical dendrite (pyramidal neurons) and some do not (spiny stellate cells).

Pyramidal Neurons. The major subtype of spiny neuron is the pyramidal neurons (Figs. 12.4 and 12.5), which constitute about two-thirds of the neurons in the neocortex. Pyra-

Fig. 12.4. Pyramidal neuron of layer 3. Note characteristic apical dendrite extending to layer 1. Many collateral branches arise from the main axon before it leaves the cortex. Labeled intracellularly *in vivo* with horseradish peroxidase. Cortical layers are as indicated. Bars = 100 μm.

midal neurons are found in all cortical layers except layer 1. Their most prominent feature is an apical dendrite that may extend through all the layers of the cortex above the soma. Pyramidal cells are the major output neurons of the neocortex. They participate both in connections between the different cortical areas and to subcortical structures such as the thalamus and superior colliculus. However, they are also a major provider of excitatory input to the area in which they are found: each pyramidal neuron has a rich collateral network that forms part of the local cortical circuitry.

Many subgroups of the pyramidal neurons can be distinguished on the basis of their morphology or functional characteristics, and in each layer pyramidal neurons can be found whose morphology and axonal projections are exclusive to that layer. For example, in layer 4, Lorente de No identified "star" pyramids, which are so-called because of their symmetrical and radially orientated basal dendrites. The most prominent pyramidal neurons in the neocortex are the Betz cells of area 4, the "motor" cortex. The Betz cells are very large pyramidal neurons located in layer 5 (see Fig. 12.5). Their axons form part of the pyramidal tract that descends to the spinal cord. The primary visual cortex also has a distinct set of exceptionally large pyramidal neurons, called the *solitary cells of Meynert*. These pyramidal neurons, which are found in the deep layers (5 or 6 depending on the species), project to other cortical areas and down to the midbrain structures such as the superior colliculus and the pons.

Fig. 12.5. Pyramidal neuron of layer 5. Note the very rich axon collateral arbor in the superficial layers. This neuron did not project out of area 17. Labeled intracellularly *in vivo* with horseradish peroxidase. Cortical layers are as indicated. Bars = 100 μm.

Within layer 5 in the visual cortex, two distinct types of pyramidal cells have been distinguished on the basis of a correlated structure–function relationship. One type has a thick apical dendrite that ascends to layer 1, where it forms a terminal tuft. These neurons have a bursting discharge of action potentials in response to a depolarizing current. The other type has a regular discharge, and their apical dendrite is thin and terminates without branching in layer 2 (Chagnac-Amitai et al., 1990; Mason and Larkman, 1990). This observation has led to theoretical work suggesting that the shape of the dendritic tree itself is a major factor in controlling the pattern of spike output from the neurons (Mainen and Sejnowski, 1996). Of course, the distribution of ion channels over the surface of the neuronal membrane also is a significant factor in determining the biophysical responses of the neuron, especially in the case of the tufted layer 5 pyramidal neuron (see later).

Pyramidal neurons can also be distinguished by their extra-areal efferent connections. For example, the thin untufted pyramids of layer 5 tend to project to the opposite hemisphere, whereas the thick tufted pyramids provide most of the output to subcortical areas. The thick tufted pyramids can be further subdivided according to the precise subcortical structure to which they project (Rumberger et al., 1998).

Spiny Stellate Neurons. A second group of spiny neurons, the spiny stellate neurons (Fig. 12.6, are found exclusively in layer 4 of the granular cortex (Cajal 1911). These
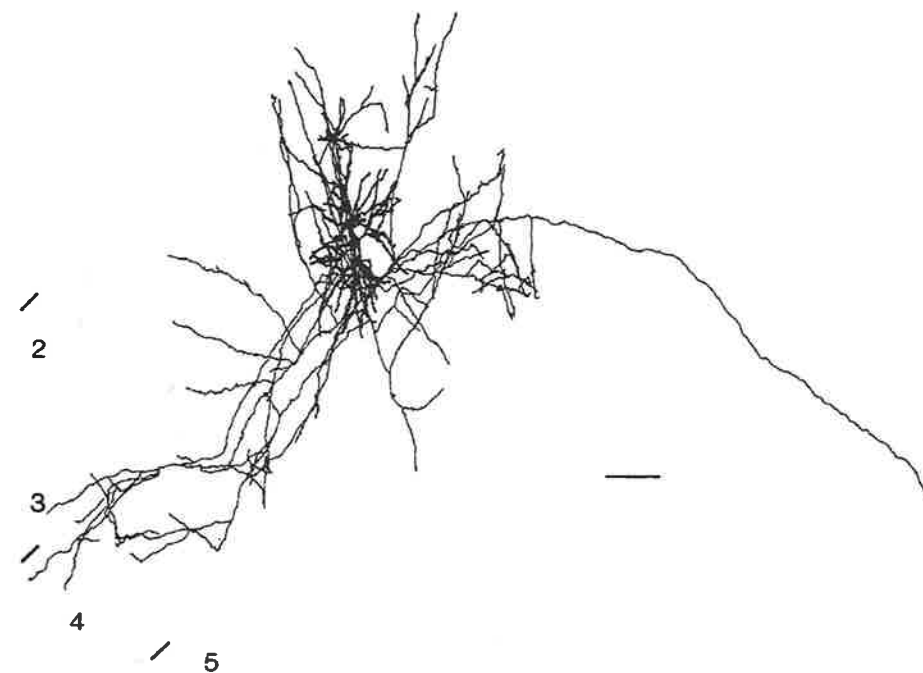

Fig. 12.6. Spiny stellate neurons of layer 4 from cat visual cortex. Labeled intracellularly *in vivo* with horseradish peroxidase. Cortical layers are as indicated. Bars = 100 μm.

also have spiny dendrites, but they do not have the apical dendrite that is characteristic of the pyramidal neurons. Instead, dendrites of approximately equal lengths radiate out from the soma and give these neurons a star-like appearance—hence their name. Occasionally, these neurons project to other areas, but most have axonal projections confined to the area in which they occur. It has been proposed that these neurons are simply pyramidal neurons without an apical dendrite. However, they differ in a number of important respects from pyramidal neurons, e.g., they have much lower spine densities and many more excitatory synapses on their dendritic tree. They should be considered as a distinct cell type confined to layer 4 of sensory cortex. Previously, the spiny stellate cells were thought to be the sole recipients of the thalamic input to the sensory cortices, but it is clear that although they probably are the major recipient, thalamic neurons also connect to the pyramidal neurons and smooth cells (Hersch and White, 1981; Hornung and Garey, 1981; Freund et al., 1985; Ahmed et al., 1994).

SMOOTH NEURONS

The class of neurons with spine-free dendrites is frequently referred to as smooth stellates, but because their dendritic morphology is rarely stellate, a more accurate term is simply *smooth neurons*. They tend to have elongated dendritic trees, in both the radial and the tangential dimension. Their dendritic morphologies have been described as multipolar, bipolar, bitufted, and stellate, but the most useful discriminator of the different types has been the axonal arbor. At least 19 different types of smooth neurons

have been described (Szentágothai, 1978; Peters and Regidor, 1981). These smooth neurons do not just have morphologically distinct axonal arbors; they also form quite specific synaptic connections, as described later.

The most prominent smooth neuron is the cortical *basket cell*, which was first described by Ramón y Cajal. As with basket cells in the cerebellum (see Chap. 7) and hippocampus (see Chap. 11), the convergence of multiple axons of the basket cells forms nests or baskets around the somata of their targets, usually pyramidal cells. Modern studies, however, have shown that basket cell boutons form most of their synapses on the dendrites and spines of pyramidal neurons. In superficial and deep layers, the main feature of the basket cell axonal arborization is the lateral extension of the axon (Fig. 12.7). However, deep basket cells often also have an arborization in the superficial layers vertically above their soma (Kisvárday et al., 1987). Similarly, superficial basket cells can have an arborization in deep layers beneath their soma.
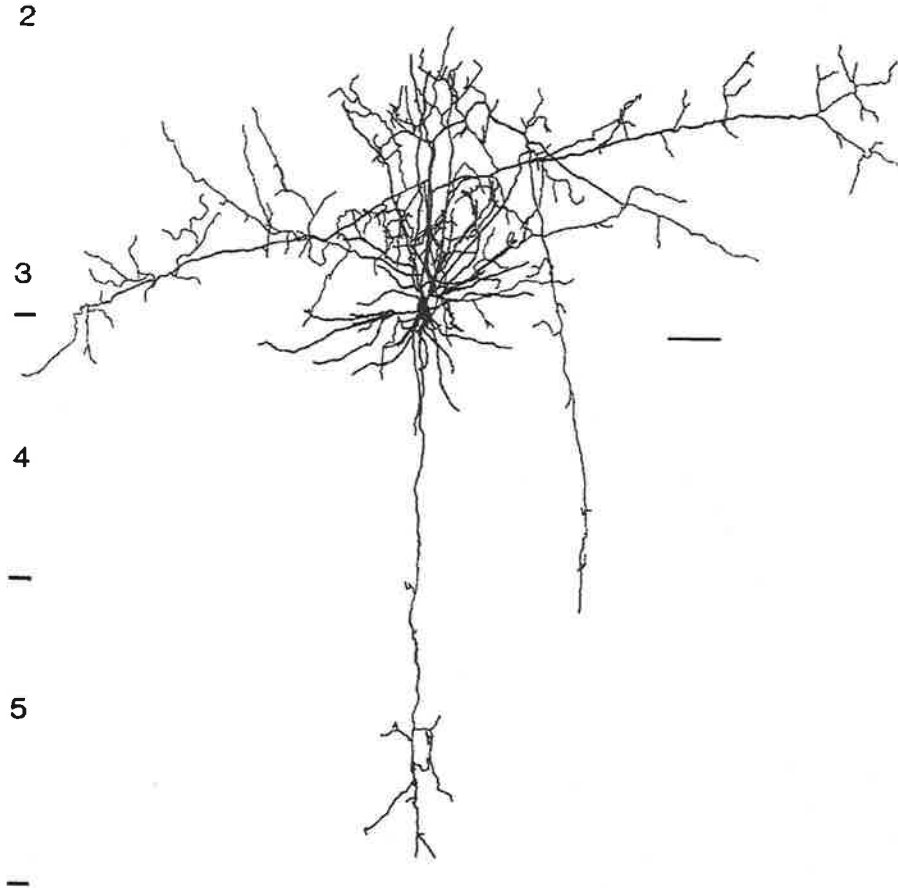
In the middle layers, the basket cells have much more compact axonal arbors (Fig. 12.8). As with the well-studied pattern of thalamic afferents to the visual cortex (see later), which underlie the functional ocular dominance columns, these differences in the axonal arborizations most probably relate to the functional architecture of the piece of cortex in which they are located.

As with the spiny cells, some morphological types of smooth neurons are found only in particular layers. Layer 1, for example, has two types that are not found in other lay-

Fig. 12.7. Large basket smooth neuron from cat visual cortex. Cortical layers are as indicated. Bar = 100 μm.
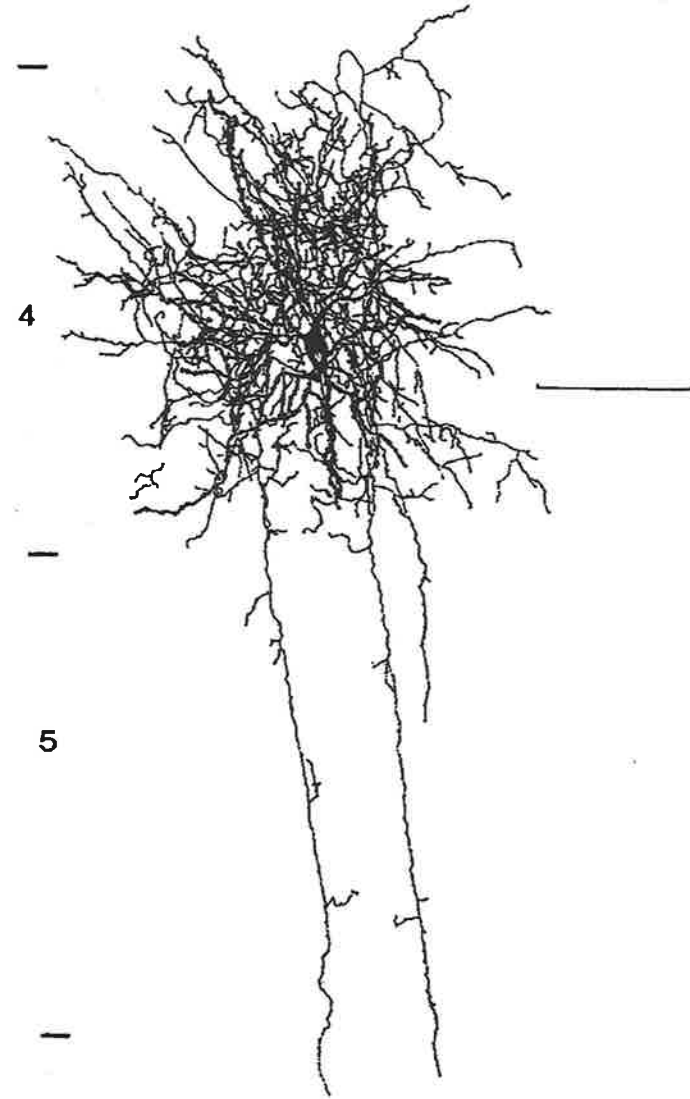
Fig. 12.8. Small basket smooth neuron from layer 4 of cat visual cortex. The major portion of the axon arbor is confined to layer 4. Cortical layers are as indicated. Bar = 100 μm.

ers: the *Retzius-Cajal neuron*, which has a horizontally elongated dendritic tree, and the *small neuron of layer 1*, which has a highly localized dendritic and axonal arbor. Many of the smooth types have descending or ascending axon collaterals in addition to their lateral extensions. Most notable of these is the *double bouquet cell* of Ramón y Cajal (Fig. 12.9), which is characterized by elongated dendrites extending radially above and below the somata and an axon that forms a cascade of vertically oriented collaterals. Another neuron with a vertical organization is the *Martinotti cell*. Originally, the Martinotti cell was described as a multipolar or bitufted neuron, with compact dendrite, that is located mainly in the deep cortical layers and whose defining axon arose from the upper

cell body or dendrites and ran vertically to layer 1, where it arborized (Faireń et al., 1984). More recently, the arborization is thought to span all layers (Faireń et al., 1984; Wahle, 1993). Martinotti cells are frequently observed in immature animals, but many degenerate during the early early postnatal period (Wahle, 1993).

Perhaps the most evocative description of a smooth cell was given by Szentágothai to the *chandelier cell*, so-named because its axonal boutons are arranged in a series of vertical "candles" that give the whole axonal arborization the appearance of a chandelier (Fig. 12.10).
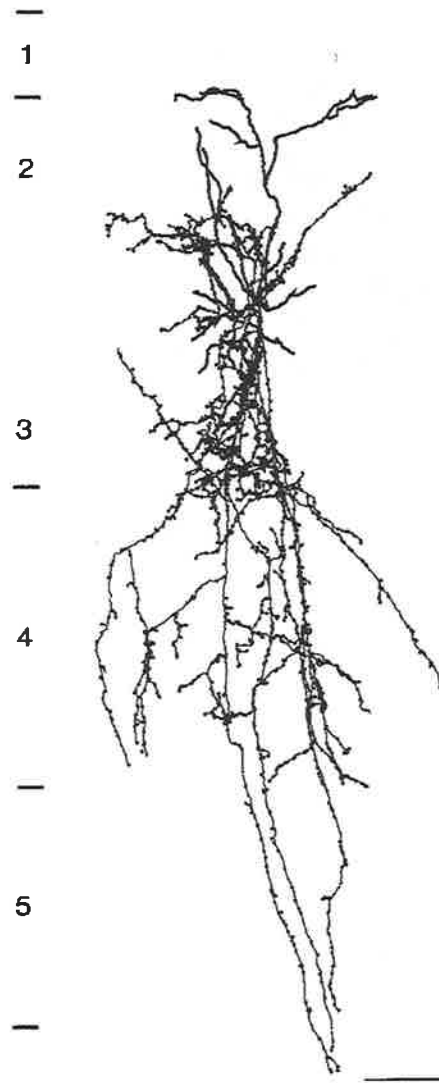

Fig. 12.9. Double bouquet smooth neuron from layer 4 of cat visual cortex. The axon collaterals run vertically. Cortical layers are as indicated. Bar = 100 μm.

Fig. 12.10. Chandelier smooth neuron from cat visual cortex. Cortical layers are as indicated. Bar = 100 μm.

Histochemical methods have revealed the existence of an additional smooth neuronal type, which is sparse in the gray matter but forms a distinct monolayer at the border of the gray and white matter. These neurons stain positively for the enzyme NADPH-diaphorase, which is a synthetic enzyme for nitric oxide gas. Although they are few in number, their axonal ramifications are immense and provide a rich plexus of axons throughout the gray matter.

There is a striking variety of smooth neuron morphological types by comparison with stellate neurons (Wang et al., 2002). The functional significance of this difference is not clear. However, the various types do show some preference for forming synapses on particular regions of their postsynaptic targets. Some types target the proximal soma and dendrite (e.g., basket cells), mid-field dendrites (e.g., bitufted, double bouquet, bipolar, neurogliaform), and distal dendritic inhibition (e.g., Martinotti cells), yet others target the axon initial segment (e.g., chandelier cells). This distinction in target regions suggests that the various inhibitory cell types might participate in different functional subsystems. For example, distal inhibition could influence local dendritic integration and modulate local synaptic modification for learning; more proximal inhibition is well placed to control overall neuronal signal gain and thresholding, and inhibition of the axon initial segment could affect the detailed timing of action potentials. Similar considerations apply to a variety of inhibitory interneurons in other regions (see especially hippocampus, Chap. 11).

AFFERENTS

Thalamus. The thalamus projects to all cortical areas and provides input to most layers of the cortex. The densest projections are to the middle layers, where they form about 5%–10% of the synapses in those layers (LeVay and Gilbert, 1976; White, 1989; Ahmed et al., 1994). The main feature of this input is that it is highly ordered. The sensory inputs are represented centrally in a way that their topographic arrangement in the periphery is preserved. This mapping is achieved by preserving the nearest-neighbor relationships of the arrangements of the sensory or motor elements in the periphery. Such topographic projections are a ubiquitous feature of the cortex. The precision of the mapping does vary between areas, however. The primary sensory and motor areas usually preserve the highest detail of the topography, which degrades progressively through secondary and tertiary and higher order areas of cortex.

An important transform in the topography from the periphery to the center is that the regions of highest sensory receptor density have the largest representation in the cortex. This transformation is described as the *magnification factor* of the projection (Daniel and Whitteridge, 1961). In the visual system of the primate, the fovea of the retina contains the highest density of photoreceptors, and the primary visual cortex represents this by devoting cortex in the ratio of 30 mm/degree of visual field to this representation. In the far periphery of the visual field, the ratio falls off to about 0.01 mm/degree of visual field. In the somatosensory system, the hand and face have high densities of touch receptors, and these parts have a magnified representation in the primary somatosensory cortex. One of the most remarkable cortical representations is that of the whiskers of rats and mice. Each whisker has a separate representation in the cortex, which has a barrel-like form when viewed in a tangential section of the somatosensory cortex (Woolsey and Van der Loos, 1970). The centers of the barrels are formed by clusters of thalamic afferents that convey input from each whisker. The cortical map of the whiskers forms a representation that is topologically equivalent to the arrangement of whiskers on the face of the rat or mouse. This whisker map dominates the representation of the sensory surface of the rodent.

