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3-D Cytoarchitectonic parcellation of human orbitofrontal cortex. Correlation with postmortem MRI

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Abstract

The orbitofrontal cortex (OFC) is located on the basal surface of the frontal lobe and is distinguished by its unique anatomical and functional features. Clinical and postmortem studies suggest the involvement of the orbitofrontal cortex in psychiatric disorders. However, the exact parcellation of this cortical region is still a matter of debate. Therefore, the goal of this study is to provide a detailed description of the extent of borders of individual orbitofrontal cortical areas using cytoarchitectonic criteria in a large sample of human brains, which could be applied by independent neuroanatomists. To make this microscopic parcellation useful to neuroimaging studies, magnetic resonance images of postmortem brains in the coronal plane were collected prior to the preparation of coronal histological sections from the same brains. A complete series of coronal sections from 6 normal human brains and partial sections from the frontal cortex of 21 normal human brains were stained with general histological and immunohistochemical methods specific for different cell-types. These sections were examined microscopically by two independent neuroanatomists (HBMU and GR) to achieve reproducible delineations. After the borders were determined, the tissue sections were superimposed on corresponding MR images. Based on our cytoarchitectonic criteria, Brodmann's areas 47 and 11 were included in the human orbitofrontal cortex. Area 47 was further subdivided into three medial (located on the medial, anterior and posterior orbital gyri) and two lateral (located on the lateral orbital gyrus) subareas. In addition, we observed an anterior-posterior gradient in the cytoarchitecture of areas 11 and 47. The transverse orbital sulcus corresponds roughly to the transition between the subregions of the anterior and posterior OFC. Finally, the present delineation is contrasted with an overview of the different published nomenclatures for the OFC parcellation.

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Keywords

Prefrontal cortex; neuroimaging and postmortem delineation; Gallyas silver staining; Nissl staining; cortical thickness; SMI-32; NF200; Parvalbumin; Calbindin

1. Introduction

The orbitofrontal cortex (OFC) is located on the basal surface of the frontal lobe and constitutes one of the subregions of the prefrontal cortex. OFC is distinguished by its unique anatomical and functional specialization (Fuster, 2008; Cavada et al., 2000; Roberts, 2006; Man et al., 2009). Behavioral, neuropsychological and functional neuroimaging studies reveal that OFC is involved in the control of emotional, motivational, cognitive flexibility and social behavior (for excellent reviews see Kringelbach and Rolls, 2004; Kringelbach, 2005; Rolls and Grabenhorst, 2008). Recent neuroimaging reports, also indicate that OFC plays a critical role in psychopathology of severe mental disorders such as schizophrenia, depression, bipolar illness, obsessive compulsive disorder and drug addiction (Blumberg et al., 1999; Crespo-Facorro et al., 2000; Baxter et al., 2000; Drevets, 2001; Bremner et al., 2002; Lacerda et al., 2004; Völlm et al., 2004; Remijnse et al., 2006; Van den Heuvel et al., 2009). Moreover, postmortem histopathological studies start to reveal that OFC is a site of neuronal and glial cell pathology in psychiatric disorders (Rajkowska et al., 1999, 2005, 2007; Cotter et al., 2002, 2005). These morphometrical and stereological studies of the OFC require a description of well defined cytoarchitectonic characteristics of individual OFC areas and borders for their delineation. Nonhuman primate studies show that OFC consists of different (sub)areas with a distinct pattern of cortical and subcortical connections and unique cytoarchitecture (Barbas and Pandya, 1989; Preuss and Goldman-Rakic, 1991; Carmichael and Price, 1994; Cavada et al., 2000; Öngür and Price, 2000; Petrides and Pandya, 2001; Barbas et al., 2002; Barbas and Zikopoulos, 2006; Barbas, 2007; Roberts et al., 2007).

Existing parcellations of the human OFC confirm its heterogeneity, but also reveal that human OFC is more complex than that of non-human primates and that simple extrapolation of subdivisions from monkey to human brain is not straightforward (Beck, 1949; Petrides and Pandya, 2001; Öngür et al., 2003; Uylings et al., 2005a; see Fig. 1). Another difficulty in understanding the parcellation of human OFC stems from the fact, that there is no agreement between different researchers on the position, extent and nomenclature of individual OFC areas. This is illustrated in Fig. 1, which shows 7 different parcellations of the human OFC. A main reason for discrepancies in the location of OFC areas between different maps is that most authors do not provide detailed description of the cytoarchitectonic criteria used to distinguish individual OFC areas as well as the location of their borders (e.g., Brodmann, 1909, 1914). These problems have been given adequate attention in three studies (von Economo and Koskinas, 1925; Kononova, 1935; Öngür et al., 2003). However, these three studies cannot be easily compared with each other since individual OFC areas on the respective maps vary in their location and extent and are specified with a different nomenclature. Therefore, the goal of the present study is to provide a set of cytoarchitectonic criteria for the delineation of individual OFC areas so that their respective borders can be reproduced by independent, experienced neuroanatomists. This is essential for stereological studies in these OFC areas applying Nissl staining for e.g. cell counting and for the interpretation of neuroimaging studies on normal and diseased brains. Herewith we prefer a nomenclature in which a cortical area is indicated with a particular Brodmann area number, while a subdivision of a cortical area is indicated with a suffix added to the pertinent Brodmann area number. This implies, that the differences between cortical areas having a different Brodmann number are larger than between the

subdivisions of a particular Brodmann area. In addition, we combine the microscopic cytoarchitectonic parcellation with immunocytochemical stainings for neurofilaments (SMI-32 and NF200) and calcium binding proteins (parvalbumin and calbindin). Moreover, the cytoarchitectonic parcellation carried out on postmortem histological sections is displayed in corresponding sections from postmortem MRI scans of the pertinent cases. These sections with delineated borders of individual OFC areas are further used for 3D reconstructions which reveal interindividual variability in the location and extent of human OFC (e.g., Uylings et al., 2005a). In addition, using our cytoarchitectonic knowledge on the microscopic location of the OFC and its individual subdivisions, we were able to define their macroscopic localization on structural MRI by using the gyral and sulcal pattern as an additional guide. We will discuss that such an approximation of OFC areas in structural MRI is preferable above a derivation of OFC areas on the basis of a Talairach-like atlases of human cerebral cortex.

2. Methods

2.1. Subjects

All procedures in this study conforms to The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Six whole and 21 partial left hemispheres from human postmortem brains were used for the cytoarchitectonic study and additional 5 right hemispheres for immunocytochemical stainings. All 32 subjects were free of neurological disorders such as: Alzheimer's disease, Parkinson's disease, epilepsy, dementia, multiple sclerosis, tumor or congenital malformation of the nervous system. Alcohol or drug dependence and mental disorders were also among exclusion criteria. The cause of death were: complications of cardiovascular disease (27 subjects), acute glomerulo-nephritis (1), rupture of duodenal ulcer (1), gun shots (2), and motor vehicle accident (1).

Subjects of both genders were included with age ranging from 23 – 86 years of age, however, only 4 subjects were older than 74 years of age. The postmortem delay in all but three subjects was 24 hours or shorter with a range of variability from 5 to 31 hrs. Serial sections of the frontal lobe from the six whole brains were obtained from the Zilles' collection (C. & O. Vogt Institute of Brain Research, Düsseldorf, Germany). Detailed information about these cases has been reported by Amunts et al., 1999. The partial blocks from 21 brains were obtained from the Cuyahoga County Coroner's Office, Cleveland, Ohio, USA (P.I. Dr. C. Stockmeier). These blocks containing the ventral and/or dorsal half of the prefrontal cortex were also used in our previous morphometric analyses on the prefrontal cortex, which reported also the detailed information about these 21 cases (Rajkowska et al, 1999, 2005; Miguel-Hidalgo et al, 2000, 2002).

2.2. Morphometric analyses

The data on cortical morphometric parameters such as, the cortical thickness, laminar width and the diameter of somata in prefrontal areas 47, 9, 46 and 12 were extracted from the 21 normal control subjects, used for the above-mentioned studies (Rajkowska and Goldman-Rakic, 1995a; Rajkowska et al, 1999, 2005; Miguel-Hidalgo et al, 2000, 2002). For these analyses the Stereo Investigator software (5.05.4 MicroBrightField, Williston, Vermont) has been applied. We reanalyzed the data to illustrate a trend in cortical thickness differences between medial and lateral part of area 47 and the above-mentioned prefrontal areas. In these new analyses, we compared each of the above-mentioned parameters between prefrontal areas 47, 9, 46, and 12 by using analysis of variance (ANOVA) followed by Tukey-test for multiple pairwise comparisons (Keppel, 1982).

2.3. Histology

The six whole brains used in the study were fixed in 4% buffered formalin, embedded in paraffin and serially sectioned into 20 µm thick coronal sections. Slide mounted sections were silver stained with a modification of Gallyas' method to visualize neuronal cell bodies (Merker, 1983; Uylings et al., 1999). This method has been reported to be more optimal for cytoarchitectonic parcellation of cortical areas in human brain than the classical Nissl staining due to the higher contrast of stained tissue (e.g., Uylings et al., 1999). Every 60th section of all paraffin sections collected from the frontal brain region (i.e., extending from the frontal pole, anteriorly to the level of the central sulcus posteriorly) was used for the cytoarchitectonic delineation of OFC areas in each brain studied.

Twenty one partial hemispheres were embedded in 12% celloidin, stained for the Nissl substance with cresyl violet and cut into 40µm thick sections (for details on this technique, see Rajkowska et al, 1999). In each brain 3–7 sections spaced by 800–1200 µm interval were selected from each prefrontal area for cytoarchitectonic and morphometric analyses. The immunohistochemical staining was obtained with SMI-32 antibody (Sternberger Monoclonals Inc., Baltimore, MD., USA: monoclonal antibody to non-phosphorylated neurofilaments, Lot Number: 11), monoclonal antibody to both phosphorylated and non-phosphorylated forms of the 200 kD protein of neurofilaments (Sigma-Aldrich, St. Louis, MO., USA: Product Number: N0142, Clone Number: N52), monoclonal anti-calbindin D-28K antibody (Sigma-Aldrich, St. Louis, MO., USA: Product Number: C-9848, Clone Number: CB-955, Lot Number: 015K4826 and Chemicon Int. Inc., Temecula, CA., USA: Product Number: AB-1778), and monoclonal anti-parvalbumin antibody (Sigma-Aldrich, St. Louis, MO., USA: Product Number: P-3171, Clone Number: PA-235, Lot Number: 026H4824). The human brain tissue was fixed with 4% buffered formaldehyde. Tissue to be stained with antiparvalbumin, anticalbindin and SMI-32 were sectioned at 50 µm by a vibratome. The antigen retrieval method of Evers and Uylings (1997) has been applied. The primary antibodies were diluted in 5% milk solution with 0.2% Triton X-100: SMI-32 at 1:1,000, parvalbumin antibody at 1: 1,000, and calbindin antibody at 1: 250. We used peroxidase-conjugated rabbit antimouse (1:100) as secondary antibody. The tissue to be stained with NF200 was embedded in celloidin and cut into 40 µm sections. The NF200 antibody was used at a 1: 400 dilution according to the immunocytochemical protocol described previously in detail (Miguel-Hidalgo and Rajkowska, 1999).

