Human Motor Cortex Excitatory–Inhibitory Neuronal Systems: Development and Cytoarchitecture

6

An essential feature of the mammalian cerebral cortex is the sequential establishment of excitatory–inhibitory neuronal assemblages between pyramidal and local-circuit inhibitory neurons. The recognition, location, and distribution of these excitatory–inhibitory neuronal assemblages are fundamental objectives in the study of the nervous system. They are recognized in all strata of the motor cortex. Most local-circuit interneurons recognized in the motor cortex are inhibitory in nature. Because, pyramidal cells represent roughly the 70% and the local-circuit interneurons the 30% of the motor cortex gray matter neurons, each inhibitory neuron establishes synaptic contacts with numerous pyramidal neurons. The excitatory pyramidal neurons are morphologically stable and functionally anchored to the first lamina. On the other hand, the inhibitory neurons are free, without first lamina attachment, capable of modifying their dendritic and axonic profiles as well as their spatial distribution in response to learned (acquired) new motor activities. Excitatory–inhibitory functional systems are essential components of the cerebral cortex normal function, and possibly of abnormal and or altered functional activity (Lewis et al. 2005). The information on inhibitory interneurons already available in the literature is considerable. In this chapter describes the developmental histories of four basic excitatory–inhibitory systems (Martinotti, basket, double-tufted, and chandelier) of the human motor cortex, using the rapid Golgi procedure. While all pyramidal neurons of the cortex are generated in the ependymal epithelium, the origin of inhibitory interneurons seems to be extracortical. Inhibitory neurons enter into the developing cerebral cortex advancing horizontally, following a proximal (ventral) to distal (dorsal) gradient and become sequentially incorporated into it, paralleling the ascending maturation of the various pyramidal cell strata (Chapter 3). In the developing human motor cortex, horizontally traversing neuronal precursors are recognized as early as the 15th week of gestation (Chapter 3). The morphology of horizontally traversing neurons is undefined and cannot be identified as distinct types. The recognition of the inhibitory neurons distinctive morphologies occurs later in development and coincides with ascending maturation of the pyramidal neurons of the various strata (Chapter 3).

Therefore, following their incorporation into the developing motor cortex, these neurons go through two stages: an early undifferentiated one as they become incorporated into a specific pyramidal cell strata and a later one when they acquire distinctive morphological and functional features. The motor cortex inhibitory interneurons are characterized by distinctive dendritic profiles, by specific axonic terminals on pyramidal neurons and by wanting first lamina dendritic attachment. Their recognition parallels the ascending functional maturation of the various pyramidal cell strata with which they establish functional contacts. They are first recognized through the lower and older pyramidal cell strata and subsequently throughout upper and younger ones. While they are recognized in lower pyramidal cell strata, they remain undifferentiated and unrecognizable in upper and younger ones. By the time of birth, various types of inhibitory local circuit interneurons are recognized throughout all-pyramidal cell strata of the motor cortex (Marín-Padilla 1969, 1990).

From the 8th to the 16th week of gestation, only pyramidal neurons anchored to first lamina by their terminal dendrites are recognized in the motor cortex. From the 16th to the 22nd week, small local-circuit

M. Marín-Padilla, *The Human Brain*,
DOI: 10.1007/978-3-642-14724-1_6, © Springer-Verlag Berlin Heidelberg 2011

neurons, without distinctive dendritic and/or axonic features, start to be recognized among the pyramidal neurons of the lower strata. At these ages, no inhibitory neurons are recognized in the upper, younger, and still undifferentiated pyramidal cell strata. Subsequently and up to birth time, four types of inhibitory neurons (Martinotti, basket, double-bouquet, and chandelier) are recognized in the human motor cortex following an ascending maturation that parallels that of pyramidal neurons.

Four distinct excitatory–inhibitory systems have been, so far, recognized in the developing human motor cortex. They include the pyramidal-basket, the pyramidal-Martinotti, the pyramidal-double-bouquet, and the pyramidal-chandelier systems, respectively. Each one is characterized by distinctive dendritic profiles, specific axonic terminals on pyramidal neurons and by a 3-D (spatial) distribution within the motor cortex. Their functional targets on pyramidal neurons include: the terminal dendritic bouquets for Martinotti cells, the soma for basket cells, the apical dendritic shaft for double-tufted cells, and the axon-hillock for chandelier cells. The developmental histories, morphological, and functional features of these four excitatory–inhibitory neuronal systems are described below. Since most inhibitory neurons appear to be spatially oriented, the rapid Golgi preparations need to be cut parallel, perpendicular, and tangential to the gyrus long axis.

It should be emphasized that there are additional short-circuit interneurons throughout the cerebral cortex as well as additional excitatory–inhibitory systems, which are not yet fully identified.

6.1 The Pyramidal-Basket System

Undoubtedly, the best-known excitatory–inhibitory system is that formed between pyramidal neurons and their corresponding inhibitory basket cells. This unique neuronal association was originally described in the mammalian cerebellum, hippocampus, and cerebral cortex (Cajal 1911; Andersen, Eccles and Løyning 1963a; Andersen et al. 1963b; Eccles 1964; Szentágothai 1965, 1975, 1978; Marín-Padilla 1969, 1990). Cajal described the formation of pericellular nests (baskets) around the cell body of cerebellar Purkinje cells, of hippocampus pyramidal cells and, eventually, on pyramidal neurons of the human visual and motor cortex (Cajal 1893, 1899). In the cerebellum and hippocampus, Cajal demonstrated the association of the pericellular baskets with a distinct type of local-circuit stellate interneuron; but did not describe the original interneuron that formed the baskets around the pyramidal neurons in the cerebral cortex (Cajal 1911). However, he suggested that large stellate neurons with horizontal axonic collaterals contiguous to pyramidal neurons could by the source of the baskets (Cajal 1911). In 1965, Szentágothai, using chronically isolated cortical slabs, with intact microvasculature, demonstrated the inhibitory function of the pericellular nest around pyramidal neurons and the local distribution of the inhibitory neurons forming them (Szentágothai 1965, 1975, 1978). Marín-Padilla, using rapid Golgi preparations, described a distinct type of stellate interneuron that form pericellular basket around the body of pyramidal neurons of the human motor and visual cortex, which since them, they are recognized as basket cells (Marín-Padilla 1969, 1970, 1972; Jones and Hendry 1984). The axonic terminals of pericellular baskets were retrograded up to these stellate interneurons and these neurons' axonic terminals were followed up to the baskets. The pyramidal-basket assemblage is the best-studied excitatory–inhibitory system of the cerebral cortex, including that of humans (Eccles 1964; Marín-Padilla 1969, Jones 1984; Marín-Padilla Jones and Hendry 1984; 1970, 1972, 1974, 1990).

The Basket Cell. The association of large stellate interneurons with large pyramidal neurons is a distinctive feature of the human motor cortex (Fig. 6.1). Basket cells are local-circuit stellate inhibitory interneurons characterized by ascending, descending, and horizontal dendrites and by an ascending and/or descending axon that branch, at various levels, into multiple long horizontal collaterals. From the long horizontal collaterals emerge numerous shorter branches that participate in the formation of the pericellular nests (baskets) around the pyramidal cells bodies (Figs. 6.1–6.3). The presence of distinct stellate basket cells among pyramidal neurons is recognized in the human motor cortex from the 30th week of gestation up to birth time. Their arrival into the developing motor cortex precedes this gestational age. At birth, these inhibitory interneurons have spiny dendrites. The terminals arising from the neuron long horizontal axonic collaterals participate in the formation of

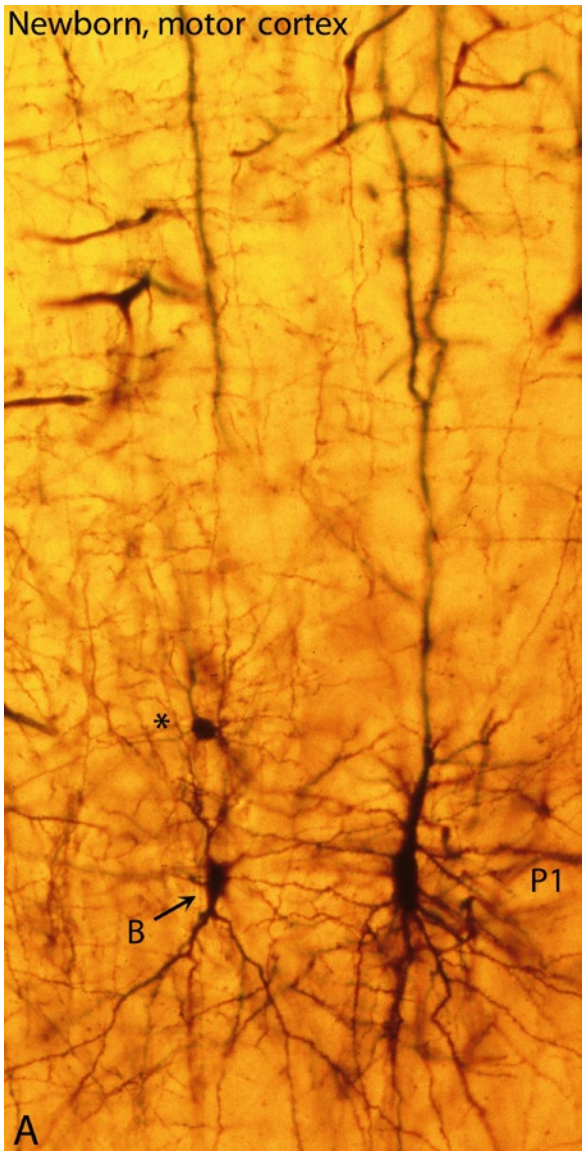

Fig. 6.1 Photomicrograph from a rapid Golgi preparation of the newborn infant motor cortex showing, comparatively, the size and dendritic profiles of a large pyramidal neuron of stratum P1 with apical dendrite reaching first lamina, a giant stellate basket cell (B) of the same stratum and a smaller (*) undetermined neuron. There are also numerous vertical and horizontal fiber terminals

multiple pericellular baskets (Figs. 6.2 and 6.3). A single basket cell participates in the formation of pericellular baskets around pyramidal neurons of its strata and of adjacent ones. Also, each pericellular basket is formed by the contributions of axonic terminals from several basket cells from its adjacent strata (Figs. 6.2 and 6.3). Basket cells are recognized in all pyramidal cell strata. Their size and location varies paralleling that of its associated pyramidal neurons (Marín-Padilla 1972). In the newborn human motor cortex, basket cells of various sizes are recognized, including: giant (P1 stratum), large (P2), medium (P3–P4), and small (P5–P6) basket cells, respectively (Marín-Padilla 1970, 1990). Regardless of their different sizes and/or location they all share similar dendritic and axonic profiles, reflecting that of its associated pyramidal neurons (Figs. 6.1–6.3).

The functional territory of a single basket cell is quite large and extends through adjacent pyramidal cell strata (Fig. 6.2 and 6.3). The basket cell size, its functional territory, and the number of pericellular baskets formed decrease from lower to upper pyramidal cell strata (Figs. 6.2 and 6.3). In the newborn motor cortex, basket cells throughout the upper pyramidal cell strata (P6–P5) are still immature and their contributing baskets are only partially constructed (Figs. 6.2a, b). Basket cells throughout lower pyramidal cell strata (P1–P2) are larger and their contributing baskets are larger and completely formed (Fig. 6.3b, c). The basket cells and the size and complexity of their baskets continue to increase during postnatal developments (Fig. 6.3b, c).

