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Parcellation of Human Temporal Polar Cortex: A Combined Analysis of Multiple Cytoarchitectonic, Chemoarchitectonic and Pathological Markers

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Abstract

Although human temporal polar cortex (TPC), anterior to the limen insulae, is heavily involved in high-order brain functions and many neurological diseases, few studies on the parcellation and extent of human TPC are available using modern neuroanatomical techniques. The present study investigated TPC with combined analysis of several different cellular, neurochemical, and pathological markers finding that this area is not homogenous as at least six different areas extend into TPC with another area being unique to the polar region. Specifically, perirhinal area 35 extends into the posterior TPC while areas 36 and TE extend more anteriorly. Dorsolaterally, an area located anterior to the typical area TA or parabelt auditory cortex is distinguishable from area TA and defined as area TAr (rostral). The polysensory cortical area located primarily in the dorsal bank of the superior temporal sulcus, separate from area TA, extends for some distance into TPC is defined as TAp (polysensory). Anterior to the limen insulae and the temporal pyriform cortex, a cortical area, characterized by its olfactory fibers in layer Ia and lack of layer IV, was defined as temporal insular cortex and named as area TI after Beck (1934). Finally, a dysgranular TPC region which capped the tip with some extension into the dorsal aspect of the TPC is defined as temporopolar area TG. Therefore, human TPC actually includes areas TAr and TI, anterior parts of areas 35, 36, TE, and TAp, and the unique temporopolar area TG.

Keywords

Medial temporal lobe; temporal area TG; auditory cortex, perirhinal cortex, insular cortex; pyriform cortex; neurofibrillary tangle

Introduction

In several classic cortical maps, the cortex covering the pole of the human temporal lobe was labeled as a single cortical area [temporal polar area by Smith (1907); area 38 by Brodmann (1909); area TG by von Economo and Koskinas (1925) and von Economo (1929)]. On the most popular and commonly used map of Brodmann, the extent of area 38 on both medial and lateral aspects of the temporal lobe is basically of similar size. This is also true for area TG of von Economo and Koskinas although their labeled area TG was

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larger than area 38 of Brodmann. In contrast, Smith (1907) labeled an asymmetric temporal polar area with its larger portion on the medial aspect and a smaller one on the lateral aspect. The large medial portion extended from the pole posterior to the level of the uncus tip while the extent of the small lateral portion is comparable to Brodmann's. It should also be pointed out that in Sarkisov et al's map (1955), although not commonly used, the temporal polar cortex (TPC) was basically treated as an anterior extension of areas 22, 21, 20 (lateral aspect), and areas 20tc and 20l (medial aspect). Areas 20tc and 20l appear to correspond to the perirhinal cortex (Sarkisov et al, 1955). Finally, Bailey and Von Bonin (1951) labeled the temporo-polar tip as a dysgranular region, similar to but smaller than Brodmann's area 38; and Vogt (see Stephan, 1975) identified subregions of the insular cortex (ai3-5) on the posterior portion of the mediodorsal aspect of the TPC.

Previous studies have shown that the TPC is heavily involved in some common neurological diseases such as Alzheimer's disease (AD) and Pick's disease, temporal lobe epilepsy and schizophrenia (Arnold et al, 1991; 1994; Wright et al, 1999; Gur et al, 2000; Semah, 2002; Crespo-Facorro et al, 2004; Chabardes et al, 2005). For instance, TPC has been shown to be one of the earliest affected in AD pathogenesis (Arnold et al, 1991) and one of the earliest regions to be involved at the onset of temporal lobe seizures (Chabardes et al, 2005). The human TPC has also attracted renewed interest because PET imaging, functional MRI (fMRI) imaging and lesion studies indicate it is critical for many higher brain functions such as retrieval of the proper names of people and landmarks, composition of sentence meaning, autobiographical memory (left TPC; Damasio et al, 1996; Maguire and Mummery, 1999; Maguire et al, 1999; Gorno-Tempini and Price, 2001; Grabowski et al, 2001; Noppeney and Price, 2002; Tsukiura et al, 2002; Vandenberghe et al, 2002; Emmorey et al, 2003), recognition of familiar people, scenes and objects, and abstract word processing (right TPC; Perani et al, 1999; Nakamura et al, 2000; Gainotti et al, 2003).

Careful examination of the above mentioned PET/fMRI results show that these higher brain functions are mapped mainly onto the lateral aspect of the TPC. On the other hand, intracortical electrical stimulation studies of the human TPC have shown that nearly all clinical responses were evoked by stimulation of the ventromedial aspect of the pole while stimulation of the dorsolateral part of the pole did not evoke these responses. These responses were mostly psychic, viscerosensory, autonomic (visceromotor) (Ostrowsky et al, 2002). Functional mapping of higher-order auditory cortex in rhesus monkey has also shown distinct separation of function by anatomical location in the dorsolateral part (for auditory processing) of the TPC and the ventromedial part (for visual processing). Interestingly, the middle portion of the TPC between the dorsal and ventral portions showed neither auditory nor visual functions suggesting additional processing capabilities (Poremba et al, 2003; Poremba and Mishkin, 2007). Collectively, these findings indicate that discrete functions can be mapped to different subareas of the human TPC.

The classic anatomical concept of treating the human TPC as a single area (area 38 or TG) is clearly inadequate to account for the results of the functional studies mentioned above, and therefore needs reevaluation since the classic studies on human cortical mapping were mainly based on Nissl preparations. Surprisingly, so far no detailed investigation has been done on human TPC using modern neuroanatomical techniques such as immunohistochemistry for different neurochemical markers. Our recent studies have suggested that Nissl preparations are not very helpful in defining some complex and transitional cortical regions, e.g. the cingulo-parahippocampal isthmus region (Ding and Rockland, 2001; Ding et al, 2003). Instead, immunochemical detection of neurochemical markers such as the calcium binding proteins parvalbumin (PV) and calbindin D-28k (CB), and the non-phosphorylated neurofilament protein (SMI-32) has proven to be a useful tool

for defining cortical area borders more precisely (Carmicheal and Price, 1994; Ding and Rockland, 2001; Ding et al, 2003; Ongur et al, 2003; Kondo et al, 2003).

The human TPC is a complex and transitional region where many different cortical regions meet. Although its typical cytoarchitectonic features such as great thickness, poor granular layer IV and well developed layers V and VI are easily observed, the extent of human TPC has been difficult to define since its transition to other areas of the various temporal convolutions has been described as “always a gradual one, and not at all distinctly marked” in Nissl preparations (von Economo, 1929). Given an increasing number of functional studies and few modern neuroanatomical studies on human TPC, the present study aims to investigate the cyto- and chemoarchitectures of this region and parcel it by means of the combined use of different neurochemical markers, in order to provide a more precise anatomical basis for increasing the accuracy and clarifying the results of clinical lesion and functional studies of the anterior temporal lobe.

Materials and Methods

Human brain tissue preparation

Anterior temporal lobes of human brains were obtained from 15 individuals (65-82 years old) at autopsy via the University of Iowa Deeded Body Program with postmortem times before fixation ranging from 3 to 6 hours. These included, as shown in Table 1, five normal cases (cases 1-5), specimens from subjects without any history of neurological or psychiatric illness and with no or few neurofibrillary tangles (NFTs) and amyloid plaques in medial temporal cortex in our routine thioflavin-S staining, six normal aging specimens (cases 6-11) from subjects without a clinical neurological history but with brain NFTs at Braak's stages I-IV and four AD specimens (cases 12-15) from subjects with apparent clinical dementia and with brain NFTs at Braak's stages V and VI (see Braak and Braak, 1992). Briefly, stages I and II were characterized by mild or severe NFT lesions in the perirhinal cortex; stages III and IV were featured by severe NFT lesions in both perirhinal and entorhinal cortices and by mild NFT lesions in hippocampus, but no NFTs were observed in neocortex. At stages V and VI, NFT distribution extended throughout all neocortical association cortices, in addition to extensive NFTs in perirhinal, entorhinal and hippocampal cortices. These NFT distribution patterns were consistent and reliable within each brain group staged as above and fulfill Braak's staging criteria, which are widely accepted.

Since Tau pathogenesis resulted in unique and reliable labeling patterns in the entorhinal cortex (EC) and areas 35 and 36 but not in other neocortical areas (i.e., the labeling patterns in other neocortical areas were basically similar and not helpful for further subdivision) of the temporal lobes in normal aging and clinical AD cases (e.g., Braak and Braak, 1992, Braak et al, 1994; Van Hoesen et al, 2000), Thioflavin-S stain was found useful in helping the parcellation of area 35 and adjoining EC and area 36 in the TPC. The staining patterns were similar to those found with Campbell's and Gallyas' stains (not described here). Thus, in the present study, Thioflavin-S staining was mainly applied to the normal aging and clinical AD cases to reveal the pathological features of tau lesions in the EC and areas 35 and 36, in addition to its use in routine pathological staging for brain sections as described above.

The anterior temporal lobes were blocked perpendicular to the anterior-posterior commissure line. They were then subdivided into two blocks and immersion-fixed in ice-cold 4% paraformaldehyde solution in 0.1 M phosphate buffer saline (PBS, pH 7.3) for 48 h. The blocks were transferred into 30% sucrose -PBS solution for 48-36 hours before they were cut into 50- μ m-thick frozen sections and collected in 10 sequential sets of sections. The first four sets of sections were stained with Thionin or myelin, Thioflavin-S,

acetylcholinesterase (AChE) and *Wisteria floribunda* agglutinin (WFA) histochemistry respectively. While one set was stored for possible future use, the remaining 5 sets of sections were stained immunohistochemically with the following primary antibodies and dilutions: PV (1:10000, Swant), CB (1:10000, Swant), SMI-32 (1: 5000, Sternberger), neuronal nuclear antigen or neuronal nuclei (NeuN, 1:1500, Chemicon), and abnormally phosphorylated tau (AT8, 1:1000, Innogenetics).

