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Sex differences in the human olfactory system

Alicia Garcia-Falgueras[a], *Carme Junque*[b], *Mónica Giménez*[b], *Xavier Caldú*[b], *Santiago Segovia*[a], *Antonio Guillamon*[a,*]

[a]*Departamento de Psicobiologia, UNED, C/ Juan del Rosal 10, Madrid 28040, Spain*
[b]*Departamento de Psiquiatría y Psicobiología Clínica, Universidad de Barcelona, C/ Casanova, 145, 08036 Barcelona, Spain*

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ABSTRACT

The olfactory system (accessory) implicated in reproductive physiology and behavior in mammals is sexually dimorphic. These brain sex differences present two main characteristics: they are seen in neural circuits related to sexual behavior and sexual physiology and they take one of two opposite morphological patterns (male>female or female>male). The present work reports sex differences in the olfactory system in a large homogeneous sample of men (40) and women (51) using of voxel-based morphology. Gray matter concentration showed sexual dimorphism in several olfactory regions. Women have a higher concentration in the orbitofrontal cortex involving Brodmann's areas 10, 11 and 25 and temporomedial cortex (bilateral hippocampus and right amygdala), as well as their left basal insular cortex. In contrast, men show a higher gray matter concentration in the left entorhinal cortex (Brodmann's area 28), right ventral pallidum, dorsal left insular cortex and a region of the orbitofrontal cortex (Brodmann's area 25). This study supports the hypothesis that the mammalian olfactory system is a sexually dimorphic network and provides a theoretical framework for the morphofunctional approach to sex differences in the human brain.

1. Introduction

The olfactory system of mammals is a very complex network that is related to reproductive behaviors. Its structure in man is inferred from experimental data obtained from nonhuman primates, rodents and other species (for a review, see Price, 2004). Fortunately rats and the monkeys present homologies (Carmichael et al., 1994). The bipolar receptor neurons from the olfactory mucosa send olfactory nerve fibers to the glomerular layer of the olfactory bulb where they synapse with the mitral and tufted cells (Price, 2004). The axons from these cells are a substantial part of the lateral olfactory tract and project to the primary olfactory cortex, which includes the anterior olfactory nucleus, piriform cortex, ventral tenia tecta, olfactory tubercle, anterior cortical nucleus of the amygdala, periamygdaloid cortex and the rostral ("olfactory") entorhinal cortex (Carmichael et al., 1994). These primary olfactory cortex structures, with the exception of the olfactory tubercle, send fibers (some of which are contralateral) back to the olfactory bulb (Carmichael et al., 1994). This pattern of connections is very similar to that reported in the rat and other species (Carmichael et al., 1994).

The primary olfactory cortex projects to limbic structures, such as the amygdala and the hippocampus (Jolkkonen et al., 2001; Price, 1986; Witter and Amaral, 1991), and also to the hypothalamus and the ventral striatum (Fuller et al., 1987; Tazawa et al., 1987), which includes the accumbens nucleus and the olfactory tubercle. The ventral striatum projects to the ventral pallidum which, in turn, projects to the mediodorsal thalamic nucleus (Price, 2004). This nucleus also receives

* *Corresponding author.*
E-mail address: aguillamon@psi.uned.es (A. Guillamon).

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projections from the olfactory cortex and subcortical structures including the amygdala (Ray and Price, 1993; Russchen et al., 1987; Velayos and Reinoso-Suarez, 1982; Yarita et al., 1980).

Neocortical analyses of olfactory information seem to be centered on the agranular insula and the posterior orbital cortex. These areas receive both direct neocortical olfactory projections from the primary olfactory cortex as well as an indirect transthalamic input through the mediodorsal nucleus (Carmichael et al., 1994; Cavada et al., 2000). There are also direct inputs from other structures like the amygdala that are reciprocal (Amaral and Price, 1984; Carmichael and Price, 1995; Cavada et al., 2000).