It is also important to point out, that a basket cell can only be visualized in its entirety (dendritic and axonic profiles and terminal baskets) in rapid Golgi preparations cut perpendicular to the pial surface and to the long axis of the gyrus. Basket cells are flat stellate neurons spatially oriented perpendicular to the gyrus long axis and, consequently, their morphological appearance might change depending of the angle of view. In rapid Golgi preparations cut perpendicular to the gyrus, it is relatively easy to locate these stellate interneurons among the pyramidal neurons (Fig. 6.1). In these preparations once the basket cell soma is localized, by moving the microscope micrometer – up and down – (5 μm each time), it is possible to follow-up its dendritic and axonic arbors in their entirety and determine their number, length, distribution, as well as the number of terminal baskets formed by them. It is also possible to determine the overall thickness of the selected basket cell. By calculating the number of micrometer moves – up and down – it is possible to estimate the basket cell thickness. The overall thickness of the large basket cells of pyramidal cell strata P1 and P2 ranges between 45 and 55 μm. The basket cell thickness and the size of their

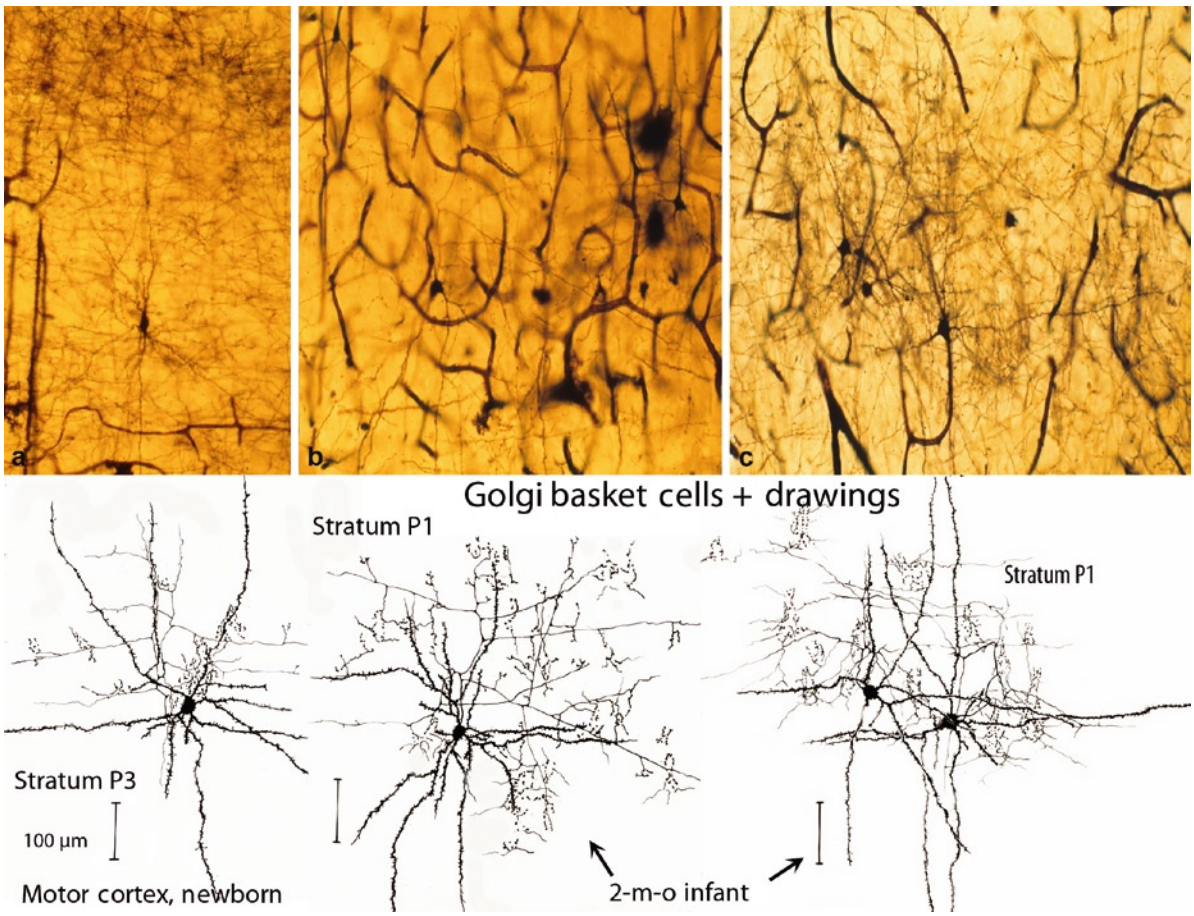

Fig. 6.2 Montage of photomicrographs and corresponding camera lucida drawings, from rapid Golgi preparations, of newborn infant's motor cortex illustrating the size, location, stellate morphology, and dendritic and axonic profiles of inhibitory basket interneurons. Basket cells from the following pyramidal cell strata are illustrated: from pyramidal strata P6 (**a**), P5 (**b**), and P4 (**c**) strata. The axon terminals of all basket cells participate in the formation of perisomatic nests (baskets); some of them are still incompletely formed

functional territories throughout the upper cortical strata are proportionally smaller.

The axonic distribution of a large basket cell could cover a rectangular functional territory that is also flat and perpendicular to the long axis of the gyrus (Figs. 6.2 and 6.3). The size of the functional territory of a large basket cell, from P1 to P2 pyramidal cell strata, measures roughly 50 µm in width, 500 µm in height, and 1,000 µm in length (Fig. 6.4b). The sizes of the functional territories of baskets cells of upper pyramidal cell strata (P3–P5) are proportionally smaller. To corroborate the basket cells spatial orientation, a computer reconstruction of a large basket cell was carried out (Fig. 6.4a). Separate and sequential camera lucida drawings, of a selected large basket cell from a 2-month-old (m-o) infant motor cortex, were made at 5 µm intervals until the entire neuron was drawn (Marín-Padilla and Stibitz 1974). Starting with the neuron body, each separate drawing records only those dendritic and axonic processes that appear in sharp focus (Fig. 6.4a). The series of incomplete drawings were digitalized individually and, with a computer program, they were reconnected to obtain a digitalized 3-D model of the neuron (Fig. 6.4a). It was then possible to rotate the digitalized basket cell and view it from different angles, ranging from 0° to 80° (Fig. 6.4a), thus confirming that basket cells have flat rectangular dendritic and axonic functional territories oriented perpendicular to the pial surface and to the long axis of the precentral gyrus (Fig. 6.4b).

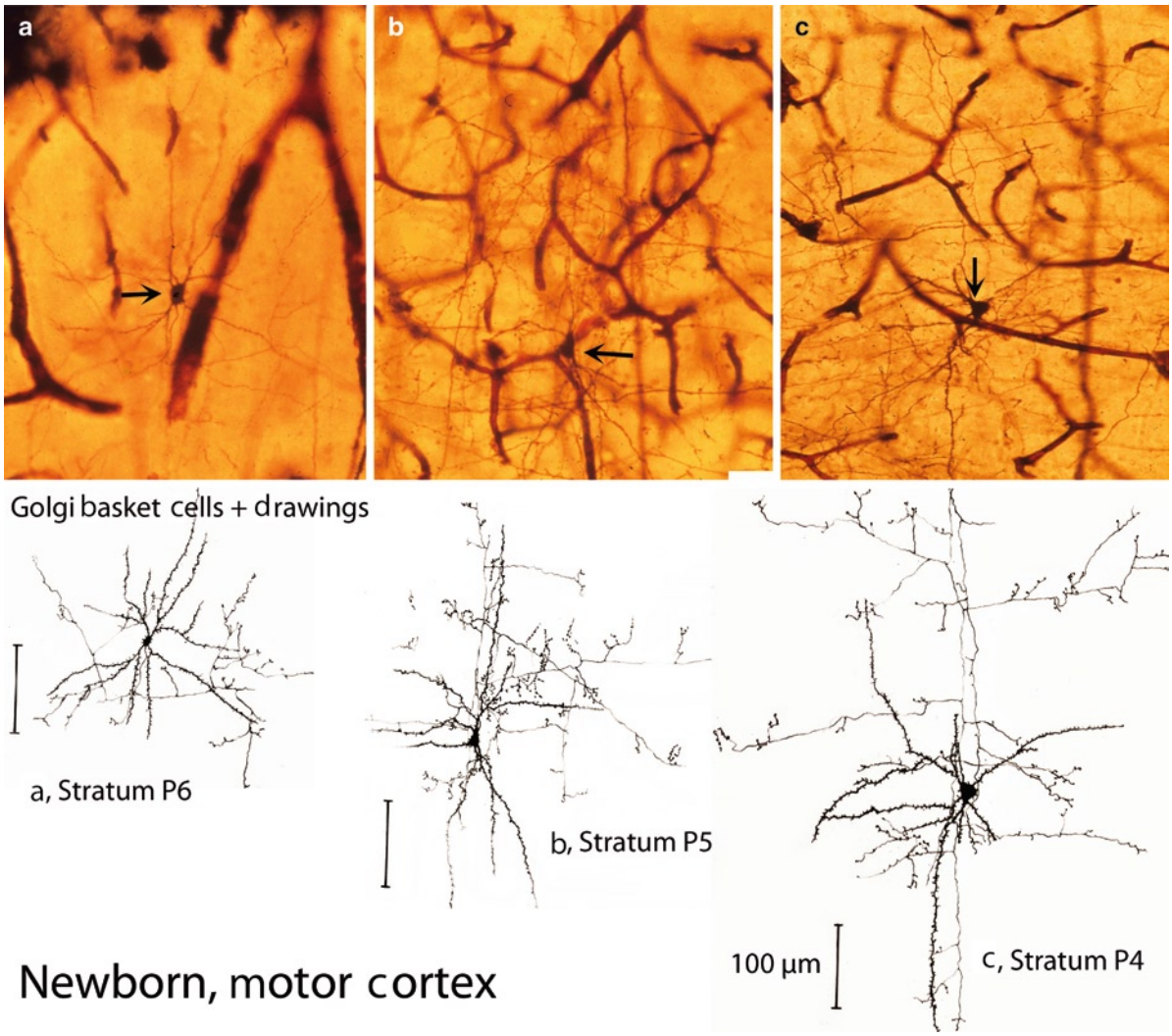

Fig. 6.3 Montage of photomicrographs and corresponding camera lucida drawings, from rapid Golgi preparations, of the motor cortex of newborns and a 2-month-old infants, illustrating the size, location, stellate morphology, and dendritic and axonic profiles of basket cells from various pyramidal cells strata, including P3 (**a**) and P1 (**b, c**) strata. The axon terminals from all basket cells participate in the formation of perisomatic baskets around pyramidal cell bodies. The number and complexity of the baskets are greater than those of the upper pyramidal cell strata (compare with those of Fig. 6.2)

Consequently, a basket cell viewed in profile (at an 80° angle) and/or in sections cut parallel to the gyrus axis assumes the morphologic features of a bipolar interneuron (Fig. 6.4a). In this context, it should be pointed out that Cajal, in his book, describes a type of double-bouquet (bipolar) neuron associated with the formation of pericellular baskets (Cajal 1911). Since the orientation of Cajal's Golgi preparations was not documented, the double-bouquet neuron he described could have actually represented a basket cell viewed in profile.