Antibody characterization

The antibodies to the two calcium-binding proteins (PV and CB) were mouse monoclonal antibodies (IgG1) from the same manufacturer (Swant, Bellinzona, Switzerland) and were both produced by hybridizing mouse spleen cells with myeloma cells lines. The PV antibody was produced by immunizing mice with PV from carp muscle (Swant product # 235). The antibody specifically stained the ⁴⁵Ca-binding “spot” of PV (MW 12,000) from rat cerebellum on two-dimensional immunoblot assays. The CB antibody was produced by immunizing mice with CB from chicken intestine (Swant product #300) and specifically stained the calcium-binding “spot” for CB (MW 28,000) from rabbit cerebellum and kidney on two-dimensional immunoblot assays. According to the manufacturer, both antibodies reacted with respective antigens from human, monkey, rabbit, rat, and mouse. PV labels subsets of non-pyramidal neurons while CB labels subsets of both pyramidal and non-pyramidal neurons in human cerebral cortex (e.g., Mikkonen et al, 1997; Ding et al, 1999).

The SMI-32 antibody was a mouse monoclonal IgG1 (Sternberger Monoclonal Inc, Baltimore, MD, USA) directed at a non-phosphorylated site on neurofilament H, and recognized a double band at MW 200,000 and 180,000, which merged into a single neurofilament H line on two-dimensional blots (Sternberger and Sternberger, 1983). In human tissue, the antibody stains a subpopulation of cortical pyramidal cells thought to be vulnerable to excitotoxic death (Campbell and Morrison, 1989; Ang et al, 1991) and had been widely used to delineate and differentiate cortical areas in primates (e.g. Hof et al, 1996).

The AT8 antibody (Innogenetics, Gent, Belgium; product #90343) was a mouse monoclonal IgG1 produced by immunizing Balb/c mice with human paired helical filament (PHF) tau protein and fusing Balb/c spleen cells with mouse myeloma SP2/0 cells. Instead of recognizing normal tau, it recognizes abnormally phosphorylated tau protein including a soluble form present in early tau pathogenesis [the pretangle stage of Braak et al (1994)]. In this stage, AT8 labeled tau was diffusely distributed in the soma, dendrites and axons, giving the labeled neurons the appearance of Golgi-like staining. In later stages (e.g. Braak's stages IV-VI), AT8+ neurons had an abundance of broken, twisted, and varicose dendrites and axons in the region of the cell bodies (Braak et al, 1994). AT8+ cells were mostly pyramidal neurons (Braak et al, 1994) but in some regions the thorn-shaped astrocytes reported by Schulz et al (2004) were found in some cases.

The NeuN antibody (Chemicon, Temecula, CA, USA; MAB377, Clone A60) was a mouse monoclonal IgG1 generated by inoculating Balb/c mice with purified cell nuclei from mouse brain and fusing cells with a myeloma cell line. Western blots indicated the antibody recognized four bands in the 46-66kDa range. NeuN is a neuron-specific DNA-binding protein and the antibody recognizes NeuN in human, monkey, rodent and other mammalian and vertebrate species. Since NeuN labels neurons but not glia, it was more useful in cytoarchitectonic analysis of cerebral cortex of human brains which were often only available at older ages when more glia cells are present. The presence of excessive glia might affect cytoarchitectonic analysis of complex cortical regions, as suggested by Vogt et al (2001).

Thioflavin-S, AChE and myelin histochemistry

For Thioflavin-S staining, sections were stained with 1-% Thioflavin-S (Sigma) following the procedure described by Lamy et al (1989). Thioflavin-S stained insoluble NFTs in the soma and neural threads (NT) in neuropil.

For AChE histochemistry, the procedure used by Ding and Rockland (2001) was adopted to reveal AChE enzyme activity in different subareas of the TPC.

Myelin histochemistry was carried out according to Solodkin and Van Hoesen (1996) to reveal the distribution of horizontal myelinated axons in layer I, to help with the identification of primary olfactory versus non-olfactory cortices. Primary olfactory cortex is the cortex receiving direct olfactory inputs in its superficial layer I (Ia) while non-olfactory cortex does not have this feature (Allison, 1954). Thus, in the present study, myelin staining results will be only described in regions associated with primary olfactory cortex and with emphasis on its layer I.

WFA histochemistry

WFA is a lectin that selectively labels N-acetylgalactosamine beta 1 residues of glycoproteins within the extracellular matrix of the neurons. It stains the perineuronal nets (PNs) that are mostly associated with non-pyramidal neurons (Hilbig et al, 2001). The basic steps for WFA staining were similar to those described by Hilbig et al (2001) but with significant modification. Briefly, sections for WFA staining were treated with 0.3% H₂O₂ in PBS for 40 min. After three rinses in PBS, the sections were incubated overnight at 4°C with biotinylated WFA (Sigma) at a concentration of 10µg/ml in PBS containing 0.3% triton X-100. After four rinses (10 min each) in PBS, the sections were incubated in ABC complex kit (Vector, 1:200) for one hour at room temperature. The reaction was revealed by 0.05% 3,3'-diaminobenzidine (DAB, Sigma)) and 0.01% H₂O₂ in 0.1 M PBS (brown color) or by SG kit (Vector) following the kit instruction (blue color) for 5–10 min.

Immunohistochemistry

Each of the five sets of sections from the anterior temporal lobes for each brain was processed for immunohistochemistry using the avidin–biotin–peroxidase complex (ABC) method as described by Ding et al (2003) with slight modification. Briefly, the sections were quenched with 0.3% H₂O₂ in 0.1 M PBS for 30 min. After washing in 0.1 M PBS, the sections were blocked with 5% normal goat serum containing 0.3% Triton X-100 in 0.1 M PBS for 1 h at room temperature followed by overnight incubation with the primary antibodies described above at 4 °C. After washing, sections were incubated sequentially with biotinylated goat anti-mouse secondary antibody (1:200, Vector), and ABC complex (ABC Elite kit, 1:200, Vector). Finally the reaction was visualized by 0.05% DAB and 0.01% H₂O₂ in 0.1 M PBS for 5–10 min, with or without adding 0.25% nickel ammonium sulfate. To detect any non-specific labeling, some negative control sections were identically incubated without the primary antibody.

Double labeling

In two normal cases (cases 1 and 2), one set of the sections stained for AT8 was double stained with NeuN in order to show that the cases used in the present study for cytoarchitectonic descriptions are truly normal (i.e., without pathological changes in cellular layers II–VI; see Result section for details). The AT8 staining was revealed by DAB reaction (brown color) as described above, then the sections were re-incubated in anti-NeuN as above but secondary antibody peroxidase activity was revealed by using the SG substrate (Vector) which produces a blue/gray color. AT8 (a pathological marker) labeled cells would not be labeled by NeuN, a normal neuronal marker. Thus, no double-labeled cells would be

observed although the relative distributions of single-labeled cells would be shown on the same tissue sections. In two further cases (cases 6 and 7 at Braak's NFT stage II), some sections containing the EC and the perirhinal cortex (areas 35 and 36) were stained with NeuN first as described above and then were re-stained with Thioflavin-S in order to reveal cell types and location of dead (NFT-containing) neurons in area 35 over the background of other surviving neurons.

Data analysis

All stained sections were examined with a Nikon Eclipse 600 light microscope under bright- and/or dark-field illuminations. Whole slide sections were scanned into Adobe Photoshop 7 at a resolution of 800 dpi via a Umax Powerlook 1100 scanner for data analysis and preparation of low magnification figures. Matching sections from individual staining sequences were identified and boundary areas delineated by staining characteristics marked. Sections were compared with each other and boundary identification confirmed. Other photomicrographs at higher magnifications to document the cyto- and chemo-architectures were taken with Nikon DCX1200 digital camera attached to the microscope. All images were transferred into Adobe Photoshop for adjustment of the brightness and contrast and for arrangement and lettering of each panel in specific figures.

Results

1. Gross neuroanatomy of the human TPC and adjoining regions

Since the defined extent of TPC was variable in previous studies (Smith, 1907; Brodmann, 1909; von Economo, 1929; Bailey and Von Bonin, 1951; Sarkissov et al, 1955), a larger region anterior to the limen insulae (LI) and roughly corresponding to the area TG of von Economo (1929) was investigated in the present study.

As shown in Fig. 1A, B, and D, there were usually two temporopolar sulci on the mediodorsal surface of the TPC, termed here as medial and lateral polar sulci (psm and psl) respectively. In some cases (Fig. 1E), only one polar sulcus (ps) was observed as shown in Fig. 2A-C. The small gyri separated by the polar sulci were termed gyri of Schwalbe (GS in Fig. 1A). On the medial surface, the collateral sulcus (cs) and inferior temporal sulcus (its) were often seen to extend only into the posterior TPC while some short, irregular and variable sulci were often observed in the anterior TPC (Fig. 1C). When the rhinal sulcus (rs) was present, its posteroventral portion usually crossed the anterior portion of the cs while its anterodorsal portion usually extended into the dorsal portion of the TPC for variable distances (Fig. 1B-D). On the dorsal surface of the anterior hippocampal uncus, two small gyri were usually identified which were separated by the semiannular sulcus (ss). Lateral to the ss was the semilunar gyrus (SG) while the ambient gyrus (AG) was located anterior and medial to the ss (Fig. 1A, B). On the lateral surface, the superior temporal sulcus (sts) often extended into the tip of the TPC but the middle temporal sulcus (mts) usually extended into the posterior part of the TPC (Fig. 1F).

2. Parcellation of Human TPC

In order to provide a consistent nomenclature to the portions of the TPC that were the anterior extensions of the typical temporal areas, we adopted the commonly used terminology of Brodmann's areas 35 and 36 for the perirhinal cortical regions and Von Economo's areas TA and TE for the superior temporal, middle-inferior temporal regions respectively. In addition, Beck's area TI (temporal insular cortex, Beck, 1934; Allison, 1954) was introduced to our parcellation of the posterior TPC, consistent with the term frontal insular cortex (FI) adopted by Ongur et al (2003) to describe the orbitofrontal insular region. Both TI and FI were merged at the anterolateral LI (limen insulae) and all three of

these regions were covered with olfactory fibers in superficial layer I (Ia) and were agranular cortex. The term agranular cortex was defined by the absence of granular cells or very few scattered granular cells typically between layers III and V, as revealed at higher (10x or above) magnification in NeuN stained sections. In addition, dysgranular cortex was defined by a thin but clear band of granular cells seen in layer IV at higher power magnification while granular cortex was defined by a thick and dense band of granular cells in layer IV that could be seen even at very low power magnification (1x-4x), in addition to higher magnification. Finally, the remaining region that covered the tip of TPC was named area TG after Von Economo (1929) although this area TG was reduced in size according to our detailed investigation in the present study.