Most mammalian species, including monkeys, have a dual olfactory system: the main (MOS) and the accessory olfactory (vomeronasal) system (AOS); these systems are interconnected at several levels (Halpern and Martinez-Marcos, 2003). The AOS is implicated in the control of sexual (Powers and Winans, 1975) and maternal behaviors (Del Cerro, 1998), as well as pheromone-mediated phenomena related to reproduction (Halpern, 1987; Halpern and Martinez-Marcos, 2003; Wysocki, 1979). Although there is good evidence for the effects of pheromones in humans (Martins et al., 2005; Preti et al., 2003; Wysocki and Preti, 2004), our vomeronasal organ is not functional (Wysocki and Preti, 2004) and we lack an accessory olfactory bulb (Meisami et al., 1998). These observations suggest that our species lack an AOS and that a "single" (main) olfactory system deals with pheromone inputs. Recently, Yoon et al. (2005) have identified in mice major olfactory projection pathways to neurons synthesizing luteinizing hormone-releasing hormone (LHRH neurons) originating from a discrete population of olfactory sensory neurons but fail to document any synaptic connectivity with the vomeronasal system. It is possible that in humans, a similar polysynaptic pathway to the hypothalamus, originated in the olfactory mucosa and related to the main olfactory system, maintain pheromone functions.

It has been shown that the AOS is a sexually dimorphic network. Indeed, the vomeronasal organ itself, and/or its primary, secondary and tertiary projections are sexually dimorphic in rodents (for a review, see Guillamon and Segovia, 1996; Halpern, 1987; Halpern and Martinez-Marcos, 2003; Segovia and Guillamon, 1986, 1993, 1996; Segovia et al., 1999) and lagomorphs (Segovia et al., 2006).

In vivo voxel-based morphometry (VBM) MRI studies of the human brain have shown the existence of sex differences. Men show larger brains and larger gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) compartments than women (Allen et al., 2003; Blatter et al., 1995; Giedd et al., 1996; Good et al., 2001; Gur et al., 1991; Lemaitre et al., 2005; Luders et al., 2002, 2005; Murphy et al., 1996; Nopoulos et al., 2000; Raz et al., 1997). However, other studies find no sex differences in the overall percentage of GM or WM (Filipek et al., 1994; Schlaepfer et al., 1995); although women show a higher proportion of GM than men (Allen et al., 2003; Gur et al., 1999; Lemaitre et al., 2005; Luders et al., 2002).

When GM concentration was measured it was found that females show higher values than males (Luders et al., 2005). Women have increased GM concentration bilaterally in the cortical mantle, parahippocampal gyri and the banks of the cingulated and calcarine sulci (Good et al., 2001), the pre- and postcentral gyri and the inferior temporal gyrus (Luders et al., 2005). In the left hemisphere, women also show a higher GM concentration than men along the border between the temporal and occipital lobes, in the inferior frontal gyrus and in a posterior region of the superior temporal gyrus (Luders et al., 2005). These findings might reflect sex differences in the underlying cytoarchitecture related to the organization of layers or the concentration of neurons (Luders et al., 2005), although it does not mean the existence of sex differences in cell packing.

However, to the best of our knowledge, no systematic study of the expression of sex differences in the human olfactory system has yet been undertaken. In this report, using a voxel-based morphometry (VBM), we searched for possible sexual dimorphism in the following olfactory reputed cerebral structures: olfactory cortex, hippocampus, hypothalamus, amygdala, ventral pallidum, accumbens nucleus, mediodorsal nucleus of the thalamus, insula and medial orbitofrontal cortex (Brodmann's area 11).

2. Results

As seen in Table 1, men show larger direct values for WM, GM, CSF and total volume (TV) than women. However, no sex differences were seen with respect to ratios.

The regional GM study of olfactory structures has two main findings. First, as in other mammals, some olfactory structures are sexually dimorphic (Tables 2 and 3), and second as reported in other species, sex differences are present in the two morphological patterns; in one pattern, women show larger morphological measurements (Table 2) whereas the other, the opposite is true (Table 3).

As can be observed in Table 2, women show higher concentration values than men in the orbitofrontal cortex (Brodmann's areas 10, 11 and 25), hippocampus, amygdala and insula whereas men have higher concentration than women in the entorhinal cortex (Brodmann's area 28), ventral and lateral pallidum and insula.

