A comparative approach to neocortical organization based on the study of the brain of the hedgehog (Erinaceus europaeus)

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Introduction

The organization of the neocortex is the result of a slow evolutionary process which might have passed through different modifications to finally become the most complex part of the nervous system. The origin of this dominating neocortex began at the reptile-mammal transition in the Triassic and continued for about 100-120 million years to reach a final prototage in primitive Insectivora in the late Cretaceous. The hedgehog (Erinaceus europaeus) has been regarded as one of the most direct descendants of these primitive placentalts that has retained, apparently with minor variations, some of the basic characteristics of its early ancestors (Fig. 1). For that reason it was chosen as the subject of a comparative study of some of the basic principles of neocortical organization.

According to a general scheme, the neocortex represents that part of the cerebral mantle in which a six-layered stratification can be recognized. This plan is not valid for the hedgehog since not only is the organization of the first two layers different from that of the higher mammals, but it also exhibits certain specific properties (Fig. 2).
from that of other mammals, but also the internal granular or the fourth layer is entirely absent. We believe that there are two fundamental characteristics which basically define the neocortical organization: 1) the existence of pyramidal cells, in the most restricted sense (neocortical pyramids), and 2) the existence of a complex,
The model of neocortical organization: the afferent fiber

The neocortex receives its major input in the form of afferent fibers from homo- and contralateral association areas and from diverse subcortical nuclei. These fibers enter the cortex from the white matter having a principal level of termination in layers III and IV. Their form and distribution, and the elements with which they make contact vary greatly in different animals.

The principles of functional neocortical organization were first described by Lorente de Nó [1]. He showed the existence of particular associations between a single afferent cortical fiber and groups of intrinsic neurons in layer IV, in the part of the somatic-sensory region of the mouse corresponding most probably to the barrel field [2]. Later, Lorente de Nó [3] advanced the concept of *elementary unit* to designate a vertical cylinder or column of cortical tissue which has a central axis formed by a specific afferent fiber containing all kinds of cortical cells capable of carrying out the entire process of nerve transmission from the afferent fiber to the afferent axon. The thesis was, however, not entirely new, for as early as 1898 Cajal [4] suggested the existence of functional systems or *isodynamic groups of neurons* in the visual cortex that could be specifically activated by elementary sensory impressions. Today the concept of columnar organization receives its strongest support from the physiological studies of Mountcastle [5] on the somatic-sensory cortex, Woolsey [6] on the auditory cortex, and Hubel and Wiesel [7] on the visual cortex (see also refs. 92 and 93).

The concurrent refinement of methods for anatomical tracing of pathways and the use of enzyme and radioactive tracers have provided evidence of the existence of regularly spaced, periodic subdivisions in the cerebral cortex and in some subcortical nuclei, readily attributable to the spatial distribution of afferent fibers. Thus, in the primate visual cortex, lesions confined to single geniculate layers showed the existence of bands of degenerating terminal fibers in layer IV of area 17, so that the input from both eyes forms a mosaic of alternating vertical subdivisions which coincides with the physiologically demonstrated ocular-dominance columns [8]. In the somatic-sensory cortex of the monkey, cell columns combine in a system of subdivisions with regions of different responsiveness to the superficial and deep somatic sensibility [5, 9]. In the somatic-sensory cortex of the mouse, each vibrissal follicle of the snout is represented in special anatomical arrangements in layer IV, forming "barrels" [2, 10]. Finally, in the auditory cortex of the cat, groups of neurons appear arranged in bands which display similar properties of tonal sensitivity and binural interaction [11—13] which, again, can be correlated with particular columnar arrangements found in layer IV of the cat's first auditory cortex [14].

This pattern of orderly partitions is not unique in the cortical primary sensory areas. Similar patterns of isolated patches of terminal distribution of corticocortical connections have been observed in almost all association cortices [15—21]. Thus, the modular concept of the association cortex also considers the neocortex as a mosaic of discrete vertical subunits of about 300 μm in diameter forming the anatomical basis in a functional design [22—26].