Based on our combined analysis of multiple markers, seven areas were recognized in human TPC. These included the most anterior part of areas 35, 36, and TE, and areas TAr, TAp, TI and TG (Figs. 1 and 2). Following the approach adopted by Price and his coworkers (Carmicheal and Price, 1994; Ongur et al, 2003), different areas were distinguished from each other if they showed divergent staining patterns with at least two stains and if they could reliably be found in the same location across multiple brains (table 2).

Anterior area 35—In order to define the most anterior portion of area 35 in the TPC, we need to briefly describe the features of area 35. Typically, the human perirhinal cortical area 35 is described as located in the collateral sulcus. As demonstrated in a recent report (Van Hoesen et al, 2000) and, as well as shown in Fig.3 of the present study, human area 35 can be subdivided into two parts (35a and 35b). Area 35a presents a distinct columnar organization in its superficial layers (Fig. 3A-C) and this distinguishes it from adjoining entorhinal cortex (EC) which always shows a patchy organization of neurons in its superficial layers. More importantly, the whole anteroposterior extent of area 35 possesses a unique layer (named layer IIIu in the present study) of large pyramidal cells, as revealed by Braak and Braak (1992; termed as descending layer II) and Van Hoesen et al (2000; termed as deep portion of layer III). Another apparent feature of area 35 is the existence of alternating vertical large-celled columns with vertical small-celled columns in layers II and III, especially in area 35a. The large cells were much more vulnerable to tau lesions than the small cells (Fig. 3C).

The unique layer IIIu (u standing for unique) was so named in the present study for the following reasons. First, layer IIIu was observed only in area 35 but not in other cortical regions; layer IIIu in area 35a often adjoined or merged with the bottom portion of the large-celled columns extending from layers II and III, and which are another important feature of area 35 (Fig. 3A, C). Second, layer IIIu was composed of large pyramidal cells that are always the earliest to be involved in Tau pathogenesis. This was clearly seen in Thioflavin-S preparations of AD brains as almost all of these unique large pyramidal cells contained NFTs and stood out as a unique layer (Fig. 3A, B). Third, layer IIIu lies just superficial to the typical layer V but deeper than the typical layer III (Fig. 3A, C); naming it as deep layer III (or IIIc) or even layer IV would misrepresent it as part of typical layer III, which itself never had the above-mentioned unique features. Fourth, naming this unique layer as Pre- α or layer II as done by Braak et al (1992) results in a confusing lamination nomenclature for human area 35: the layers of area 35 would be sequentially layers I, II, III, Pre- α or II, V and VI. We believe naming this layer as IIIu is preferable to placing a Pre- α or layer II between typical layers III and V.

Anterior area 35 was localized anterior to the most anterior portion [mainly Eo (the olfactory part) and Elr (the rostral lateral part) of the entorhinal cortex (EC), posterior to the anterior portion of area 36 and medial to area TI (Fig. 1B). In practice, when a deep rhinal sulcus (rs) existed, anterior area 35 was mainly located in the fundus and the lateral/anterior bank of the

rs while the anterior EC was located posterior/medial to the RS. This is similar to the relative positions of the perirhinal and entorhinal cortices in rodents (Shi and Cassell, 1999). When the rs is shallow, anterior area 35 often extended further forward on the TPC surface than anterior EC (Fig. 1B).

Cytoarchitecture: Anterior area 35 in NeuN-stained sections was characterized by a either lack of granular layer IV (area 35a) or the presence of a few granular cells scattered in layer IIIu (area 35b) (Figs. 4A; 5D, I). Layer II was characteristically thin and irregular, and contained both large and small cells (Figs. 4A; 5D, I). Layer III in area 35a often displayed alternating large- and small-celled columns while a relatively evenly distributed population of medium to large-sized pyramidal cells was found in area 35b (Figs. 4A; 5D, I). Layer IIIu in both 35a and 35b was characterized by large pyramidal cells (Figs. 4A; 5I). Layer V mainly contained larger and more darkly stained pyramidal cells compared to layer III (Fig. 5D, I). Layer VI consisted of a mix of relatively smaller pyramidal and fusiform neurons (Fig. 5D).

Chemoarchitecture: PV immunostaining in anterior area 35 appeared to be relatively lighter than in adjoining regions (Figs. 6A2, B2 and C2; 7A2, B2, C2 and D2). A lightly stained PV+ plexus was found in layer IIIu and the deep part of layer III. Only a small number of PV+ neurons were observed in layers II-VI (Fig. 8A and B). CB immunostaining in area 35 was noticeably darker than adjacent areas (Fig. 6A3, B3 and C3) and at higher magnification, both pyramidal and non-pyramidal neurons were found to contain CB. CB+ pyramidal neurons were mainly found in layers II, III and IIIu while CB+ non pyramidal neurons were mainly scattered in layers II and III (Fig. 6E). SMI-32 staining in anterior area 35 was weak and mainly localized to layers V and VI (Fig. 9B and C). Compared to the intense staining in the amygdala and caudate nucleus and putamen (data not shown), AChE staining intensity in area 35 was moderate in layers II and III, relatively light in layer IIIu and very light in layers V and VI (Figs. 10H, I; 11C). WFA labeling in area 35 was generally lighter than adjoining EC and area 36 (Fig. 7A4, B4, C4 and D4; 12 A, C and E). WFA labeling in area 35 was mainly found in layers III and IIIu. Within area 35, area 35a had much clearer vertical columns than area 35b in PV and CB labeled sections (Figs. 6A2, B2, A3 and B3; 7A and B; Table 2) although these differences between areas 35a and 35b were not apparent in SMI-32, AChE and WFA stained sections.

Tau pathology: In AT8 stained sections from the same cases at Braak's stages I-IV, the density and staining intensity of AT8+ neurons and processes could be compared across different regions (Fig. 7). As shown in Figure 10A, area 35 displayed the highest regional density of AT8 immunostaining and intensely stained AT8+ neurons and processes were present throughout layers II-VI. Within area 35, the AT8 staining intensity in area 35a was higher than in area 35b (Fig. 7; Table 2).

Comparison with the anterior EC: Overall, neurons in area 35 were much more intensely stained by NeuN than those in the EC in all five normal brains, making the boundary between these two areas distinctly demarcated (Fig. 6A1, B1 and C1). In CB stained sections, layer II labeling in area 35 was lighter than in the anterior portion of the EC (Eo; Fig. 6D). AChE staining in area 35 was relatively lighter than in the EC (Figs. 10H; 11A, C). In addition, the Eo had darker AChE staining mainly in layers V and VI while area 35 had the darker staining mainly in layers II and III (Fig. 11A, C). The Eo showed intense AT8 immunostaining in layers II and V, and relatively lighter AT8 labeling of layers III and VI while area 35 displayed homogeneously dark AT8 staining (Fig. 7A3, B3 and C3; 10A). More importantly, area 35 had a unique layer IIIu which did not extend into the EC (Table 2).

Anterior area 36—Anterior area 36 was located anterior to anterior area 35, ventral/medial to area TG and ventral/anterior to area TE (Fig. 1B-D). Anterior area 36 was identified as a continuation of the typical area 36 located lateral to the collateral sulcus (Figs. 2-inset and 6). Unlike area 35, this region did not have vertical cell columns in the superficial layers. It also did not have the special layer IIIu characteristic of area 35.

Cytoarchitecture: In NeuN stained sections, area 36 appeared to be a dysgranular region with a thin but clear layer IV containing modest numbers of granular cells and identifiable as a single layer in low power microphotographs (Figs. 5A, B and E; 6A1, B1 and C1; 9A). Layer II was thicker and more densely packed with small round cells and pyramidal cells than area 35. Layer III appeared less densely packed and mainly consisted of small- to medium-sized pyramidal neurons in its upper and deeper portions respectively. Layer V contained small- and medium-sized pyramidal neurons and was slightly less densely packed than in layer VI, thus the border between these two layers was not clear. Layer VI contained medium to large neurons in its superficial portion but mainly smaller fusiform neurons in its deep portion (Figs. 5E; 13F).

Chemoarchitecture: In PV stained sections, area 36 had moderately densely labeled fibers and terminals in layer IV and deep layer III but few in other layers (Figs. 6A2, B2 and C2; 7A2, B2, C2 and D2). More PV labeled neurons were seen in area 36 than in area 35 especially in layer III (Fig. 8C). CB staining in area 36 was also moderate (Fig. 6A3, B3 and C3). Many CB+ pyramidal neurons were found in deep layer III while some CB+ non pyramidal neurons were scattered throughout layers II-IV (Fig. 6F) with many fewer in layers V and VI. SMI-32 stained neurons and processes in area 36 were mainly present in layer V and less staining was observed in layer VI and deep layer III (Fig. 9B and D). Light AChE staining was observed in deep layer III and in layers V and VI in area 36 (Figs. 10H, I; 11E). WFA labeling in area 36 was mainly located in deep layer III and layer IV (Figs. 7A4, B4, C4 and D4; 12E).

Tau pathology: Compared to area 35 in the same case, the density and staining intensity of AT8+ neurons and processes in area 36 were moderate (Figs. 7A3, B3, C3 and D3; 10B). AT8 labeled neurons and processes were mainly seen in layers III and V (Fig. 10B).

Comparisons with area 35: In comparison with area 35, area 36 had generally darker PV, SMI-32 and WFA staining and generally lighter CB, AChE and AT8 labeling. A combination of these markers made the areas 35/36 border easy to identify (Figs. 6, 7). Based on the above described features revealed with different markers, anterior area 36 was found to extend only as far as the ventromedial part of the TPC but did not extend into the anterior tip of the TPC which was included in area TG (Fig. 1; Table 2).

