The Parahippocampal Gyrus in the Baboon: Anatomical, Cytoarchitectonic and Magnetic Resonance Imaging (MRI) Studies

The parahippocampal gyrus, located at the medial temporal lobe, is a key structure in declarative memory processing. We have analyzed the general organization of the parahippocampal gyrus in the baboon, a nonhuman primate species relatively close to human. This region is rostrocaudally made up of the temporopolar, perirhinal, entorhinal (divided into seven subfields) and posterior parahippocampal (areas TH and TF) cortices. The basic analysis has been performed in three brains, serially sectioned and stained with thionin, myelin stain, acetylcholinesterase and parvalbumin, to determine cytoarchitectonic boundaries. Borders of all subfields were charted onto camera lucida drawings, and two-dimensional maps of the surface and topography of the parahippocampal gyrus were made. Finally, the limits of each parahippocampal area were then transposed on corresponding MR images (commonly used for in vivo PET or functional MRI activation studies) of two animals for precise identification. The general cytoarchitectonic features of the baboon parahippocampal gyrus are similar to macaques, but the size of temporopolar cortex and the laminar organization of perirhinal and posterior parahippocampal cortices resemble humans more than macaque species. In conclusion, the size and structure of the baboon parahippocampal cortex makes this species very appropriate for studies on memory function.

Keywords: entorhinal, functional neuroanatomy, magnetic resonance imaging, memory, monkey, perirhinal, posterior parahippocampal, temporal pole

Introduction

The strategic importance of the medial temporal lobe in human memory mechanisms for facts and events (declarative memory) has been widely demonstrated, especially in neuropsychological studies performed in amnesic subjects (Rempel-Clower et al., 1996; Corkin et al., 1997; Mishkin et al., 1997; Reed and Squire, 1997) as well as in positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies in healthy subjects and Alzheimer’s disease patients (Roland and Gulyas, 1995; Desgranges et al., 1998; Eustache et al., 2000; Rombouts et al., 2000). However, precise knowledge of the neuroanatomy of possible subdivisions of the parahippocampal gyrus in the human brain is still unclear, thereby the anatomical systems of declarative memory lacking from characterization. It is now accepted that it is not only the hippocampus that is the key structure in memory processing, but also the surrounding areas (entorhinal, perirhinal and posterior parahippocampal cortices) as well (for a review, see Fletcher et al., 1997; Tulving et al., 1999). Their implication in memory has also been investigated in lesion and electrophysiological studies in nonhuman primates (see for a review, see Squire and Zola, 1996). Indeed, several studies based on the lesion of the hippocampus and/or amygdala plus surrounding cortical areas, i.e. the entorhinal, perirhinal and/or posterior parahippocampal cortices, all led to declarative memory impairment (Squire and Zola-Morgan, 1991; Suzuki et al., 1993; Alvarez et al., 1995). Since research has focused on these brain areas, it has been shown that bilateral lesions of cortex lying the rhinal sulcus (including the lateral entorhinal cortex and perirhinal cortex) lead to an impairment in visual recognition memory tests (Meunier et al., 1993; Gaffan, 1994; Chavoix et al., 2002). Lesions of perirhinal cortex alone are sufficient to produce severe memory impairment (Malkova et al., 2001; Millien et al., 2002), while lesions limited to the entorhinal cortex alone result in mild visual recognition memory deficit (Leonard et al., 1995). Concerning the posterior parahippocampal cortex, the few available reports suggest that this brain area would be more involved in spatial than visual declarative memory (for a review, see Squire and Zola, 1996). Activation studies performed in the monkey with 2-deoxyglucose (2-DG) autoradiography (Davachi and Goldman-Rakic, 2001) or 18F-fluorodeoxyglucose (18FDG) PET (Blaizot et al., 2000) methods also revealed the implication of perirhinal cortex in recognition memory. It is interesting to note that patients with Alzheimer’s disease (AD), in whom declarative memory deficits are predominant (for a review, see Eustache et al., 1994), the transentorhinal area of Braak and Braak (1985) is the earliest cortical area and the most affected by neurofibrillary tangles. In the same way, a recent study of this research team revealed an age-related progression of tau pathology in baboon’s brains, thus providing a unique potential model of neurodegenerative disorders afflicting the human brain, such as AD (Schultz et al., 2000). Interestingly, we have recently shown in the baboon, that impairment in visual recognition memory observed after rhinal cortex lesion is correlated with a decrease in glucose consumption as measured by PET in several brain regions, also hypometabolic in AD (Blaizot et al., 2002). Taken together, these data do not only stress the importance of the parahippocampal gyrus and especially the rhinal cortex in memory, but also point out the need to elucidate the anatomy of this brain area to determine declarative memory at level systems. However, if experimental data have revealed precious information about the role of the medial temporal lobe in memory, the extrapolation to humans is more difficult. Therefore it seems relevant to assess a comparative anatomical evaluation among primate species to be used in neuroimaging techniques such as PET (Takechi et al., 1997; Blaizot et al., 2000) or fMRI (Logothetis et al., 1999) that can be applied, as in humans, to nonhuman primates to perform activation studies. Note that performing activation studies in nonhuman primates allow to assess plasticity of memory systems and synaptic reorganization after selective
brain lesions, which is not possible to perform in humans. Such activation studies require MRI as a morphological reference to identify brain regions significantly activated during a cognitive task, thereby making necessary to have the best anatomical interpretation of MRI images based on the histological correlation. Thus, the knowledge and use of the cytoarchitectonic data to determine the limits of the parahippocampal areas on the corresponding MR images of the same animal can be very useful to place approximately the limits of the parahippocampal areas on primates’ MR images, without the need for histological verification.