One of the best known afferent cortical systems is the geniculocortical input into the primary visual cortex. Ascending fibers from the diencephalon reach the intermediate zone of the developing cortex [27] in the hedgehog embryo (Fig. 3). The entrance of these thalamic afferents constitutes an important issue from a phylogenetic point of view. These afferents not only will contribute to the differentiation of the developing cortex into a neocortical type favouring the development of neocortical pyramidal cells, but their maturation will also shape different patterns of intrinsic connectivity in the adult brain [28, 29].

In the hedgehog, the pattern of cortical ramifications of presumably specific thalamicocortical fibers is very simple, but it becomes progressively more complicated in more advanced mammals. In Golgi-stained parasagittal sections of young hedgehogs, cortical afferents to the occipital region were seen entering in an anteroposterior direction (Fig. 4). They ascend very obliquely through layers VI and V and develop cortical fans forming a plexus in the middle of the cortical thickness, which we will label layer III—IV. According to Gould et al. [30] the geniculocortical fibers in the hedgehog end in layers VI and III—IV having some degree of topographical organization. These authors found a strong fiber system projecting to...
layer I in both striate and parastriate cortices. O'Leary and Bishop [31] have described, in the rabbit, Golgi-stained afferent fibers distributing in layer IV and mainly in layer III of the visual cortex. In the cat, cortical afferents to the visual area were described by Cajal [32] and O'Leary [33] using the Golgi method; recent studies using the same procedure in kittens [34] revealed additional aspects of the tangential distribution of geniculocortical fibers.

Ferster and Le Vay [35] have studied the morphology and laminar distribution of afferent fibers in the visual cortex of the cat after injections of horseradish peroxidase (HRP), while Gilbert and Wiesel [36] injected individual axons entering the visual cortex. The studies from the Harvard group demonstrated that two different types of geniculocortical fibers enter layer IV of the cat, and showed that these correspond to the Y- and X- geniculate cells [37, 38]. They arborize independently in the lower part of layer III and upper layer IV, and in the lower part of layer IV. In addition, these fibers emit collaterals ramifying in layer VI, while some presumed geniculate fibers can also be traced to layer I.

In the monkey, ostensibly geniculocortical fibers were observed in Golgi material ending in the lower part of layer III and sublayers IVa and IVc [34, 39, 40]. The terminal ramifications appeared with numerous tightly packed, short-side appendages, resembling dendritic spines and giving a picture characteristic of these fibers (Fig. 3 of ref. 34), which suggests a high density of synaptic endings covering densely, restricted portions of target cells. A single afferent fiber can project to both subdivisions and, furthermore, other afferent fibers can be traced to layers I and VI.

A comparison of the intrinsic organization and afferent and efferent fiber patterns between the monkey and the cat has been made by Lund et al. [41].
The targets for cortical afferents: specificity versus occasionality

The study of target cells for cortical afferents is important because their identification, not only in a given animal, but also in different species, will reveal important issues of phylogenetic development and may show particular details about the functional circuits and processing organization in the neocortex [42].

In the hedgehog, layer III—IV contains pyramidal cells and various types of elements with intrinsic axons (Fig. 4, cells b through e). Of these, the most frequently found element corresponds to a type of large multipolar cell with smooth and unusually long dendrites that may traverse the entire thickness of neocortex (Fig. 5). The axons of these cells are very thick and show no preferential orientation. They develop a number of long horizontal and vertical collaterals covering large volumes of cortical tissue through layers II and III—IV. There are other cell varieties with shorter, smooth dendrites, but which have similar axonal distribution (Fig. 6, cells d and e). Less frequently, we have found another distinct variety in which the axon forms a dense local plexus (Fig. 7, cell a), with numerous twisted secondary branches, and characteristically, three to five main dendritic trunks resolved into tufts of short spinous dendrites. These neuronal varieties, as well as the apical shafts and basal dendrites of pyramidal cells in layers II and III—IV, might equally represent potential recipients of cortical afferents.