Area TG—Area TG mainly occupied the tip and the anterior portion of the mediodorsal aspect of the TPC. It merged with area TI posteriorly on the mediodorsal surface, with area 36 ventromedially, with area TAr dorsally, and with area TE posteriorly on the lateroventral aspect of the TPC (Fig. 1A-F).

Cytoarchitectonic features: Like anterior area 36, area TG also appeared to be a dysgranular region though with a thinner and weaker layer IV, at least in NeuN stained sections (Figs. 5C; 9A). Compared to area 36, layer II of area TG was thin and less densely packed. Layer III was relatively densely packed and mainly consisted of medium-sized pyramidal neurons, especially in its deeper two-thirds. Its superficial one-third mainly consisted of slightly smaller pyramidal neurons and it gradually merged with layer II,

leaving an indistinct border between layers II and III. Layers V and VI of area TG were thick and similar to those of area 36 in cell composition (Fig. 5C).

Chemoarchitecture: In PV stained sections, area TG had a pattern of weakly labeled fibers and terminals in layer IV and deep layer III with a much sparser distribution of immunoreactivity in other layers (Figs. 6C2; 7D2, E2 and F2). Very few PV labeled neurons were seen in area TG (Fig. 8D). Overall CB immunostaining in area TG was generally dark, similar to that found in area 35b (Fig. 6C3). Many CB+ pyramidal neurons were found in deep layer III while some CB+ non pyramidal neurons were mainly seen in layers II-III (data not shown). Dark SMI-32 immunostained neurons and processes in area TG were mainly localized to layer V while less overall SMI-32 staining was seen in layer VI and deep layer III (Fig. 9B, G). AChE labeling in area TG was detected in layers III-VI and was a little darker than in area 36 (see Fig. 11D, E). WFA labeling in area TG was mainly observed in deep layer III with much less in layers II and IV (Fig. 7D4, E4, F4; 12D).

Tau pathology: The density of AT8 labeled neurons in area TG was higher than in area 36 while the density of AT8 stained processes in area TG was lower than in area 36 (Figs. 7D3, E3 and F3; 10C). AT8 labeled neurons and processes were mainly seen in layers II and III with many fewer found in layers V and VI (Fig. 10C). Consistent with this AT8 staining pattern, NFTs were only found at stages IV-VI in AD cases and area TG was much less affected than area 36. For example, in early AD cases, area TG displayed few or no Thioflavin-S stained NFT cells in layers II/III while area 36 had many more although both areas contained many NFT cells in layer V. In severe AD cases, the density of NFT cells in the superficial layers of area TG was much lower than in area 36.

Comparison with area 36: As described above, area TG had overall less dense PV, WFA and AT8 labeling but more intense CB and AChE staining compared to anterior area 36, in addition to specific differences in cytoarchitecture, especially in layer III. Thus, although both areas TG and 36 were dysgranular cortical regions, a combination of multiple markers made these two areas distinguishable (Table 2).

In summary, the combination of markers employed here defined an extent of area TG much reduced from the original description even though the original defining features described by Von Economo (1929) were retained. These features included (1) a dysgranular layer IV; (2) a thin layer II and thick layers III and VI; and (3) medium-sized pyramidal cells homogenously packed in layer III. It should be noted that laminar staining patterns in area TG were fairly heterogeneous across its whole extent. For example, from the most anterior tip (which adjoined granular areas TE and TAr) to the mediodorsal surface (which merged with the agranular area TI), dysgranular layer IV and the intensity of staining produced by the PV antibody and WFA became gradually, but noticeably, reduced while the AT8 labeling very gradually increased in area TG (Figs. 2A-C and 7).

Area TI—According to Allison (1954), the term area TI was used by Beck (1934; see also Stephan, 1975 for discussion) to describe the posterior portion of the TPC that is covered with olfactory fibers running in its layer Ia as revealed in myelin stained sections (see Fig. 5L). Although Allison (1954) included this area TI in his pre-piriform area because of a similar layer I in both regions (Fig. 5H, J, L), we have opted to separate them because with Nissl and NeuN stains, the real pre-pyriform (or simply pyriform) area was additionally characterized by a typical three-layer lamination, i.e., molecular (I), granular (II) and polymorph (III) layers as shown in Figure 5H. The granular layer or layer II was composed of very densely packed small cells and was very easily identifiable even in low power photomicrographs (Fig. 5B, H). In contrast, area TI was seen to be an agranular region of multiple layers and lacking only layer IV (Fig. 5J).

Cytoarchitecture: Area TI appeared to be an agranular region lacking a layer IV in NeuN stained sections. Layer I of area TI was very thick and could be subdivided into upper (Ia) and deeper (Ib) portions. Layer Ia consisted of many densely packed horizontal olfactory fibers as clearly demonstrated in the myelin preparations (Fig. 5L) and many of its glial cells were stained in some cases by AT8 (Figs. 5J; 10F, G). Layer Ib was an apparent neuron-free zone with fewer fibers and glia. Layer II was thin and less densely packed with small round and pyramidal cells, whereas layer III was thick and relatively densely packed mainly with small-sized pyramidal neurons. Layer V was also thick and contained loosely packed small to medium-sized pyramidal neurons. Layer VI was very thick and also contained small to medium-sized neurons (Fig. 5J).

Chemoarchitecture: In PV stained sections, area TI had a modest density of PV-immunopositive fibers and terminals in deep layer III (IIIb) but little in other layers (Figs. 6B2; 7B2 and C2) and few labeled neurons were seen in layers II-VI. Overall CB immunostaining in area TI was also moderate (Fig. 6B3) though many CB+ pyramidal neurons were found in deep layer III. Some CB+ non pyramidal neurons were found scattered in layers II-III with few in layers V and VI. SMI-32 immunostaining of neurons and processes in area TI was light and mainly localized to layers V and VI. Darker AChE staining in area TI was observed in layers III, V and VI than in area TG (Fig. 10H; 11B, D). WFA labeling in area TI was very light and mainly located in deep layer III (Fig. 7B4 and C4; 12B).

Tau pathology: The density of AT8+ neurons and processes in area TI was relatively high (Figs. 7B3 and C3; 10E). AT8 labeled neurons and processes were mainly seen in layers III and VI with less seen in layers II and V (Fig. 10E).

Comparison with areas 35, TG, PI and the EC: Area TI had overall light PV, SMI-32 and WFA staining and generally dark CB staining; this is similar to that found in adjoining area 35. However, AChE staining in area TI was generally denser than in area 35 (Fig. 11B, C) while AT8 labeling in area TI was generally lighter than in area 35 (Fig. 10A, E). In addition, area TI had a unique layer I while area 35 had a unique layer IIIu as described above. All these morphological differences suggested that these two areas are distinct although both are agranular regions. Compared to adjoining dysgranular area TG, area TI lacked a granular layer IV and had olfactory fibers in its layer Ia. Area TI also exhibited lighter PV and WFA staining, but darker CB, AChE and AT8 staining than area TG (Figs. 7, 10, 11 and 12). Area TI merged with the agranular part of the insula at the LI and adjoined area PI (parainsular area) lateroposteriorly. Area PI is a dysgranular region mainly localized in the fundus of the circular sulcus of the insula. In PV, WFA, and SMI-32 stained sections, area TI had less labeling compared to area PI. In contrast, the former had darker staining in CB, AChE and AT8 stains when compared to the latter (Figs. 6, 7). Area TI also adjoined the anterior EC medioposteriorly. As described above, the anterior EC contained large cells in layer II which were vulnerable to early tau lesion and formed a clear patch-like organization while area TI lacked this type of arrangement (Table 2).

Area TAr—Area TAr (defined as the area rostral to Von Economo's area TA) was located dorsal to area TG and the anterior areas TAp and TE, and anterior to area TA (Fig. 1A, B, D-F).

Cytoarchitecture: Area TAr should be classified as a granular cortical region as it has a thick and prominent layer IV which was identifiable as a single layer even in low power microphotographs (Figs. 5A, B, G and 6 A1, B1, C1; 9A). Layer II was relatively thicker and more densely packed while layer III could be subdivided into an upper IIIa with higher

cell density and mainly consisting of smaller pyramidal neurons, and a deeper IIIb with a lower cell density and mainly consisting of medium-sized pyramidal neurons. Layer V contained small to medium-sized pyramidal neurons and had slightly lower cell density than layer VI. Layer VI mainly contained medium-sized neurons in its superficial portion and smaller fusiform or spindle cells in its deep portion (Fig. 5G).

Chemoarchitecture: In PV immunostained sections, area TAr had strong PV labeling of fibers and terminals in layers IV and IIIb but moderate labeling in layers II, IIIa, V and VI (Figs. 2A-C; D, F; 6A2, B2 and C2; 7-Column PV; 8H). More PV immunoreactive neurons were seen in layers II, III, V and VI of area TAr than in area 36 (Fig. 8H). CB immunoreactivity in area TAr was moderately dense in deep layer III (IIIb; Fig. 6A3, B3 and C3). CB+ neurons found in deep layer III resembled pyramidal neurons while those scattered in layers II-IV were mostly CB immunoreactive non-pyramidal neuron types. Strong SMI-32 immunoreactivity in area TAr was localized to layers V and VI while moderate labeling was seen in deep layer III (IIIb; Fig. 9B, F). AChE staining in the deep layers of area TAr appeared lighter than in areas TI and TG but darker than in areas 36 and TE (Fig. 10I). WFA labeling in area TAr was intense and mainly located in deep layer III and layer IV (Fig. 7-column WFA).

Tau pathology: As in area TEv (Fig. 10D), few AT8 labeled neurons and fibers were observed in area TAr.

Comparison with areas TG, TA and PI: Area TAr had a much thicker layer IV, generally stronger PV, SMI-32 and WFA immunoreactivity, and lighter CB immunoreactivity and AChE labeling, and much lighter AT8 immunoreactivity, compared to area TG (Figs. 2-5). Area TAr could be easily distinguished from area TA by its relatively lighter overall PV staining compared to the very dark PV labeling found over area TA (or roughly parabelt auditory cortex), as demonstrated in Figure 2-Inset, D and F. The posterior portion of area TAr was also easily distinguishable from adjoining dysgranular area PI by the lighter overall appearance of PV and WFA staining in PI (Figs. 6A2 and B2; 7; Table 2).

