

## Pigment Architecture of the Human Telencephalic Cortex

### IV. Regio Retrosplenialis\*

Heiko Braak

Zentrum für Morphologie, Johann Wolfgang Goethe-Universität, Frankfurt a.M., Bundesrepublik Deutschland

**Summary.** Cortical lamination and parcellation of the retrosplenial region in the human brain is evaluated with the aid of frontal serial sections stained for nerve cells (15  $\mu\text{m}$ ), myelin sheaths (100  $\mu\text{m}$ ), and lipofuscin granules (800  $\mu\text{m}$ ).

For the most part, the retrosplenial region is buried in the depth of the sulcus corporis callosi covering the posterior parts of the cingulate gyrus. It lies between the supracallosal derivatives of the allocortex (fascia dentata, cornu ammonis, subiculum) and the mature parietal isocortex.

The region can be subdivided into five areas. The transitory periallocortical *area ectosplenialis* is followed by a richly differentiated proisocortical core displaying extremely externopyramidal, externoteniate, and astriate to unistriate characteristics. The parvocellular core is averagely poor in pigment (*typus clarus*) and rich in myelinated fibres (*typus dives*). Minor structural differences allow for its subdivision into a lateral, an intermediate, and a medial retrosplenial field. The accompanying *area parasplenialis* is adjacent to the equoteniate parietal isocortex. It is only weakly externopyramidal, externoteniate, and propebistriate. The already homotypical field shows an average pigmentation and myelin content. These structural features permit its classification as a belt area of the retrosplenial core.

**Key words:** Cerebral cortex – Man – Limbic lobe – Retrosplenial region – Architectonics – Lipofuscin.

The retrosplenial region of man is located in the posterior cingulate gyrus where it is wedged between laterally-situated allocortical structures and medially-situated isocortical areas of the parietal lobe.

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Send offprint requests to: Prof. Dr. med. Heiko Braak, Zentrum für Morphologie, Theodor Stern Kai 7, 6000 Frankfurt a.M., Federal Republic of Germany

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As to the architectonics of the region one must rely upon the studies of Brodmann (1908, 1909, 1912, 1914), von Economo and Koskinas (1925), von Economo (1926, 1927), and Rose (1928, 1935) which are partly contradictory.

Pigment preparations generally reveal a clear lamination pattern of the cortex. They also greatly facilitate delineation of cortical areas (Braak, 1978a; in preparation). It is the purpose of the present study to suggest a new parcellation of the retrosplenial region which is based on the analysis of pigment preparations corroborated by the analysis of preparations stained for nerve cells and myelin sheaths.

## Materials and Methods<sup>1</sup>

The observations were made on brains of adult humans. Tissue obtained from autopsy material<sup>2</sup> was fixed by immersion in formaldehyde 1:9 for several months. Frozen sections were cut at 800 µm for pigment preparations. Eleven uninterrupted series of coronal sections through both the left and the right hemispheres were at our disposal (for details see Braak, 1979). The sections were oxidized with performic acid (30 min), washed under running tap-water, transferred to 70% ethanol, and stained with aldehyde-fuchsin (Braak, 1978d). After washing with 70% ethanol, the sections were dehydrated, cleared with toluene and mounted with a synthetic resin (Permount, Fisher).

Smaller blocks including the splenium corporis callosi and posterior parts of the cingulate gyrus were cut at 800 µm for pigment preparations, at 100 µm for myelin preparations, and again at 800 µm for Nissl preparations. The pigment preparations were processed as described above. The 100 µm thick sections were stained for myelin sheaths according to the technique of Schroeder (1939; Romeis, 1968). The subsequent 800 µm thick section was embedded in paraffin, cut at 15 µm, and stained with cresyl violet (Romeis, 1968).

Photographs of myelin preparations were taken on Ilford FP4 film and developed with Neofin blue (Tetenal) for 4 min. Photographs of pigment preparations and Nissl-preparations were taken on Agfa-ortho 25 professional film and developed with Agfa Rodinal 1:20 for 10 min.

The nomenclature recommended by Vogt and Vogt (1919) is applied in our architectural descriptions. The isocortical laminae are denominated as follows (from the pial surface); molecular layer (I), corpuscular layer (II), pyramidal layer (III), granular layer (IV), ganglionic layer (V), multiform layer (VI).

A *parvocellular* cortex is on the average formed of small nerve cells. Predominance of the fourth layer distinguishes the *hypergranular* cortex. *Dysgranular* fields with a rather thin granular layer are intermediate between *agranular* and *eugranular* areas. An *externopyramidal* field shows a predominance of the lower reaches of the pyramidal layer (IIIc) in comparison with the ganglionic layer (V).