Control sections incubated according to the same procedures described above, but omitting the primary antibody were all negative.

2.4. Cytoarchitectonic Parcellation

2.4.1. Cytoarchitectonic criteria—In order to uniformly characterize and distinguish individual cytoarchitectonic areas within the OFC region a set of cytoarchitectonic criteria was developed based on: (a) granularity of layer IV, (b) cell density and relative soma sizes in the different layers and when present, sublayers and (c) absolute and relative thickness of cortical layers. Application of these criteria permitted specification of microscopic borders between individual OFC areas. These borders were delineated in each of the six complete brains on a series of coronal histological sections by two independent researchers (G.R. and H.B.M.U) to obtain reproducible delineations using a Leica stereomicroscope MZ6 at a final magnification of 20 – 40×. In these analyses, we took into account the possible occurrence of the cortical changes associated with aging (e.g., increase in density of glial cells in gray and white matter) or the preclinical stages of mental disease (reduction of cortical thickness) as much as possible in this type of study.

2.4.2. Image analysis—Between fixation and embedding, 3-D MRI imaging of six brains was performed using a Siemens 1.5 Tesla Vision Scanner (Erlangen, Germany) with a T1-weighted 3-D FLASH sequence covering the whole brain (flip angle (FA) = 40°, repetition time TR = 40 msec, echo time TE = 5 msec for each image). Each volume consisted of 128 sagittal sections. The spatial resolution was $1 \times 1 \times 1.17$ mm and each voxel had a resolution of 8 bits corresponding to 256 gray values. After using MRI scanning, these six brains were embedded in paraffin and serially sectioned in the coronal plane (20 μ m thick sections, see above). Each 60th section of the entire series was used for microscopic analysis. Three data sets were available for each brain: 1) the data set with images of histological sections digitized with a CCD camera), 2) a data set with low contrast images of the histological section made before sectioning for the 3-D integrity of the histological volume, and 3) the structural MR-data set. These three data sets enabled the corrections for deformations and shrinkage caused by histological techniques to be made, and were used for three-dimensionally reconstructions.

Microscopically determined cytoarchitectonic OFC borders were interactively traced onto the digital images of the histological sections using an image analysis system (Kontron IBAS, Munich, Germany). Then, these digital delineations were automatically transformed into the pertinent MRI images from the same brains. The planar orientation of each MRI section was made similar to the plane of sectioning of histological sections. Finally, a combination was applied of linear affine transformation, grey level normalization and non-linear elastic alignment was applied (Schormann and Zilles, 1998; Amunts et al, 2004).

3. Results

3.1. Orbital Sulci and Gyri

Orbitofrontal cortex (OFC) is a cortical region located on the orbital surface of the hemisphere. In OFC, we and others (Ono et al, 1990; Chiavaras and Petrides, 2000) distinguish the following main sulci: the olfactory sulcus (OLF), medial orbital sulcus (MOS), transverse orbital sulcus (TOS), lateral orbital sulcus (LOS) (Fig. 2). The medial, lateral and transverse orbital sulci form, in many cases, roughly an H-like pattern on the basal surface of the brain (Fig. 2, brain 7186, L; 14686, L). However, in other cases, other shapes such as: “X” (Fig. 2, brain 6895, R), “K”, “L”, or “T”) can be found (Retzius, 1896, Ono et al, 1990; Chiavaras and Petrides, 2000).

The following gyri associated with the above mentioned sulci are recognized: lateral orbital gyrus, located lateral to the LOS; medial orbital gyrus, located medial to the MOS; anterior orbital gyrus, and posterior orbital gyrus, located anterior and posterior, respectively to the TOS; and gyrus rectus, located medial to the OLF.

In all brains analyzed in this study, the lateral orbital sulcus and olfactory sulcus were not ramified and always found at the similar, constant position (Fig. 2). In contrast, medial orbital sulcus was represented in a ramified form (Fig.2, for further details see Ono et al., 1990; Chiavaras and Petrides, 2000). Accordingly, the gyri related to the mentioned sulci, i.e., lateral orbital gyrus, and gyrus rectus were represented in a form of a single gyrus in all brains examined. In contrast, the medial orbital gyrus, and anterior and posterior orbital gyri were represented by different subgyri.

3.2. Common Cytoarchitectonic Features of OFC cortex

Cytoarchitectonic areas 47 and 11 possess common cytoarchitectonic features that distinguish them from surrounding cortical areas. These features include: (1) narrow cortex, (2) a relative thin, discernible layer IV, (3) narrow layer III, 4) wide infragranular layers V

and VI, (4) small size of neurons. The cortical thickness of OFC cortex is generally less than that of other prefrontal areas, such as Brodmann area 9, 46, 12 (see Fig. 3 and Rajkowska and Goldman-Rakic, 1995a), and 45 or 10. For example, the mean $\pm$ standard error (SE) of the cortical width of the combined subareas 47 m¹ and 47l¹ is 1.90 ± 0.07 mm, whereas in area 9 it is 2.38 ± 0.09 mm, in area 46 2.47 ± 0.12 mm, and in area 12 2.34 ± 0.13 mm (ANOVA, $F(4,43) = 9.49$, $P < 0.0001$). The granular layer IV is significantly narrower in OFC than in the surrounding prefrontal areas 12, 9 or 46 (Fig.3; ANOVA, $F(4, 43) = 7.62$, $P < 0.0001$). Although layer IV can be distinguished across OFC, its width and prominence varies between different parts of OFC according to two different gradients: a medial-lateral and a posterior-anterior one. For example, layer IV forms a clearly delineated band in the lateral part of area 47, whereas it is represented by a narrow band with zigzag-like borders in the medial part of area 47 and in area 11. Layer IV becomes even less discernible being interrupted and not visible as a continuous layer at the posterior levels of medial part of area 47 and area 11. This gives these areas at the posterior levels a dysgranular appearance. Supragranular layer III and especially its sublayer IIIc in OFC cortex is less prominent than the corresponding layer in the adjacent area 45 and in dorsolateral prefrontal areas 9 and 46 (ANOVA, $F(4, 43) = 6.965$, $P < 0.0001$). For example, sublayer IIIc in area 47 m¹ is significantly narrower than sublayer IIIc in areas 9 ($P = 0.001$ according to the post-hoc Tukey-test) and 46 ($P = 0.018$, Tukey-test). The width of sublayer IIIc in subarea 47 m¹ is 0.64 ± 0.03 mm, whereas in area 46 it is 0.81 ± 0.04 mm and in area 9 0.85 ± 0.04 mm. There is a non-significant trend for the width of sublayer IIIc in subarea 47 m¹ to be narrower than in subarea 47l¹, 0.75 ± 0.04 mm, while it is similar to the width of sublayer IIIc in area 12 (0.63 ± 0.05 mm).

Moreover, the diameter of neuronal cell bodies in sublayer IIIc are significantly smaller in subarea 47 m¹ (14 ± 0.4 μ m) than that in area 9 (16 ± 0.3 μ m; $P = 0.038$, Tukey-test), but not different from those in area 12 (14 ± 0.4 μ m) or in subarea 47 l¹ (15 ± 0.4 μ m). In contrast to supragranular layers, infragranular layers V and VI are more prominent in OFC than in dorsolateral prefrontal areas 9 and 46 and in area 45. In addition to common cytoarchitectonic features shared by areas 47 and 11, each of these areas is distinguished by its own unique cytoarchitectonic characteristics described below.

3.3. Cytoarchitectonic Characteristics of Individual OFC Areas

In OFC we distinguish two main cytoarchitectonic areas, area 47 and area 11 which are labeled according to the Brodmann's nomenclature. Area 47 is located on the orbital surface of the hemisphere, whereas area 11 is located on the ventromedial edge of the orbital surface covering the crown of the gyrus rectus. Area 47 is further subdivided into two major cytoarchitectonic subdivisions: medial (47m) and lateral (47l). The medial subdivision is located on the medial orbital gyrus (MOG) and on the anterior and posterior orbital gyri and it is further subdivided into three smaller subareas: 47m¹, 47m² and 47m³. The lateral subdivision is located on the lateral orbital gyrus (LOG) and it is further subdivided into two smaller subareas: 47l¹ and 47l². Thus, area 47 is composed of five smaller cytoarchitectonic subareas: 47m¹, 47m², 47m³, 47l¹ and 47l². In addition, we recognize the anterior-posterior gradient in the cytoarchitectonic features of areas 47 and 11 (see below).

3.4. Area 47

This is the largest and most complex area in the OFC. It can be distinguished from the neighboring OFC area 11 by the following general cytoarchitectonic features: 1) the size of neuronal cell bodies in all layers of area 47 is generally larger than those in area 11; 2) layer III and V in area 47 are more differentiated than those in area 11 due to differentiation in size of neuronal cell bodies; 3) granular layer IV in area 47 is more clearly delineated than that in area 11.

We divided area 47 into two major subdivisions, i.e., medial (area 47-medial) and lateral (area 47-lateral) based on the differences in the pattern of their cytoarchitectonic features such as width and discernability of individual cortical layers, differences in the cell packing density and size of neuronal cell bodies and the presence of radial striations. The medial subdivision of area 47 can be generally distinguished from the lateral subdivision by the presence of two darkly stained horizontal bands in infragranular layers. These bands are particularly visible at low magnification and they are composed of densely packed cells located in sublayer Va (upper band) and in the upper part of layer VI (lower band), see Figs. 4–6).