In the cat visual cortex, the terminal arbors of each individual thalamic afferent may extend over 1–5 mm of the cortical surface (Fig. 12.11) so that each point in layer 4 is covered by the arbors of at least 1000 separate thalamic relay cells. Thus, the dendritic tree of an average layer 4 neuron, which extends for 200–300 μm, could receive input from many more thalamic afferents. However, the connections are not made randomly between the geniculate afferents and the cortical neurons. Selectivity is expressed in several ways. For example, there is a high degree of precision in the visuotopic map recorded in the first-order cortical neurons in the input layer, i.e., those receiving monosynaptic activation by the thalamic afferents. This clustering is made according to the eye preference of the arbors. The afferents of those thalamic relay neurons that are driven by the right eye cluster together in regions about 0.5 mm in diameter and are partially segregated from the afferents that are driven by the left eye. This segregation forms the basis of ocular dominance columns. In addition, there is some clustering of the afferents according to whether they are ON or OFF center. This clustering of inputs forms the basis of the ON and OFF subfields of the simple cells. In the somato-
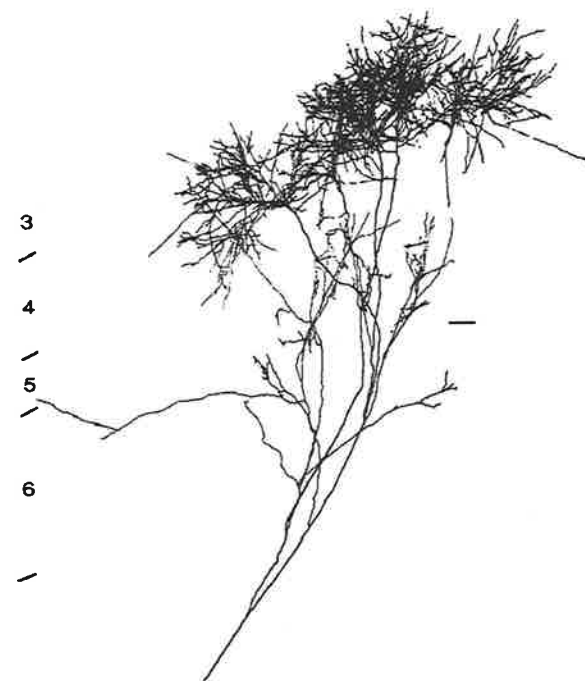

Fig. 12.11. Y-type thalamic afferent from cat visual cortex. Note extensive but patchy arbor in layer 4. This axon formed over 8000 boutons. Labeled intracellularly *in vivo* with horseradish peroxidase. Cortical layers are as indicated. Bars = 100 μm.

sensory pathway, there are segregations according to the modality of the sensory information, e.g., light touch is segregated from deep pressure, and so on.

Other Subcortical Regions. Although the thalamus is a major source of input to the neocortex, it is not the only one. More than 20 different subcortical structures projecting to the neocortex have been identified (Tigges and Tigges, 1985). These structures include the claustrum, locus coeruleus, basal forebrain, the dorsal and median raphe, and the pontine reticular system. As has been pointed out in other chapters, these pathways have distinct neurochemical signatures, which has made the analysis of their cortical targets more tractable. The contributions of these different pathways vary from one cortical area to the next and among species for a homologous cortical area. There are also wide differences in the laminar projections of the terminals of these neurons between areas, and in very few cases have the synaptic targets of these projections been identified. Thus, it is as yet not possible to offer a simple schematic of these pathways, but there are a few whose role in plasticity and development have been examined.

Monoamines. Because of their relative ease of identification, the monoaminergic innervation of the cerebral cortex has been studied most intensively. These systems are generally thought to be diffuse and nonspecific, both in terms of the information they carry and in terms of their lack of spatial specificity and anatomical organization. Physiological examinations of these neurons are rare, but closer examinations of the anatomy have generally revealed a higher degree of specificity (Parnevelas and Papadopoulos, 1989). Three main types of monoamine-containing cortical afferents have been described: the dopamine-positive axons arising from the rostral mesencephalon, the axons containing norepinephrine (also called noradrenaline) originating from the locus coeruleus, and the serotonin (5-hydroxytryptamine [5-HT]) axons that originate from the mesencephalic raphae nuclei. There has been some doubt as to the mode of release of the transmitter, because early studies failed to find clear ultrastructural evidence of synapses. This was consistent with an older concept of the brain as a complex neuroendocrine organ where neurosecretion was the means by which brain activity was modulated. However, it is now clear that monoaminergic axons in the neocortex do form conventional synapses and can show a high degree of anatomical specificity, both for particular cortical areas and for particular laminae within a single cortical area.

Norepinephrine (Noradrenaline). The projections of the locus coeruleus, which lies in the dorsal pons, have been relatively well studied. The nucleus is small, but it projects to most of the neocortex in a roughly topographic arrangement (Waterhouse et al., 1983). Neurons in the dorsal portion project to posterior regions of the neocortex, such as the visual regions, whereas neurons in the ventral portion project to frontal cortical areas. In primates, the strongest projections are to the primary motor and somatosensory cortices and their related association areas in frontal and parietal lobes (Tigges and Tigges, 1985). The fine, unmyelinated axons ramify horizontally, most prominently in layer 6, and form synapses with spine shafts and somata (Papadopoulos et al., 1987). The neurons synthesize norepinephrine, which is thought to be involved in the development and plasticity of thalamocortical projections in the visual cortex. These fibers develop early, and their removal by neurotoxins prevents plasticity of the columns

formed by the thalamic afferents arbors driven by left and right eye (Pettigrew, 1982; Daw et al., 1983). Activity in locus coeruleus neurons correlate with changes in the EEG, which suggests that it is involved in the arousal response induced by sensory stimuli.

Serotonin. The raphe nuclei and pontine reticular formation are a complex of nuclei that contain the highest density of neurons that synthesize serotonin. These neurons project to all cortical areas with varying degrees of laminar specificity (Tigges and Tigges, 1985; Mulligan and Tork, 1988). There are clear differences between projections to the homologous areas in different species that make generalizations impossible, e.g., the strongest projections in the monkey are to the thalamorecipient layers of area 17, whereas these layers are relatively poorly innervated in the adult cat. In the kitten, however, there is a transient surge of serotonergic innervation of the thalamo-recipient layer 4, which may indicate a relationship to the critical period (Gu et al., 1990).

Dopamine. The third monoamine projection to cortex is the dopaminergic pathway. It has been suggested that a dysfunction of the dopaminergic innervation of the prefrontal cortex is one of the factors in the pathogenesis of schizophrenia. The dopaminergic projection to the frontal cortex originates from ventral tegmental area, the rostral mesencephalic groups, and the nucleus linearis. They form symmetrical synapses with the dendrites of pyramidal neurons and with GABAergic smooth neurons. All layers except layer 4 receive dopaminergic input. Dopaminergic projections are strongest to the rostral cortical areas, especially the prefrontal cortex. Here, they target pyramidal neurons, particularly spines, which they share with an excitatory synapse (Goldman-Rakic et al., 2000).

Acetylcholine. Although there are intrinsic sources of acetylcholine (ACh) from neurons within the cortex, the major sources of the acetylcholinergic fibers in the cortex are extrinsic. These fibers originate from the nucleus basalis of Meynert and the diagonal band of Broca, which constitute the nuclei of the basal forebrain. These cholinergic projections to the neocortex have been of particular interest because of their possible involvement in the pathology of Alzheimer's disease. The terminals of the acetylcholinergic fibers distribute through all cortical layers, with the most dense innervation in layer 1 and relatively sparse innervation of the deep layers (De Lima and Singer, 1986; Aoki and Kabak, 1992). They form synapses with dendritic shaft and spines but show some bias for the GABAergic neurons.

GABA. Although it was previously thought that all GABAergic synapses were derived from intrinsic sources in the cortex, it has been demonstrated that there are GABAergic projections from subcortical nuclei to the cortex. These afferents arise from the basal forebrain, the ventral tegmental area, and the zona incerta. The GABAergic neurons of the zona incerta project to sensory and motor cortex but not to the frontal cortex. As with the acetylcholinergic fibers from the same source, the GABAergic neurons of the visual cortex are a major target of the GABAergic afferents of the basal forebrain (Beaulieu and Somogyi, 1991).

Claustrum. The claustrum is also a nucleus of the basal forebrain. It connects recip-rocally and topographically with the cerebral cortex (LeVay and Sherk, 1981; Tigges and Tigges, 1985). However, the reciprocal connections do not form a single continu-ous map but are segregated into function-specific divisions. Thus, the claustrum is not strictly multimodal, although it contains representations of visual, auditory, somato-sensory, limbic, and perhaps motor functions. These functions are represented sepa-rately in the nuclear mass of the claustrum, and there is no evidence of integration of the different modalities. In this respect, the claustrum follows the principle of separate representation of these modalities adopted by the neocortex and thalamus. In the case of the visual system, it is clear that many, perhaps all, of the retinotopically organized visual areas converge on the claustrum, and it in turn projects divergently back to them. In the primary visual cortex the projection to the claustrum arises from a subset of the layer 6 pyramidal neurons. The claustrum sends a sparse projection back to all layers of visual cortex, where it forms excitatory connections mainly with the spines of ex-citatory neurons, except in layer 4, where synapses with dendritic shafts form about half the targets (LeVay and Sherk, 1981).

Corticocortical Connections. Although anatomists have emphasized the long fiber tracts between the neocortex and their subcortical targets and suppliers, the major in-put to any cortical area is from other cortical areas. Braitenberg and Schüz (1991) have calculated that only 1 in 100 or even 1000 fibers in the white matter is involved in sub-cortical projection; the majority of the fibers in the white matter are involved in the in-trahemispheric connections and interhemispheric connections. Since the 1980s, these connections have been intensively studied, particularly in the primate visual cortex. The pattern that has emerged is that the pyramidal neurons of the superficial layers project to the middle layers (principally layer 4) of their cortical target area, whereas the deep layer pyramids project outside the middle layers to superficial and deep layers. These patterns have been used to classify connections as feedforward (projecting to layer 4) or feedback (projecting outside layer 4) (Felleman and Van Essen, 1991). All cortical areas are reciprocally connected by these "feedforward" and "feedback" pathways. In the face of multiple parallel pathways projecting to and from cortical and subcortical areas, such simple classifications of feedforward and feedback may not translate into functional significance. However, it is clear that the substantial majority of the neu-ronal targets of these corticocortical connections are pyramidal neurons.

CONSIDERATIONS OF CORTICAL WIRING

One of the most important principles of a cortical circuit is the need to save "wire," i.e., to reduce the length of the axons that interconnect neurons (Mead, 1990; Mitchi-son, 1992). The dimensions of this problem are evident in the statistics of the amount of wire involved. Each cubic millimeter of white matter contains about 9 m of axon, and an equal volume of gray matter contains about 3 km of axon. The volume occu-pied by axons would be greatly increased if each neuron had to connect to every other neuron in a given area or if every neuron were involved in long distance connections between cortical areas. Instead, the design principles of cortex are that neurons are sparsely connected, that most connections are local, and that only restricted subsets of neurons are involved in long distance connections.

The constraint on volume can also lead to multiple cortical areas. If separate areas are fused into a single area that preserves the total cortical thickness of 2 mm, then the components of the original areas must spread over a larger area (Mitchison, 1992). The original connections between neurons now have to span larger distances and so con-tribute to a larger volume for the same number of neurons. Mitchison has shown that fusing 100 cortical areas leads to a 10-fold increase in the cortical volume. Of course, if all the cortical areas are fused into one area, then much of the white matter can be eliminated. However, the increase in the volume of the *intra*-areal axons far exceeds the reduction of the *inter*areal axons. Similar arguments can be raised for the patchi-ness of connections within a cortical area that are a cardinal feature of cortical orga-nization. A given cluster of neurons projects to a number of sites within a given cortical area. These clusters tend to link areas of common functional properties. The size of the clusters—about 400 μm—is remarkably uniform between cortical areas. This size is similar to the spread of the dendritic arbor. Malach (1992) has shown theoretically that such an organization increased the diversity of sampling across a cortical area that has a nonuniform distribution of functional properties.

SYNAPTIC CONNECTIONS

TYPES

As discussed in Chap. 1, there are two basic types of synapses in the neocortex, which Gray called type 1 and type 2 (Gray, 1959). The synapses made by the vast majority of the cortical spiny neurons and by some of the subcortical projections such as the thalamic and claustral afferents are type 1. Type 2 synapses are made by smooth neu-rons and some of the subcortical projections such as the noradrenergic fibers.

Both types of synapses are found throughout the cortex in approximately constant proportions. In each cubic millimeter of neocortex, there are 2.78×10^8 synapses, of which 84% are type 1 and 16% are type 2 synapses (Beaulieu and Colonnier, 1985). Both types are found on all cortical neurons, but their locations on the dendritic trees of the different types differ (see Fig. 12.12; Gray, 1959; Szentágothai, 1978; White and Rock, 1980; Beaulieu and Colonnier, 1985; White, 1989). Pyramidal neurons receive few type 1 synapses on their dendritic shafts and none at all on their somata or initial segment of the axon. Conversely, type 2 synapses are found on the proximal dendritic shafts, on the somata, and on the axon initial segment of pyramidal cells. Nearly every spine of pyramidal neurons forms a type 1 synapse, but only about 7% of spines form an additional type 2 synapse. Similar distributions have been reported for the spiny stellate cells of the mouse somatosensory cortex, but the pattern for the spiny stellate cells in layer 4 of the primary visual cortex is different. About 60% of the type 1 synapses are formed with the proximal and distal dendritic shafts; the remainder are formed on the heads of the dendritic spines. Type 2 synapses are found on the somata, but synapses are rarely found on the axon initial segment. Although the type 2 synapses are clustered in higher density on the proximal dendrites, about 40% of the type 2 synapses are on distal portions of the dendrites (i.e., more than 50 μm from the soma).

The smooth neurons by definition do not bear spines, so both type 1 and type 2 synapses are formed on the beaded dendrites that are a characteristic feature of smooth neurons (Fig. 12.13). The beads themselves are the sites of clusters of synaptic inputs.
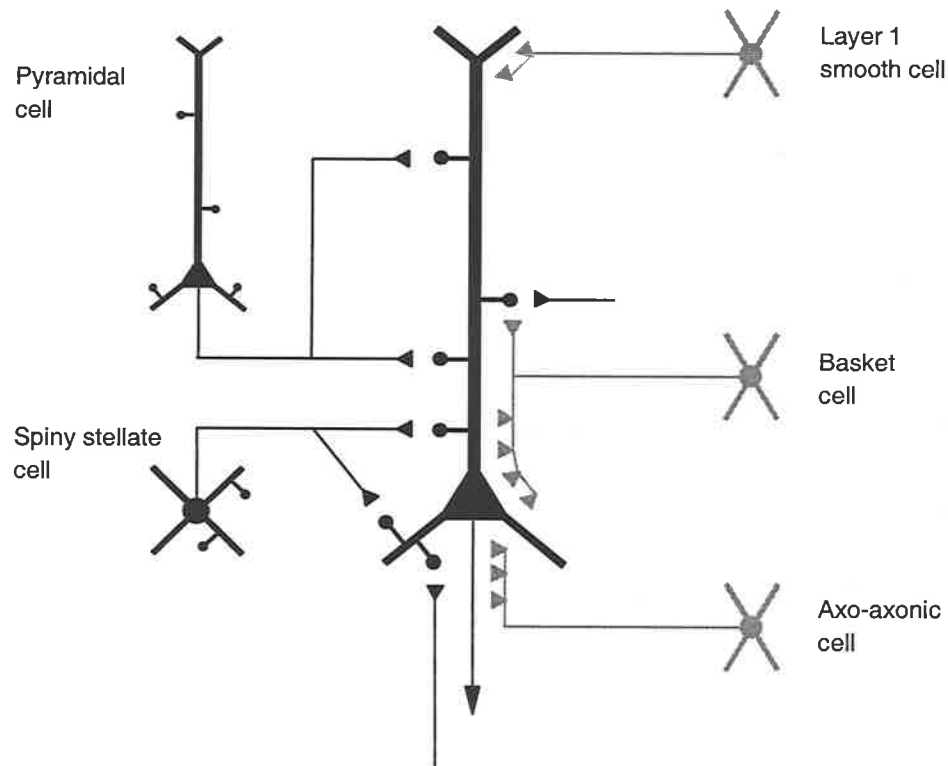
Fig. 12.12. Synaptic connections in neocortex. Configurations of excitatory and inhibitory connections made onto a typical pyramidal neuron (center). The excitatory (spiny) cells (black, left) and thalamic afferents (black, bottom) form synapses with the spines of the target pyramidal neuron. Layer 6 pyramidal neurons (not shown) are the exception to this rule. They form synapses mainly with the dendritic shafts of target spiny cells. There are a number of different classes of inhibitory (smooth) cells (gray, right), each with a characteristic pattern of connection to the pyramidal neuron. Examples of three of the many classes of inhibitory neurons are shown. Each inhibitory cell makes multiple contacts with its target. The superficial smooth cell forms synapses with the apical tuft. The basket cell forms synapses with the soma, dendritic trunk, and dendritic spines. The chandelier cell forms synapses with the initial segment of the axon.

The pattern of input to the smooth neurons is quite different from that of the spiny neurons: both types of synapses cluster on the proximal dendrites and somata at about 2–3 times the density found for spiny dendrites. The type 2 synapses are rarely found on the distal regions of the dendritic tree, and the density of type 1 synapses on the distal dendrites is less than that on the proximal dendrites. The initial segment of the axon of smooth neurons does not form synapses.

Gap junctions have been observed between smooth neurons in an electron microscope study of primate neocortex (Sloper, 1972). Later electrophysiological studies established that there are electrical synapses in the neocortex created by low-resistance pathways, or gap junctions, between dendrites or between dendrites and somata of particular GABAergic neurons, which contain parvalbumin or somatostatin (Galarreta and
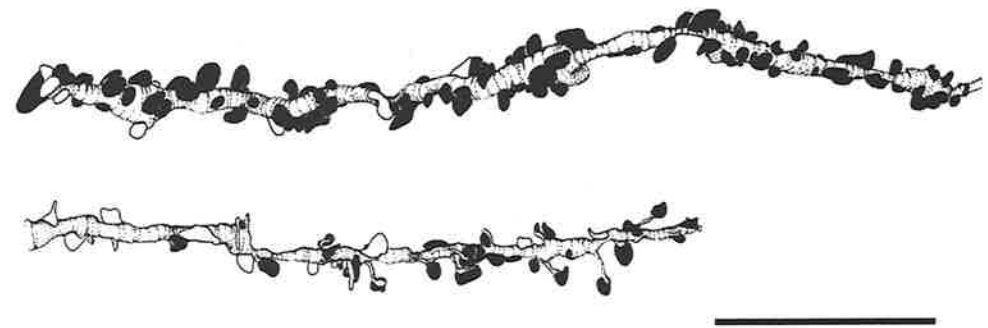

Fig. 12.13. Reconstruction of serial electron microscopic sections showing the complete synaptic input to the dendrite of a small basket cell (top) and the proximal dendrite of a spiny stellate cell (bottom). The leftmost end is connected to the cell body. Both were located in layer 4 of cat visual cortex. The dark shapes are presynaptic boutons that formed asymmetrical (excitatory) synapses, and the open shapes indicate boutons that formed symmetrical (inhibitory) synapses. Scale bar is 10 μm.