A single basket cell forms pericellular baskets around the bodies of all pyramidal neurons within its rectangular functional territory. The functional territory of any basket cell overlaps with contiguous ones, above and below it, with a similar spatial orientation (Fig. 6.4b). From superficial P6 to deep P1 pyramidal cell strata, the motor cortex is subdivided into a series of overlapping rectangular functional territories perpendicular to both pial surface and the long axis of the gyrus, which are interconnected by their basket cells (Fig. 6.4b). The motor cortex may be viewed as a series of perpendicular

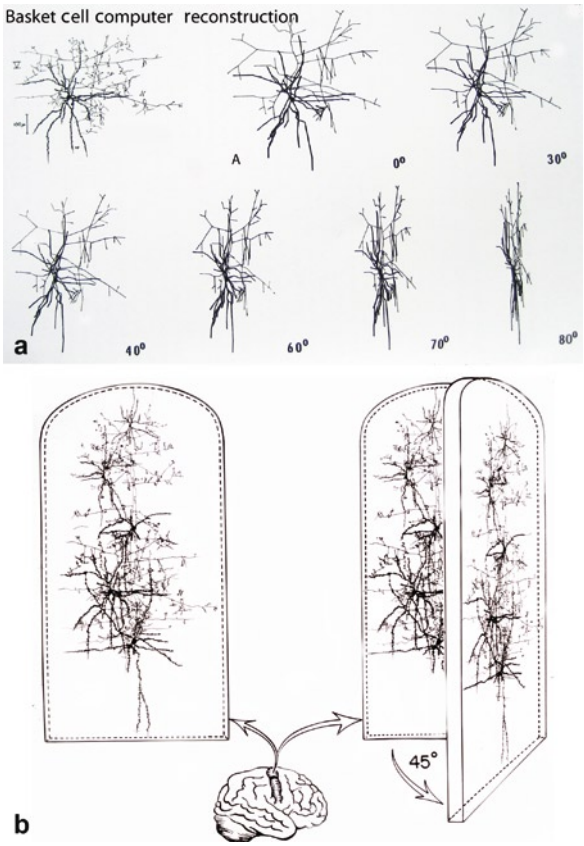

Fig. 6.4 Schematic representations of (**a**) the rotation of a computer reconstructed basket cell of P1 pyramidal cell stratum of the motor cortex of a 2-month-old infant and of (**b**) the vertical and rectangular pyramidal-basket functional assemblages established in all pyramidal cell strata of the motor cortex, which extend throughout the entire gyrus like the pages of a book. The progressive rotation of the basket cell (A) changes its stellate morphology into a bipolar one. Basket cells are flat and spatially oriented neurons and their morphologic appearance varies depending on the angle of view (see also Figs. 6.2 and 6.3).

and spatially oriented rectangular functional territories composed of excitatory–inhibitory pyramidal-basket systems extending throughout the entire precentral gyrus, like the pages of a book. The size and orientation of these pyramidal-basket functional territories are not unlike the narrow and vertical functional fields established by some specific corticipetal fibers systems (Blakemore and Tobin 1972; Hubel and Wiesel 1977; Jones 1984; Jones and Hendry 1984).

The fact that basket cells are spatially oriented and that their morphologic appearance may changes as the angle of observation changes is an important observation with significant implications. Specific spatial orientations may also be applicable to other excitatory–inhibitory systems. Possibly, all local-circuit interneurons of the mammalian cortex have spatially oriented functional territories. Therefore, the need to establish the spatial orientation of any brain section is mandatory as the appearance and distribution of some local-circuit interneurons could change depending on the angle of view. On the other hand, all pyramidal neurons, throughout the cerebral cortex, lack specific spatial orientation and their morphologic appearance remains unchanged from any angle of view. However, all pyramidal neurons throughout the cerebral cortex are interconnected by spatially oriented functional systems, represented by the inhibitory neurons of its various strata. The bland and uniform appearance of the pyramidal neurons of the cerebral cortex, from any angle of view, gain considerable functional relevance by its association with the spatially oriented inhibitory interneurons.

The C-R cells that target the pyramidal terminal dendrites throughout the neocortex represent another important and spatially oriented functional system of the cerebral cortex (Chapter 5).

The mammalian neocortex can be thought of as a complex organization composed of superimposed strata of fixed (unchangeable) excitatory pyramidal neurons bounded and functionally interconnected by spatially oriented inhibitory systems capable of establishing functional contacts only with neurons within their functional territory, such that, a single pyramidal cell column may receive functional contacts from differently oriented inhibitory interneurons. In this context, the motor cortex may be thought of as a forest (paraphrasing Cajal's idea) with innumerable immovable tree trunks (pyramidal cells apical dendrites) and by a series of moving shadows that make contacts with different tree trunks. As the shadows move, different tree trunks become contacted. While the immovable tree trunks represent the neocortex immovable projective pyramidal neurons, the moving shadows represent the spatially oriented local-circuit inhibitory neurons. During the subsequent development and functional maturation of the neocortex, the numbers of different spatially oriented inhibitory neuronal systems contacting pyramidal neurons and their extent could be extraordinary and unimaginable, thus multiplying many folds the functional possibilities of the same neurons. The need for further explorations into the morphology and function of these inhibitory neuronal are important goals in the study of the human brain.

The Pericellular Nest or Basket. The presence of stellate interneurons among the large pyramidal neurons of P1 stratum is first recognized around the 25th week of gestation. However, at this stage, the pericellular baskets are not yet recognizable as distinct structures and these interneurons have not yet developed distinct dendritic profiles. During the neocortex subsequent ascending functional stratification, stellate neurons (no yet fully developed) forming terminals pericellular baskets are progressively recognized among the ascending maturation of the various pyramidal cells strata. Fully developed pericellular baskets have been described in the human motor and visual cortex of newborn infants, using the rapid Golgi procedure (Marín-Padilla 1969, 1970, 1972, 1974, 1990).

By the time of birth, pericellular baskets are recognized throughout all pyramidal cell strata (Figs. 6.2 and 6.3). A pericellular basket is a prominent, triangular shaped, and complex structure composed of numerous axonic terminals that establish axo-somatic contacts with the pyramidal cell body (Fig. 6.5a, b). The triangular (pyramidal) shapes of baskets mimic that of pyramidal cell bodies (Figs. 6.6–6.8). It is important to point out that, in rapid Golgi preparations, a pericellular basket can only be visualized if the pyramidal cell body is not stained (Figs. 6.5a, b and 6.6a, b). In rapid Golgi preparations that the pyramidal cell body is stained, the pericellular baskets will be unrecognizable (Fig. 6.1; and Figs. 4.1, 4.9 and 9.3). Contrarily, if the pyramidal cell body is not stained the entire pericellular basket can be visualized (Figs. 6.6–6.9). This rapid Golgi procedure welcome idiosyncrasy has permitted the visualization of the entire pericellular basket and has facilitated its 3-D reconstruction. This welcome rapid Golgi behavior remains inexplicable and cannot be preplanned, which further supports the idea of making as many Golgi preparations as is possible. Perhaps one of those preparations could offer an unexpected but extraordinary view of the nervous tissue (Chapter 12).

At low magnification, the pericellular baskets throughout the lower pyramidal cell strata (P1 and P2) are quite prominent and contact sharply with the surrounding tissue (Figs. 6.5a, b). The concentration of pericellular baskets throughout the upper pyramidal cell strata is less conspicuous (Figs. 6.2 and 6.3). The staining of the pyramidal cell body will obscure the whole pericellular axonic nest visualizing only its lateral elements. Contrarily, if the neurons body is unstained, the pericellular basket anterior, laterals and posterior walls

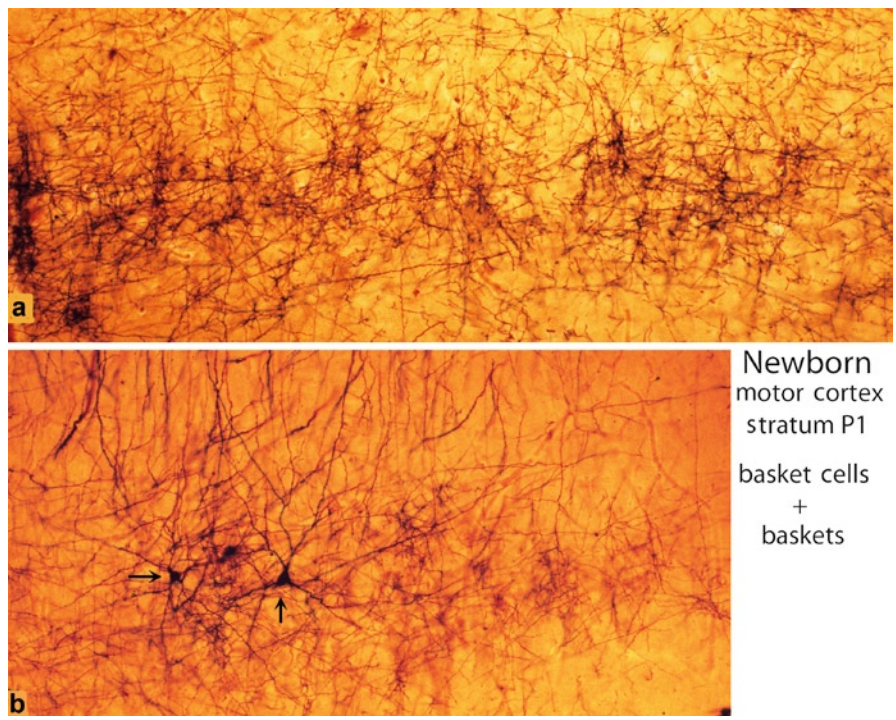

Fig. 6.5 Montage of photomicrographs, from rapid Golgi preparation of newborn infant's motor cortex, showing (**a**) the number and structural complexity of pericellular baskets from around the bodies of unstained pyramidal neurons of stratum P1 and of a few basket cells (**b**) associated with them. In Golgi preparations, pericellular baskets can only be visualized if the pyramidal neurons are unstained. Numerous axonic terminals from basket cells concentrate around the unstained pyramidal cell somata forming roughly triangular-shaped complex pericellular nests or baskets that mimic the neuron's body size and shape. Both illustrations also show numerous fine horizontal fiber terminals representing basket cells horizontal axonic fibers

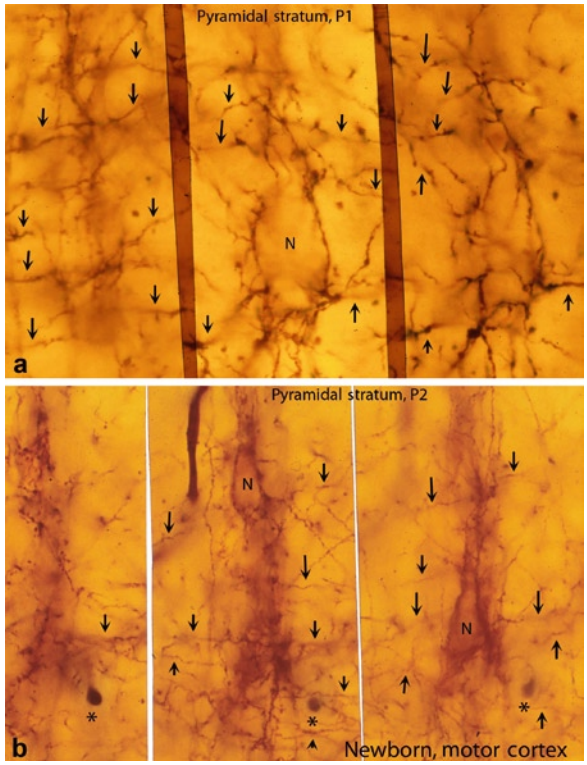

Fig. 6.6 Montage of photomicrographs, from rapid Golgi preparations of the motor cortex of newborn infants, showing, at a higher magnification, three levels (superior, middle, and inferior) of pericellular baskets formed around the unstained body of P1 (**a**) and of P2 (**b**) pyramidal neurons. The illustrations demonstrate the baskets 3-D structural organization, its fiber terminals complexity, and its apparently empty center occupied by the pyramidal neuron unstained (N) body. Arrows identify the large number of horizontal axonic terminals that participate in the basket formation. Asterisks (*) identify a blood capillary coursing near the basket (**b**). The illustrations offer three views of the same basket, which covers a vertical and rectangular area, roughly 45–55 μm thick. The illustrations also demonstrate that good rapid Golgi preparations permits very high resolution without losing the fine structural details of the nervous tissue, in clarity as well as in authenticity