The cytoarchitecture of area 47 changes gradually in anterior-posterior direction. The anterior subdivision extends from the anterior part of area 47 to approximately the level of the genu of corpus callosum or the transverse orbital sulcus (TOS) (Fig. 2). The posterior subdivision is an immediate continuation of the anterior one and extends to the level of the insula (Fig. 2 & 7A). This posterior subdivision is characterized by the gradual disappearance of layer IV and gradual prominence of layer V (Compare Figs. 4, 5 & 6).

Anteriorly, area 47 is replaced by granular area 10, caudally by agranular area 25 and insular cortex, laterally by area 45 and medially by area 11 (Fig. 7). Below we compare cytoarchitectonic features of areas 47 and 10. For a description of agranular area 25 see Vogt et al., 1995. For details of the cytoarchitecture of area 45 see Amunts et al., 1999; and Uylings et al., 2005b.

3.5. Comparison of cytoarchitecture between area 47 and area 10

Cellular elements in area 10 are larger than those in area 47, which is especially noticeable in supragranular layers II and III (see Figs. 4 & 5). Layer IV in area 47 is narrower and its borders are less clear than the corresponding layer in area 10. Infragranular layers in area 47 are generally distinguished from corresponding layers in area 10 by the presence of two dark bands corresponding to densely packed cells in sublayers Va and in the upper part of layer VI. These bands in area 47 are separated by a cell poor sublayer Vb appearing as a light band. In contrast, in area 10 the cell density in sublayer Vb is not clearly different from the cell density of adjacent sublayer Va and layer VI.

At the border of areas 10 and 47 we noted a small area with mixed cytoarchitectonic features of areas 10 and 47. We refer to this subarea as 10–47 (see Fig. 4). This area is consistently located on the lower bank of the frontomarginal sulcus and it is recognized only at the anterior levels of the OFC and it disappears more caudally together with a disappearance of the frontomarginal sulcus. In subarea 10–47 as in area 10 there is no cytoarchitectonic differentiation of layer III into separate sublayers due to uniform cell size and density. At the same time, infragranular layers V and VI in subarea 10–47 are differentiated into sublayers as they are in area 47. Finally, layer IV in subarea 10–47 is much narrower and has less prominent borders than the corresponding layer in area 10.

3.6. Subdivisions of area 47

As mentioned above the medial subdivision of area 47 consists of three subareas: 47m¹, 47m² and 47m³ whereas the lateral subdivision is subdivided into two subareas: 47l¹ and 47l² (Fig. 7).

3.6.1. Subarea 47m¹—In this subarea layer II is easily distinguishable from layer III because of its high cell packing density. Layer II is darker and its cells are more densely packed in subarea 47m¹ than in adjacent subarea 47m². Layers III and V are relatively wide. In layer III two parts can be distinguished, the upper one consisting of sublayers IIIa and IIIb

contain similar size neurons, and the lower one (sublayer IIIc) has larger neurons mixed with the smaller ones. Layer IV is narrow, zigzag-like due to penetration of cells from neighboring layers III and V. Layer V has two sublayers, Va with larger, more densely packed cells and sublayer Vb with smaller less densely packed cells organized in small clusters. Large cells in sublayers IIIc and Va are not homogeneously distributed and they are organized in clusters of several cells. Layer VI is not very wide, rather homogenous and densely packed, especially in the upper part, what makes its border with the underlying white matter clearly visible.

The cytoarchitectonic differences between the anterior and posterior parts of subarea 47m¹ are visible in Fig. 5. In the anterior part of subarea 47m¹ sublayer Va is less prominent and its cells are generally smaller in size than the cells in sublayer IIIc. At more posterior levels, however, cells in sublayer Va become larger and are more densely packed than the cells of sublayer IIIc. On the other hand, layer IV is more pronounced and wider in more anterior parts of 47m¹ and is getting thinner and hardly distinguishable at more posteriorly located parts (Fig. 5).

3.6.2. Subarea 47m²—Cell packing density in the layers of subarea 47m² is lower than in subarea 47m¹ (see Fig. 5). Supragranular layer II in subarea 47m² is less clearly delineated than that of subarea 47m¹ due to a similar packing density between this layer and neighboring layer III. Layer III also has a more uniform appearance in subarea 47m² than in subarea 47m¹ due to a lack of cell clusters. Layer IV is wider in 47m² than in 47m¹ and its borders are more distinct. Layer V in subarea 47m² is more differentiated into cell-dense sublayer Va, and cell-sparse sublayer Vb, than in subarea 47m¹. Therefore, sublayer Vb has a relatively thicker and lighter appearance in 47m² than in 47m¹. Layer VI is wider in subarea 47m² than in subarea 47m¹ and its border with the underlying white matter is also less demarcated. In subarea 47m², as was observed in subarea 47m¹ an anterior-posterior gradient exists in cytoarchitectonic features. The most prominent difference is visible in layer V where cells are less densely packed and more clustered at the anterior level as compared to the posterior one (Fig 5). Also, the size of sublayer Va neurons is smaller and this sublayer is thinner at the anterior level as compared to the caudal one.

3.6.3. Subarea 47m³—This subarea is the largest and most variable among the subareas of area 47. Moreover, in subarea 47m³ as compared to other subareas of area 47 the cytoarchitectonic variations between medial and lateral parts are more prominent than changes in the anterior-posterior direction. Nonetheless, subarea 47m³ can be distinguished from the surrounding areas by its unique cytoarchitectonic features.

Granular layers II and IV are more demarcated in subarea 47m³ than in subarea 47m². Moreover, layer IV in subarea 47m³ is more prominent at the anterior than the posterior level (Fig 5). Cell sizes in layer III are also more differentiated in subarea 47m³ than in subarea 47m² which makes its subdivision into the three sublayers: IIIa, IIIb and IIIc less difficult. Size and packing density of cells in layer V varies between different parts of subarea 47m³ both in a medial to lateral and in a posterior to anterior gradient. For example, in the most medial parts of this area, cells of sublayer Va are not larger than cells of sublayer IIIc. In contrast, in the more lateral parts the sublayer Va cells are larger than cells in sublayer IIIc. In subarea 47m³ like in subarea 47m² two dark bands of densely packed cells can be observed in infragranular layers however, these bands are not equally prominent in all parts of this subarea.

3.6.4. Subarea 47l¹—Subarea 47l¹ belongs to the lateral subdivision of area 47 and it is consistently found around the lateral orbital sulcus (LOS) mostly covering its lateral wall. The most characteristic cytoarchitectonic feature of this subarea is the large size of neurons

in sublayer IIIc (Figs. 6 & 8). This feature is more pronounced at mid-posterior levels than at the most posterior levels of subarea 47¹, from where (Fig. 7a) we took the picture presented in Fig. 6. At the mid-posterior levels of subarea 47¹ layer IIIc cells are larger than those of the adjacent subareas 47² and 47^{m3}. Another prominent feature of subarea 47¹ is that both granular layers, layer II and layer IV are clearly distinguishable from adjacent layers and they are wider in subarea 47¹ than in the neighboring subarea 47^{m3} (Fig. 6). Layer V in subarea 47¹ is not well differentiated due to low cell packing density and small cell sizes across both sublayers. Sublayer Va appears to be less distinct in subarea 47¹ than in the neighboring areas. Layer VI is rather narrow but its upper part is still densely packed with cells, which at low magnification, appears as a dark band. As mentioned above, the cytoarchitecture of the anterior part of subarea 47¹ differs from its caudal part with regard to size of sublayer IIIc neurons. However, the size of sublayer Va neurons is not different between anterior and posterior parts of 47¹ and therefore, the neurons of sublayer IIIc are larger than sublayer Va neurons in both the anterior and posterior parts of subarea 47¹. In this respect, subarea 47¹ differs from all other subareas of area 47 in which layer Va neurons are larger than neurons in sublayer IIIc at the posterior but not anterior levels. The prominence of layer IV changes while moving from anterior to posterior levels of subarea 47¹. This layer is wider and more clearly delineated at the anterior levels but not at the caudal ones (Figs. 6 & 8).

3.6.5. Subarea 47²—Layer III in subarea 47² is more clearly differentiated into three sublayers than the corresponding layer in subarea 47¹. Cells in sublayer IIIb in subarea 47² are smaller and less densely packed than the cells in sublayer IIIc (Fig. 6). Layer IV in subarea 47² is wider and more clearly demarcated from its neighboring layers than layer IV in subarea 47¹. Sublayer Va in subarea 47² contains a higher number of cells, which are larger and more homogeneously distributed than those in sublayer Va of subarea 47¹. Sublayer Vb contains more cells, which are larger than in other subareas of area 47. In layer VI no clear sublayering is visible.

Anterior-posterior changes in the cytoarchitecture of subarea 47² are obvious in layer III. In anterior parts of subarea 47² sublayer IIIc cells are larger than those in sublayer Va (Fig. 6). In contrast, in the posterior parts of this area layer Va cells are larger than those in sublayer IIIc (Fig. 6). This anterior-posterior trend in the cytoarchitecture of subarea 47² is similar to all other subareas of area 47 except of subarea 47¹. Another anterior-posterior difference is the width of layer IV. This layer is wider in the anterior parts of subarea 47² than in the caudal parts (Figs. 6)

3.7. Comparison of cytoarchitecture between area 47 and area 45

Lateral to subarea 47² is Brodmann area 45. The cytoarchitecture of area 45 is clearly distinguishable from that of area 47. In area 45 layer III is wide and its neurons, especially those in sublayer IIIc, are larger than those in area 47. Layer IV is clearly distinguishable from adjacent layers and it is more prominent than in area 47. Infragranular layers in area 45 in contrast to area 47, are relatively narrow (for further details on area 45 see Amunts et al., 1999; and Uylings et al., 2005b).

3.8. Area 11

This is a small area located on the crown of the gyrus rectus. Area 11 is surrounded by subarea 47^{m1} laterally, by area 12 medially, by area 10 anteriorly and by agranular cortex of area 25 caudally.

This area is distinguished from the surrounding cortex by the following cytoarchitectonic features: 1) small size of neuronal cell bodies in all layers; 2) the lack of a clear border

between layers II and III; 3) a non-differentiated layer III; 4) narrow and not clearly delineated granular layer IV; 5). Poorly demarcated border between layer VI and underlying white matter (Figs. 4).

