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Regional sex differences in grey matter volume are associated with sex hormones in the young adult human brain

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ABSTRACT

Previous studies suggest organizing effects of sex hormones on brain structure during early life and puberty, yet little is known about the adult period. The aim of the present study was to elucidate the role of 17β-estradiol, progesterone, and testosterone on cortical sex differences in grey matter volume (GM) of the adult human brain. To assess sexual dimorphism, voxel-based morphometry (VBM) was applied on structural magnetic resonance images of 34 healthy, young adult humans (17 women, 17 men, 26.6 ± 5 years) using analyses of covariance. Subsequently, circulating levels of sex hormones were associated with regional GM using linear regression analyses. After adjustment for sex and total GM, significant associations of regional GM and 17β-estradiol were observed in the left inferior frontal gyrus ($\beta = 0.39$, $p = 0.02$). Regional GM was inversely associated with testosterone in the left inferior frontal gyrus ($\beta = -0.16$, $p = 0.04$), and with progesterone in the right temporal pole ($\beta = -0.39$, $p = 0.008$). Our findings indicate that even in young adulthood, sex hormones exert organizing effects on regional GM. This might help to shed further light on the underlying mechanisms of both functional diversities and congruence between female and male brains.

Introduction

Structural sex differences in the developing and adult brain have been described by a large number of studies, pointing to both functional diversities and compensation mechanisms between women and men (reviewed, for example, in Cahill, 2006; Cosgrove et al., 2007; De Vries, 2004). Using post-mortem techniques and *in vivo* magnetic resonance imaging (MRI), it has been consistently shown that even to some extent independent from body size, female brains are on average smaller compared to males (Filipek et al., 1994; Gur et al., 1999; Nopoulos et al., 2000; Passe et al., 1997; Peters, 1991; Rabinowicz et al., 1999; Witelson et al., 1995). In addition, relative to cerebrum size, cerebral spinal fluid (CSF) volume (Agartz et al., 1992; Giedd et al., 1997; Grant et al., 1987; Gur et al., 1999; Lemaître et al., 2005) and white matter (WM) volume (Filipek et al., 1994; Gur et al., 1999; Lemaître et al., 2005) are greater in males than in females, whereas females exhibit greater cortical grey matter (GM) volume (Allen et al., 2003; Gur et al., 1999; Lemaître et al., 2005). In addition, distinct cortical and subcortical regions have been identified to be sexually dimorphic. For example, females tend to have larger volumes or cell packing density in language-related fields such as Boca's and Wernicke's areas (Harasty et al., 1997; Jacobs et al., 1993; Schlaepfer et al., 1995; Witelson et al., 1995), but also in the right hemisphere in the parietal and temporal association cortices (Good et al., 2001; Sowell et al., 2007), in the dorsolateral prefrontal (Schlaepfer et al., 1995), orbitofrontal, superior frontal, and lingual gyri (Goldstein et al., 2001), right inferior parietal cortex (Nopoulos et al., 2000), anterior cingulate cortex (Good et al., 2001; Paus et al., 1996), as well as in the hippocampus (Filipek et al., 1994; Giedd et al., 1996; Murphy et al., 1996), whereas males exhibit greater volumes or neuronal densities in the medial frontal cortex (Goldstein et al., 2001; Zhou et al., 1995), amygdala (Giedd et al., 1996), hypothalamus (Allen et al., 1989; Swaab and Fliers, 1985), mesial temporal lobes (Good et al., 2001), paracingulate gyrus (Paus et al., 1996), and cerebellum (Carne et al., 2006; Good et al., 2001). However, results are partially conflicting, e.g., regarding the corpus callosum (Allen et al., 1991; Bishop and Wahlsten, 1997). These divergent findings might be explained by different age range of the respective subjects investigated (Raz et al., 2004; Sowell et al., 2007) and on the different methods used (Chen et al., 2007; Shah et al., 2004; Sowell et al., 2007; Witelson et al., 1995), for example, considering post-mortem evaluation, *in vivo* region-of-interest (ROI) analysis, or voxel-based morphometry (VBM).

Structural sex differences in the adult brain have been suggested to depend on "activating" (i.e., facilitation of reproductive behaviour) and "organizing" (i.e., irreversible neuronal changes) effects of circulating sex steroid hormones, both during neonatal differentiation, but also later in life (Cooke et al., 1998; Kawata, 1995). The original hypothesis limited organizational effects to the embryonic period, whereas in recent considerations, steroid hormones are thought to organize neuronal circuits also during puberty (Sisk and Foster, 2004). For a long time it is known that exposure to testosterone during gestation induces masculinization of the brain by aromatization to estrogens (Arnold and Gorski, 1984; MacLusky

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and Naftolin, 1981; McEwen et al., 1977). Recently, Neufang et al. (2009) found that increasing levels of circulating testosterone during puberty in boys may contribute to the emerging sex differences in the amygdala and hippocampus region during adolescence, whereas Peper et al. (2009) found grey matter changes to be associated with increasing levels of 17β-estradiol in pubertal girls.

In addition, animal studies have demonstrated that hippocampal synaptic plasticity and neurogenesis in the adult brain are dependent on testosterone and estrogens (Galea et al., 2006; MacLusky et al., 2006). However, whether circulating steroid hormones exert organizational influence on brain morphology and structure in the adult period of humans is to date rather unknown (e.g., De Vries, 2004; Stein et al., 2008), but first recent studies indicated that pharmacologically induced changes in steroid levels lead to changes in neocortical volume in young adult transsexuals (Pol et al., 2006) and after menopause in women (Boccardi et al., 2006a; Eberling et al., 2003; Lord et al., 2008).

