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THE PYRAMIDAL NEURON OF THE CEREBRAL CORTEX: MORPHOLOGICAL AND CHEMICAL CHARACTERISTICS OF THE SYNAPTIC INPUTS

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1. INTRODUCTION

1.1. INTRODUCTORY REMARKS

Information in the cerebral cortex flows through synapses in a finely organized network, formed between the extrinsic cortical afferent fibers on the one hand, and the cell bodies, dendrites and axons of a variety of morphological types of cortical neurons, on the other. Processed information leaves the cortex through the axons of the so-called pyramidal cells to reach other cortical areas or subcortical nuclei. Current understanding of the synaptic organization of the cerebral cortex and of how this information flow occurs depends to a large extent on knowledge of the synaptic inputs to the pyramidal cells. Although many morphological, chemical and functional characteristics are common to the pyramidal cells of the three major divisions of the cerebral cortex (palaeocortex, archicortex and neocortex), in this review we shall deal only with the pyramidal neurons of the neocortex. The term "cerebral cortex" will thus be used as a synonym of "neocortex".

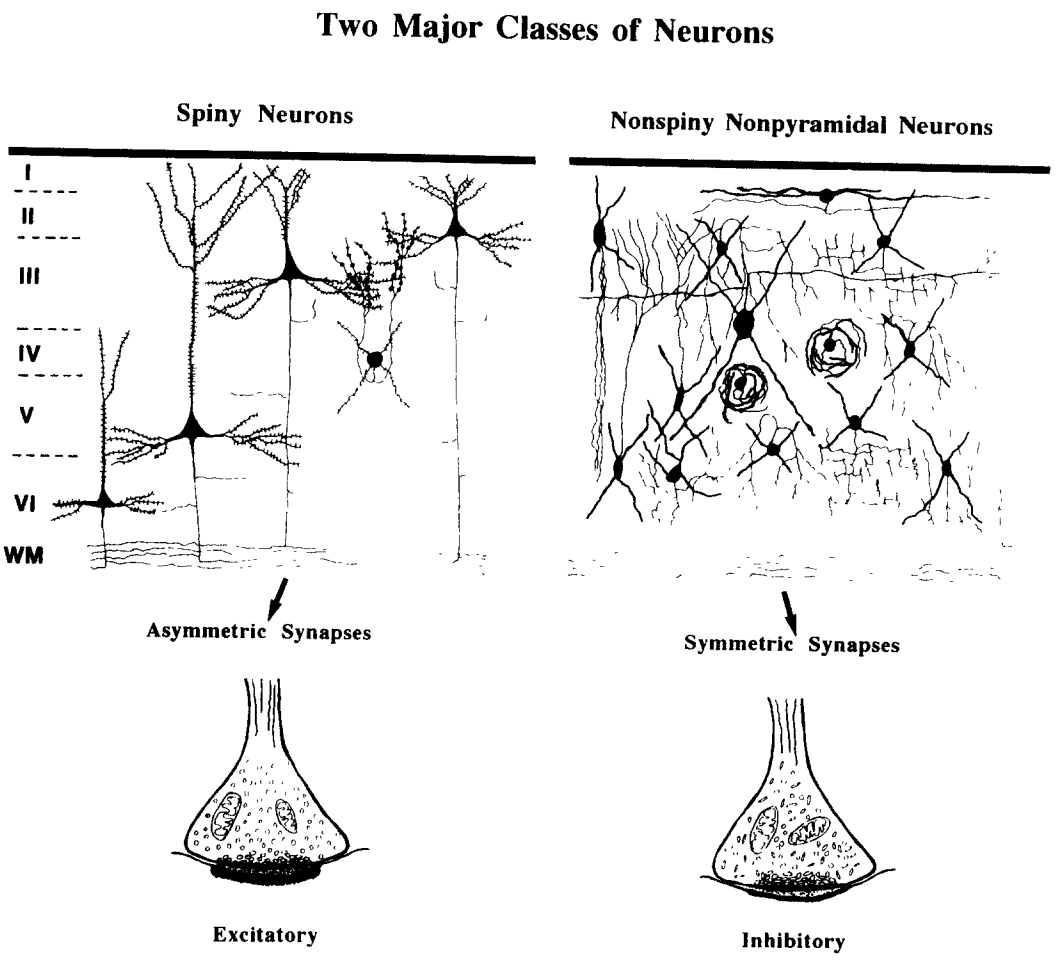

FIG. 1. Schematic diagram showing the classes of neurons and synapses in the cerebral cortex.

Because of the extraordinary complexity of the synaptic organization of the cerebral cortex, this review is not intended to be complete, and the unavoidable limitations in the selection of data and their interpretation reveal, in many cases, the personal views and interests of the authors.

1.2. Classes of Neurons and Synapses in the Cerebral Cortex

In the cerebral cortex there are two major classes of neurons: spiny neurons and aspiny nonpyramidal neurons (Fig. 1). *Spiny neurons* are subdivided in to pyramidal cells and spiny nonpyramidal cells. Pyramidal cells are long-axon cells and they are located in all layers except layer I. These cells are very abundant in all cortical areas (they constitute approximately 70–85% of the total population of neurons). Spiny nonpyramidal cells are short-axon cells (interneurons); they are confined to the middle layers of the cortex (especially layer IV) and different morphological types can be recognized, depending on the species, cortical area and sublayer in which they are found (e.g. Fairén *et al.*, 1984; Lund, 1984). The typical spiny nonpyramidal cell is the spiny stellate cell, which is a small neuron with rounded soma sending off spinous dendrites at all angles. Spiny stellate cells have been described mainly in the primary visual cortex, somatosensory cortex, and in the auditory cortex where they are apparently very abundant (for a review see Lund, 1984). *Aspiny nonpyramidal neurons* are also short-axon cells with smooth or sparsely spiny dendrites (in this review we will use the term "aspiny"). They are present in all cortical areas and layers and represent approximately 15–30% of the total population of neurons. Aspiny nonpyramidal neurons constitute a morphologically heterogeneous group of neurons, and different morphological types are recognized on the basis of their patterns of axonal arborization (e.g. Fairén *et al.*, 1984).

There are also two main morphological types of synapses (Fig. 1) in the cerebral cortex: *asymmetric and symmetric synapses* (Colonnier, 1968), which correspond, respectively, to Gray's type I and II (Gray, 1959). The major morphological feature that characterizes these two types of synapse is the nature of the density in the cytoplasmic face of the postsynaptic element. Asymmetric synapses are characterized by a prominent postsynaptic density, and symmetric synapses by a very thin postsynaptic density. Quantitative electron microscopic investigations have shown that the number of symmetric synapses is considerably less than that of asymmetric synapses. In a

variety of species and cortical areas, the values range between approximately 75–95% for synapses of the asymmetric type and 10–25% for those of the symmetric type (e.g. Beaulieu and Colonnier, 1985; Schüz and Palm, 1989; Zecevic *et al.*, 1989). In addition, in aldehyde-fixed material, axon terminals forming asymmetric synapses contain round or spherical vesicles, whereas most axon terminals forming symmetric synapses contain pleomorphic vesicles (e.g. Peters *et al.*, 1991). The major sources of asymmetric synapses are spiny neurons (pyramidal and spiny nonpyramidal cells) and extrinsic cortical afferents, whereas the vast majority of symmetric synapses are of intrinsic origin (Fig. 1) and are formed by the population of aspiny nonpyramidal neurons (Peters and Jones, 1984; Peters, 1987a; White, 1989). Since spiny neurons and the major cortical afferent systems are excitatory in function (Gilbert, 1983; Jones, 1985; White, 1989) and many aspiny nonpyramidal cells are GABAergic and thus, inhibitory (e.g. Houser *et al.*, 1984), it is generally assumed that axon terminals forming asymmetric synapses are excitatory and those forming symmetric synapses are inhibitory (e.g. Colonnier, 1981).

1.3. WHAT IS A PYRAMIDAL CELL?

With few clear exceptions (e.g. some large spiny stellate cells of layer IV of the visual cortex; see Lund, 1984), pyramidal cells are the only projection neurons of the cerebral cortex and they are commonly subdivided according to their projection site (e.g. Jones, 1984; White, 1989) (Fig. 2). They have traditionally been considered to be the principal neuron of the cerebral cortex because: they are the most abundant neuron; they have a unique morphology; and because they are found exclusively in the cerebral cortex. However, pyramidal cells are heterogeneous with regard to somal size and shape, dendritic branching, spine density, pattern of axonal collaterals and

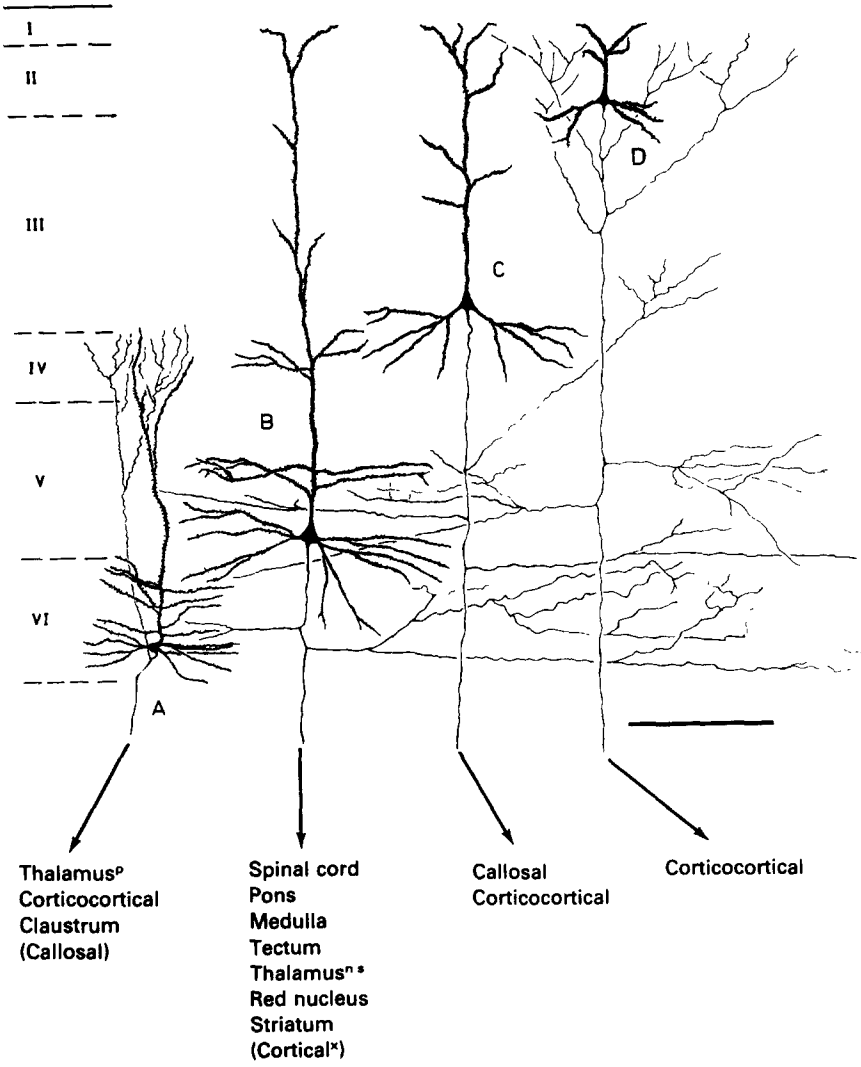

FIG. 2. Schematic diagram of laminar origins of efferent projections, based mainly on data from monkeys. Parentheses indicate that a projection may not arise from the layer indicated in all species or all areas. *n.s.*, nonspecific thalamic nuclei; *p*, principal thalamic relay nuclei; *x*, from some cortical areas only. (From Jones, 1984.)

projection site; so, it is difficult to make a simple definition and/or description of an archetypical pyramidal neuron. Moreover, as the body of evidence supporting the heterogeneity of the pyramidal cells grows, it becomes clear that there are significant differences in the synaptic inputs to different populations of pyramidal cells. Given the difficulty of exhaustive electron microscope studies and the lack of a convenient catalog of pyramidal cell types, little is known yet about the extent and significance of these differences (see Section 5). The purpose of this section is to provide a brief summary of the characteristics of different pyramidal cell types described in the literature. For the sake of simplicity, pyramidal neurons will be divided into pyramidal neurons with typical and atypical morphologies.

Typical pyramidal cells. There are three main distinguishing, though not exclusive, characteristics that define a typical pyramidal cell (Fig. 3): (1) The shape of the cell soma is pyramidal or ovoid. From the upper pole arises a prominent apical dendrite directed radially towards the pia mater and which gives off a number of oblique branches. From the base there emerges a system of large basal dendrites directed laterally or downward. It is also frequent to observe thinner dendrites arising from the soma which are commonly horizontally oriented. In general, the apical dendrite reaches layer I, where it forms a tuft of branches. Therefore, the length of the apical dendrite depends on the depth of the cell soma from which it emerges. However, there are many exceptions to this. For example, the apical dendrites of the majority of pyramidal cells located in layer VI and some pyramidal cells located in layer V do not reach layer I (Feldman, 1984). (2) From the base of the cell or from the origin of a basal dendrite comes the axon

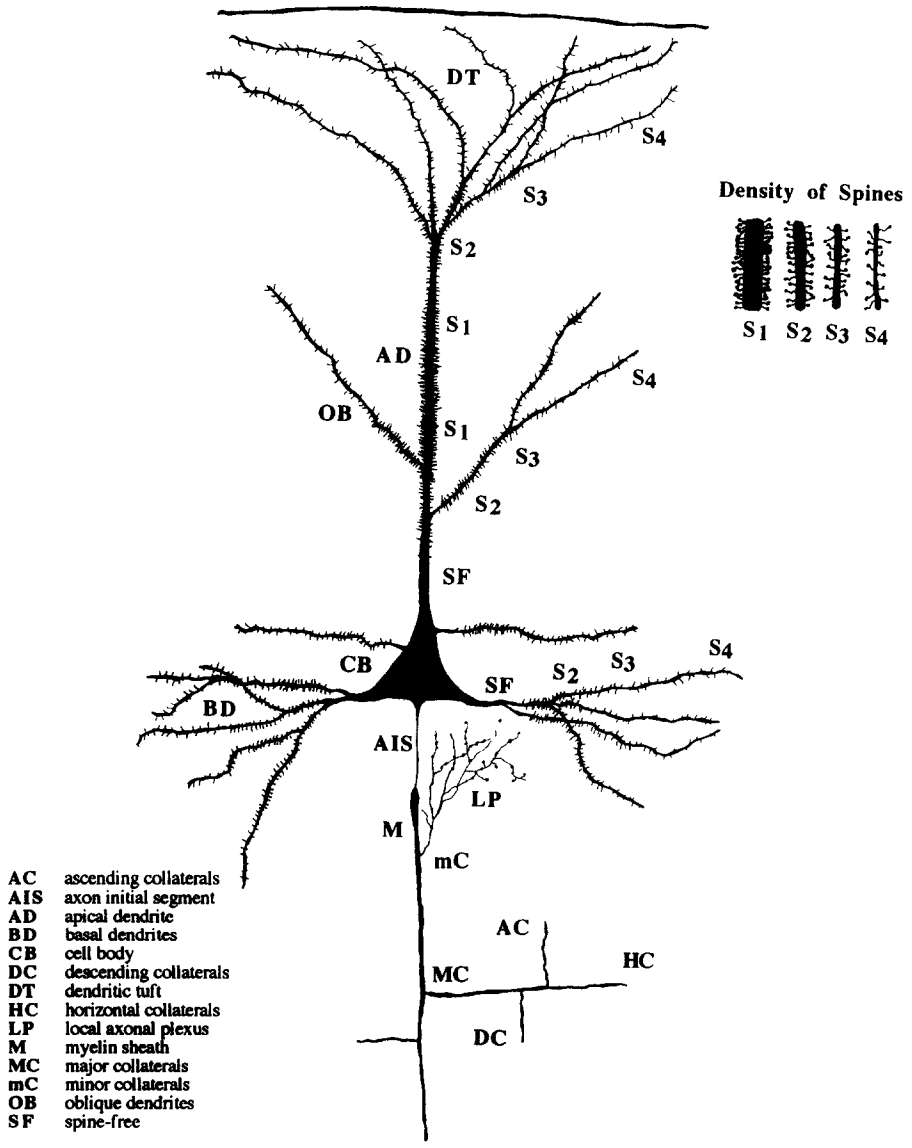

FIG. 3. Schematic diagram showing a typical pyramidal cell. Minor collaterals (mC) are thin, nonmyelinated and give rise to a terminal axonal plexus near the cell body. Major collaterals are thick, myelinated and give off several branches that form terminal axonal plexuses near the cell body or at a distance.

that is directed downwards and leaves the cortex to terminate in other cortical or subcortical regions. The axon during its descending course through the cortex, gives off several minor and major collaterals (Fig. 3) that give rise to terminal axonal arborizations which constitute one of the main components of the intracortical circuitry. These terminal axonal arborizations are distributed locally (near the parent cell soma), vertically (above or below the cortical layer in which the parent soma lies) or horizontally (in the same or an adjacent layer, but at a distance from the parent soma in the horizontal dimension). (3) All dendritic surfaces are covered by spines, except the proximal segments arising directly from the cell soma which are generally spine-free.

Atypical pyramidal cells. Most atypical pyramidal cells are considered so because of special features of the dendritic branching pattern, cell body shape, density of spines, and of axonal characteristics. What follows is a list of the most common examples of atypical pyramidal cells (Fig. 4), most of them have already been described by Cajal (see DeFelipe and Jones, 1988b).

(1) Modified pyramidal cells of layer II. These are neurons located in the upper part of layer II which either lack or have a very short apical dendritic shaft and often a polygonal or stellate shape, giving them a slight similarity to the spiny stellate neurons of layer IV (for example, see Einstein and Fitzpatrick, 1991), but their somata are generally larger than those of layer IV cells.

(2) Pyramidal cells with multiple "apical" dendrites. These cells are commonly found in layer V. The most frequent case is that in which the apical dendrite divides near the cell soma, giving rise to two or more main radial branches with the same, or similar, caliber and morphological characteristics as the primary apical dendrite (Deschênes *et al.*, 1979; Yamamoto *et al.*, 1987a, b; Ghosh *et al.*, 1988; Hübener *et al.*, 1990). Much less frequently, two apical dendrites arise directly from the soma (for example, see Fig. 282 of DeFelipe and Jones, 1988b).

(3) Modified pyramidal cells of the infragranular layers. Their somata display a large variety of forms (ovoid, triangular or fusiform) and, in general, they are located mainly in layer VI (Tömböl, 1984; de Lima *et al.*, 1990). Some of them exhibit an ascending dendrite that makes frequent turns and that usually does not extend above layer IV. The ascending dendrite is considered by some authors as the apical dendrite, regardless of its caliber; other authors use the term "apical" for the main dendritic shaft which can be oriented at various angles, and from this comes the differences in naming these cells by different authors. The common characteristics of these cells are that they have spiny dendrites and are projection neurons. The most usual forms are the inverted pyramidal cells, the horizontal pyramidal cells, the horizontal fusiform neuron (also called deep bipolar neuron by Peters and Regidor, 1981), and the vertical fusiform neuron. Inverted pyramidal cells are those pyramids whose main dendrite trunk is directed towards the white matter. They are located in layers V and VI (for a recent study, see Bueno-López *et al.*, 1991). In the horizontal pyramidal cells (Tömböl, 1984), which are similar to the asymmetrical pyramids of de Lima *et al.* (1990), the main dendritic shaft is oriented horizontally. Finally, the horizontal and vertical fusiform neurons are characterized by the fusiform shape of the cell body and because from each of the two opposite poles arises a main dendritic shaft; they are oriented either horizontally (horizontal fusiform neuron) or vertically (vertical fusiform neuron).

(4) Pyramidal cells whose dendrites lack, or have very few, dendritic spines. It has been reported that some layer V pyramidal cells are almost devoid of dendritic spines. These cells have been described in the monkey and cat motor cortices (Hamada *et al.*, 1981; Labelle and Deschênes, 1979; Deschênes *et al.*, 1979; Liu *et al.*, 1991), and in the cat somatosensory (Yamamoto *et al.*, 1987b, 1990), parietal (Samejima *et al.*, 1985; Yamamoto *et al.*, 1987a) and visual (Gabbott *et al.*, 1987; Hübener *et al.*, 1990) cortices. All of these pyramidal cells were identified by intracellular injections of dyes and, in the case of the cat visual cortex, some of the spine-free neurons (Hübener *et al.*, 1990) were previously identified by retrograde labelling as corticotectal projection neurons. In the monkey motor cortex and cat sensorimotor and parietal cortices, layer V pyramidal neurons were first identified with antidromic activation upon stimulation of the medullary pyramid, pontine nuclei or cerebral peduncles, and then stained intracellularly with horseradish peroxidase. These spine-free pyramidal cells were electrophysiologically characterized as neurons with axons having fast conduction velocity, whereas the spiny pyramidal cells had axons of slow conduction velocity. However, not all corticotectal neurons in the cat visual cortex, nor all neurons with fast conduction velocity in the cat motor cortex (Ghosh *et al.*, 1988), were spine-free, only a subpopulation of them.

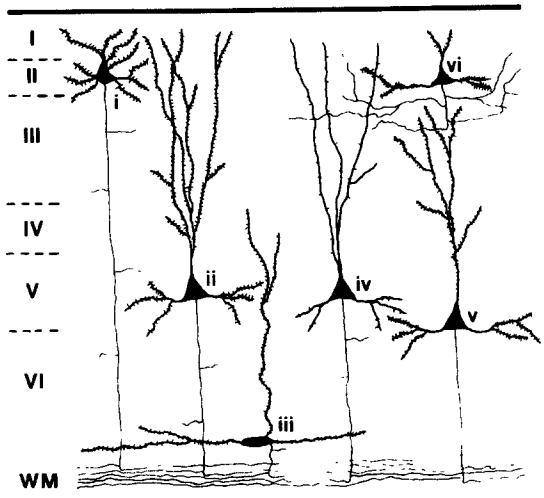

FIG. 4. Schematic diagram of different types of atypical pyramidal cells. (i) Modified pyramidal cells of layer II. (ii) Pyramidal cells with multiple "apical" dendrites. (iii) Modified pyramidal cells of the infragranular layers. (iv) Pyramidal cells whose dendrites lack, or have very few, dendritic spines. (v) "Pure" projection pyramidal cells. (vi) Pyramidal cells whose axons do not leave the cortex.

(5) "Pure" projection pyramidal cells. These are pyramidal cells whose axons are devoid of intracortical axonal collaterals. Following the classical studies of Cajal (see DeFelipe and Jones, 1988b) using the Golgi method, and recently confirmed by Katz (1991) by means of intracellular fluorescent dye injections in brain slices, intracortical axonal collaterals of most pyramidal cells appear during the first or second postnatal week of development. According to Cajal, the postnatal appearance and development of the axonal collaterals could probably be the reason why in early studies some authors like Kölliker, who made his observations in embryonic material, were not able to find the collaterals of pyramidal cell axons. However, in the visual cortex of 3-week-old kittens, a strikingly large number of Golgi-impregnated pyramidal cells with unbranched axons to the white matter were described by Sholl (1955a). At present, it is a popular belief that all pyramidal cell axons have intracortical collaterals. However, Ghosh *et al.* (1988), by using the method of intracellular injection of horseradish peroxidase in area 4γ of the cat motor cortex, found that the axons of certain filled pyramidal cells (located in layer VI) did not emit any intracortical collaterals, even though the axons were traced from their origin to their entrance into the white matter. This observation suggests that pure projection pyramidal cells indeed exist.

(6) Pyramidal cells whose axons do not leave the cortex (or intrinsic cortical pyramidal neurons). Although it is a frequent observation that the axons of some pyramidal cells disappear within the gray matter, it is seemed to be correctly assumed that this is due to either incomplete staining or to sectioning of the axon by the microtome knife. However, pyramidal cells with well-stained axons, but not leaving the cortex, have been described in layers II, III and V of the cat visual cortex (Gilbert and Wiesel, 1979; Martin and Whitteridge, 1984). These cells are different to what Cajal called "small pyramidal cells with arciform (recurrent) axon" which are confined to the middle layers of the cortex (especially layer IV; see also Sholl, 1955a). The latter neurons possess a rudimentary apical dendrite and a poorly developed system of basal dendrites. They have been considered by some authors as a common morphological variety of spiny stellate cells, and by others as a variety of pyramidal cells.