Hestrin, 2001). These voltage-dependent gap junctions are formed by a class of proteins called *connexins*. The electrical synapses act as low pass filters. However, because spikes are transmitted through the gap junctions, a spike in one neuron can lead to a fast depolarization in coupled cells, thus providing a means of synchronizing a network of GABAergic neurons (Galarreta and Hestrin, 1999; Gibson et al., 1999). This interpretation is supported by experiments showing that electrical coupling is virtually absent when connexin36 was knocked out, leading to a reduction in gamma frequency synchronization (Gibson et al., 2001).

SPINY NEURONS

The axons of the spiny neurons form the vast majority of the synapses in the cortex. The synapses they form are type 1 in morphology, and almost all are on spines (80%–90% of targets) (Sloper and Powell, 1979a; Martin, 1988). One type of spiny neuron, a layer 6 pyramidal neuron, is the exception to this general rule: it forms most of its synapses preferentially with the shafts of spiny neurons in layer 4 (see White, 1989). The axonal boutons come in two basic types. One is like a bead on the axon; these are called *en passant* boutons. The other bouton looks like a small drumstick and in appearance and dimension closely resembles a dendritic spine. These are called boutons *terminaux*. Some synapses of the spiny stellate or pyramidal neurons are formed with dendrites or somata of smooth neurons, but these constitute only about 10% of their output. A feature of the output of the spiny neurons is that they contribute only a few synapses to any individual postsynaptic target. Conversely, any single neuron must receive its excitatory input from the convergence of many thousands of neurons, most of which are in the same cortical area. The thalamic afferents, which provide the principal sensory input to cortex, form about 10% or less of the synapses in layer 4, the main thalamorecipient layer (White, 1989; Ahmed et al., 1994) (Fig. 12.14).
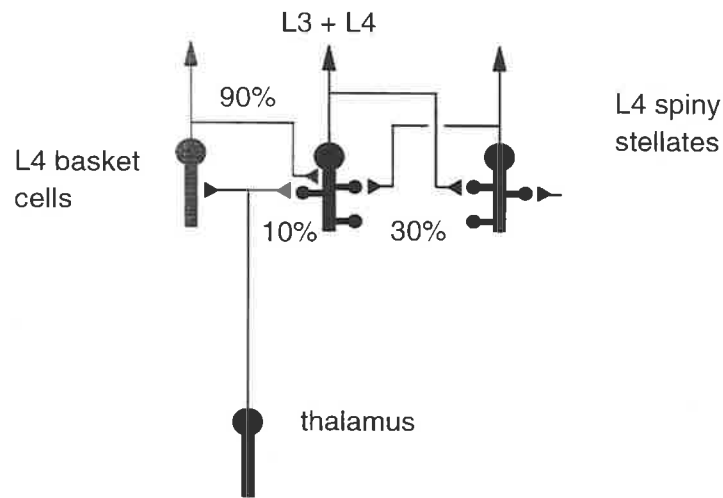
Fig. 12.14. Fractions of synaptic connections onto spiny stellate neurons in layer 4 of cat striate cortex. [From Ahmed et al., 1994.] Only about 10% of the excitatory synapses are derived from the lateral geniculate nucleus. Thirty percent arise from other spiny stellate neurons in layer 4, and an additional 40% arise from the layer 6 pyramidal neurons (not shown). Ninety percent of the inhibitory inputs arise from layer 4 GABAergic neurons, like the basket cells (gray).

SMOOTH NEURONS

The synaptic connections of the smooth neurons differ in a number of significant respects from the spiny neurons. Their axons are less extensive than the spiny neurons, and they make multiple synapses on their targets. This means they contact many fewer targets on average than do spiny neurons. Although the spiny neurons generally form synapses with dendritic spines and shafts, the smooth neurons target these and the soma and axon initial segment. In addition, different smooth neuron types form synapses specifically with different portions of the neuron (see Fig. 12.12). The major output of the smooth neurons, however, is to spiny neurons. The smooth neurons form no more than 15% of the targets of the spiny neurons.

These neurons again fall into several characteristic classes: large basket cells, small basket cells (clutch cells), chandelier cells, double bouquet cells, bipolar cells, neuroglialform cells, Martinotti cells, and Cajal Retzius cells. Smooth neurons use GABA as a transmitter and hence act to inhibit those neurons to which they connect.

Basket cells (see Fig. 12.7) are the most frequently encountered smooth neuron in Golgi preparations and in intracellular physiological studies *in vivo*. Kisvárday (1992) has estimated that they form at least 20% of all GABAergic neurons. They have a very characteristic axon that forms the most extensive lateral connections of any of the smooth cell types. The basket cells of the superficial and deep layers have axons that radiate from the soma up to distances of 1–2 mm. In layer 4, the small basket cell (see Fig. 12.8) axon is more localized and extends about 0.5 mm laterally in most cases (Mates and Lund, 1983; Kisvárday et al., 1985). Each basket cell forms multiple synapses with about 300–500 target neurons and makes about 10 synapses on average

with each target. Ramón y Cajal originally provided the descriptive name *basket cells* because in the Golgi preparations the axons of the basket cells form pericellular *nests*, or baskets, around the soma of the pyramidal neurons (Cajal, 1911). Modern light and electron microscopic studies on the axonal boutons of intracellularly labeled basket cells has revealed, as noted earlier, that the major targets of the basket cell axons are the dendritic shafts and spines of pyramidal and spiny stellate cells (Somogyi et al., 1983; Kisvárday, 1992). The "basket" seen by Ramón y Cajal is formed by the convergence of about 10–30 basket cells, each contributing a twiglet to the perisomatic nest. Superficial and deep basket cells and clutch cells make about 20%–40% of their synapses with spines, 20%–40% with dendritic shafts, and the remainder with somata.

Chandelier cells (see Fig. 12.10) are rarely encountered in Golgi preparation and in intracellular recordings *in vitro* and *in vivo*. However, they have been a focus of interest because their sole output is to the initial segment of the axons of pyramidal neurons. Such specificity is not seen with any other neocortical cell, although it is common elsewhere in the brain (see earlier chapters). The chandelier cells seem to be found only in the superficial layers and layer 4, but some have a descending axon collateral that innervates the deep layer pyramidal neurons. Correspondingly, electron microscopic examination of the initial segments of the pyramidal neurons has indicated that there are about 3 times as many synapses along the initial segment of the axon of superficial layer pyramidal neurons as in deep layer pyramidal cells (Somogyi, 1977; Sloper et al., 1979; Peters, 1984). In the superficial layers, the axon initial segment forms about 40 type 2 synapses with the boutons of the chandelier cell. Each pyramidal neuron receives input from three to five chandelier cells, and each chandelier cell forms synapses with about 300 pyramidal neurons over a surface of about 200–400 μm (Somogyi et al., 1982; Peters, 1984).

Double bouquet neurons (see Fig. 12.9) are smooth neurons that are found in the superficial layers and have a "bitufted" axonal system that spans several layers (Cajal, 1911; Somogyi and Cowey, 1981). In contrast to the laterally directed axons of the basket cell, the predominant orientation of the double bouquet cell's axon is vertical. For this reason it was originally thought that the vertically oriented apical dendrites of the pyramidal neurons were the major target of multiple synapses from the pallisades of double bouquet axons. There is no clear evidence of multiple synapses between double bouquet axons and apical dendrites, but the pyramidal neurons are nevertheless major targets. In the cat, about 70% of the type 2 synapses of double bouquet cells are formed with dendritic spines, and most of the remainder are formed with dendritic shafts (Tamas et al., 1998).

The synaptic connections formed by the axons of layer 1 neurons have been studied rarely. The small neurons of layer 1 have as their major target the spines and dendritic shafts of pyramidal neurons apical dendritic tufts that form most of the neuropil of layer 1. The connections made by the Cajal-Retzius cells are unknown. Similarly, the connections made by other smooth neurons of the neocortex have yet to be determined.

Many GABAergic cells are immunoreactive to one or more of the calcium binding proteins parvalbumin, calbindin, and calretinin, as well as to neuropeptides such as cholecystokinin, somatostatin (SSt), vasoactive intestinal polypeptide (VIP), neuropeptide Y, and corticotropin-releasing factor (Demeulemeester et al., 1988, 1991). The profile of immunoreactivity expressed by a neuron depends on its laminar location and

morphological type. There is considerable overlap between cell types, and the profile also depends on the cortex's state of embryological development. Nevertheless, these provide a useful basis for the classification of smooth cell subtypes (Wang et al., 2002). Some spiny neurons also express immunoreactivity for calbindin, cholecystokinin, and SSt. However, their reactivity for these molecules is weaker, particularly in more mature animals.

BASIC CIRCUIT

An article of faith among neuroanatomists from the beginning of the study of the cortical circuits was that there was an elementary pattern of cortical organization. Anatomists studying Golgi-stained material were generally convinced that there were structural details that remained constant despite variations in cell number, form, size, and type of neurons. This constant, according to Lorente de Nó, was the "arrangement of the plexuses of dendrites and axonal branches," by which he meant the synaptic connections between cortical neurons. However, subsequent examination of the details of cortical circuitry still leave considerable leeway in interpretation of the pattern of connections. Any attempt to suggest a common basic pattern of connections necessarily will be open to the criticism that such models are based on the intensive study of a very small number of areas, mainly primary sensory areas (White, 1989). Nevertheless, the great advantage of having some hypothetical circuit is that it focuses ideas and gives form to otherwise simply descriptive accounts of cells and connections within a given area that have been the standard works in the anatomical field.

Most modern models of cortical circuits are derived from functional studies. In contradistinction to the great diversity of cell types and interconnections that characterize the anatomical descriptions, the circuits derived from physiological experiments strip off all of the embellishment and detail: simple circuits of excitatory cells make up the core of these models. The Hubel-Wiesel models of the local circuits of visual cortex are the best known examples (Hubel and Wiesel, 1977). In these circuits, the inhibitory neurons are added as a means of providing the lateral inhibition that is such a feature of sensory processing at all levels. Two basic designs have emerged. In the dominant model, the processing is strictly serial: input arrives in the cortical circuit, it is fed forward through a short chain of two or three neurons within the local area, and then is transmitted to other areas by the output neurons. This feedforward model follows from the simple idea that sensory input must pass through several processing stages in the neocortex before it arrives at a motor output.

An alternative view was first given form by Lorente de Nó (1949). He supposed that the rich interconnections between the different cortical layers made the cortical circuit a unitary system, with no clear basis for a distinction between input, association, and output layers. In his view, impulses circulate through these recurrent circuits, and the activity in the cortical circuit is modified by the action of the association fibers arriving at critical points within the circuit. In turn, the effect of the incoming input depends on the activity in the circuit at that point in time.

Both the feedforward and the recurrent model agree, however, that the processing that occurs in the neocortex is essentially local and vertical. In this arrangement, the anatomy and the physiology agree. In most cortical areas, the function being represented is laid out topographically, as in the retinotopic maps of the visual cortex or the motor maps in the motor cortex. For example, portions of the sensory surface that will receive related input are nearest neighbors in their cortical representation. The vertical connectivity within the cortex is similarly local. The axons of cortical neurons do not extend more than a few millimeters laterally in any area; thus, the monosynaptic connections at least are local. This corresponds with the physiological findings of Mountcastle, Hubel, Wiesel, and others who discovered that neurons with similar functional properties are organized in "columns" that extend for the cortical surface to the white matter (Powell and Mountcastle, 1959; Hubel and Wiesel, 1977).

In fact, with few exceptions, most arrangements of neurons are only strictly columnar when viewed with the one-dimensional tool of the microelectrode. The clearest view arises from optical imaging in which the activity of large numbers of nerve cells can be viewed by optical imaging (Sengpiel and Bonhoeffer, 2002) and fMRI (Duong et al., 2001). Such imaging techniques have been used to show that in the lateral dimension, the different functional maps take the form of slabs or pinwheels. The widths of the slabs vary according to the particular property being mapped but are of the order of 0.5 mm in the case of the best-studied system—the ocular dominance system of the primary visual cortex. In this system, the afferents of the lateral geniculate nucleus representing the left and right eye map into a series of parallel slabs that look like a zebra's stripes when viewed from the surface. Such segregation and patchiness are seen at the level of single axonal arbors and appear to be the means by which the cortex maps multiple processes into a single area. In the few cases in which it has been examined, the rule of connectivity between patches is that "like connects to like." For example, a number of different functional dimensions are represented within the retinotopic map of the primary visual cortex of primates. These dimensions are seen physically in the ocular dominance, the orientation slabs, and the cytochrome oxidase columns, which are called *blobs* because of their appearance when viewed in tangentially cut sections of the cortex (Hendrickson et al., 1981; Horton and Hubel, 1981; Wong-Riley and Carroll, 1984). In each of these systems, neurons of like function interconnect.

CORTICAL OUTPUT

All projection neurons have recurrent collaterals that participate in local cortical circuits, so there are no layers that have exclusively output functions. The output neurons from the cortex are generally pyramidal neurons. These same cells, however, may also be *input* neurons in that they may receive direct input from the thalamus. There is a laminar-specific organization of the output according to the location of their targets. A simplified view of the laminar organization is provided in the summary diagram in Fig. 12.15. The general rule of thumb is that corticocortical connections arise mainly from the superficial cortical layers and the subcortical projections arise from the deep layers. Within the deep layers, there is an output to regions that have a motor-related function, e.g., the superior colliculus, basal ganglia, brainstem nuclei, and spinal cord. These regions receive their cortical output from a relatively small number of layer 5 pyramidal neurons. There is also an output to the subcortical relay nuclei in the thalamus, which are the source of the primary sensory input to the cortex. This corticothalamic projection generally arises from the layer 6 pyramidal neurons. However,
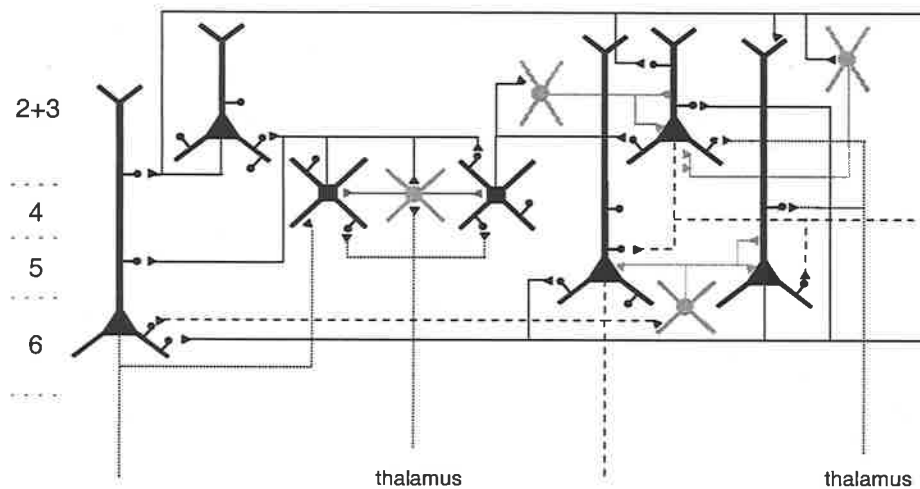
Fig. 12.15. Basic circuit for visual cortex. Smooth, GABAergic neurons and their connections are indicated in gray. Spiny neurons and their connections are indicated in black. Cortical layers as indicated.

there are clear exceptions to this rule of thumb. In area 4 the projections that form the pyramidal tract, which supplies the spinal cord and cerebellum, arise from both layer 5 and layer 3 pyramidal cells. The corticocortical connections may also arise from neurons in the deep layers. However, the simplifications are not extreme and offer a useful constraint on the connections that can be made within the basic circuits. For example, if a circuit in cat visual cortex requires an output to the eye-movement maps of the superior colliculus, then it necessarily will have to connect to the output pyramidal neurons of layer 5. Although particular laminae are the source of the outputs to these different cortical and subcortical regions, the set of output neurons within a given lamina may not be uniform. Thus, within layer 6, the pyramidal neurons that give rise to the corticothalamic projection are morphologically different from those that give rise to the corticoclaustral projection (Katz, 1987). These two groups of pyramidal neurons also have different local projection patterns: the corticothalamic pyramidal neurons have a rich projection to layer 4, whereas the corticoclaustral cells project within layer 6 itself. The receptive fields of the corticothalamic neurons are significantly smaller than those of the corticoclaustral neurons (Grieve and Sillito, 1995).

As with the local intra-areal connectivity, the output neurons that project to other cortical areas also appear to be organized in patchy systems. One of the most elaborate discovered so far is the output from primary (V1) to the secondary visual area (V2), which arises from at least three specific subgroups of neurons in V1. These neurons project to a stripe system in area V2 in the monkey. These pathways may be visualized by the pattern of cytochrome oxidase staining (Gilbert and Kelly, 1975; Gilbert and Wiesel, 1979). Neurons located in the cytochrome oxidase blobs in V1 project to a series of thin cytochrome oxidase blobs in V2. The neurons in layer 3 outside the blobs project to pale stripe (interstripes) in V2, whereas the third group of projection neurons located in layer 4B of V1 project to a series of thick cytochrome stripes in V2.

The stripes formed by these projections themselves reveal the organization of output from V2 to other cortical areas: the thin cytochrome stripes project to visual area 4 (V4), the interstripes to V3 and V4, and the thick stripes to area MT.

SYNAPTIC ACTIONS

Release of neurotransmitter at synapses is triggered by the membrane depolarization associated with the arrival of an action potential. Consequently, the pattern of arrival of action potentials at the synaptic bouton is one of the fundamental factors governing the interaction of presynaptic and postsynaptic neurons. It is usually assumed that the pattern of action potentials seen by the presynaptic terminal is exactly the pattern that was generated at the beginning of the presynaptic axon. That is, we assume that the axon acts as a simple transmission line for action potentials and that there are no factors that selectively alter its transmission characteristics over moderate time intervals. The presynaptic axon begins at the initial segment, which is also the site at which the axonal action potential transmission begins. The initial segment is electrotonically close to the soma, and therefore we assume the electrical events of the initial segment can be recorded from the soma, which is where most intracellular recordings are made with patch or sharp pipettes. Most of our knowledge about interneuronal communication rests on interpretations of electrical events in the soma and in particular on the assumption that the action potentials that we observe in the soma will ultimately affect postsynaptic targets.

NEURONAL EXCITABILITY

In considering the action of synapses, there are two key issues. One is the effect of the synapses on the neuron at the site of the synapse; the other, the response of the whole neuron to these local synaptic actions. The former issue includes the attributes of the synapse such as the kinetics of a single synaptic response and the dynamics of a series of synaptic transmissions, whereas the latter includes the attributes of the neuron such as its membrane properties, the ionic currents involved, and the shape and cable properties of the neuron.

Sodium Currents. Action potential generation entails regenerative depolarization followed by a restorative repolarization. In cortical neurons, as in most other neurons, these two phases are mediated by a fast, voltage-dependent, inactivating sodium current (Connors et al., 1982) and a delayed, voltage-dependent potassium current (Prince and Huguenard, 1988), respectively. In addition to the inactivating sodium current, cortical neurons also exhibit a noninactivating, voltage-dependent sodium current (Stafstrom et al., 1982, 1984) similar to that observed in cerebellar Purkinje cells (Llinas and Sugimori, 1980a,b) (see Chap. 7) and hippocampal pyramidal neurons (Hotson et al., 1979; Connors et al., 1982) (see Chap. 11) and analogous to the slow inward calcium current (I_i) seen in spinal motoneurons (Schwindt and Crill, 1980) (see Chap. 3). In cortical neurons, this "persistent" sodium current ($I_{Na,P}$) is activated about 10–20 mV positive to the resting potential and attains steady state conductance within about 4 ms. It remains persistent and large up to at least 50 mV above resting potential (Stafstrom et al., 1984). These properties suggest that $I_{Na,P}$ can be activated by EPSPs and that $I_{Na,P}$ acts as a current

amplifier for depolarizing inputs. Indeed, $I_{Na,P}$ can itself provide regenerative depolarization that is able to drive the membrane to the level where the larger spike-generating sodium current is activated (Stafstrom et al., 1982). The difference in kinetics between these two regenerative sodium currents is probably responsible for the indistinct transition between the subthreshold rise of membrane potential and the rapid initial rise of the action potential (Stafstrom et al., 1984). On the other hand, the deactivation of $I_{Na,P}$ during an IPSP removes its contribution to regeneration and thereby enhances the effect of inhibition when the cell is relatively depolarized.