The purpose of the present study was to determine the structural organization of the parahippocampal gyrus in the baboon, specifically aiming at the correlation of histological sections and MR images. The data are compared to those observed in macaques and humans to see correspondence between those three primate species.

Materials and Methods
Three young-adult male Papio anubis baboons (14–16 kg) were used in this study according to the European Union rules for care and use of laboratory animals (UE 86/609/CEE).

Experimental Protocol
Two animals underwent a MRI examination for which the MR scanning methodology has been described elsewhere (for details, see Blaizot et al., 1999). Briefly, the animals were premedicated with a mixture of ketamine-xylazine (6–0.6 mg/kg i.m.), intubated and then ventilated with N2O/O2 (2:1 v/v). The anesthesia was completed with ketamine-xylazine (3.20–0.32 mg/kg/40 min i.m.) plus enflurane (0.5–1.5%). The head of the baboon was placed in a non-ferromagnetic stereotactic frame with the animal in a sphinx position. Heart rate, arterial pressure, body temperature and end-tidal CO2 were continuously monitored.

MR Scanning
MR scanning was performed using a GE Signa 1.5 T scanner with a 12.7 cm (5 inch) general purpose receive-only surface coil. MR-T1-weighted images were obtained in the coronal plane using the inversion-recovery technique (TI = 600 ms, TE = 3.8 ms, TR = 15.8 ms, FOV = 18 cm, matrix = 256 × 256, thickness = 1.5 mm).

Histological Procedure
Animals were deeply anesthetized with enflurane (0.5–2%) and N2O/O2 (2:1 v/v) plus atracurium (0.5 ml i.v.), and perfused transcardially after clamping the descending aorta. Blood was washed with 500 ml of saline. Fixation started with a series of 1% paraformaldehyde in 0.1 M phosphate buffer (4°C, pH 7.4) at rate of 250 ml/min for 6 min, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (4°C, pH 7.4) at the same rate for 15 min, and subsequently at 100 ml/min for 75 min. The series finalized with 5% sucrose in 0.2 M phosphate buffer (4°C, pH 7.4) at rate of 100 ml/min for 30 min. Brains were cut into five blocks along the rostrocaudal axis with the baboon’s head fixed in the stereotaxic apparatus. Blocks were then removed and cryoprotected in 10% glycerol and 2% dimethylsulfoxide in 0.1 M phosphate buffer during 24 h followed by 20% glycerol in 0.1 M phosphate buffer and 2% dimethylsulfoxide for 2 days. They were then sectioned coronally at 50 µm in a freezing, sliding microtome. One in five sections was immediately mounted onto gelatin-coated slides and stored for cytoarchitectonic analysis after thionin staining. The adjacent section was also mounted and stained for the demonstration of myelin series (modification of Heidenham stain; Hutchins and Weber, 1983). Additional series were prepared for the demonstration of acetylcholinesterase by the method of Hedreen et al. (1985). Immunohistochemical series were prepared for demonstration of the calcium binding protein parvalbumin (Swant, Bellizona, Switzerland) at a working dilution of 1:10 000, revealed with 3,3′-diaminobenzidine (25 mg in 100 ml Tris-HCl, pH 7.4 and 0.002% H2O2). Analysis was performed under a Leica Q500W stereomicroscope. Drawings of coronal sections every 1.25 mm apart from the beginning of the temporal pole until the end of the posterior parahippocampal cortex (average distance of 17.5 mm along the rostrocaudal axis). Boundaries of each parahippocampal area were first determined under detailed microscopic analysis (Insauti et al., 1995) of thionin stained sections as well as in the additional histochemical and immunohistochemical series. They were then charted on these drawings to appreciate the extent and topographical organization of each field in the parahippocampal gyrus. We performed a two-dimensional unfolded map based on unfolding the line corresponding to layer IV or the interval between layers III and V (see Fig. 5), using the fundus of the rhinal sulcus at rostral level and the transition between areas TH and TF (Bonin and Bailey, 1947) more caudally as base line. The unfolding method has been described previously (Insauti and Muñoz, 2001); see also the method followed by Insauti et al. (1987a, Fig. 15), for additional details on the unfolding of the temporal pole.