In summary, a neuron would be named pyramidal cell if it is located in any layer except layer I and possesses at least one of the following three features: (1) if it is a projection cell located outside layer IV; (2) if it is a spiny neuron located outside layer IV; (3) if it displays a prominent apical dendrite. Most data about the morphological and chemical characteristics of the synaptic inputs on pyramidal cells have been obtained from typical pyramidal cells. Therefore, unless otherwise specified, in this review we shall refer to the typical pyramidal cells.

1.4. Neurotransmitters in the Cerebral Cortex

This is not the place for a detailed review of the neurotransmitters present in the cerebral cortex and what follows is only a brief survey.

There is a large number of compounds found in the vertebrate nervous system which are believed to operate as neurotransmitters (e.g. Krnjević, 1974). In the cerebral cortex, on the basis of work using immunocytochemical methods, the following putative transmitters (or their synthesizing enzymes) have been found: The *"classical" or conventional transmitters* γ-aminobutyric acid (GABA), glutamate (and/or aspartate), noradrenaline, dopamine, serotonin, and acetylcholine; and the *neuroactive peptides*—cholecystokinin (CCK), somatostatin (SRIF), neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), tachykinins, opioid peptides (dynorphin and enkephalin), corticotropin releasing factor (CRF), calcitonin gene-related peptide and neurotensin. Although some of these putative transmitters have been localized in significant populations of both extrinsic cortical afferent fibers (arising in the brainstem and basal forebrain) and cortical neurons (for instance, acetylcholine in the rat cerebral cortex; e.g. Houser *et al.*, 1985), most of them have been localized exclusively or to a large extent in either extrinsic afferent fibers or cortical neurons (Jones and Hendry, 1986a). There are differences between areas and species with regard to the pattern of immunoreactive staining (that is, number and laminar distribution of immunoreactive neurons, fibers and terminal-like puncta) for most above-mentioned compounds. The reported differences appear to be due, in some cases, to technical considerations. For example, numerous VIP immunoreactive neurons are found in the cerebral cortex of the rat, whereas in the monkey cerebral cortex there are no clear examples of neurons immunoreactive for VIP (Jones and Hendry, 1986b). However, using *in situ* hybridization, Benson *et al.* (1991) found numerous VIP mRNA-labeled neurons (thus capable of synthesizing VIP) in both rat and monkey cortices.

GABA is the major inhibitory neurotransmitter of the cerebral cortex and is almost entirely of intrinsic origin (Emson and Lindvall, 1979) although a few hypothalamic GABAergic cells have been found to project to the rat cortex (Vincent *et al.*, 1983). Immunocytochemical studies have shown that only aspiny nonpyramidal cells are immunoreactive for GABA or for its synthesizing enzyme, glutamic acid decarboxylase (GAD) (e.g. Ribak, 1978); and it appears that virtually all the morphologically well-characterized types of aspiny nonpyramidal cells are GABAergic (Houser *et al.*, 1984). Glutamate (and/or aspartate), which is thought to be the major cortical excitatory neurotransmitter, appears to be the transmitter used by pyramidal cells (e.g. Conti *et al.*, 1987). Noradrenaline, dopamine and serotonin are contained in extrinsic afferent fibers that arise in the brainstem; however, some neurons immunoreactive for tyrosine-hydroxylase (the rate-limiting enzyme in the biosynthesis of catecholamines) have been found in the mouse, rat and human cortices (Gaspar *et al.*, 1987; Kosaka *et al.*, 1987a, c; Hornung *et al.*, 1989; Satoh and Suzuki, 1990). The tyrosine-hydroxylase immunoreactive neurons display a nonpyramidal morphology, and a significant population of them also displays immunoreactivity for GAD or GABA (Kosaka *et al.*, 1987c; Trottier *et al.*, 1989). Finally, the neuropeptides are found mainly in aspiny

nonpyramidal neurons (Jones and Hendry, 1986a) and, although there are some reports describing neuropeptide-immunoreactive pyramidal cells (e.g. Ong and Garey, 1990), most authors find that the neuropeptide-immunoreactive neurons possess a nonpyramidal morphology, which is in line with the finding that a large proportion of the peptide-immunoreactive neurons are also immunoreactive for GABA or GAD (Jones and Hendry, 1986a).

Axon terminals immunoreactive for GABA (or GAD) form synapses exclusively of the symmetric type (Ribak, 1978; Peters *et al.*, 1982; Freund *et al.*, 1983; Hendry *et al.*, 1983a; DeFelipe *et al.*, 1985; Somogyi and Soltész, 1986; Verney *et al.*, 1990; Beaulieu and Somogyi, 1990, 1991; DeFelipe and Jones, 1992), whereas glutamate has been localized in axon terminals forming asymmetric synapses (DeFelipe *et al.*, 1988; Conti *et al.*, 1989; Dori *et al.*, 1989). This is in keeping with the suggestion that symmetric synapses are inhibitory and asymmetric synapses excitatory in function (see Section 1.2). The terminals immunoreactive for the peptides studied so far at the electron microscope level (CCK, SRIF, NPY, VIP and tachykinins) form predominantly, if not exclusively, symmetric synapses (Freund *et al.*, 1986; Hendry *et al.*, 1983b, 1984b; Connor and Peters, 1984; Hajós *et al.*, 1988; Jones *et al.*, 1988; de Lima and Morrison, 1989; Peters *et al.*, 1987; DeFelipe *et al.*, 1990; Peters, 1990). Finally, cholinergic and monoaminergic axon terminals have been described as forming asymmetric or symmetric synapses, both types of synapses, or not forming synapses at all (Houser *et al.*, 1985; Parnavelas *et al.*, 1986; Papadopoulos *et al.*, 1987, 1989a, b; de Lima and Singer, 1986; de Lima *et al.*, 1988; DeFelipe and Jones, 1988a; Goldman-Rakic *et al.*, 1989; Séguéla *et al.*, 1989, 1990; Verney *et al.*, 1990; Beaulieu and Somogyi, 1991; DeFelipe *et al.*, 1991).

1.5. Synaptic Circuitry: Limitations of Electron Microscopy

The complexity of the organization of the cerebral cortex is, of course, the major limitation to the study of synaptic circuitry. For example, in the mouse cerebral cortex it has been estimated that there is approximately 3 km of axonal length per mm^3 and an average density of neurons and synapses of approximately $9 \times 10^4/mm^3$ and $7 \times 10^8/mm^3$, respectively (Schüz and Palm, 1989). But regardless of the complexity of organization of the cortex, one of the most important limitations to the study of synaptic circuitry is the thinness of the sections used for electron microscopy. While it is true that some cellular components such as cell bodies or large dendrites are relatively easy to follow in serial thin sections, it is extremely difficult and time-consuming to follow in serial sections a single, small process, such as a distal dendrite or an axonal profile, back to its parent cell soma. Thus, it is much more difficult (if not virtually impossible) to follow all distal dendritic profiles that are postsynaptic to a given cell, in order to know how many of those targets belong to the same or different parent cells. In addition, the precise identification of the recipient cell is needed for each case if a clear scheme of the synaptic relationships among cortical elements is to be achieved. The development of methods to examine individual neurons in the light microscope and, subsequently, in the electron microscope—e.g. the Golgi–electron microscope technique of Fairén *et al.* (1977)—has permitted us to analyze synaptic relationships of identified neurons. However, the sample sizes used in most studies are relatively small and, in addition, the identification of the postsynaptic cells of a given neuron is, in many cases, very difficult to make, and only at a rather superficial level (for example, identification of the postsynaptic cell as pyramidal or nonpyramidal, without further details enabling one to ascribe them to a particular morphological type).

Keeping in mind the above-mentioned limitations, the data available in the cerebral cortex strongly suggest that a characteristic feature of the synaptic relationships among cortical neurons is that a given nonpyramidal neuron (e.g. Peters and Fairén, 1978; Fairén and Valverde, 1980; Somogyi and Cowey, 1981; Somogyi *et al.*, 1982, 1983; Peters *et al.*, 1982; Kisvárday *et al.*, 1985, 1987; DeFelipe and Fairén, 1988) or pyramidal cell (Winfield *et al.*, 1981; McGuire *et al.*, 1984, 1991; Kisvárday *et al.*, 1986; Gabbott *et al.*, 1987) forms relatively few synapses with other neurons. The same can be said with regard to the extrinsic cortical afferent fibers (e.g. Freund *et al.*, 1989). Many investigators who currently study synaptic circuitry in the cerebral cortex generally consider that, if the axon of a given cortical neuron or an afferent fiber forms say, ten or twenty synapses with the same postsynaptic cell (such as is the case of chandelier cells or basket cells; see below), then this is a *high* number of synapses. Since it has been estimated that the number of synapses that a cortical neuron may receive is of the order of thousands (approximately 5,000 to 60,000 synapses per neuron, depending on the cortical area and species; Cragg (1967), Peters (1987b)), this implies that the synaptic connections of individual cortical neurons are highly divergent.

1.6. Synaptic Inputs on Pyramidal Cells

As we will see in this review, there are many types of cortical neurons and extrinsic cortical afferent fibers that innervate the pyramidal cells. There is now a good deal of evidence to indicate that different portions of a pyramidal cell are innervated by different types of neurons and different extrinsic afferent fibers. Clear examples of this differential innervation are represented by the synaptic connections of the large or classical basket cells, chandelier cells and double bouquet cells (Fig. 5) which so far constitute the best morphologically and chemically characterized types of aspiny nonpyramidal neurons. The selective innervation pattern appears to be an important characteristic, since it is currently thought that the region of a neuron where the synaptic junction is made has an important functional significance. For example, it seems obvious that an inhibitory synapse close to the cell body of the postsynaptic cell has a greater effect than an inhibitory synapse located more distally on the dendrite. In Sections 2, 3 and 4, we will see that the above-mentioned three types of neurons are important sources of synaptic inputs on

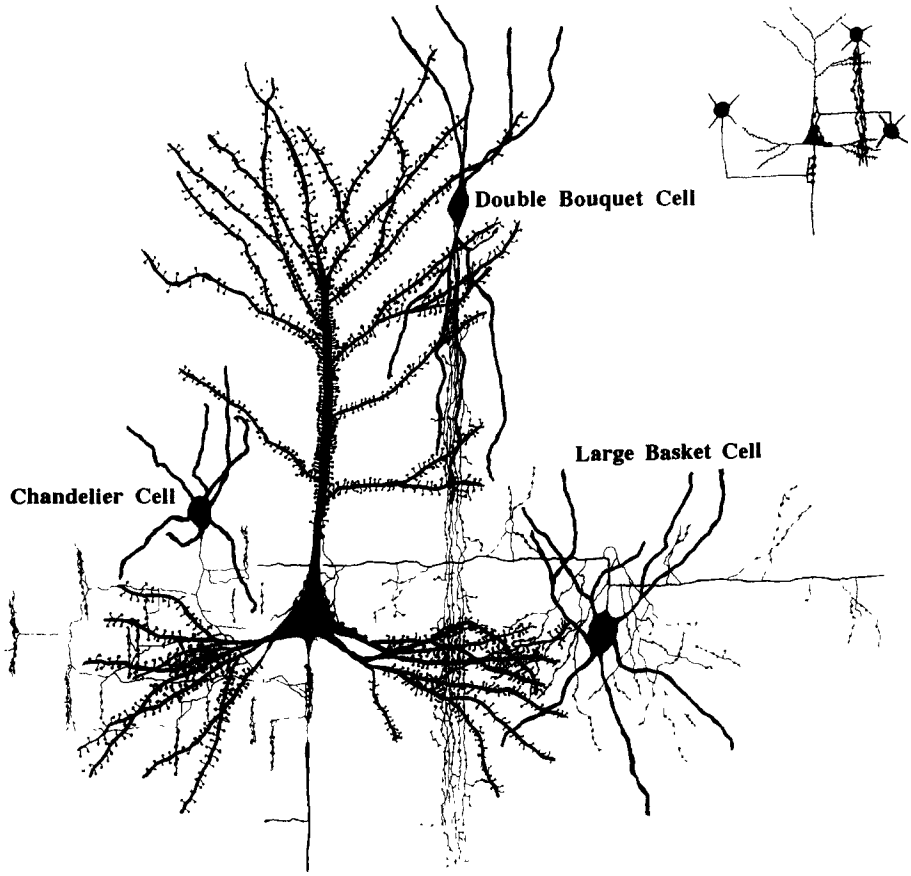

Fig. 5. Drawing to illustrate the synaptic relationships between double bouquet cells, chandelier cells and large basket cells with pyramidal cells. These cells constitute the best morphologically and chemically characterized types of aspiny nonpyramidal neurons. Inset is a schematic diagram to illustrate the synaptic connections between the three nonpyramidal cells and the pyramidal cell. Notice that each type of nonpyramidal neuron innervates a different region of the pyramidal cell.

pyramidal cells and, thus, their synaptic relationships with the pyramidal cells and their chemical characteristics will be one of the major aspects of this review. Another example of the regional specialization of the synaptic inputs on pyramidal cells is that their dendritic shafts and spines are the only postsynaptic sites for axon terminals forming asymmetric synapses and, thus, the only sites where the major cortical afferent systems and spiny neurons terminate. This will be another major subject of the present work and we shall focus on this in Section 4.

2. SYNAPSES ON THE CELL SOMA

2.1. Morphology of the Axosomatic Synapses

As far as we know, in all electron microscope studies reported until now, the axon terminals forming synapses with the cell somata (axosomatic synapses) of pyramidal cells have been found to be exclusively of the symmetric type (e.g. Peters *et al.*, 1991).

Recently, Peters and colleagues (Peters and Harriman, 1990; Peters *et al.*, 1990) have further morphologically characterized the axon terminals synapsing with the cell bodies of layers II/III pyramidal cells in the rat visual cortex. These authors distinguished three types of axon terminals forming symmetric axosomatic synapses: Large terminals, medium-sized terminals and dense terminals. *Large terminals.* They are the largest axon terminals (1.5 μm long and 0.8 μm wide) forming axosomatic synapses. Their shape is ovoid, they contain one or two mitochondria, and they frequently synapse with another neuronal element adjacent to the pyramidal cell. The packing density of the synaptic vesicles is 80–160 synaptic vesicles per μm^2 and the vesicles have a mean diameter of 33.8 nm. *Medium-sized terminals.* These are the most common axon terminals forming axosomatic synapses. They resemble the large terminals, but their size is smaller (1 μm long and 0.5–0.8 μm wide). The packing density of the synaptic vesicles is usually higher (80–200 synaptic vesicles per μm^2) and they have a mean diameter of 33.5 nm. They only occasionally form synapses with an adjacent neuronal element. *Dense terminals.* Not all pyramidal cells receive dense terminals on their somatic surface. These terminals are usually flattened against the cell body, have irregular shapes and their size is approximately 1 μm long and 0.2–0.4 μm wide. The packing density of the synaptic vesicles is higher (120–240

synaptic vesicles per μm^2) than in the other two types of terminals, and mitochondria are not frequently found. They usually display only one synaptic junction. The vesicles are larger and rounder than the vesicles contained in the large and medium-sized terminals and, although the majority of these terminals have mean vesicle diameters of 34.8–40.0 nm, some contain very large vesicles with mean vesicle diameters of 43.4–47.6 nm. Dense terminals also frequently contain dense-core vesicles with diameters of approximately 80 nm.

The range of values for mean eccentricity of the vesicles, length of the synaptic junction and width of the synaptic clefts are similar in the three types of terminals, but the dense terminals display more variability in the length of their synaptic junctions (0.1 μm–0.4 μm). In addition, Peters *et al.* (1990) made reconstructions from serial thin sections of large, medium-sized and dense terminals to analyze the area of the interface between the axon terminal and the pyramidal cell surface, the number and areas of synaptic junctions, the proportion of the interface occupied by synaptic junctions, as well as by *puncta adhaerentia*. They found that there can be multiple synaptic junctions between the axon terminals and the pyramidal cells and usually one or more *puncta adhaerentia*. They reported that the synaptic junctions occupy areas of 12 to 26% for dense terminals, 10–15% for medium-sized terminals and 8% for the one large terminal reconstructed.

It has been reported that some of the axosomatic synapses on layer V pyramidal cells projecting from the visual cortex to the superior colliculus of the cat have morphological features that are characteristic of excitatory synapses (Kennedy, 1982). This author reported that corticotectal cells receive two types of axosomatic synapses on the basis only of the shape of the synaptic vesicles, one from axon terminals containing spherical vesicles and the other from axon terminals containing flattened vesicles. Axon terminals containing spherical vesicles would form excitatory synapses, while the terminals containing flattened vesicles would form inhibitory synapses. However, it has been shown that some GABAergic (inhibitory) axon terminals forming symmetric synapses contain round or spherical vesicles (Beaulieu and Somogyi, 1990). Thus, the shape of the vesicles may be a valid characteristic to distinguish between different morphological types of axon terminals, but not to classify them as excitatory or inhibitory.

2.2. NUMBER OF AXOSOMATIC SYNAPSES

There is a considerable lack of data about the total number of axosomatic synapses on pyramidal cells. The majority of authors agree that the greater part of the somata of pyramidal cells are, to a large extent, free of axosomatic synapses (e.g. Colonnier, 1968; Peters and Kaiserman-Abramof, 1970; Jones and Powell, 1970a) and that the number of axosomatic synapses in a single section can vary from 1 to 14; but few quantitative studies are available. The variable number of axosomatic synapses is often related to the variability that appears to exist in the number of nonsynaptic cellular processes (neuroglial processes, dendrites, myelinated axons, etc) that are apposed to the somatic surface of the pyramidal cells. Although it is not known whether these nonsynaptic perisomatic processes come in contact with the pyramidal cell because there are no axon terminals, or whether axon terminals are excluded because the spaces are "occupied" by those processes, we will see that, at least in some cases, the first alternative appears to be the most plausible.

Most quantitative or semi-quantitative studies about this subject have been made in the visual cortex and most of these have been made on unidentified (unknown projection site) pyramidal cells. Partial, although extensive, reconstructions of somata of identified callosally projecting pyramidal cells (callosal cells) and corticothalamic projecting pyramidal cells (corticothalamic cells) in the cat, area 17 (Figs 6 and 7), showed that within each population of pyramidal cells there was variability in the number and density of axosomatic synapses (Fariñas and DeFelipe, 1991a). However, callosal cells received a greater number and higher density of axosomatic synapses than corticothalamic cells (Tables 1 and 2). Within each class of pyramidal cell (callosal and corticothalamic cells) there was variability in the number of axosomatic synapses, but the synaptic density was quite homogeneous within each population ($19.6 \pm 3.9/100\ \mu m^2$ for callosal cells, and $10.6 \pm 3.7/100\ \mu m^2$ for corticothalamic cells; Fariñas and DeFelipe, 1991a). Therefore, the variability in the number of axosomatic synapses within each population seems to be better explained by differences in the size of the cell somata, whereas the differences in the density of synapses between the two populations seems to be better explained by differences in the arrangement of the intracortical circuits involved.

The total number of axosomatic synapses is estimated to be of an order of less than one hundred to a few hundred (see Tables 13 and 14). Colonnier and Rossignol (1969) estimated that the number of axosomatic synapses on pyramidal cells in layers II and III of the cat area 17 is of the order of fifty to one hundred. Davis and Sterling (1979) reported a synaptic density of approximately 11 synapses per 100 μm^2 on three pyramidal cells located at the border of layers III–IV of cat area 17 that were partially, but extensively, reconstructed. These authors estimated that the total number of synaptic inputs on these pyramidal cells is probably of an order of a few hundred. A similar density of synapses on somata of pyramidal neurons (13 synapses/100 μm^2) was obtained by Müller *et al.* (1984) for pyramidal cells located in area 17 of the rabbit visual cortex. An estimate of the total number of axosomatic synapses received by three layer III callosal cells and four layer VI corticothalamic cells in the cat area 17 (Fariñas and DeFelipe, 1991a) gave values of 162, 269 and 326 for the callosal cells (with surface areas of 1,010, 1,120 and 1,417 μm^2, respectively), and 33, 43, 60 and 120 for the corticothalamic cells (with surface areas of 657, 613, 600 and 858 μm^2, respectively) (Table 3).

The total number of axosomatic synapses in these cases are much lower than the 870 axon terminals estimated by Kaiserman-Abramof and Peters (1972) to synapse on the cell soma of the average Betz pyramidal cell (with a surface area of 6,700 μm^2) of

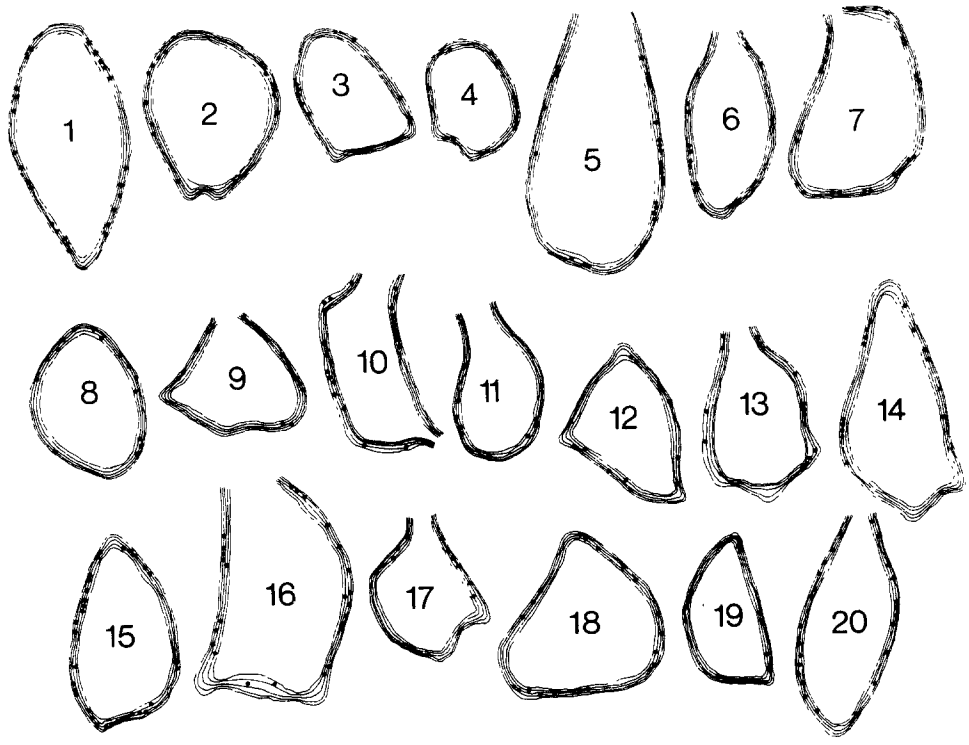

FIG. 6. Partial reconstructions of callosal cell bodies (numbered from 1 to 20) in area 17 of the cat. Dots represent synapses (data listed in Table 1). (From Fariñas and DeFelipe, 1991a.)

the cat motor cortex. However, these authors obtained this figure by calculating that there are approximately 13 axosomatic synapses per 100 μm^2 of somatic surface, which is a figure similar to the density of axosomatic synapses found by Müller *et al.* (1984) for pyramidal cells located in layers II–IV, and by Fariñas and DeFelipe (1991a) for (layer VI) corticothalamic cells, and by Davis and Sterling

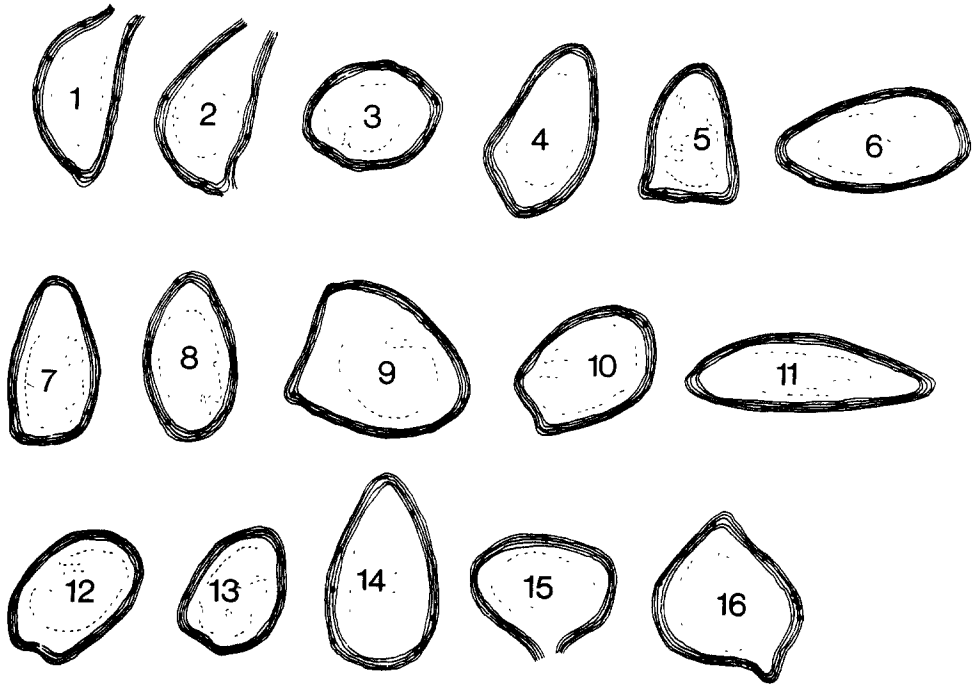

FIG. 7. Partial reconstructions of corticothalamic cell bodies (numbered from 1 to 16) in area 17 of the cat. Dots represent synapses (data listed in Table 2). (From Fariñas and DeFelipe, 1991a.)