As shown in Fig. 1, the areas of higher concentration in women's gray matter practically outline the orbital and medial regions of the olfactory sulcus. In addition, a large cluster bilaterally involves Brodmann's areas 11 and 10. There

Table 1 – Volumetric sex differences in cm^3

	Females	Males
Direct values (cm^3)		
Gray matter (GM)	723±65.3	844±63.3*
White matter (WM)	372±76.7	444±40.4*
Cerebrospinal fluid (CFS)	342±48.3	390±41.8*
Total volume	1437±103.6	1678±119.1*
Ratios		
GM/total volume	0.504±0.04	0.503±0.02
WM/total volume	0.257±0.04	0.264±0.01
CSF/total volume	0.237±0.02	0.232±0.02
GM/WM	2.01±0.43	1.906±0.11

* $p<0.001$.

Table 2 – Cerebral regions in which concentration is greater in females than in males

Regions	T	MNI coordinates			Cluster size (voxels)
Brodmann's 10 (frontal superior gyrus)	5	−16	40	−22	37
Brodmann's 11 (frontal superior gyrus)	7.3	−10	66	−16	107
	6.6	8	68	−10	124
Brodmann's 25 (central superior gyrus)	5.9	12	38	−24	40
Hippocampus	5.7	−16	40	−22	37
	5.6	−34	−32	−12	41
	5.2	40	−32	−12	52
Amygdala	5.3	30	−4	−26	26
Insula	4.2	−42	6	−10	18

is another significant cluster caudally placed in the olfactory cortex that corresponds to the superior frontal gyrus (Brodmann's area 25). The medial temporal region shows a large cluster of increased concentration that bilaterally involves the hippocampus (Fig. 1). There is a laterality effect in the amygdala; the region of increased concentration corresponds to the right lateral division of this structure. There is a high concentration cluster in the left insula.

Men show fewer significant high concentration clusters than women and their clusters are also smaller than the women's (see Table 3 and Fig. 2). Interestingly, men have greater concentration than women in the entorhinal cortex. In Brodmann's area 25, of the left hemisphere, there is also a cluster in which men have a greater concentration than women. This cluster is dorsally located in men compared to its more basal position in women (compare Figs. 1 and 2). The same happens with the left insula, the region of higher concentration in men is more dorsally located than in women. In the right ventral pallidum men show a cluster with greater concentration than women (Fig. 2).

3. Discussion

As in other mammals (Guillamon and Segovia, 1996; Segovia and Guillamon, 1986, 1993, 1996), the human olfactory system is sexually dimorphic. Sex differences have been reported in the rat accessory olfactory pathway and are presumed to exist in all rodents (Segovia and Guillamon, 1993). The rabbit, as a representative of the lagomorphs, also shows sex differences in its accessory olfactory system (Segovia et al., 2006). Sex differences in the brain are strain and species-specific (Garcia-Falgueras et al., 2005; Segovia et al., 2006) and are also so in

Table 3 – Cerebral regions in which concentration is greater in males than in females

Regions	T	Coordinates MNI			Cluster size (voxels)
Brodmann's 25	4.4	−4	20	−12	23
Brodmann's 28 (enthorrinal cortex)	4.2	−26	−14	−4	40
Ventral pallidum	4.3	18	0	−6	44
Lateral pallidum	3.7	−20	−8	−4	40
Insula	4.6	−32	−18	14	53