The cytoarchitecture of area 11 differs between its anterior and posterior parts (Fig. 4). Size of sublayer Va cells is smaller in anterior area 11 as compared to the posterior one. This feature makes the subdivision of layer V into two sublayers (Va and Vb) more clearly visible at the posterior than anterior levels of area 11. Also, in the posterior area 11 cells are larger in sublayer Va than those in sublayer IIIc whereas, in the anterior area 11 cell sizes in both sublayers have approximately similar sizes.

All the cytoarchitectonic features of OFC described above on the basis of Gallyas' stained sections are also visible and similar in the Nissl stained material (compare Figs. 4–6 with Fig. 8).

3.9. Comparison of cytoarchitecture between area 47 and insular cortex

Area 47 is bordered posteriorly by the agranular insular cortex. This cortex, in contrast to area 47, is located above the claustrum (e.g., Brockhaus, 1940; Öngür et al., 2003) and it is characterized by a lack of layer IV and less clearly differentiated layers.

3.10. 3D Reconstruction in MR Images

The delineations of the orbital subregions in the serial sections of the frontal lobe from the six whole brains from the Zilles' collection have been transformed into the corresponding MRI images of the respective brains of which the planar orientation is similar to the plane of sectioning of histological sections (compare Fig. 7B with Fig. 7C). These MRI delineations are used for the 3D reconstruction and visualization of the microscopically defined orbital subregions (Fig. 9).

3.11. Immunocytochemical characteristics of OFC areas

The SMI-32 staining of non-phosphorylated neurofilaments shows clearly 2 darkly stained bands in the infragranular layers in both anterior and posterior parts of areas 11, and subareas 47m and 47l. Area 11 differs from both the 47m and the 47l areas especially in the lower frequency of SMI-32 positive neurons in layer III (Figs. 10 & 11). In the SMI-32 staining only minor differences between the subareas of area 47 are detectable (Figs. 10 & 11), but obvious differences are noticeable between area 47 and adjacent agranular insular cortex (Fig. 11). In the agranular insular cortex a clear, darkly stained population of SMI-32 positive pyramidal neurons is located in layer III. Another clear band of darkly stained immunoreactive neurons is located in layer V. Thus, insular cortex differs from the adjacent area 47 by the presence of only one instead of two darkly stained bands of pyramidal neurons in its infragranular layers (Fig. 11).

The NF200 staining of both phosphorylated and non-phosphorylated forms of the 200 kD protein of neurofilaments does not show the two dark bands in the infragranular layers (Fig. 12). This staining shows more clearly the layer III positive neurons than the SMI-32 staining does. Both neurofilament stainings show no clear differences between subareas 47m and 47l (Figs. 10, 11 & 12).

Furthermore, the parvalbumin staining also does not reveal obvious differences between subareas 47m and 47l, as well between the anterior and posterior parts of area 11 (Fig. 13 & 14). There is only a relatively small difference between these areas in calbindin-positive neurons of layer II (Fig. 15). Area 11 has less frequently stained layer II calbindin-positive neurons than subareas 47m and 47l do (Fig. 15). In the parvalbumin staining the intensity

and frequency of parvalbumin-positivitive neurons in the agranular insular cortex is largely increased in all layers as compared with those in the subareas of area 47 (Figs. 13 & 14).

4. Discussion

The present study provides the delineation and description of the orbitofrontal cortical (OFC) areas. Using a set of cytoarchitectonic criteria the borders between individual OFC areas were defined microscopically in two different stainings (Gallyas and Nissl) by two independent researchers (G.R. and H.B.M.U). In addition, we compared the individual OFC areas in immunocytochemically stained sections. Finally, we transformed our microscopic delineations into 3-D MRI images of the pertinent brains for 3D reconstruction and visualization (Figs. 7 and 9).

We distinguished two main areas within the OFC, area 47 and area 11, mainly on the basis of cytoarchitectonic characteristics, and small immunocytochemical differences. These areas are labeled according to the Brodmann's nomenclature. Although we adopted the widely used Brodmann's nomenclature, our parcellation differs in many aspects from that of Brodmann (1909, 1914). For example, our area 11 is smaller than area 11 on Brodmann's map (Fig. 1) and restricted to the crown of the gyrus rectus. Consequently, the extent of the neighboring area 47 also differs between our and Brodmann's parcellations. Unfortunately, we cannot compare our cytoarchitectonic criteria with those used by Brodmann, since his papers do not provide a description of the cytoarchitectonic criteria used.

Comparison of our OFC parcellation to the maps of Von Economo and Koskinas (1925), Sarkisov et al. (1955), Öngür et al. (2003) and Mackey and Petrides (2009) reveals the existence of both similarities and differences. Our delineation of OFC area 47 resembles that of Von Economo and Koskinas (1925) whereas our area 11 is more restricted than those of Von Economo and Koskinas (i.e., area FG and FH) and Sarkisov map (Fig.1). Area 11 in our parcellation is restricted to the crown of the gyrus rectus and its posterior part corresponds to area 14 on the map of Öngür et al (2003), see Fig.1, Table 1.

In our study, area 47 was further subdivided into two major cytoarchitectonic subdivisions: medial (47m) and lateral (47l). The medial subdivision was further parcellated into three smaller subareas: 47m¹, 47m² and 47m³ whereas, the lateral subdivision was subdivided into two smaller subareas: 47l¹ and 47l² based on the differences in their cytoarchitecture. In addition, we recognize the anterior-posterior gradient in the OFC cytoarchitecture. The cytoarchitectonic features of areas 47 and 11 change gradually in the anterior-posterior direction. We define the anterior subdivision as extending from the anterior part of area 47 to approximately the level of the genu of corpus callosum or the transverse orbital sulcus. The posterior subdivision is a continuation of the anterior one and extends to the level of the insula. There is not a clear-cut border detectable between the anterior and the posterior parts, but a very wide (several millimeters) transitional zone, which possesses mixed features of both parts. A similar observation was made by other researchers (Von Economo and Koskinas, 1925; Beck, 1949; Hof et al, 1995). This anterior –posterior trend is mostly noticeable in the cytoarchitectonic features of three different layers, i.e., III, IV, and V (see also Von Economo and Koskinas, 1925) and is such a gradual one that Von Economo and Koskinas (1925) and also ourselves refrained here from subdividing OFC into clear-cut anterior and posterior areas. For simplicity in Fig. 9, we draw a geometric line around the transverse orbital sulcus to indicate roughly the middle of the wide transition zone between the anterior and posterior OFC subdivisions. On the other hand, Beck (1949) made a distinction between anterior and posterior areas. Although she mentioned explicitly, that area 13 is a subdivision of area 11, she specified them with a different number, e.g., area 11 for the anterior subdivision and area 13 for the posterior one. She also indicated a large

transitional zone between these subdivisions in her map (see Fig.1). As others (Von Economo and Koskinas (1925) and Beck (1949)) we have observed that the reduction in the granularity of the posterior parts of OFC (as compared to the anterior one) is quite variable between different brains. In many cases layer IV becomes dysgranular, whereas in some other brains posterior parts of OFC are practically agranular. This observation is of importance when different studies are compared (see Hof et al., 1995; Van Hoesen et al, 2000).

In addition to the anterior-posterior trend, a medial-lateral trend is reported in the cytoarchitectonic map of Sarkisov et al.(1955) and Öngür et al. (2003). This is developed further in our parcellation. When we position the anterior-posterior border zone around the transverse orbital sulcus, then we find more agreement between different researchers for the medial-lateral subdivision of the posterior part of the OFC, than for the anterior part (Fig.1). This might be also caused by the larger variability in major and minor sulci in the anterior part of OFC (Chiavaras and Petrides, 2000), since cortical layering looks different at the bottom of OFC sulci as compared to the convexity of the OFC gyri (e.g., Von Economo and Koskinas, 1925; Bok,1959). Our parcellation of the posterior OFC is most similar to that of Öngür et al. (2003) in spite of a different nomenclature used (Fig.1; Table 1). Only three minor aspects are different between our parcellation of the posterior OFC and that of Öngür et al. (2003): (1) We distinguish two subareas within the lateral orbital gyrus (located lateral to LOS) (47l¹ and 47l²), like Sarkisov et al. (1955) whereas Öngür et al. (2003), distinguishes only one area (47/12l) in that gyrus; (2) We distinguish one subarea (47m¹) in the olfactory sulcus, whereas Öngür et al. (2003), subdivides the lateral and medial walls of this sulcus into two different subareas (13b and 13m) (see Fig.1); and (3) we do not subdivide the posterior area 11, whereas Öngür et al. (2003) divides this area into area 14r and 14c. Our subdivision of the anterior part of OFC, however, is quite different from that of Öngür et al. (2003), and more like the one of Von Economo and Koskinas (1925). Öngür et al. (2003) consider that all three gyri, gyrus rectus, medial orbital gyrus and anterior orbital gyrus are covered by one cytoarchitectonic area 11 (with two subareas, 11m and 11l, see Fig.1). For this part of the orbitofrontal cortex this is also largely the view of Mackey and Petrides (2009), although they include here a part of area 14 r too. In contrast, we distinguish two different cytoarchitectonic areas in this region, area 11 and area 47 (subdivided into three subareas, 47m¹, 47m² and 47m³). In addition, Öngür et al. (2003), consider that the lateral orbital gyrus consists of area 47/12r, whereas we subdivide this gyrus into two subareas, 47l¹ and 47l². With regard to the anterior part of the OFC, it is conspicuous that Sarkisov et al. (1955) consider that most of this area is covered by a different cortical type, i.e., area 10. This is in contrast to our and other parcellations (Von Economo and Koskinas, 1925; Beck, 1949; Hof et al., 1995; Öngür et al., 2003; Mackey and Petrides, 2009; see Fig. 1). Finally, we have to mention that in our study the subarea 47m³, both in the anterior part and the posterior part of OFC is quite heterogeneous. In the posterior part of OFC Sarkisov et al. (1955) subdivided this heterogeneous 47m³ into 3 subareas (Fig.1). In the anterior part, this subarea 47m³ was not differentiated at all by Sarkisov et al. (1955), but they considered this area to be within area 10, as mentioned above, see Fig.1.