Particularly rich endowment of myelinated fibres is referred to as *typus dives*. The *bistriate* type of cortex displays two bands of Baillarger. In the *unitostriate* areas the lines of Baillarger cannot be distinguished from one another because of the high myelin content of the intrastriate layer. Provided that the substrate layer is also filled with myelinated fibres this pattern is denoted as the *astriate* characteristic.

The *typus clarus* characterizes a cortex mainly formed of sparsely pigmented pyramidal cells. *Biteniate* areas display both teniae. Accentuation of the outer tenia is referred to as the *externoteniate* characteristic. For detailed remarks on pigmentoarchitectonics the reader is referred to Braak (1978a, b, 1979, in preparation).

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## Results

The induseum griseum covering the corpus callosum is formed medially of isolated remnants of the fascia dentata which abut on strands of Ammon's horn constituents. These are either filled with pigment or contain only a few disseminated lipofuscin granules (Braak, 1974). The band of cells lying immediately lateral to those consists of deeply stained nerve cells containing lipofuscin granules agglomerated in a clump. This band is continuous with the layer of internal subicular pyramidal cells (Braak, 1972). These allocortical derivatives of the fascia dentata, the cornu ammonis, and the subiculum represent the supracommissural parts of the hippocampal formation (Stephan, 1975).

The adjoining *area ectosplenialis* (es; periallocortex; Fig. 1) hardly displays a recognizable lamination and can therefore be classified with the propeateniate cortex. The cortical band broadens gradually. The molecular layer is filled with a great number of tightly packed myelinated fibres. Several rows of almost uniformly pigmented pyramids follow. Here and there a blurred lightening allows for the distinction of an outer main stratum (III) from an inner one which seemingly consists of only the multiform layer (VI).

The cortex of the following retrosplenial areas is already formed of isocortical laminae which nevertheless display some primitive traits. The territory constitutes one of the main regions of the proisocortex. The border with the ectosplenial field is a sharp one (Fig. 1).

Within the limits of the *area retrosplenialis lateralis* (rsl; proisocortex; Fig. 1) the molecular layer is still conspicuously broad and filled with myelinated fibres. Pigment preparations display a narrow superficial band subjacent to the molecular layer which consists of small and tightly packed pyramids ( $P_{III}$ ). The amount of pigment stored within the cells decreases from the top of the layer to the bottom. The following external tenia (Te) is extremely broad and can be considered a hallmark of the field. The outer tenia as well as  $P_{III}$  are filled with small stellate cells and pyramids, the latter predominating. There is no granular layer (IV). The lower pyramids of the outer main stratum are devoid of pigment deposits. There is a broad and dense plexus of myelinated fibres with a particularly blurred upper border spreading over the lower portions of the outer main stratum (Fig. 2).

Irregular rows of modestly pigmented widely spaced pyramids indicate the formation of lamina  $P_{Va}$ . The layer is poorly represented and often becomes too tenuous to permit recognition. It is followed by a narrow and cell-sparse stripe – the internal tenia (Ti) – that is poor in pigment. Both  $P_{Va}$  and the inner tenia are filled with myelinated fibres giving the cortex an astriate character. The multiform layer (VI) is continuous with the deep stratum of the ectosplenial field. It shows clear-cut upper and lower boundaries. Its main constituents are rich in pigment. The relatively abrupt appearance of a markedly pigmented multiform layer allows one to draw a clear borderline between the ectosplenial field and the lateral retrosplenial field (Fig. 1).

The cortex of *area retrosplenialis intermedia* (rsi; proisocortex; Figs. 1, 2) is broader than in the foregoing field. It is still extremely extenotenate, poor in overall pigmentation (*typus clarus*) and rich in myelinated fibres (*typus dives*).

The thick plexus of myelinated fibres within the molecular layer gradually narrows down as one proceeds in the medial direction. The breadth of the subjacent band of cells by far exceeds that of the inner layers. In preparations stained for nerve cells it appears composed of the larger constituents of the pyramidal layer (III) with a fair number of particularly small pyramids interspersed. A real granular layer is still absent. The pigmented zone of the outer main stratum (pIII) has broadened a trifle at the expense of the outer tenia. It retains its characteristic decrease in pigmentation from top to bottom. This results in a blurred upper border of the pallid outer tenia which is still remarkably broad (Figs. 1, 2). Myelin preparations display a rich fibre plexus in the lower reaches of the outer main stratum indicating an extremely broadened outer stripe of Baillarger.