By recording directly from the apical dendrites of the pyramidal neurons (Stuart and Sakmann, 1994; Stuart et al., 1997), it has been shown that the dendrites contain active sodium conductances. This permits the action potential to propagate back along the apical dendrite. However, the dendritic sodium conductances appear to be at a much lower density than at the soma or axon initial segment, which has the highest density and is the main site of initiation of the action potential as was originally proposed from recordings from the motoneuron (Eccles, 1957; Fuortes et al., 1957) (but see Colbert, 2001) in hippocampus.

One effect of the dendritic sodium conductances is that they support propagation of the action potential from the soma backward into the dendritic tree. However, because their concentration is relatively low in the dendrites, the gain for depolarization there is not necessarily regenerative, and so the back propagation into the dendrites is passive. This renders the dendritic action potential a graded action potential, the amplitude of which depends on the local input resistance, which can, for example, be reduced by dendritic inhibition. Such local changes in the gain for depolarization may be crucial for controlling the amount of calcium influx evoked by the action potential into the dendrites, and thereby scale the degree of synaptic plasticity induced at a synapse as a function of background dendritic activity.

Potassium Currents. The inward sodium currents that accompany spike depolarization are opposed by an increase in outward potassium currents, and these currents ultimately restore the neuronal membrane to its resting level. The classic action potential mechanism provides a restorative outward current by just one delayed voltage-dependent potassium conductance, but the restorative outward current of cortical neurons is enhanced by several additional potassium currents. These currents affect the dynamics of membrane during postspike recovery and also during the subthreshold response to depolarizing inputs. Consequently, they affect the neuron's repetitive discharge behavior.

In the simplest case, a suprathreshold sustained depolarizing input current will evoke a train of action potentials. Each action potential ends with a repolarization that drives the membrane potential below threshold. The subsequent interspike interval will depend on the rate of postspike depolarization, because this will determine the interval to the next threshold crossing. If the time constants of the membrane currents are all short (i.e., of the order of an action potential duration), then the interspike intervals will be of equal duration and the neuron will exhibit sustained regular discharge. But some of the potassium conductances have much longer time constants, so their outward currents can be active throughout successive interspike intervals.

Because these outward potassium currents oppose the depolarizing input currents, they retard threshold crossing and so increase the interspike interval. These interactions are the basis of adaptation, often referred to as "spike frequency adaptation," the progressive lengthening of interspike interval that occurs during a sustained depolarizing input to some cortical neurons. The process of adaptation in cortical cells is interesting because it imposes an intrinsic restriction on their discharge. It is calcium dependent, it can be modified by neurotransmission, and adaptation characteristics correlate with morphological cell type.

The outward potassium currents that underly the impulse afterhyperpolarizations (AHPs) are seen in cortical neurons both *in vivo* and *in vitro* (Connors et al., 1982). Three separate APHs have been identified in layer 5 neurons of sensorimotor cortex: fast, medium, and slow (Schwindt et al., 1988). The fast AHP has a duration of milliseconds and follows spike repolarization. It is often followed by a transient delayed afterdepolarization (ADP). The medium AHP follows a brief train of spikes. It has a duration of tens of milliseconds, and its amplitude and duration are increased by the frequency and number of spikes in the train. The slow AHP is evoked by sustained discharge and has a duration of seconds. All three hyperpolarizations are sensitive to extracellular potassium concentration, but they have different sensitivities to divalent ion substitutions and pharmacological manipulations (Schwindt et al., 1988). This suggests that they are mediated by at least three distinct potassium conductances. However, the individual potassium conductances have not been identified completely. This is partly because of the difficulty in comparing the characteristics of the many potassium conductance types found in various excitable cells, the many different regimens of investigation, and inconsistent nomenclature.

There is evidence that neocortical cells have at least four potassium conductances: (*1*) a delayed rectifier, (*2*) a fast transient voltage-dependent (A-like) current, (*3*) a slow calcium-mediated (APH-like) current, and (*4*) a slow receptor-modulated voltage-dependent (M-like, mAHP) current (Connors et al., 1982; Schwindt et al., 1988). Thus, the potassium currents of neocortical neurons appear qualitatively similar to those reported in hippocampal neurons of archicortex (see Chap. 11). However, the situation is rather more complicated than this, as the following examples illustrate. The transient fast current of cortex is TEA sensitive (Schwindt et al., 1988). The mAHP current is due to a calcium-mediated potassium conductance and so is superficially similar to AHP currents seen in hippocampal neurons, but the cortical conductance mechanism is not sensitive to TEA, whereas the hippocampal current is. The cortical conductance is sensitive to apamin, whereas the hippocampal current is not (Schwindt et al., 1988). Muscarine and beta-adrenergic agonists abolish the sAHP but have no effect on the mAHP (Schwindt et al., 1988), whereas in hippocampus acetylcholine affects both the M and AHP currents (Madison and Nicoll, 1984). These and other conductance differences may be due to important functional constraints on the discharge of neocortical neurons that are different from the discharge requirements of hippocampal neurons. An alternative view is that the differences have less to do with unique discharge requirements than with the variations of parallel evolution.

Some outward current conductances can be modulated by neurotransmitters (see Chap. 2). The outward potassium M current of cortical pyramids is reduced by activation of muscarinic receptors (McCormick and Prince, 1985; Brown, 1988). Because the outward current is reduced, the effect of depolarizing currents is enhanced. The slow Ca^{++}-activated potassium (AHP) current of cortical pyramids is also decreased

by ACh (McCormick and Prince, 1986a). These modulations of slow outward currents are the means whereby ACh enhances discharge frequency and decreases adaptation. Similar effects have been noted in hippocampal neurons (Benardo and Prince, 1982; Cole and Nicoll, 1984; Madison and Nicoll, 1984). Neurotransmitters may also modulate currents that interact with the slow hyperpolarizing potassium currents. Schwindt et al. (1988) have shown that low concentrations of muscarine abolish the sAHP, but at higher concentrations the sAHP is replaced by an sADP that is not potassium sensitive, nor is it sensitive to the sodium channel blocker TTX. The mechanism of the sADP is unknown.

In addition to these effects, acetylcholine also evokes a transient early inhibition of pyramidal neurons. However, two findings indicate that this inhibition is probably an indirect effect of the excitation of inhibitory interneurons. First, the inhibition is mediated by a chloride conductance similar to that activated by GABA. Second, ACh has a rapid excitatory effect on the fast-spiking (presumably GABAergic) cortical neurons (McCormick and Prince, 1986a,b), and smooth cells are known to have cholinergic afferents (Houser et al., 1985).

Calcium Conductances. Calcium currents also contribute to the dynamics of cortical neurons. For example, depolarization of the dendrites, by dendritic action potentials, can evoke calcium influx through multiple calcium channels (Yuste et al., 1994; Markram et al., 1995; Schiller et al., 1995). These calcium currents may affect the dynamics directly by contributing to the electrical behavior of the membrane or indirectly by changing the internal calcium concentration, which in turn affects potassium conductance (described earlier) and also by regulating multiple intracellular metabolic pathways and receptor kinetics, as well as synaptic plasticity.

Somatic recordings from antidromically activated pyramidal tract neurons *in vivo* indicated the existence of fast prepotentials (Deschenês, 1981). Blocking the sodium channel blocker with QX-314 left these prepotentials intact, suggesting they were mediated by calcium channels in the dendrites (Hirsch et al., 1995). Where calcium currents are voltage dependent, they operate as sodium currents do and so could contribute to spike generation. However, the calcium currents appear to be relatively small in cortical neurons and must be unmasked by both blocking the sodium currents and depressing the potassium currents. Under these conditions, a Ca^{2+} spike can be elicited from some cortical neurons (Connors et al., 1982; Stafstrom et al., 1985). The threshold for this spike is about 30–40 mV positive to the resting potential and therefore well above the activation thresholds for the sodium currents, $I_{Na,P}$ and I_{Na}.

Calcium spikes have been observed in hippocampal pyramidal neurons (see Chap. 11) and elsewhere, and in these cases they can be evoked after blockade of the sodium currents alone (Schwartzkroin and Slawsky, 1977; Wong et al., 1979). To initiate a calcium spike, the conductance for calcium must be much larger than that for potassium. Presumably, g_K is large in cortical cells and must be depressed to obtain a conductance ratio favorable for calcium spike initiation. Therefore, the need to depress the potassium conductance in cortical cells implies either that g_K is larger in cortical neurons than other calcium-spiking cells or that the calcium conductance is smaller. An alternative explanation is that the site of the calcium conductance is located in the dendrites, electronically distant from the soma. In this case, a depolarization large enough to drive the distant site to the activation threshold of the calcium conductance would also strongly activate the more proximal voltage-dependent potassium conductances. The resulting increase in potassium conductance would shunt depolarizing current injected into the soma, and so prevent the dendritic membrane from reaching the threshold for calcium current activation.

Stafstrom et al. (1985) suggest that there are two calcium conductances in cortical neurons and that these are distributed along the soma-dendrite. The somatic calcium conductance is slow and small and has a high threshold. The dendritic conductance is both faster and larger than its somatic counterpart. Its threshold is also high, but this may be partly due to electrotonic distance from the soma, which makes it relatively difficult to activate from an electrode in the soma. Both somatic and dendritic currents contribute to the calcium spike. Somatic depolarization activates the somatic calcium current, and that in turn activates the more distal dendritic calcium conductance that powers the calcium spike. Both currents are probably persistent, so they require activation of an outward current to effect the recovery phase of the spike. This outward current is provided by the slow potassium (AHP) current that is activated by the influx of calcium in cortical (Hotson and Prince, 1980) and hippocampal (Lancaster and Adams, 1986; Madison and Nicoll, 1984) neurons.

Direct evidence for the existence of dendritic voltage-sensitive calcium channels has come from membrane patches of apical dendrites (Huguenard et al., 1989) and by calcium imaging of the dendrites (Yuste et al., 1994). The imaging studies showed that the dendritic accumulation of calcium took place immediately after calcium spikes were triggered, followed by a slower diffusion of intracellular calcium. Confocal and two-photon microscopic imaging of calcium has revealed the sites of calcium channels in the dendritic shaft (Markram and Sakmann, 1994) and in spine heads (Yuste and Denk, 1995; Holthoff et al., 2002). It appears that calcium channels are distributed over the whole dendritic tree, but the distribution has hot spots where activation can be regenerative. For example, in the tuft of the layer 5 pyramidal neurons, the sodium-dependent action potential can propagate back into the terminal tuft of layer 5 pyramidal neurons and evoke a calcium-dependent action potential, which then propagates forward toward the soma (Schiller et al., 1997). This is due to a spatially restricted low-threshold zone on the apical dendrite, located 550–900 μm from the soma, at which calcium-dependent action potentials can be evoked. This zone appears to be active *in vivo* during synaptic stimulation (Larkum and Zhu, 2002).

The issue of the role of spines in the compartmentalization of calcium has also been addressed by a number of studies. The first studies that used optical methods to image the spines in hippocampal pyramidal neurons indicated that individual spines could have quite different calcium dynamics to their parent dendrites (Guthrie et al., 1991; Muller and Connor, 1991). However, further studies in the hippocampal pyramids in which two-photon microscopy was used to image the spines of hippocampal pyramidal neurons loaded with a calcium-sensitive dye indicate that individual spines are only activated under subthreshold conditions. If the neuron fires an action potential, then calcium enters the spines (Denk et al., 1996). In cortical pyramidal cells, the spine calcium kinetics are controlled by the diameter of the parent dendrites, the length of the spine neck, and the strength of the spine calcium pumps. The importance of the spine neck is that it is motile and thus the calcium dynamics of the spine can be regulated

by rapid spine motility (Majewska et al., 2000). The morphological constraints also mean that the calcium dynamics of spines depends on their location on the dendritic tree (Holthoff et al., 2002). Theoretically, the restriction of calcium in the spine during subthreshold synaptic activation could serve to segregate the potential that occurs on spines during coactivation of presynaptic and postsynaptic neuron (Rall, 1974a).

Repetitive Discharge. The repetitive discharge properties of neocortical cells has been investigated both *in vivo* (e.g., Calvin and Sypert, 1976) and *in vitro* (e.g., Ogawa et al., 1981). McCormick et al. (1985) originally reported three electrophysiological cell types in neocortex: fast-spiking, regular spiking, and bursting cells. The fast-spiking cells were sparsely spiny or aspiny neurons. These "smooth cells" are the GABA-containing, inhibitory neurons of cortex. The regular and bursting cells were both pyramidal neurons. "Regular firing," which is somewhat of a misnomer, refers to the adapting pattern of discharge in response to an injection of constant current into the soma. This was the predominant behavior of most pyramidal neurons. Only a small percentage of these pyramidal neurons exhibited a bursting discharge, and they were located mainly in the deep cortical layers (Connors and Gutnick, 1990). However, it is now clear that there are exceptions to the general function–structure relationships described, and smooth neurons are found that have the adapting pattern that was thought to be a characteristic of pyramidal neurons. Kawaguchi (1995), for example, has found chandelier cells, double bouquet cells, and neurogliaform cells with adapting patterns of discharge that are more commonly associated with spiny neurons.

The discharge of regular spiking neurons showed various degrees of adaptation, and the presence of both AHP and M currents could be demonstrated in these cells. A transient fast voltage-dependent (A) current is present in pyramids, and this may contribute to their adaptation (Schwindt et al., 1988). The structure–function correlations of the burst/nonburst firing pyramidal neurons of layer 5 have been determined (Chagnac-Amitai et al., 1990; Connors and Gutnick, 1990; Mason and Larkman, 1990; Kim and Connors, 1993). The regular firing pyramids have thin apical dendrites that do not branch extensively in layer 1. The burst-firing pyramids, by contrast, have thick apical dendrites and an extensive tuft in layer 1. Multipolar and bitufted neurons with bursting patterns have been described by Kawaguchi (1995), who called them "low-threshold spike" cells. These neurons would respond with a burst of action potentials when the neuron was depolarized from a hyperpolarized potential. Their dendrites had few spines.

Bursting neurons (Connors et al., 1982; McCormick et al., 1985; Kawaguchi, 1995) respond to depolarization by generating a short burst of about three spikes. McCormick and Gray (1996) have reported another class of bursting cell, which they called a "chattering cell." This fast-spiking spiny cell produces a rhythmic series of high-frequency bursts during sustained depolarization. The exact mechanism of bursting in cortical neurons is unknown, but it has been explained by various mechanisms including the activation of low- and high-threshold calcium currents (McCormick et al., 1985; Jahnsen, 1986; McCormick, 1996), by a calcium-dependent potassium current (Berman et al., 1989), and by the distribution of sodium channels in the dendritic tree (Mainen and Sejnowski, 1996). In theory, it is possible to achieve a short burst in a neuron that has limited fast outward current and a dominant AHP current (Berman et al., 1989).

The reduced fast outward current encourages a short interspike interval and consequently a rapid discharge. The discharge would be terminated by the growing AHP current. Thus, variations in the parameters of the same outward current conductances could determine whether a pyramidal neuron discharges in regular or burst mode. In the model of Mainen and Sejnowski (1996), the dendritic sodium conductances promote propagation of the somatic action potential back into the dendrites. When the soma has repolarized, current returns from the dendrites to produce a late depolarization and some maintained action potential discharge. This effect was enhanced by high-threshold voltage-gated Ca^{2+} channels. Schwindt et al. (1988) have shown that reduction of the transient fast potassium conductance converts normal firing into burst firing, whereas specific reduction of mAHP increases the instantaneous discharge rate but does not affect adaptation.

The "fast spiking" cells encompass a variety of smooth neuron types (Kawaguchi, 1995) (Fig. 12.16). The action potentials of fast-spiking cells are brief by comparison with pyramidal neurons. The repolarization phase of the action potential is rapid and followed by a significant undershoot. This indicates the presence of an unusually large and fast repolarizing potassium current. Indeed, Hamill et al. (1991) were able to demonstrate that fast-spiking cells have a higher density of "delayed rectifier" potassium currents than do pyramidal neurons. The spike repolarization is followed
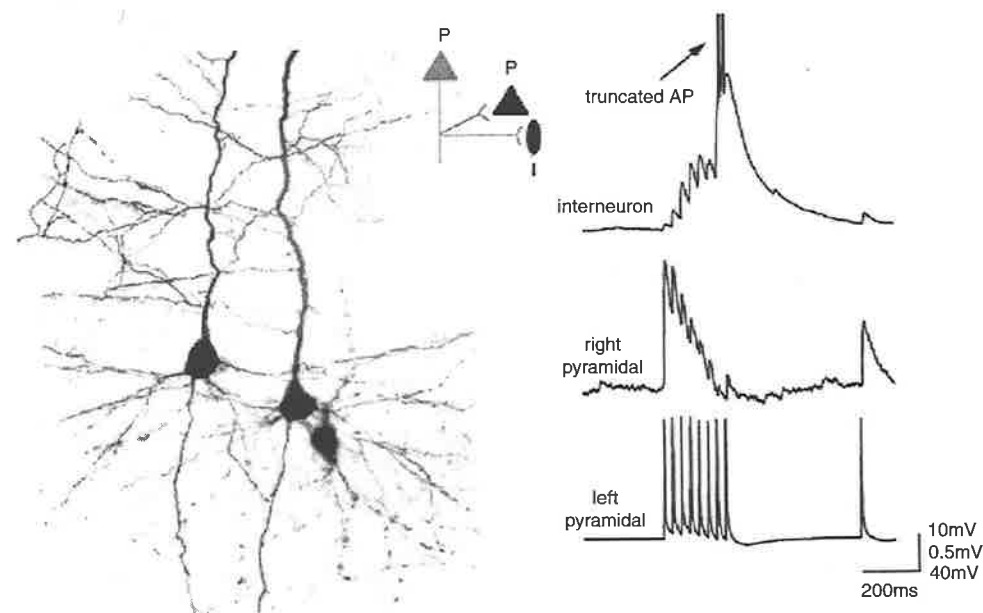
Fig. 12.16. An example of differential facilitation and depression, in a network of three biocytin-labeled cortical neurons (two pyramidal neurons and an interneuron) that were recorded simultaneously using the whole-cell patch-clamp technique. Trains of action potentials evoked in the right most pyramidal neuron by direct current injection elicited a facilitating synaptic response in the interneuron (upper voltage trace) but a depressing synaptic response in the neighboring pyramidal neuron (lower voltage trace).

lowed by a transient afterhyperpolarization. These cells showed little adaptation. The initial slopes of their current–discharge relation were steeper, and their maximum discharge frequencies were higher than those of pyramidal neurons. There was no evidence of either the AHP or M potassium currents in these neurons, and the absence of these longer time-constant outward currents in fast-spiking cells would explain their lack of adaptation.

Studies have attempted to classify the different electrical properties of interneurons in more detail. Five main classes have been identified; adapting, nonadapting, stuttering, irregular spiking, and bursting (Kawaguchi and Kubota, 1993; Kawaguchi, 1995; Porter et al., 1998; Gupta et al., 2000). Different behaviors at the onset of a depolarizing pulse have also been reported: delayed discharge, transient bursting, or no special onset response.