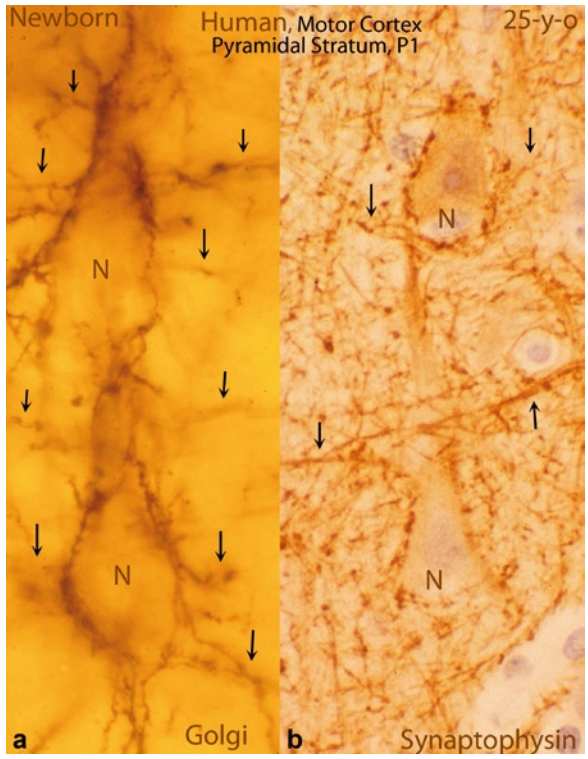

Fig. 6.7 Montage of photomicrographs comparing, at a very high magnification, the structural complexity and remarkable similarities of pericellular baskets from the motor cortex of (**a**) a newborn infant (rapid Golgi preparations) and (**b**) from a 25-year-old man (synaptophysin stain preparations). The arrows indicate some of the horizontal fiber terminals that participate in the basket formation. The baskets apparently empty center is occupied by the unstained pyramidal cells (N) bodies (Golgi preparations), which are partially stained in the synaptophysin stain

are clearly recognized (Figs. 6.6a, b, 6.7a, b, and 6.8a–c). In good rapid Golgi preparations, it is possible to demonstrate the three-dimensional complex organization of the basket and its apparently empty center occupied by the unstained pyramidal cell body (Figs. 6.6a, b, 6.7a, and 6.8a–c). Separate photomicrographs taken above, center, and below a pericellular basket demonstrate its structural complexity, the numerous –terminal, and en-passant-axo-somatic contacts and its apparently empty center (Figs. 6.6–6.8). Each basket is composed of axonic terminals from several adjacent basket cells and any basket cell provides axo-somatic contacts to several pyramidal cell bodies (Figs. 6.6a, b and 6.7a, arrows). The structural organization of a pericellular basket can also be demonstrated, in the adult cerebral cortex, using synaptophysin stain preparations (Fig. 6.7b). These preparations demonstrate the basket numerous axo-somatic lateral contacts around the pyramidal cell body but fail to demonstrate its overall 3-D organization (Fig. 6.7b).

Montages of camera lucida drawings can be made by superimposing three separate ones taken from the basket upper, middle, and lower levels (Fig. 6.8a–c). These montages demonstrate the basket extraordinary structural complexity, the number of axonic terminals reaching it, the number axo-somatic (terminals and in-passant) contacts, as well as their apparently empty center (Fig. 6.8a–c). Electron microscopic and immunocytochemical studies have confirmed the basket's

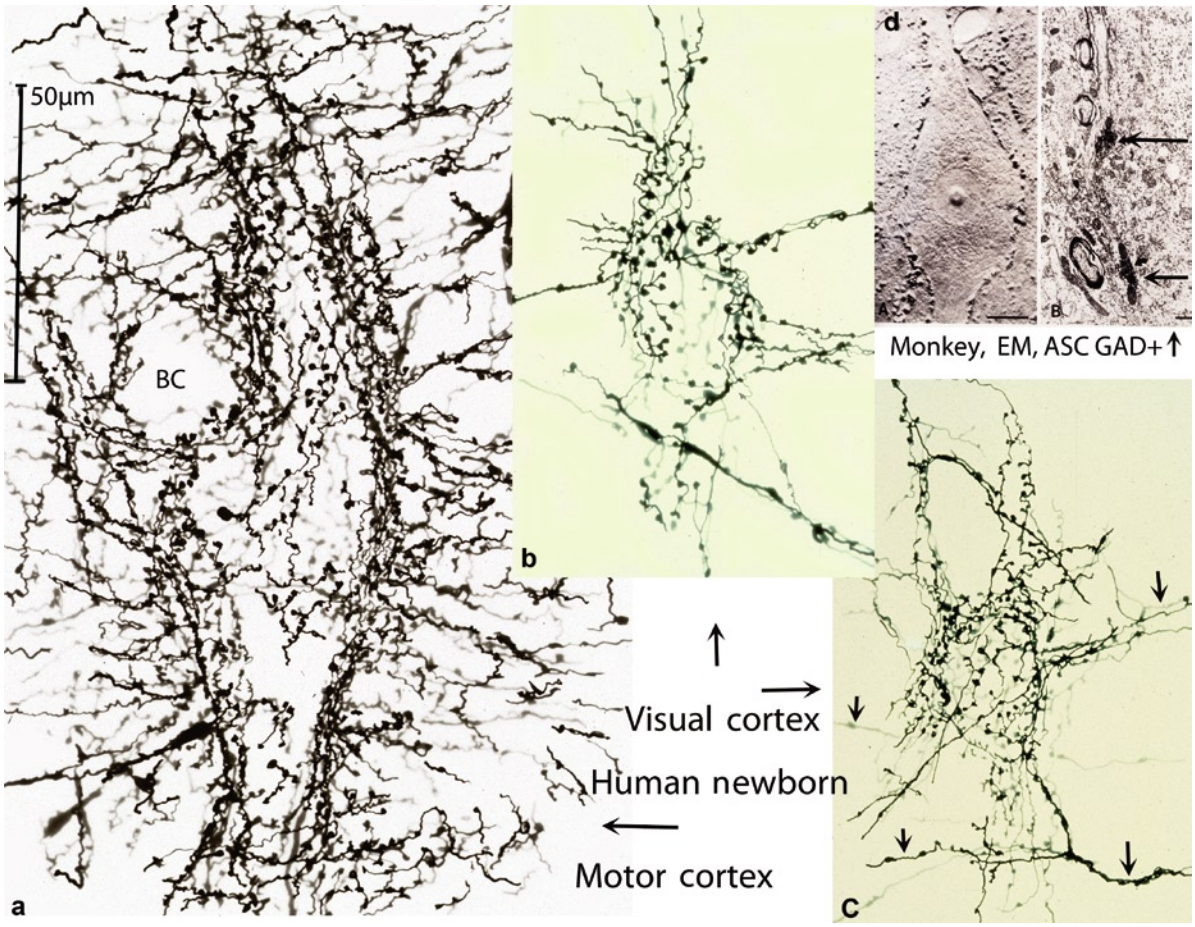

Fig. 6.8 Montage (**a**, **b**, **c**) of camera lucida drawings, showing the basket 3-D structural organization, from rapid Golgi preparations of the motor (**a**) and the visual (**b**, **c**) cortex of newborn infants. Superimposing three separate drawings of each basket anterior, middle, and posterior levels makes the illustrations. The reconstructions also show the numerous, terminal and en-passant, synaptic axo-somatic contacts established on each basket. (**d**) Illustrate an electron microscopic and immunohistochemical (ASC and GAD+) views of baskets from the monkey cerebral cortex showing the numerous axo-somatic synaptic contacts of the pyramidal neuron body as well as their inhibitory nature. (From Jones and Hendry 1984)

numerous axo-somatic contacts as well as their inhibitory nature (Jones and Hendry 1984). These studies, however, failed to convey the basket's 3-D structural organization (Fig. 6.8d), a fact that further encourages the need to use the rapid Golgi procedure to study of the brain's cytoarchitectural organization.

6.2 The Pyramidal-Martinotti System

Martinotti cells with ascending axons that reach and branch within the first lamina are among the earlier recognized local-circuit interneuron in the mammalian developing neocortex (Chapters 2 and 3). These early Martinotti cells are essential components of the neocortex subplate (SP) zone. Together with the SP pyramidal-like neurons and first lamina Cajal–Retzius (C-R) cells, they represent the elements of the primordial cortical organization, prior to the appearance of the mammalian pyramidal cell plate (PCP) (Chapters 2, 3, and 5). Their ascending axon fans into a terminal bouquet that mimics the dendritic bouquets of SP pyramidal-like neurons. These early Martinotti cells are local-circuit interneurons, which interconnect structurally and functionally the SP zone pyramidal-like neurons and the first lamina C-R neurons.

These early SP Martinotti cells are recognized up to around the 15th week of gestation (Chapter 3). Afterward, their axonic terminals start to lose their first

lamina contacts and to regress. During subsequent development, these early SP interneurons (together with pyramidal-like neurons) are progressively displaced downward and transformed into deep interstitial neurons. Subsequently, new Martinotti inhibitory interneurons are progressively incorporated into the cortex PCP paralleling the ascending maturation of its pyramidal cell strata. Martinotti cells are bipolar interneurons characterized by long ascending and descending dendrites with spine-like excrescences and by a long ascending axon that crosses the maturing PCP and reaches the first lamina (see Fig. 3.14). Because of their length, the deep (P1 stratum) Martinotti cells with long axon are difficult to see in a single Golgi preparation, although their location, dendritic profiles, and the axon proximal segment can be recognized at various pyramidal cells strata. Similarly their distinct terminal axonic bouquets are also easily recognizable within the first lamina (Fig. 6.9a–c).

The most distinguishing feature of these local-circuit neurons is their ascending axon that reaches and fan into the first lamina forming a terminal bouquet with spine-like projections (Fig. 6.9a–c and Fig. 3.14a). These neurons terminal axonic bouquets mimic the size of the dendritic bouquets of pyramidal neurons of their own strata, which suggest both structural as well as functional

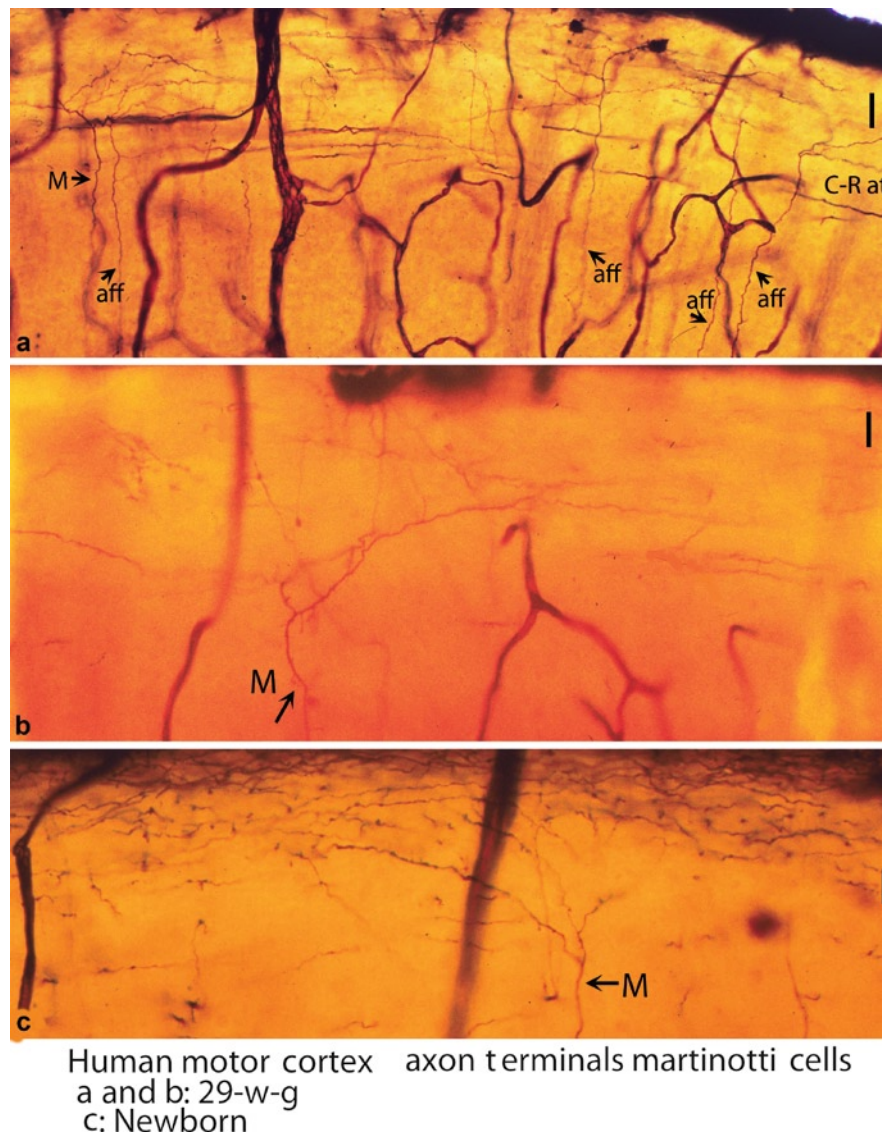

Fig. 6.9 Montage of photomicrographs, from Golgi preparations, of the motor cortex of 29-week-gestation fetuses (**a**, **b**) and of a newborn infant (**c**) showing the distinctive terminal axonic bouquets of deep Martinotti neurons (M) as they branch within first lamina (I), some afferent (aff) fiber terminals (**a**) and the C-R cells axon terminals (C-R at). The size and distribution of Martinotti neurons terminal axonic bouquets mimic the terminal dendritic bouquets of pyramidal neurons of their cortical stratum