Results
After a brief description of the topography of the entorhinal, perirhinal and posterior parahippocampal cortices (Fig. 1), cytoarchitectonic characteristics of these subfields, used for delimitation, will be discussed and are shown in Figures 2 and 3. MRI–histological correlations are presented in Figure 4, in which parahippocampal subfields are delimited on both thionin stained sections (represented as drawings) and T1-weighted MR images of the same animal. Finally, a two-dimensional reconstruction of the ‘unfolded’ parahippocampal gyrus is presented in Figure 5. Because of the very high similarity in the parahippocampal gyrus between macaques and baboons, topography and cytoarchitectonics will be described using the same terminology as used by Amaral et al. (1987) and Suzuki and Amaral (1994) for the macaque. Although cytoarchitectonic features of the parahippocampal region is quite similar across species, we noticed differences on the general neuronal organization, especially in the temporal pole, where neuronal layers are less defined in baboons compared to macaques.

Gross Anatomy of the Parahippocampal Gyrus
The parahippocampal gyrus is a gross morphological term that describes the ventromedial area of the temporal lobe. It is composed of three different cortical areas, namely entorhinal, perirhinal and posterior parahippocampal cortices (see Figs 1 and 2).

We first observed several macroscopic characteristics in the baboon: the anterior and dorsal extent of the rhinal sulcus is limited as compared to macaque in which it does extend as far as the dorsal aspect of the temporal pole. Additionally, in the macaque the course of the rhinal sulcus and the hippocampal fissure are roughly parallel; in contrast, in the baboon, the larger extension to the entorhinal cortex forms an angle. In the human brain, the rhinal sulcus is rather short and almost perpendicular to the hippocampal fissure (Insauti et al., 1995). These characteristics also contribute to the expansion of the temporal pole in baboons as compared to macaques, although it remains smaller than in humans. Indeed, our calculations from 2D unfolded maps measurements of each area in the baboon, indicate that the temporal pole’s surface represents ~24% of the whole parahippocampal gyrus, while it represents 17% in macaques and 45% in humans (Burwell et al., 1996; data not shown).

The rostralmost portion is the temporal pole. It has been partitioned according to criteria described previously for

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Macaca fascicularis into areas 36pm and 36pl (Insausti et al., 1987b). Behind this region, area 35 of the perirhinal cortex occupies the fundus of the rhinal sulcus as it comes around the medial aspect of the anterior temporal lobe (Fig. 1). Ventrally and rostrally, area 35 borders laterally the rostral entorhinal cortex and medially area 36r of the perirhinal cortex. Area 35 tapers off and finally disappears at the caudal part of the entorhinal cortex (at the beginning of the hippocampal fissure approximately). Area 36 resembles more closely the lateral adjacent visual association area TE, especially at its caudal level (36c) as it presents columnar organization. More caudally, at the end of the rhinal sulcus, area 36 borders area TF of the posterior parahippocampal cortex.

The caudal part of the parahippocampal gyrus is formed by areas TH and TF of the posterior parahippocampal cortex, in caudal continuation with the entorhinal and perirhinal cortices respectively. At the end of the rhinal sulcus, it lies beneath the body and tail of the hippocampus. TH borders the subfield E_{CL} rostrally and area TF laterally (except for a short tongue of parasubiculum, not included in the unfolded map of Fig. 5).
This latter is bordered laterally by the visual associative areas TE and more caudally TEO. Rostral to TF, lies the area 36c and caudally is bordered by the visual area OA (Bonin and Bailey, 1947) or V4 (Zeki, 1971).

Cytoarchitectonic Organization of the Entorhinal Cortex

The entorhinal cortex is located at the ventromedial part of the temporal lobe. About one-half of the rostral extent of the entorhinal cortex lies under the amygdaloid complex, while the caudal half, starting at the hippocampal fissure, is associated with the uncal portion of the hippocampus. The entorhinal cortex is bordered medially and rostrally by the vertical extension of the rhinal sulcus (Figs 1 and 2). Rostrally and dorsally, the piriform and the periamygdaloid (area 51; Brodmann, 1909) cortices border the rostral entorhinal cortex. The posterior portion of this limit is indicated by a shallow groove, the sulcus semiannularis, that separates the entorhinal and periamygdaloid cortices. Laterally, the entorhinal cortex extends as far as to the fundus of the rhinal sulcus, bordering area 35 rostrally and 36c caudally.