TABLE 1. SYNAPSES ON CALLOSAL PYRAMIDAL CELL SOMATA ($n = 20$, SEE FIG. 6) LOCATED IN LAYER III OF THE CAT VISUAL CORTEX (AREA 17)

Cell body	Maximum diameter (μm)	Perimeter (μm)	Synapses*	Synaptic density†
1	28	70	25	2.1
2	19	48	17	2.0
3	18	43	12	1.6
4	14	36	15	2.4
5	30	75	28	2.1
6	21	55	15	1.5
7	22	65	26	2.3
8	19	55	15	1.6
9	15	44	14	1.8
10	21	55	23	2.4
11	17	47	13	1.6
12	21	44	12	1.6
13	20	47	15	1.8
14	25	65	17	1.5
15	23	60	33	3.1
16	27	73	25	2.0
17	16	39	14	2.1
18	21	63	19	1.7
19	18	47	15	1.8
20	27	64	25	2.2
Mean ± SEM	21.10 ± 0.96	54.75 ± 2.53	18.90 ± 1.34	1.96 ± 0.39

Reconstructions of each cell body through its maximum diameter were made for a thickness of approximately 1.75 μm and the number of synapses counted. (From Fariñas and DeFelipe, 1991a.)

* Counted through a depth of 1.75 μm.

† Number of synapses per 10 μm^2: (10 × synapses)/(1.75 × perimeter).

(1979) for layer V pyramidal cells, but lower than for (layer III) callosal cells (Fariñas and DeFelipe, 1991a). Therefore, it seems that the number of synapses not only depends on the total surface but also on a population specific synaptic density.

In conclusion, the available data suggest that there is variability in the number and density of synaptic inputs on pyramidal cell somata (between approximately 10 and 20 synapses per 100 μm^2), but each population of pyramidal cells seems to have a relatively characteristic number and density of axosomatic synapses. These differences might be of great functional importance given that most of the axosomatic synapses are likely to be inhibitory (see Section 2.4), and inhibitory synapses located on the soma are thought to be optimally positioned for inhibiting input arriving from all the cell dendrites (e.g. Jack *et al.*, 1975).

TABLE 2. SYNAPSES ON CORTICOTHALAMIC PYRAMIDAL CELL SOMATA ($n = 16$, SEE FIG. 7) LOCATED IN LAYER VI OF THE CAT VISUAL CORTEX (AREA 17)

Cell body	Maximum diameter (μm)	Perimeter (μm)	Synapses*	Synaptic density†
1	16	39	8	1.2
2	16	23	3	0.7
3	12	34	11	1.8
4	16	40	4	0.5
5	12	29	3	0.5
6	17	46	12	1.5
7	15	29	4	0.8
8	16	35	9	1.5
9	15	47	12	1.4
10	14	39	7	1.0
11	22	49	6	0.7
12	14	38	7	1.0
13	13	33	7	1.2
14	14	34	6	1.0
15	11	33	8	1.4
16	10	29	4	0.8
Mean ± SEM	14.56 ± 0.69	36.06 ± 1.74	6.94 ± 0.72	1.06 ± 0.37

Reconstructions of each cell body through its maximum diameter were made for a thickness of approximately 1.75 μm and the number of synapses counted. (From Fariñas and DeFelipe, 1991a.)

* Counted through a depth of 1.75 μm.

† Number of synapses per 10 μm^2: (10 × synapses)/(1.75 × perimeter).

Table 3. Estimation of the Total Somatic Innervation of Three Callosal Pyramidal Cells (Cells 7, 10 and 12 of Table 1) and Four Corticothalamic Pyramidal Cells (Cells 4, 9, 10 and 11 of Table 2)

Cell*	Surface (μm^2)	Synaptic density*	Total synapses
Callosal			
7	1417	2.3	326
10	1120	2.4	269
12	1010	1.6	162
Corticothalamic			
4	657	0.5	33
9	858	1.4	120
10	600	1 0	60
11	613	0.7	43

Synaptic density was assumed to remain constant through the entire cell soma surface. Total cell surface was approximately calculated by summing the products of cell perimeter and section thickness for each of the semithin sections of the complete series containing these cells. (From Fariñas and DeFelipe, 1991a.)

* See Tables 1 and 2.

2.3. Sources of Axosomatic Synapses

At present, it seems clear that the sources of axosomatic synapses on pyramidal cells are largely, if not exclusively, of intrinsic origin. This is because: (1) the axosomatic synapses on pyramidal cells are of the symmetric type, whereas the major cortical afferent fibers form asymmetric synapses; and (2) after undercutting the cortex, the reduction in the number of axosomatic synapses appears to be insignificant (Szentágothai, 1965; Gruner *et al.*, 1974). Therefore, the source of axosomatic synapses must originate in cortical neurons.

Traditionally, the large basket cell of Cajal (Figs 8 and 9) has been considered to be a major source of pericellular axonal plexuses on the somata and proximal dendrites of pyramidal cells (Marin-Padilla, 1969, 1974; Jones and Hendry, 1984; Fairén *et al.*, 1984). The synaptic connection between the large basket cells and pyramidal cells has been demonstrated by studying the axon terminals of horseradish peroxidase-intracellularly-filled large basket cells in the cat visual cortex (Somogyi *et al.*, 1983; Kisvárday *et al.*, 1987; see also Fairén and Smith-Fernández, 1992). Somogyi *et al.* examined a total of 177 synapses formed by the axons of two large basket cells in layer III (66 boutons from one basket cell and 89 from the other) and found that approximately 33% of the synapses formed were with the somata of pyramidal cells (22 axosomatic synapses from the first basket cell and 38 axosomatic synapses from the second); 20% and 24% of the synapses were with spines and with the apical and basal dendritic shafts, respectively, of pyramidal cells. Kisvárday *et al.* (1987) examined one horseradish peroxidase-filled basket cell whose soma was located in lower layer V and whose axon formed two main plexuses; a horizontal plexus in layer V and upper layer VI and another plexus in layer III. The total number of boutons was 3,773 and, in a random sample of 199 postsynaptic elements contacted in layers III, V, and VI, 20.1% were somata, 38.2% dendritic shafts and 41.2% dendritic spines. The majority of the postsynaptic elements were interpreted as belonging to pyramidal cells.

Several other cell types have also been found to form relatively large numbers of axosomatic synapses with pyramidal cells (see below). However, there is some terminological confusion since any cortical cell forming a relatively large number of axosomatic synapses is often called a "basket cell" (see DeFelipe *et al.*, 1986 for further discussion), which has led many authors to think that a single cell type ("the basket cell") is *the* major source of axosomatic synapses. Correlative light and electron microscopy studies, using a variety of techniques to analyze the synaptic connections of identified neurons, have shown that many types of aspiny nonpyramidal cells other than large basket cells contribute in a greater or lesser extent to the somatic and proximal dendritic innervation of pyramidal cells. These aspiny nonpyramidal cells include, among others, the so-called smooth and sparsely-spined stellate cells, clutch cells, small basket cells and neurons with axonal arcades (Peters and Fairén, 1978; Peters and Proskauer, 1980; DeFelipe and Fairén, 1982, 1988; Freund *et al.*, 1986; Kisvárday *et al.*, 1985, 1987). This implies that convergence of many types of neurons is probably involved in building up the pericellular plexuses on the somata and proximal dendrites of the pyramidal cells. In Section 5 we shall discuss whether or not different types of pyramidal cells are preferentially innervated by certain aspiny nonpyramidal cells.

2.4. Chemical Characteristics of Axosomatic Synapses

Immunocytochemical studies indicate that the vast majority of synapses on pyramidal cell somata (about 90–95%) are formed by GABAergic axon terminals (Ribak, 1978; Hendry *et al.*, 1983a; Freund *et al.*, 1983; Fariñas and DeFelipe, 1991a) (see Fig. 10 and Table 4). In addition, a number of other compounds have been immunocytochemically identified in boutons on the cell bodies of pyramidal cells. These include the peptides, VIP (Peters *et al.*, 1987), CCK (Hendry *et al.*, 1983b; Freund *et al.*, 1986), SRIF (de Lima and Morrison, 1989), tachykinins (Jones *et al.*, 1988; DeFelipe *et al.*, 1990) and the calcium binding protein, parvalbumin (Celio, 1986; Hendry *et al.*, 1989; DeFelipe *et al.*, 1989a; Blümcke *et al.*, 1991). All of these compounds, with the exception of VIP, have been found to be largely colocalized with GAD or GABA, but little colocalization has been found between peptides and parvalbumin (e.g. Hendry *et al.*, 1984a, 1989; Somogyi *et al.*, 1984; Celio, 1986; Kosaka *et al.*, 1987b; Jones *et al.*, 1988; Demeulemester *et al.*, 1989, 1991). In addition, not all pyramidal cells are surrounded by axon terminals immunoreactive for these substances and, when they are, the number of immunoreactive terminals is variable (see Section 5). Thus, it seems that different chemical subpopulations of GABAergic neurons innervate selectively and, with different degrees of affinity, certain pyramidal cell somata.

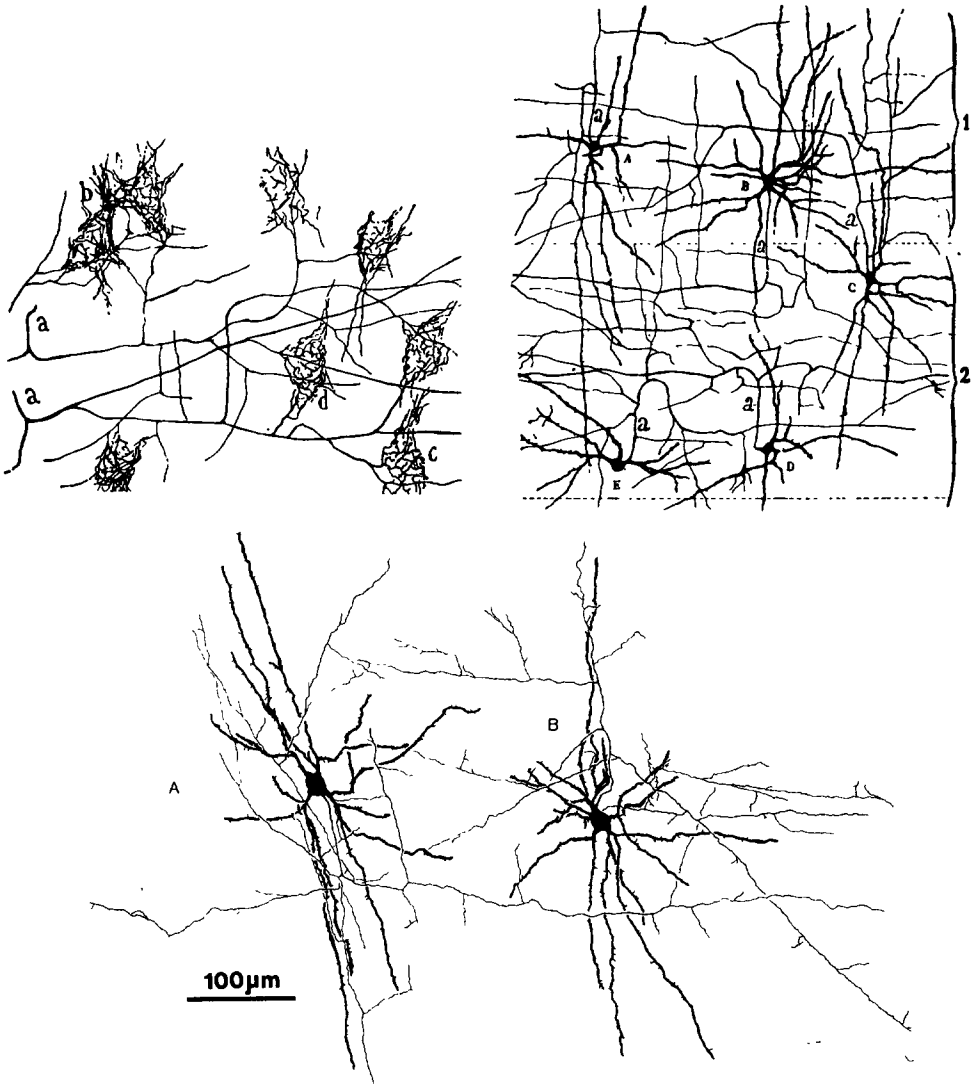

FIG. 8. Upper left and right, drawings from Cajal (1899c) of Golgi-impregnated axonal plexuses and cells of the human motor cortex. Upper left, "Pericellular arborizations of the layers of the external medium and giant pyramids of the motor cortex of a child of twenty-five days. a, axons dividing into long horizontal branches; b, c, d, pericellular baskets". Cajal pointed out that the large nonpyramidal cells that are illustrated in the upper right of the figure, which possess long dendrites and an axon giving rise to very long horizontal branches, *probably* represent the origin of the pericellular baskets. These cells are now called classical or large basket cells after the studies of Marin-Padilla (1969, 1974). See text for further details. Bottom, two large basket cells (notice the long horizontal axons) drawn from original preparations of Cajal. Cell A is from deep layer III of the visual cortex of a 27-day-old infant, and B from layer III of the motor cortex of a newborn. (From Fairén *et al.*, 1984.)

3. SYNAPSES ON THE AXON INITIAL SEGMENTS

3.1. MORPHOLOGY OF THE INITIAL SEGMENT SYNAPSES

The axon initial segment of pyramidal cells commonly receives synapses from axon terminals. Other types of axoaxonic synapses, such as those found in the spinal cord between an axon terminal of one neuron and an axon terminal of another neuron, have not been described in the cerebral cortex. We will use the term "initial segment synapses" to refer to the synapses on the axon initial segments. The axon initial segments of nonpyramidal cells receive none or, in some cases, very few (1 or 2) synapses. Only exceptionally do they receive more than three synapses. This is the case of the large basket cells, which have been found to receive as many as six or eight initial segment synapses (DeFelipe *et al.*, 1986). Therefore, the synaptic innervation of the axon initial segments appears to be a typical characteristic of pyramidal cells.

In all electron microscope studies on the synaptic innervation of the initial segments of pyramidal cells, the terminals have been found to be of the symmetric type. Most of the terminals are flattened against the initial segment, contain one or two mitochondria and pleomorphic synaptic vesicles. In serial thin section

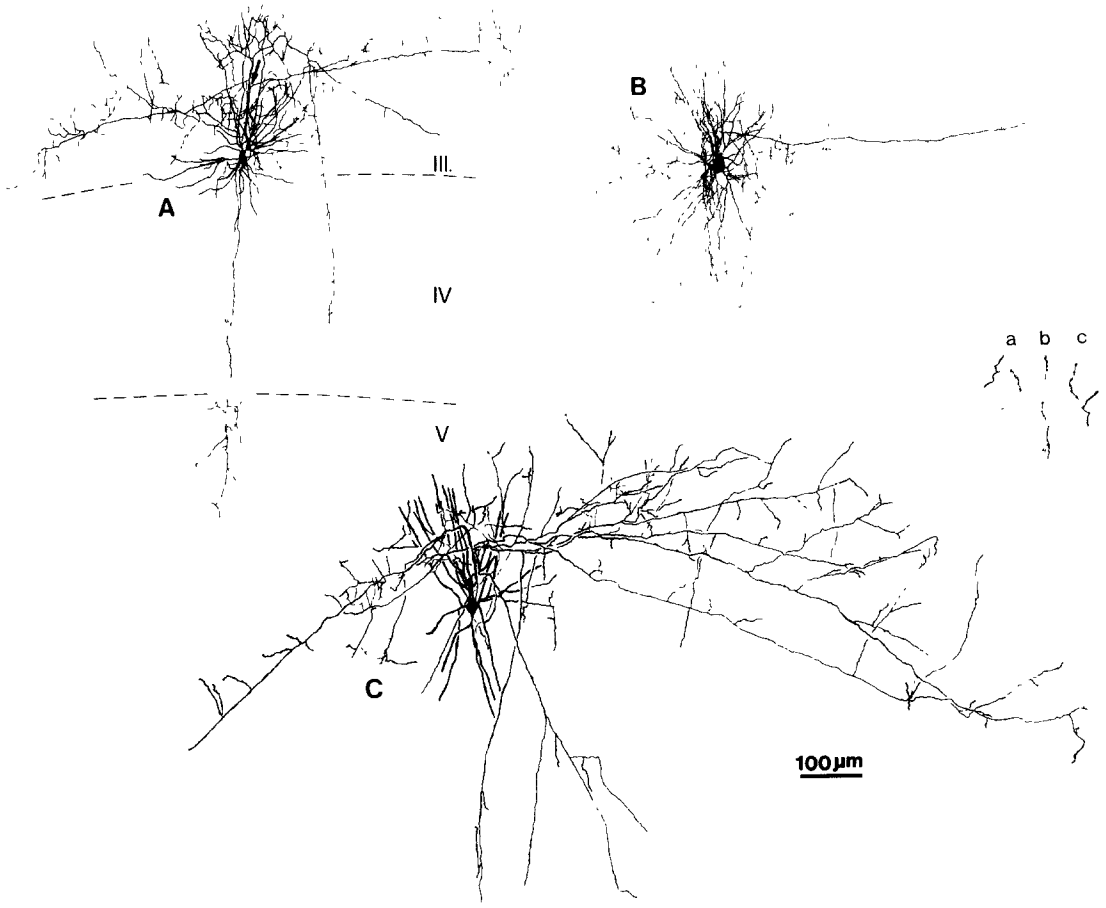

Fig. 9. Camera lucida drawings of large basket cells. (A) Basket cell labeled after intracellular injection of horseradish peroxidase (cat visual cortex; from Somogyi *et al.*, 1983). (B) Basket cell labeled after Golgi impregnation (cat visual cortex; from Fairén and Smith-Fernández, 1992). (C) Basket cell labeled after extracellular injection of horseradish peroxidase (monkey somatosensory cortex; from DeFelipe *et al.*, 1986). Inset (in the right), a, b and c are examples of terminal branches from neuron C, which are in apparent contact with lightly labeled neurons.

analyses, it is common to find the axoaxonic terminals joined together by cytoplasmic bridges with each axon terminal forming a single synaptic junction with the initial segment. Synapses with another adjacent neuronal element are only occasionally found. For example, of the 227 initial segment terminals examined in serial reconstructions, only six formed an additional synapse with an adjacent element (DeFelipe *et al.*, 1985). In the rat visual cortex, Peters and Harriman (1990) made a morphometric analysis of 25 chandelier cell axon terminals ending on pyramidal cell initial segments. They found that the diameters of the synaptic vesicles vary between 20 and 48 nm (mean 33.5 nm). The packing density of synaptic vesicles within individual terminals, varied from 109 to 299 vesicles per μm^2 (most had between 120 and 200 vesicles per μm^2), and the mean eccentricity of the synaptic vesicles varied between 1.21 and 1.44.

3.2. Number and Distribution of Initial Segment Synapses

The first estimation of the synaptic density and total number of synapses on pyramidal cell axon initial segments, using quantitative methods, was made by Sloper and Powell (1979a) in the monkey motor and somatic sensory cortices. These authors reported that the density of synapses on the axon initial segments of supragranular pyramidal cells was approximately three times larger than that on the initial segments of infragranular pyramids (mean synaptic densities in synapses/μm^2: supragranular 0.48; infragranular 0.15). These data were, in part, confirmed by DeFelipe *et al.* (1985) who made complete serial reconstructions from axons of pyramidal cells in layers II–III and V of monkey motor and somatic sensory cortices (Fig. 11). These authors found that the initial segments of layers II–III pyramids receive, on average, more synapses than those of layer V pyramids. In addition, for a given layer, the number of initial segment synapses was variable (from 2 to 52 for layers II and III and from 2 to 26 for layer V). A variability in the number of axoaxonic synapses was also found by Fairén and Valverde (1980) on the axon initial segments of pyramidal cells in layers II and III of the cat visual cortex. However, by examining the synaptology of the completely reconstructed axon initial segments of

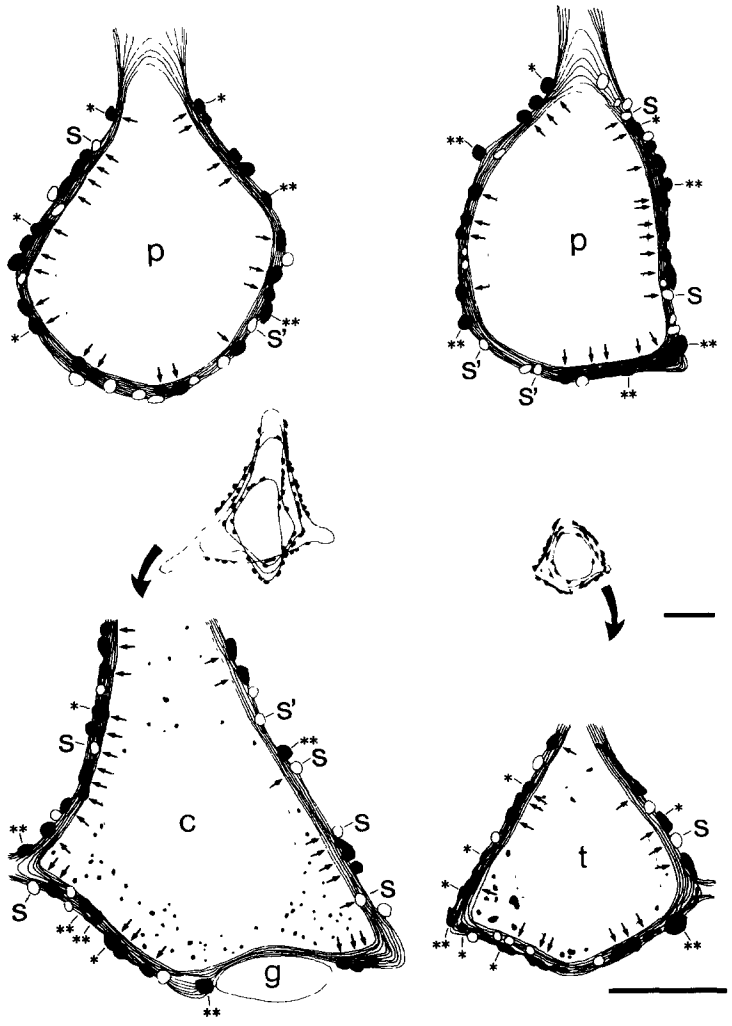

FIG. 10. Reconstructions of the cell bodies of two unidentified layer III pyramidal cells (p), a callosal cell (c) and a corticothalamic cell (t) in area 17 of the cat. The insets are reconstructions of the semithin (2 μm thick) sections of cells c and t, accounting for about half of each neuron. In both cases the semithin section through the cell maximum diameter was resectioned into thin sections. All GABA-positive boutons (black dots) and all nonimmunoreactive synaptic boutons (white dots) adjacent to the cell body were drawn. Each synapse on the cell body is marked by an arrow. Only a few were made by nonimmunoreactive axon terminals (S). Other nonimmunoreactive axon terminals (S′ and white dots without marks) established symmetric synapses (S′) or asymmetric synapses (white dots without marks) with elements distinct from the cell body. One asterisk indicates a GABA-positive terminal making synapse with an element of the neuropil and two asterisks indicate GABA-positive terminals that were not seen forming synapses through the series. g, neuroglial cell. Large bar = 6 μm; small bar = 10 μm (inset). (From Fariñas and DeFelipe, 1991a.)

three populations of retrogradely-horseradish peroxidase-labeled pyramidal cells (Figs 12 and 13) in the cat area 17 (11 layer III callosal cells, 8 layer VI corticothalamic cells and 7 layer III ipsilateral cortico-cortical cells projecting to area 19), it was found that each population of pyramidal cells examined received a characteristic and rather homogeneous number of initial segment synapses (Fariñas and DeFelipe

TABLE 4. RELATIONSHIP BETWEEN GABAERGIC PERISOMATIC AXON TERMINALS AND AXOSOMATIC SYNAPSES ON FOUR PYRAMIDAL CELLS OF CAT VISUAL CORTEX (AREA 17) ILLUSTRATED IN FIG. 10

Cell*	Maximum diameter (μm)	GABA terminals	GABA synapses	Total synapses	Synaptic GABA terminals	GABA synapses
pyramidal (p; left)	20	24	21	22	88%	96%
pyramidal (p; right)	18	26	20	22	77%	91%
callosal (c)	21	26	24	27	92%	89%
corticothalamic (d)	14	16	14	15	88%	93%

* See Fig. 10. (From Fariñas and DeFelipe, 1991a.)