interrelationships between them (Fig. 6.9b, c). The new Martinotti neurons are first recognized through the lower pyramidal cells strata and subsequently, during prenatal development, in upper strata. By birth time, Martinotti neurons are recognized in most pyramidal neurons strata (Marín-Padilla 1984). Their size and extent of their terminal axonic bouquets varies with their cortical location, mimicking that of corresponding pyramidal neurons of similar cortical depth. Deep-sited Martinotti neurons form larger axonic terminal bouquets than superficial ones, further suggesting structural as well as functional interrelationship between them.

Probably, Martinotti neurons terminal axonic bouquets establish inhibitory synaptic contacts with the dendritic bouquets of the corresponding pyramidal neurons. Consequently, the functional territory of a single Martinotti cell is local and limited to the terminal dendritic bouquets of pyramidal neurons from its cortical strata. This excitatory–inhibitory neuronal system is the first one established and recognized in cortical development. Additional pyramidal-Martinotti systems are progressively incorporated during the ascending maturation of its various pyramidal cells strata (Marín-Padilla 1984).

6.3 The Pyramidal-Double-Bouquet System

Double-bouquet (Cajal bipenachada) local-circuit interneurons are the most frequently recognized and described inhibitory neurons in the mammalian neocortex (Cajal 1911; Somogyi and Cowey 1984). In rapid Golgi preparations cut perpendicular to the pial surface and the long axis of the precentral gyrus, these cells appears as bipolar neuron characterized by several long and closely arranged ascending and descending dendrites (Figs. 6.10a–d and 6.11a, b). Their size varies according to their location and/or cortical strata: in the newborn motor cortex, ranging from small of P5 stratum (Figs. 6.10a), to medium of P4 stratum (Fig. 6.10b, c), to large of P3–P2 strata (Figs. 6.10d and 6.11a) to giant of P1 stratum (Fig. 6.11c). Their size corresponds to that of pyramidal neurons of similar cortical strata. The incorporation, recognition, and morphological and functional maturations follow as ascending and stratified progression that parallels that of the various pyramidal cell strata.

The neuron primary dendrites emerge from the top and bottom poles of their body and branch into several long ascending and/or descending cascading branches (Figs. 6.10 and 6.11). The dendrites are closely arranged and extend for a considerable distance on either direction. The length and distribution of the neuron ascending and descending dendrites are similar, appearing as mirror image of each other (Figs. 6.10a–d and 6.11a, b). These neurons are the most commonly recognized in the developing human motor cortex. The morphology of many still undifferentiated local-circuit interneurons is essentially bipolar.

These interneurons axonic terminal profiles are also quite characteristic. The axon emerges from the neuronal body and branches into a series of long and cascading ascending and descending terminals, which are also closely arranged (Figs. 6.10a–d and 6.11a, b). Their axonic distribution covers a vertical cylindrical functional territory. The distance between contiguous axonic terminals corresponds, roughly, with the thickness and the separation of the apical dendrites of its associate pyramidal neurons (Fig. 6.11c). The functional territories of these interneurons vary in size, and decreases from lower to upper cortical strata. Moreover, the distance between its long axonic terminals also decreases from lower to upper strata reflecting the size and thickness of the apical dendrites of their corresponding pyramidal neurons (Figs. 6.10 and 6.11). In rapid Golgi preparations (as in the case of basket cells), if the pyramidal neuron's apical dendrites are stained, the double-bouquet neurons axonic terminals will not be adequately visualized or recognized. Contrarily, if the apical dendrites of pyramidal neurons are not stained, the long cascading axonic terminals of these interneurons are clearly visible (Fig. 6.11c). Their ascending and descending axonic processes have spine-like excrescences (Fig. 6.11c, arrows). These excrescences will facilitate direct contact with the dendritic shaft, between the numerous spines. These axonic excrescences could represent the double-bouquet interneurons with specialized presynaptic units (Fig. 6.11c).

By the time of birth, double-bouquet interneurons are recognized in all pyramidal cell strata (Figs. 6.10a–d and 6.11a, b). Both, the interneuron size and that of its functional territory increase progressively from upper to lower pyramidal cell strata, paralleling that of their pyramidal neurons functional cohorts (Figs. 6.10 and 6.11). Based on rapid Golgi observations, the functional target of each double-bouquet interneuron

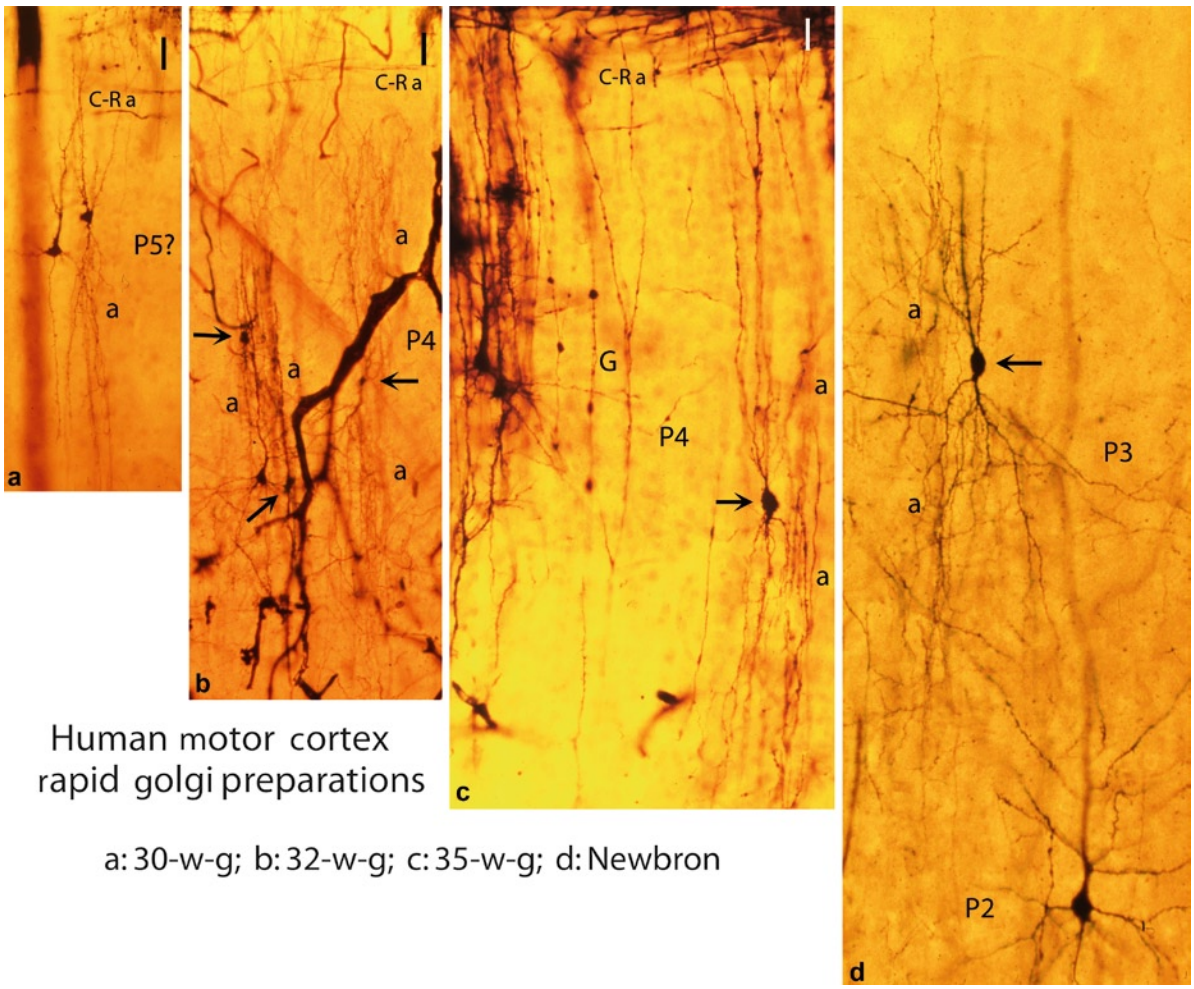

Fig. 6.10 Montage of photomicrographs, from rapid Golgi preparations of the motor cortex of 30- (**a**), 32- (**b**) and 32-week-old (**c**) fetuses and of a newborn infant (**d**), illustrating the location and morphologic features of double-tufted inhibitory interneurons (*arrows*) of pyramidal cell strata P5 (**a**), P4 (**b**), P4 (**c**), and P3 (**d**), respectively. Also illustrated are the C-R cells (C-R a) horizontal axon terminals within first lamina (**a**–**c**), the double-tufted neurons ascending and descending axonic terminals cascades (**a**–**b**), and pyramidal neurons of stratum P2 (**a**), P4 (**c**), and P2 (**d**). The size and functional territories of double-tufted interneuron increases from upper to lower pyramidal cell strata paralleling that of the pyramidal neurons of their corresponding strata

appears to be the apical dendritic shaft of several contiguous pyramidal neurons of the same strata. The double-bouquet neurons functional territory covered by its ascending and descending axonic terminals is vertical and cylindrical. Each one may include a cluster of 6–12 apical dendrites of neighboring pyramidal neurons. Moreover, in tangential rapid Golgi preparations of the human motor cortex, the presence of small (6–8) and large (10–12) apical dendrites clusters is quite common finding. The size of these tangentially cut cylindrical clusters of apical dendrites also decreases from lower to upper pyramidal cell strata and may overlap with each other, throughout the motor cortex. The diameter of the apical dendrites within these clusters also decreases from lower to upper pyramidal cell strata. It is important to point out that the morphologic appearance of double-bouquet neurons remains essentially unchanged in rapid Golgi preparation cut parallel and/or perpendicular to the precentral gyrus long axis.