The ganglionic layer is split into an upper zone relatively rich in cells and a cell-sparse lower one. Layer pVa, though still a tenuous band, is sharply delimited and becomes clearly recognizable. The inner tenia (Ti) has broadened a little and appears more pallid than the outer one. The band-like multiform layer (VI) is still the most densely pigmented part of the cortex. Both the ganglionic layer and the multiform layer are filled with myelinated fibres. The field displays an astriate to propeunitostriate character because of the high myelin density, not only of the intrastriate layer (5a) but also of the substriate zone (6) (Figs. 1, 2).

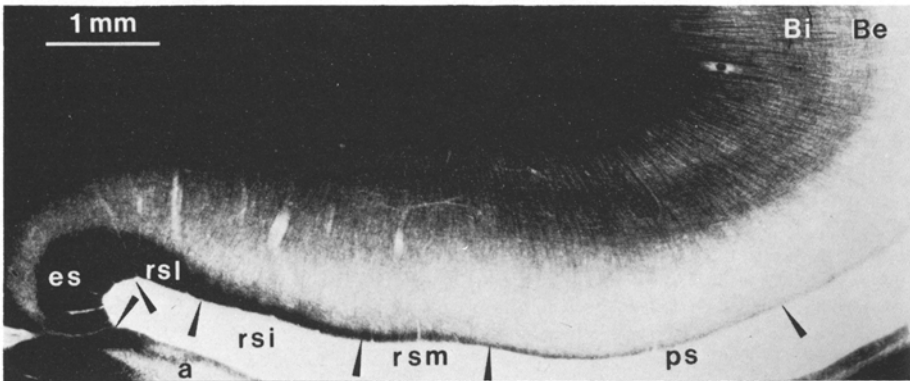
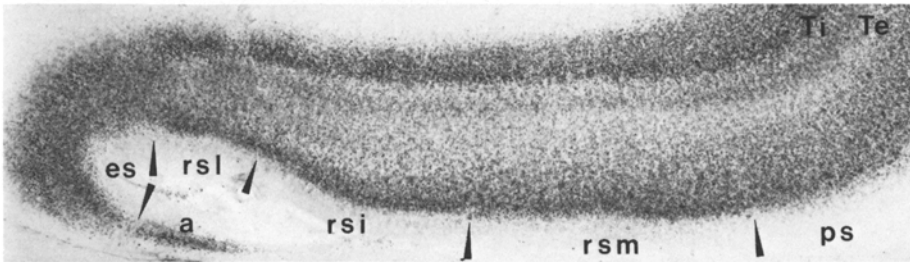
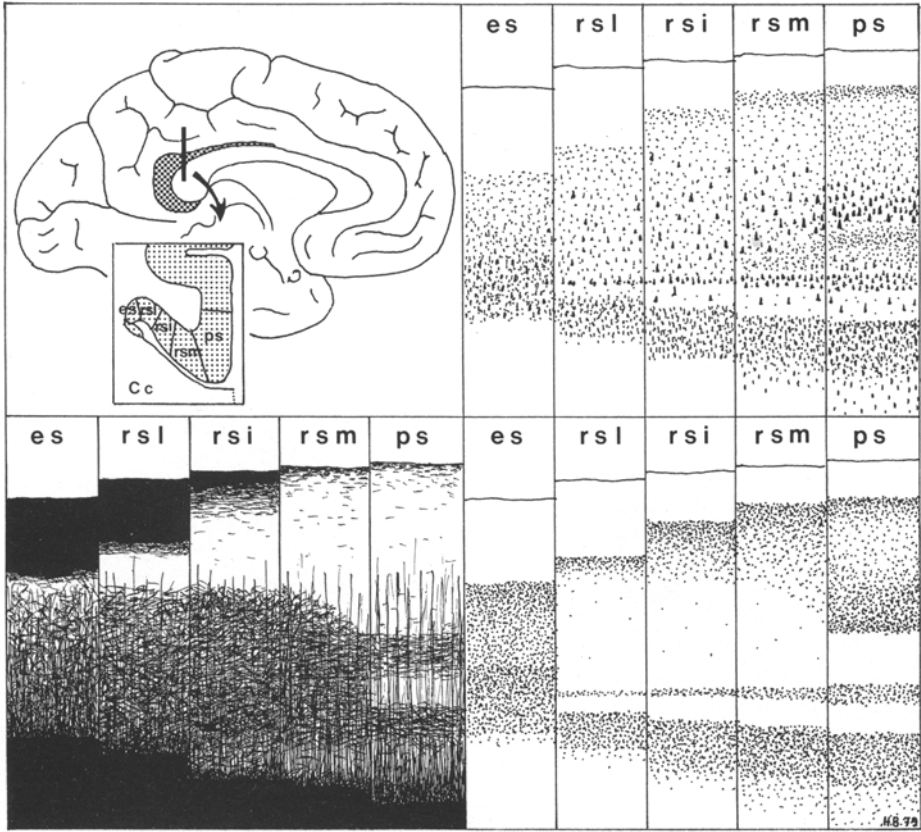
*Area retrosplenialis medialis* (rsm; proisocortex; Figs. 1, 2) is still of the markedly externoteniate, poorly pigmented and richly myelinated type.