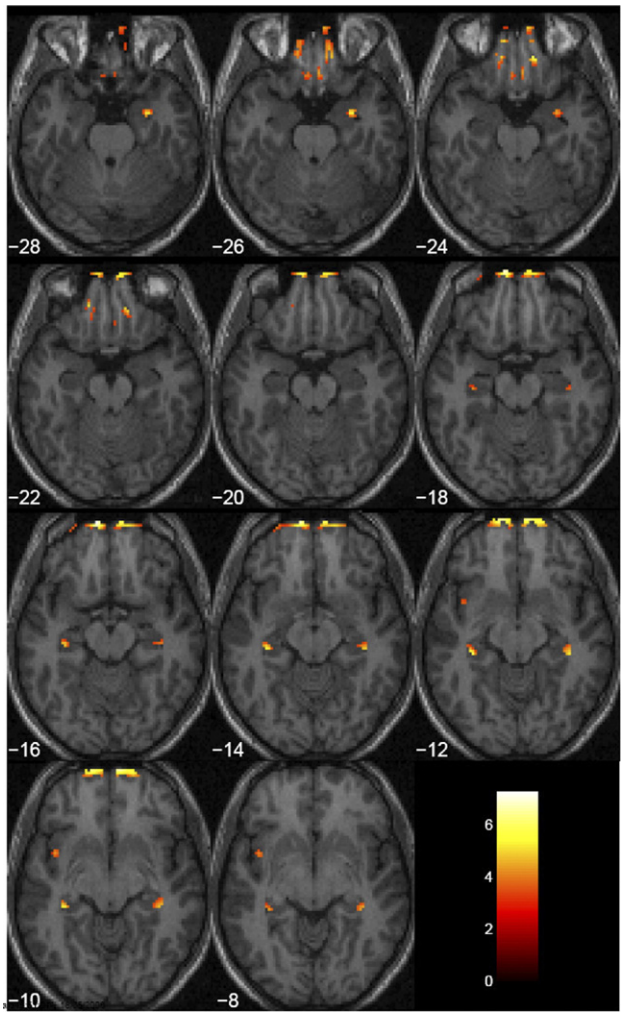

Fig. 1 – The figure represents horizontal sections of the regions of the human brain in which women show higher gray matter concentration than men. Gray matter concentration increases in females versus males. Regions of increased gray matter concentration are superimposed on a normalized single subject structural image. Magnetic resonance images are in the horizontal plane. The color bar represents the *t* score. Yellow color indicates greater statistical significance than orange or red. The statistical parametric maps follow the standard neuroradiological presentation: left side of the images corresponds to the left hemisphere. The number of the slices corresponds to the relative position in the *Z* axis. The point 0 corresponds to the anterior–posterior commissure line. Negative values are inferior to this line and positive values superior to this line. We can see statistically significant voxels in the frontal orbital region involving bilaterally the olfactory cortex (slices from −28 to −20). The areas on increased concentration are located around the olfactory sulcus. In the medial temporal lobe the regions that achieved statistical significance are the right amygdala (slices −28 to −24) and the bilateral hippocampus (slices −18 to −8). We also observed increased concentration in the left insular cortex (slices −14 to −8).