SYNAPTIC DYNAMICS

The effect of a presynaptic action potential on a particular postsynaptic target is complex, and depends on a functional context in both the presynaptic and postsynaptic components of the synapse which the action potential excites. This context is governed by many time-scales, from milliseconds to years.

Transient Changes in Effect. The arrival of an action potential at a presynaptic terminal triggers an increase in the intracellular concentration of calcium. This rise in Ca^{2+} enables a process whereby transmitter-laden vesicles fuse with the synaptic membrane and release their contents into the synaptic cleft. A precondition for release is that the vesicles be docked at a restricted number of sites on the presynaptic membrane. However, not every vesicle releases its contents in response to an action potential. Some sites are refractory following previous release; of all of the n vesicles that are primed to release their contents, any particular vesicle will react to a given action potential with probability P, as discussed in Chap. 1. This probability varies from less than 0.01 at specific pyramidal neuron–interneuron connections (Thomson et al., 1995) to 0.3–0.6 for typical pyramidal–pyramidal neuron connections (Markram et al., 1998; Thomson and Bannister, 1999). The value for thalamic synapses is even higher, perhaps as much as 1.0 (Stratford et al., 1996). The value of P depends also on the prevailing synaptic calcium dynamics.

Because the overall amount of transmitter released by an action potential depends on prevailing n and P and they depend on the evolving state of the synapse, the effect of a given action potential depends on its temporal relationship to preceding spikes. Consequently, the precise dynamics of transmission displayed at a synapse depends on the probability of release, time constants of recovery from synaptic facilitation, and synaptic depression (Markram et al., 1998).

If P, is high, then the synapse will exhibit depression: The first action potential of a sufficiently closely spaced train will exhaust the synapse so that subsequent spikes in a train of action potentials will evoke successively smaller effects. If P is low, then the synapse will exhibit facilitation: the first action potential will release only a few of the available vesicles and will prime the others by increasing the intracellular calcium concentrations. Subsequent spikes in a train will evoke successively larger effects until a steady-state effect is reached (Thomson, 2000).

Long-Term Potentiation. Unlike the short time-scale of facilitation and depression, loner tetanic stimulation potentiates synapses in hippocampal excitatory synapses for many hours (Bliss and Lömo, 1973). The mechanisms of this process have been intensively studied in the hippocampus (see Chap. 11) and the same processes are found in neocortical neurons. Homosynaptic (specific to the stimulated pathway) long-term potentiation (LTP) was first seen in neocortex *in vivo*, along with the converse phenomenon of heterosynaptic (affecting nonstimulated pathways) long-term depression (LTD) in which the synapses become weaker (Tsumoto and Suda, 1979). Subsequent investigation *in vitro* confirmed the presence of both LTP and LTD in neocortical neurons of young rats and kittens (Komatsu et al., 1981; Artola and Singer, 1987; Bindman et al., 1988; Artola et al., 1990; Aroniadou and Teyler, 1992). However, although LTP could be readily induced in neocortical neurons that showed bursting behavior (Artola and Singer, 1987), LTP was only induced in other cortical neurons in the presence of bicuculline, the $GABA_A$ antagonist (Artola and Singer, 1987).

Artola et al. (1990) provided evidence that identical stimulations could produce either LTD or LTP, depending on the level of depolarization of the postsynaptic neurons. They suggested that if the EPSPs produced a depolarization that exceeds a certain level but remains below the threshold for activation of the NMDA receptor, then LTD results. If, however, the threshold for the NMDA receptor is reached, then LTP results. This is essentially an experimental verification of the theoretical model of Bienenstock et al. (1982). Kimura et al. (1990) found that tetanic stimulation that would otherwise produce LTP will produce LTD if postsynaptic calcium ions are chelated. This indicates that the prevailing calcium concentration may be important for the production of LTP or LTD.

Kirkwood et al. (1993) showed that one of the most effective means of producing LTD is by low frequency (1 Hz) stimulation and that the induction of LTD was dependent on NMDA receptors. This suggests that the actual level of calcium might be critical for determining whether LTP (high postsynaptic calcium) or LTD (low postsynaptic calcium) is induced.

Recent studies have refined the conditions for inducing LTP/LTD by showing that relative millisecond timing of presynaptic and postsynaptic activity determines whether LTP or LTD is induced (Markram et al., 1997a). When the presynaptic input arrives to assist the postsynaptic neuron to discharge action potentials, LTP is observed, and when the presynaptic input arrives after the postsynaptic neuron discharges, LTD is induced, revealing a causal-reward/acausal-punishment. This form of plasticity is referred to as spike-time–dependent plasticity (STDP) and has also been reported in several other brain regions, indicating that it is not unique to neocortical synapses (see Abbot, 2001).

Recent studies indicate that the form of modification of synapses between pyramidal neurons in the neocortex, which are typically depressing, is not a uniform change but rather a redistribution of synaptic efficacy that is caused when the probability of release is increased (Markram and Tsodyks, 1996). This observation introduces a new notion of the plasticity of synaptic dynamics, as opposed to the plasticity of simple synaptic gain.

LTP of inhibitory synapses has also been observed in the visual cortex of developing rat (Komatsu and Iwakiri, 1993). Tetanic stimulation of an inhibitory pathway onto layer 5 pyramidal neurons leads to a long (more than 1 hr) potentiation of the IPSPs.

Weaker stimuli led to a short-term potentiation. The effects were specific to the stimulated pathway. Relative timing of presynaptic and postsynaptic activity determines the direction of synaptic modification also at inhibitory synapses (Holmgren and Zilberter, 2001).

The precise roles of LTP and LTD in general and plasticity of synaptic gain and synaptic dynamics in particular remain a matter of speculation—the widely held belief that they have something to do with memory remains the central dogma. The existence of a clear "critical period" of development in the sensory cortex, in particular, has led to the obvious hypothesis that these synaptic processes are part of the cascade that leads to the formation and modification by experience of nerve connections. Investigations of mouse barrel cortex and rat visual cortex have indicated that LTP, induced without the aid of GABA antagonists, has a critical period. For example, in barrel cortex, it was possible to potentiate the thalamocortical synapses during the first week of life but not the second (Crair and Malenka, 1995). This matches approximately the time course of structural plasticity of the thalamocortical afferents (Schlaggar et al., 1993). The LTP was dependent on NMDA-receptor activation and on increases in postsynaptic calcium.

In rat visual cortex, Kirkwood et al. (1995) also demonstrated that there is a critical period for LTP that corresponds to the falling phase for the plasticity of left and right eye inputs to cortex (ocular dominance plasticity). LTP was evoked in layer 3 by tetanic stimulation in the white matter. Unlike in adult rat cortex (described earlier), the LTP could be induced without blocking the GABAa receptors. LTP could, however, be preserved beyond the normal end of the critical period by dark-rearing the pups. This procedure is known to delay the critical period in cats (Cynader and Mitchell, 1980) and appears to have some effect in rats. This dark-rearing paradigm has also been used to study the model of Bienenstock et al. (1982), which predicts that synapses that are used a lot will be more prone to LTD, whereas synapses that are used less will potentiate more readily. The history of synapse use therefore is an important factor in deciding whether a particular stimulation will lead to LTD or LTP. Kirkwood et al. (1996) found that in dark-reared rats, LTP was enhanced, whereas LTD was hard to evoke. The effect was reversed after dark-reared rats were exposed to light after just 2 days.

Understanding the function of synaptic plasticity may also require considering differences in synapses within local microcircuits, between cortical areas, and between neocortex and subcortical regions. The processes of LTP and LTD have been studied in a number of different cortical areas and for thalamocortical fibers. In the motor cortex (area 5a) of young adult cats, brief tetanic stimulation of the same area or area 1 and 2 could evoke LTP (Keller et al., 1990). This study was one of few to examine the phenomenon *in vivo* and to identify the neurons being recorded. They found that LTP could be evoked in both spiny (pyramidal neurons) and smooth neurons. However, LTP was induced only in those neurons that produced monosynaptic EPSPs in response to stimulation. Thalamic input to the motor cortex could also be potentiated *in vivo* by co-activation with the corticocortical pathway (Iriki et al., 1991). Tetanic stimulation of the ventrobasal thalamus alone did not produce LTP.

There are, however, some differences in the plasticity seen in rat sensory (granular) cortex and motor (agranular) cortex *in vitro* (Castro-Alamancos et al., 1995). Although both cortical areas could reliably generate homosynaptic LTD, LTP was more reliably generated in sensory cortex than in motor cortex, unless inhibition was reduced by application of GABA receptor antagonists. In both areas, the application of NMDA antagonists blocked the induction of both LTP and LTD. However, the kainate/AMPA receptor–mediated responses are also potentiated (Aroniadou and Keller, 1995) in rat motor cortex *in vitro*.

Homeostatic Plasticity. Development and learning mold cortical neuronal circuits by changing the number and strength of synapses. During this process, individual neurons change their morphology, synapses and the profile of their dendrosomatic ion channels. Nevertheless, the functional expression of the neurons and networks remain relatively stable.

In cultured cortical neurons, the strength of excitatory synaptic connections between pyramidal neurons scales as a function of firing rate. The scaling is due to changes in the quantal amplitude of AMPA receptor–mediated excitatory neurotransmission (Turrigiano et al., 1998). The changes are mediated by brain derived neurotrophic factor (BDNF), which is produced by pyramidal neurons and acts on a high-affinity receptor, trkB, found on both pyramidal and nonpyramidal neurons. BDNF has opposite effects on pyramidal neurons and interneurons. It reduces the strength of pyramidal–pyramidal connections but increases the strength of inhibitory interneuron to pyramidal neuron connections. Thus, increases in network pyramidal activity that enhance BDNF secretion are followed by a compensatory reduction in interpyramidal connection strength and increase in pyramidal neuron inhibition (Turrigiano, 1999).

EXCITATORY SYNAPSES

In the neocortex, the main excitatory neurotransmitter is the amino acid glutamate. The postsynaptic membrane of glutamate synapses contains a collection of different receptor types. The amino-acid sequences of many of these receptor proteins have now been identified and specific antibodies have been raised that recognize subunits of the receptors (see Chap. 2). Although additional species of glutamate receptor may well be identified, the immediate goal is to discover the role of these different receptor subtypes in the different cortical circuits.

Glutamate Receptors. As discussed in Chap. 2, the glutamate receptors have been divided into three major types: AMPA/kainate receptor, NMDA receptor, and metabotropic.

In the neocortex, studies of the action of metabotropic receptors in the neocortex are in their infancy and have mainly addressed issues of development and plasticity. Few have considered their functional roles. The direct action of the mGluR on pyramidal neurons, after blocking AMPA and NMDA receptors, was to produce a slow depolarization after evoked spikes (Greene et al., 1994). In burst-firing neurons that project to the superior colliculus or pons, application of mGluR agonists inhibited the burst firing and changed the neurons to a tonic mode of firing (McCormick et al., 1993; Wang and McCormick, 1993). This effect is mediated by a decrease in a potassium conductance. In isolated neocortical neurons, Sayer et al. (1992) found that mGluR activation reduced the high-threshold Ca^{2+} current mediated by L-type calcium channels.

In slices of frontal cortex of immature rats, Burke and Hablitz (1995) provided evidence that mGlu receptors are located on both presynaptic and postsynaptic terminals. It appeared from their pharmacological dissection that different receptor subtypes

were localized at the presynaptic and postsynaptic sites. When GABAa receptors were blocked, mGluR agonists increased epileptiform discharges, whereas the antagonist RS-α-methyl-4-carboxyphenylglycine (MCPG suppressed epileptiform activity. Iono-tophoretic application of mGluR agonists in rat barrel cortex *in vivo* produced disin-hibition in response to natural stimulation of the vibrissae, whereas application of the antagonists reversed these disinhibitory effects (Wan and Cahusac, 1995). The effect might be mediated by a presynaptic receptor that depresses the release of GABA.

Locations of Excitatory Synapses. The major fraction (65%–85%) of excitatory synapses made on pyramidal cells are on their spines, with the remainder being on den-dritic shafts. No excitatory synapses are made on the somata of pyramidal neurons. It was previously supposed that spiny stellate cells followed the pattern of innervation of pyramidal neurons. This is true for spiny stellate cells of the mouse barrel cortex (White, 1989), but it is not true for spiny stellates of layer 4a in area 17 of the cat (Ahmed et al., 1994). It also may not be true for monkey spiny stellates. In the cat, about 60% of the excitatory input arrives on shafts of dendrites. The excitatory inputs to smooth neu-rons are onto both the dendritic shafts and the soma. In the case of the cat, at least some of the layer 4 smooth neurons form somatic synapses with the thalamic afferents (see Synaptic Connections).

The strength of the excitatory synaptic coupling between excitatory neurons has been studied in a variety of cortical areas in rat and cat. Mason et al. (1991) made the first recordings from pairs of pyramidal neurons in the superficial layers of the rat visual cortex. They reported that the synapses produced small-amplitude EPSPs, about 0.1–0.4 mV as recorded in the soma. Thomson et al. (1993) studied the connections be-tween pyramidal neurons in the deep layers of the rat's motor cortex. They found that synaptic transmission was mediated by both NMDA and non-NMDA glutamate re-ceptors. These synapses produce an EPSP with an amplitude of 1–2 mV, recorded in the soma, which was depressed by repetitive stimulation. Similar findings have been made by Markram and Tsodyks (1996), recording from pairs of neighboring layer 5 pyramidal neurons in rat somatosensory cortex.

Stratford et al. (1996) examined the excitatory input to spiny stellate neurons in layer 4 of cat visual cortex. The advantage of the spiny stellate neuron for these studies is that its dendritic tree is symmetrical and electrotonically compact. Thus, variations in ampli-tude and time course of the epsps are largely due to synaptic properties rather than to the cable properties of the dendrites. The sources of excitation were spiny stellate neurons similar to the target, as well as layer 6 pyramidal neurons. These two types of cortical neu-ron had very different synaptic physiologies. The spiny stellate to spiny stellate synapse produced EPSPs with an amplitude recorded in the soma of about 1.5 mV, which de-pressed slightly with repetitive stimulation. The layer 6 pyramid synapses produced com-paratively small amplitude EPSPs (0.4 mV), which showed strong facilitation with repetitive stimulation. In addition, they were able to demonstrate large-amplitude (2.0 mV) EPSPs from putative single thalamic fiber inputs to these same spiny stellate cells. Unlike the EPSPs of cortical origin, these putative thalamic EPSPs showed remarkably little vari-ance in amplitude from trial to trial and only slight depression with repetitive stimulation. Thus, the excitatory synapses formed with a single type of neuron can show a variety of static and dynamic properties, according to their source.

INHIBITORY SYNAPSES

The existence of inhibition in the cortex has been demonstrated repeatedly using in-tracellular recording. The first recordings were made in the Betz cells of the cat mo-tor cortex *in vivo* (Phillips, 1959). By antidromically activating the pyramidal tract neurons, Phillips demonstrated the presence of a recurrent inhibitory pathway in the cortex. Later studies were made in visual cortex (Li et al., 1960; Creutzfeldt et al., 1966; Pollen and Lux, 1966; Krnjévic and Schwartz, 1967; Toyama et al., 1974). These confirmed Phillips' (1959) observation that every neuron received an inhibitory input. Electrical stimulation of either the subcortical thalamic nuclei or local cortical stimu-lation produced a long-lasting (100–200 msec) IPSP. The role and mode of operation of inhibition in generating the stimulus-specific responses of neurons in the visual cor-tex remain questions of intense interest (Douglas et al., 1995; Somers et al., 1995; Fer-ster et al., 1996).

A number of chemical substances have inhibitory effects on cortical neurons, but the most dominant inhibitor appears to be GABA. Krnjévic and Schwartz (1967) performed a direct comparison between the membrane effects of GABA and naturally occurring IPSPs in mammalian cortex. They used surface stimulation to evoke IPSPs in neurons of pericruciate cortex and recorded IPSPs that reached peaks at about 20–30 ms, had durations of 200–300 ms, and had amplitudes of about −10 mV at the resting mem-brane potential. These IPSPs could be reversed by current injection or intracellular Cl⁻ injection (Krnjévic and Schwartz, 1967; Dreifuss et al., 1969). Application of GABA usually hyperpolarized the cells and reduced the amplitude of the IPSPs (Krn-jévic and Schwartz, 1967). The reduction in IPSP amplitude was dependent on the GABA ejection current. The highest ejection currents flattened the IPSP (Krnjévic and Schwartz, 1967) and sometimes slightly inverted them (Dreifuss et al., 1969). The ap-plied GABA increased the input conductance, whose time course was similar to that of the IPSP voltage response and decayed with a time constant of about 50 ms. Direct application of GABA also gave a marked increase in input conductance, together with hyperpolarization in most instances. The reversal potentials for the direct GABA ef-fect and the IPSP were similar; Dreifuss et al. (1969) therefore concluded that GABA was the source of the cortical IPSP. The development of a specific GABA receptor an-tagonist, bicuculline, confirmed that cortical inhibitory processes were GABA medi-ated and had an important functional role in shaping cortical responses (Sillito, 1975; Tsumoto et al., 1979). Subsequent identification of the structure of the GABA recep-tor (Barnard et al., 1987) has allowed specific antibodies to be developed for the GABA receptor subunits and so enabled the regional distribution of GABA receptor subunits to be mapped (Fritschy and Mohler, 1995).

Receptor Types. In their original paper on the heterogeneity of hippocampal responses to GABA, Alger and Nicoll (1982) proposed that there were two different mechanisms mediating GABA inhibition and that these two mechanisms were activated by two dif-ferent GABA receptor types. However, the details of their hypothesis differed consid-erably from current models of GABA action in hippocampus. Alger and Nicoll (1982) suggested that there was only one hyperpolarizing mechanism and that it was distrib-uted throughout both soma and dendrites. This hyperpolarization was Cl⁻ dependent. Their second mechanism was depolarizing. They were uncertain of the ion conduc-

tance involved, but it was slightly sensitive to chloride. However, the presence of both hyperpolarizing and depolarizing responses on the dendrite, and both sensitive to chloride, did not seem attractive! A solution to this paradox is the notion that the chloride transporters (chloride/bicarbonate exchangers, $Na^+/K^+/Cl^-$ cotransporters, and an ATP-driven chloride pump) are unevenly distributed between soma and dendrites, resulting in a gradient of chloride concentration (Hara et al., 1992). This gradient would shift the reversal potential of the $GABA_A$ synapse according to its location (Staley, 1995).

$GABA_A$. In early studies it was found that the conductance changes, reversal potential, and sensitivity to chloride of GABA ionophoresis and IPSPs were similar (Eccles, 1964), suggesting that they were both mediated by chloride channels. The GABA receptor associated with the chloride conductance is now known as the $GABA_A$ receptor. It is the receptor that also binds benzodiazepine and barbiturate (see Matsumoto, 1989). The $GABA_A$ receptor is selectively blocked by bicuculline. There are 16 known $GABA_A$ receptor subunits that may assemble in various combinations of 5 (pentamers) that form the functional chloride channels (Barnard et al., 1987; Nayeem et al., 1994). The beta subunit contains the $GABA_A$ receptor site, whereas the alpha subunit contains the benzodiazepine receptor site. Benzodiazepines increase the effect of GABA by increasing the frequency of channel opening in the presence of GABA. Picrotoxin acts by interference with the chloride ionophore (Barker et al., 1983). At low concentrations, barbiturates prolong the duration of $GABA_A$ channel opening without affecting conductance, and at concentrations of the order 50 μm, they directly activate chloride channels (Study and Barker, 1981). Alphaxalone has similar effects (Cottrell et al., 1987). The $GABA_A$ receptor sensitivity is reduced in the presence of the raised intracellular calcium associated with the calcium spike (Inoue et al., 1986).