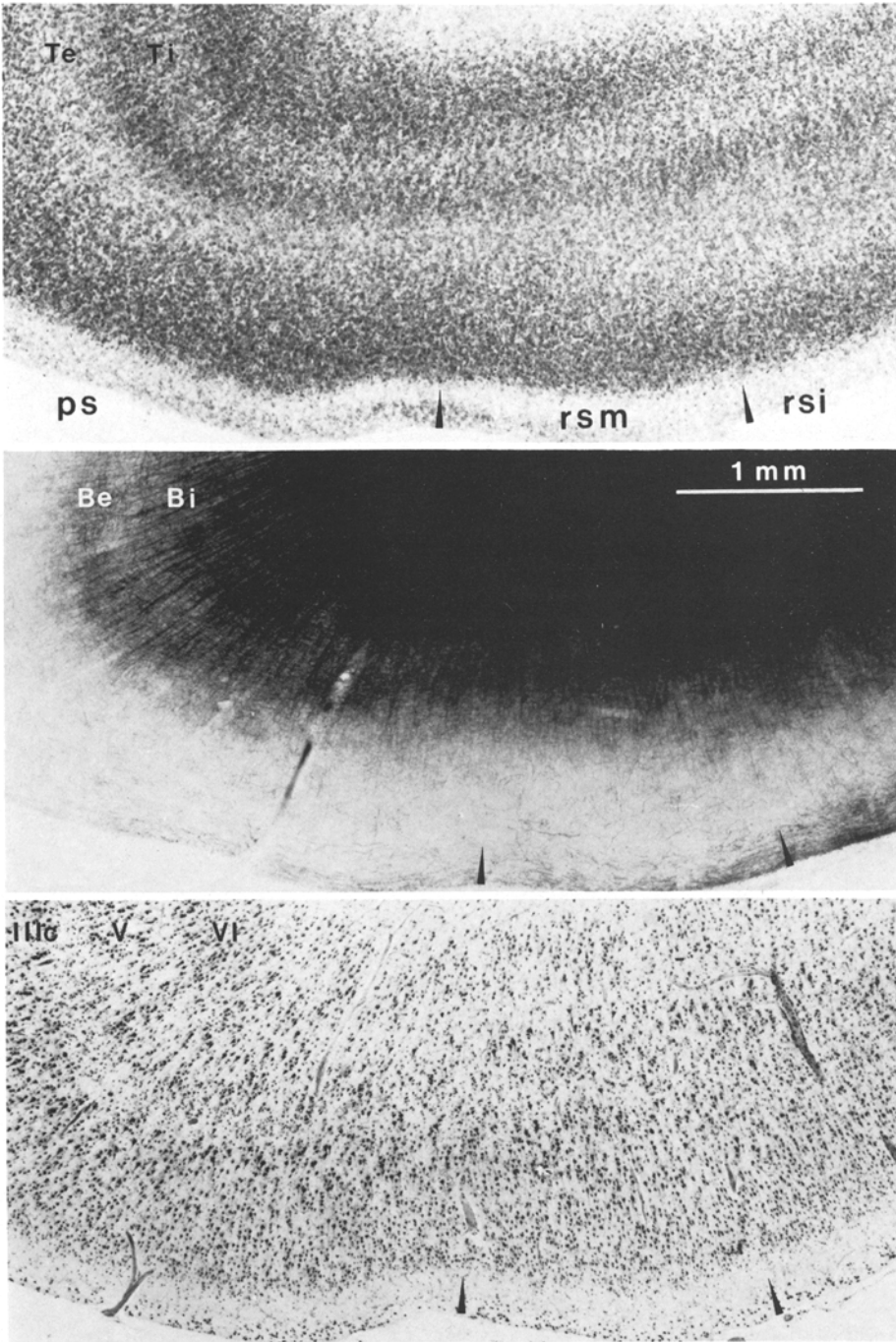
The molecular layer contains a fair number of myelinated fibres. The breadth of the outer main stratum exceeds that of the inner one. The average size of its constituents appears slightly increased. There is a progressive increase in the size of the pyramidal cells with depth in layer III. Sublayer IIIab is composed predominantly of small pyramids and is distinguishable from sublayer IIIc. An incipient granular layer (IV) becomes recognizable. In pigment preparations the breadth of the pigmented outer zone (pIII) shows a progressive increase as one proceeds medially. This leads to a considerable attenuation of the outer tenia (Te), a fact which corresponds with a similarly gradual attenuation of the outer stripe of Baillarger in myelin preparations (Fig. 2).