The baboon entorhinal cortex as in the fascicularis monkey (Amaral et al., 1987) can be partitioned into seven distinct subfields, based on their cytoarchitectonic features. The distinction among subfields is based on the changing cytoarchitectonic features along the rostrocaudal and mediolateral axes, thus defining olfactory (E_O), rostral (E_R), intermediate (E_I), caudal (E_C) and caudal limiting (E_CL) subfields. Those subfields are bordered laterally by two subfields, lateral rostral (E_Lr) and lateral caudal (E_Lc), both located at the medial bank of the rostral half of the rhinal sulcus, up to the point where the rhinal sulcus becomes shallow. Of all the subdivisions, E_O is the...
only one characterized by its cytoarchitectonic features and its connectivity; as in the case of the *M. fascicularis* monkey it is likely that *E*$_{O}$ receives direct connections from the olfactory bulb as already described (Amaral et al., 1987; Insausti et al., 2002).

We recognize in the baboon six layers in the entorhinal cortex (Fig. 5a) following the scheme of layering described in macaques by Amaral et al. (1987), who, in turn, followed the concept of Lorente de Nó (1934). Although layers are designated I–VI, they are not transposable to those of the neocortex. Layer I is a plexiform layer that tends to be thicker at posterior levels. Layer II is one of the most characteristic of the entorhinal cortex. It is a layer made up of cell aggregates (islands) of relatively large and darkly stained pyramidal and stellate cells. The islands are distributed all over the entorhinal cortex, although they tend to be thinner rostrally. There is usually an acellular gap between layers II and III that tends to increase caudally. Layer III is made up of a relatively homogenous population of medium-size pyramidal cells, with an increasingly more columnar organization laterally as well as caudally. In contrast, rostral and medial portions of the entorhinal cortex are characterized by the arrangement of layer III pyramids into clusters. One of the most typical features of the entorhinal cortex is the absence of an internal granular layer (layer IV), being replaced by a cell-sparse zone called *lamina dissecans*, especially noticeable at mid-rostrocaudal levels. Layer V is made up of large and darkly stained pyramidal neurons; it merges with deep layer III at rostral levels, while they are separated by the *lamina dissecans* at mid and caudal levels. More caudally, layer V gets thicker and can be divided into three sublayers: Va, the most superficial, is made up of the largest pyramidal neurons of the whole entorhinal cortex; Vb, the middle sublayer, contains a larger proportion of smaller cells; and Vc contains only few neurons and looks like an acellular band. Layer VI is characterized by the presence of polymorphic neurons, easier to identify caudally. Interestingly, it does not present a coiled appearance in the baboon, in contrast to layer VI in the entorhinal cortex of the *fascicularis* monkey, thereby being more akin to layer VI of the human.
entorhinal cortex. In the same way, the border between layer VI and the white matter is quite clear caudally, while it becomes more blurred at rostral levels.

As mentioned above, the entorhinal cortex of the baboon can be partitioned rostrocaudally into seven subfields based on the cytoarchitectonic analysis. (Fig. 3a):

EO (Olfactory Subfield of the Entorhinal Cortex). Layer II is very thin or absent in patches. Layer III is made up of clusters of neurons. No lamina dissecans is present and layers V and VI are fused and not easy to differentiate.

ER (Rostral Subfield of the Entorhinal Cortex). Layer II is thicker than in EO, with islands of multipolar, darkly stained cells separated by wide cell-sparse zones. As in EO, layer III is made up of large and irregular patches of neurons (darker and bigger in the outer part) separated by cell-sparse areas. Layer IV is absent except for the caudal most portion, where it can be demonstrated by myelin stain while layer V presents an incomplete sublamination.

ELr and ELc (Lateral Rostral and Lateral Caudal Subfields of the Entorhinal Cortex). Along the rhinal sulcus and close to area 35, EL is different from the more medial areas ER and EI at the level of the layers V and VI, that are quite similar to those observed in area 35 with big and darkly stained neurons. Furthermore, sublamination of layers V and VI in EL is less evident than in EI, Eo or EC. EL can be divided in two sub-fields ELr and ELc because of the differences observed in layer II and III: Layer II in ELr, near the rostral border of the entorhinal cortex, is made up of wide islands of darkly stained neurons. These islands become wider at more caudal levels, in ELc, and tend to form a continuous and homogenous band. In the same way, layer III of ELc is notably more homogenous because of the presence of small cells that fill in the gaps seen in ELr, thus increasing the cell density.