Therefore, the human motor cortex pyramidal neurons are also interconnected by a series of overlapping cylindrical (columnar) functional territories established by the double-bouquet inhibitory interneurons. These

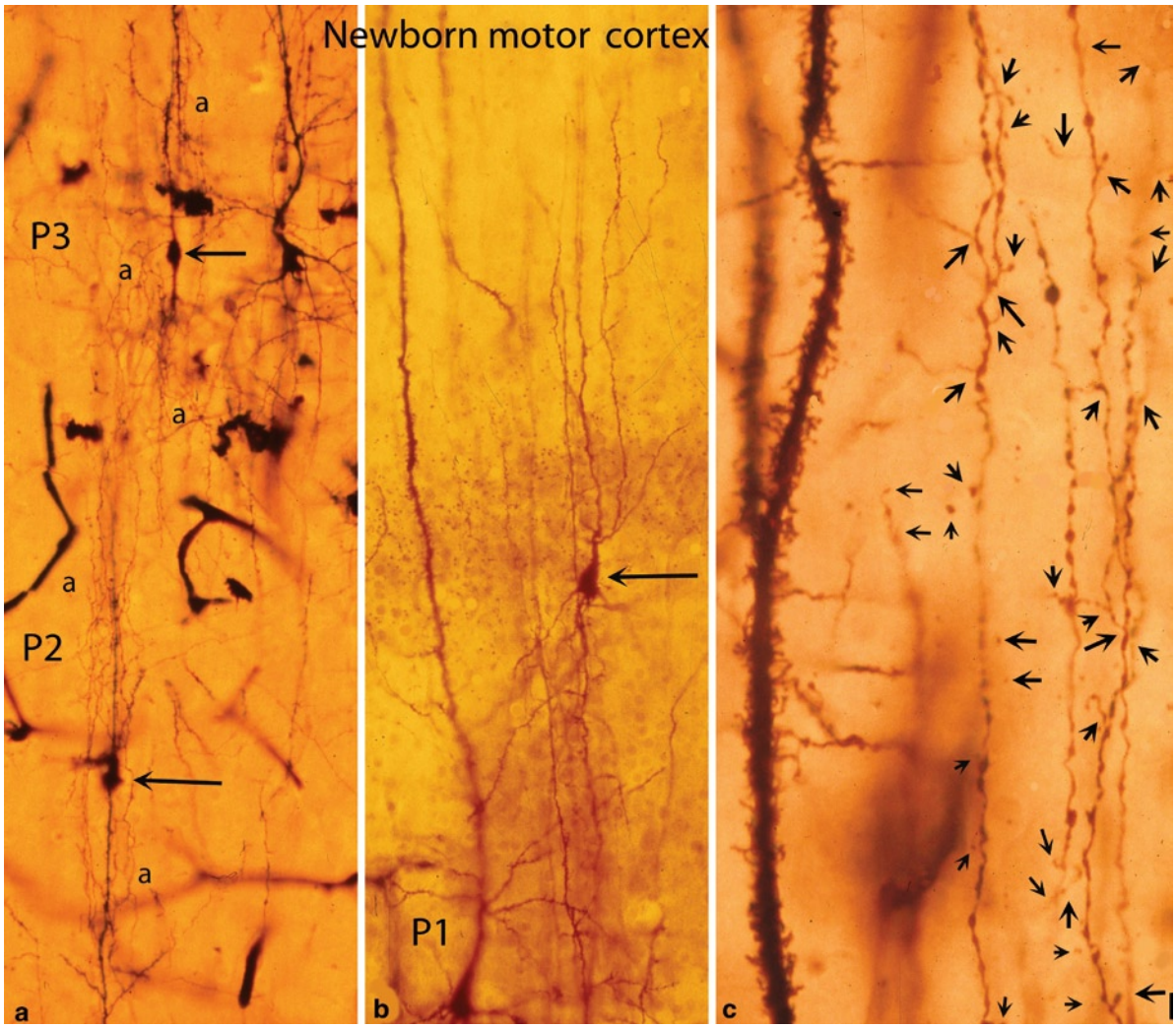

Fig. 6.11 Montage of photomicrographs, from rapid Golgi preparations of newborn infants, illustrating the size, location, and morphologic features of double-tufted inhibitory interneurons of pyramidal cell strata P3 and P2 (**a**) and P1 (**b**). Also illustrated, at a high magnification (**c**), are these interneuron's vertically and closely arranged axonic terminals with numerous spines-like short projections (*arrows*) as well as a segment of a P1 pyramidal neuron apical dendrite for comparison. The distance between these interneurons vertical axonic terminals is roughly comparable to the thickness of pyramidal neurons apical dendrites (**c**) of similar cortical strata. The double-tufted interneurons axonic terminals inter-distance decreases from lower to upper cortical strata reflecting their decreasing size as well as that of their functional cohorts, the pyramidal neurons

columnar functional territories extend throughout the entire thickness of the gray matter, overlap with each other and parallel that of their functional cohorts, the pyramidal neurons (Chapter 3). These functional territories are also sequentially stratified from older, lower, and larger ones to younger, smaller, and superficial ones. The appearance of the double-bouquet inhibitory interneurons and that of their dendritic and axonic profiles and cylindrical functional territories remains unchanged from any angle of view.

6.4 The Pyramidal-Chandelier System

The chandelier (axo-axonic) neuron is the smallest of all known inhibitory interneurons. They are quite common in the human motor and visual cortex (Marín-Padilla 1987). In one of the cases studied (an 8-month-old child), chandelier neurons were quite abundant in the visual cortex striate (area 17) and parastriate (area 18) regions, being particularly numerous at their transitional zone (Fig. 6.12d). This

particular location for chandelier cells has been also mentioned of the visual cortex of rats (Somogyi 1977; Peters 1984), cats (Somogyi 1979; Fairen and Valverde 1980), rabbits (Müller-Paschinger et al. 1983) and monkeys (Somogyi et al. 1982). Although these interneurons seem to be particularly abundant at the visual cortex; they are also found in other cortical regions including the newborn motor cortex (Fig. 6.12a). There are no reasons why they should not be also present in other cortical regions that remain essentially unexplored.

The chandelier neuron is a small multipolar cell with round or globular body (ca. 20 μm diameter), several (six–nine) ascending and descending dendrites with a reduced lateral extension and by an axon with specific axo-axonic terminals (Fig. 6.12a–c). The dendrites are thin, irregular, have focal dilatations and a few long spines-like excrescences (Fig. 6.12d). They also have a "wavy" appearance and short "recurring" collaterals (Fig. 6.12d). The dendritic arbor covers a smaller functional territory than the axon (Fig. 6.12b–c).

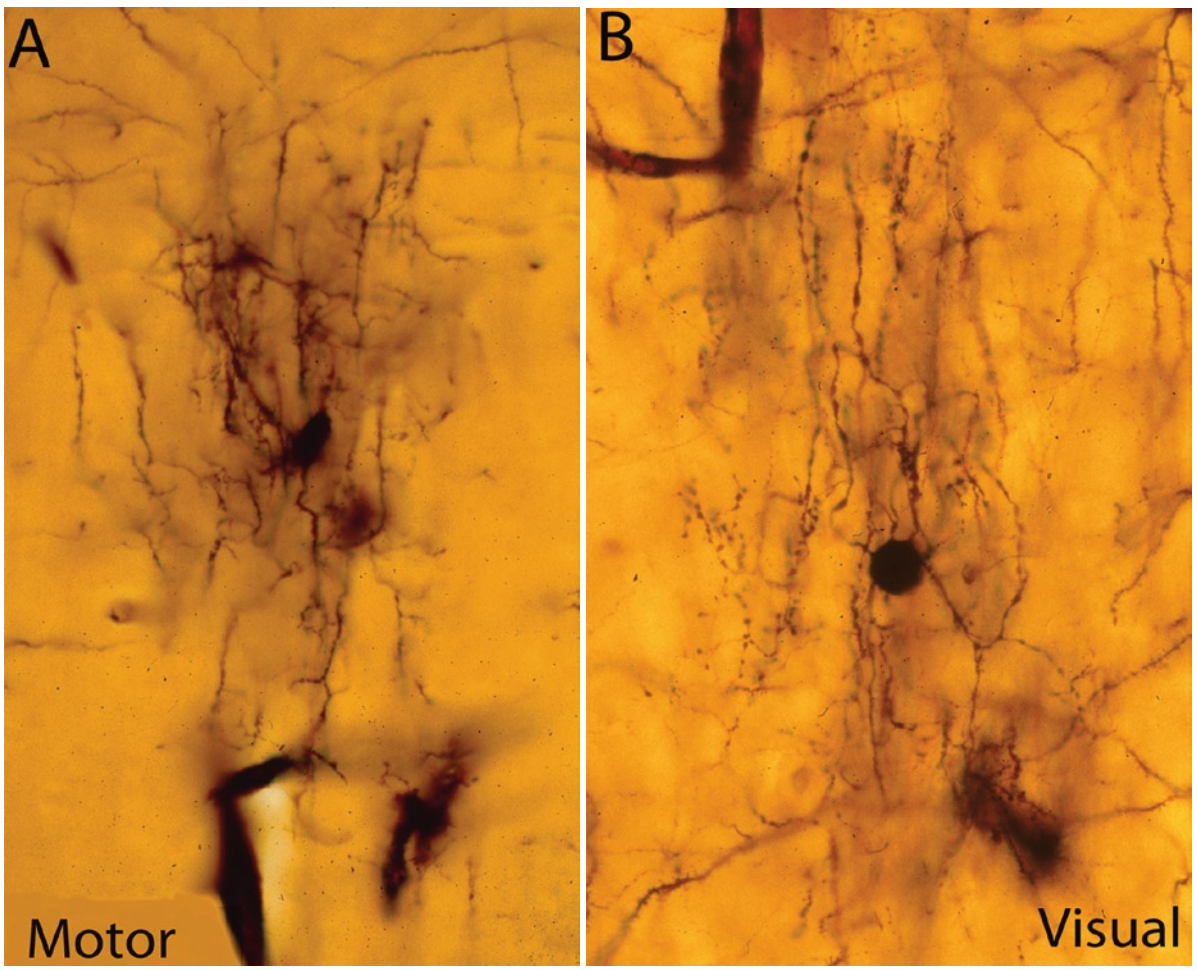

Fig. 6.12 Composite figure of photomicrographs, from rapid Golgi preparations of newborn infants motor (**a**) and visual cortex (**b**, **c**), illustrating the small size and concentrate dendritic and axonic profiles of chandelier inhibitory interneurons. Also illustrated is a camera lucida drawing (**d**) illustrating, in the human primary visual cortex (intersection of areas 17 and 18), the number, size, location, dendritic and axonic profiles, and the small rectangular functional territory of these inhibitory cells. These interneurons axo-axonic terminals form specific and short terminals arrays of axo-axonic synaptic contacts (candles) with the pyramidal neurons axon first segment. In Golgi preparation, in order to visualize these inhibitory interneurons specific axo-axonic terminals (candles) their pyramidal neuron functional cohort must be unstained (**a**–**d**)