The outer part of the ganglionic layer (Va) has broadened slightly. The internal tenia (Ti) remains a narrow and pallid stripe. The multiform layer (VI) is now divisible into a densely pigmented upper zone (pVIa) and a lighter lower one (pVIb). The substriate zone (6) becomes recognizable in myelin preparations. The area therefore attains a unitostriate character.

As the cortex is traced forward out of the sulcus into the free surface of the cingulate gyrus the only weakly externoteniate *area parasplenialis* (ps; isocortex;

**Fig. 1.** *Upper half:* Location of the retrosplenial region of man. Bar indicates the position of the frontal section seen in greater detail below. Diagrammatic representation of the various areas forming the retrosplenial region in the Nissl-, myelin-, and pigment preparations. *Lower half:* Frontal sections through posterior parts of the cingulate gyrus. The boundaries between the various areas of the retrosplenial region are marked by triangles. Pigment preparation, aldehyde-fuchsin, 800  $\mu\text{m}$  (above) myelin preparation, hematoxylin, 100  $\mu\text{m}$ . *a* Allocortical derivatives; *Be* outer line of Baillarger; *Bi* inner line of Baillarger; *Cc* corpus callosum; *es* area ectosplenialis; *ps* area parasplenialis; *rsi* area retrosplenialis intermedia; *rsl* area retrosplenialis lateralis; *rsm* area retrosplenialis medialis; *Te* external tenia; *Ti* internal tenia





**Fig. 2.** Pigment-, myelin-, and Nissl preparation successively cut from the same block for comparison of the architectonic features revealed by the different methods (♀ aged 84, myocardial infarction). The boundaries between the retrosplenial areas are indicated by triangles. Within the limits of the medial retrosplenial field the breadth of both the outer tenia and the outer band of Baillarger decreases gradually. Pigment preparation, aldehyde-fuchsin, 800 μm; myelin preparation, hematoxylin, 100 μm; Nissl preparation, cresyl violet, 15 μm. *Be* outer line of Baillarger; *Bi* inner line of Baillarger; *ps* area parasplenialis; *rsi* area retrosplenialis intermedia; *rsm* area retrosplenialis medialis; *Te* external tenia; *Ti* internal tenia; *IIIc*, *V*, *VI* isocortical layers. Magnification bar applies also for the pigment and Nissl preparations

Figs. 1, 2) appears, which lies adjacent to the equoteniate parietal cortex. It is also intermediate in both its myelin content and overall pigmentation.

The molecular layer still contains some scattered myelinated fibres. Nissl preparations reveal a clear separation of sublayer IIIab from IIIc. Additionally a granular layer (IV) appears. The layer III-pyramidal cells show the common pattern of pigmentation, i.e., the deeper their position the greater amounts of pigment they store. This leads to a relatively clear-cut lower border of <sup>p</sup>III. The external tenia (Te) accordingly appears markedly attenuated. It is nevertheless still wider than the inner one. The outer band of Baillarger is narrow with a relatively sharp upper border.

The outer half of the ganglionic layer (Va) is filled with medium-sized pyramidal cells. In pigment preparations the layer (<sup>p</sup>Va) is sharply delineated and broader than in the foregoing fields. The internal tenia (Ti) and the multiform layer (<sup>p</sup>VI) remain unchanged. As one proceeds medially, the intrastriate layer (5a) becomes recognizable in addition to the substriate zone (6). The intrastriate band is relatively poor in myelinated fibres, thereby contrasting with the two bordering lines of Baillarger (Figs. 1, 2). The cortex attains a propebistriate characteristic.