The anterior area TAp—The anterior area TAp (meaning polysensory region separated from Von Economo's area TA) is also a granular cortical region and situated mainly on the dorsal bank of the anterior STS. Anterior area TAp was located ventral to area TAr, dorsal to anterior area TE and posterior to area TG.

Cytoarchitecture—Area TAp presented a thick and clear layer IV with a prominent columnar organization (Figs. 5A, B, K; 6A1, B1, C 1; 9A). Layer II was relatively thicker and more densely packed with small round neurons than other parts of TA. Layer III was loosely packed and could also be subdivided into a superficial part (IIIa) mainly consisting of smaller pyramidal neurons, and a deeper part (IIIb) mainly consisting of medium to large pyramidal neurons. Layer V contained small to medium-sized pyramidal neurons which appeared to be arranged in small vertical columns. Layer VI was densely packed and mainly contained medium-sized neurons. A columnar organization was not evident in layer VI (Fig. 5K).

Chemoarchitecture: Area TAp had a dense and darkly stained distribution of PV immunoreactive fibers and terminals in layers IV and IIIb with much more modest labeling in layers IIIa and V (Figs. 2C, E; 7A2, B2, C2 and D2; 8G). Many PV immunoreactive neurons were found in layers III-IV (Fig. 8G). CB labeling in area TAp was light (Fig. 6A3 and B3) but while only a few CB+ pyramidal neurons were found in deep layer III, many CB+ non pyramidal neurons were seen in layer II with moderate labeling in layers II-VI.

Darkly stained SMI-32 immunoreactive neurons and processes were found in deep layer III and layers V and VI (Fig. 9B). AChE staining in area TAp was generally light (Fig. 10H, I) in sharp contrast to WFA labeling in area TAp which was strong and mainly located in deep layer III and layer IV (Fig. 7-column WFA).

Tau pathology: In common with areas TEv (Fig. 10D) and TAr, few AT8 labeled neurons and fibers were observed in area TAp.

Comparison with area TAr: Although area TAp had a pattern of PV and WFA staining similar to area TAr, lighter CB staining clearly set it apart (Fig. 6A3 and B3). In addition, although both areas showed similar dark SMI-32 staining in the deep layers V and VI, area TAp showed many more darkly stained SMI-32 immunoreactive neurons in its deep layer III than area TAr did (Fig. 9B). Following NeuN staining, area TAp had a thicker layer IV and a less densely packed layer III compared with area TAr (Table 2).

The anterior area TE—Area TE is a typical neocortical region with a clear and thick layer IV, dark staining for PV, WFA and SMI-32, and overall lighter staining for AChE and CB. Area TE could be further divided into area TEv, which mainly occupied the inferior temporal gyrus, and area TEd which mainly occupied the middle temporal gyrus.

Cytoarchitecture: Area TE had a thick layer IV with a prominent columnar organization within it (Figs. 5A, B, F; 6A1, B1, C1; 9A). Layer II was relatively thick and densely packed with small neurons. Layer III was loosely packed and could be subdivided into a superficial part (IIIa) with mostly smaller pyramidal neurons, and a deeper part (IIIb) with mostly large pyramidal neurons. Layer V contained mainly medium-sized pyramidal neurons and was less densely packed than layer VIa, making the layers V/VI boundary clearly identifiable. Layer VI was densely packed and mainly contained medium to large-sized neurons (Fig. 5K).

Chemoarchitecture: Strongly PV immunoreactive fibers and terminals in layers IV and IIIb with more moderate labeling in layers IIIa and V were observed in area TE (Figs. 2, 6, 7, 8E, F). Numerous PV immunoreactive neurons were found in layers III-IV (Fig. 8E, F). Overall CB immunostaining in area TE was lighter compared to areas 35 and TAr (Fig. 6A3, B3, C3; G). Specifically, few or no CB+ pyramidal-like neurons were found in layers II-III and IV-VI of area TE, whereas large to moderate numbers of CB+ non-pyramidal neurons were seen in layer II and layers III-VI respectively. Strongly SMI-32 immunoreactive neurons and processes were found in deep layer III and layers V and VI (Fig. 9E) though AChE staining in area TE was light (Fig. 10H, I). WFA labeling in area TE was strong and mainly present in deep layer III and layer IV (Fig. 7-column WFA; 12F) with relatively less strong in layers IIIa, V and VI (Fig. 12F).

Tau pathology: Few AT8 labeled neurons and fibers were observed in area TE (Fig. 10D).

Distinction between areas TEv and TEd: Although both regions had similar overall immunostaining with CB and AT8 antibodies, and similar densities of WFA and AChE labeling, subtle architectonic differences between them could be discerned. TEv had a higher cell density in layers II and III and more clear radial columns in layer V than area TEd. Further, area TEv had fewer PV immunoreactive neurons and fibers/terminals compared to area TEd (Fig. 8E, F) and had fewer neurons strongly labeled by SMI-32 in deep layer III and layers V/VI (Fig. 9B).

Comparison with adjoining areas 36, TG, TAp and TAr: Area TE appeared generally more strongly stained by PV and SMI-32 antibodies, generally weaker by CB antibody and much lighter by AT8 antibody than adjoining area 36 though the border between area 36 and area TE was particularly clear-cut in AT8 immunostained material (Fig. 7). The differences between anterior area TE and area TG were very evident with immunostaining/staining for NeuN, PV, WFA, SMI-32 and AT8. For example, Area TE had a much thicker layer IV than area TG and much lower cell densities in layers III and V. More large cells were identifiable in layers IIIb and VI of area TE compared to area TG (Fig. 5C, F, G). PV, WFA, SMI-32 labeling was generally darker in TE while CB, AChE and AT8 staining was much lighter. Area TE also adjoined TAp and the anterior area TAr (Fig. 7-level D). Compared to area TAp, area TE had slightly more intense (Figs. 2; 7) or similar PV immunoreactivity and WFA staining (Figs. 6, 7), and similar CB and SMI-32 immunoreactivity. In addition to a darker overall PV immunoreactivity, area TE had lighter AChE staining than area TAp (Fig. 10H, I) and a higher cell density in layer VI (Figs. 5F, K). Compared to area TAr, the neuronal density in layer III of area TE appeared noticeably lower, the superficial layers in area TE were generally thinner, and the pyramidal cells in layer III were larger (Fig. 5F, G). In addition, the SMI-32 labeling in layer III of area TE was much darker than in area TAr (Fig. 9B, E, F).

3. The adjoining entorhinal, pyriform and peri-amygdaloid cortices

The anterior EC—The most anterior part of the EC (mainly Eo and Elr) adjoined anterior area 35 posteriorly. In the EC, only layer II and the deep layers (V and VI) were darkly stained with NeuN antibodies; the wide layer III was lightly stained (Figs. 5B; 6B1). This contrasted well with area 35 where overall dark NeuN immunoreactivity was seen in layers II-VI (Figs. 5B; 6A1, B1). A similar pattern was observed in classic Nissl stained preparations, but the low-power appearance of staining of layer II neurons was much darker than seen with NeuN stain probably due to the additional labeling of glia cells by Nissl stain. Although both Eo (or Elr) and area 35 have cell islands in the superficial layers II and III, the shapes and patterns of the cell islands were different in these two areas. The cell islands in layer II of the Eo (or Elr) were usually ovoid with their long axes parallel to the pia while those in superficial layer III were more irregularly-shaped and “patch-like” (Fig. 13A, B). However, the cell islands in the superficial layers of anterior area 35 were vertically oriented with their long axes perpendicular to the pia and these cell islands (or columns) were composed of alternating large- and small-celled islands (or columns, Figs. 3,4). In early AD cases, the cell islands in layer II but not those in superficial layer III of the Eo (or Elr) contained Thioflavin-S stained NFTs (data not shown). However, only the large-celled columns and not the small-celled columns of area 35 contained NFTs. More importantly, the whole anteroposterior extent of area 35 had the characteristic layer IIIu, as described above.

The pyriform cortex—The pyriform cortex (PC or prepyriform cortex by some authors) was located posterior to area TI and anterior to the peri-amygdaloid cortex (PAC, Fig. 1B). The pyriform cortex had a wide layer I, which was divisible into a superficial part (Ia) containing many glia and a deep part (Ib) containing few glia (Figs. 5B, H). In some cases examined in the present study, many AT8-positive, thorn-shaped astrocytes (Schultz et al, 2004) were found in layer Ia but not in Ib (Fig. 5H). A unique feature of the PC was that its layer II was composed of uniformly darkly and densely stained small granular cells. Its layer III contained lightly stained and loosely packed cells without forming clear cell patches (Fig. 5H). The PC was thus distinguishable from the EC and area TI by its unique layer II and three-layered structure.

The subarea of peri-amygdaloid cortex (PACo)—a special subarea of the PAC was found amidst the PC, EC (Eo) and the main part of the PAC (Fig. 1B). This subarea was

named as PACo following Price (1990) and appeared to be much more evident in human than in monkey brains. In the present study, PACo was found to be clearly distinct from the PC, EC and the main part of the PAC. A unique feature of the PACo was its distinctly patchy layer III located under a thin layer II (Fig. 13C). Following NeuN immunocytochemistry, the patches in layer III were seen to consist of dark densely packed small cells distributed through the whole depth of layer III. The patches were of round, oval and irregular shape and separated from each other by spaces containing lightly stained and loosely distributed cells (Fig. 13C). Whether this PACo can be treated as a distinct region requires further evidence from connectional, neurochemical and functional studies. This region appears to have been included in a variety of different cortical regions including EC, PC, the amygdalopiriform transition area (Apir) and the periamygdaloid cortex by different authors (e.g., Allison, 1954; Stephan 1975; De Olmos, 1990; Price, 1990; Insausti et al, 1995).