$GABA_B$. The failure of the specific $GABA_A$-receptor antagonist bicuculline to block the long-duration IPSP in cortex (Curtis et al., 1970; Godfraind et al., 1970; Curtis and Felix, 1971) indicated the presence of another GABA-mediated response. Bowery and colleagues (Hill and Bowery, 1981; Bowery et al., 1987) discovered a second class of GABA receptor, which was not sensitive to barbiturates or benzodiazepine (Alger and Nicoll, 1982; Blaxter et al., 1986; Bormann, 1988). These $GABA_B$ receptors are activated by the antispastic drug baclofen, which is ineffective at $GABA_A$ receptors (Bowery et al., 1984). The GABAb receptor, which was cloned in 1997 (Kaupmann et al., 1997), forms part of the G-protein–coupled receptor superfamily (Bowery, 1993). The $GABA_B$ receptor is indirectly coupled to calcium and potassium channels via GTP-binding proteins and perhaps protein kinase C (Dolphin and Scott, 1986; Dutar and Nicoll, 1988). In frontal cortex, G-proteins are involved in the postsynaptic response, and the short latency of the $GABA_B$ IPSPs suggests a close coupling between receptor and ionophore (Hablitz and Thalmann, 1987). The $GABA_B$ receptors are also found presynaptically, where they activate potassium channels or inhibit calcium conductances. This may reduce the GABA released and reduce the overall level of GABA-mediated inhibition. On excitatory terminals, the $GABA_B$ receptors may also reduce the release of excitatory neurotransmitter (Thomson et al., 1993). Connors et al. (1988) showed in cortical slices that baclofen, the GABAb agonist, activated a long time course

hyperpolarization with a reversal potential around the potassium reversal potential, and similar observations were made in cat visual cortex *in vivo* by Douglas et al. (1988) and Douglas and Martin (1991).

Two low-potency $GABA_B$ antagonists, phaclofen and saclophen, have been used as $GABA_B$ receptor blockers (Kerr et al., 1987, 1988). They block the long-duration late component of the IPSPs *in vitro* in frontal cortex (Karlsson et al., 1988) and in visual cortex (Connors et al., 1988; Hirsch and Gilbert, 1991). Due to the low potency of phaclofen, its effects on orientation and direction selectivity of visual cortical neurons *in vivo* have proved to be inconclusive (Baumfalk and Albus, 1988), whereas blocking $GABA_A$ receptors with *n-m*-bicuculline produces a marked reduction in the selectivity of visual cortical neurons to visual stimuli (Sillito, 1975).

In addition to their role in postsynaptic inhibitory process, $GABA_B$ receptors inhibit transmitter release in neocortical neurons via a presynaptic mechanism (Deisz and Prince, 1989). The mechanism is probably by reducing the entry of calcium into the presynaptic terminal and thus lowering the probability of transmitter release. Phaclophen is ineffective at the presynaptic $GABA_B$ sites (Dutar and Nicoll, 1988).

Neocortical GABAergic synapses also display frequency-dependent dynamics (Thomson and Deuchars, 1994). Three classes of synapses have been defined according to the relative amount of facilitation and depression they exhibit (Gupta et al., 2000). The type of synapse deployed at an inhibitory connection depends on the nature of the presynaptic interneuron and its target neuron.

ELECTRICAL PROPERTIES OF THE IPSP

Responses to GABA. There are at least three distinct responses to direct GABA applications to cortical neurons *in vitro* (Scharfman and Sarvey, 1987). The first response is a fast hyperpolarization; this predominated when the GABA was ejected close to the soma. It produced a large increase in the input conductance. The second was a longer-lasting depolarization, which was evoked most readily by application of GABA to the distal dendrites. It was associated with a moderate increase in the input conductance. The third was a slow hyperpolarization that appeared on the trailing edge of the depolarization. It was a prolonged response that decayed over many seconds and was associated with a moderate increase in the input conductance. The three components are often mixed. For example, ejection in the vicinity of soma may show early somatic (hyperpolarizing response) followed by a relatively late depolarization (as GABA diffuses onto dendrites and evokes a dendritic response). GABA ejected in the vicinity of proximal dendrites evokes both a somatic and a dendritic response.

The somatic response had a reversal potential of -65 mV, was chloride dependent, and was blocked by bicuculline (Scharfman and Sarvey, 1987; Connors et al., 1988). The dendritic depolarization is probably also mediated by GABAa receptors because it is blocked by bicuculline and picrotoxin and is potentiated by benzodiazapines (Blaxter and Cottrell, 1985). The differences in the response between somatic and dendritic activation of the same receptor have been explained by possible differences in the chloride concentrations in dendrites and soma (Thomson et al., 1988). Lambert et al. (1991) have proposed that the $GABA_A$ receptors in the hippocampus mediate their dendritic responses via a different ionophore. By contrast, the dendritic hyperpolarization evoked by GABA is mimicked by baclofen, the specific $GABA_B$ agonist. This hyperpolariza-

tion reverses at −90 mV (Ogawa et al., 1986; Scharfman and Sarvey, 1987; Connors et al., 1988). The GABA hyperpolarization is potassium dependent and can be evoked alone by small doses of GABA applied to the dendrites (Wong and Watkins, 1982) or by blocking the depolarizing component with bicuculline (Inoue et al., 1985a,b). This dendritic hyperpolarization is blocked by phaclofen and thus is probably mediated by the GABA$_B$ receptor (Dutar and Nicoll, 1988).

In neocortical slices, long and short IPSPs have been observed (Ogawa et al., 1981; Connors et al., 1982). The stimulus threshold for the late, long IPSPs mediated by the GABA$_B$ receptor is higher than that for the early, short IPSPs, which are mediated by the GABA$_A$ receptors (Connors et al., 1982). In most neurons, the early IPSPs were depolarizing because the resting potential of the cortical neurons was more negative than the reversal potential of chloride (Connors et al., 1982; McCormick et al., 1985).

Sites of Action of Inhibitory Synapses. The soma or the proximal dendrites are the regions where anatomical and immunocytochemical studies have revealed the major concentration of symmetrical (Gray type II), GABAergic synapses (LeVay, 1973; Ribak, 1978; White and Rock, 1980; Freund et al., 1983; Peters, 1987). In brain slice preparations, large conductance changes occur only transiently at the onset of an electrically evoked IPSP and last for 15–25 ms. The long phase of the IPSP is associated with a small conductance change (Ogawa et al., 1981). Intracellular recordings from visual cortical neurons *in vivo* revealed hyperpolarization during a long period of visually evoked inhibition (Douglas et al., 1988; Ferster, 1988; Ferster and Jagadeesh, 1992). Large conductance changes have been observed *in vitro* studies and have been reported in cat visual cortex (Borg-Graham et al., 1998) in response to natural stimulation. A more distal location would enhance the shunting effect of the synapse (see "shunting synapses" in Chaps. 1 and 2), because the input conductance of the trunk dendrite decreases relative to the active conductance of the inhibitory synapse. Inputs to the apical dendrite of large layer 5 pyramidal neurons in the rat from a single inhibitory neuron are strong enough to block the dendritic Ca^{2+} action potential (Larkum et al., 1999).

An extreme example of dendritic inhibition is where the shunting inhibitory synapse is located on the spine head or neck. Under these circumstances, a large increase in conductance evoked in the spine head would provide very specific shunting of an excitatory synapse on the spine head, but the conductance change would be masked from the soma by the high axial resistance of the spine neck. However, the present neuroanatomical data suggest that relatively few excitatory synapses could be influenced in this way: only 7% of spines have both synaptic types (Beaulieu and Colonnier, 1985). Even if this figure is an underestimate, there remain a large number of spines without inhibitory input. Because the major excitatory input to pyramidal neurons is thought to arrive on spines (Colonnier, 1968; LeVay, 1973; Szentágothai, 1973; Peters, 1987), most of this input could not be selectively inhibited.

NEUROTRANSMITTERS

AMINO-ACID TRANSMITTERS

The establishment of the identity of cortical neurotransmitters has been one of the most tortuous activities of the past 40 years.

Glutamate. The failure of the specific Hayashi (1954) first proposed the amino acids L-glutamate and L-aspartate as candidates for the excitatory neurotransmitters in the cerebral cortex. This was supported by Krnjévic and Phillis (1963) and by the superfusion studies of Jasper et al. (1965), who found that glutamate, aspartate, glycine, and taurine were released during activation of the cortex. Clark and Collins (1976) showed that the release of glutamate, aspartate, and GABA were calcium dependent. However, resistance to accepting glutamate as a neurotransmitter was strong because glutamate is distributed throughout the brain in high concentrations, a quite different picture from the restricted location and lower concentrations of acknowledged neurotransmitters such as ACh and catecholamines.

Other amino acids also exert an excitatory effect of neurons. Some of these are more potent than the endogenous amino acids. The D-isomer of N-methyl aspartate is much more potent than L-glutamate (Curtis and Watkins, 1963). The extraction of kainate and quisqualate from plants provided more agents that had stronger excitatory effects on neurons than L-glutamate (Shinozaki and Konishi, 1970). An antagonist, L-glutamic acid diethylester (GDEE) proved to be more effective against L-glutamate than other excitatory amino acids and indicated that there may be more than one type of excitatory amino acid receptor (Haldeman et al., 1972; Haldeman and McLennan, 1972). With the recent effort devoted to the characterization of receptor subunits L-glutamate has emerged as the major excitatory amino acid transmitter of the cerebral cortex.

GABA. The acceptance of the amino acid GABA as the major inhibitory neurotransmitter has been as slow as that for glutamate. This occurred despite the demonstration by Krnjévic and Schwartz (1967) that ionophoretically applied GABA profoundly inhibited cortical neurons and the evidence that GABA was released from active cortical synapses (Iversen et al., 1971). Application of n-m-bicuculline, the GABAs receptor antagonist, has a marked effect on the receptive field structure of visual cortical cells (Sillito, 1975; Tsumoto et al., 1979) and on the shape of the electrically evoked IPSP (Connors et al., 1988; Douglas et al., 1989). Antibodies directed against the synthetic enzyme for GABA, glutamate decarboxylase, or against the amino acid itself, indicate that about 20% of the neocortical neurons synthesize and contain GABA (Naegele and Barnstable, 1989).

Acetylcholine. Ionophoretic application of ACh modifies the response to visual stimulation of most neurons in the cat visual cortex (Sillito and Kemp, 1983). The effect is usually facilitatory and seems to enhance the signal-to-noise ratio, rather than being generally excitatory. In deep layer pyramidal neurons, ACh induces a depolarization accompanied by an increase in resistance. The reversal potential is above that for potassium, suggesting that the action of ACh is to decrease the conductance for potassium (Krnjévic et al., 1971) by modulation of the slow outward potassium M current. The ACh response is mediated by a muscarinic receptor. The slow depolarization is preceded by a short-latency hyperpolarization and a decrease in resistance that is probably due to the rapid muscarinic excitation of the inhibitory neurons (McCormick and Prince, 1985, 1986a). The inhibitory neurons are innervated by cholinergic afferents (Houser et al., 1985).

The onset of the depolarizing muscarinic excitation is slow and the response is sustained for many seconds. Some of the effects of ACh are mediated by second mes-

sengers (Stone and Taylor, 1977). Low concentrations of muscarine abolish the sAHP, but at higher concentrations the sAHP is replaced by a slow afterdepolarization (sADP) that is not mediated by potassium or sodium (Schwindt et al., 1988). ACh-induced excitation can be enhanced selectively by SSt, although SSt itself inhibits spontaneous firing (Mancillas et al., 1986).

Biogenic Amines. Norepinephrine depresses the spontaneous extracellular activity of most cortical neurons (Reader et al., 1979; Armstrong-James and Fox, 1983). Some cortical cells in the deep layers are excited by low concentration of norepinephrine but inhibited by higher concentrations (Armstrong-James and Fox, 1983). Waterhouse et al. (1990) found that visual cortical cells in the rat showed enhanced responses to visual stimuli during ionophoresis of norepinephrine but depressed responses during serotonin ionophoresis.

Neuropeptides. The GABAergic neurons of the neocortex co-localize various peptides, including SSt, cholecystokinin, neuropeptide Y, VIP, and substance P (Hendry et al., 1984; Schmechel et al., 1984; Somogyi et al., 1984; Demeulemeester et al., 1988). VIP and substance P are also associated with cholinergic axons (Vincent et al., 1983; Eckenstein and Baughman, 1984).

The physiological role of neuropeptides remains obscure. Salt and Sillito (1984) showed that SSt could inhibit or excite cortical neurons. They were unable to demonstrate a modulatory effect on either GABAergic or cholinergic transmission. Mancillas et al. (1986) found that SSt inhibited rat cortical neurons. Cholecystokinin and VIP (Grieve et al., 1985a,b) produce mild excitation in some neurons. The difficulty in detecting effects, and the variety of effects produced, suggests that the role of these peptides is not primarily neurotransmission. Possibly they are part of some cascade of effects acting over time-courses of many hours or days, rather than 1 hour or so for conventional experiments that require receptive field mapping.

DENDRITES

The surface area of the dendrites is one to two orders of magnitude larger than that of the soma, and the dendrites receive the vast majority of the synaptic inputs to the neuron, as has been shown for most other neurons in preceding chapters. This arrangement suggests that the role of the dendrites is to integrate synaptic input, the result of which the neuron then expresses as the discharge activity of the soma. Unfortunately, in most cases the diameters of the dendrites of typical cortical neurons are too small to obtain stable recordings using available electrophysiological techniques, so most of our understanding of their electrical behavior is derived indirectly, from recordings made from the somata and apical dendrites (Stuart and Sakmann, 1994) (see Fig. 1.9B). These methods are now being supplemented by sophisticated imaging techniques such as calcium imaging and two-photon microscopy (Denk et al., 1995).

The simplest electrical model of a dendrite is the passive electrotonic structure (Rall, 1977, 1989; Johnston and Wu, 1995; Segev et al., 1995). In this *cable model*, the dendrites are composed of cylindrical segments of membrane that are linked in a tree-like structure. The membranous walls of the cylinders have capacitance and linear conductance, and the interior of the cylinders present a linear axial conductance to the longitudinal passage of current. Such cylinders are electrically distributed (nonisopotential) structures, and an input current injected at a point will establish voltage gradients along the dendrite. For simple cylindrical dendrites, the voltages at any point along their length is specified by the *cable equation* (see Jack et al., 1975). The solution of this equation, and so the voltage profile, depends on the boundary conditions at the ends of the dendritic cylinder. These boundaries are usually approximated as either an infinite cable, a cut (short circuit) end, or sealed (open circuit) end. Rall (1959) showed that the cable equation could also be solved for branching cylindrical dendrites, provided that there was a "3/2 power" relationship between the parent and daughter branch diameters. When this relationship and a few other restrictions hold, then the entire dendrite can be reduced to a single equivalent cable of constant diameter.

In real neurons, the synaptic voltages attenuate more rapidly toward the soma than toward the dendritic terminations. The electrical asymmetry of the dendritic tree arises because the terminations are sealed ends and little synaptic current is lost from them, whereas the somatic end has many other dendrites attached and so presents a large conductance load to the source synapse located on one of the dendrites. The voltage attenuation from dendrites to soma may be as much as a few hundred-fold. This implies that a number of EPSPs must occur within a membrane time constant to displace the somatic potential across a 10- to 20-mV threshold. If the effect of a synapse depended only on its peak voltage, then the response of the neuron would be very sensitive to the displacement along the dendrite of the synapse. But, if the entire EPSP is considered, the situation is different. The passive dendrite behaves as a low-pass filter, and so the temporal form of the synaptic potentials become significantly broader as they spread from distal synaptic sites toward the soma. Although the *peak* synaptic voltages are attenuated, the attenuation of the time integral of the EPSP at the soma is smaller and not much affected by synaptic location. This is also true of the integral of the synaptic current at the soma (synaptic charge delivery). Because the synapses exert their effect collectively, by sustained depolarization of the somatic membrane, it is the charge delivery to the soma that best expresses synaptic efficacy (Bernander et al., 1994).

The broadening of the EPSP as it spreads centripetally has the effect of delaying the signal and makes the response at the soma sensitive to the temporal order of synaptic events applied in the dendrites. This means that the dendrite can usefully compute functions such as direction of motion (Rall, 1964; Koch et al., 1982). Passive dendrites can also act on different time-scales. For example, extensively branched distal dendrites provide a large area for charge equilibration, so the time constant for synaptic integration is much shorter (about $0.1\ \tau_m$) there than closer to the soma. The briefer synaptic events in the distal arbors interact as coincidence detectors, whereas the longer events closer to the soma integrate (Agmon-Snir and Segev, 1992).

The morphology of the dendritic tree is important, at least in so far as it affects the passive spread of currents from the synapses to the initial segment (Mainen and Sejnowski, 1996). Most cortical neurons are electronically compact, whether measured electrophysiologically (Stafstrom et al., 1985) or anatomically (Douglas and Martin, 1992). However, the apical dendrite of the pyramidal neurons is a special case. The electrotonic length of the apical dendrite is about 2–3 times greater than that of the basal dendrites, and the synaptic inputs injected into the "apical tuft" at the distal end of

the apical dendrite are greatly attenuated en route to the soma (Bernander et al., 1994). This apparent ineffectiveness of the distal apical input is counterintuitive. Important interareal projections make their synapses there, and there has been no phylogenetic trend to dispense with apical dendrites (with the possible exception of layer 4 spiny neurons). One possibility is that the apical dendrite makes use of active currents to enhance selectively signal transmission to the soma (Bernander et al., 1994). Where the dendrites are long and narrow, and so electrotonically short, the dendrite can decompose into electrotonically separate subunits, each of which can compute a relatively independent function (Koch et al., 1982). It is unlikely that such conditions exist in cortical neurons, except possibly in the apical tufts.

ACTIVE PROPERTIES

The cable model has been extremely useful in obtaining qualitative insights into the behavior of quiescent dendrites. However, it has two significant failings that limit its application to cortical neurons. First, the approximation to a cable across the branches in a dendrite requires that a particular relationship of diameters hold between the parent and daughter segments of the branch. This relationship is only rarely true across the dendritic branches of cortical neurons. Second, it is clear that the majority of cortical neurons have many active conductances in their dendrites (Stuart and Sakmann, 1994), so the linear cable approximation is only useful under very restricted conditions. Active dendritic conductances include voltage-dependent sodium, potassium, and calcium currents (Stuart and Sakmann, 1994; Markram and Sakmann, 1994; Johnston et al., 1996). When the nonlinearities due to the active conductances are included, the dendritic models have mathematical descriptions that cannot be solved analytically. The models must then be investigated by numerical simulations of compartmental approximations to the dendrites.

The active conductances are able to generate a variety of subthreshold nonlinearities and may cross the thresholds for calcium or sodium action potentials. The exact interactions of the various active dendritic conductances are unknown, but they support a number of interesting functions. In the case of subthreshold synaptic potentials, the apical tuft of the layer 5 pyramidal neuron has a high potassium channel (Ih) density that increases the attenuation of EPSPs, and could uncouple the apical tuft dendrites from their basal counterparts, by decreasing the somatopetal length constant (Berger et al., 2001). However, action potentials initiated in the somata of layer 5 pyramidal neurons are able to propagate actively backward into their dendritic tree. This retrograde propagation is probably due partly to the amplification of depolarizing currents by the relatively high density of sodium channels found in the dendrites of those neurons (Stuart et al., 1997). The action potential propagates more reliably centrifugally than it does centripetally, because in the latter case the branching dendritic tree presents a large impedance load to the small action potential currents generated in the narrow peripheral dendrites. On the other hand, the degree and pattern of back propagation can be affected by the spatiotemporal conductance profile of the dendritic tree due to activation of intrinsic conductances or high conductance synaptic inputs.