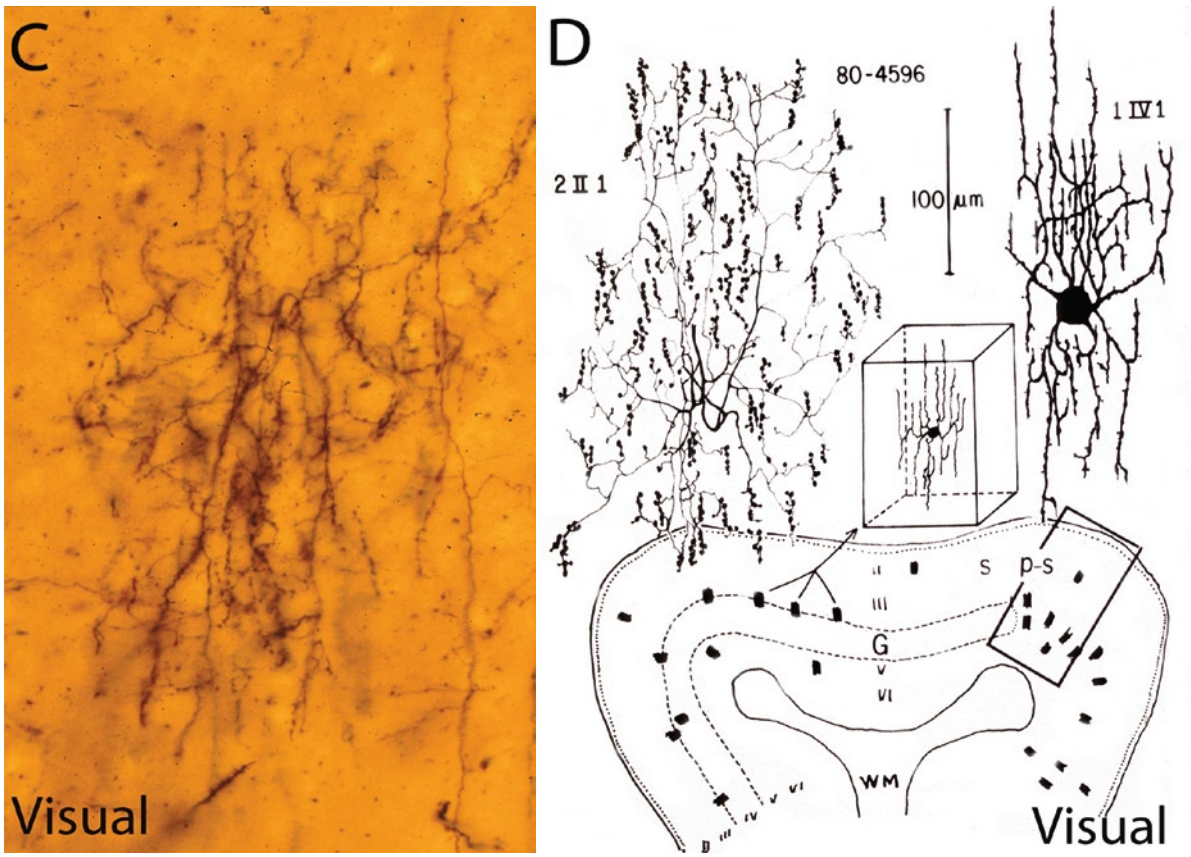

Fig. 6.12 (continued)

The most distinguishing feature of the neuron is its specialized axo-axonic terminals, referred to as candles, and hence its colorful nomination of chandelier cells (Figs. 6.13a–d, asterisks). The axon emerges from the top or bottom poles of the neuron body and immediately bifurcates into several ascending and descending collateral (Fig. 6.12c, d). From these collaterals emerge numerous ascending and descending short vertical branches that carry the neuron specialized synaptic terminals (Fig. 6.13a–d). These neurons specialized axonic terminals are unique structures characterized by short vertical arrays of presynaptic buttons or "candles" (Fig. 6.13, asterisks). In rapid Golgi preparation, these specialized axo-axonic terminals (candles) can only be visualized if the contacted and functional cohort pyramidal neurons are unstained. If the pyramidal neuron is stained, these fine axo-axonic terminals will not be adequately visualized. The structural complexity of these axo-axonic "candles" terminals depends on the number of contacts and could range from simple to complex ones. Simple ones are composed of a few axo-axonic "en-passant" contacts united by a very fine axonic fiber. The complex ones are longer, often have double arrays of presynaptic contacts interconnected by fine fibers (Figs. 6.13a–d, asterisks). In complex terminals, the number of axo-axonic buttons could ranges up to 10.

These inhibitory interneurons terminal axo-axonic arrays (candles) are essentially identical in all mammals so far investigated (Valverde 1983; Marín-Padilla 1987). However, the numbers of "candles" per neuron varies significantly among different mammals. In the human visual cortex, the number of specialized terminals ranges from 60 to 80 "candles." This low number of presynaptic contacts and the neuron's size will make the human chandelier cell the smaller among all mammalian species studied (Lund et al. 1979; Lund 1981; Valverde 1983; Marín-Padilla 1987).

Within the thickness of a rapid Golgi preparation (up to 250 μm) it is possible to visualize the chandelier cells in toto, including their complete dendritic and axonic profiles and their overall functional territory (Fig. 6.12a, b, d). The neuron occupies the center of a relatively small rectangular functional territory, which measures, roughly, 300 × 200 × 100 μm (Fig. 6.12d). Their roughly rectangular functional territory remains unchanged in both perpendicular and parallel cut rapid Golgi preparations.

Although, the human chandelier cell size and functional territory seems to be comparable to that of the monkey (Lund et al. 1979; Lund 1981; Valverde 1983), the neuron size and functional territory within the much larger human brain should be comparatively smaller than of the monkey. Moreover, the overall size of chandelier neuron's functional territories seems to decrease progressively in the course of mammalian phylogeny (Valverde 1983). It appears that the size and functional territory of these inhibitory interneurons decreases progressively through the hedgehog, mouse, rabbit, cat, and monkey (Valverde 1983). A possible interpretation of this phenomenon could be that, in the course of mammalian phylogeny, chandelier cells increase their functional specialization by reducing both the number of pyramidal neurons contacted and the size of their functional territory. During mammalian phylogeny, this type of inhibitory interneurons

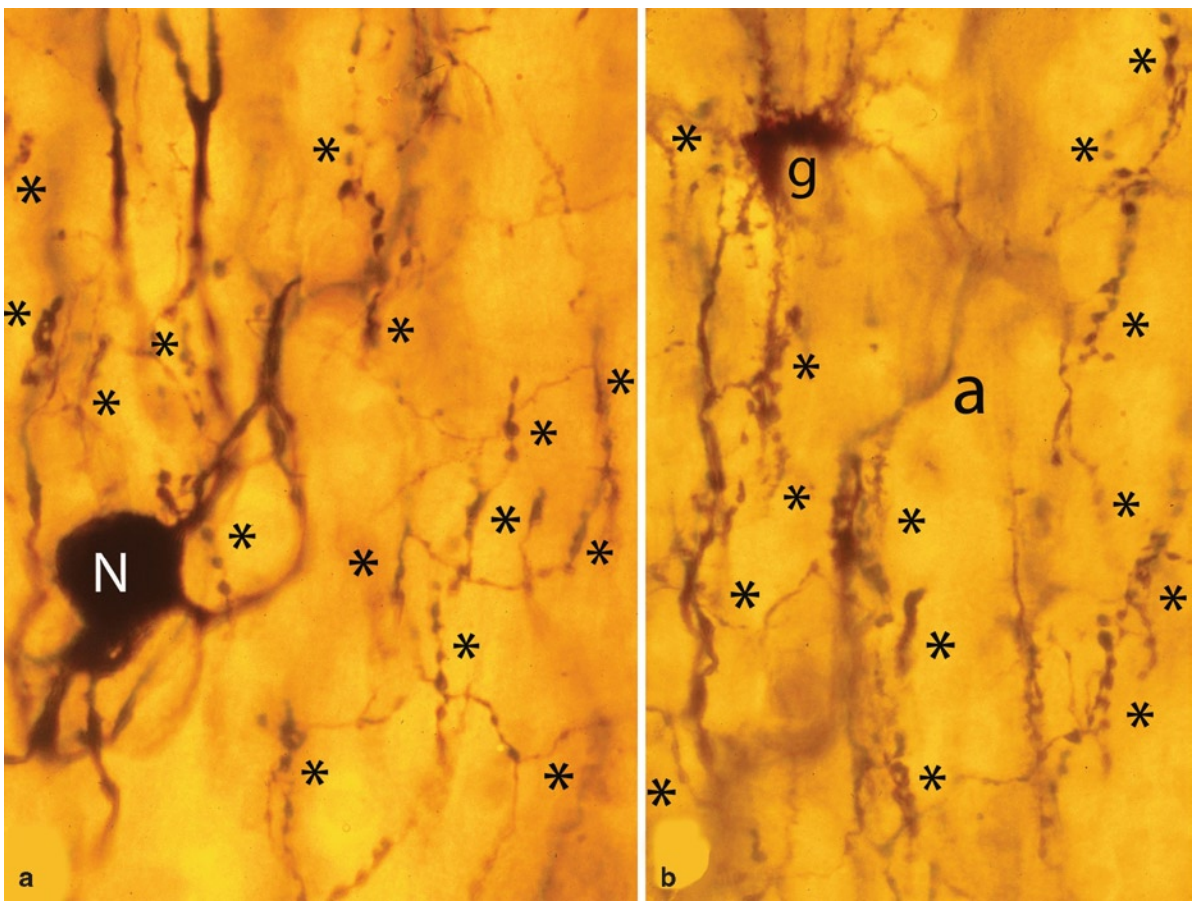

Fig. 6.13 Montage of photomicrographs (**a**–**d**), from rapid Golgi preparation of newborn's primary visual cortex, illustrating, at a higher magnification, the morphological features of the specific axo-axonic terminals (candles) of chandelier inhibitory interneurons. In these preparations, to visualize these specific terminals (*) the pyramidal neurons (functional cohorts) must be unstained. The composition, size, distribution, and number of synaptic axo-axonic contacts (*) of chandelier cells axon (**a**) are clearly demonstrated in these (**a**–**d**) illustrations. A chandelier cell small and globular body and its smooth wavy dendrites are also illustrated (**a**)

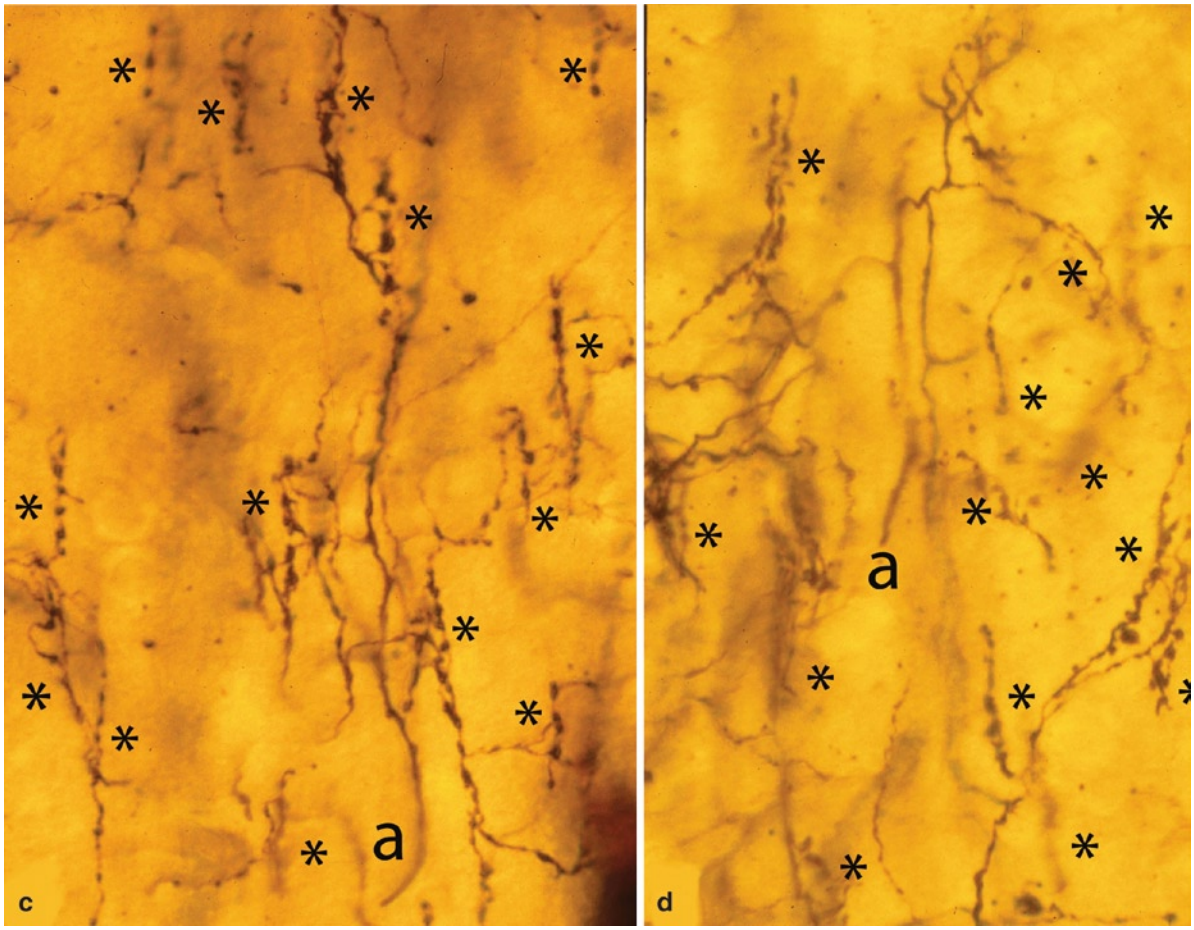

Fig. 6.13 (continued)

shows a tendency toward a reduction of its dendritic and axonic processes. A concentrated pattern of dendrites and axonic distribution has been considered to connote functional specialization (Ramón-Molliner and Nauta 1966; Szentágothai and Arbib 1974).