Discussion

The present study has provided strong cell distribution and chemoarchitectonic evidence that the human TPC region, area TG as originally labeled by Van Economo (1929), is not a homogenous cortical region but contains several distinct areas. These include the most anterior portion of areas 35, 36, TE, areas TAr, TAp and TI, and area TG which is smaller than that originally defined by Von Economo (Figs. 1A-C; 12). The present mapping of human TPC, which is based on a combined analysis of multiple cellular, phenotypic and pathological markers, differs substantially from previous attempts at mapping this cortical region (Smith, 1907; Brodmann, 1909; Van Economo 1929; Sarkissov et al, 1955; Insausti et al, 1998ab). The different cytoarchitectural scheme reported here is mainly attributable to the more effective delineation of boundaries provided by the use of multiple stains. Previous studies mostly relied on the cytoarchitectonic appearance in classic Nissl stain which may not have brought out subtle changes in architecture. In addition, the TPC itself is a complex, transitional region where many different cortical regions meet and the transition between areas of the various temporal convolutions was “always a gradual one, and not at all distinctly marked” in Nissl preparations, as Von Economo (1929) pointed out. This has made the extent of the TPC vary widely among different maps based mainly on Nissl preparations. Below we discuss some of the major findings and differences of the present study, primarily in comparison with previous studies in humans.

1. Area 35 is anterior to the entorhinal cortex in TPC

In the present study, the most anterior area 35 was found to adjoin the anterior EC (mainly Eo and Elr) and to be co-extensive with it regardless of the existence of the rhinal sulcus (rs). Area 35 roughly corresponds to the anterior portion of area TGa of Von Economo (1929). This area TGa was described as an agranular region and labeled in his map as located anterior to his area HA, which roughly corresponds to the anterior EC. However, it should be pointed out that on the mediodorsal aspect of the TPC, Von Economo's area TGa also surrounds the insular area Ic and appears to include our area TI, which is also an agranular region. In the present study, area TI was designated as distinct from area 35 because it has a dense arrangement of olfactory fibers in its layer Ia and is less darkly stained with anti-AT8, while area 35 lacks olfactory fibers in its layer Ia and has the darkest AT8 immunostaining in the region. A further critical feature is that area TI does not have the large pyramidal cell columns and the unique layer IIIu vulnerable to tau pathology characteristic of area 35.

Other authors have labeled anterior area 35 in different ways. In a recent study of human medial temporal lobe (Insausti et al, 1998a, their Figure 1 section 2), the area corresponding region to our area TI is labeled as area 35d, based on its agranular appearance in Nissl

preparations. However, we observed clear differences between areas 35 and TI. In Brodmann's map (1909), area 35 is not shown to extend to the TPC region anterior to the EC (his areas 34 and 28) although the present study shows a clear extension into TPC. Finally, in the map of Sarkissov et al (1955), their area 20l appears to correspond roughly to area 35 except for the most anterior portion which does not appear to be located anterior to the EC and instead extends more anteriorly to occupy the majority of the ventral aspect of the TPC. Most of this area was included in area 36 in the present study.

In recent Nissl-based studies (Insausti et al, 1998ab), the anterior EC at and anterior to the level of the limen insulae appears to have been regarded as the anterior area 35 (e.g., Insausti et al, 1998b, their Fig. 2C, D). This differs from the present study where the anterior EC was found to be very easily distinguishable from anterior area 35 in NeuN, AT8 and Thioflavin S preparations. Among the many differences between EC and area 35 are the different shape, pattern and cell composition of the cell islands in the superficial layers in many stains and overall staining intensity in NeuN and AT8 preparations, as described above. For instance, the wide layer III in anterior EC is lightly stained while layer II and deep layers V and VI were darkly labeled in NeuN and AT8 preparations; whereas area 35 is more evenly and darkly stained (e.g., Figs. 5B; 6B1; 7).

Establishing the anterior limit of area 35 at the same point where the EC (area Eo) ends also brings the situation in human cortex in line with the boundaries usually delineated in non-primate species. For example, in rodents, perirhinal cortex (area 35) is co-extensive with the entorhinal cortex and when entorhinal cortex transitions to piriform cortex, so area 35 transitions into the posterior (parietal) insular cortex (Burwell et al., 1995; Shi and Cassell, 1998).

2. Area 36 extends to the ventral portion of the TPC

In the present study, area 36 was found to extend to the ventral portion but not the most anterior tip of the TPC. Thus our anterior area 36 roughly corresponds to Brodmann's although the extent of his area 36 in the TPC region is a little smaller than the one identified here. In the maps of Smith (1907) and Von Economo (1929), the region corresponding to our area 36 appears to be included in their larger temporal polar area or area TG. The area 20tc of Sarkissov et al (1955) appears to correspond to the typical area 36 lateral to the collateral sulcus, but this area 20tc was not shown to extend into the ventromedial aspect of the TPC.

It appears to have been difficult in previous Nissl studies to distinguish areas 36 and TG in the TPC region (e.g., Insausti et al, 1998a, b). The inconsistent labeling of the human TPC within this group and among different groups also indicates how difficult it is to define the boundaries in the TPC without a combination of multiple staining techniques. In the present study, however, differences were consistently found between areas 36 and TG in the TPC region with a combination of multiple marker stainings although it can not be completely ruled out that individual or natural variation may partly contribute to the difference.

3. Areas TAr and TAp are different from areas TA and TG

The STGr (i.e., the rostral part of the superior temporal gyrus; roughly corresponding to area TAr in the present study) has different connections compared with the posteriorly located para-belt auditory association cortex (roughly corresponding to area TA in the present study) in monkeys (e.g., Hackett et al, 1999; Romanski et al, 1999). Consistent with this finding is the evidence presented here showing area TAr to be less darkly stained in PV and SMI-32 immunostaining, and WFA preparations (Figs. 2A-D, F; 7 and 9; Table 1) while the posteriorly located area TA is very darkly stained with these same markers (Fig. 2D, F and

the Inset; Table 1). Area TAr is also different from area TG since the former area has a distinct layer IV while that layer in the latter is dysgranular. Area TAr showed relatively darker PV, SMI-32 and WFA labeling compared with area TG. In previous studies, the region of our area TAr was variously included in the dorsal part of temporal polar area (Smith, 1907), area 38 (Brodmann, 1909) or area TG (von Economo, 1929). Since area TAr has not been well defined, detailed information about its organization and connections in monkey is limited although this region has been shown to be a high order auditory association cortical region (Poremba et al, 2003).

Area TAp is the cortical region located in the upper bank and fundus of the superior temporal sulcus. Based on connectional and physiological studies of non-human primates, this area is regarded as a polysensory association cortex (e.g., Seltzer and Pandya, 1978, 1991; Bruce et al, 1981). Area TAp in monkeys shows responses to both auditory and visual stimuli, thus confirming its polysensory nature (Poremba et al, 2003). In the present study, area TAp clearly differed from areas TAr and TG in its very heavy staining of pyramidal neurons in both layers IIIb and V/VI in SMI-32 stained sections (Fig. 9B). Differences between areas TAp and TAr or TG were also observed with the NeuN stain (Figs. 5, 6).

4. Area TE extends farther into the TPC region than previously thought

In the present study, the granular cortical area TE was shown to extend more anteriorly than previously labeled by Von Economo (1929), though it is roughly similar to the areas 21 and 20 of Brodmann (1909). This extension is consistent with many connectional studies in monkey suggesting area TE continues far into the anterior TPC on the lateral surface (e.g., Iwai and Yukie, 1987; Webster et al, 1991; Webster et al, 1994; Saleem and Tanaka, 1996; Saleem et al, 2000). According to connectional and functional studies in monkeys, anterior area TE is a high order visual association cortical region (Ungerleider and Haxby, 1994).

5. Olfactory cortex extends farther into human TPC

Although the temporal part of the pyriform cortex has been identified in the posterior TPC region of both monkey and human brains (Allison, 1954; Stephan, 1975; Price, 1990), the identification of area TI anterior to the pyriform cortex (PC) in the present study suggests that human olfactory cortex also extends far into the TPC. Area TI has an important feature in common with the PC, that is, layer Ia in both areas is covered with thick olfactory fibers and contains many glial cells. However, area TI is easily distinguished from the PC by its mesocortical lamination pattern and the difference in composition of layer II, as detailed in the Result section and shown in Fig. 5H and J. In addition to the presence of olfactory fibers in layer Ia, another key landmark defining agranular insular cortex is the anterior rhinal sulcus, which clearly marks the boundary between the PC and insular cortex in non-primates such as rodents (Shi and Cassell, 1998; Paxinos and Franklin, 2001). Although in some human brains the rhinal sulcus can not be identified as located between the PC and area TI (e.g., Fig. 1B), a shallow extension of the rhinal sulcus was observed to separate PC and area TI in others (e.g., Fig. 1D). This is strongly suggestive of a consistency with the PC/insula boundary in non-primates and also suggests our labeling of area TI is accurate. It is not clear if a similar temporal insular cortex exists in monkey TPC since so far no such an area has been identified although the area TGa of Kondo et al (2003) is a possible homolog of human area TI.

Thus, the region commonly designated as human 'olfactory cortex' (e.g. Afifi and Bergman, 1998) may have to incorporate the insular cortex of the limen, the pyriform cortex and areas TI, PACo and Eo in the temporal lobe, as well as the pyriform cortex and area FI in the frontal lobe since all these regions were found to be covered with olfactory fibers derived from the olfactory tract running in layer Ia.