The retrograde spikes could provide a signal to Hebbian synapses that the postsynaptic cell is active, and so trigger synaptic changes mediating learning. For example, Yuste and Denk (1995) used two-photon microscopy of hippocampal pyramidal neu-

rons to show that the centrifugal action potential invades the dendritic spines and leads to a local rise in their calcium ion concentration. The increase in calcium concentration can induce synaptic plasticity, modify NMDA receptor responses, and modify the conductance profile of the dendritic tree by activating dendritic potassium conductance (Stuart et al., 1997; Koch, 1999). The relative timing of a presynaptic spike and the retrograde postsynaptic spike can be used to drive causal associative learning (Gerstner, 1999).

The introduction of active conductances in the apical dendrite could also support anterograde signal transmission by providing amplification and linearization of synaptic inputs to the apical tuft (Bernander et al., 1994), by decomposing the dendritic tree into a number of distinct multiplicative subunits (Mel, 1993), or by supporting localized dendritic action potentials (Stuart et al., 1997). The active conductances could also amplify selective combinations of input by nonlinear multiplicative interactions (Mel, 1993). Because these effects are usually associated with increases in conductance, increasing stimulation will cause the multiplicative and subregion effects to become more localized in space and time (Mel, 1993).

Dendrites with slow active currents that are partly decoupled from the fast spike generating currents at the soma can produce a wide repertoire of temporal patterns of output spikes, including bursting (Pinsky and Rinzel, 1994; Mainen and Sejnowski, 1996). Activation of dendritic potassium conductances could also offset large dendritic input currents, so providing an adaptive mechanism to keep the dendrite in a favorable operating range (Bernander et al., 1991, 1994).

SPINES

One of the most prominent features of cortical neurons are their dendritic spines (reviewed in Chap. 1). They are the major recipients of excitatory input and play an important role in activity-dependent modification of synaptic efficacy such as LTD and LTP (for a review, see Shepherd, 1996). Although these structures have been extensively examined by light and electron microscopy, physiological data have been more difficult to obtain because of their tiny dimensions, and so their functional role is still not entirely understood. Fortunately, recent advances in imaging techniques are making it possible to measure calcium dynamics in individual spines with high time resolution (Denk et al., 1996).

The simplest views of spine function were mechanical. They were thought to be convenient physical connections whereby *en passant* axonal boutons could more easily connect to dendrites (Peters and Kaiserman-Abramof, 1970; Swindale, 1981; Anderson and Martin, 2001). More elaborate views have considered the electrical and chemical properties of the spinous connection.

ELECTRICAL MODELS

The membrane area of the spine neck is very small, and consequently little synaptic current flows through the neck membrane; most of the synaptic current injected into the spine head reaches the trunk dendrite via the spine neck. Nevertheless, the resistance to current flow through the neck is high, on the order of 100 $M\Omega$ or more (Segev and Rall, 1988). This is roughly the input resistance of a typical spiny dendrite about

half a length-constant from the soma. Therefore, the spine neck will attenuate by about half of the voltage applied at the spine head. Thus, the neck resistance could be used to control the efficacy of the synapse (Rall, 1962) and so provide a basis for synaptic plasticity (Fifkova and Anderson, 1981). The resistance could be changed by modifying the neck diameter or length (Rall, 1974a,b) or by partial occlusion of the neck by the spine apparatus (Rall and Segev, 1987).

The "twitching spine" hypothesis of Crick (1982) proposed that a change in spine length could be achieved quickly, through calcium activation of myosin and actin localized in the spine neck (Fifkova and Delay, 1982; Markham and Fifkova, 1986). In theory, a burst discharge of the excitatory afferent could raise the free calcium concentration in a spine to the level required to activate the actin (Gamble and Koch, 1987). Although it has now been shown with modern imaging methods that spines are motile on very short (seconds) time-scales (Fischer et al., 1998; Dunaevsky et al., 1999), the function of this motility is still not clear.

As discussed earlier, the calcium dynamics between spine and dendritic shaft are certainly affected by changes in spine neck dimensions (Majewska et al., 2000). Attempts to correlate changes in spine dimensions in relation to, for example, LTP induction have indicated that spines become larger but do not increase in number (Fifkova and van Harreveld, 1977; Andersen et al., 1987; Desmond and Levy, 1990). More recent studies of "on-line" imaging of spines have shown that new spines appear about 30 min after the induction of LTP (Yuste and Bonhoeffer, 2001).

Saturating Spines. The flow of synaptic current through the spine input resistance will shift the spine head potential toward the EPSP reversal potential and reduce the driving potential for the synaptic current. For small synaptic conductances, the synaptic current increases approximately linearly, but for larger synaptic conductances, the synaptic current increases approximately linearly, but for larger synaptic conductances, the synaptic current saturates. This interdependence gives rise to a sigmoidal relationship between synaptic conductance and synaptic current. We do not know exactly where on this relationship the operating range of the neocortical synapse lies.

The spines generally receive only one excitatory synapse can be interpreted in two opposing ways, in this context. It may reflect the need to avoid saturating the synapse, or it may indicate that a single synapse on a spine always saturates the synapse, so that additional inputs would be redundant. If the synapse is driven into saturation, then the spine potential will be relatively insensitive to the exact amount of neurotransmitter delivered to the synapse. The spine head will simply turn on to a repeatable voltage level. Moreover, because the spine neck resistance is at least as large as that of the parent dendritic trunk, the spine approximates a constant current source attached to the dendrite. The synapse on the spine head is less susceptible to changes in the dendritic input resistance than is a synapse located on the dendritic trunk.

Spine Action Potentials. A special case of saturation behavior arises if the spine head membrane contains active conductances that could amplify the synaptic signal (Jack et al., 1975; Miller et al., 1985; Perkel and Perkel, 1985; Rall and Segev, 1987; Segev and Rall, 1988). Shepherd et al. (1985) and others (Rall and Segev, 1987; Baer and Rinzel, 1991) have suggested that this amplification could lead to spinous action potentials. The saltatory transmission of these action potentials from spine to spine, conditional on their synaptic input, could form the basis of Boolean algebraic-like processing along the dendrite. Although these notions are attractive, the experimental evidence consistent with spiking spines has been obtained only in cerebellar Purkinje neurons (Denk et al., 1995).

Nonsaturating Spines. If the synapse on the spine is not driven into saturation but operates instead within its linear range of sensitivity, then the synapse will be particularly susceptible to nonlinear interactions with other spines. Because the resistance of the spine head membrane is much greater than the axial resistance of the neck, the dendritic potential is transferred to the spine head with little attenuation. Consequently, a depolarization of the dendritic trunk will reduce the driving potential of the spine synapse and mediate nonlinear interactions between neighboring spines. These interactions are only possible if the spines operate in their linear range. If the spines operate in saturation, then their synapse will be insensitive to modulations of local driving potential.

Inhibition on Spines. A small proportion (about 10%) of neocortical spines receive input from a type 2 (GABAergic) synapse in addition to the type 1 (excitatory) synapse (Jones and Powell, 1969; Peters and Kaiserman-Abramof, 1970; Sloper and Powell, 1979b; Somogyi et al., 1983; Beaulieu and Colonnier, 1985; Dehay et al., 1991). This arrangement raises the possibility that some excitatory inputs receive selective inhibition. Nonlinear inhibition of excitatory inputs on the same spine can be large and are essentially limited to the affected spine (Koch and Poggio, 1983). The large series resistance of the spine neck masks changes that occur in the head from the trunk dendrite and so may effectively restrict the inhibitory control to the affected spine. This specific effect has attracted much theoretical interest (Koch and Poggio, 1983; Segev and Rall, 1988) because of its computational possibilities, but only a small percentage of the excitatory input onto a single neuron could be gated in this way. It is possible that all of the spines that are controlled by an inhibitory synapse receive input from a strategically important class of afferents, such as the thalamocortical inputs for example. However, Dehay et al. (1991) have shown that this selective inhibition does not occur.

Less selective locations of inhibitory synapses may also permit strong nonlinear effects. For example, inhibitory synapses on the trunk dendrite would reduce interspine communication and could control saltatory conduction between spines (Shepherd and Brayton, 1987). This raises the possibility of enabling or disabling selected branches of dendrites. However, logical computations in spines do not necessitate inhibitory inputs. Triggering an action potential could also be conditioned by activation of the spine head synapse (e.g., the NMDA receptor is voltage dependent only if gated by neurotransmitter). If saltatory conduction were conditional on excitatory input, then this arrangement would provide an elegant means of signal gating that depends on the coincidence of excitatory inputs, rather than the interaction of excitatory and inhibitory inputs.

BIOCHEMICAL COMPARTMENTS

The strong role of calcium in synaptic plasticity and the need to localize plasticity to the activated synapse have led to the suggestion that the spines provide the necessary isolated biochemical compartment (Koch and Zador, 1993; Zador and Koch, 1994).

The spine neck limits the diffusion of calcium between the head and the dendrite. It is proposed that restriction in calcium movement through the neck arises from the calcium sink created by the calcium pumps in the neck membrane. Their activity has the effect of shortening the calcium space constant in the neck, leading to significant calcium attenuation across the neck.

Calcium could enter the head through NMDA channels, voltage-dependent calcium channels, and second-messenger channels. Studies in hippocampal neurons have provided evidence that calcium levels in the spine head are to some extent uncoupled from those in the parent dendrite (Guthrie et al., 1991; Muller and Connor, 1991; Yuste and Denk, 1995; Yuste et al., 2000). In neocortical pyramidal neurons, the decay kinetics of calcium in spines are controlled by calcium pumps in the spine and by diffusion through the neck of the spine (see earlier) (Majewska et al., 2000).

FUNCTIONAL OPERATIONS

SINGLE NEURONS OR NEURAL NETWORKS

The history of ideas of cortical function makes a fascinating account of the interplay of hypothesis and experiment (Martin, 1988). In particular, the experimental results from microelectrode recordings from single cortical units (neurons) have had a deep influence on our ideas of cortical function. Much of the motivation for studying the functional properties of single units in the cortex in such detail arises from the fact that the activities of cortical neurons are thought to describe the world and so to reflect our subjective experiences. However, the nature of the encoding used by the neurons to represent the world is still a matter of intense and interesting debate.

The encoding problem is important because it determines to a large extent the success with which the nervous system can interact with the world. It is clear that the attributes of the world must be encoded in the variables of the nervous system. If the neural encoding is suitable, then the nervous system will be able to well represent the world, and the efficiency interactions with the physical world will be enhanced. For example, in artificial neural networks, learning and generalization improve with the quality of data representation.

One central question is whether the nervous system uses a data representation in which the encoding of objects is distributed across many neurons or whether the representation is localized. This debate is usually couched in the domain of perception. There the question is whether the discharge of a combination of neurons, or the discharge of just one neuron, reflects the experience of a percept. These opposing views of the operation of cortex have a long and distinguished history. Sherrington (1941), for example, contrasted the notion of "one ultimate pontifical nerve-cell . . . [as] the climax of the whole system of integration [with the concept of mind as] a million-fold democracy, whose each unit is a cell."

The case for localized encoding has been formalized in the *neuron doctrine* proposed by Barlow (1972). He proposed five dogmas that encapsulate the powerful idea that percepts are the product of the activity of certain individual cortical neurons, rather than by some more complex (and obscure) properties of the combinatorial rules of the usage of nerve cells. The force of Barlow's thesis in molding our ideas is evident in most textbooks of psychology and neurobiology, which are well stocked with illustra-

tions showing how the specificity of neurons arises from a hierarchical sequence of processing through the cortical circuits.

Recently, the pendulum has begun to swing back. The antithetical proposition that perceptual processing occurs through the collective properties of parallel cortical networks rather than through the activity of single units has been receiving close attention from theoreticians working on *neural networks* or *connectionist* models of cortical function. Results obtained from computer simulations of these hypothetical nerve circuits have led to a model of cortical function that is quite different from that proposed in the neuron doctrine.

The dialectic of the one versus many neurons is best considered in the context of the visual system, where the physiology and anatomy are known in greatest detail and where the behavioral performance is well established.

SINGLE NEURONS OR NEURAL NETWORKS?

It is evident that visual perception is a complex task. We need not only to determine the form, movement, and position in space of the objects we encounter but also to recognize them as being particular objects. Solving this key problem was central to Barlow's development of the neuron doctrine. He proposed that the primary visual cortex dealt only with the elemental building blocks of perception, the detection of oriented line segments, or the local motion of these segments, for example. To build these responses into neurons that were selective for a cat, chair, or grandmother, he proposed a hierarchical sequence of processing within single cortical areas and through the many visual areas. Thus, the *grandmother* cell scheme is essentially a classification network in which the input is classified according to which output neuron is activated. In nervous systems, the classification occurs in a hierarchical network. The neurons at each stage of the hierarchy become progressively more selective to the attributes of the stimulus, so that while the neurons in the primary visual cortex would respond to many objects, neurons at the highest level of the hierarchy would respond only to particular objects. Barlow (1972) suggested that the activity of about 1000 of these high-level *cardinal* neurons would be sufficient to represent a single visual scene. Because the number of possible percepts is very large, however, the total number of cardinal cells would have to be a substantial fraction of the 10^{10} cells of the human brain (Barlow, 1972).

The single neuron representation faces two major difficulties: poor generalization and limited encoding capacity. If individual objects are very specifically encoded by single neurons, then it is difficult for the neurons to generalize their classification to novel intermediate cases. For example, given only a "red apple" neuron and a "green apple" neuron, how does the nervous system respond to a yellow apple? Either it must quickly recruit a new neuron with very similar connections and assign it to yellow apples, or the yellow apple percept must arise from some combination of the activity of the "red" and "green" neurons, in which case the single cell–encoding hypothesis is weakened. Moreover, if new neurons must be recruited for each new feature (such as yellow) that is added to the classification scheme, then the number of neurons required to encode selectively the various combinations of features increases explosively and soon exceeds the number of neurons available.

Despite these difficulties, selective encoding representation has remained a popular implicit hypothesis in experimental neuroscience. Since 1972, many of the visual areas

beyond area 17 have been explored in some detail. Efforts to discover whether cardinal cells reside in these visual areas have met with mixed success. In most areas, the stimulus requirements for activating neurons are not very different in quality from those for area 17. If anything, the requirements are less restrictive, in that only a single property of the stimulus might be important, such as its direction of motion, or color, or depth in visual space. Only in the primate inferotemporal region of cortex have neurons with higher-order properties been found (Gross et al., 1972). These neurons respond preferentially to parts of the body, especially faces, although they respond to other visual stimuli as well (Gross et al., 1972; Bruce et al., 1981; Richmond et al., 1983; Young and Yamane, 1992). Other cells in the inferotemporal region have large receptive fields that respond quite specifically to complex shapes, but close neighbors tend to respond to similar features (Miyashita, 1988; Miyashita and Chang, 1988; Tanaka et al., 1991; Fujita et al., 1992). Neurons in these areas appear to "learn" specific complex stimuli.

Direct examination of neuronal responses involved in the perceptual foundation of a decision process (Salzman and Newsome, 1994; Shadlen and Newsome, 1996) have also brought some support for the cardinal cell view. It appears that in the motion discrimination task, the reliability of the animal's decision is not much better than that of a single observed neuron, which argues against the view that the animal bases its decision on an average across many neurons.

Nevertheless, the general conclusion from the many studies that have examined encoding is that individual neurons do not respond completely selectively to single *trigger features*. Instead, each neuron is sensitive to a number of different stimulus characteristics, such as contrast, dimension, depth, and orientation. Single cortical neurons appear unable to signal unambiguously the presence of a particular stimulus, and therefore cannot act as cardinal cells. An important reason why such cardinal cells are not found may lie in the basic organization of the cortical circuitry, which expresses much stronger lateral and recurrent interactions between neurons than is expected of a feedforward classification network.

NEW DESIGNS FOR THE VISUAL CIRCUITS

When Barlow proposed his neuron doctrine in 1972, the modern study of cortical microcircuitry was in its infancy. Anatomical studies had emphasized the vertical, columnar structure of cortex. This view was reinforced by many electrophysiological studies, which showed vertical functional columns. Technical advances since the late 1970s have resulted in a wealth of new information about the cortical microcircuitry. The technique of intracellular labeling of neurons has revealed an extensive system of horizontal connections within the cortex. Certain markers such as horseradish peroxidase or biocytin fill the entire axonal arborization, including the boutons, and so estimates of the number and spatial distribution of the synapses made by a single neuron are now evident for the first time. The horizontal spread of connections means that each point in cortex is covered by axons of a very large number of neurons. For example, estimates for the number of geniculate X cells (see Chap. 8) that provide input to any point in cat area 17 range from 400 to 800 (Freund et al., 1985), whereas the figure for Y cells may be even higher. The geniculate axons form less than 10% of the excitatory synapses on spiny stellate neurons in layer 4 (Ahmed et al., 1994) (see Synaptic Con-

nections), so the number of cortical neurons providing the input to a single point must be considerably higher. Because one cortical neuron supplies only a few synapses to any other cortical neuron, each neuron can potentially be activated by hundreds of other neurons. It is this highly divergent and convergent connectivity that is the feature of neocortex, and it differs considerably from that of the lateral geniculate nucleus (see Chap. 8), where there is a much tighter coupling between neurons.

The widespread and rich connections of the thalamic afferents ensure that even the smallest detectable disturbance of the retinal receptor layer—for example, that induced by a dim flash of light—alters the probability of firing of thousands of cortical neurons in the primary visual cortex. The signal is then amplified by the divergent axonal arbors of the cortical neurons, which ensure that many thousands more neurons are activated both within area 17 and in the other cortical areas to which these neurons project. Thus, although there certainly is the convergence of many inputs that is required to create the cardinal cells, the considerable divergence of the connections of each neuron ensures the simultaneous activation of many neurons. In such a context it is difficult to see that the activity of any single neuron can be completely isolated from that of its companions to signal a unique percept. Instead, the combined activity of large numbers of cortical neurons seems more likely to be the basis of our perceptual experience. However, distributed representations have problems of their own, such as the ambiguity of interpretation when a particular neuron is permitted to respond in more than a single context. For example, many neurons may implement the distributed encoding of apples, and some common fraction of these will be activated by particular red, yellow, and green apples. If this common fraction is activated in a number of different contexts, what is the unique neural object that defines a particular apple? von der Malsburg (1981) has proposed that the population of neurons activated by the stimuli of a particular physical object are bound together transiently by a common physiological process. One possibility is that the 40-Hz oscillation of discharge observed in cortical neurons reflects such a binding process (Gray and Singer, 1989; Crick and Koch, 1992; Singer, 1994).

PARALLEL PROCESSING IN NEURAL NETWORKS

It is evident from the preceding discussion that normal vision involves the activity of very large numbers of cortical neurons. These large numbers do not simply reflect redundancy, which an efficient coding must avoid, but rather are a necessary part of perception. This is evident in the example of color vision, where both behavioral and theoretical studies show that the relative stability of the perceived color of objects in the face of changing illumination (e.g., moving from indoors to outdoors) requires the comparison of the reflected wavelengths over a large region of the visual field (Jameson and Hurvich, 1959; Land, 1959a,b; Land, 1983). This phenomenon of color constancy necessarily involves the coordinated activity of large numbers of cortical neurons. Similar considerations apply in the case of binocular vision, where two slightly different views of the same complex scene must be fused to produce a single vision and stereopsis. Attempts to replicate this performance have shown that it is a difficult task (Marr and Poggio, 1979; Mahowald, 1994), yet we fuse the image and extract the exact three-dimensional information effortlessly and far more rapidly than can any computer. One reason for this difference is that the strict hierarchy of serial processing used

by computers with von Neumann architecture is slow. In the cerebral cortex, by contrast, the higher degree of divergence in the connections makes it likely that much of the processing occurs simultaneously through parallel pathways. Of course, computers have transistors that can generate digital impulses at very high rates and transmit them at the speed of light to perform their computations. By comparison, neurons generate impulses at very low rates and transmit them at speeds of meters per second. However, the neocortex makes many connections to and from each neuron, whereas the limitations of size and thickness mean that silicon components have limited connectivity. Thus, advantages of speed are offset against limited connectivity.