In previous studies [45, 44] we observed in the visual cortex of the mouse specific geniculo-cortical afferents ending on cells having smooth dendrites with intrinsic axons in layer IV (compare cell of Fig. 5 with cell b in Fig. 2 in Vaverde and Ruiz-Marcos [44]; see also ref. 45), as well as on the basal dendrites of pyramidal cells of layer III. A possible basic input—output neocortical circuit will then include such large multipolar cells with smooth dendrites, intercalated between theafferent cortical fiber and the layer V pyramidal cell axon. This circuit seems to be entirely comparable in the hedgehog and mouse. However, in the mouse and rat, small multipolar cells with thin spinous dendrites located in layer IV, which were not found in the hedgehog, appear to represent a new cell variety which substitutes the large multipolar cells, resulting in an increasing "granularization" of neocortex in advanced mammals. Small spinous stellate cells abound in the cat and in the monkey; they have been considered one of the principal recipients of thalamic fibers [34, 36, 46, 47].

Target cells receiving primary afferent inputs have been identified, with the aid of the electron microscope, in the somatic sensory and visual areas of the mouse, cat, and monkey [48–55] and, although the complete characterization of all cell varieties and their peripheral processes receiving direct thalamic input is still lacking, these studies confirm implicitly that all cell bodies and dendrites located within the specific afferent domain in layers III and IV, and having asymmetric synapses (i.e., admittedly excitatory), could be considered potential recipients of thalamic fibers, as we have previously suggested [40]. Therefore it is evident that the specificity of
Fig. 6. Frontal section through the parietal region of the neocortex of the hedgehog showing examples of two cells in layer II with a pyramidal shape (a, b); one cell (c) with spiny dendrites and two opposite, radially directed dendrites and an ascending axon (d); two large multipolar cells (d, e) with intrinsic axons (f, g); a large multipolar cell (f) with smooth dendrites in layer VI; another polymorphic cell (h) in the same layer with spiny dendrites and apical dendritic branch ascending toward layer I; and a typical pyramidal cell (i) in layer V. Golgi method, camera lucida drawing.

Fig. 7. Frontal section through the parietal region of the neocortex of the hedgehog showing a type of cell (a) with a smooth local axon and two small, smooth dendrites. Compare with a multipolar cell with long smooth dendrites (b) whose axon was not stained. Examples of superficial (c, d) pyramid-shaped bodies in layer III and large pyramidal bodies (e) through h) in layer III—IV are also included in this picture. Golgi method, camera lucida drawing.
connections established by a given cortical afferent fiber will turn out to be the occasionality [23] of neurons, pair and present along the evolutionary scale, which occurred in the domain of such afferent fibers. From a comparative point of view, we notice that the increase in the number of cells with local axons entails a reduction in the volume of neuropil interlinked by them. The cells become smaller, but at the same time, the number of possible intracortical circuits increases almost exponentially.

**Neurons with intrinsic axons: the local circuitry**

Two recent reviews [56, 57] have been devoted to the study of neurons with local axons in various parts of the brain confirming that these neurons, and the local circuits they form, increase in number and variety along the phylogenetic scale. Cells with local axons constitute a heterogeneous population and several attempts have been made to define them in various neocortical sensory areas [39–41, 49, 55–66]. Besides the presence or absence of dendritic spines, we recently proposed a classification which considers neurons with local axons as having either generalized or specialized axonal arbor [34]. Generalized axons (defined by exclusion) lack specific pretenuous arborizations; their axons extend through larger volumes of cortical tissue, and they are most commonly found in the hedgehog (e.g., Fig. 5), mouse [44] and rat [61]. There is evidence that some of them form symmetric synapses (admittedly inhibitory) with the bodies and apical dendrites of pyramidal neurons, and bodies and dendritic branches of other similar neurons in the visual cortex of the rat [67].