6. Area TG is a unique area in TPC

Although area TG or area 38 was originally labeled as a cortical region capping the TPC in both human and monkeys (though to different extents), some studies have challenged this idea on the basis of connectional findings in monkey and the dysgranular nature of this area. Most have treated the TPC as a continuation of the perirhinal cortical area 36 while area TG was not referred to (e.g., Insausti et al, 1987; Suzuki and Amaral, 1994, 2003). However, other authors have suggested that anterior area 36 extends only into the ventral portion of the TPC but not to the most anterior tip and dorsal portion in monkeys (Iwai and Yukie, 1987; Preuss and Goldman-Rakic, 1991; Webster et al, 1991; Saleem and Tanaka, 1996; Saleem et al, 2000, 2007; Hoistad and Barbas, 2008). Here, an area TG was identified in the tip of the monkey TPC although it is smaller in extent than area TG as originally labeled by Von Bonin and Bailey (1947) (Krieg 1975; Iwai and Yukie, 1987; Preuss and Goldman-Rakic, 1991; Webster et al, 1991; Saleem and Tanaka, 1996; Saleem et al, 2000; Hoistad and Barbas, 2008). Consistent with these reports is the finding here that in humans the ventral portion of the TPC belongs to area 36 while the dorsal portion appears to belong to area TAr. Furthermore, there is a unique area TG which is located amidst areas 36, TE and TAr and roughly corresponds to the dysgranular temporopolar tip outlined by Bailey and von Bonin (1951). Connectional studies in monkey have revealed differences between the dorsal and ventral temporal pole (Galaburda and Pandya, 1983; Moran et al, 1987; Barbas, 1988; Webster et al, 1991; Suzuki and Amaral, 1994; Carmichael and Price, 1995; Saleem and Tanaka, 1996; Barbas et al, 1999; Kondo et al, 2003; Saleem et al, 2008) and between the medial and lateral temporal pole (Stefanacci et al, 1996; Hoistad and Barbas, 2008). Also, a functional study in monkey has shown differences between the ventral and dorsal portions of the temporal pole wherein the ventral portion is associated with visual function while the dorsal one is associated with auditory function (Poremba et al, 2003). Collectively, these findings suggest that our division of the TPC is the more accurate as regards the location of area TG. Further studies are still needed to clarify the monkey homolog to human area TG.

7. A general comparison with monkey TPC subdivisions

As in humans, three different types of cortex are recognized in the TPC of monkeys. These include agranular, dysgranular and granular cortices (Moran et al, 1987; Gower, 1989; Carmichael and Price, 1995; Suzuki and Amaral, 2003; Kondo et al, 2003; Saleem et al, 2007). The dysgranular areas are lightly stained by PV and SMI-32 while the granular areas are relatively darkly labeled (Suzuki and Amaral, 2003; Kondo et al, 2003; Saleem et al, 2007). In the agranular region [area TGa of Kondo et al (2003) and Saleem et al (2007)], less PV and SMI-32 staining has been observed. Although a detailed comparative study may be needed to compare the small TPC of monkeys with the large TPC of the humans, currently available chemoarchitectonic, connectional, and functional data suggests that areas TGa, TGvd, TGdd and TGdg of the monkey TPC (Kondo et al, 2003) may correspond to areas TI, 36, TG and TAr of the human TPC, respectively. Area TGvg plus the lower portion of the TGsts, and the upper two portions of the area TGsts of the monkey TPC appear to roughly correspond to anterior areas TE and TAp of the human TPC, respectively. Different TPC subareas in monkey have been shown by many reports to have differential connections with prefrontal cortex, temporal cortex, amygdala, and insula (Van Hoesen, 1981; Mufson and Mesulam, 1982; Mesulam and Mufson 1982; Galaburda and Pandya, 1983; Moran et al, 1987; Barbas, 1988; Webster et al, 1991; Barbas, 1993; Seltzer and Pandya, 1994; Suzuki and Amaral, 1994; Carmichael and Price, 1995; Stefanacci et al, 1996; Barbas et al, 1999; Romanski et al, 1999; Kondo et al, 2003; Munoz and Insausti, 2005; Saleem et al, 2007, 2008; Hoistad and Barbas, 2008). The many common architectural features of human and monkey TPC suggest that connectional data may be reliably transferred from primate to human.

8. Functional consideration of different TPC regions

Overall, the TPC appears to be heavily involved in social and emotional processing including face processing, recognition, and semantic memory (Nakamura and Kubota, 1996; Hodges and Patterson, 2007; Olson et al, 2007; Hoistad and Barbas, 2008). However, since the present study has identified at least seven different areas in the human TPC, more detailed investigation will be needed to clarify the specific role of these areas. In the present study, perirhinal cortical areas 35 and 36 and area TE were shown to extend to the ventromedial portion of the TPC. Anterior areas 35, 36 and TE are higher-order visual association cortical regions and may be involved in abstract visual processing and recognition memory (see Nakamura and Kubota, 1996; Holdstock, 2005; Olson et al, 2007). Area TAr occupies the dorsolateral part of the TPC and if it does correspond to area TS2 plus a large part of area TS1 (Galaburda and Pandya, 1983) and to the STGr (rostral superior temporal gyrus; Hackett et al, 1999; Saleem et al, 2008) in monkey, then it may be involved in higher order auditory processing as functional studies in monkeys have shown (Hackett et al, 1999; Romanski et al, 1999; Poremba et al, 2003). Area TI seems clearly to be an olfactory-related cortex and may be homologous to the posterior pyriform cortex of lower mammals which has been identified as an association-like olfactory cortex because of connectivity patterns similar to association areas of neocortex (Johnson et al, 2000). Alternatively, area TI may be more closely affiliated with the parietal insular cortex of rodents which not only separates the gustatory insular region from perirhinal cortex but has strong connections with first and second order somatosensory cortical areas (Shi and Cassell, 1998).

Besides the above areas identified in the TPC, the remaining dysgranular region in the TPC has been treated as the archetypal area TG in the present study since this region still met the criteria for area TG as defined by Von Economo (1929). This area TG occupies only the most anterior tip of TPC but with a relatively wider posterior extension on the mediodorsal surface of the TPC. The functional significance of this area TG is not known, but it may bind complex, highly processed perceptual inputs to visceral emotional responses, as Olson et al (2007) recently suggested. This is consistent with our finding that area TG is situated at the junction of high order visual (areas 36 and TE), auditory (area TAr), and olfactory/insular (area TI) association cortices.

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Abbreviations

35, 36	temporal areas 35 and 36 based on Brodmann (1909)
AChE	acetylcholinesterase
AD	Alzheimer's disease
AG	ambiens gyrus
Am	amygdala
AT8	abnormally phosphorylated tau
CB	calbindin D-28k

Cl	claustrum
cs, CS	collateral sulcus
EC	entorhinal cortex
Elr	rostral lateral part of entorhinal cortex
Eo	olfactory part of entorhinal cortex
FI	frontal insular area
GS	gyri of Schwalbe
HG1, HG2	Heschl's gyrus 1 and 2
In	insulae
its	inferior temporal sulcus
LI	limen insulae
mts	middle temporal sulcus
NeuN	neuronal nuclear antigen or neuronal nuclei
NFT	neurofibrillary tangle
NT	neural thread
OT	olfactory tract
PAC	periamygdaloid cortex
PI	parainsular cortex
Pir or PC	piriform or pyriform cortex
psm, psl	medial and lateral temporopolar sulci
PV	parvalbumin
rs, RS	rhinal sulcus
SG	semilunar gyrus
SMI-32	non-phosphorylated neurofilament protein
ss, SS	semiannular sulcus
sts, STS	superior temporal sulcus
TA	temporal area TA based on Von Economo (1929)
TAr	temporal area TAr, the area rostral to area TA
TAp	temporal area TAp, the polysensory area in the dorsal bank of the STS
TE	temporal area TE based on von Economo (1929)
TEd, TEv	dorsal and ventral parts of area TE
TG	temporopolar area TG, the area caps the tip of temporal pole
TI	temporal insular area based on Beck (1934)
TPC	temporal polar cortex
U	uncus
WFA	<i>Wisteria floribunda</i> agglutinin

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Fig. 1.

Macrophotographs of human temporal lobes showing different aspects of the human TPC. The anatomical landmarks and the major subdivisions (anterior areas 35, 36, TE, TAp and areas TAr, TI, and TG) and their relationship in the TPC are shown. **A-C:** Dorsal (A), mediodorsal (B) and medioventral (C) aspects of the same temporal lobe from case 1. Solid line 7A in B indicates the imaging plane of the level A (A1-A4) in Fig.7. **D:** Dorsal view of the TPC in another case (case 4) showing how the limen insulae (LI) connects the temporal (TI) and frontal (FI) parts of the agranular insular cortex. Note in this case the shallow rhinal sulcus (rs) extends between the pyriform cortex (Pir) and area TI. **E:** Anterior view of the TPC from case 6. The dashed lines 2A, 2B and 2C indicate the planes of the coronal sections cut from the same brain block and showed in Fig. 2A, B and C, respectively. **F:** Lateral view of the TPC from another case. The solid lines 2D and 2E indicate the planes of the horizontal sections shown in Fig.2D and 2E, respectively. The dashed line 2F in panel F indicates the sagittal plane shown in Fig.2F. For abbreviations see the list for this figure and for the following figures). Scale bar: 1cm (for A-F).

Fig.2.

Coronal (A-C), horizontal (D and E) and sagittal (F) sections through the TPC of a normal aging case (case 6) showing the different patterns of PV immunoreactivity across TPC regions. Note that only one temporal polar sulcus (ps) was observed in the case shown in A-C. The inset shows a posterior section as a comparison for the TPC regions. The orientation of the coronal sections in A-C and in the inset is indicated by the compass in C (d: dorsal; m: medial); that of the horizontal sections in D and E is shown by the compass in E (m: medial; p: posterior); and of the sagittal section in F (d: dorsal; P: posterior). The short solid lines along the cortical surface mark the borders of different cortical areas (the same marks are used in the following figures). The approximate locations of the sections A-C and D-F are indicated in Fig. 1E and F. Scale bar: 6 mm (for A-F).

Fig. 3.

Typical features of area 35 as revealed by Thioflavin-S staining. **A and B:** lower (A) and higher (B) power views of the unique layer IIIu in area 35 in a severe AD case (**case 12**). Note the vertical columns in area 35a. The marks * (in A and the inset) and # (in A and B) point to the corresponding regions. Note in layer IIIu many large pyramidal cells were stained. The imaging location of section A was shown in the inset of Fig.4 by the dashed line 3A. **C:** A section from a normal aging case (case 7) and double-stained with NeuN (black) and Thioflavin-S (white) shows how layer IIIu and the vertical large-celled columns stained with Thioflavin-S stand out in area 35 against other neurons stained with NeuN. Note the clear alternating large- (white) and small- (black) celled columns in area 35a in this double-stained section. Roman numbers indicate different cortical layers (the same marks are used in the following figures). Scale bars in A-C: 200 μ m.