EI (Intermediate Subfield of the Entorhinal Cortex). EI is the level of the entorhinal cortex more representative of the entorhinal cortex. Layer II is made up of islands of multipolar cells. The inner cells of layer II merge in columns with the...
superficial layer III neurons, while the deep portion of layer III is more continuous in appearance, what is reminiscent of layer III of EI in humans. Lamina dissecans is clearly present as an acellular band. Furthermore, EI presents a marked sublamination in layer V with a clear sublayer Vc.

EI (Caudal Subfield of the Entorhinal Cortex). Overall, EI has a more columnar appearance. Layer II presents wide islands of big, stellate neurons. By contrast, layer III has a rather homogenous appearance with a hint of columnar organization. Lamina dissecans is no longer noticeable in Nissl stain, although very rostrally in EI, myelin stain produces a band that corresponds to lamina dissecans. Finally, as in EI, sublayers of layer V in EC can be easily distinguishable.

EC (Caudal Subfield of the Entorhinal Cortex). EC is located at the caudal extreme of the entorhinal cortex and is followed caudally by a medial extension of the parasubiculum for a short distance, to be replaced by area TH of the posterior parahippocampal cortex. Layer II is thicker and more continuous (wider cell islands) than any other portion of the entorhinal cortex. Layer III is thinner and more columnar than EC and lamina dissecans is totally absent. In contrast to EC, the sublamination in layer V is less clear, especially at the level of sublayer Vc.

ECL (Caudal Limiting Subfield of the Entorhinal Cortex). ECL is included as the most rostral and dorsal part of the perirhinal cortex (Insausti et al., 1987a). Ventrally, it is bordered by area 36r of the perirhinal cortex, laterally and dorsally by the superior temporal gyrus, while medially and dorsally it is replaced by piriform cortex (adjacent to the limen insulae) and dorsal portion of area 35 of the perirhinal cortex (Fig. 1).

Temporal Pole. The temporal pole is included as the most rostral and dorsal part of the perirhinal cortex (Insausti et al., 1987a). Ventrally, it is bordered by area 36r of the perirhinal cortex, laterally and dorsally by the superior temporal gyrus, while medially and dorsally it is replaced by piriform cortex (adjacent to the limen insulae) and dorsal portion of area 35 of the perirhinal cortex (Fig. 1).

The temporopolar cortex or area 36p in the baboon is characterized by a rather thin layer II, which is discontinuous
at the ventromedial portion and forms small clusters of darkly stained neurons, while it becomes quite homogeneous in the dorsolateral field. This feature is the basis to recognize a polar-medial division (area 36pm) that contains clusters in layer II, and a polar-lateral (36pl) division with a continuous layer II (Fig. 3b). Both areas, 36pm and 36pl display the same cytoarchitectonic features in the remainder of the layers. Layer III is thick, populated by medium to big pyramids that often present a gradient from superficial to deep levels. Layer IV is thin but recognizable, while layer V is thick and populated by darkly stained, large pyramids. The limit between layers V and VI is blurred especially rostrally.

Area 35. Area 35 is the agranular portion of perirhinal cortex. It parallels closely the course of the rhinal sulcus both at its ventral portion — where it is bordered medially by the
entorhinal cortex — and at its dorsal portion, where the rhinal sulcus takes a vertical course in the medial aspect of the temporal pole and it is bordered by area 36pm (see previous section).

While area 35 keeps a basic cytoarchitectonic scheme that gives unity to the area, it is by no means uniform throughout and although it does not seem to justify further subdivisions, a hint of the areal specification found in the human area 35 (Insausti et al., 1995) can be recognized. Layer II, is prominent and made up of clumps of darkly stained neurons. Layer III is narrow and patchy and separated from a robust layer V by an acellular band of fibers, darkly stained in myelin stain. Layer V is very outstanding because of the presence of large darkly stained pyramids. At the vertical portion of the rhinal sulcus, layer V of area 35 is tangentially cut and takes the shape of a dark ribbon under the superficial layers of the rostral subfields of the entorhinal cortex. Layer VI is poorly populated and little prominent. Further confirmation of the border of area 35 with

Figure 3. [Image]
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the entorhinal cortex (E_LR and E_LC) is provided by the histochemical (AchE) and immunohistochemical (parvalbumin) preparations as shown in Figure 2h.

Area 36. Area 36 is the granular portion of the perirhinal cortex. Rostrally, area 36 (area 36r, see below) borders the ventral portion of area 36pm of the temporal pole, while caudally it runs along the lateral bank of the rhinal sulcus. Medially, it is bordered by area 35 and laterally by the infero-temporal cortex or area TE of Bonin and Bailey (1947). Overall, area 36 has a more columnar appearance, reminiscent of the lateral neighboring area TE. The clear separation between layers V and VI in TE allows the delimitation from area 36.