Neurons with local axons having specialized axonal arbor include several categories whose terminal branches adopt specific patterns. These include basket cells [31, 63, 64, 68–70], cholinergic cells [40, 59, 66], cells with vertical, striped axonal bundles [59, 66, 71], and chandelier cells [26, 72, 73]. Some of them have been considered important key pieces in conceptual models of neocortical operation [22]. Although this classification is incomplete because both subdivisions are broadly defined, it supports the idea that specialized axons abound in higher species (cat, monkey, man), yet both generalized and specialized axons have been found to exist in lower mammals (hedgehog). However, to name a few significant differences (within the limitations of the Golgi method) between different species, concerning cells which seem to be main targets of specific thalamocortical afferents [40, 65], we shall mention that the large multipolar cells with smooth dendrites which we found in layer III—I of the neocortex in the hedgehog are morphologically different from the minute cholinergic cell occurring in sublayer IVC of the monkey's areas 17 and 18: spiny and smooth stellate cells in layer IV of the cat's visual cortex (e.g., ref. 41), some of which have long-projecting axons [32, 34], are different from small spiny stellate cells in sublayer IVC of the monkey's visual cortex which have recurrent ascending axons (compare Figs. 2 and 3 in Falch and Valverde [34]). Double-tufted cells having long, stranded vertical axonal bundles appear well represented in man [68], cat and monkey [59, 66, 71], while they seem to be absent in the hedgehog and mouse.

In contrast, two of the most elaborate types of specialized neurons, basket and chandelier cells, are present in the neocortex of the hedgehog. Basket cells were first described by Cajal [68] as specialized neurons whose axon terminals form baskets around the bodies of pyramidal cells (Fig. 8). They have been found in layers III and V of the human motor and visual cortices [68–70], and in layers II, III and V of the cat's striate area [63, 74]. The pericellular baskets are formed by the convergence of several terminal endings of a number of basket axons from different neurons. For this reason, and also due to the selectivity of the Golgi impregnation, it has always been difficult to obtain impregnations of complete neocortical baskets, except under particular conditions [69–71]. Thus, experimental hypoxia in infant monkeys shows degenerating axon terminals making symmetrical synapses selectively located around the bodies and proximal dendrites of layer V pyramidal cells [73]. This observation might account for the selective staining of some baskets that I have observed in examining old Golgi preparations made by Cajal in 1900 from human infants who died of cardiopulmonary failure (Fig. 8), and suggests that the baskets may be very sensitive to hypoxia which, for unknown reasons, favours their impregnation.

The morphology of basket cells is variable. A recent study [76] shows a type of interneuron in layers II and III of the cat's visual cortex, whose axons were found to form multiple symmetrical contacts on cell bodies of pyramidal and nonpyramidal neurons. There is now evidence that symmetrical synapses are found on the bodies and proximal parts of dendrites of pyramidal cells [67, 77–79] and that these synapses are inhibitory [80–82]. Thus the basket cell appears as an important, phylogenetically old, inhibitory interneuron, probably present early in neocortical evolution to control the output of a newly acquired pyramidal cell system. We found in the neocortex of the hedgehog some examples of cells with axons forming terminal ramifications which resemble baskets (Fig. 9). They are located in the upper part of layer III–IV, have a few long and smooth dendrites and, in several cases, show rather elaborate axonal tracts extending for long distances. It was not difficult to demonstrate that some terminal branches approach unmyelinated cell bodies, rendered faintly visible when the microscope condenser was lowered.

Chandelier cells, first observed by Szentagothai and Arbib [26], represent an interesting neuronal variety whose axon develops a local plexus of collateral branches, mainly in layers II and III, that resolves into vertically oriented long bouton aggregates. In the rat, Somogyi [73] described similar cells under the name "axon-axonic cells" and showed that these vertically arranged bouton terminals
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Fig. 8. The basket outlining an unstimulated large pyramidal cell of layer V in the human motor cortex. Camera lucida drawing from a Golgi preparation made by S. Ramón y Cajal. The author believes that it corresponds to the pericellular net labelled b in Cajal's Histologie (66) figure 362.