Fig.4.

Three adjacent horizontal sections from a normal aging case (case 8) and stained for NeuN (**A**), NFT (thioflavin-S, **B**) and PV (**C**) show the typical staining patterns found in anterior area 35. The orientation of section **C** is shown in the Inset. **A and B** are higher power views of area 35 from adjacent sections, as indicated in **C**. Note in **C** that the anterior and posterior area 35 are positioned anterior and posterior to the EC, respectively. **D**. A high power view of NFT-containing neurons in the marked region of **B** (the arrow **D** in **B** and the arrow in **D** point to the corresponding location). The dashed line 3A in the inset indicates the imaging coronal plane where section **A** in Fig.3A was taken. Scale bars: **A** and **B**, 800 μ m; **C** and inset, 1cm; **D**, 200 μ m.

Fig. 5.

Sequential AT8/NeuN double-labeled sections from the anterior (**A**) and posterior (**B**) part of the TPC from a normal case (case 2) show that heavy AT8+ immunostaining was only observed in layer Ia (the dark bands) of pyriform cortex (Pir) and area TI (**B**) but not in other regions. The dark AT8+ band in layer Ia (in **B**, **H** and **J**) of the pyriform cortex (Pir) and area TI (TI) is composed of AT8+ thorn-shaped astrocytes. High power views of layer Ia in area TI are shown in Fig. 10F and G. Cytoarchitectonic features of the TPC areas TG (**C**), 35b (**D**), 36 (**E**), TE (**F**), TAr (**G**), Pir (**H**), 35a (**I**), TI (**J**), and TAp (**K**) were clearly revealed by NeuN immunostaining in this normal case. The locations of C-K are shown in A and B by the black bars with corresponding letters. Section **L** shows the dense olfactory fibers in layer Ia of area TI. For detailed explanations, see text. Scale bars: 6 mm (A and B); 200 μ m (C-L).

Fig.6.

Low power views of the staining pattern revealed by three different markers at three anteroposterior levels (A-C) of the anterior temporal lobe from a normal case (case 3). At each level, adjacent coronal sections are shown for NeuN, PV and CB labeling. Higher power views of the CB staining in areas Eo, 35a, 36 and TE are shown in D, E, F and G, respectively. Note that the staining intensity in CB+ pyramidal cells (arrows) is generally lower than that in CB+ non-pyramidal cells (arrowheads; D-G). The locations of these areas are marked by black bars with corresponding letters. For detailed explanations, see text and table 2. Scale bars: 10mm (A-C); 200µm (D-G).

Fig.7.

Low power views of the staining pattern revealed by four different markers at six anteroposterior levels (**A-F**) of the anterior temporal lobe from a normal aging case (case 9). At each level, adjacent coronal sections are shown for NeuN, PV, AT8 and WFA staining. Higher power views of the PV staining in areas 35a, 35b, 36, TG, TEv, TEd, TAp and TAR are shown in panels A, B, C, D, E, F, G and H of Fig.8, respectively. The locations of these areas are marked by black bars 8A-8H in **C2** and **D2**. In addition, higher power views of the AT8 staining in areas 35b, 36, TG, TE and TI were shown in A, B, C, D and E of Fig.10, respectively. The locations of these areas are marked by black bars A-E in **C3** and **D3**. Finally, higher power views of the locations indicated by black bars 13A-E in **B1**, **A1**, **C1** (for NeuN) are shown in Fig.13A-E, respectively. Scale bar: 6 mm.

Fig.8.

Higher power views of PV immunostaining patterns in different layers of areas 35a (A), 35b (B), 36 (C), TG (D), TEv (E), TED (F), TAP (G) and TAr (H) from the case shown in Fig.7. The locations of these images are indicated in sections C2 and D2 of Fig.7. Note the differences in density and staining intensity of PV+ neurons and neuropil across different areas. Scale bar: 200 μ m (A-H).

Fig.9.

Two adjacent sections from the anterior TPC immunostained with NeuN (**A**) and SMI-32 (**B**) antibodies in a normal case (case 5) show typical cytoarchitectonic and SMI-32+ staining patterns at different locations in the TPC. Higher power views of SMI-32+ labeling in areas 35b, 36, TE, TAr and TG in section **B** are shown in **C**, **D**, **E**, **F** and **G**, respectively. Scale bars: 6 mm (A and B); 300 μ m (C-G).

Fig.10.

A-E: Higher power views of AT8 staining patterns in different layers of areas 35b (A), 36 (B), TG (C), TE (D) and TI (E) from the case shown in Fig.7. The locations of these images are indicated in sections C3 and D3 of Fig.7. Note the apparent difference in density of AT8+ neurons and neuropil across different areas. **F and G:** An AT8 and NeuN double-stained section from area TI from the case shown in Fig. 5 shows densely packed AT8+ astrocytes in layer Ia (brown color) but not in other layers. Thus, layer II only contains NeuN immunostained neurons (blue color). **H and I:** Low power views of the two AChE stained sections at levels C and D of Fig.7 (from the same case) show the general AChE staining patterns found in TPC areas. Higher power views of the AChE staining in the EC (Eo) and areas TI, 35, TG, 36 and TE (locations indicated by the black bars) are shown in Fig. 11A-F, respectively. Scale bars: 200 μ m (A-F); 50 μ m (G); 6mm (H and I).

Fig.11.

Higher power views of AChE staining patterns in different layers of the Eo (A) and areas TI (B), 35 (C), TG (D), 36 (E) and TE (F) from the locations shown in Fig. 10H and I. Note that relatively darker AChE staining was seen in layers V and VI than in layers II and III of all areas except area 35 where relatively darker labeling was observed in layers II and III than in layers V and VI. Scale bar: 200 μ m.

Fig.12.

Higher power views of typical WFA staining patterns found in different layers of the Eo (A) and areas TI (B), 35 (C), TG (D), 36 (E) and TE (F) from the locations shown in Fig. 7C4, D4. Note the different density and staining intensity of WFA labeling in different areas. Scale bar: 200 μ m.

Fig.13.

Higher power views of NeuN staining in areas Eo (**A** and **B**), **PACo** (**C**), TEv (**D**), and TED (**E**) from the same case as shown in Fig.7. The locations of these images are shown in sections B1, A1 and C1 of Fig. 7 by black bars 13A-13E, respectively. The cell islands in layer II of the Eo in A and B are circled while the patches in layer III of area PEA in C are indicated by arrows. In addition, NeuN labeled neurons in layers IV, V and VI of area 36 are shown in F at high magnification while CB immunoreactive cells and neuropil in both superficial and deep layers of area 36 is shown in G. Note in G that layers IV –VI have many fewer CB labeled neurons than layers II-III and that arrows and arrowheads point to pyramidal and nonpyramidal neurons respectively. Scale bars: 200 μ m (A-E, G); 100 μ m (F).

Fig.14. Summary of the locations of different TPC areas and their relationships on the lateral (A), dorsal (B) and medial (C) aspects of the human temporal lobe.

Table 1

Case Demographics

Case Number	Age(y)	Sex	Brain Weight (g)	Duration of Postmortem (h)	Cause of Death	Braak's NFT Stages
1	80	M	1440	6.0	pneumonia	no NFTs
2	76	M	1306	3.0	pneumonia	no NFTs
3	72	M	1424	4.5	pneumonia	no NFTs
4	81	M	1255	3.5	pneumonia	few NFTs
5	65	M	1421	5.5	acute carotid block	no NFTs
6	77	F	1208	5.0	cardiac arrest	stage II
7	77	M	1267	3.0	sepsis	stage II
8	82	M	1303	4.0	subdural hematoma	stage III
9	77	M	1270	4.5	pneumonia	stage I
10	80	M	1440	6.0	pneumonia	stage III
11	79	F	1158	5.0	pneumonia	stage IV
12	82	F	1130	3.0	Alzheimer's disease	stage V
13	77	M	873	3.0	Alzheimer's Disease	stage V
14	71	M	942	5.0	Alzheimer's Disease	stage VI
15	76	F	1010	4.0	Alzheimer's Disease	stage VI

Table 2

Overall staining intensity and patterns of different TPC areas by different markers

Areas	NeuN	PV	AT8	CB	SMI-32	WFA	Thioflavin S (NFT)	myelin (Ia)	AChE
Eo	agranular; II patches; IIIa clusters; thick IIIb	(+)	++	++++	(+)	+	II patches(+++)	++	+++
anterior area 35	agranular; II vertical Columns; IIIu	+	+++	++++	(+)	(+)	IIIu & II Col (+++)	–	++
area TI	agranular; thick I&III, III smaller cells	(+)	++	+++	(+)	(+)	IIIu (–); II Col(–)	+++	++
anterior area 36	IVdg; thick III; III&V middle-sized cells	++	++	++	+	+	IIIu (–); II Col(–)	–	+
area TG	IVdg; thick III; III&V middle-sized cells	++	+	++	+	+	–	–	+
area TAr	IVg; thick III; V middle-sized cells; IIIb large cells	+++	–	+	++	++	–	–	(+)
anterior area TAp	IVg; thinner III; III-V columns	+++	–	+	+++	++	–	–	(+)
anterior area TE	IVg; thin III; IIIb large cells; IV columns	++++	–	+	++++	++++	–	–	(+)
area TA	IVg; Thick III, V&VI; III-IV columns	++++	–	+	+++	+++	–	–	(+)

AT8 scale is based on brains at Braak's NFT stage I. Thioflavin-S scale is based on brains at Braak's NFT stage II. Staining intensity: negative/few (–), very weak [(+)], weak (+), intermediate (++) , strong (+++) and very strong (++++). Ia, II, III, IIIu, IV, V and VI: cortical layers; IVdg and IVg: dysgranular and granular layer IV; Col: cell columns; NFT: neurofibrillary tangle. See text for detailed description.