The increase in speed offered by parallel processing has been exploited in a number of models of visual processing based on feedforward artificial neural networks. The most common form of feedforward network is composed of three layers of highly abstract neurons whose activity typically varies between 0 and 1. A first layer, or *input layer*, connects extensively to units of a second, *hidden layer*, which in turn sends its output to the third, *output layer*. The sensory input is applied via the input layer. The responses of units in the hidden and output layers are determined by summing activities of all the units in the previous layer. The effect of each of the inputs is governed by a synaptic *weight*, which may be positive (excitatory) or negative (inhibitory). The values of these weights determine what functions of the input the network can compute, and so what overall task the network performs. These weights may be specified directly, but more often they are organized by a learning algorithm, such as *back propagation* (Rumelhart and McClelland, 1986; Anderson, 1995; Hertz et al., 1991). Such networks turn out to be powerful and are capable of solving some of the perceptual problems of depth, form, and motion perception (Lehky and Sejnowski, 1988; Zipser and Andersen, 1988).

ARE NEURAL NETWORKS LIKE CORTICAL CIRCUITS?

The neural network models, besides being functionally successful, have a strong appeal because of their superficial resemblance to the structure of the cortex: they are layered and have highly interconnected units. However, this superficial resemblance should be examined more critically in the light of our knowledge of the structure of area 17. The basic circuit of Fig. 12.18 illustrates some of the main neuronal components and their connections within area 17. Comparing this figure with the feedforward neural network of Fig. 12.17 shows a number of similarities. The first layer in the neural network corresponds to the map of the geniculate terminals, the second layer units to the neurons in layer 4 that project to the superficial layers, and the third to the pyramidal neurons projecting from cortical layers 2 and 3. In respect of this laminar organization, the pattern of the model corresponds to that of cortex in that very few neurons of layer 4 provide an output to other cortical areas, whereas a large proportion (70%) of the pyramidal neurons in layers 2 and 3 do project to other areas. However, it is evident at a glance that the organization of cortex shown in Fig. 12.18 is in many important respects different from the neural network circuit of Fig. 12.17.

Unlike the neural network, the primate visual cortex receives at least two physiologically and anatomically distinct inputs from the thalamus, which are laminar specific. The cortex has twice as many or more layers, particularly if the subdivisions of the six basic layers are taken into account. This may be a requirement of the cortex to
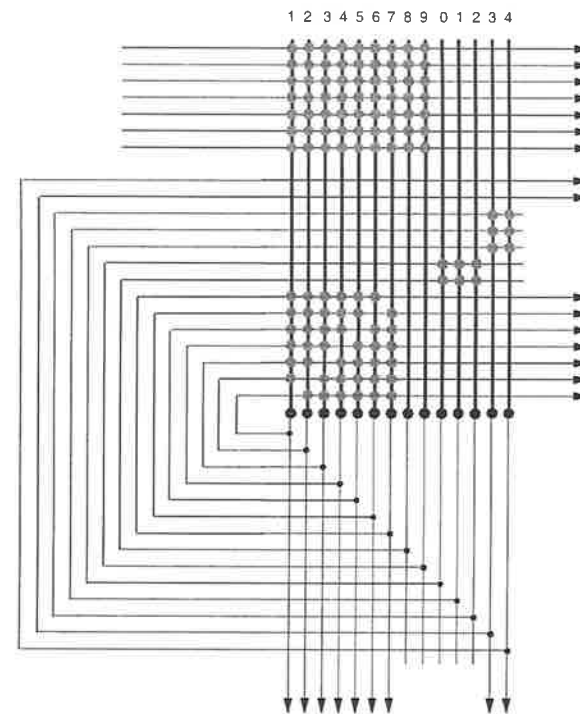

Fig. 12.17. A hypothetical cortical neuronal network composed of two subnetworks; one feedforward and the other feedback. Neurons of the network (black) receive input synapses (gray circles) onto their dendrites (thick black vertical lines). The effects of these neurons are integrated into the neuronal somata (black circles), and their outputs are transmitted along their axons (thin black lines). Branch points of the axons are indicated as small black dots on the axons. The neurons are numbered from 1 to 14. Feedforward inputs enter via the seven horizontal axons above. Feedforward network: the feedforward inputs synapse with the first layer of cortical neurons (8 and 9), which project to the second layer (10–12) and thence to the final layer (13 and 14) whose outputs project out of this region of cortex. The intermediate computations of this entirely feedforward network are unaffected by connections between cells in the same layer or by backward projections from later layers. Feedback network: The feedforward inputs synapse with the distal dendrites of the recurrently connected population of neurons (1–7). Their axons synapse with all other members of their population (but not with themselves). In this case, the evolving response of each neuron comes to influence the computations of its fellows. The overall computation is iterative in quality and finds a solution that is, in some sense, a consensus among the cooperating neurons. The state of the population is transmitted out of this region of cortex.

divide its output destinations ("addresses") into laminar specific zones. However, the internal connectivity and physiology differ in different layers, indicating that there may be important differences in the processing within layers. In contrast to the units within a single layer of the neural network, there are extensive lateral connections within a single lamina or sublamina of cortex. Thus, the local connectivity of cortical neurons resembles that of a "Hopfield" recurrent network, but with the notable difference that synaptic interactions between two neurons are not symmetrical as required for a pure
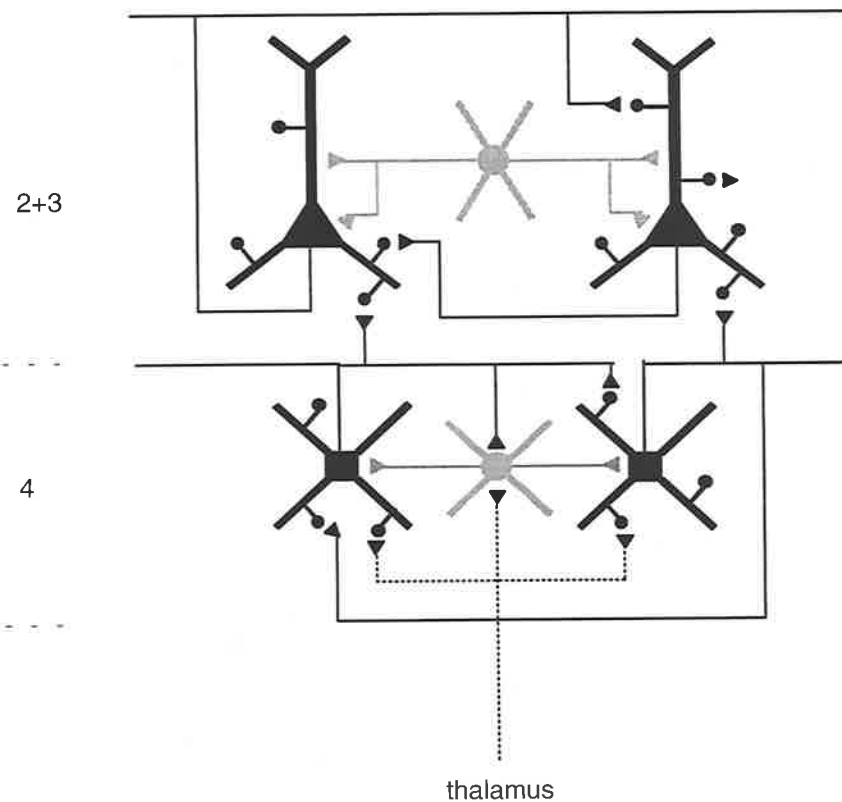
Fig. 12.18. Possible recurrent connections in neocortex. This figure is an elaboration of Figs. 12.15 and 12.14. The feedforward thalamic inputs synapse with spiny stellate neurons and a small basket cell in layer 4. Some of the spiny stellate connections are recurrent to other spiny stellates. They also synapse onto superficial pyramidal neurons that in turn have recurrent connections with one another.

Hopfield network (Hopfield, 1982, 1984). This similarity of cortical networks to artificial recurrent networks is useful, because it offers a partial explanation for how cortical circuits could interpret their inputs. The model shows how output patterns can be stored as the dynamical attractors of the network by setting the strengths of all the network connections appropriately. External input initializes the network dynamics, which then retrieves a stored memory by converging to the attractor that most nearly matches the pattern of external input.

Unlike typical recurrent artificial neural networks the cortical ones are not fully connected. Instead, they are quite sparsely connected. Similarly, the interconnections between cortical laminae are highly specific and do not simply connect adjacent layers as in the units of the neural network. Both the vertical and the lateral connections in the cortex are clustered, focusing on discrete zones. This columnar structure is a feature of cortical organization, but it is the exception rather than the rule to incorporate such horizontal organization within network models.

STATIC AND DYNAMIC CONNECTIVITY

Both the neuroanatomical diagram shown in Fig. 12.18 and the neural network circuit of Fig. 12.17 depict the static connectivity between neurons in the respective networks. It is clear from the above discussions of the physiology of the various cortical neuronal types and their synapses that the static connectivity only sets the most fundamental constraint on what kinds of interactions are *possible* within the network. The dynamic, functional connectivity of the network may differ very significantly from its static counterpart. These dynamic circuit changes have important consequences for signal processing.

For example, the spike discharge gain of a neuron with respect to a presynaptic neuron depends not only on its intrinsic feedforward gain but also on feedback activation that it receives from the network in which it is embedded. If the feedback signal is well correlated with the input, then the presynaptic signal can be strongly amplified. Thus, the neurons response to presynaptic input depends on a context offered by the embedding network. Even if all synaptic strengths between neurons of the network are kept fixed, the effective strength of feedback is variable. The variation arises because the neurons of the network are partitioned into two disjoint sets: those that are above threshold and active and those that are below threshold and inactive. The effective strength of feedback depends only on the synaptic connections between active neurons, as only they participate in feedback loops (Hahnloser et al., 2000). However, the synaptic strengths of active neurons are not fixed: dynamic synapses transmit different aspects of presynaptic activity depending on the pattern of activity and synaptic parameters (Markram, 1998; Tsodyks et al., 1998; Tarczy-Hornoch et al., 1998). Thus, the effective connectivity of the network changes with time, because individual neurons fall beneath an inhibitory threshold and so no longer activate their group output synapses, and also because the effect of an individual activated synapse depends also on the pattern of its past activations (Fig. 12.19). Novel network scale activity detection methods with high temporal and spatial resolution will be required to characterize the relationship between the static and dynamic connectivity.

It is somewhat surprising, given the intensity of cortical research, that the rules of even the static connectivity for neocortex have still to be discovered. At this stage we know that the different types of neurons connect with some degree of specificity to particular regions of other neurons, e.g., dendritic shafts or spines, but whether single neuron–to–single neuron connections are specified is still quite unclear. At this stage it seems likely that neurons do not connect on a point-to-point basis. Rather, they connect on a point-to-zone basis, targeting particular subsets of neurons within a zone.

The point is readily made that these and other differences show that the artificial neural networks are different in important respects from the cerebral cortex. Finally, artificial *neural* networks are not really very neural. They are just networks operating according to a specific algorithm, and it would be rash to press their analogy to cortical circuits too far. Nevertheless, the potential usefulness of network models that are biologically based cannot be overestimated. The major problem lies in trying to bridge the gap between experimental data and theory. Our knowledge of the structure of the cortical microcircuitry outstrips our understanding of the function of these circuits. This disparity, together with the sheer complexity of the cortical circuits, is a significant barrier to moving from networks that are simply *neurally inspired* to those that actually
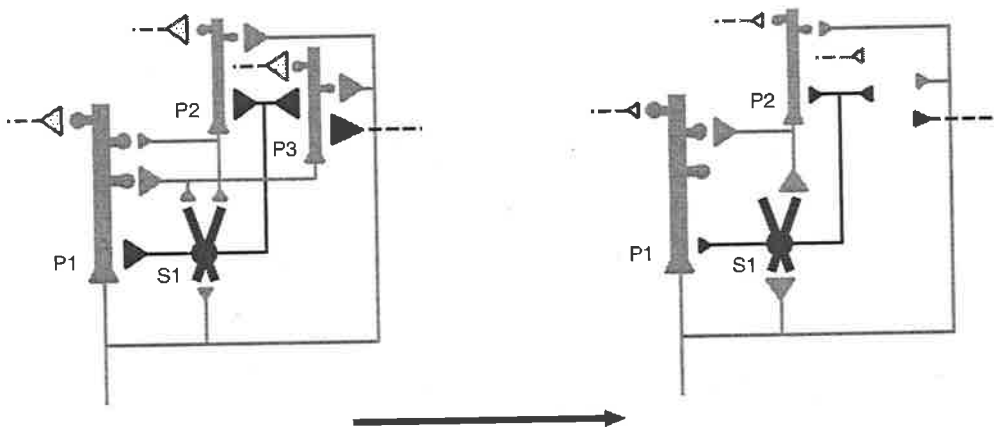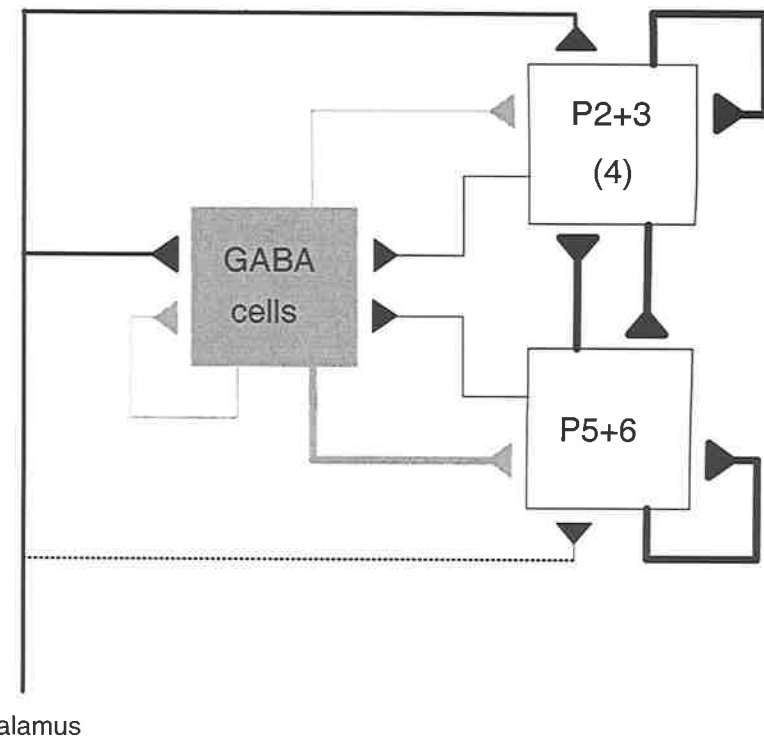
Fig. 12.19. Simplified schematic representation of dynamical changes in the functional connectivity of a hypothetical cortical network, showing how a circuit might reconfigure itself during processing. P1–3, pyramidal neurons; S1, smooth neuron. Left initial state; right, final state, later in time. The size of a synapse represents its strength. Input is delivered via the three stippled synapses, which depress over time. The connections between neuron P1 and its pyramidal targets (P2, P3) also depress, whereas its synapse with the smooth neuron S1 facilitates. P3 is similar to P1. However, the combination of local and external inhibition drive this neuron below threshold, and so it no longer participates in the circuit. P2 is an example of a pyramidal neuron whose P-to-P synapse facilitates. In this example, all of the smooth cell synapses depress.

thalamus

Fig. 12.20. The canonical microcircuit for striate cortex. Three populations of neurons interact with one another: One population is inhibitory (GABAergic cells, gray synapses), and two are excitatory (black synapses) representing superficial (P2 + 3) and deep (P5 + 6) pyramidal neurons. The properties of layer 4 stellates (4), which contribute 10% of neurons in granular cortex (less elsewhere), are similar to those of the superficial pyramids. The thickness of the connecting lines indicates the functional strength of the input. Note that the dominant connection is between excitatory neurons, so that a relatively weak thalamic input can be greatly amplified by the recurrent excitation of the spiny neurons.

incorporate basic features of the biology. To achieve this step, the cortical connections shown in Fig. 12.15, and their associated physiology, have to be simplified. Given the outline of the preceding sections, one such simplification can now be suggested (Fig. 12.20). The form of this "canonical" circuit was arrived at from an analysis of the structure and function of local circuits in the visual cortex (Douglas et al., 1989, 1995; Douglas and Martin, 1991). However, an analysis of the circuits of other cortical structures such as the olfactory cortex (paleocortex) and hippocampus (archicortex) reveals that they, too, bear many resemblances to the circuits of the neocortex (Shepherd, 1988b,a). Thus, it is tempting to suppose that there may be some common basic principles that underly the organization and operation of all cortical circuits, reflecting the principles of synaptic circuit design outlined in Chap. 1.

A CANONICAL CORTICAL CIRCUIT

From the anatomy, several components and connections seem to dominate in most cortical areas (see Fig. 12.20). Any realistic model must separate inhibitory (GABAergic) and excitatory neurons into distinct populations. The excitatory group (80% of the cortical neurons) can be subdivided into two major pools, one being found in the granular and supragranular layers (layers 2–4), and the other in the deep layers (layers 5 and 6). Although these groups are extensively interconnected, this division is made because their outputs are distinct, and because inhibition appears to be stronger in the deep layers (Douglas et al., 1989). The different types of GABAergic smooth neurons cannot

yet be distinguished on functional grounds; they are therefore represented in the diagram of Fig. 12.20 by a single population.

Neurons within each division form connections with other members of that division. The dominant interlaminar connections are between the superficial and deep layer groups of spiny neurons, whereas the inhibitory neurons connect across the laminae to both groups of spiny neurons. All three groups receive direct activation from the thalamic afferents, but because the thalamic input provides only about 10% of the excitatory input, 90% of the excitation is provided here by intracortical connections between pyramidal neurons. This recurrent excitation may provide selective amplification of geniculate input (Douglas et al., 1989, 1995). Such intracortical amplification provides the basis for a number of recent models of cortical computation (Mahowald, 1994; Ben-Yishai et al., 1995; Douglas et al., 1995; Somers et al., 1995; Suarez et al., 1995).

it may be relatively weak (Douglas et al., 1995).

The excitatory neurotransmitters act on two major receptor types, the NMDA and non-NMDA receptors. The inhibitory neurotransmitter GABA acts via the GABAa and the GABAb receptors. These distinctions are made because the receptor types have distinctly different kinetics. The biophysical characteristics of the cortical neurons, as outlined in the previous sections, also need to be incorporated to give the appropriate response characteristics.

This recurrent excitation model provides the minimum specifications that seem necessary for basic cortical circuits, based on our present level of knowledge. The form of this simplified model is sufficiently general that it can be applied equally well to visual cortex as to motor cortex, and as such has the properties of a canonical circuit. This circuit forms only a basic building block. Obviously, each cortical area has individual features that need to be incorporated. However, simplicity encourages the convergence of theory and biology through common models, and such convergence is imperative if we are to understand how the synaptic organization of the neocortex produces the complexity of cortical function.