Different nomenclatures are now in use for the frontal lobe. A stable and unequivocal nomenclature is desired (e.g., Paxinos and Watson, 2005), although neuroanatomical nomenclature should remain flexible in order to incorporate new insights (Swanson, 1992). Unfortunately, a generally accepted nomenclature for the prefrontal cortical areas in the human brain is still lacking. The most logical and flexible nomenclature of Von Economo and Koskinas (1925), appears to be too difficult to be apprehended by the majority of scientists. Therefore, the simpler number system of Brodmann (1909, 1914) has been widely adopted and incorporated in the atlas of Talaraich & Tournoux (1988), which is frequently used by neuroimagers (Uylings et al., 2005a). The same number system was also applied by

Brodmann to monkey brain, although he himself felt uncertain about the homologies implied by using the same numbers (Brodmann, 1909). The cytoarchitectonic study of macaque prefrontal cortex by Walker (1940) tried to bring the number system for the cortical areas distinguished in macaque prefrontal cortex in line with the human brain as far as specified by Brodmann (1909). The final map of Brodmann (1914), -which is widely reproduced in textbooks, but not in the atlas of Talaraich and Tournoux (1988) – distinguishes an area 12 at the ventral medial wall of the frontal lobe, which was not specified in Brodmann's (1909) prefinal map. This is clearly a different area from Walker's area 12 in the macaque. Walker's study has influenced later cytoarchitectonic studies on the prefrontal cortex of monkey brains (e.g., Barbas and Pandya, 1989; Preuss and Goldman-Rakic, 1991; Carmichael and Price, 1994; Petrides and Pandya, 2001; Barbas et al., 2002). Extrapolation from monkey data on connectivity circuits and invasive functional studies is desirable for many reasons. Therefore, several studies have been undertaken to compare human and macaque prefrontal cortex to have one (viz. Walker) numbering system for both species to facilitate extrapolations (Beck, 1949; Petrides and Pandya, 1994, 2001; Öngür and Price, 2000; Öngür et al., 2003; Mackey and Petrides, 2009). This has turned out to be a very difficult task, since the results are not similar between these well acknowledged researchers (see Fig. 1 and Uylings et al., 2005a). For example, area 13 is located in Öngür et al. (2003) medially to the medial orbital sulcus (MOS), while in Mackey and Petrides (2009) area 13 is located laterally to the MOS in the posterior orbital gyrus. In addition, areas which are considered to be subdivisions of a given cortical area by one of these authors (Beck, 1949) are given a different number (see above). For reasons mentioned above we did not apply the nomenclature of Von Economo and Koskinas (1925), but the one of Brodmann (1914). We also did not use one of the extrapolated Walker's number systems either, as we prefer to specify subdivisions of a same area with a similar number. A literature search based on key words for prefrontal areas should take this into account. Therefore we include Table 1, in which a summary of the different nomenclatures is presented to facilitate their comparison.

Subdivision of the frontal lobe in MR images is relevant to several neuropsychiatric disorders (e.g., Lacerda et al., 2003). Different MRI parcellation schemes of the frontal cortex have been proposed (e.g., Caviness et al., 1996; Crespo-Facorro et al., 1999; Tisserand et al., 2002; Howard et al., 2003; Lacerda et al., 2003; Croxson et al., 2005; Desikan et al., 2006; John et al., 2006, 2007; Nakamura et al., 2008). These parcellation schemes (with the exception of Howard et al., 2003; and Lacerda et al., 2003) have been based upon the sulcal pattern, which is visible in MR imaging. Only a gross comparison can be made between the different macroscopic MRI parcellations and our cytoarchitectonic subdivision, as the microscopically defined cytoarchitectonic areas cannot be discerned on MR images of the frontal lobe. In the parcellation scheme of Caviness et al. (1996) the orbitofrontal cortex is located in the 'frontal medial cortex' (FMC), the 'frontal orbital cortex' (FOC), and the 'subcallosal cortex' (SC). Their FMC includes an anterior part of the gyrus rectus (i.e., part of area 11 and medial part of 47m¹), and also area 12. The SC includes a posterior part of gyrus rectus, area 12 and ventral area 24. The medial border of FOC is the bottom of the olfactory sulcus and the lateral border is the anterior horizontal ramus of the Sylvian fissure. Thus, the FOC includes the lateral part of 47m¹, and subareas 47m², 47m³, 47l¹ and 47l², and most likely also a small part of area 45 (Amunts et al., 1999; Uylings et al., 2005b).

The anterior horizontal ramus of the Sylvian fissure was also defined as the lateral border for the OFC in MR images by Tisserand et al. (2002). They divided the OFC into a ventral medial and a lateral orbital part. The (medial) dorsal border of the 'ventral medial' part is at the 'subgenual'/ventral cingulate sulcus. The lateral border is positioned at the lateral crown of the gyrus rectus, which thus constitutes the medial border of the lateral 'orbital' part. Thus, the 'ventral medial' part of Tisserand et al. (2002) corresponds roughly with

Brodmann areas 12 and 11 whereas, their lateral orbital part is roughly comparable to our subareas 47m¹, 47m², 47m³, 47l¹, 47l² and probably it also contains a small portion of area 45.

Crespo-Facorro et al. (1999) defined, however, the lateral border at the lateral orbital sulcus. Thus, our subareas 47l¹ and 47l² are excluded from their orbitofrontal cortex. The medial border of the OFC was defined after Caviness et al. (1996) at the bottom of the olfactory sulcus. In the parcellation of Crespo-Facorro the area 11 is incorporated into the gyrus rectus, together with parts of area 12 and the medial part of 47m¹.

Desikan et al. (2006) subdivided the OFC into a medial and a lateral division. Given the course of the lateral boundary of their lateral division, i.e., the lateral bank of the lateral orbital sulcus, our area 47l² and likely a lateral portion of area 47l¹ are not included in their lateral division. However, our area 47m², 47m³, a lateral part of 47m¹, and a medial part of 47l¹ are included in the lateral OFC division of Desikan et al, who position the boundary between their lateral and medial divisions at the fundus of the olfactory sulcus. The medial boundary of the medial division of Desikan and colleagues is located at the ventral part of the cingulate sulcus and on the superior frontal gyrus. Moreover, both divisions contain a large portion of area 10, because their posterior boundary of the frontal pole runs on the coronal plane through the anterior tip of the horizontal ramus of the lateral/Sylvian fissure.

Croxson et al. (2005) subdivided the OFC into 3 parts which are a 'medial orbital', a 'central orbital', and a 'lateral orbital' part. Their medial orbital part has a dorsal border at the subgenual cingulate sulcus and lateral border at the medial orbital sulcus (MOS). Therefore, it also comprises of Brodmann areas 12, 11, our areas 47m¹, 47m², and a medial part of area 47m³. Their 'central orbital' part is defined as being between the medial orbital sulcus (MOS) and the lateral orbital sulcus (LOS), thus mainly encompassing our area 47m³ and a medial part of area 47l¹. Their 'lateral orbital' part which is positioned between the LOS and the horizontal ramus of the lateral (Sylvian) fissure corresponds to our area 47l², the lateral part of area 47l¹ and a small part of area 45.

Nakamura et al. (2008) also divided the OFC into three parts, which are the lateral orbital, the middle orbital and the 'gyrus rectus'. Their three parts differ from the subdivisions of Croxson et al. (2005). Given the variability in the sulcal pattern, Nakamura et al. use the bottom of the olfactory sulcus and not the MOS as the lateral landmark for the medial part, which they call gyrus rectus. The medial boundary of the gyrus rectus is set by Nakamura et al. (2008) at the bottom of the inferior rostral sulcus (Retzius, 1896; Ono et al. 1990), also called supraorbital sulcus (Nakamura et al., 2008).

Nakamura and colleagues did not position the dorsal border at the ventral cingulate sulcus. Thus, the 'gyrus rectus' includes area 11, a medial wall of our area 47m¹, and a ventral part of area 12. The boundary between their middle and lateral orbital part runs at the fundus of the lateral orbital sulcus. Therefore, their middle orbital part includes our areas 47m², 47m³, the lateral wall of area 47m¹, and a small medial portion of area 47l¹. This middle part also includes a posterior part of area 10 given the course of their anterior boundary.

The lateral boundary of their lateral orbital part is put at the horizontal ramus of the lateral fissure, referred to as the ventrolateral orbital sulcus. Thus, the location of the lateral orbital part of Nakamura et al. (2008) is similar to that of the lateral orbital part defined by Croxson et al. (2005) and includes a large portion of our area 47l¹, area 47l², and a small part of area 45.

Lacerda et al. (2003) proposed to apply a geometrical approach instead of a sulcal pattern approach, in which only one sulcus, i.e., the bottom of the olfactory sulcus, separates the

`medial' from the `lateral' orbitofrontal cortex. Their `medial' orbitofrontal cortex incorporates a large part of Brodmann area 12 and the gyrus rectus, whereas the lateral border of the `lateral orbitofrontal cortex' is only geometrically defined and cannot be specified with a particular sulcus. Lacerda et al. (2003) prefer the geometrical border as the lateral border, since the delineation is faster and they noticed that neuroimagers have often difficulties in detecting the lateral orbital sulcus. The question to be considered in the future is which macroscopic parcellation deviates the least from the variable cytoarchitectonic areas: the one based upon the variability of sulcal patterns or the one based upon a geometrical approach.

Our cytoarchitectonic studies (e.g., Rajkowska & Goldman-Rakic, 1995b; Uylings et al., 2005a,b) and those of Zilles and Amunts (e.g., Amunts et al., 1999) demonstrate that even after 3-D transformation of brains to a reference brain, a high inter-individual variability in the extent and position of microscopically defined Brodmann's areas still remains. Therefore, we concluded that the use and specification of Brodmann's area numbers in macroscopic images derived from Talairach's atlas (Talairach and Tournoux 1988; Lancaster et al. 2000), in addition to `Talairach' or MNI coordinates, is not appropriate (Uylings et al., 2005a; Devlin and Poldrack, 2007). Since we cannot distinguish individual cortical cytoarchitectonic areas on neuroimaging scans, an estimation of cortical regions on the basis of the sulcal and gyral pattern is the best possible approximation at the present time (Fischl et al., 2008).

Based upon our cytoarchitectonic study and the observed inter-individual structural variability, we propose the following macroscopic demarcation of the orbital areas (Fig. 16). The lateral boundary of OFC is at the midpoint of the ventral wall of the horizontal ramus of the Sylvian fissure, which excludes part of Broca's area 45 (Uylings et al., 2005b; Keller et al. 2009) and more posteriorly at the circular sulcus of the insula to exclude insular cortex. The medial boundary is determined at the medial side of the crown of the gyrus rectus thus, including area 11 and excluding area 12. When area 12 is selected to be included in the OFC (e.g., Burgmans et al. 2009), then the medial border is positioned more dorsally and runs along the bottom of the subgenual cingulate sulcus and the bottom of the superior frontal gyrus located between the anterior tip of the cingulate sulcus and the posterior border of the frontal pole.