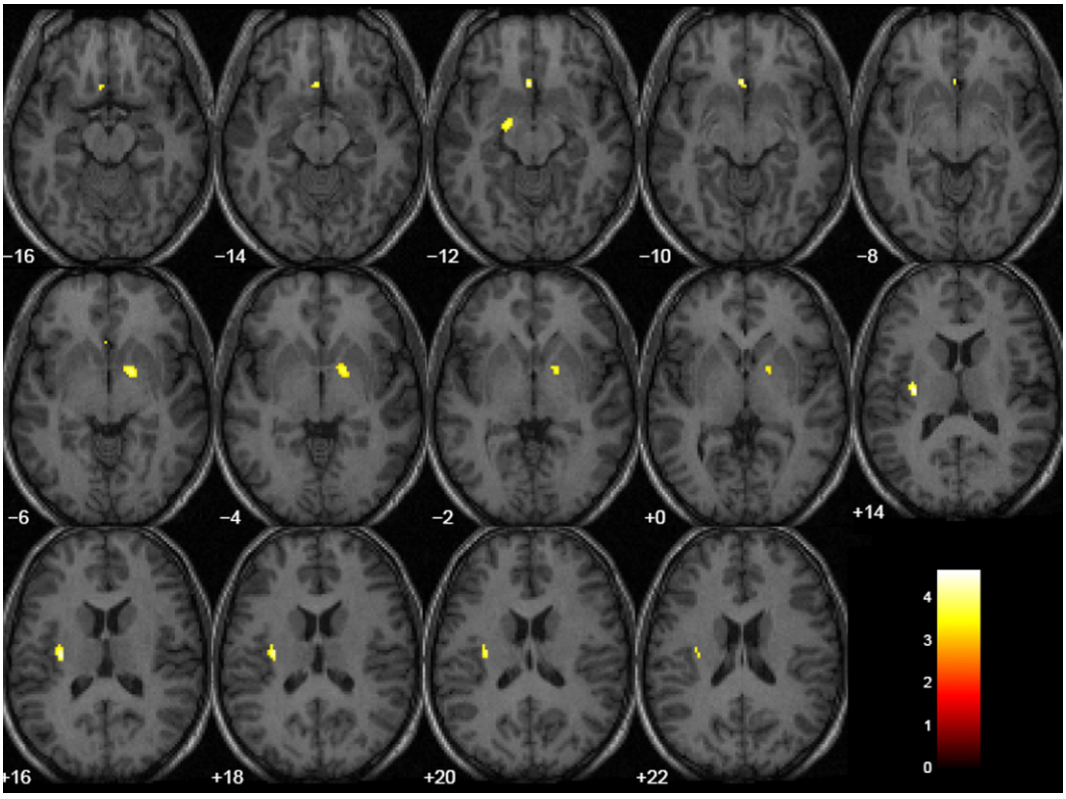

Fig. 2 – The figure represents horizontal sections of the regions of the human brain in which men show higher gray matter concentration than women. Gray matter concentration increases in males versus females. Regions of increased gray matter concentration are superimposed on a normalized single subject structural image. Magnetic resonance images are in the horizontal plane. The color bar represents the *t* score. Yellow color indicates greater statistical significance than orange or red. The statistical parametric maps follow the standard neuroradiological presentation: left side of the images corresponds to the left hemisphere. The number of the slices corresponds to the relative position in the *Z* axis. The point 0 corresponds to the anterior–posterior commissure line. Negative values are inferior to this line, and positive values superior to this line. We can see statistically significant clusters of increased concentration in the left Brodmann's area 25 (slices −16 to −8) the right pallidum slices (−6 to +0) and the left insula (slices +14 to +22). Comparison with the slices of Fig. 1 shows that sex differences in the insula and the Brodmann's area 25 of men are more dorsally located than those sex differences favoring women in the same structure.

humans, in which we have seen that some structures of the olfactory system are sexually dimorphic.

Interestingly, the results of the regional gray matter analysis resemble reports from neurohistological studies in other mammal species. First, not all olfactory structures present sexual dimorphism, and second, sexual dimorphism takes two morphological patterns: male>female and female>male (Guillamon and Segovia, 1996; Segovia and Guillamon, 1993; Segovia et al., 2006). Our MRI data show two patterns of sexual dimorphism in the human olfactory system (males>females; females>males). These patterns can occur in different regions of the same structure, as we saw in the insula. In the rat the medial anterior and lateral anterior subdivisions of the bed nucleus of the stria terminalis, the anteroventral periventricular nucleus, the parastrial nucleus, the arcuate nucleus and the locus caeruleus are larger in females than in males, whereas the opposite is found in the accessory olfactory bulb, bed nucleus of the accessory olfactory tract, medial posterior subdivision of the bed nucleus, the sexually dimorphic nucleus of the preoptic area, medial amygdala, posteromedial cortical amygdala, ventromedial hypothalamic nucleus, the spinal nucleus of the bulbocavernosus and the dorsolateral nucleus (for a review, see Guillamon and Segovia, 1996; Segovia and Guillamon, 1993; Segovia et al., 1999). Thus, the regional olfactory sex differences reported here using MRI resembles those reported in the literature using neurohistological methods. The fact that sex differences take either pattern and are structure specific support the proposal that the sex differences found here are independent from the whole cerebral volume in which males exceed females.

Similarly to other studies in the literature, we have found that adult men have larger brains, and larger WM, GM and CSF compartments than women (Allen et al., 2003; Blatter et al., 1995; Giedd et al., 1996; Good et al., 2001; Gur et al., 1991; Lemaitre et al., 2005; Luders et al., 2002, 2005; Murphy et al., 1996; Nopoulos et al., 2000; Raz et al., 1997). However, no sex differences were found with respect to ratios.

Our results are difficult to compare with previous MRI studies because methodological differences. However, Goldstein et al. (2001) tested regional volumetric differences in 45 brain regions and found that women had larger volumes

related to cerebral size particularly in frontal and medial paralimbic cortices, whereas men had larger volumes related to cerebrum size in frontomedial cortex, the amygdala and hypothalamus, but they did not tested concentration. Gur et al. (2002), using a quantified measure of the total volume of the orbitofrontal cortex, including the olfactory areas studied by us, found that women have larger orbitofrontal cortices than men. With a similar methodology, Tisserand et al. (2002) reported that women have a larger volume than men in the frontal pole (Brodmann's area 10).