The aim of the present study was to investigate the role of levels of circulating estrogen, progesterone, and testosterone on regional sex differences in GM volume, using VBM on structural magnetic resonance (MR) images of healthy, young adult humans. Considering not only differences between women and men in brain-related domains including specific cognitive abilities (e.g., Kimura, 1996), the prevalence of some psychiatric and neurological diseases (e.g., Kessler et al., 1993), but also exciting congruence within the sexes (De Vries, 2004), our findings might help to shed further light on the functional and structural relevance of sex steroid hormones on the human brain in adulthood.

Materials and methods

Subjects

Thirty-four healthy young subjects (17 women and 17 men; aged 26.6 ± 5 years, mean ± SD) in a narrow age range and with similar educational levels (≥12 years of education) were recruited via advertisement at the Medical University of Vienna, Austria. All subjects underwent a medical examination at the screening visit including medical history, electrocardiogram, and routine laboratory tests. Exclusion criteria were history of severe disease, any psychiatric or neurological disorder, drug abuse including anabolic steroids or psychiatric medication, use of hormonal contraceptives for the past 6 months including oral contraceptives, and a positive urine pregnancy test. Six women and eight men (41.2%) were tobacco smokers with an average of 7.08 ± 1.5 cigarettes/day in women and 7.13 ± 0.5 cigarettes/day in men (range: 2–20). VBM and hormone data acquisition and analyses were done by investigators blinded to the study question. Demographic details are given in Table 1. All subjects provided written informed consent and received reimbursement after participation. The study was approved by the Ethics Committee of the Medical University of Vienna.

Hormone measurements

Venous blood samples were collected in the morning to assess plasma levels of sex hormones. Analyses of 17β-estradiol, progesterone, testosterone, as well as luteinizing hormone (LH) to check for menstrual cycle phase in females were performed for women and men by the Clinical Institute for Medical and Chemical Laboratory Diagnostics at the Medical University of Vienna (for details of standards and references, see http://www.kimcl.at). Briefly, for quantification of sex steroid hormones we used electrochemiluminescence (ECLIA). Assays were performed using the E170 Module (Roche E170 Modular Analytical System®) as published elsewhere (Bieglmayer et al., 2004). All women were measured in the (mid-)follicular phase to avoid bias due to menstrual circle phase. In this phase, levels of progesterone and 17β-estradiol remain relatively stable at low levels until the beginning of the ovulation phase and the luteal phase, which are characterized by highly fluctuating levels of LH, progesterone, and 17β-estradiol. Adherence was monitored by follicular reference ranges of 0.5–1.0 ng/ml for progesterone, 22–215 pg/ml for 17β-estradiol, and 2.4–12.6 mU/ml for LH. Details of hormonal plasma levels are given in Table 1 (LH not shown).

Table 1
Demographic parameters and hormone plasma levels.

	Mean ± SD [min–max]	
	Women	Men
Age [years]	24.5 ± 2.4 [21.7–29.2]	28.8 ± 6.1 [21.3–35.8, 47.1]
Testosterone [μg/l]	0.53 ± 0.04	6.42 ± 0.5
17β-Estradiol [ng/l]	58.47 ± 8	30.47 ± 2
Progesterone [ng/l]	0.76 ± 0.05	0.98 ± 0.08

Magnetic resonance imaging

Structural magnetic resonance imaging was performed with a 3 Tesla whole-body MEDSPEC S300 MR-scanner (Bruker BioSpin, Ettlingen, Germany) using a magnetization-prepared rapid gradient-echo (MPRAGE, T1-weigthed) sequence (128 slices, 256 × 256 matrix, slice thickness 1.56 mm, voxel size 0.78 × 0.86 mm).

Voxel-based morphometry

Similar to previous studies by our group (e.g., Riederer et al., 2008), VBM analysis (Good et al., 2001) was performed using the Gaser toolbox (http://dbm.neuro.uni-jena.de/vbm/) with SPM 5 (The Wellcome Department of Imaging Neuroscience, University College London; www.fil.ion.ucl.ac.uk/spm/) and Matlab 7.3 (Math Works, Natick, MA). For a detailed description of VBM procedures, please see Good et al. (2001). Briefly, inhomogeneity corrected MR images were first coregistered to the MNI-template. The resulting image data was segmented into partitions of GM, WM, and CSF applying a set of proprietary tissue-priors (study-specific templates), which were created from the set of structural scans itself. The output was a set of brain GM volume maps, one for each subject. Volumetric artifacts due to normalization during the segmentation were compensated for by modulation using the Jacobian Determinant. Subsequently, grey matter segments were smoothed with an 8 mm full-width at half maximum (FWHM) Gaussian kernel and subjected to voxel-wise statistical analysis with the SPM package.

Statistical analysis

Hormone levels and total volumes of GM, WM, and CSF were assessed for normal or near-normal distribution.

Independent t-tests (two-tailed) or Mann–Whitney U-tests were calculated as appropriate to detect potential sex differences in total volumes of GM, WM, and CSF.

Statistical parametric maps were created to detect regional differences in GM volumes between women and men. Therefore, an analysis of covariance (ANCOVA) was run in SPM/MATLAB with main effect factor 'sex' (female, male) and 'total GM volume' as covariate ("nuisance variable"), to adjust for individual differences and potential sex dimorphism in GM size. Two post-hoc t-contrasts ('female>male' and 'male>female') were created to delineate significant regions. Significance was set at $p<0.05$ using false discovery rate (FDR) as correction for multiple comparisons and a cluster size of 50 voxels.

These cluster regions were used to reveal whether significant sex-different regions in GM volume were correlated with adult levels of circulating sex hormones. Therefore, regional GM volumes of these clusters were read out of the pre-processed individual images