Consistent sulci are lacking for the anterior and posterior OFC boundaries. Therefore, on basis of our cytoarchitectonic OFC study a pragmatic cut-off approach is selected. For the posterior boundary of the frontal pole, we selected a vertical plane drawn from the middle between the anterior tip of the cingulate sulcus and the anterior tip of the olfactory sulcus, which allows us to exclude a majority of area 10 (Fig. 16). Therefore, in this definition, the frontal pole is larger than the one defined by Desikan et al. (2006); Croxson et al. (2005); John et al. (2006, 2007); and Nakamura et al. (2008), and smaller than the one defined by Caviness et al. (1996); and Tisserand et al. (2002). John et al. (2007) position the posterior limit of the frontal pole at the coronal plane through the anterior tip of the olfactory sulcus. Such a position is often too anterior (see Fig. 16) so that their OFC region includes often a significant part of area 10. Crespo-Facorro et al. (1999) didn't have a frontal pole in their parcellation; thus a large part of area 10 is incorporated in their OFC region.

The pragmatic posterior border of OFC is defined for our purposes as a coronal plane drawn downward from the inner curvature of the corpus callosum, which excludes agranular insular cortex (Tisserand et al., 2002).

Our study can further subdivide the OFC into a medial-lateral division in 3–4 zones, or also, when an additional anterior-posterior division is applied, in 6–8 zones. We have to take into

account herewith, that the anterior-posterior subdivision is less precise due to the absence of a clear border between anterior and posterior parts (see Results and above). In our OFC subdivision into the three zones, we prefer to separate the crown of the gyrus rectus (i.e., area 11) from the medial wall of the olfactory sulcus. The second zone is located between the lateral side of the crown of the gyrus rectus and the bottom of the lateral orbital sulcus (LOS), which comprises our areas 47m¹, 47m² and 47m³. The third zone is located between LOS and the lateral border of OFC, thus containing areas 47l¹ and 47l². Two reasons can be used to justify the above subdivision: 1) this is the subdivision separating area 47m from area 47l and, 2) Nakamura et al. (2008) indicates that neuroimagers can distinguish LOS better than MOS. Scientists, who are capable of delineating MOS or the interrupted MOS well in the cases they study, can decide to subdivide the second zone into two zones with a middle border at the bottom of MOS. Next, the medial part of the second zone contains our areas 47m¹, 47m² and the lateral part contains 47m³. This further subdivision of the second zone might be of interest in particular psychiatric conditions (e.g., Schwartz et al, 2010).

When it is important that the anterior-posterior subdivision is identified, then, the medial-lateral zone are subdivided by the transverse orbital sulcus (TOS) and extrapolated to both, the medial OFC and the dorsal OFC boundary.

So far it appears that the two different trends we see in our cytoarchitectonic parcellation of the OFC can comply with the two different trends distinguished by functional imaging: a medial-lateral and an anterior-posterior one (Kringelbach and Rolls, 2004; Kringelbach, 2005). The ventromedial cortex is related to monitoring, learning and memory of the reward value of many different reinforcers, whereas the more lateral OFC areas are involved in the evaluation of punishers, which may lead to a change in ongoing behaviour. In the anterior-posterior trend, more complex or abstract reinforcers, such as monetary gain and loss, activate the anterior areas, while the simpler reinforcers such as taste or pain activate the more posterior areas (Kringelbach and Rolls, 2004; Kringelbach, 2005). These functional data support our view to specify the subdivisions of area 47 as subdivisions of one area rather than assigning them with different area numbers.

A detailed cytoarchitectonic description is still of importance so that other researchers can use these characteristics to delineate OFC areas for morphometric and stereological studies in Nissl stained sections (Rajkowska et al, 2005; Cotter et al., 2005) and thus arrive at a total number of neurons or glia cells in well defined cortical regions (Uylings et al., 2005b). In the present study we also applied immunocytochemical stainings, which support our cytoarchitectonic parcellation of the OFC into the two main areas (47 and 11). The immunocytochemical borders between individual subareas of area 47 were less obvious than their cytoarchitectonic borders and thus don't support a division of area 47 into areas specified with a different areal number. This study provides a useful tool also for postmortem neurochemical and molecular studies and can serve as a guidance for neuroimaging analyses.

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ABBREVIATIONS

LOG	lateral orbital gyrus
LOS	lateral orbital sulcus
MOG	medial orbital gyrus
MOS	medial orbital sulcus
OFC	orbitofrontal cortex
OLF	olfactory sulcus
TOS	transverse orbital sulcus

REFERENCES

- Amunts K, Schleicher A, Bürgel U, Mohlberg H, Uylings HBM, Zilles K. Broca's region re-visited: Cytoarchitecture and intersubject variability. *Journal of Comparative Neurology*. 1999; 412:319–341. [PubMed: 10441759]
- Amunts K, Weiss PH, Mohlberg H, Pieperhoff P, Gurd J, Shah JN, Marshall CJ, Fink GR, Zilles K. Analysis of the neural mechanisms underlying verbal fluency in cytoarchitectonically defined stereotaxic space - The role of Brodmann's areas 44 and 45. *Neuroimage*. 2004; 22:42–56. [PubMed: 15109996]
- Barbas H. Specialized elements of orbitofrontal cortex in primates. *Annals N.Y. Academy of Sciences*. 2007; 1121:10–32.
- Barbas H, Pandya DN. Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. *Journal of Comparative Neurology*. 1989; 286:353–375. [PubMed: 2768563]
- Barbas, H.; Zikopoulos, B. Sequential and parallel circuits for emotional processing in primate orbitofrontal cortex. In: Zald, DH.; Rauch, SL., editors. *The Orbitofrontal Cortex*. Oxford University Press; Oxford: 2006. p. 57-91.
- Barbas, H.; Ghasghaei, HT.; Rempel-Clower, NL.; Xiao, D. Anatomic basis of functional specialization in prefrontal cortices in primates. In: Grafman, J., editor. *Handbook of Neuropsychology*. 2nd ed.. Vol. vol. 7. Elsevier; Amsterdam: 2002. p. 1-27.
- Baxter MG, Parker A, Lindner CC, Izquierdo AD, Murray EA. Control of response selection by reinforcer value requires interaction of amygdala and orbital prefrontal cortex. *Journal of Neuroscience*. 2000; 20:4311–4319. [PubMed: 10818166]
- Beck E. A cytoarchitectural investigation into the boundaries of cortical areas 13 and 14 in the human brain. *Journal of Anatomy*. 1949; 83:147–157.
- Blumberg HP, Stern E, Ricketts S, Martinez D, de Asis J, White T, Epstein J, Isenberg N, McBride PA, Kemperman I, Emmerich S, Dhawan V, Eidelberg D, Kocsis J, Silbersweig D. Rostral and orbital prefrontal dysfunction in the manic state of bipolar disorder. *American Journal of Psychiatry*. 1999; 156:1986–1988. [PubMed: 10588416]
- Bok, ST. *Histonomy of the Cerebral Cortex*. Elsevier Publ. Cy.; 1959.
- Bremner JD, Vythilingam M, Vermetten E, Nazeer A, Adil J, Khan S, Staib LH, Charney DS. Reduced volume of orbitofrontal cortex in major depression. *Biological Psychiatry*. 2002; 51:274–279.
- Brockhaus H. Die Cyto- und Myeloarchitektonik des Cortex Claustralis und das Claustrum beim Menschen. *Journal für Psychologisches Neurologie*. 1940; 49:249–348.
- Brodmann, K. Vergleichende Lokalisationslehre der Grosshirnrinde. Barth Verlag; Leipzig: 1909.
- Brodmann, K. Physiologie des Gehirns. In: Von Bruns, P., editor. *Neue Deutsche Chirurgie*. Vol. vol. 11. Enke; Stuttgart: 1914. p. 85-426.
- Burgmans S, Van Boxtel MPJ, Vuurman EFPM, Smeets F, Gronenschild EHBM, Uylings HBM, Jolles J. The prevalence of cortical gray matter atrophy may be overestimated in the healthy aging brain. *Neuropsychology*. 2009; 23:541–550. [PubMed: 19702408]