The measurements of gray matter concentration (or density) in VBM might reflect the underlying cytoarchitecture related to the organization of layers or the density (concentration) of neurons (Luders et al., 2005), although should not be confused with cell packing density measured cytoarchitectonically (Mechelli et al., 2005). In our work, women show greater concentration of GM than men in the olfactory orbitofrontal cortex (Brodmann's areas 10, 11 and 25), similar results were reported by Good et al. (2001). These authors, using VBM analyzed volume and gray matter concentration of the whole brain, found increased gray mater concentration in the frontal, posterior temporal and parietal cortical mantle, in addition to parahypoccampal gyrus, caudate head and calcarine and cingulated sulci. They did not show any region of significant increased gray matter concentration in males. Moreover, Good et al. (2001) tested the whole brain, but they threshold at $P<0.05$ corrected for multiple comparisons whereas we used significance at uncorrected $P<0.001$. In addition, our findings are partially in agreement with those reported by Luders et al. (2005) who found that women have a higher concentration of gray matter in the pars orbitalis of the inferior frontal gyrus. However, these authors did not test subcortical structures and they did not report the male>female pattern of gray matter concentration. Our larger and more homogeneous sample might have a greater statistical power. Functional neuroimaging has already generated important new insights into the neural substrates of olfactory processing. In a review of imaging studies of olfactory perception in humans, Savic (2002) found that in 8 over 9 PET or fMRI studies described the activation of the orbitofrontal cortex.

An important finding here is that men have greater concentration than females in the entorhinal cortex, a region that it is considered to be primary olfactory cortex that receives direct input from the olfactory bulb (Price, 2004), and this supports the proposal that the human olfactory cortex is sexually dimorphic, as it is in other mammals. The primary olfactory cortex is reciprocally connected to the insula/posterior orbital cortex (Carmichael et al., 1994). Although MRI techniques cannot distinguish the olfactory regions of the insula, we did detect two patterns of sex differences in this structure. In men, the areas of greater concentration with respect to women are located more dorsally that the densest areas in women. There is a report that the total volume of the insula is greater in men than in women (Allen et al., 2003); however, the method used in that work precludes the identification of regional sex differences in concentration within the insula itself. A similar reasoning could be applied to the amygdala, in which global volumetric analyses show greater measures for men (for a review, see Brierley et al., 2002). To our knowledge, our study is the first to show sex differences in the concentration of the gray matter in the amygdala (right). Right hemisphere dominance for olfactory functions has been reported (Jones-Gotman and Zatorre, 1993). In addition, putative pheromones activate the amygdala (Savic, 2002).

The hippocampus is bilaterally denser in women than in men. The classic volumetric studies show that women have a larger hippocampus than men (Filipek et al., 1994; Giedd et al., 1996; Murphy et al., 1996). We have observed gender differences in gray matter concentration in several regions that form part of the olfactory system. However, we cannot isolate the specific subregions involved in olfactory functions. For example, we have just comment we observed a widespread cluster of increased gray matter concentration in the hippocampus, a structure mainly related to declarative memory processes (Squire and Zola, 1996).

Voxel-based morphometry has been found to be sensitive enough to detect brain changes due to hormonal effects. It has been demonstrated that substitutive estrogen therapy in postmenopausal women precludes cerebral atrophy in several brain regions, including the hippocampus (Erickson et al., 2005). Moreover, using manual tracing of the medial temporal region, Kesler et al. (2004) found a reduction of the volume of amygdala and hippocampus in persons with Turner's syndrome, who have low levels of estrogens (Hojbjerg Gravholt et al., 1999). Using a similar MRI procedure, Merke et al. (2003) found a significant decrease in the amygdala volume of both male and female children with congenital adrenal hyperplasia. As in other mammalian species, the human brain is sensitive to the actions of sex steroids. The human brain shows estrogen (ER) and androgen (AR) receptors in some olfactory structures (Abdelgadir et al., 1999; Österlund and Hurd, 2001). ER alpha messenger (m) RNA has been found in the human amygdala, hypothalamus and globus pallidus, whereas ER beta mRNA was detected in the hippocampus, entorhinal cortex and thalamus (Donahue et al., 2000; Österlund and Hurd, 2001).