While area 36 shows a basic cytoarchitectonic pattern unified by the presence of a clear internal granular layer (layer IV), it shows enough differences between rostral and caudal portions that warrants a subdivision into a rostral 36r and a caudal 36c areas (Fig. 3b). Area 36r is characterized by a

Figure 3. (c).
bilaminar layer II with small clumps of darkly stained neurons. Layer III is populated with small, medium pyramids that present a gradient size, more accentuated than in temporopolar cortex areas 36pm and 36pl. Layer IV is present and thicker than in the temporopolar cortex, but thinner than in area 36c. Layer V is very outstanding, with the presence of large, darkly stained pyramids. Layer VI contains neurons of various sizes and shapes extending into the white matter.

Area 36c presents differences compared to area 36r in various layers. Layer II is thinner and more continuous than in area 36r. Layer III is more columnar than in area 36r and layer IV is well developed. Layer V is thick and presents big pyramids, darkly stained, also oriented in columns. Layer VI does not present differences compared to area 36r.

In contrast to the *fascicularis* monkey, where Suzuki and Amaral (1994) describe medial and lateral portions in both areas 36r and 36c, this difference could not be conclusively observed in the baboon.

**Cytoarchitectonic Organization of the Posterior Parahippocampal Cortex — Areas TH and TF of Bonin and Bailey**

The posterior parahippocampal cortex was described in the rhesus monkey by Bonin and Bailey (1947) and divided into two distinct areas, TF and TH. Both together make up the caudal portion of the parahippocampal gyrus and they continue caudally both the entorhinal and perirhinal cortices, behind the rhinal sulcus as far as the transition with visual association areas (Fig. 1).

As in perirhinal cortex, the cytoarchitectonic features of the posterior parahippocampal cortex in the baboon are comparable, although less defined, to the *fascicularis* monkey as described by Suzuki and Amaral (1994).

The main distinction between area TH and TF is the presence of an inner granular layer in area TF. Area TH is the more medially located of the two and it is bordered medially by the presubiculum. Layer II is thick and continuous; layer III is rather homogeneous and densely packed with small pyramids. Layer IV is absent and layer V is very outstanding by the presence of large pyramids, also densely packed, in contrast with layer VI that is more sparsely populated.

Area TF has in general a closer appearance to the adjacent neocortex. Layer II is thin and uniform. Layer III is more columnar that in area TH and less densely populated. Layer IV, although thin, is unmistakable. Layer V is not as conspicuous as in area TH, but still contains large and darkly stained neurons. Layer VI is fused with layer V and extends into the white matter.

The distinction between THr (rostral) and THc (caudal) is not as clear as in the macaque and although a gradient toward a more neocortical appearance at caudal levels is noticeable, we did not subdivide areas TH and TF into rostral and caudal divisions as in the *fascicularis* monkey (Suzuki and Amaral, 1994).

**Inferotemporal — Area TE of Bonin and Bailey — Visual Association Cortex**

The visual association area TE (Bonin and Bailey, 1947) borders laterally the perirhinal cortex and forms a wide band of typical neocortex, with distinct cytoarchitectonic features, neurons organized into clear-cut columns (see Figs 2 and 3c). Layer IV is much thicker than in proisocortical regions and layers V and VI are well separated.

**Delimitation of the Parahippocampal Regions on MR Images**

Figure 4 shows all the subdivisions of the parahippocampal region in case AB-3. The different subfields of the temporal pole, perirhinal, entorhinal and posterior parahippocampal cortices are approximately placed onto MRI cuts according to the precise rostrocaudal histological level of the same case. After analyzing the cytoarchitectonics of the parahippocampal region every 0.25 mm on thionin-stained sections of each brain, 15 slices, taken on average every 1.25 mm, were used for coregistration with the 15 corresponding 1.5 mm thickness MR images, on which we correlated the histologically defined borders with the MR image of the medial temporal lobe. The total rostrocaudal extent of parahippocampal region, calculated from MR images, is ~21 mm (14 × 1.5 mm). Histologically, it extended for ~17.5 mm (14 × 1.25 mm), the difference due to the shrinkage occurred during histological processing and staining, estimated at 16.6% and comparable to shrinkage obtained in other studies. Real rostro-caudal distances, i.e. calculated from MR-images, of each subfield of the parahippocampal region as well as characteristic landmarks from the
beginning of the temporal pole (that can be helpful in the delimitation of these subfields) are presented in Table 1.