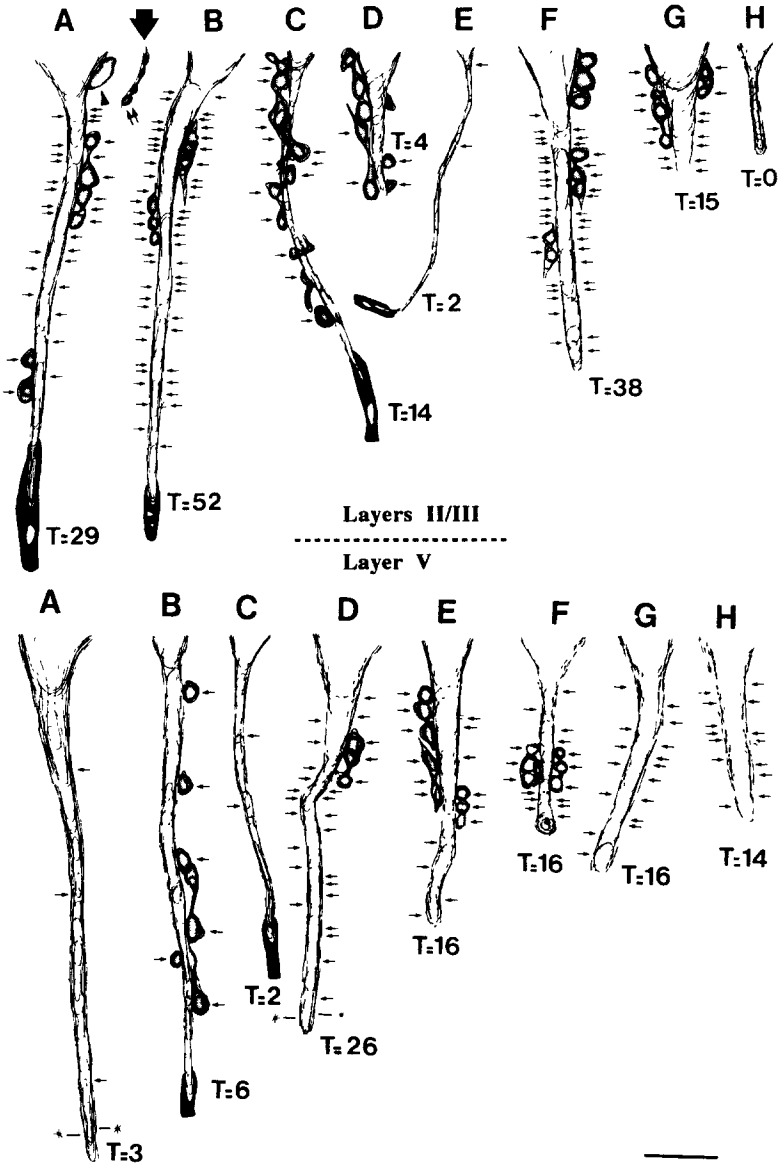

FIG. 11. Complete reconstructions of the axon hillocks and axon initial segments of pyramidal cells in the monkey sensory–motor cortex. Top, pyramidal cells located in layers II–III of area 3b (A, C, F, G) and area 4 (B, D, E, H). Bottom, pyramidal cells located in layer V of area 3b (A, F) and area 4 (B–E, G, H). The shaded boutons are GAD-positive boutons, except in the case of F and G in the top, in which immunocytochemistry was not done. These axon initial segments (F and G, top) are from callosally projecting pyramidal cells retrogradely labeled with horseradish peroxidase. Arrows indicate synaptic contacts only on the axon initial segment. The total number of synapses on the axon initial segment (GAD-positive and unlabeled) is indicated by T in each case. Arrowhead in A is to indicate a GAD-positive bouton (double arrow) that is part of a multiterminal pericellular basket (large arrow) contacting the soma of the pyramidal cell. Stars represent the loss of the dense undercoating. Note the lower density of synapses on the axon initial segments of layer V pyramidal cells in comparison with the axon initial segments in layers II–III. Bar = 5 μm. (From DeFelipe *et al.*, 1985.)

1991b): pyramidal cells projecting to the thalamus receive an extremely low number of synapses (from 1 to 5; mean $\pm$ SEM, 2.75 $\pm$ 0.52; see Table 5) on their axon initial segments, compared to callosal pyramidal cells (from 16 to 23; mean $\pm$ SEM 20.00 $\pm$ 0.61; see Table 6) and ipsilateral corticocortical pyramidal cells (from 22 to 28; mean $\pm$ SEM 24.43 $\pm$ 0.78; see Table 7).

The total number of synapses on the axon initial segments of layer III pyramidal cells (callosal and ipsilateral corticocortical cells) of the cat visual cortex found by Fariñas and DeFelipe (1991b) is similar to the value of 24 synapses obtained by Fairén and Valverde (1980) for the completely reconstructed axon initial segment of a layer III pyramidal cell in the cat visual cortex. These values are smaller than those of 44, 42 and 44 obtained by Somogyi *et al.* (1982) for the total number of synapses on the axon initial segments of three layer III pyramidal cells of

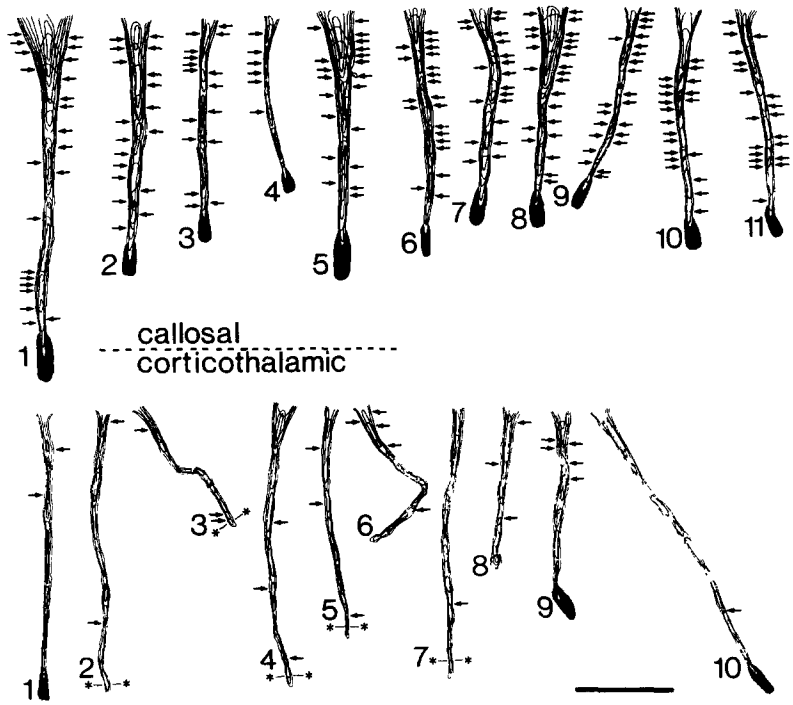

FIG. 12. Complete reconstructions of axon initial segments of callosal (top) and corticothalamic (bottom) cells in area 17 of the cat. Numbers indicate the parent cell body to which they belong (see Figs 6 and 7). Each synapse is marked by an arrow. Asterisks represent the loss of the dense axolemmal undercoating. Note the low density of synapses on the axon initial segments of corticothalamic cells (see Tables 5 and 6). Bar = 10 μm. (From Fariñas and DeFelipe, 1991b.)

the cat visual cortex. In addition, Fariñas and DeFelipe (1991b) found a callosal neuron in layer III (cell number 4 in Fig. 12) that received only six axon initial segment synapses. Therefore, there is a laminar variability in the number of synapses on the axon initial segments of pyramidal cells in all species and cortical areas studied, but this variability is, in general, insignificant among pyramidal cells projecting to the same target. On average, the axon initial segments of pyramidal cells located in layers II–III receive more synapses than those located in layers V and VI. This implies that differences in the arrangement of intracortical circuits exist between supragranular and infragranular layers.

With regard to the distribution of synapses, Sloper and Powell (1979a) reported that the initial segment synapses are evenly distributed along the length of the axon initial segments, whereas Jones and Powell (1969a) and Freund *et al.* (1983) reported an increase in the number of synapses distally. However, when the axon initial segments are completely reconstructed it is observed that the number of these synapses is more numerous in the proximal third of the axon initial segments (Table 8) and decrease distally (DeFelipe *et al.*, 1985; Fariñas and DeFelipe, 1991b; see also Fairén and Valverde, 1980).

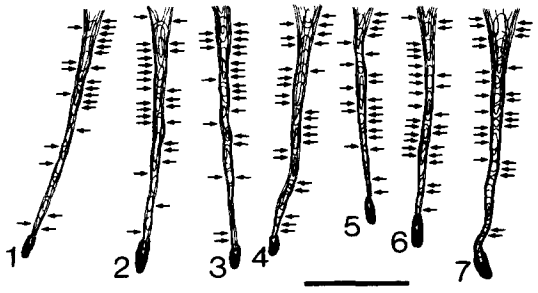

FIG. 13. Complete reconstructions of axon initial segments of ipsilateral corticocortical cells in area 17 of the cat projecting to area 19. Each synapse is marked by an arrow (see Table 7). Bar = 10 μm. (From Fariñas and DeFelipe, 1991b.)

TABLE 5. INNERVATION OF CORTICOTHALAMIC PYRAMIDAL CELL AXON INITIAL SEGMENTS (AIS)

AIS number	Diameter (μm)	Length (μm)	Synapses
1	0.8	26	2
2	0.6	29*	2
3	0.7	17*	3
4	0.7	29*	3
5	0.5	22	3(p)
6	0.8	19	5
7	0.7	28*	1
8	0.8	16	3(p)
9	0.9	19	5
10	0.7	31	1
Mean $\pm$ SEM	0.74 $\pm$ 0.03	24.75 $\pm$ 1.83	2.75 $\pm$ 0.52

Total number of axoaxonic synapses were counted in complete reconstructed AIS of ten pyramidal cells (see Fig. 12) except for the two marked with (p) that were partially reconstructed for the length indicated. The latter two AIS were not considered in any calculation or statistical test. (From Fariñas and DeFelipe, 1991b.)

* Does not enter myelin sheath.

Table 6. Innervation of Callosal Pyramidal Cell Axon Initial Segments (AIS)

AIS number	Diameter (μm)	Length (μm)	Synapses
1	1.2	33	22
2	1 4	23	18
3	0.9	19	16
4*	0.5	16	6
5	1.2	22	20
6	1.0	22	20
7	1.2	18	19
8	1.1	19	23
9	0.8	19	22
10	0.9	21	20
11	0.9	20	20
Mean $\pm$ SEM	1.06 ± 0.06	21.60 ± 1.30	20.00 ± 0.61

Total number of axoaxonic synapses were counted in complete reconstructed AIS of eleven pyramidal cells (see Fig. 12). (From Fariñas and DeFelipe, 1991b.)

* Not considered in any calculation or statistical test.

Table 7. Innervation of the Axon Initial Segments (AIS) of Ipsilateral Corticocortical Cells

AIS number	Diameter (μm)	Length (μm)	Synapses
1	0.9	23	22
2	1.1	23	25
3	1.0	23	23
4	1 1	23	25
5	1.0	19	22
6	1.0	21	28
7	1.3	24	26
Mean $\pm$ SEM	1.06 ± 0.05	22.24 ± 0.60	24.43 ± 0.78

Total number of axoaxonic synapses were counted in complete reconstructed AIS of seven pyramidal cells (see Fig. 13). (From Fariñas and DeFelipe, 1991b.)

3.3. Sources of Initial Segment Synapses

Although the first description of axoaxonic synapses in the cerebral cortex was given by Westrum (1966) in the prepyriform cortex of the rat, this author did not determine whether the postsynaptic axons were axon initial segments or preterminal axons. The first clear demonstrations that the axon initial segments of pyramidal cells receive synapses from axon terminals were published in 1968 by Palay *et al.* (1968) and Peters *et al.* (1968). Jones and Powell (1969a) suggested that the source of these synapses should be cells that are intrinsic to the cortex, because: (1) The different morphology of the synaptic junctions of the main cortical afferent fibers (asymmetric type, see Section 1.2) and; (2) the lack of degenerating endings on the axon initial segments found after the experimental interruption of the main cortical afferent fibers or after undercutting the cortex. However, the type(s) of interneuron forming

Table 8. All Axon Initial Segments (AIS) Included in Tables 5, 6 and 7 Were Divided into Three Equal Parts and Synapses Counted From Each Division. Significant Differences in the Number of Synapses Among the Three Divisions were Proved Using a One-Way Analysis of Variance Within Each Population and for all AIS

Cell population	AIS number	AIS length	Number of synapses proximal third	Number of synapses middle third	Number of synapses distal third
Callosal	1	33	11	4	7
	2	23	7	5	6
	3	19	9	4	3
	4	16	4	2	0
	5	22	13	4	3
	6	22	9	8	3
	7	18	10	7	2
	8	19	12	7	4
	9	19	9	6	7
	10	21	2	12	6
	11	20	6	5	9
			8.36 ± 3.35	5.82 ± 2.68	4.55 ± 2.66
Ipsilateral	1	23	16	3	3
	2	23	12	10	3
	3	23	13	6	4
	4	23	11	10	5
	5	19	7	8	7
	6	21	11	10	7
	7	24	10	14	2
			11.43 ± 2.76	8.71 ± 3.50	4.43 ± 1.99
Corticothalamic	1	26	1	1	0
	2	29	1	0	1
	3	17	1	0	2
	4	29	0	2	1
	5	22	1	1	0
	7	28	0	0	1
	9	19	4	1	0
	10	31	0	0	1
			1.00 ± 1.31	0.63 ± 0.74	0.75 ± 0.71

initial segment synapses was not identified until the Golgi-electron microscopic study of Somogyi (1977), who described an "axoaxonal" interneuron in the rat visual cortex that formed synapses specifically with the axon initial segments of pyramidal cells. This type of interneuron is now generally referred to as the chandelier cell, which was the name given by Szentágothai and Arbib in 1974 (see also Szentágothai, 1975) to a type of interneuron whose terminal portions of its axon form short vertical rows of boutons resembling candlesticks. At that time, it was argued (Szentágothai and Arbib, 1974; Szentágothai, 1975) that the chandelier cells were likely to form multiple symmetrical synapses with the apical dendrites of pyramidal cells. The presumed connections of the chandelier cells with the apical dendrites of pyramidal cells, together with the fact that Somogyi (1977) found his axoaxonal interneuron morphologically more similar to Jones' type 4 interneuron (whose axon also forms short vertical rows of boutons; Jones, 1975) than to the chandelier cell of Szentágothai, were perhaps the main causes why Somogyi (1977) did not identify his axoaxonal interneuron as a chandelier cell. At present, most authors agree that the chandelier cells of Szentágothai, the axoaxonal interneurons of Somogyi and the type 4 of Jones are all the same type of interneuron whose most distinctive feature is the short vertical rows of boutons formed by the terminal portions of its axon (Fig. 14) (e.g. Fairén and Valverde, 1980; Somogyi *et al.*, 1982; Peters *et al.*, 1982; DeFelipe *et al.*, 1985). These rows of boutons vary in their complexity; within a single chandelier cell's axon terminations there are single rows of 5 or 10 boutons, while others are so complex that they form tight braid-like structures made up of more than 20 boutons; see Fairén and Valverde, 1980 and DeFelipe *et al.*, 1985).

Chandelier cells represent a unique example of synaptic specificity in the cerebral cortex, since, unlike other types of cortical neurons which form synapses with a variety of postsynaptic elements, the chandelier axon terminals form synapses only with the axon initial segments (Fig. 14) of pyramidal cells (Somogyi, 1977, 1979; Fairén and Valverde, 1980; Somogyi *et al.*, 1982; Peters *et al.*, 1982; Freund *et al.*, 1983; DeFelipe *et al.*, 1985). Chandelier cells are thought to be the major source of synapses on pyramidal cell axon initial segments for two main reasons: (1) Each single chandelier cell axon can give off several hundred short vertical rows of boutons (Fig. 15) (e.g. Freund *et al.*, 1983; DeFelipe *et al.*, 1985) and the number of initial segment synapses formed by each row of boutons can be similar to the total number of synapses found on the axon initial segments of pyramidal cells (up to 52; see Section

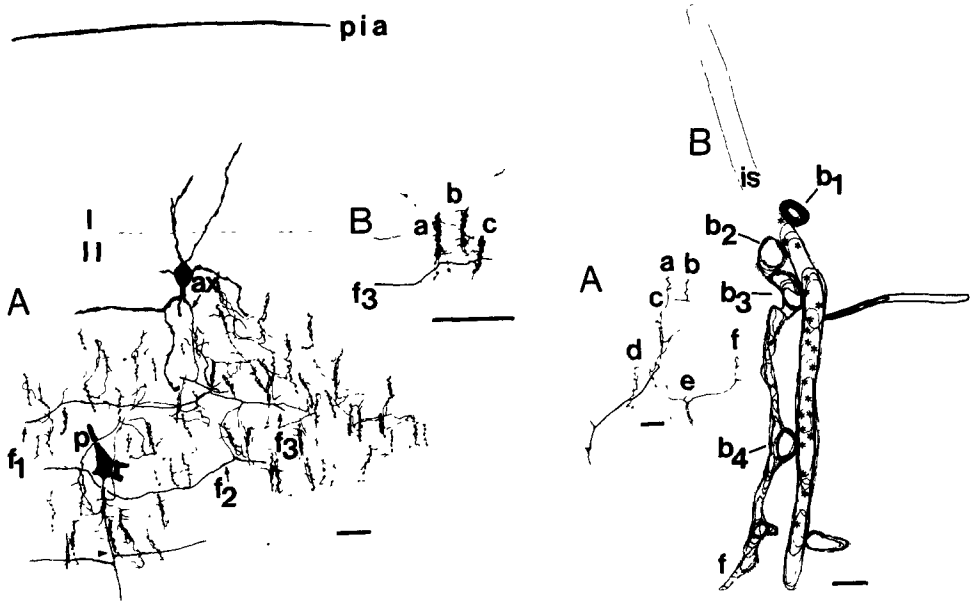

FIG. 14. **A** (left) Camera lucida drawing of a Golgi-impregnated chandelier cell in layer II of the motor cortex of the monkey. Only those parts of the axonal plexus within a plane of approximately 50 μm thick are drawn. The axon (ax) originates from a dendrite. Note the horizontally and obliquely oriented major collaterals (f_1, f_2, f_3). Inside the axonal field, the axon initial segment of a Golgi-impregnated pyramidal cell (p) is contacted by a complex termination before giving off the first collateral (arrowhead). Bar = 20 μm. **B** (left) Camera lucida drawing of part of the collateral f_3 and a number of other collaterals in a deeper focal plane, illustrating three complex terminations (a, b, c) arising from different preterminal branches of the same chandelier axon and forming a typical cluster. Bar = 10 μm. **A** (right) Camera lucida drawing from a Golgi-rapid preparation showing the part of a chandelier cell axonal plexus contained within a slab approximately 6 μm thick. Six terminal rows of boutons are shown (a–f). After gold toning and resectioning at 2 μm, these six terminations were found to be located beneath pyramidal somata. Bar = 20 μm. **B** (right) Reconstruction from serial electron micrographs obtained by resectioning one of the 2 μm sections, showing part of the axon initial segment (is) of one of the pyramidal cells and the contacts made by the termination f (boutons 1–4) in A. Stars represent synaptic contacts. Bar = 1 μm. (From DeFelipe *et al.*, 1985.)

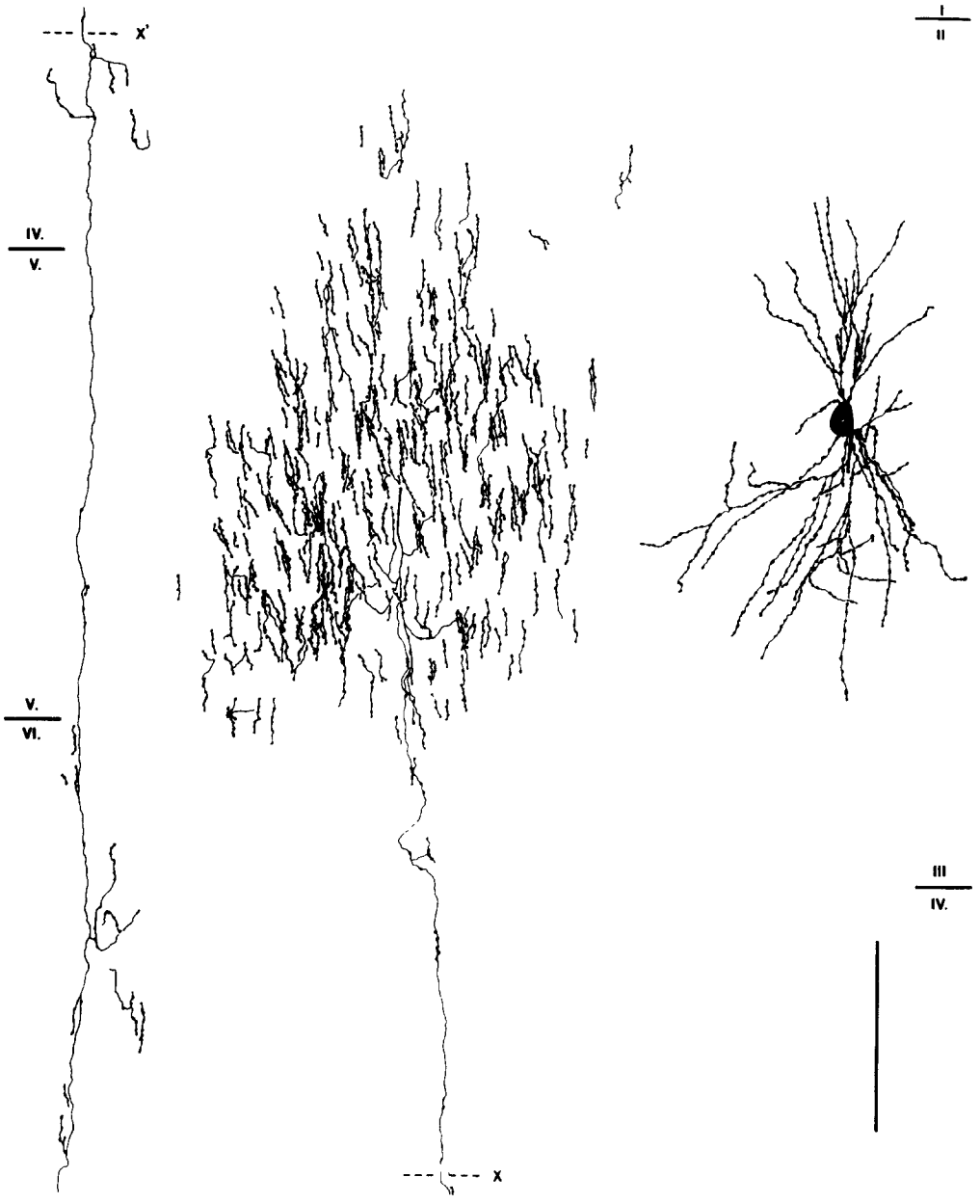

FIG. 15. Camera lucida drawing of an axoaxonic (chandelier) cell in the cat's visual cortex that had been filled with horseradish peroxidase by iontophoresis. The main axon (drawn separately, but at the same level in the cortex) passes through all layers below layer II; it gives rise to 330 specialized terminal segments through its axon collaterals in layers II and III, and to occasional segments in layer IV and 10 in layers V–VI. The broken lines marked with X and X′ indicate the common part of the axon, which had to be drawn in two separate parts. Bar = 100 μm. (From Freund *et al.*, 1983.)