Fig. 9. Frontal section through the motor region of the neocortex of the hedgehog. One cell (a) with smooth dendrites has a descending axon (14) which emits several collaterals ending in basket-like terminal ramifications (2a through 5a), probably devoted to contact the bodies of pyramidal cells like b and c. Golgi method, camera lucida drawing.
make symmetrical (inhibitory) synapses specifically on the initial axonal segments of superficial pyramidal cells. In the cat's visual areas 17 and 18, chandelier cells develop their terminal portions in synaptic contact (symmetrical synapses) with the initial axonal segments of layer III pyramidal cells, with occasional branches descending to layer V [72]. Also, in the visual cortex of the rabbit, we recently found evidence, using a combined Golgi-EM technique [83], that the vertically oriented terminal portions of the same cells form symmetrical synapses with the initial segments of axons of superficial pyramidal cells.

Thus far, chandelier or "xono-axonic cells" represent an outstanding example of target selectivity [72, 84], for they not only select the postsynaptic partner (superficial pyramidal cells), but also a well-defined part thereof (the initial axon segment). We found chandelier cells in various cortical areas in a number of our Golgi preparations. Fig. 10 shows five cells with chandelier axons in hedgehog, mouse, rabbit, cat and monkey drawn at the same magnification to facilitate comparisons. In the hedgehog they were observed in the interhemispheric cortex (Brodmann's areas 24 and 32 [83]), entorhinal cortex (area 28p) and in the parietal region (area 5p-7p).

There is some evidence that chandelier cells, together with their companions the basket cells, compose a powerful inhibitory apparatus for pyramidal cells. The inhibitory nature of chandelier axon terminals seems to be supported by the observation of GAD-positive reaction terminals contacting initial segments of axons [61, 81]. The interaction of both cells with respect to the pyramidal cell's output is not clear at present, although it has been suggested that basket cells acting at the periphery of cortical modules tend to depress adjacent modules [22, 61, 70, 93].

**Tangential organization of the neocortex of the hedgehog: a remnant of a paleocortical structure**

In the hedgehog neocortex the most distinctive feature is the organization of layers I and II. Layer I (Figs. 11 and 12) is exceedingly thick and contains a dense palisade of peripheral spinous dendrites from all neurons located in layer II; it also contains the ascending, superficial ramifications of apical dendrites of deeper pyramidal cells as well as a large number of tangential fibers derived from the ascending axons of Martinotti cells located in layer VI. From bipolar cells in layers III—IV and V, and from collateral branches of the large multipolar cells with smooth dendrites which run horizontally for quite a long distance and whose bodies are in layer III—IV. In addition, the first layer contains intrinsic cells (Fig. 11, cell a) with complicated axonal ramifications contacting all invading dendrites of underlying cells as well as a system of horizontally disposed small bundles of myelinated fibers (Fig. 11, F) passing through its lower half. They correspond to Flores' [86] radii longi and probably contain association fibers from distant regions, probably from secondary or tertiary olfactory centres.
Layer II is formed by a stratum of densely packed large cell bodies. Santides and Santides [87] observed this characteristic, "accentuated layer II", in insectivora and considered it a common primitive architec tonic feature. Some cells in layer II have a pyramidal shape with short apical dendrites, quickly branching into a bush (Fig. 6, cell a), while others appear as if some of their basal dendrites were directed up toward layer I (Figs. 6 and 11, cells b). Most commonly they have three to five main, obliquely ascending, dendritic trunks which branch out profusely in layer I, and also they have a basal bunch of descending dendrites (Fig. 12, upper left corner). The axons descend to the white matter with only a few horizontal and obliquely ascending collaterals given off as they pass through the upper part of layer III-IV.

In general the organization of the first and second neocortical layers is similar to that of the allocortex. In Nissl-stained preparations layer II shows discontinuities resulting from the clustering of their large stellate cells which leaves free spaces for ascending apical dendrites of pyramidal cells located below, as if these were growing inside a dominant palaeocortical organization.

Based on trends of comparative neuroanatomy [88-91], we believe that in the neocortex of the hedgehog we are seeing the culmination of early development which originated when thalamic afferent fibers entered a primitive cortical matrix shaping a population of new pyramidal (neocortical) cells, and that, as we ascend the phylogenetic scale, the association circuitry of local axons becomes increasingly complicated.

References

Structural substrates and nervous function