**Bidimensional Reconstruction of the Parahippocampal Region**

Figure 5 represents an unfolded, two-dimensional map of the surface of the whole parahippocampal gyrus, including the temporal pole, perirhinal, entorhinal and posterior parahippocampal cortices in the baboon. We used the fundus of the rhinal sulcus as a reference from which the profile of layer IV or the interval between layers III and V have been unfolded. This line has been represented in the map as the line between entorhinal and perirhinal cortices, artificially elongated (dotted line) rostrally as far as the dorsal portion of the rhinal sulcus (section 4 in Fig. 5) and caudally between areas TH and TF.

As revealed in the unfolded map, the temporal pole occupies a large proportion of the parahippocampal region, especially area 36pm, as compared to the macaque, although the rostro-caudal extent of the temporal pole is limited (~5 mm for area 36p). Other than that, the topography and shape of the entorhinal, perirhinal and posterior parahippocampal cortices are quite comparable to those observed in the macaque.

**Discussion**

In this work we studied the anatomical and cytoarchitectonic characteristics of the brain areas in the parahippocampal gyrus of the baboon, i.e. the entorhinal, temporo-polar, perirhinal, and posterior parahippocampal cortices. Overall, the general organization of the parahippocampal region in the baboon is comparable to macaques (Suzuki and Amaral, 2003) and humans (Insausti et al., 1994, 1995). However, the lamination of the different areas in this region is less defined in the baboon compared to macaques, in particular the fascicularis monkey, but more evident than in humans.

The baboon entorhinal cortex presents a characteristic cytoarchitectonic organization in seven sub-fields, EBO, EBR, ELC, E, ECI, and ECL, as already described both in the fascicularis monkey (Amaral et al., 1987) and in humans (Insausti et al., 1995).

In the same way, the cytoarchitectonic organization of the temporo-polar, perirhinal and posterior parahippocampal cortices in the baboon is quite comparable in the three primate species. The rostral portion of the perirhinal cortex constitutes the temporo-polar region made up of areas 36pm and 36pl, that corresponds to area TG of Bonin and Bailey (1947) and to Brodmann’s area 38. We included this region as a part of the perirhinal cortex because of the similarities of its cytoarchitectonic organization with the area 36 at more caudal levels, in particular area 36r. Area 36pl, more dorsal and lateral than 36pm and for which layer II is more homogenous, would be roughly equivalent to area 36d of Suzuki and Amaral (1994, 2003), while the ventromedial portion 36pm would be included in the rostral portion of area 36r of the perirhinal cortex. Although we consider the temporo-polar cortex as a
rostral extension of the perirhinal cortex, its cytoarchitectonic characteristics are not as well defined as in 36r and 36c. Area 35 of the perirhinal cortex extends along the fundus of the rhinal sulcus. This agranular cortex between the entorhinal cortex and area 36r is present both in humans -where it has been also called transentorhinal cortex by Braak and Braak (1985) as well as in nonhuman primates. The cytoarchitectonics of the lateral adjacent perirhinal area 36 resemble to those seen in visual association area TE (Bonin and Bailey, 1947), especially with its granular layer IV and its columnar appearance.

The posterior parahippocampal cortex extends from the end of the rhinal sulcus as far as the visual association area TEO. Two areas can be distinguished, i.e. the agranular area TH medially and area TF laterally, with a well defined layer IV, as described by Suzuki and Amaral (1994, 2003). In this study, the authors distinguished the rostral portion of TH (THr) from the caudal one (THc) as well as the medial part of TF (TFm) from the lateral one (TFl). In the baboon, differences along the rostrocaudal and mediolateral axes are not very clear, and therefore, as in humans, we have not partitioned them.

MRI Study

Nowadays, nonhuman primate species are commonly used in functional studies such as PET (Perlmutter et al., 1991; Takechi et al., 1994; Eberling et al., 1995; Tsukada et al., 1997; Blaizot et al., 2000) or fMRI (Dubowitz et al., 1998; Stefanacci et al., 1998; Logothetis et al., 1999). We thus found it interesting to delimitate the different regions that compose the parahippocampal gyrus on MR images as it has been already done in humans by Insausti et al. (1998). Indeed, although MRI is usually used for the anatomical identification of the brain areas in activation studies, after coregistration with PET images, its resolution does not allow one to distinguish the limits between cytoarchitectonically different areas such as, for example, the limit between TH and TF of the posterior parahippocampal cortex.

Several neuroanatomical functional studies performed in humans reveal the activation of the parahippocampal region during episodic encoding and retrieval (for reviews, see Dolan and Fletcher, 1999; Schacter and Wagner, 1999). However, the identification of the activated brain areas is often vague, because of the lack of precise anatomical data concerning this region and of the nomenclature used. Furthermore, the interpretation of the neuronal networks revealed during activation in human studies could be strengthened by the knowledge and the use of nonhuman primates data for which anatomical connections have been demonstrated (Insausti et al., 1987a,b; Suzuki, 1996; Saleem and Hashikawa, 1998; Insausti and Muñoz, 2001) because of this high similarity between human and monkeys as also previously shown, for example, for the entorhinal cortex (Insausti, 1993).