3.2). In other words, some axon initial segments of pyramidal cells may be innervated by only one row of boutons from a single chandelier cell. In other cases, convergence of two or three rows of boutons originating from one, two or three chandelier cells would be sufficient to account for the total number of synapses on the axon initial segment of a single pyramidal cell. (2) It is true that there are other types of interneurons that form occasional synapses with the axon initial segments of pyramidal cells (Peters and Fairén, 1978; Peters and Proskauer, 1980; Somogyi *et al.*, 1983; Kisvárday *et al.*, 1985, 1987; DeFelipe and Fairén, 1988), but as far as we know, there is no example of a cortical neuron whose terminal axonal arborization forms short vertical rows of boutons as conspicuously as those of the chandelier cells. Since the axon initial segments of pyramidal cells are short (approximately 15–40 μm in length) vertically oriented structures, a cell that would form multiple synapses with axon initial segments will necessarily have an axon with short vertical rows of terminal boutons. Therefore, the fact

that the axon of a given neuron lacks these vertical rows of boutons could be taken as indicative that the cell does not form multiple initial segment synapses. In conclusion, chandelier cells are by far the major source of synapses on pyramidal cell axon initial segment.

3.4. CHEMICAL CHARACTERISTICS OF INITIAL SEGMENT SYNAPSES

Since chandelier cells are the major source of synapses on pyramidal cell axon initial segments, data on the chemical nature of chandelier cell axons can be extrapolated to the initial segment synapses in general. It has been shown by combining Golgi-impregnation and immunocytochemistry for GAD or GABA, or by correlative light and electron microscopy, in a variety of cortical areas and species, that chandelier cells are very likely to be GABAergic (Peters *et al.*, 1982; Freund *et al.*, 1983; Somogyi *et al.*, 1985; DeFelipe *et al.*, 1985). So far, with probably the only exception being the neuropeptide, corticotropin-releasing factor (Lewis *et al.*, 1989; Lewis and Lund, 1990), all immunocytochemical studies have failed to show the presence of any neuropeptide in chandelier cell axons. The neuropeptides tested include: VIP (Peters *et al.*, 1987), CCK (Hendry *et al.*, 1983b; Freund *et al.*, 1986), SRIF (de Lima and Morrison, 1989), and tachykinins (Jones *et al.*, 1988). Furthermore, it has been shown that in certain areas and layers of the monkey cerebral cortex, the terminal portions of the chandelier cell axons (vertical rows of boutons) are immunocytochemically stained for the calcium-binding protein parvalbumin (DeFelipe *et al.*, 1989a; Lewis and Lund, 1990; Hendrickson *et al.*, 1991); in the monkey, cat and rat cerebral cortex, parvalbumin immunoreactive cells have been found to be immunoreactive also for GABA (Celio, 1986; Hendry *et al.*, 1989; Demeulemeester *et al.*, 1991). In addition, in the rat and cat cerebral cortex (Kosaka *et al.*, 1987b; Demeulemeester *et al.*, 1991), parvalbumin shows little or no colocalization with the neuropeptides CCK, SRIF and neuropeptide Y. Nor do chandelier cell axons seem to contain calbindin D-28K, another calcium-binding protein (Hendry *et al.*, 1989; DeFelipe *et al.*, 1989b).

Therefore, chandelier cells axons and, thus, axon terminals forming axoaxonic synapses with the axon initial segments of pyramidal cells, could be chemically defined as axons that are very likely to use GABA as their conventional neurotransmitter, to contain parvalbumin, but not calbindin, and to contain the neuropeptide corticotropin-releasing factor, but not the neuropeptides CCK, SRIF, neuropeptide Y, VIP and tachykinins. However, not all chandelier cell axons are immunoreactive for parvalbumin or corticotropin-releasing factor, only a subpopulation of such axons (Lewis *et al.*, 1989; Lewis and Lund, 1990; see also DeFelipe and Jones, 1991). In motor, somatosensory, prefrontal and occipital association cortices of macaque and squirrel monkeys, numerous chandelier cell axons are stained for parvalbumin, mainly in layers II and III, whereas few chandelier axon terminals are immunoreactive in the primary visual cortex. On the other hand, chandelier cells immunoreactive for corticotropin-releasing factor have been found mainly in layer IV of the prefrontal cortex of the squirrel monkey. In area 18, relatively few chandelier cell axons (located in the superficial layers) are immunoreactive for corticotropin-releasing factor , and in area 17 no chandelier cell axons were found to be immunoreactive. In macaque monkeys no chandelier cell axons immunoreactive for corticotropin-releasing factor have been observed (see Lewis and Lund, 1990). These data suggest a chemical heterogeneity of chandelier cell axons (see Fig. 29) and, thus, of initial segment synapses. Nevertheless, it is important to point out that in all areas and species studied so far, GABA is apparently present in all initial segment synapses, while the more abundant cortical neuropeptides are absent.

4. SYNAPSES ON THE DENDRITIC SHAFTS AND DENDRITIC SPINES

4.1. MORPHOLOGY OF THE AXODENDRITIC AND AXOSPINOUS SYNAPSES

The dendrites constitute, undoubtedly, the largest receptive surface of cortical neurons. Sholl (1955b) estimated that dendrites formed approximately 90–95% of the receptive surface of the neurons in the cat sensorimotor and visual cortices. Similar values were obtained by Mungai (1967) in the cat somatic sensory cortex. He found (for ten pyramidal cells examined) that the cell body surface accounted for approximately 2–4% of the total neuronal surface (values ranging from 616 to 1,135 μm^2), dendritic shafts for 61–71% (from 12,636 to 24,980 μm^2) and dendritic spines for 25–43% (from 4,887 to 12,414 μm^2). The total surface area of the neurons examined ranged from 20,518 to 35,797 μm^2. Thus, cell bodies represent a minor amount of the total surface, while that corresponding to dendrites (shafts and spines) accounts for the largest surface area (96–98%). However, only a strikingly small portion of the neuronal surface area is occupied by synaptic junctions. For example, Müller *et al.* (1984) estimated that approximately 2% of the total surface of the pyramidal cells (mean $\pm$ SEM; 1.9 ± 0.3 for apical dendrites; 1.8 ± 0.3 for somata and, 2.6 ± 0.4 for basal dendrites) of the rabbit visual cortex is covered with synaptic junctions.

The dendritic shafts and spines of pyramidal cells represent the only postsynaptic sites that receive axon terminals forming asymmetric (excitatory) synapses. The detailed and extensive studies of White and colleagues on the synaptic inputs on dendrites of pyramidal cells (White and Hersch, 1981, 1982; Hersch and White, 1981a, 1982; Porter and White, 1986) have shown that the majority of the synapses on the apical dendrites (and other dendritic regions) are found on the dendritic spines, and that the vast majority of these axospinous synapses are asymmetric. Therefore, the dendrites and spines appear to be the exclusive synaptic sites for the synaptic relationship to be established between pyramidal cells and the major cortical afferent systems, and between pyramidal cells and the axons of spiny nonpyramidal and other pyramidal cells (all of which form asymmetric

synapses, see Section 1.2). Numerous axon terminals forming symmetric synapses are found on the dendrites, especially on the dendritic shafts. White and colleagues showed that synapses on the dendritic shafts were fewer in number than those on spines and (with the exception of some callosal projection neurons) were mainly of the symmetric type.

When the dendritic spines were first described by Cajal in 1890 (Cajal, 1890), a period of time followed in which it was discussed whether they were real anatomical dispositions or simply artifacts (see Cajal, 1896 for an interesting discussion on the subject; Fig. 16). In recent years, a large number of theories about the significance and possible functional role of dendritic spines have been proposed (e.g. Chang, 1952; Diamond *et al.*, 1970; Swindale, 1981; Gray, 1982; Crick, 1982; Fifková and Delay, 1982; Shepherd and Greer, 1988; Segev and Rall, 1988; Rall and Segev, 1988). Most authors have given great functional importance to the spines, although their precise significance is not entirely clear. It is unlikely that they are devices to increase the amount of receptive surface, as originally proposed by Cajal, since most of the cell surface is devoid of synapses (see above). Gray (1959) was the first to demonstrate with the electron microscope that spines are postsynaptic elements of axon terminals forming synapses. This study was followed by others confirming the findings of Gray (e.g. Colonnier, 1968; Jones and Powell, 1969b; Peters and Kaiserman-Abramof, 1969, 1970). Dendritic spines usually consist of two parts, a stalk protruding from the dendritic surface and a small end-bulb (e.g. Peters *et al.*, 1991). A range of morphological varieties of spines (Figs 17 and 18) are detected along the length of the same dendrite which might be related to the reception of particular inputs (Jones and Powell, 1969b; Peters and Kaiserman-Abramof, 1969). Moreover, Jones and Powell (1969b) reported that shape complexity and size are inversely related to the diameter of the parent dendrite (Fig. 18).

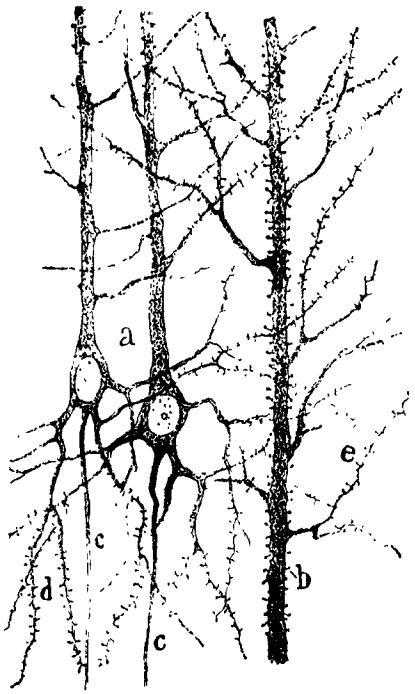

FIG. 16. Drawing from Cajal (1891) of pyramidal cells of the cerebrum of an adult common rabbit, stained with methylene blue (method of Ehrlich modified by Cajal). Notice that all dendritic surfaces are covered by spines (b, d, e), except the initial segments arising directly from the cell soma, which are spine-free. In the time when dendritic spines were discovered by Cajal (see text) with the method of Golgi, the spines were only visualized with this method. Some authors (including Golgi himself) interpreted the spines as artifacts produced by the Golgi method; " ... a superficial precipitate, like a crystallization of needles, fortuitously deposited on the dendritic surface" (see Chapter 9 in DeFelipe and Jones, 1988b). Therefore, Cajal used a different method of staining to demonstrate that the dendritic spines were real morphological dispositions, that could be observed with different methods.

It seems that all spines receive at least one asymmetric synapse, usually on the expanded part (e.g. Peters and Kaiserman-Abramof, 1969, 1970; White and Hersch, 1981, 1982). The postsynaptic density is usually more conspicuous than that found at asymmetric synapses occurring on dendritic shafts of the pyramidal and nonpyramidal cells, or cell bodies of aspiny nonpyramidal cells. Peters and Kaiserman-Abramof (1969) observed that when the axospinous synapses are examined in *en face* plane section, the postsynaptic density in the smallet dendritic spines has the form of a disc, and in larger spines the disc displays one or several perforations (Fig. 19). As seen in Fig. 19, the synaptic junctions occupy most of the apposition between the spine and the axon terminal. In addition, Peters (1987a) pointed out that the profile of the head of the spine and of the axon terminal are frequently similar in size and, thus, small spines would receive small axon terminals, whereas large spines would receive large terminals. It is not known whether this feature of the axospinous synapses has any functional significance.

4.2 Number and Distribution of Axodendritic and Axospinous Synapses

It seems clear that the vast majority of the synapses a pyramidal cell receives are on the dendritic spines because the cell body surface represents a minor part of the total neuronal surface (see previous section) and has few axosomatic synapses (see Section 2.2); relatively few synapses are found on the dendritic shafts (see below) while there are thousands of spines and each spine receives at least one synapse. In the cat visual cortex (area 17) it has been estimated that 84% of the total synapses are of the asymmetric type, with the remaining 16% of the symmetric type; only 7% of all symmetric synapses are found on cell somata (Beaulieu and Colonnier, 1985). Since pyramidal cells have only symmetric synapses on their somata, it might then be said that the majority of the symmetric synapses (at least 93%) and all the asymmetric synapses on pyramidal cells are on the dendrites. From the studies of White and colleagues, it is found that approximately 70–95% of dendritic synapses on pyramidal cells are axospinous, while 5–30% occur on the shafts (Hersch and White, 1981a, 1982; White and Hersch, 1981, 1982; see also Feldman, 1984).

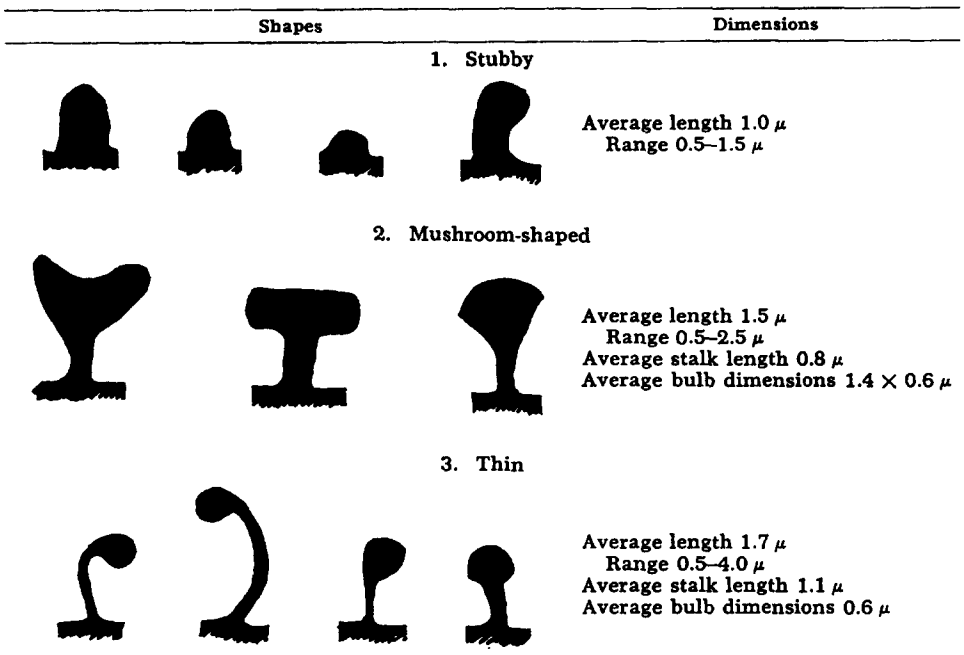

FIG. 17. Shapes and lengths of dendritic spines. (From Peters and Kaiserman-Abramof, 1970.)

It is a traditional belief that the majority of dendritic spines establish a synaptic junction (asymmetric) with only one axon terminal and that only occasionally the spines may synapse with two separate axon terminals (e.g. Jones and Powell, 1969b estimated that 10–20% of the spines receive a second terminal; see also Feldman, 1984), and that only infrequently does this second axon terminal make a symmetric synapse (e.g. Colonnier, 1981; Feldman, 1984; but see Beaulieu and Colonnier (1985), who estimated that 31% of the symmetric synapses are on spines). It is true that symmetric synapses on spines appear to be rather scarce (Table 9), as shown by those studies in which relatively large numbers of axospinous synapses were analyzed (e.g. White and Hersch, 1981, 1982). However, the dendritic spines are an important target of the basket cell axons and, especially, double bouquet cell axons, at least in some species, and both types of interneurons form symmetric synapses (see Section 4.3.1.3). As we will see, the dendritic spines postsynaptic to double bouquet cells appear to be mainly those originating from basal dendrites and oblique branches of the apical dendrite rather than from the apical trunk. Thus, it is possible that the spines receiving a symmetric synapse are located mainly in these dendritic regions rather than on the apical dendrite. Furthermore, in these studies it was observed that many of the spines postsynaptic to a symmetric synapse receive, in addition, an asymmetric synapse. However, it is not known how many spines overall possess such a dual innervation. In summary, the following alternatives are possible: Some spines (the majority) receive only one axon terminal that forms an asymmetric synapse; others receive two terminals, with both forming asymmetric synapses, or one forming an asymmetric synapse and the other a symmetric synapse; and still others might receive only a symmetric synapse (not proved).

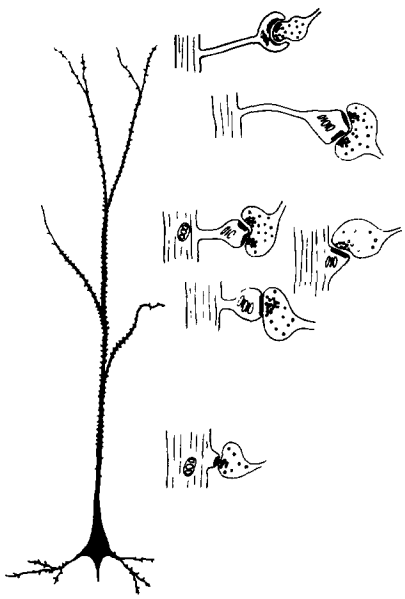

FIG. 18. Schematic illustration showing the main variations in the morphology of the dendritic spines with regard to their location in different parts of the pyramidal cell. Notice the long pedicles of the spines on the small terminal dendritic branches (top). (From Jones and Powell, 1969b.)

Spine density is variable within and between different pyramidal cell types and between different dendritic regions (Fig. 3) of a given pyramidal cell (e.g. see Feldman, 1984). In a recent study made by Larkman (1991), the total numbers and the distribution of spines were estimated on the dendrites of individual pyramidal cells ($n = 39$) which were

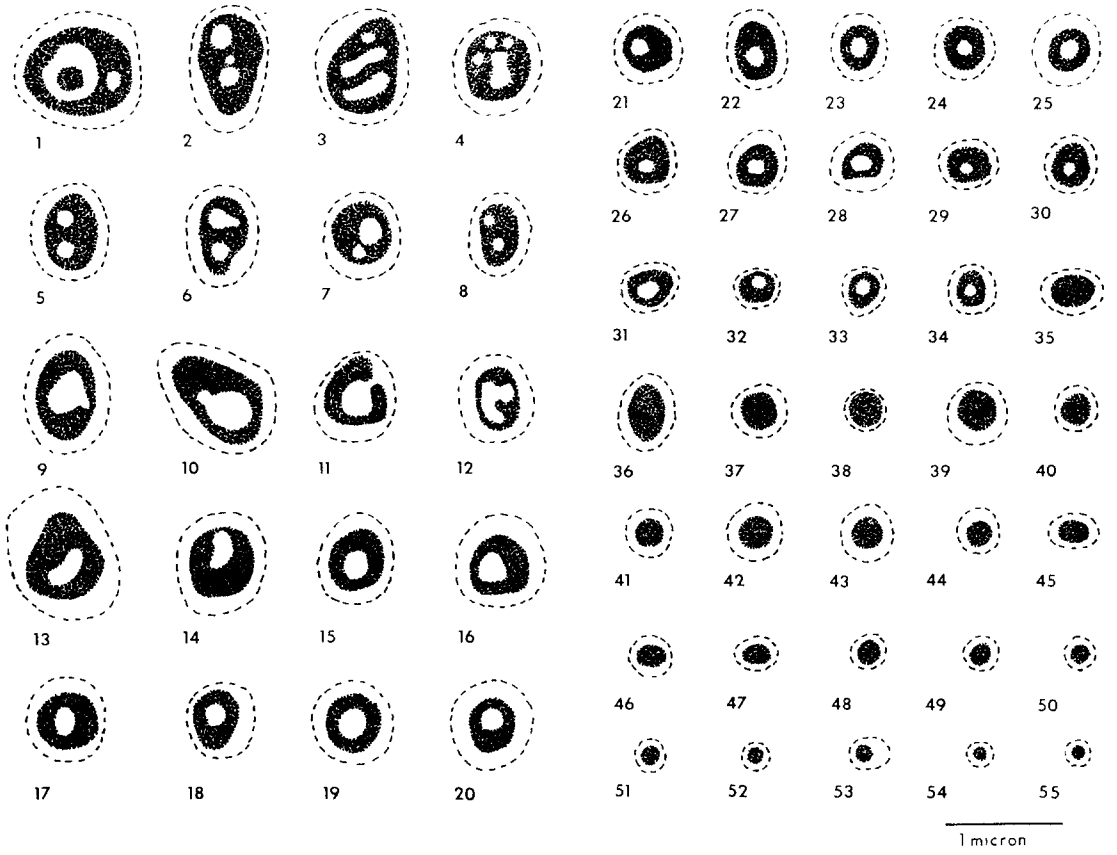

FIG. 19. Reconstructions from serial sections of the patterns formed by 55 different postsynaptic densities. The stippled areas represent the densities and the broken lines the extent of the apposition between the axon terminals and the dendritic spines. (From Peters and Kaiserman-Abramof, 1969.)

injected intracellularly with horseradish peroxidase in layers II/III and layer V of the rat visual cortex (Figs 20 and 21). In layer V two classes of pyramidal cells were distinguished; thick cells characterized by a thick apical dendrite that terminated by forming a terminal arbor in layer I, and slender cells that were characterized by a thin apical trunk that terminated before reaching layer I and without forming a terminal arbor. The mean total numbers of spines per cell were 7,965 for layer II/III cells, 8,647 for slender layer V cells and 14,932 for thick layer V cells (Table 10). As seen in Table 10, there are between 1 and 2 spines per 1 μm of dendritic length for the three classes of pyramidal cells examined. The highest density of spines is found on the apical trunks (spines/μm: 0.71 to 5.91 for layer II/III cells; 0.37 to 4.82 for slender layer V cells; 2.69 to 8.71 for thick layer V cells), whereas the lowest density is on the terminal dendritic arbors (0.14 to 1.17 for layer II/III cells and 0.27 to 2.16 for thick layer V cells). However, for all cell classes (Figs 20 and 21), the greatest number of spines is found on the basal dendrites (approximately 60% of spines for layer II/III cells and slender layer V cells, and 40% for thick layer V cells), the next highest

TABLE 9. NUMBER AND PROPORTIONS OF SYNAPSES ON RECONSTRUCTED DENDRITIC SEGMENTS IN LAYER IV OF DIFFERENT TYPES OF PYRAMIDAL CELLS (THE LOCATION OF THE CELL BODIES IS INDICATED IN PARENTHESES)

	Spine number	Symmetric Synapses		Asymmetric Synapses		TC (%)
		On shafts	On spines	On shafts	On spines	
Corticothalamic (VI)	2.30 ± 0.27*	0.48 ± 0.10	0.02 ± 0.01	0.12 ± 0.04	2.30 ± 0.27	13.21 ± 5.06
Corticocortical (III, IV)	6.01 ± 0.62†	1.16 ± 0.15	0.07 ± 0.05	0.18 ± 0.09	6.01 ± 0.62	4.02 ± 2.14
Corticostriatal (VI)	14.79 ± 0.80‡	1.19 ± 0.07		0.19 ± 0.26	15.11 ± 0.83	0.55 ± 0.15

The reconstructed dendrites of corticothalamic and corticostriatal cells are portions of apical dendrites, whereas for corticocortical cells are portions of their basal dendrites except one apical dendritic segment that was 70 μm of length. TC, thalamocortical synapses, calculated from all asymmetric synapses formed with the reconstructed dendrites. The numbers of spines and symmetric and asymmetric synapses are values (mean ± S.E.M.) per 5 μm length of reconstructed dendrites. Data obtained from White and Hersch (1981, 1982) and Hersch and White (1982).

* Total dendritic length reconstructed, 648 μm from 7 cells. (From White and Hersch, 1982.)

† Total dendritic length reconstructed, 760 μm from 6 cells. (From White and Hersch, 1981.)

‡ Total dendritic length reconstructed, 645 μm from 7 cells. (From Hersch and White, 1982.)