It has been suggested that in rodents and lagomorphs estradiol aromatized from testosterone is involved in sexually differentiating the males from females and is responsible for some structures in the former being larger than in the latter (for a review, see Guillamon and Segovia, 1996; Segovia and Guillamon, 1993; Segovia et al., 2006). However, the picture appears to be quite different in humans. It seems that androgens might play a major role in brain sexual differentiation and sexual behavior, whereas the aromatization of testosterone to estradiol would be less relevant (Swaab, 2004).

Sex differences in the central nervous system of mammals are known to be strain- (Garcia-Falgueras et al., 2005) and species-specific (Segovia et al., 2006), and the results of the present work show the existence of a specific pattern of sex differences in the human olfactory system. This system, as in other mammalian species, is also related to sexual physiology and sexual behavior. Some structures of the human olfactory system are activated by visual erotic stimuli (Karama et al., 2002) as well as by physical sexual stimulation (Georgiadis and Holstege, 2005). Scent is also active in the human species. Odor discrimination and preferences are influenced by gender and sexual orientation (Wysocki and Preti, 2004). Extracts of male axillary secretions have a direct effect upon LH-pulses and mood in women (Preti et al., 2003), and the menstrual cycle is

Table 4 – Characteristics of the sample

Group	Mean ± SD age	Age range	N
Females	19.7 ± 2.3	18–19	51
Males	20.55 ± 3.3	18–33	40

influenced by axillary secretions from other women (Russell et al., 1980) and men (Cutler et al., 1986). These reports strongly suggest that, as in other mammalian species, the human olfactory system still retains functions related to sexual physiology and sexual behavior.

The accessory olfactory system of rodents and lagomorphs is sexually dimorphic and we just have seen that the same occurs in the human olfactory system. It seems that sex differences in the olfactory system are maintained within the mammalian class with differences among species due to adaptation. Moreover, our findings indicate the relevance of employing MR imaging to assess sex differences in the comprehensive framework provided by the olfactory system. We believe that this approach to the study of human brain sex differences will help us to exploit the abundant literature of animal data on sex differences and sexual behavior and facilitate the construction of a morphofunctional motivational model that could explain sex differences in the human brain.

4. Experimental procedures

4.1. *Subjects*

The subject sample was composed of 91 undergraduate students from the School of Psychology at the University of Barcelona (51 young adult women and 40 young adult men) aged between 18 and 33 years old (Table 4). All subjects were right-handed and were screened by interviews using the Structured Clinical Interview for DSM-IV non-patient version questionnaires to rule out any possible psychopathology. The study was approved by the Ethics Committee of the University of Barcelona. Written consent was obtained from all participants.

4.2. *MRI acquisition and VBM protocol*

Data were obtained on a 1.5-T scanner (NV/Cv1 8.4 General Electric, Milwaukee, WI). A set of high-resolution T1-weighted images was acquired with a Fast Spoiled Gradient 3d sequence (TR/TE = 12/5.2; TI 300 1 nex; FOV = 24 × 24 cm; 256 × 256 matrix), yielding contiguous 1.5 mm thick coronal slices.

Automated image processing was done using SPM2, running in Matlab (MathWorks, Natick, MA). A single investigator (AG-F) performed the prior manual steps for image preparation (anterior–posterior commissure line determination and image reorienting).

In recent years, a number of unbiased objective techniques have been developed to characterize morphological differences in vivo using structural magnetic resonance images. The voxel-based morphometry (VBM) is a technique that compares different brains on a voxel-by-voxel basis after spatially normalization of the images. Using this technique, the brain can be examined in an unbiased and objective manner. VBM is very sensitive for localizing small-scale regional differences in gray or white matter. The aim of VBM is to identify differences in the local composition of brain tissue while discounting large-scale differences in gross anatomy and position. This is achieved by spatially normalizing all the structural images to the same stereotaxic space, segmenting the normalized images into gray and white matter, smoothing the gray and white matter images and finally performing a statistical analysis to localize significant differences between two or more groups. The output is a statistical parametric map (SPM) where gray or white matter differs significantly among the groups (Mechelli et al., 2005).