- Carmichael ST, Price JL. Architectonic subdivision of the orbital and medial prefrontal cortex in the macaque monkey. *Journal of Comparative Neurology*. 1994; 346:366–402. [PubMed: 7527805]
- Cavada C, Company T, Tejedor J, Cruz Rizzolo RJ, Reinoso Suarez F. The anatomical connections of the macaque monkey orbitofrontal cortex. *Cerebral Cortex*. 2000; 10:220–242. A review. [PubMed: 10731218]
- Caviness VS, Meyer J, Makris N, Kennedy DN. MRI-based topographic parcellation of human neocortex: an anatomically specified method with estimate of reliability. *Journal of Cognitive Neuroscience*. 1996; 8:566–587.
- Chiavaras MM, Petrides M. Orbitofrontal sulci of the human and macaque monkey brain. *Journal of Comparative Neurology*. 2000; 422:35–54. [PubMed: 10842217]
- Cotter, D.; Pariante, C.; Rajkowska, G. Glial pathology in major psychiatric disorders. In: Agam, G.; Belmaker, RH.; Everall, I., editors. *The Postmortem Brain in Psychiatric Research*. Kluwer Acad Publ; Dordrecht: 2002. p. 291-324.
- Cotter D, Hudson L, Landau S. Evidence for orbitofrontal pathology in bipolar disorder and major depression, but not in schizophrenia. *Bipolar Disorders*. 2005; 7:358–369. [PubMed: 16026489]
- Crespo- Facorro B, Kim J-J, Andreasen NC, O'Leary DS, Wiser AK, Bailey JM, Harris G, Magnotta VA. Human frontal cortex: An MRI-based parcellation method. *NeuroImage*. 1999; 10:500–519. [PubMed: 10547328]
- Crespo- Facorro B, Kim J-J, Andreasen NC, O'Leary DS, Magnotta VA. Regional frontal abnormalities in schizophrenia: a quantitative gray matter volume and cortical surface size study. *Biological Psychiatry*. 2000; 48:110–119. [PubMed: 10903407]
- Croxson PL, Johansen-Berg H, Behrens TEJ, Robson MD, Pinski MA, Gross CG, Richter W, Richter MC, Kastner S, Rushworth MFS. Quantitative investigation of connections of the prefrontal cortex in the human and macaque using probabilistic diffusion tractography. *J Neurosci*. 2005; 25:8854–8866. [PubMed: 16192375]
- Devlin JT, Poldrack RA. In praise of tedious anatomy. *Neuroimage*. 2007; 37:1033–1041. [PubMed: 17870621]
- Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker Buckner, R.L. Dale AM, Maguire RP, Hyman BT, Albert MS, Killiany RJ. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage*. 2006; 31:968–980. [PubMed: 16530430]
- Drevets WC. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. *Current Opinion in Neurobiology*. 2001; 11:240–249. [PubMed: 11301246]
- Evers P, Uylings HBM. An optimal antigen retrieval method suitable for different antibodies on human brain tissue stored for several years in formaldehyde fixative. *Journal of Neuroscience Methods*. 1997; 72:197–207. [PubMed: 9133585]
- Fischl B, Rajendran N, Busa E, Augustinack J, Hinds O, Yeo BTT, Mohlberg H, Amunts K, Zilles K. Cortical folding patterns and predicting cytoarchitecture. *Cerebral Cortex*. 2008; 18:1973–1980. [PubMed: 18079129]
- Fuster, JM. *The Prefrontal Cortex*. 4th Ed. Academic Press; San Diego: 2008.
- Hof PR, Mufson EJ, Morrison JH. Human orbitofrontal cortex: cytoarchitecture and quantitative immunohistochemical parcellation. *Journal of Comparative Neurology*. 1995; 359:48–68. [PubMed: 8557847]
- Howard MA, Roberts N, García-Fiñana M, Cowell PE. Volume estimation of prefrontal cortical subfields using MRI and stereology. *Brain Research Protocols*. 2003; 10:125–138. [PubMed: 12565683]
- John JP, Wang L, Moffitt AJ, Singh HK, Gado MH, Csernansky JG. Inter-rater reliability of manual segmentation of the superior, inferior and middle frontal gyri. *Psychiatry Research*. 2006; 148:151–163. [PubMed: 17088050]
- John JP, Yashavantha B,S, Gado M, Veena R, Jain S, Ravishankar S, Csernansky JG. A proposal for MRI-based parcellation of the frontal pole. *Brain Structure & Function*. 2007; 212:245–253. [PubMed: 17929054]

- Keller SS, Crow T, Foundas A, Amunts K, Roberts N. Broca's area: nomenclature, anatomy, typology and asymmetry. *Brain & Language*. 2009; 109:29–48. [PubMed: 19155059]
- Keppel, G. *A Researcher's Handbook*. 2nd Ed. Prentice-Hall Inc.; Englewood Cliffs: 1982. Design and Analysis.
- Kringelbach ML. The human orbitofrontal cortex: linking reward to hedonic experience. *Nature Reviews Neuroscience*. 2005; 6:691–702.
- Kringelbach ML, Rolls ET. The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. *Progress in Neurobiology*. 2004; 72:341–372. [PubMed: 15157726]
- Kononova EP. The variability of structure of the cortex of the brain: inferior frontal gyrus of adult man. *Brain Research Institute Publications*. 1935; 1:49–118. in Russian.
- Lacerda ALT, Hardan AY, Yorbik O, Keshavan MS. Measurement of the orbitofrontal cortex: a validation study of a new method. *Neuroimage*. 2003; 19:665–673. [PubMed: 12880797]
- Lacerda ALT, Keshavan MS, Hardan AY, Yorbik O, Brambilla P, Sassi RB, Nicoletti M, Mallinger AG, Frank E, Kupfer DJ, Soares JC. Anatomic evaluation of orbitofrontal cortex in major depressive disorder. *Biological Psychiatry*. 2004; 55:353–358. [PubMed: 14960287]
- Lancaster JL, Woldorff MG, Parsons LM, Liotti M, Freitas CS, Rainey L, Kochunov PV, Nickerson D, Mikiten SA, Fox PT. Automated Talairach atlas labels for functional brain mapping. *Human Brain Mapping*. 2000; 10:120–131. [PubMed: 10912591]
- Mackey S, Petrides M. Architectonic mapping of the medial region of the human orbitofrontal cortex by density profiles. *Neuroscience*. 2009; 159:1089–1107. [PubMed: 19356690]
- Man MS, Clarke HF, Roberts AC. The role of the orbitofrontal cortex and medial striatum in the regulation of prepotent responses to food rewards. *Cerebral Cortex*. 2009; 19:899–906. [PubMed: 18689858]
- Merker B. Silver staining of cell bodies by means of the physical development. *Journal of Neuroscience Methods*. 1983; 9:235–241. [PubMed: 6198563]
- Miguel-Hidalgo JJ, Rajkowska G. Immunohistochemistry of neural markers for the study of the laminar architecture in celloidin sections from the human cerebral cortex. *Journal of Neuroscience Methods*. 1999; 93:69–79. [PubMed: 10598866]
- Miguel-Hidalgo JJ, Baucom C, Dilley G, Overholser JC, Meltzer HY, Stockmeier CA, Rajkowska G. Glial fibrillary acidic protein immunoreactivity in the dorsolateral prefrontal cortex separates young from old adults with major depressive disorder. *Biological Psychiatry*. 2000; 48:861–873. [PubMed: 11063981]
- Miguel-Hidalgo JJ, Wei J, Andrew M, Overholser JC, Jurjus G, Stockmeier CA, Rajkowska G. Glia pathology in the prefrontal cortex in alcohol dependence with and without depressive symptoms. *Biological Psychiatry*. 2002; 52:1121–1133. [PubMed: 12488057]
- Nakamura M, Nestor PG, Levitt JJ, Cohen AS, Kawashima T, Shenton ME, McCarley RW. Orbitofrontal volume deficit in schizophrenia and thought disorder. *Brain*. 2008; 131:180–195. [PubMed: 18056163]
- Öngür D, Price JL. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cerebral Cortex*. 2000; 10:206–219. [PubMed: 10731217]
- Öngür D, Ferry AT, Price JL. Architectonic subdivision of the human orbital and medial prefrontal cortex. *Journal of Comparative Neurology*. 2003; 460:425–449. [PubMed: 12692859]
- Ono, M.; Kubik, S.; Abernathy, CD. *Atlas of the Cerebral Sulci*. Georg Thieme Verlag; Stuttgart: 1990.
- Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*. 5th ed.. Academic Press, Elsevier; San Diego: 2005.
- Petrides, M.; Pandya, DN. Comparative architectonic analysis of the human and the macaque frontal cortex. In: Boller, F.; Grafman, J., editors. *Handbook of Neuropsychology*. Vol. vol. 9. Elsevier; Amsterdam: 1994. p. 17-58.
- Petrides M, Pandya DN. Comparative analysis of the human and macaque ventrolateral prefrontal cortex and corticocortical connection patterns in the monkey. *European Journal of Neuroscience*. 2001; 16:291–310. [PubMed: 12169111]

- Preuss TM, Goldman-Rakic PS. Myelo- and cytoarchitecture of the granular frontal cortex and surrounding regions in the strepsirrhine primate *Galago* and the anthropoid primate *Macaca*. *Journal of Comparative Neurology*. 1991; 310:429–474. [PubMed: 1939732]
- Rajkowska G, Goldman-Rakic PS. Cytoarchitectonic definition of prefrontal areas in the normal human cortex: I. Quantitative criteria for distinguishing areas 9 and 46. *Cerebral Cortex*. 1995a; 4:307–322.
- Rajkowska G, Goldman-Rakic PS. Cytoarchitectonic definition of prefrontal areas in the normal human cortex: II. Variability in locations of areas 9 and 46. *Cerebral Cortex*. 1995b; 4:323–337.
- Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, Meltzer HY, Overholser JC, Roth BL, Stockmeier CA. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biological Psychiatry*. 1999; 45:1085–1098. [PubMed: 10331101]
- Rajkowska G, Miguel-Hidalgo JJ, Dubey P, Stockmeier CA, Krishnan RR. Prominent reduction in pyramidal neuron density in the orbitofrontal cortex of elderly depressed patients. *Biological Psychiatry*. 2005; 58:297–306. [PubMed: 15953590]
- Rajkowska G, O'Dwyer G, Teleki Z, Stockmeier CA, Miguel-Hidalgo JJ. Reduction in calbindin-immunoreactive GABA interneurons in the prefrontal cortex in major depression. *Neuropsychopharmacology*. 2007; 32:471–482. [PubMed: 17063153]
- Remijnse PL, Nielen MMA, Van Balkom AJLM, Cath DC, Van Oppen P, Uylings HBM, Veltman DJ. Reduced OFC-striatal responsiveness on a reversal learning task in Obsessive-Compulsive Disorder. *Archives of General Psychiatry*. 2006; 63:1225–1236. [PubMed: 17088503]
- Retzius, G. Teil 1 Text. Norstedt & Söner; Stockholm: 1896. *Das Menschen Hirn*.
- Roberts AC. Primate orbitofrontal cortex and adaptive behaviour. *Trends in Cognitive Science*. 2006; 10:83–90.
- Roberts AC, Tomic DL, Parkinson CH, Roeling TA, Cutter DJ, Robbins TW, Everitt BJ. Forebrain connectivity of the prefrontal cortex in the marmoset monkey (*Callithrix jacchus*): an anterograde and retrograde tract-tracing study. *Journal of Comparative Neurology*. 2007; 502:86–112. [PubMed: 17335041]
- Rolls ET, Grabenhorst F. The orbitofrontal cortex and beyond: From affect to decision-making. *Progress in Neurobiology*. 2008; 86:216–244. [PubMed: 18824074]
- Sarkisov, SA.; Filimonov, IN.; Kononova, EP.; Preobraschenskaja, IS.; Kukuev, LA. Atlas of cytoarchitectonics of the human cerebral cortex. Medzig; Moscow: 1955. in Russian
- Schormann T, Zilles K. Three-dimensional linear and nonlinear transformations: an integration of light microscopical and MRI data. *Human Brain Mapping*. 1998; 6:339–347. [PubMed: 9788070]
- Schwartz CE, Kunwar PS, Greve DN, Moran LR, Viner JC, Covino JM, Kagan J, Stewart SE, Snidman NC, Vangel MG, Wallace SR. Structural differences in adult orbital and ventromedial prefrontal cortex predicted by infant temperament at 4 months of age. *Arch Gen Psychiatry*. 2010; 67:78–84. [PubMed: 20048225]
- Swanson, LW. Brain maps: structure of the rat brain. Elsevier; Amsterdam: 1992.
- Talairach, J.; Tournoux, P. 3-D proportioned system: an approach to cerebral imaging. Thieme-Verlag; Stuttgart: 1988. Co-Planar Stereotaxic Atlas of the Human Brain.
- Tisserand DJ, Pruessner JC, Sanz Arigita EJ, Van Boxtel MPJ, Evans AC, Jolles J, Uylings HBM. Regional frontal cortical volumes decrease differentially in aging: An MRI study to compare volumetric approaches and Voxel-Based Morphometry. *NeuroImage*. 2002; 17:657–669. [PubMed: 12377141]
- Uylings HBM, Zilles K, Rajkowska G. Optimal staining methods for delineation of cortical areas and neuron counts in human brains. *NeuroImage*. 1999; 9:439–445. [PubMed: 10191172]
- Uylings HBM, Rajkowska G, Sanz-Arigita E, Amunts K, Zilles K. Consequences of large interindividual variability for human brain atlases: converging macroscopical imaging and microscopical neuroanatomy. *Anatomy Embryology (Berl)*. 2005a; 210:423–431.
- Uylings HBM, Malofeeva LI, Bogolepova IN, Jacobsen AM, Amunts K, Zilles K. No postnatal doubling of number of neurons in human Broca's area (BA 44 and 45)? A stereological study. *Neuroscience*. 2005b; 136:715–728. [PubMed: 16344146]