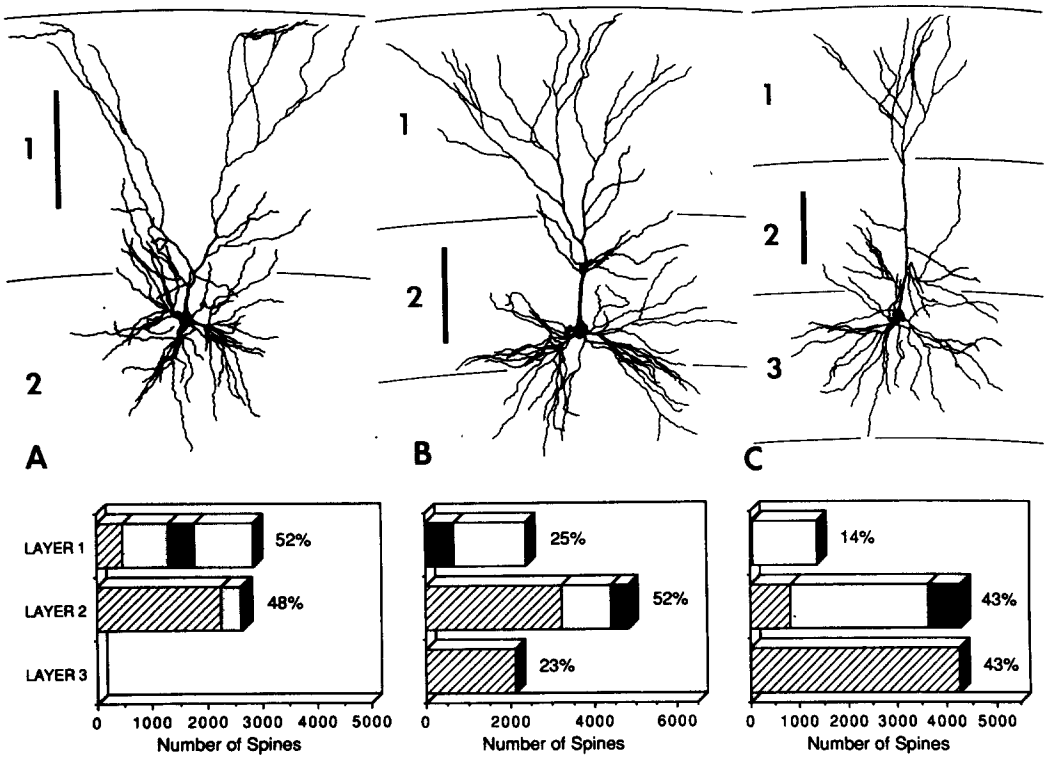

FIG. 20. Camera lucida drawings and bar graphs showing the absolute numbers and percentages of spines in the various cortical layers for individual cells with their somata at different depths in layer 2/3 (visual cortex of the rat). The contributions made by the different types of dendrite are shown in the bar graphs as follows: basal dendrites—hatched; oblique dendrites—stippled; apical trunk—black; terminal arbor—plain. **A**, cell with soma in the upper part of layer 2. **B**, cell with soma in the lower part of layer 2. **C**, cell with soma in the upper part of layer 3. Bars = 100 μm. Mean numbers and percentages of spines in each layer are given in Table 12. (From Larkman, 1991.)

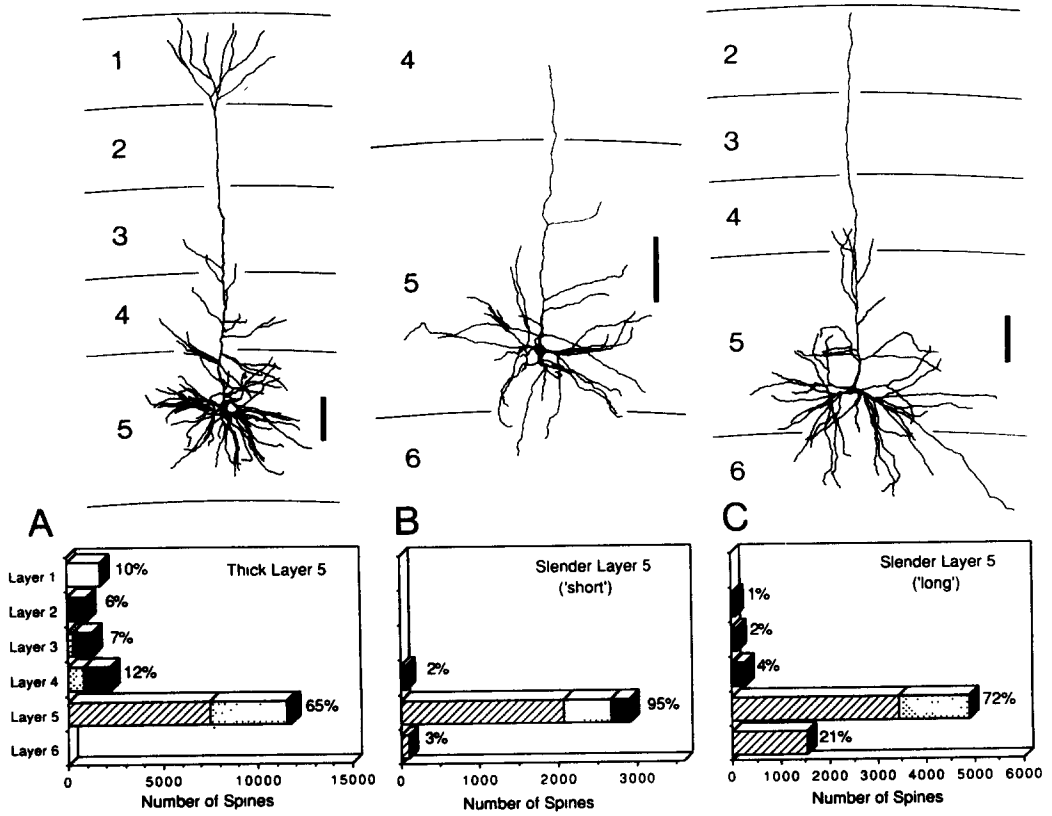

FIG. 21. Camera lucida drawings and bar graphs showing the absolute numbers and percentages of spines in the various cortical layers of individual layer V cells. Conventions as in Fig. 20. **A**, thick L5 cell. **B**, slender L5 cell with its apical dendrite terminating near the top of layer 4. **C**, slender L5 cell with a longer apical dendrite extending into layer 2. Mean numbers and percentages of spines in each layer are given in Table 12. (From Larkman, 1991.)

Table 10. Spine Numbers and Densities*

	Layer 2/3	Slender L5	Thick L5
n Cells	18	10	11
n Segments	205	99	113
Spine linear densities (spines/μm dendritic segment length)			
Basal segments	1.40 ± 0.32	1.29 ± 0.60	1.43 ± 0.29
	(0.38–2.06)	(0.34–2.65)	(0.78–2.26)
Oblique segments	1.30 ± 0.44	1.28 ± 0.55	1.50 ± 0.35
	(0.40–2.62)	(0.36–2.46)	(0.88–2.46)
Apical trunk segments	2.54 ± 1.25	2.10 ± 1.29	6.30 ± 1.60
	(0.71–5.91)	(0.37–4.82)	(2.69–8.71)
Terminal arbor segments	0.56 ± 0.25		0.91 ± 0.60
	(0.14–1.17)		(0.27–2.16)
Overall spine numbers and densities			
Total spines per cell	7965 ± 2723	8647 ± 3097	14932 ± 3371
	(3606–16042)	(3088–14536)	(8058–18085)
Total spines/total dendritic length (μm-1)	1.33 ± 0.19	1.31 ± 0.41	1.65 ± 0.26
	(1.06–1.72)	(0.64–1.92)	(1.05–1.90)
Total spines/total shaft membrane Area (μm-2)	0.62 ± 0.07	0.53 ± 0.17	0.58 ± 0.10
	(0.54–0.78)	(0.30–0.81)	(0.45–0.73)

* Expressed as class mean ± S.D. with range in brackets. (From Larkman, 1991.)

Table 11. Spine Distribution by Dendrite Type*

	Layer 2/3	Slender L5	Thick L5
Basal dendrites	4741 ± 1622	5324 ± 2260	6566 ± 1908
	(60.0 ± 9.3%)	(61.6 ± 11.4%)	(42.4 ± 5.5%)
Oblique dendrites	1605 ± 876	2307 ± 1081	4096 ± 1059
	(21.0 ± 7.4%)	(26.7 ± 9.5%)	(26.5 ± 4.8%)
Apical trunk	773 ± 325	1009 ± 373	3133 ± 725
	(10.0 ± 3.5%)	(11.8 ± 1.9%)	(20.2 ± 3.3%)
Terminal arbor	768 ± 699		1678 ± 452
	(9.0 ± 5.9%)		(10.9 ± 2.4%)†

* Total number of spines per cell on dendrites of each type; expressed as class mean ± S.D. with percentages in brackets.

† Thick 5 terminal arbors were underestimated due to losses during slice preparation. The values shown are for the 5 cells with most complete terminal arbors. Values for all cells in this class were: 1,152 ± 622 spines (7.5 ± 3.1%).

(From Larkman, 1991.)

number of spines on the apical oblique dendrites, followed by the apical trunks, and the least number is found on the terminal arbors (Table 11). Larkman also examined the distribution of spines with regard to the layers (Table 12) and found that all cells had a high proportion (for most cells, a majority) of their spines in the layer in which the cell soma is located.

In general, given that the majority of synapses that a pyramidal cell receives are axospinous and that each spine receives at least one asymmetric synapse, one might take the variations in the number of spines within and between different pyramidal cell classes as

Table 12. Laminar Distribution of Spines*

	Layer 2 cells	Layer 3 cells	Slender L5 cells	Thick L5 cells
Layer 1	33.1 ± 16.5	7.3 ± 4.8	0	9.6 ± 2.2
	(2,823 ± 1,099)	(522 ± 449)	(42.4 ± 5.5%)	(1,482 ± 473)†
Layer 2	60.9 ± 14.1	22.6 ± 15.6	0.6 ± 0.5	5.7 ± 1.4
	(5,864 ± 3,334)	(1,555 ± 1,098)	(57 ± 46)	(880 ± 282)
Layer 3	6.0 ± 8.5	67.1 ± 17	1.8 ± 1.4	7.0 ± 2.0
	(581 ± 787)	(4,963 ± 1,882)	(167 ± 130)	(1,089 ± 421)
Layer 4	0	3.0 ± 7.3	7.0 ± 4.1	8.9 ± 3.0
		(247 ± 584)	(652 ± 488)	(1,384 ± 543)
Layer 5	0	0	84.9 ± 7.8	68.8 ± 6.6
			(7,239 ± 2,408)	(10,654 ± 2,534)
Layer 6	0	0	5.7 ± 7.1	0
			(526 ± 680)	

* Expressed as class mean% ± S.D. with absolute numbers in brackets.

† Thick 5 terminal arbors in layer 1 were underestimated due to losses during slice preparation. The values shown are for the 5 cells with most complete terminal arbors. Values for all cells in this class were: 6.3 ± 2.9% (969 ± 592).

(From Larkman, 1991.)

indicative of differences in the number and distribution of asymmetric synapses they receive (Larkman, 1991). By using this approach and other estimations, the total number of asymmetric and symmetric synapses that a given pyramidal cell receives can be estimated (Tables 13 and 14). However, this approach does not take into account the possible variations within and between cell types in the proportion of symmetric and asymmetric synapses on the dendritic shafts. In the mouse primary somatosensory cortex, the percentage of symmetric synapses on the reconstructed dendritic shaft segments of apical dendrites of corticostriatal cells (Hersch and White, 1982) was rather uniform (approximately 84–90%), and also the ratio of the total asymmetric to symmetric synapses was uniform (from 12.4:1 to 12.8:1). By contrast, the percentage of symmetric synapses on the dendritic shafts of corticothalamic projecting cells (White and Hersch, 1982) ranged from approximately 43% to 100% and the ratio of asymmetric to symmetric synapses from 3:1 to 12:1. Even more remarkable differences have been found in the case of the distribution of synapses on identified fast and slow pyramidal tract neurons (Fig. 22) in the cat motor cortex (Liu *et al.*, 1991). These authors studied, at the electron microscope level, two fast (with few dendritic spines; see Section 1.3) and two slow (with numerous spines) pyramidal tract cells, all located in layer V. For the fast pyramidal tract cells, a sample of 86 synapses on the apical dendrites and 26 on the basal dendrites was studied. Approximately 98% of the synapses located on the apical dendrites and 92% on the basal dendrites were on the dendritic shafts, and approximately 71% of the synapses on the apical dendrites and 92% on the basal dendrites were of the asymmetric type. For the slow pyramidal tract cells, the sample was 78 synapses on the apical dendrites and 24 on the basal dendrites. Approximately 9% of the synapses on the apical dendrites and 69% on the basal dendrites were on the dendritic shafts, and approximately 97% of the synapses on the apical dendrites and 72% on the basal dendrites were of the asymmetric type. Therefore, these two morphological and physiological classes of pyramidal tract cells are distinguished by their differential synaptic organization. In summary, it is very important to study identified populations of pyramidal cells rather than random samples of such cells if their detailed synaptic organization is to be related to functional differences.

4.3. SOURCES OF AXODENDRITIC AND AXOSPINOUS SYNAPSES

There are many kinds of axon terminals of both intrinsic and extrinsic origin that form synapses with dendritic shafts and spines. Since only the pyramidal cells and the small spiny nonpyramidal neurons of layer IV display significant numbers of spines, it is

TABLE 13. DISTRIBUTION OF SYNAPSES BY DENDRITE TYPE OF THREE LAYER II/III CELLS (A, B AND C FROM FIG. 20)

	Total number spines*	Total synapses†	Total asymmetric‡	Total symmetric§
Basal dendrites	A 2667	A 3228–4381	A 2711–3680	A 516–701
	B 5929	B 7177–9740	B 6029–8181	B 1148–1558
	C 6437	C 7793–10576	C 6546–8884	C 1247–1692
Oblique dendrites	A 1111	A 1345–1826	A 1130–1534	A 215–292
	B 1143	B 1383–1877	B 1162–1577	B 221–300
	C 2875	C 3480–4723	C 2923–3967	C 556–756
Apical trunk	A 444	A 538–730	A 452–613	A 86–117
	B 1071	B 1297–1760	B 1089–1478	B 207–282
	C 687	C 832–1129	C 699–948	C 133–181
Terminal arbor	A 1000	A 1211–1643	A 1017–1380	A 194–263
	B 1643	B 1988–2699	B 1670–2267	B 318–432
	C 1312	C 1588–2156	C 1334–1811	C 254–345
Total	A 5222	A 6322–8580	A 5310–7207	A 1011–1373
	B 9786	B 11675–16076	B 9808–13503	B 1867–2572
	C 11311	C 13693–18584	C 11502–15610	C 2190–2974
Total synapses on cell soma‖	A 71–96			
	B 131–180			
	C 153–208			
Total synapses per cell¶	A 6393–8676			
	B 11806–16256			
	C 13846–18792			

* Number of spines (from Larkman, 1991, See Fig. 20).

† Assuming that (i) each spine receives one synapse (ii) on average 15% of spines receive an additional synapse (Jones and Powell, 1969b), and (iii) approximately 70–95% of the dendritic synapses are axospinous (Hersch and White, 1981a, 1982; White and Hersch, 1981, 1982). We assumed also that the estimated values given by different authors are similar in all cortical areas and species. See text for further details.

‡§ Assuming that 84% of all synapses are asymmetric and 16% symmetric (Beaulieu and Colonnier, 1985).

‖ Assuming that 7% of all symmetric synapses are on the cell soma (Beaulieu and Colonnier, 1985).

¶ Total synapses on dendrites plus total synapses on cell soma.

Table 14. Distribution of Synapses by Dendrite Type of Three Layer V Cells (A, B and C from Fig. 21)

	Total number spines*	Total synapses†	Total asymmetric‡	Total symmetric§
Basal dendrites	A 7667	A 9281–12596	A 7796–10581	A 1485–2015
	B 2154	B 2607–3539	B 2190–2973	B 417–566
	C 4937	C 5977–8111	C 5021–6813	C 956–1298
Oblique dendrites	A 4333	A 5245–7119	A 4406–5980	A 839–1139
	B 577	B 699–949	B 587–797	B 112–152
	C 1462	C 1769–2401	C 1486–2017	C 283–384
Apical trunk	A 3333	A 4035–5476	A 3389–4600	A 646–876
	B 1346	B 1629–2211	B 1368–1857	B 261–354
	C 437	C 529–719	C 444–604	C 85–115
Terminal arbor	A 1667	A 2018–2739	A 1695–2301	A 323–438
	B —	B —	B —	B —
	C —	C —	C —	C —
Total	A 17000	A 20579–27930	A 17286–23462	A 3293–4468
	B 3077	B 4935–6699	B 4145–5627	B 790–1072
	C 6836	C 8277–11231	C 6951–9434	C 1324–1797
Total synapses on cell soma ‖		A 230–313		
		B 55–75		
		C 93–126		
Total synapses per cell¶		A 20809–28243		
		B 4990–6774		
		C 8370–11357		

*–¶ As in Table 13.

assumed that virtually all dendritic spines located outside layer IV belong to pyramidal cells. However, in relatively few studies of synaptic connectivity, the postsynaptic dendritic shafts for a given identified cortical cell or afferent fiber have been positively identified as belonging to pyramidal cells. Thus, little is known about the sources of the innervation of the pyramidal dendritic shafts.

4.3.1. *Cortical origin*

4.3.1.1. *Other pyramidal cells*

Most pyramidal cells give rise to an extensive and elaborate system of intracortical axon collaterals (Gilbert and Wiesel, 1979, 1983; Martin and Whitteridge, 1984; DeFelipe *et al.*, 1986; Schwark and Jones, 1989; Ojima *et al.*, 1991). At the electron microscope level, it has been shown that the terminals of the local axon collaterals of pyramidal cells form synapses only of the asymmetric type (LeVay, 1973; Parnavelas *et al.*, 1977; Somogyi, 1978; White *et al.*, 1980; White and Hersch, 1981; Winfield *et al.*, 1981; McGuire *et al.*, 1984; Kisvárday *et al.*, 1986; Gabbott *et al.*, 1987; White and Keller, 1987; Elhanany and White, 1990; White and Czeiger, 1991; McGuire *et al.*, 1991) with dendritic spines and with dendritic shafts belonging to both spiny and aspiny neurons. Because pyramidal cells are very numerous, and virtually the only source of corticocortical connections whose axons end in asymmetric synapses (see Section 4.3.2.2), they are without doubt a most important source of axon

Table 15. Positively Identified Pyramidal Cells Forming Synapses with Major Afferent Fibers

Origin of afferents	Species	Cortical region	Soma location (layer)	Projection site	References
thalamus	mouse	somatic sensory	III, V	?	White, 1978
thalamus	mouse	somatic sensory	V, VI	?	Hersch and White, 1981a,b
thalamus	mouse	somatic sensory	V, VI	thalamus	White and Hersch, 1982
			V, VI	thalamus	Keller and White, 1989
thalamus	mouse	somatic sensory	III, IV	ipsilateral cortex	White and Hersch, 1981
thalamus	mouse	somatic sensory	V–VI	caudate-putamen nucleus	Hersch and White, 1982
thalamus	rat	visual	III, V	?	Peters *et al.*, 1979
thalamus	rat		IV	?	Somogyi, 1978
thalamus	cat	visual	III, V	?	Freund *et al.*, 1985
			III, V	?	Hornung and Garey, 1981
thalamus	cat	visual	III	contralateral cortex	Hornung and Garey, 1981
thalamus	cat	motor	III, V	?	Ichikawa *et al.*, 1985
thalamus	monkey	somatic sensory	III	contralateral cortex	Hendry and Jones, 1983b
contralateral cortex	rat	auditory	II–VI	?	Cipolloni and Peters, 1983
contralateral cortex	mouse	motor	II–III, V	contralateral cortex	Porter and White, 1986
ipsilateral cortex	cat	motor	III, V	?	Ichikawa *et al.*, 1985

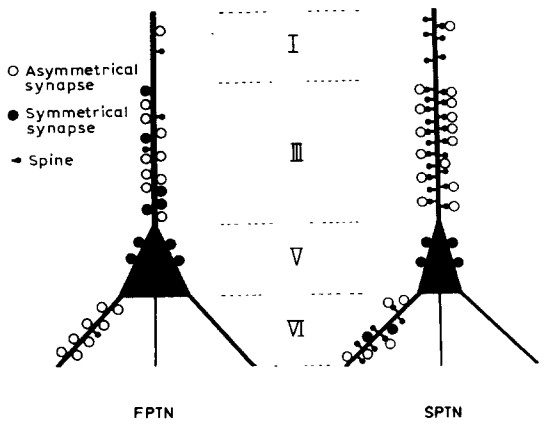

FIG. 22. Schematic diagram showing distributions of synapses on fast (FPTN) and slow (SPTN) pyramidal tract neurons in the cat motor cortex. (From Liu *et al.*, 1991.)

terminals forming asymmetric synapses in the cerebral cortex and, in a given small cortical region, both the local axon collaterals of the pyramids located within that region and the primary axons of pyramidal cells located in other cortical areas should contribute to this synaptic population. Since each corticocortical afferent system terminates specifically in certain cortical areas and layers (depending on the species), and since in the layer(s) of termination the density of terminals can vary, one would expect that the proportions of axon terminals coming from the local axon collaterals and from the primary corticocortical axons would vary depending on the cortical layer, the cytoarchitectonic area and the species under consideration.

Most quantitative data about the synaptic connectivity of single pyramidal cells have been obtained in the visual cortex (area 17), after labeling pyramidal cells by intracellular injections of horseradish peroxidase (Kisvárday *et al.*, 1986; Gabbott *et al.*, 1987; McGuire *et al.*, 1984, 1991). These studies, together with other less extensive analyses of the axons of pyramidal cells labeled by the Golgi method or by extracellular injections of horseradish peroxidase in different cortical areas and species, have shown that, with some exceptions, dendritic spines are major targets of pyramidal cell axons, but apparently different pyramidal cells can form either similar or different proportions of their synapses on spines. For instance, Kisvárday *et al.* (1986) examined in layers III and V of cat area 17 a total of 191 elements postsynaptic to the axons of two layer III pyramidal cells (70 from one pyramidal cell axon and 121 from the other), and found that 84% and 87%, respectively, were on dendritic spines; only one bouton formed a synapse with a cell body and the remainder were on dendritic shafts (some of them probably belonging to other pyramidal cells). Similar results were reported by Gabbot *et al.* (1987) who analyzed a total of 313 boutons in layers V and VI and arising from the axons of two pyramidal cells located in layer V of the cat area 17 (229 boutons from one pyramidal cell and 84 from the other). They found that 81% of the boutons of one and 78% of the boutons of the other formed synapses with dendritic spines while the remainder ended on dendritic shafts (mainly of small caliber). Again, of the 117 synaptic targets of the axons of two layer III pyramidal cells in monkey area 17 (65 targets of one cell and 52 of the other) examined by McGuire *et al.* (1991), 78% and 73%, respectively, were on dendritic spines and the remainder on dendritic shafts (some of them belonging to pyramidal cells). Therefore, all of the axons of pyramidal cells studied in layers III–V in the visual cortex form a rather similar high proportion of their synapses on dendritic spines.

Lower proportions of axospinous synapses formed by axons of pyramidal cells, along with a preference for dendrites of aspinous nonpyramidal cells, have been reported in other areas and in other layers of the visual cortex. Winfield *et al.* (1981) found that the majority (60%) of the 62 synapses formed by the axon of a layer III pyramidal cell in the somatic sensory cortex of the monkey were on dendritic shafts (46% of them arising from aspiny cells), and the remainder on dendritic spines. An even lower proportion of synapses was formed on dendritic spines by axons of two layer VI pyramidal cells studied by McGuire *et al.* (1984) in the cat area 17. These authors examined a total of 151 synapses in layer IV (101 from the axon of one neuron and 50 from the other) and found that 72% of the synapses were on dendritic shafts (many of them belonging to aspiny nonpyramidal cells) and 28% were on dendritic spines. Of the 101 synapses from the axon of the first neuron, 68 (67%) were on dendritic shafts and the rest (33%) were on spines, whereas, of the 50 synapses of the axon of the second neuron, 41 (82%) were on dendritic shafts and the rest (18%) were on spines.

White and colleagues (White and Keller, 1987; Elhanany and White, 1990; White and Czeiger, 1991) used a different approach to study the synaptic connections made by local axon collaterals of relatively large numbers of identified pyramidal cells that were filled by the retrograde transport of horseradish peroxidase after injection of the tracer into different parts of the mouse brain. These authors studied three populations of pyramidal cells in the somatosensory cortex: Corticothalamic (White and Keller, 1987), corticocortical (Elhanany and White, 1990) and callosal (White and Czeiger, 1991) projection neurons. For the corticothalamic cells (with cell bodies were located in lower layer V or upper layer VI), White and Keller found that of 190 synapses made by the local axon collaterals of these cells and examined in layers IV–VI, 175 were with dendritic shafts (92%) and the remainder (8%) with spines. They concluded that many of the postsynaptic dendritic shafts belonged to aspiny cells. For ipsilateral corticocortical cells with cell bodies located in layers II through V, Elhanany and White (1990) examined 139 labeled synapses in layer III and 104 in layer V, and found that in both layers about 87% were formed with dendritic spines and the remainder 13% with dendritic shafts which belong to either spiny or aspiny neurons. Finally, for callosal cells with cell bodies located in layers II, III, V and VI, White and Czeiger (1991) examined 1,215 labeled synapses and found that 1,174 (about 97%) were with dendritic spines and only 41 (about 3%) were on dendritic

shafts originating from both spiny and aspiny neurons.