For voxel-based morphometry (VBM) analysis we selected the standard VBM procedure which included the following steps: (a) normalization of original MRI images

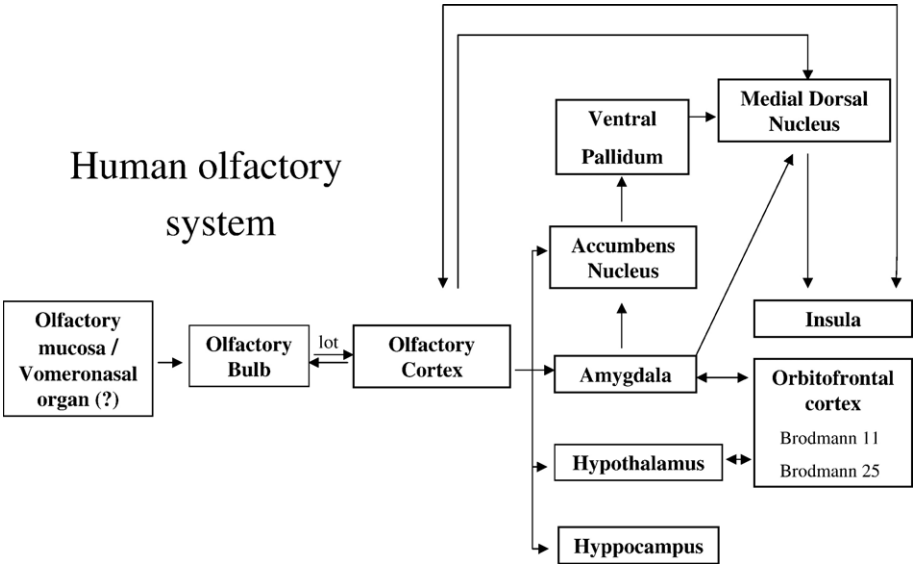

Fig. 3 – The diagram showing the olfactory system in humans is an adapted version from Price (2004). It should be kept in mind that the vomeronasal organ in humans is vestigial.

into a standardized coordinate system. This was achieved by registering each of the images on to the SPM T1 template; (b) the spatially normalized images were automatically partitioned into separate images representing probability maps for gray matter, white matter, and cerebral spinal fluid; and (c) segmented gray matter images were smoothed with a 6-mm full-width at half-maximum isotropic Gaussian kernel. The kernel size was determined considering the brain structures where differences were expected. We used the current standard of the Montreal Neurological Institute (MNI) template adopted by the International Consortium of Brain Mapping, known as ICBM152 (Brett et al., 2002). The analysis of segmented images indicates regional differences in gray matter concentration (or density) (Mechelli et al., 2005).

We performed region of interest (ROI) analyses of the cerebral structures involved in the olfactory system according to the schema presented in Fig. 3 [adapted from Price (2004) description of the olfactory system in humans]. We used the WFU-Pick atlas toolbox software for SPM (Maldjian et al., 2003) to analyze the selected ROI. The included structures were as follows: olfactory cortex, pallidum, accumbens nucleus (Talarach coordinates: 6 8 −6; −6 8 −6; $r=2$), amygdala, hippocampus, hypothalamus, medial–dorsal nucleus of the thalamus, insula and Brodmann's area 11. The olfactory cortex included the olfactory tubercle, lying in the caudal side of the gyrus rectus within two branches of the fourth frontal sulcus and part of Broca's olfactory cortex located under the corpus callosum genu (Tzourio-Mazoyer et al., 2002).

4.3. *Statistical analysis*

The processed gray matter images were analyzed using the SPM2 two-sample *t* test group comparison. We performed two-sided comparisons to evaluate gray matter brain concentration. We performed "males>females" and "females>males" comparisons.

We used a threshold at uncorrected voxel *P* value of <0.001 and we only report clusters of more than 10 voxels that were significant at the corrected cluster level of $P<0.05$.

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