## Discussion

In the human brain the retrosplenial region is for its most parts buried in the depth of the sulcus corporis callosi. Only a part of the parasplenial field extends onto the free surface of the cingulate gyrus (Fig. 1). The outlining of the retrosplenial core in the maps of Brodmann (1909) and von Economo and Koskinas (1925) does not coincide with its real extent.

Most authors agree in the subdivision of the region into a hypergranular part which abuts on the supracallosal allocortex and an agranular part adjacent to the parietal isocortex. Table 1 gives a synopsis of the nomenclature used by different authors for the designation of the various subdivisions of the retrosplenial region.

**Table 1.** Synopsis of nomenclature concerning the retrosplenial region of man according to various authors

Brodman: 1908, 1909 (cyto), Stephan: 1975 (cyto, myelo)	ectospl.  (26)		retrospl. gran. (29)	retrospl. agran. (30)	
v. Economo and Koskinas: 1925, v. Economo: 1926, 1927 (cyto)	retrospl. gran. inf. (LE2)		retrospl. gran. sup. (LE1)	retrospl. agran. (LD)	
Rose: 1928, 1935 (cyto, myelo)	retrospl. gran. med. (RSg $\alpha$ )	retrospl. gran. interm. (RSg $\beta$ )	retrospl. gran. lat. (RSg $\gamma$ )	retrospl. agran. (RSag)	
Present study (cyto, myelo, pigmento)	ectospl.  (periallo)	retrospl. lat. (proiso)	retrospl. interm. (proiso)	retrospl. med. (proiso)	paraspl.  (iso)

Examination of preparations stained for nerve cells reveals that the so-called "granular" part of the retrosplenial region is devoid of both the corpuscular (II) and the granular (IV) layer (Vogt, 1976). It is merely the small size of the constituents of the pyramidal layer (III) which accounts for the parvocellular character of the cortex. Its characterization as granular or hypergranular seems therefore incorrect. The territory under consideration reveals rather an agranular character.

As is evident from the foregoing description, the so-called "agranular" part of the retrosplenial region, on the contrary, is endowed with an incipient granular layer and should therefore correctly be designated as dysgranular or intermedio-granular cortex (Sanides, 1962; Vogt, 1976).

Pigment preparations greatly facilitate the delineation of the cortical layers. In the retrosplenial areas the ganglionic layer in particular appears only as a tenuous stripe. Nissl preparations display a less clear-cut lamination pattern and, accordingly, Rose (1928, 1935) erroneously designates the lower reaches of the pyramidal layer as "layer Va". Nevertheless, it seems rather unlikely that a pronouncedly parvocellular cortex would be endowed with a strikingly broad ganglionic layer. Pigment preparations clearly reveal the narrow line of <sup>P</sup>Va and the marked predominance of the outer cellular layers. The externopyramidal characteristic of the retrosplenial areas is comparable to only that of sensory core fields such as the striate area or the postcentral coniocortex (Braak, 1976, in preparation).

Within the isocortex, markedly externoteniate fields occur only within the limits of the visual, acoustic, somatosensory and somatomotor cores (Braak, 1977, 1978c, 1979). Hence it is tempting to consider that the extremely externoteniate retrosplenial fields constitute a core within the proisocortex.

The isocortical cores are furthermore partly or totally surrounded by belt areas, which are less pronouncedly externoteniate and which separate them from the usually equoteniate areas of the generalized isocortex. The parasplenial field also shows such a weakly externoteniate character and therefore can be looked on as a belt area accompanying the retrosplenial core.

Most types of cortical nerve cells the somata of which lie within a thick plexus of axonal terminal branches remain almost devoid of pigment deposits, provided that the plexus is formed of axon collaterals of cortical pyramidal cells or of terminal branches of amygdalo-cortical or thalamo-cortical fibres (Braak, in preparation).

The isocortical core fields are known to attract a particularly massive thalamic input. The thalamo-cortical fibres ramify preferentially within the lower reaches of the outer main stratum, a localization which corresponds well to the markedly externoteniate characteristic of the isocortical core fields.

It has long been thought that the so-called "limbic lobe", which includes anterior and posterior parts of the cingulate gyrus, receives a massive input from anterior nuclei of the thalamus (Yakovlev et al., 1960; Locke et al., 1964). From recent investigations, however, it appears that only the retrosplenial region is under the influence of the anterior thalamic nuclei (Vogt et al., 1979). This finding is in agreement with the fact that, within the cingulate gyrus, the extremely externoteniate characteristic is also confined to the retrosplenial areas.



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