In conclusion, the axons of pyramidal cells constitute a major source of axospinous synapses and, thus, a major source of synaptic inputs on other pyramidal cells. Different populations of pyramidal cells show different patterns of intracortical synaptic connectivity. The proportions of axospinous synapses formed by different pyramidal cells vary within a wide range of values, but apparently each population of pyramidal cells forms a characteristic proportion of their synapses with spines.

4.3.1.2. *Spiny nonpyramidal neurons*

The cell body and dendrites of spiny nonpyramidal neurons are, in most cases, restricted to layer IV (see Section 1.2). However, their axons tend to ramify in supragranular layers as well (Lund, 1973; Lund and Boothe, 1975; Jones, 1975; Lund *et al.*, 1979; Gilbert and Wiesel, 1979; Martin and Whitteridge, 1984). In all cases, spiny stellate cells have been found to form asymmetric synapses mainly with dendritic spines (Le Vay, 1973; Mates and Lund, 1983; Somogyi, 1978; Saint Marie and Peters, 1985). Saint Marie and Peters (1985) reported that 66% of the asymmetric synapses formed by these cells in layer IV of the visual cortex of the monkey were on dendritic spines. These authors concluded that the majority of the postsynaptic spines belonged to other spiny stellate cells. However, it is likely that the axons of spiny stellate cells that project to supragranular or infragranular layers (e.g. see Lund, 1984) also form synapses with dendritic spines arising from pyramidal cell dendrites. A direct demonstration of such connectivity, however, has not yet been made.

4.3.1.3. *Aspiny nonpyramidal neurons*

The vast majority of symmetric synapses in the cerebral cortex are of intrinsic origin and are formed by the axons of the aspiny nonpyramidal neurons (see Section 1.2). This implies that aspiny neurons are the major source of terminals forming symmetric axospinous and axodendritic synapses with pyramidal cells. However, not all aspiny nonpyramidal cells form symmetric synapses, since it has been shown that certain (presumably few) neurons, usually called bipolar cells, form asymmetric synapses mainly with dendritic spines that are likely to belong to pyramidal cells (Peters and Kimerer, 1981; Fairén *et al.*, 1984; Peters and Harriman, 1988); but they also form synapses with the shafts of both pyramidal and nonpyramidal cells (Peters and Kimerer, 1981; Peters and Harriman, 1988).

Several types of aspiny nonpyramidal cells have been found to form symmetric synapses with dendritic shafts, but only in relatively few instances has the postsynaptic element been positively identified as belonging to a pyramidal cell. One of the known sources of symmetric synapses on the apical and basal dendritic shafts of pyramidal cells (Fig. 23) is the classical or large basket cell (Somogyi *et al.*, 1983; Kisvárday *et al.*, 1987). These authors found that large basket cells in layer III and layer V of the cat visual cortex make approximately 24% and 38% of their synapses, respectively, with dendritic shafts, the majority of which originated from pyramidal cells (see Section 2.3).

It seems clear that the majority of dendritic spines located outside layer IV belong to pyramidal cells and, therefore, it may not be absolutely necessary to follow in serial thin sections the spines in order to determine whether their origin is from pyramidal cells. As mentioned in Section 4.1, it has been demonstrated that a major source of symmetric synapses on

FIG. 23. Camera lucida tracing of one of the descending axon collaterals of basket cell illustrated in Fig. 9, A. The collateral had few varicosities before it approached the ascending apical dendrite of a giant layer V pyramidal cell. Sixteen varicosities of the basket cell axons were directly apposed to the apical dendrite. Bar = 50 μm. (From Somogyi *et al.*, 1983.)

dendritic spines of pyramidal cells is the double bouquet cell. These cells were first described by Cajal in 1899 (Cajal, 1899a, b) in the human cerebral cortex (Fig. 24) and are characterized by axons which form tightly intertwined bundles of vertical collaterals that transverse several layers. Somogyi and Cowey (1981) studied 5 Golgi impregnated double bouquet cell axons at the electron microscope level (3 from cat area 17, 1 from cat area 18 and 1 from monkey area 17). They found that 4.6% of the boutons in cat area 17 (total boutons examined 66) and 26% of the boutons in cat area 18 (total boutons examined 19) formed axospinous synapses, whereas in monkey area 17 it was 40% (total boutons examined 35). DeFelipe and colleagues (DeFelipe *et al.*, 1989b; DeFelipe *et al.*, 1990) examined the synapses formed by double bouquet cell axons visualized by calbindin ($n = 7$) or tachykinin ($n = 9$) immunoreactivity in the monkey somatosensory or auditory cortex (Fig. 25). These authors found that, of the total 276 synapses formed by the calbindin double bouquet cell axons and of the total 337 synapses formed by the tachykinin double bouquet cell axons, 105 (38%) and 144 (43%), respectively, were on dendritic spines that likely belonged to pyramidal cells. A similar distribution of synapses was formed by the somatostatin immunoreactive double bouquet cell axons studied in the temporal cortex of the monkey by de Lima and Morrison (1989). Of the total number of 100 boutons examined by de Lima and Morrison, 138 were on spines (38%). Double bouquet cells are very abundant: In tangential sections passing through layer III of the monkey cortex, there are 7–15 double bouquet cell axons/10,000 μm^2 (DeFelipe *et al.*, 1990), and each individual axon forms hundreds of synapses. Therefore, double bouquet cells can be considered as one of the most important sources of symmetric axospinous synapses on pyramidal cells, at least in the primate cerebral cortex. In addition, basket cells have been found to form a relatively high proportion of their synapses on dendritic spines that are likely to belong to pyramidal cells (Somogyi *et al.*, 1983; Kisvárday *et al.*, 1987). These authors found that approximately between 20% and 41% of the synapses formed by the axons of the basket cells examined were with dendritic spines (see Section 2.3).

Finally, it is possible that different parts of their dendritic arbor (apical dendrites, oblique dendritic branches, primary basal dendrites, etc.) are selectively or preferentially innervated by certain aspiny non-pyramidal cells. For example, double bouquet cells appear not to form synapses with apical dendrites (Fig. 5), but with basal dendrites and oblique branches of the apical dendrites (Somogyi and Cowey, 1981;

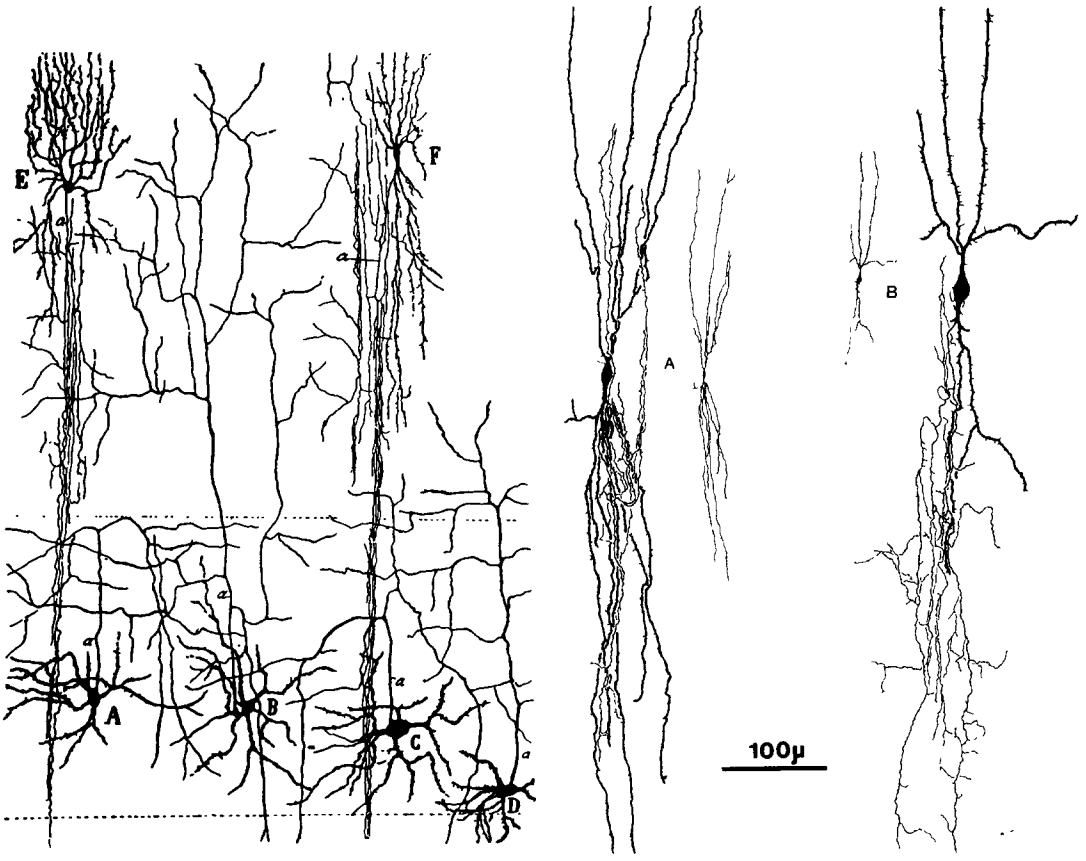

FIG. 24. Left, drawing from Cajal (1899b) showing two double bouquet cells (E, F), impregnated by the Golgi method, in the human visual cortex. Right, two double bouquet cells (A, B) drawn from original Cajal preparations of the somatosensory cortex of infants, impregnated by the Golgi method. Both have their perikarya in layer III. Insets show the total span of their dendritic arborizations. (From Fairén *et al.*, 1984.)

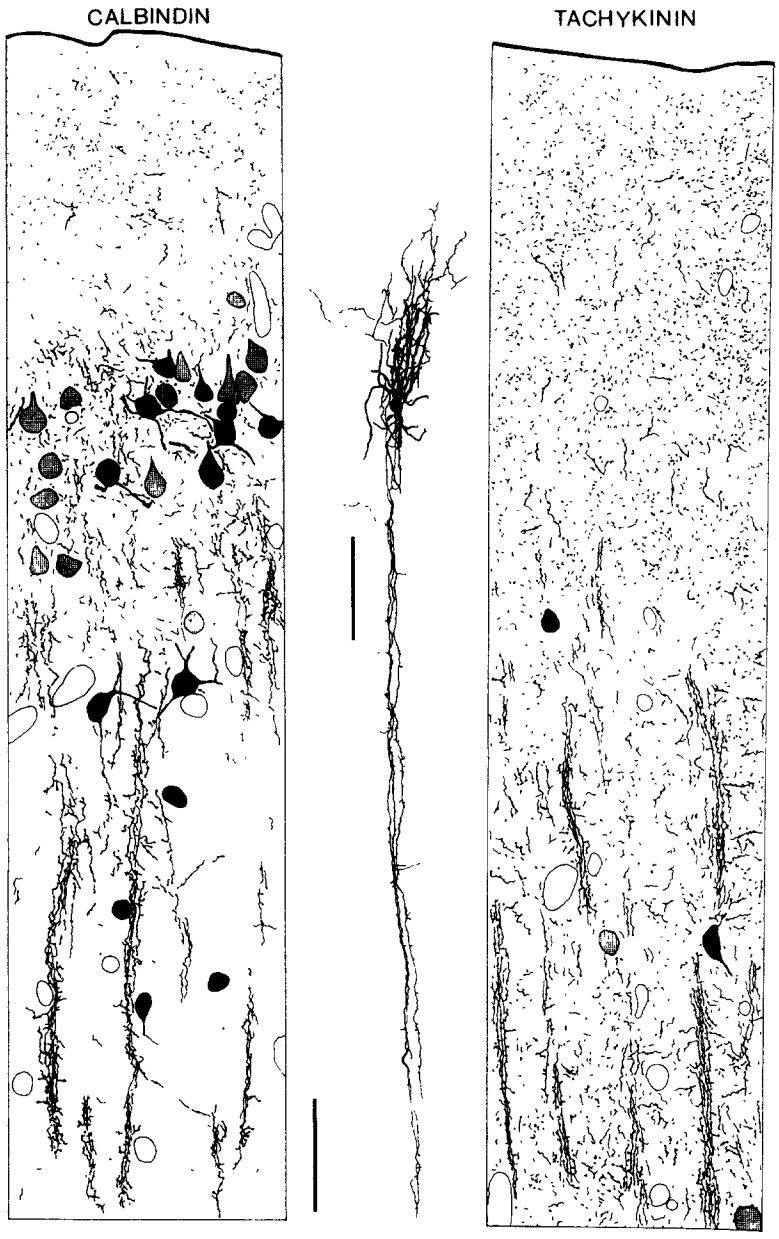

FIG. 25. Camera lucida drawings showing the distribution of calbindin- and tachykinin-immunoreactive somata, punctate profiles and bundles of processes (double bouquet cell axons) in a region extending from layer I through approximately the upper two thirds of layer III in area 18 of the monkey cortex. The solid black profiles are darkly stained neuronal somata, and the shaded profiles are lightly or moderately stained somata. Open outlines represent blood vessels. In the inset is shown a camera lucida drawing of a Golgi-impregnated, double bouquet cell taken from Jones (1975). Bar = 50 μm (lower); 100 μm (upper, for the Golgi-impregnated cell). (From DeFelipe *et al.*, 1991.)

DeFelipe *et al.*, 1989b, 1990), whereas other types of aspiny nonpyramidal cells, such as large basket cells (Somogyi *et al.*, 1983; Kisvárday *et al.*, 1987), form a considerable number of synapses with apical dendrites.

4.3.2. *Cortical afferent systems*

4.3.2.1. *Thalamocortical afferent fibers*

Thalamocortical fibers terminate primarily in layer IV and the lower part of layer III in many cortical areas. Additional thalamic fiber terminations are found at the junction of layers V and VI and in layer I (for a review see Jones, 1985). Over the last 20–30 years, many efforts have been made in the identification of cortical cells which receive input from the thalamus. It has been found that thalamic axon terminals always form asymmetric synapses and that postsynaptic elements are mainly dendritic spines (reviewed in White, 1989). Garey and Powell (1971) studied the distribution of a total of 1,027 degenerating thalamic axon terminals on different postsynaptic

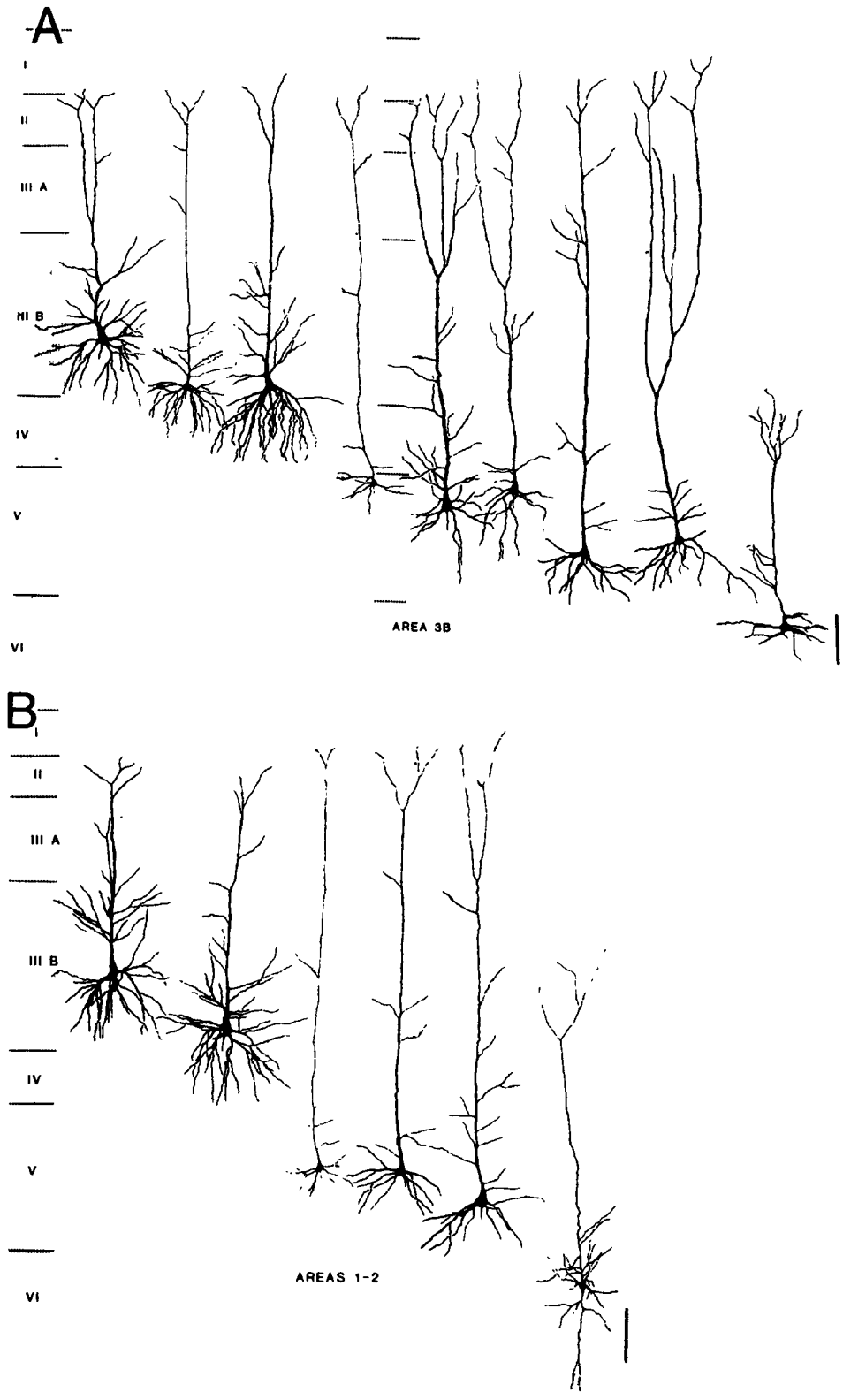

FIG. 26. Varieties of pyramidal cells from layers IIIb, V, and VI of areas 3b (A) and 1–2 (B) of monkey somatic sensory cortex showing relationships of their dendritic branching patterns to layers of termination of thalamic afferents (stipple). Bars = 100 μm. (From Jones, 1984, after Hendry and Jones, 1983a.)

profiles in layer IV of monkey area 17 and cat areas 17, 18 and 19, after lesions of the lateral geniculate nucleus. They found that the majority of the degenerating terminals ($n = 825$, which represent approximately 80% of the total) ended on dendritic spines, whereas approximately 15% were on dendritic shafts and 4% on cell somata. Similarly, Peters and Feldman (1976) found that, of the 277 degenerating thalamic terminals in layer IV of the rat area 17, 83% were on dendritic spines, 15% on dendritic shafts and 2% on cell somata.

Extensive reconstructions from serial thin sections in conventional electron microscopy (Peters and Feldman, 1976; Davis and Sterling, 1979), or by using the Golgi-electron microscope technique of Fairén *et al.* (1977), have been necessary to determine which morphological types of cortical cells are implicated in the reception of the thalamic input and the possible variations in the pattern of thalamic input to the different types of cells. The data obtained in these studies have shown that input from the thalamus is not restricted to a single class of cortical neuron (e.g. White, 1978; Somogyi, 1978; Peters *et al.*, 1979; Hornung and Garey, 1981; Hersch and White, 1981b; Davis and Sterling, 1979; Ichikawa *et al.*, 1985; Keller and White, 1987; Cipolloni and Keller, 1989) but, with few exceptions, every neuron examined having a dendrite in layer IV receives thalamocortical synapses (White, 1989). Moreover, the studies of White and colleagues, have clearly shown that there is a wide variation in the number and proportion of thalamic axon terminals synapsing on different types of cells.

Within layer IV, the apical dendrites and basal dendrites of pyramidal cells whose cell bodies are located in infragranular layers (layers V and VI) and in layer III, respectively, receive thalamic afferents in all species studied (Table 15). Although the majority of pyramidal cells have significant parts of their dendritic fields in the layers where thalamocortical axons terminate (Hendry and Jones, 1983a) (Fig. 26), there is a great variability in the number and proportion of thalamic synapses that different pyramidal cells receive (Fig. 27) which is independent of the proportion of thalamic synapses found in the surrounding neuropil or of the number of spines born by the dendrites (Hersch and White, 1981a, b). In addition, dendrites belonging to the same parent cell, independent of the cell type being considered, form, in general, a similar number and proportion of thalamic synapses (White, 1989).

A number of studies have tried to relate the above-mentioned individual variations, found even

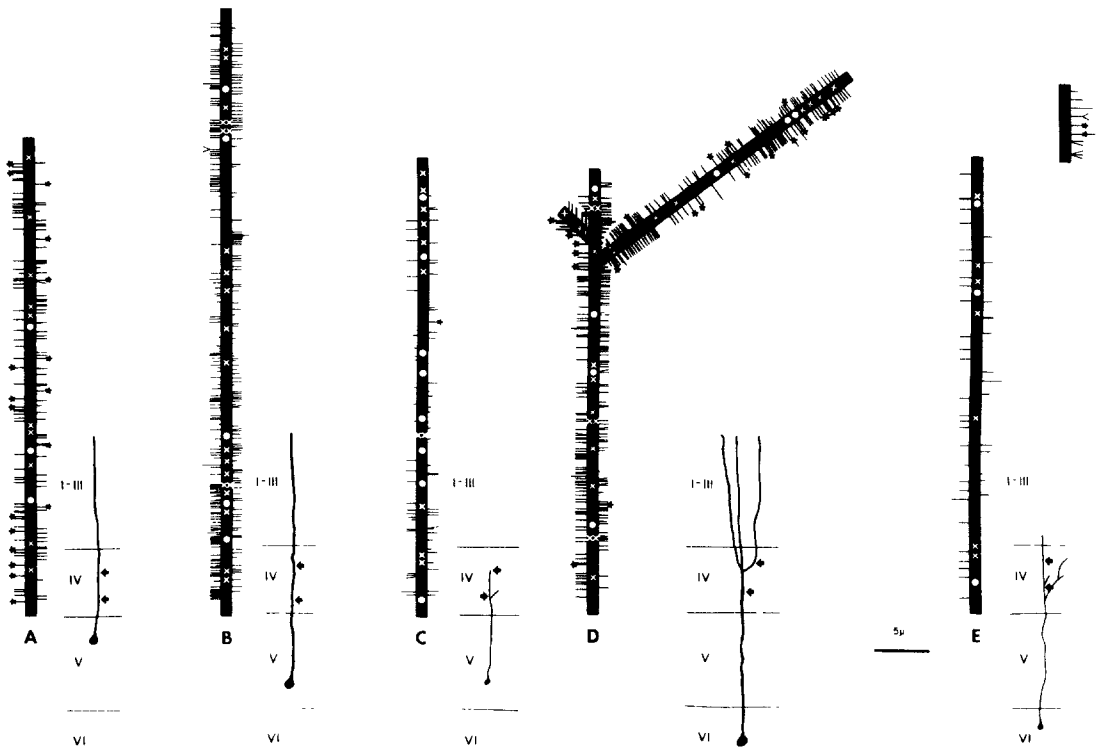

FIG. 27. Graphic reconstructions showing the distribution of synapses with the reconstructed pyramidal cell dendritic segments (mouse somatic sensory cortex). Shaft synapses (x, symmetrical; O, asymmetrical) are indicated on the thick lines which represent the long axes of the dendritic segments. Thin lines indicate the points of origin of spines from their parent shafts. Axospinous synapses (all asymmetrical) are depicted using the symbols shown in the key at the top far right of the figure. The explanation of the key symbols from top to bottom is as follows: spine forming no synapse, 1, 2, 3 synapses, synapse with a degenerating thalamocortical axon terminal, synapse with a thalamocortical axon terminal, and with a normal axon terminal, double headed spine with each head forming one synapse, triple headed spine with each head forming 1 synapse. At the lower right of each reconstruction is a drawing of its neuron of origin showing the region from which the reconstruction was made (arrows). Dendritic spines, basal dendrites, and apical dendritic tufts are not shown. (From Hersch and White, 1981a.)

among pyramidal cells located in the same layer with the projection site of the cell, by combining anterograde degeneration of thalamic axonal fibers with the retrograde transport of horseradish peroxidase (White *et al.*, 1980). By using this approach (Fig. 28), quantitative data have been obtained in the mouse primary somatosensory cortex on pyramidal cells projecting to ipsilateral cortical areas (White and Hersch, 1981; White *et al.*, 1980), to the thalamus (White and Hersch, 1982; Keller and White, 1989) and to the striatum (Hersch and White, 1982). These studies have shown that each of these populations of pyramidal cells receives a characteristic proportion of their layer IV dendritic synapses from thalamocortical axon terminals (Table 9). As shown in Table 9, corticothalamic cells receive the greatest number of thalamocortical synapses ($13.21 \pm 5.06\%$ of all axospinous synapses), corticocortical cells the next highest number ($4.02 \pm 2.14\%$), and corticostriatal the least ($0.55 \pm 0.15\%$).