A functional parameter that supports the idea that chandelier cells might undergo a progressive specialization is the reduced number of axo-axonic terminals (candles) per neuron observed among different mammals. The number of axo-axonic contacts "candles," (or the number of pyramidal neurons contacted) reported in the literature decreases from 220–260 "candles" in rabbits, to 150–250 in cats, to around 100 in monkeys, to 60–80 in the humans visual cortex, per chandelier cell (Tömböl 1976, 1978; Somogyi et al. 1982; Müller-Paschinger et al. 1983; Valverde 1983; Marín-Padilla 1987). Moreover, the size of these inhibitory neuron's functional territory also decreases proportionally. A possible functional specialization of chandelier cells could be related to the increasing visual acuity and motor dexterity that characterize the evolution of mammals.

The functional territories of inhibitory neurons become delineated by the extension of its axonic terminals and hence the number of contacted pyramidal neurons within (Fig. 6.14). The functional territory of a basket cell is a large rectangular slab perpendicular to the pia and the long axis of the precentral gyrus (Fig. 6.14b); that of a double-bouquet interneuron is a vertical and cylindrical one (Fig. 6.14d–t); and, that of a chandelier cell is a small rectangular one (Fig. 6.14c). The number of pyramidal neurons (functional cohorts) contacted by a single basket cell of P1 stratum is probably a hundred (Figs. 6.2, 6.3, 6.5, and 6.14b). The apical dendrites of pyramidal neurons contacted within a single double-bouquet inhibitory interneuron functional

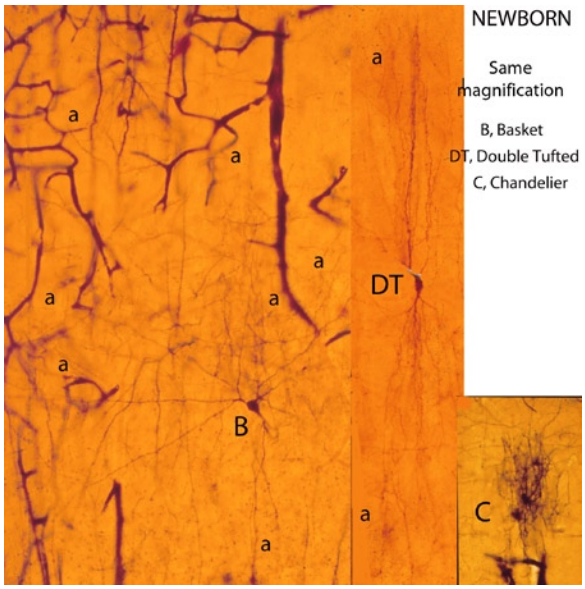

Fig. 6.14 This montage includes three photomicrographs of equal magnification, from rapid Golgi preparations of motor and visual cortex of newborn infants, illustrates comparatively the neuron size and extent of its corresponding functional territory of three inhibitory interneurons, namely: a deep basket (B), a double-tufted (DT), and a chandelier (C) cell. It is important to recognize and to appreciate the significant morphological, functional differences, and spatial orientation among some of the inhibitory interneurons of the human cerebral cortex. The functional implications of these differences are of paramount importance for understanding the function of the human brain

territory may range between from 6 to10 on the upper strata and from 12 to 16 on the lower ones (Figs. 6.10, 6.11, and 6.14d–t). The functional territory of a single chandelier cell of the human visual cortex could establish synaptic contacts with 60–80 pyramidal cells axons (Figs. 6.12, 6.13, and 6.14c). A Martinotti cell may establish synaptic contacts with the terminals dendritic bouquets of several pyramidal neurons of its strata. Possibly, a single Martinotti inhibitory cell may contacts with between 6 and 12 pyramidal neurons.

These significant differences are important contributing factors in the final functional output of the cerebral cortex pyramidal cells. It should be emphasized that the incorporation and functional maturation of these excitatory–inhibitory systems follows an ascending and stratified sequence that parallel that of their functional cohorts, the pyramidal neurons. While the developmental maturation of these inhibitory systems is an ascending process, their functional inputs may follow a descending pathway, from upper to lower strata, paralleling the pyramidal neurons descending functional network (Chapter 4). The motor capabilities and proficiency of each mammalian species will depend on the number, and extent of the excitatory–inhibitory neuronal systems established in their corresponding motor cortex.

The remarkable morphological (different sizes), functional (extend of functional territories), and spatial orientation differences among the inhibitory interneurons of the cerebral cortex are important observations (Fig. 6.14) which must be considered for the understanding and interpretation of the functions of the human brain.

It should be pointed out that besides the four excitatory–inhibitory systems described herein, there are additional ones throughout the cerebral cortex, targeting the pyramidal neurons of the various strata, which remain essentially unidentified. These additional systems should also be investigated, hopefully using the rapid Golgi procedure.

References

Andersen P, Eccles JC, Løyning Y (1963a) Recurrent inhibition in the hippocampus with identification of the inhibitory cells and its synapses. Nature 198:540–542

Andersen P, Eccles JC, Voorhoeve PE (1963b) Inhibitory synapses on somas of purkinje cells in the cerebellum. Nature 199:655–665

Blakemore C, Tobin EA (1972) Lateral inhibition between orientation detectors in the cat's visual cortex. Exp Brain Res 15:439–440

Cajal SyR (1893) Estructura asta de Ammon y fascia dentate. Anales de la Sociedad Española de Historia Natural 22:30–46

Cajal SyR (1899) Estudios sobre la corteza cerebral humana Estructura de la corteza motriz del hombre y mamíferos. Revista Trimestral Micrográfica 4:117–200

Cajal SyR (1911) Histologie du systéme nerveux de l'homme et des vertébrés, vol 2. Maloine, Paris

Eccles JC (1964) The physiology of the synapses. Academic, New York

Fairen A, Valverde F (1980) A special type of neuron in the visual cortex of the cat: a Golgi and electron microscopic study of chandelier cells. J Comp Neurol 194:761–779

Hubel DH, Wiesel TN (1977) Functional architecture of macaque monkey visual cortex. Proc R Soc Lond Ser B 189:1–59

Jones EG (1984) Laminar distribution of cortical efferent cells. In: Peters A, Jones EG (eds) Cerebral cortex, vol 1. Plenum Press, New York, pp 521–553

Jones EG, Hendry SH (1984) Basket cells. In: Peters A, Jones EG (eds) Cerebral cortex, vol 1. Plenum Press, New York, pp 309–336

Lewis DAT, Hashimoto T, Volk DW (2005) Cortical inhibitory neurons and Schizophrenia. Nat Rev Neurosci 6:312–324

Lund JS (1981) ntrinsic organization of the primate visual cortex, area 17 as seem in Golgi preparations. In: Schmitt FO, Worden FG, Adelman G, Dennis SG (eds) Organization of the cerebral cortex. MIT Press, Cambridge, MA, pp 105–124

Lund JS, Henry GH, Mac Queen CI, Harvey AR (1979) Anatomical organization of the primate visual cortex (area 17) of the cat: A comparison with area 17 of the macaque monkey. J Comp Neurol 148:599–618

Marín-Padilla M (1969) Origin of the pericellular baskets of the pyramidal neurons of the human motor cortex. Brain Res 14:633–646

Marín-Padilla M (1970) Prenatal and early postnatal ontogenesis of the human motor cortex. A Golgi study. II. The basket pyramidal system. Brain Res 23:185–191

Marín-Padilla M (1972) Double origin of the pericellular baskets of pyramidal neurons of the human motor cortex. A Golgi study. Brain Res 38:1–12

Marín-Padilla M (1974) Three-dimensional reconstruction of the pericellular baskets of the motor (area 4) and visual (area 17) areas of the human cerebral cortex. Zeitschrift für Anatomie und Entwicklungeschichte 144:123–135

Marín-Padilla M (1984) Neurons of layer I. A developmental study. In: Peters A, Jones EG (eds) Cerebral cortex, vol 1. Plenum Press, New York, pp 447–478

Marín-Padilla M (1987) The chandelier cell of the human visual cortex. A Golgi study. J Comp Neurol 265:61–70

Marín-Padilla M (1990) The pyramidal cell and its local-circuit interneurons: a hypothetical unit of the mammalian cerebral cortex. J Cognitive Neurosci 2:180–194

Marín-Padilla M, Stibitz G (1974) Three-dimensional reconstruction of the baskets of the human motor cortex. Brain Res 70:511–514

Müller-Paschinger IB, Tömböl T, Petsche H (1983) Chandelier neurons within the rabbit cerebral cortex. A Golgi study. Anat Embryol 166:149–154

Peters A (1984) Chandelier cells. In: Peters A, Jones EG (eds) Cerebral cortex, vol 1. Plenum Press, New York, pp 361–380

Ramón-Molliner R, Nauta WJH (1966) The idiodendritic core of the brain stem neurons. J Comp Neurol 126:311–335

Somogyi P (1977) A specific 'axo-axonic' interneuron in the visual cortex of the rat. Brain Res 136:345–350

Somogyi P (1979) An interneuron making synapses especially on the axon initial segment of pyramidal neurons in the cerebral cortex of the cat. J Physiol (Lond) 296:18–19

Somogyi P, Freund TF, Cowey A (1982) The axo-axonic interneuron in the cerebral cortex of the cat, rat, and monkey. Neurosciences 7:2577–2609

Somogyi P, Cowey A (1984) Double bouquet cells. In: Peters A, Jones GE (eds) Cerebral cortex, vol 1. Plenum Press, New York, pp 337–358

Szentágothai J (1965) The synapses of short local neurons in the cerebral cortex. Symp Biol Hungary 5:251–276

Szentágothai J (1975) The "module concept" in the cerebral cortex architecture. Brain Res 95:475–496

Szentágothai J (1978) The neuron network of the cerebral cortex: a functional interpretation. Proc R Soc Lond Ser B 201:219–248

Szentágothai J, Arbib MA (1974) Conceptual models of neural organization. Neurosci Res Prog Bull 12:383–286

Tömböl T (1976) Golgi analysis of the internal layers (V–VI) of the cat visual cortex. Exp Brain Res 1:292–295

Tömböl T (1978) Comparative data on Golgi architecture of interneurons of different cortical areas in the cat and rabbit. In: Brazier MAB, Petsche H (eds) Architectonics of the cerebral cortex. Raven Press, New York, pp 59–76

Valverde F (1983) A comparative approach to neocortical organization based on the study of the brain of the hedgehog (Erinaceous europeus). In: Grisolia S, Guerry CC, Samson F, Norton S, Reinoso-Suarez F (eds) Ramón y Cajal contribution to neurosciences. Elsevier, Amsterdam, pp 149–170