- Van den Heuvel OA, Remijnse PL, Mataix-Cols D, Vrenken H, Groenewegen HJ, Uylings HBM, van Balkom AJ, Veltman DJ. The major symptom dimensions of obsessive-compulsive disorder are mediated by partially distinct neural systems. *Brain*. 2009; 132:853–868. [PubMed: 18952675]
- Van Hoesen GW, Parvizi J, Chu C-C. Orbitofrontal cortex pathology in Alzheimer's disease. *Cerebral Cortex*. 2000; 10:243–251. [PubMed: 10731219]
- Vogt BA, Nimchinsky EA, Vogt LJ, Hof PR. Human cingulate cortex: surface features, flat maps, and cytoarchitecture. *Journal of Comparative Neurology*. 1995; 359:490–506. [PubMed: 7499543]
- Von Economo, C.; Koskinas, GN. *Die Cytoarchitektonik der Hirnrinde des Erwachsenen Menschen*. Springer Verlag; Berlin: 1925.
- Völlm BA, de Araujo IE, Cowen PJ, Rolls ET, Kringelbach ML, Smith KA, Jezzard P, Heal RJ, Matthews PM. Methamphetamine activates reward circuitry in drug naïve human subjects. *Neuropsychopharmacology*. 2004; 29:1715–1722. [PubMed: 15138439]
- Walker EA. A cytoarchitectural study of the prefrontal area of the macaque monkey. *Journal of Comparative Neurology*. 1940; 73:59–86.

Fig. 1.

Maps illustrating differences in the position, extent and nomenclature of different areas in the orbitofrontal cortex region. Adapted from Brodmann, 1914; Von Economo and Koskinas, 1925; Beck, 1949; Sarkisov et al., 1955; Petrides and Pandya, 1994, 2001; Hof et al., 1995; and Öngür et al., 2003. HR: horizontal ramus of the Sylvian fissure; LOS: lateral orbital sulcus; MOS: medial orbital sulcus; OLF: olfactory sulcus; TOS: transverse orbital sulcus.

Fig. 2.

Pattern of sulci on the orbitofrontal surface of the hemisphere in four different brains (cases 6895, 7186, 14686 and 1696). R: right hemisphere; L: left hemisphere. For abbreviations: LOS, MOS, OLF, and TOS see Fig.1.

Fig. 3.

Comparison of the histograms of the overall thickness of cortical gray matter, and thickness of layer IV between orbitofrontal subareas 47m^l, 47l^l, and medial prefrontal area 12, and dorsolateral prefrontal areas 9, and 46.

Fig. 4.

Comparison of the cytoarchitecture of anterior and posterior subregions of the orbitofrontal cortex. High power photomicrograph of Gallyas staining of subarea 10–47, and areas 10, and 11. Scale bar: 200 μ m

Fig. 5.
Photomicrograph of anterior and posterior parts of subareas 47m¹, 47m², and 47m³ of the orbitofrontal cortex, Gallyas staining (high power). Scale bar: 200μm

Fig. 6.
Photomicrograph of anterior and posterior parts of subareas 47I¹, and 47I² of the orbitofrontal cortex, Gallyas staining (high power). Scale bar: 200μm

Fig. 7.

A. Series of coronal section drawings (left panel) with delineations of orbitofrontal cortical subareas in case # 6895. FMS: frontomarginal sulcus, IFS: inferior frontal sulcus, and POS: posterior orbital sulcus, and OLF: olfactory sulcus Sfh: horizontal ramus of Sylvian fissure. For abbreviations LOS, and MOS, see Fig.1. The lines indicate the exact location, where the cytoarchitectonic pictures displayed in Figs 4–6 were taken. B. Series of coronal histological

images from anterior to posterior sections of brain # 6895 displaying delineations of orbitofrontal cortical subareas. Color coded as in A. Scale bar is 5 mm. C. Series of coronal MR images from anterior to posterior sections of brain # 6895 with the transformed delineations; color coded as in A.

Fig. 8.

Cytoarchitecture of three anterior regions: area 11, and subareas 47 m¹ and 47l¹ of the orbitofrontal cortex stained for Nissl. Note that general features of laminar architecture are similar to those visible with Gallyas staining. Scale bar: 210µm.

Fig. 9.

3-D reconstructions of orbitofrontal subareas as visible on the ventral (left panel) and lateral (right panel) view of three brains: 1696; 7186, and 14686. The dotted line at the transverse orbital sulcus (TOS) indicates the middle of the wide transition zone between the anterior and posterior region of area 47.

Fig. 10.
Overview and details of SMI-32 staining of the anterior part of orbitofrontal cortex (sub)areas. Lower scale bar: 500 μm.

Fig.11.

SMI-32 staining of posterior parts of area 11 and subareas 47m of orbitofrontal cortex shows only minor differences. A major difference between the subareas 47m and the agranular insular cortex is noticeable. Scale bars: 300 μ m.

Fig. 12.
Comparison of NF200 staining of area 10, subarea 10-47 and anterior parts of subareas 47m and 47l. Scale bar: 250 μ m.

Fig. 13.
Overview and details of parvalbumin-staining of anterior parts of orbitofrontal cortex (sub)areas. Lower scale bar: 500 µm.

Fig. 14.

Parvalbumin staining of posterior parts of subareas 47m of orbitofrontal cortex shows only minor differences between the subareas of 47m. In contrast, subareas 47m and the agranular insular cortex are different in this staining. Scale bars: 300 μ m.

Fig.15. Comparison of calbindin-staining of the anterior part of area 11 and subareas 47m and 47l. Scale bar: 300 μ m.

Fig.16.

Proposed macroscopic demarcation of the orbitofrontal areas shown from a medial-ventral perspective of the right hemisphere (panel A) and a basal surface of the brain (panel B). A: Microscopically defined cytoarchitectonic areas of the orbitofrontal cortex were superimposed onto the macroscopic picture of the anterior part of the brain shown in Fig. 17 from Duvernoy, H.M. (1999) "The Human Brain. Surface, Blood Supply, and 3-D sectional Anatomy, p.34, 2nd edition, Springer-Verlag Wien/New York (with permission) using gyral and sulcal landmarks for orientation. B: Delineations of orbitofrontal cortical areas are specified on the picture of the brain which is used for the macroscopical neuroanatomy of human brain and displayed on the website

http://www.anatomie-amsterdam.nl/onderwijs/anatomie-zenuwwerking/new_site_2006/anatomie_123_neuro/start.htm.

LOS: lateral orbital sulcus; MOS: medial orbital sulcus; TOS: transverse orbital sulcus; CS: cingulate sulcus; FMS: frontomarginal sulcus; OLF: olfactory sulcus; OB: olfactory bulb; OT: olfactory tract; cc: corpus callosum; *: the most anterior tip of the olfactory sulcus. Individual areas are depicted in different colors: orange-brown: area 12, purple: area 11, dark blue: area 47m¹, light blue: area 47m², pink: area 47m³, dark grey: area 47l¹, dark brown: area 47l².

Table 1

Comparison of parcellations and nomenclatures for human orbitofrontal areas

Present study	Brodmann (1914)	Sarkisov et al. (1955)	Von Economo & Koskinas (1925)	Hof et al. (1995)	Beck (1949)	Petrides & Pandya (1994; 2001)	Öngür et al. (2003)
<i>Anterior</i>							
11	<<<11	<<11	<FG	<AM	<A.Recta _a	<14	<11m
47m1	<<<11	<<11	<FG	<AM	<A.Recta _a	<14	<11m
47m2	<<47	<<11	<<FF	<AM&MO	=11	<11&13	<111
47m3	<<47	<<<10	<<FF	<MO	<<47	<11&13	<111
4711	<<47	<<<10	<<FF	<<<AL	<<47	<47/12	<47/12r
4712	?<<47	<<<10	<<FF	<<<AL	<<47	<47/12	<47/12r
<i>Posterior</i>							
11	<11	<<11	<FH	<<PM	<A.Recta _p	<14	=14r+c
47m1	<11	<<11	<<FH,FF	<<PM	<A.Recta _p	<14	=13a+b
47m2	<<47	<<11	<<FF	<<PM	=13	? <13*	=131
47m3	<<47	=47-1+2+3	<<FF	<<PL	<<47	<13*	=47/12m
4711	<<47	=47-4	<<FF	<<PL	<<47	<47/12	<47/121
4712	?<<47	=47-5	<<FF	<<PL	<<47	<47/12	<47/121

<<< : minor part of large region

<< : small part of undivided orbitofrontal area

< : part of undivided area

? : not evident from original map

= : equal to

* Cf. Mackey & Petrides(2009): 47m3 = 13