4.3.2.2. *Nonthalamic afferent fibers*

One of the major systems of nonthalamic afferent fibers to the cortex is represented by the corticocortical fibers. Several studies have been aimed at identifying the types of cortical neurons postsynaptic to axon terminals coming from other areas. Association and callosal fibers form asymmetric synapses (Jones and Powell, 1970b; Lund and Lund, 1970; Sloper, 1973; Fisken *et al.*, 1975; Sloper and Powell, 1979b; Voight *et al.*, 1988; Cipolloni and Peters, 1983; Ichikawa *et al.*, 1985; Porter and White, 1986; Porter and Sakamoto, 1988; Lowenstein and Somogyi, 1991), in conformity with their origin from pyramidal cells. In these studies, it has been demonstrated that the vast majority of corticocortical terminals form synapses with dendritic spines, most of which probably belong to pyramidal cells. Therefore, corticocortical fibers are a major source of axospinous synapses on pyramidal cells. The percentage of synapses on dendritic spines varies from approximately 70 to 95%, depending on the type of projection, cortical area and species. For example, Porter and Sakamoto (1988) made iontophoretic injections of the lectin PHA-L in area 2 of the cat somatosensory cortex in order to label anterogradely the axons that project from this area to area 4 of the motor cortex. They found that 72% of the synapses (sample of 215 labeled terminals) formed axospinous synapses. On the other hand, Cipolloni and Peters (1983), combining the Golgi-electron microscope technique and lesion-induced degeneration procedures, found that 95% of callosal axon terminals (sample of 200 degenerating terminals) in the rat auditory cortex formed synapses with dendritic spines of pyramidal cells that had cell bodies in layers II to VI. However, pyramidal cells located in different layers are not equally innervated. Porter and White (1986) studied the distribution of callosal synapses on identified callosal pyramidal cells in the mouse primary motor cortex, by combining retrograde transport of horseradish peroxidase and lesion-induced degeneration. Segments of apical and basal dendrites of layer II/III callosal cells and apical dendrites of layer V callosal cells were reconstructed and synapses counted. It was found that the percentage of callosal synapses on dendrites of layer II/III callosal cells ($4.19 \pm 1.64\%$) is higher than that on dendrites of layer V callosal cells ($1.99 \pm 1.88\%$), although in both cases the concentration of degenerating callosal terminals in the surrounding neuropil was similar.

Other afferent systems (forming asymmetric or symmetric synapses) also appear to constitute a substantial input to pyramidal cells. These include the claustrocortical afferents (LeVay, 1986; forming asymmetric synapses) and dopaminergic afferents (e.g. Goldman-Rakic *et al.*, 1989; forming symmetric synapses). In both cases, pyramidal cells are considered as important targets.

Finally, in a given cortical area, the distribution pattern of the majority of the various afferent systems (thalamic and nonthalamic) is nonhomogeneous throughout the cortical layers and often different, depending on the areas and species under consideration (for example, see the review of Foote and Morrison (1987) on extrathalamic afferents to the cortex). Therefore, one might expect that pyramidal cells located in different layers and cortical areas of the same or different species would receive different numbers and proportions of synapses from the various cortical afferents. In addition, one might also expect that the apical dendrites of the pyramidal cells during their ascending trajectory through the cortex would receive on each dendritic (laminar) segment different numbers and proportions of synapses from different afferent systems. However, as White and colleagues have demonstrated, within the target region of a given afferent system, there is great variability in the number and proportion of the synapses formed by the afferents with different cell types. Therefore, quantitative studies are necessary to determine the synaptic relationships of each type of pyramidal cell with the various afferent systems.

4.4. CHEMICAL CHARACTERISTICS OF AXODENDRITIC AND AXOSPINOUS SYNAPSES

4.4.1. *Asymmetric synapses*

Axon terminals forming asymmetric axodendritic and axospinous synapses have been stained immunocytochemically using antibodies against glutamate (DeFelipe *et al.*, 1988; Conti *et al.*, 1989; Dori *et al.*, 1989). Glutamate is thought to be the major excitatory neurotransmitter in the neocortex (see Krnjević, 1974), and its localization in asymmetric synapses is in line with the belief that this morphological type of synapse is excitatory (Colonnier, 1981). Several lines of evidence indicate that a large proportion of pyramidal cells use glutamate and/or aspartate as neurotransmitters (e.g. see Conti *et al.*, 1987, 1989), and it is thought that one of the major sources of glutamate-positive axon terminals is the system of intracortical collaterals arising from pyramidal cell axons (DeFelipe *et al.*, 1988; Conti *et al.*, 1989; Dori *et al.*, 1989). However, a large number of axon terminals forming asymmetric synapses does not stain for glutamate. Possible explanations for this are technical problems and/or that they contain other neurotransmitters (e.g. see Conti *et al.*, 1989). In

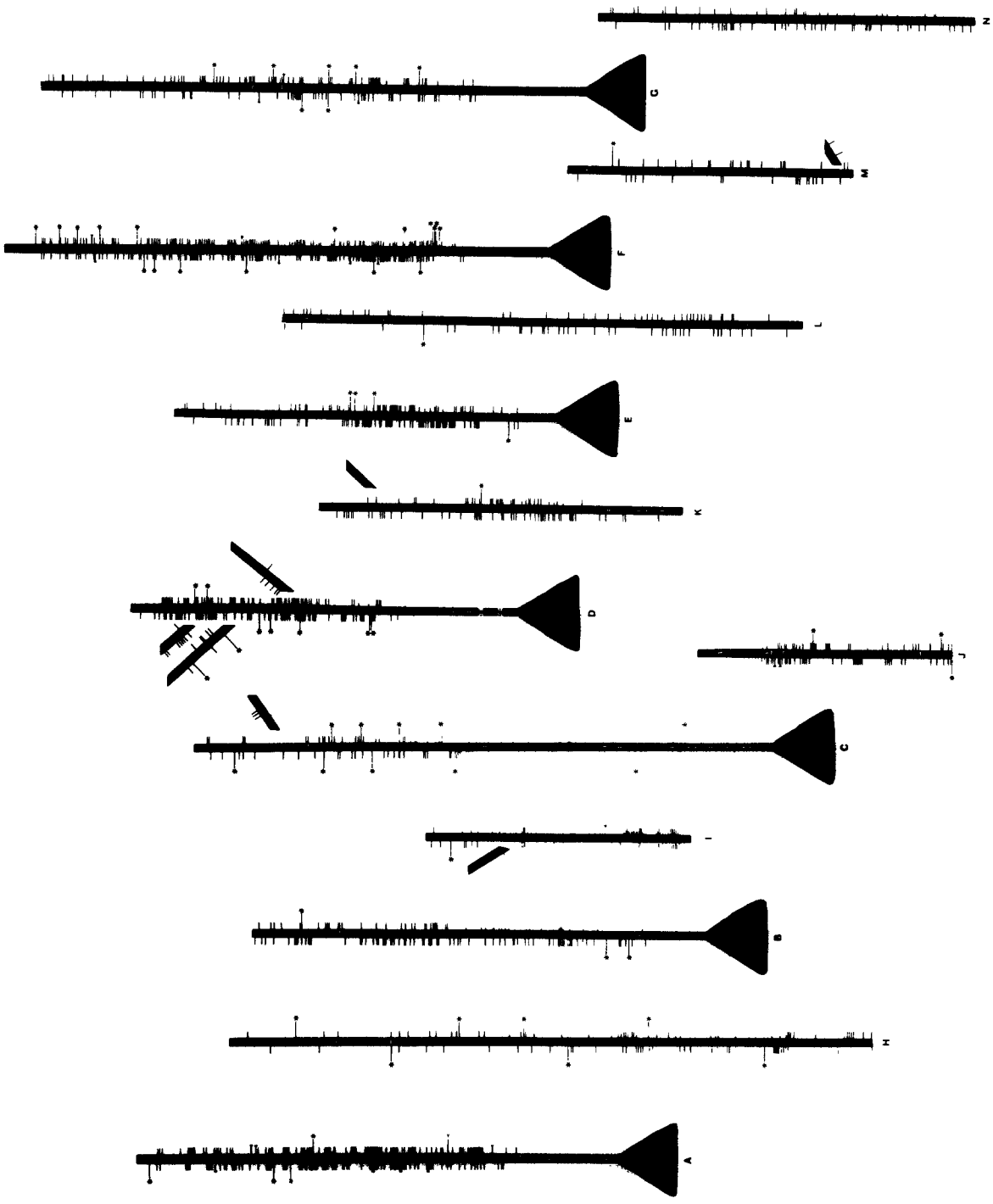

Fig. 28. Caption on facing page.

addition, a number of other substances (that include noradrenaline, dopamine, serotonin, choline-acetyltransferase, somatostatin, neuropeptide Y and tachykinins) have been detected immunocytochemically in relatively many or very few (depending on the authors and on the compound analyzed) axon terminals forming asymmetric synapses with dendritic spines and dendritic shafts, some of them probably belonging to pyramidal cells (e.g. see Hendry *et al.*, 1984b; Jones *et al.*, 1988; Houser *et al.*, 1985; Papadopoulos *et al.*, 1989a, b; Parnavelas *et al.*, 1986; DeFelipe and Jones, 1988a). However, it is clear that in the cerebral cortex a very large proportion of axon terminals forming asymmetric synapses arise from pyramidal cell axons, and since a large proportion of pyramidal cells are likely to use glutamate as a neurotransmitter, it follows that many of these terminals are glutamatergic.

4.4.2. *Symmetric synapses*

The dendritic shafts and spines of pyramidal cells receive numerous GABAergic synapses. At the light microscope level, it is observed that the proximal dendrites of many pyramidal cells are outlined by GABAergic terminal-like puncta. At the electron microscope level, it is confirmed that these puncta are GABAergic axon terminals forming symmetric synapses and, thus, the proximal dendrites of many pyramidal cells are strongly innervated by GABAergic axon terminals.

Dendritic spines are considered to be major postsynaptic targets of excitatory axon terminals (forming asymmetric synapses). However, immunocytochemical studies at the electron microscope level have shown that dendritic spines (presumably arising from pyramidal cells) are common postsynaptic targets of GABAergic axon terminals. In these immunocytochemical studies, it is frequently found that the dendritic spines that are postsynaptic to GABAergic axon terminals also receive an asymmetric synapse from a nonimmunoreactive terminal (e.g. Ribak, 1978; Hendry *et al.*, 1983a).

As described in Section 4.3.1.3, one of the most important sources of axospinous synapses appears to be double bouquet cells whose axons form approximately 5–43% of their synapses (depending on the species and cortical area) with dendritic spines (Somogyi and Cowey, 1981; de Lima and Morrison, 1989; DeFelipe *et al.*, 1989b, 1990). In the monkey cortex, the axons of double bouquet cells have been shown to be immunoreactive for GABA (DeFelipe and Jones, 1992) and, in certain cortical areas, for the peptides SRIF and tachykinin (de Lima and Morrison, 1989; DeFelipe *et al.*, 1990) and for the calcium-binding protein calbindin (Hendry *et al.*, 1989; DeFelipe *et al.*, 1989b, 1990). In addition, in the cat visual cortex, certain neurons immunoreactive for CCK, whose axons form approximately 20% of their synapses with spines, have been interpreted as double bouquet cells (Freund *et al.*, 1986).

In addition to the differences in the number and density of symmetric axodendritic and axospinous synapses on different types of pyramidal cells or on different parts of the dendritic tree of a given pyramidal cell (see Tables 13 and 14), there seems to be differences in the chemical characteristics of these synapses. For example, double bouquet cells are chemically heterogeneous (depending on the area where they are located, they are immunoreactive or nonimmunoreactive for several substances; see above); therefore, it is likely that there is a differential chemically-definable innervation of pyramidal cells located in different areas (Fig. 29).

Several classes of axon terminals other than those belonging to double bouquet cells, have been found to form significant numbers of symmetric synapses with dendritic spines. For example, in the cat visual cortex, approximately 9% of the synapses formed by certain small basket neurons immunoreactive for CCK are formed with dendritic spines (Freund *et al.*, 1986). Approximately 21% of tachykinin immunoreactive terminals in the monkey motor and somatic sensory cortex, and approximately 5–34% of SRIF immunoreactive terminals in the monkey temporal and occipital cortex, form axospinous synapses (Jones *et al.*, 1988; de Lima and Morrison, 1989). The majority of neurons immunoreactive for the peptides, SRIF, tachykinins and CCK, are also immunoreactive for GABA or GAD (e.g. Hendry *et al.*, 1984a; Schmechel *et al.*, 1984; Somogyi *et al.*, 1984; Lin *et al.*, 1986; Demeulemeester *et al.*, 1988; Jones *et al.*, 1988), and a significant proportion of the neurons immunoreactive for the calcium-binding protein calbindin is also immunoreactive for GABA (Demeulemeester *et al.*, 1988, 1991; Hendry *et al.*, 1989; Hendry and Jones, 1991; DeFelipe and Jones, 1992), but there is little colocalization of these neuropeptides and calbindin in cortical neurons (Demeulemeester *et al.*, 1988, 1991; DeFelipe *et al.*, 1990). Therefore, several morphological and chemical types of interneurons form a considerable number of symmetric axospinous synapses and, since some of these interneurons preferentially innervate certain dendritic

FIG. 28. Graphic representations of the reconstructed segments of apical dendrites of layer II/III and layer *V* callosar neurons show the distribution and location of spines and synapses along the dendritic shafts (mouse somatic sensory cortex). Oblique bars represent portions of side branches which were included in the reconstructions. Synapses formed between callosal axon terminals and spines of callosal projection neurons are dispersed along the shaft with no apparent periodicity. Synapses formed between the labeled shafts and nondegenerating axon terminals are preferentially located in that most symmetric synapses occur closer to the somata than do asymmetric synapses. Occasionally, the postsynaptic density of contacts with dendrites were obscured by reaction product and difficult to identify. Key: black bar (——), labeled spine that forms an asymmetric synapse; black bar with filled triangle (—◀), labeled spine that forms two synapses; asterisk (*), synapse between callosal axon terminal and labeled spine; white bar (▭), asymmetric synapse with labeled shaft; white x, symmetric synapse with labeled shaft; white ○, synapse, with labeled shaft, of uncertain identity. (From Porter and White, 1986.)

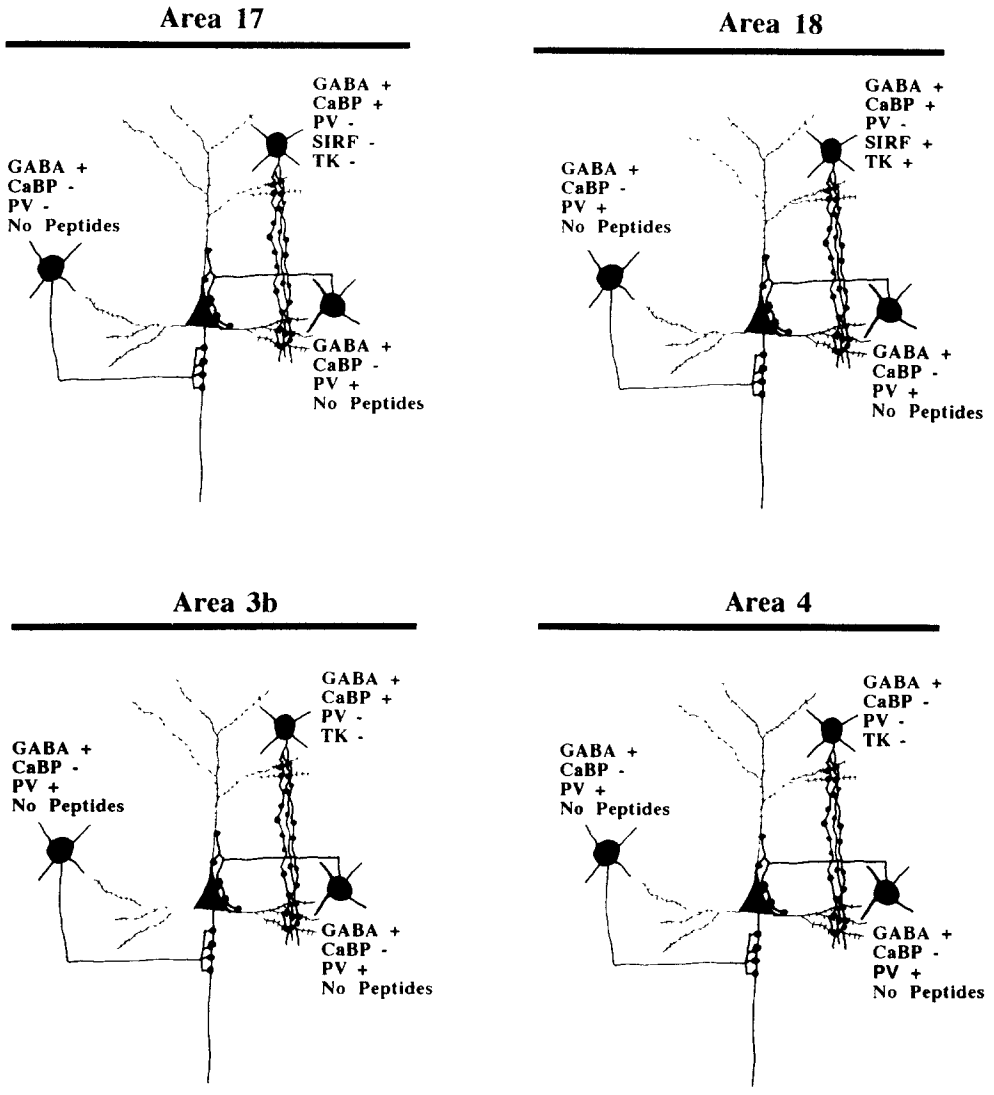

FIG. 29. Schematic diagram illustrating the chemical characteristics and the synaptic connections of double bouquet cells, chandelier cells and large basket cells with pyramidal cells (see Fig. 5), in layers II and III of different areas of the monkey cortex. The three types of nonpyramidal cells are involved in the same basic synaptic circuit in all areas, and use the same "main" neurotransmitter (GABA), but possess different neuropeptides and calcium-binding proteins, depending on the neuronal type and/or cortical area where they are located. GABA: γ-aminobutyric acid; SRIF: somatostatin; TK: tachykinin; CaBP: the calcium-binding protein calbindin D-28K; PV the calcium-binding protein parvalbumin.

regions of the pyramidal cells, this suggests a differential chemical innervation of individual pyramidal cells.

5. ARE ANATOMICALLY-DEFINED OR CHEMICALLY-DEFINED POPULATIONS OF PYRAMIDAL CELLS INNERVATED PREFERENTIALLY BY CERTAIN TYPES OF ASPINY NONPYRAMIDAL CELLS?

Very little is known about this subject; however, recent studies suggest that different classes of pyramidal cells can be innervated either *preferentially* or *exclusively* by certain types of aspiny nonpyramidal cells. For example, it is clear that, within the axonal field of a single classical basket cell or a single chandelier cell, there are pyramidal cells that are postsynaptic targets to the basket or chandelier cell axons, whereas many other pyramidal cells are not, and among the target neurons some are more heavily innervated than others (e.g. see Fairén and Valverde, 1980; Somogyi *et al.*, 1983; DeFelipe *et al.*, 1985). Thus, each single basket or chandelier cell seems to show a preference for certain pyramidal cells and an avoidance of certain other pyramidal cells. However, in these studies it is not known whether the target pyramidal cells belong to the same or a different anatomical class (for example, on the basis of their projection site or their pattern of intrinsic axonal arborization) or to a particular chemical class (immunoreactivity for glutamate or aspartate). Nor is it known whether those other pyramidal cells that are not targets of the basket or chandelier cell axons

examined are the targets of neighbouring basket or chandelier cells. If so, the selective innervation of pyramidal cells displayed by adjacent basket or chandelier cells could have its origins in the phenomenon of competition for synaptic space during development. However, the latter possibility seems to be unlikely, since approximately 98% of the soma surface is free of synaptic junctions (see Section 4.1) and, therefore, the "available space" is potentially very large.

As described in Section 3.2, it has been shown in the cat visual cortex (area 17) that the axon initial segments of ipsilateral corticocortical pyramidal cells receive the greatest number of synapses (22–28), callosal pyramidal cells the next highest number (16–23), and corticothalamic pyramidal cells the least (1–5). Since chandelier cell axons are the major source of axon terminals synapsing on the axon initial segments of pyramidal cells (Section 3.3) the data suggest that certain pyramidal cells are preferred (with different degrees of affinity) or avoided as targets by the chandelier axons (for example, corticothalamic cells are unlikely to be major postsynaptic targets of chandelier cells since they have very few initial segment synapses). Chandelier cells, though probably all GABAergic, appear to be heterogenous with regard to their content of other substances such as parvalbumin (see Section 3.4). Similarly, double bouquet cells, which are an important source of symmetric synapses on the spines of pyramidal cells (Sections 4.1 and 4.3.1.3), are also heterogeneous with regard to their content of certain peptides or calbindin (de Lima and Morrison, 1989; DeFelipe *et al.*, 1990). Thus, pyramidal cells may not only be innervated by certain morphological types of nonpyramidal cells, but also by certain chemical subclasses of a given morphological type (Fig. 29).

With regard to the innervation of the pyramidal cell soma, there are no studies in which a morphologically characterized type of aspiny nonpyramidal cell forming axosomatic synapses has been shown to innervate preferentially, or exclusively, a particular population of pyramidal cells, as appears to occur with chandelier cells. It is true that layer III pyramidal cells projecting callosally receive a greater number and higher density of axosomatic synapses than do layer VI corticothalamic pyramidal cells in the cat visual cortex (Section 2.2), but it does necessarily not mean that different morphological types of interneurons are involved in this differential innervation.

Immunocytochemical studies, however, suggest that there may exist aspiny nonpyramidal cells that are specialized in forming axosomatic synapses preferentially with certain populations of pyramidal cells. Jones *et al.* (1988), using immunocytochemistry to localize the tachykinin family of neuropeptides (Section 1.4), found that the cell somata and proximal dendrites of some pyramidal cells in the monkey cerebral cortex were surrounded by many tachykinin-immunoreactive terminals, whereas the somata and proximal dendrites of most pyramidal cells had few or no terminals around them. This variability appears to occur within single classes of pyramidal cells. In area 4 of the monkey, some pyramidal cells, which could be identified as Betz cells (because of their very large size), were surrounded by many tachykinin-immunoreactive terminals, while others received none. Since the tachykinin-immunoreactive neurons in the monkey cerebral cortex consist of aspiny nonpyramidal cells (Jones *et al.*, 1988), this differential innervation of pyramidal cells could be taken as an indication that certain aspiny nonpyramidal cells form synapses preferentially or specifically with certain pyramidal cells, but independently of the projection site of the latter.

Nevertheless, the "projection site" is not the only important criterion for classifying pyramidal cells. For example, by combining retrograde transport of tracers and immunocytochemistry for glutamate or aspartate, it has been shown that cortical projection cells are chemically heterogeneous (Conti *et al.*, 1988; Giuffrida and Rustioni, 1988, 1989; Dinopoulos *et al.*, 1989). It is an open question whether these further subdivided cortical projection cells are characterized by a particular number and density of initial segment and axosomatic synapses.

6. CONCLUDING REMARKS

Pyramidal cells constitute a heterogeneous group of cortical neurons. This has been shown by the use of a variety of techniques that include Golgi impregnations, intracellular injection of dyes, tract-tracing methods and immunocytochemical methods to localize a number of compounds including neurotransmitters and related substances. At the light microscope level, there are observed a variety of cell body shapes and sizes, dendritic morphologies, local axonal patterns, projection sites and chemical characteristics. At the electron microscope level, variability is found with regard to the local and distant synaptic connectivity of pyramidal cell axons, and with regard to chemical characteristics, number, proportion and distribution of asymmetric and symmetric synapses on the different parts of pyramidal cells.

It seems clear that pyramidal cells located in different layers and/or areas participate in different synaptic circuits and, since pyramidal cells whose somata occupy different layers largely project to different sites, the projection site can be considered as one of the most important features for distinguishing between pyramidal cells. When the population of pyramidal cells is subdivided according to this criterion, it is observed that a number of characteristics are shared by all members of each class with relatively little variation; this could be a clue for the correlation with their physiological properties. However, many of these common characteristics are often not exclusive for the class, but are shared among one or several classes of pyramidal cells. It is usually the ensemble of several common properties that better defines a single class of pyramidal cells. Therefore, it is necessary to study in detail the synaptic organization, morphology and chemical characteristics of the various classes of pyramidal cells in order to better understanding the synaptic basis of their distinct functional attributes.

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