Therefore, the overall anatomy and cytoarchitectonics of the parahippocampal region is comparable between human and nonhuman primates.

Despite of this homogeneity, we have found differences in the general organization, especially in the rostral part of the perirhinal cortex, the temporal pole. We first observed several macroscopic characteristics in the baboon: the anterior and dorsal extent of the rhinal sulcus is limited as compared to macaque in which it does extend as far as the dorsal side of the
temporal pole. Additionally, in the macaque the course of the rhinal sulcus and the hippocampal fissure are roughly parallel; in contrast, in the baboon they form an angle that gives a larger extension to the entorhinal cortex. In the human brain, the rhinal sulcus is rather short and almost perpendicular to the hippocampal fissure (Insausti et al., 1995). But the main macroscopic feature is the proportional extent of the temporal pole, that is greater in baboons as compared to macaques but smaller than in humans, as revealed by the 2D-unfolded maps of the parahippocampal region. Indeed, area 36p and especially 36pm, tends to expand laterally in baboons as compared to macaques and in a bigger extent in humans. In parallel, cytoarchitectonics of this area, is more diffuse, as compared to the macaque, displaying the absence of a clear-cut lamination and

Table 1
Distances in millimetres of various landmarks from the beginning of the temporal pole

<table>
<thead>
<tr>
<th></th>
<th>Beginning of the entorhinal cortex</th>
<th>Limen insula</th>
<th>Beginning of the amygdala</th>
<th>Anterior commissure</th>
<th>Transition between amygdala and hippocampus</th>
<th>End of hippocampus</th>
<th>Beginning of the posterior parahippocampal cortex</th>
<th>End of the posterior parahippocampal cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baboon 1</td>
<td>4.5</td>
<td>5.5</td>
<td>7</td>
<td>9</td>
<td>10.5</td>
<td>25</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>Baboon 2</td>
<td>6</td>
<td>7.5</td>
<td>9</td>
<td>12</td>
<td>12</td>
<td>27</td>
<td>18</td>
<td>25</td>
</tr>
<tr>
<td>Mean</td>
<td>5.25</td>
<td>6.5</td>
<td>8</td>
<td>10.5</td>
<td>11.25</td>
<td>26</td>
<td>17</td>
<td>23</td>
</tr>
</tbody>
</table>
a general neural organization closer to the neighboring neocortex. Patches of layer II in the entorhinal cortex are in general less evident than in the macaque; the variation in neuron size as well as in staining intensity between layers (for example, between the superficial and deep levels of layer III of the entorhinal cortex) is not clear in the baboon. Because of these characteristics, the delimitation of the parahippocampal areas in the baboon is thus harder than in the macaque and easier than in the human species in which differences among cytoarchitectonic features of the parahippocampal areas are less pronounced.

This cytoarchitectonic analysis seems thus to reveal a gradient of complexity among primates, especially at the level of the temporal pole which looks more developed in phylogenetically higher species, taking into account the cyriification degree that is higher in baboons than in macaques (Zilles et al., 1989). Interestingly, several lesion or functional experimental studies have revealed the particular implication of the temporal pole in declarative memory in primates (Murray and Mishkin, 1986; Meunier et al., 1993; Blaizot et al., 2000). Furthermore, this region is strongly connected to the entorhinal cortex as well as with the field CA1 of the hippocampus, or the orbitofrontal cortex, all known to be largely implicated in memory (Witter et al., 1989; Suzuki and Amaral, 1990). Considering these data altogether, the following hypothesis can be suggested: does the phylogenetic development of the temporal pole contribute to the specialization of declarative memory?

**Conclusion**

In this work, we have described the anatomy and cytoarchitecture of the parahippocampal region in the baboon. Globally, its neuronal organization is comparable to macaques and humans. These observations allow thus to compare directly data obtained in these three primate species in terms of neuroanatomy to explain memory circuits and neuronal networks as revealed by PET or fMRI. The advantage of the use of nonhuman primates in this kind of study is the possibility to investigate, for example, the consequences of specific brain lesions (such as lateral entorhinal and perirhinal cortices) on brain metabolism to explore neuronal mechanisms of reorganization and/or plasticity, that will allow better understanding of neurodegenerative disorders such as Alzheimer's disease. The analysis of the parahippocampal region also revealed that its cytoarchitecture has an overall organization in baboons closer to humans than to macaques, especially in its rostral part, the temporal pole, suggesting that this region could represent an important site for the processing of declarative memory information, by means of its connections with the hippocampal formation and cortical association areas.

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