Normal Sexual Dimorphism of the Adult Human Brain Assessed by *In Vivo* Magnetic Resonance Imaging

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The etiology and consistency of findings on normal sexual dimorphisms of the adult human brain are unresolved. In this study, we present a comprehensive evaluation of normal sexual dimorphisms of cortical and subcortical brain regions, using *in vivo* magnetic resonance imaging, in a community sample of 48 normal adults. The men and women were similar in age, education, ethnicity, socioeconomic status, general intelligence and handedness. Forty-five brain regions were assessed based on T_1-weighted three-dimensional images acquired from a 1.5 T magnet. Sexual dimorphisms of adult brain volumes were more evident in the cortex, with women having larger volumes, relative to cerebrum size, particularly in frontal and medial paralimbic cortices. Men had larger volumes, relative to cerebrum size, in frontomedial cortex, the amygdala and hypothalamus. A permutation test showed that, compared to other brain areas assessed in this study, there was greater sexual dimorphism among brain areas that are homologous with those identified in animal studies showing greater levels of sex steroid receptors during critical periods of brain development. These findings have implications for developmental studies that would directly test hypotheses about mechanisms relating sex steroid hormones to sexual dimorphisms in humans.

Introduction

A number of animal and human studies have demonstrated normal sexual dimorphisms of the brain (Allen and Gorski, 1986, 1990; MacLusky *et al.*, 1987; Witelson, 1989; Benes *et al.*, 1994; Filipek *et al.*, 1994; Kulynych *et al.*, 1994; Schlaepfer *et al.*, 1995; Witelson *et al.*, 1995; Caviness *et al.*, 1996a; Giedd *et al.*, 1996; Paus *et al.*, 1996; Harasty *et al.*, 1997; Passe *et al.*, 1997; Gur *et al.*, 1999; Highley *et al.*, 1999; Rabinowicz *et al.*, 1999). Early work in this area, primarily in rats, focused on the effects of sex steroid hormones on brain morphology during critical periods of early development [reviewed by McEwen (McEwen, 1983) and Pilgrim and Hutchison (Pilgrim and Hutchison, 1994)]. Postmortem work in humans also identified sexual dimorphisms in brain regions involved in the neural control of sexual and maternal behavior and gonadotropin secretion (Allen and Gorski, 1987, 1990; Allen *et al.*, 1989; Witelson, 1989; Highley *et al.*, 1999). With the advent of magnetic resonance imaging (MRI) to examine *in vivo* brain anatomy and increased acceptance of the idea of sex differences in the human brain, there are a growing number of *in vivo* studies on sexual dimorphisms in human adults.

In vivo imaging and postmortem studies of sexual dimorphisms in humans report that the cerebrum is larger in men than women by ~8–10% (Filipek *et al.*, 1994; Witelson *et al.*, 1995; Passe *et al.*, 1997; Rabinowicz *et al.*, 1999; Nopoulos *et al.*, 2000), a finding that is not wholly attributed to body size. However, regionally specific sex differences, relative to size of cerebrum, have been reported, and the direction of the sex effects differs depending on the brain region. These studies have reported, in women, relative to cerebrum size, greater cortical gray matter volume (Gur *et al.*, 1999), larger volumes of regions associated with language functions [e.g. Broca's area (Harasty *et al.*, 1997)] and superior temporal cortex, in particular planum temporale (Jacobs *et al.*, 1993; Schlaepfer *et al.*, 1995; Harasty *et al.*, 1997)], and larger volumes of the hippocampus (Filipek *et al.*, 1994; Giedd *et al.*, 1996; Murphy *et al.*, 1996), caudate (Filipek *et al.*, 1994; Murphy *et al.*, 1996), thalamic nuclei (Murphy *et al.*, 1996), anterior cingulate gyrus (Paus *et al.*, 1996), dorsolateral prefrontal cortex (Schlaepfer *et al.*, 1995), right inferior parietal lobe (Nopoulos *et al.*, 2000), and white matter involved in interhemispheric connectivity (Allen and Gorski, 1987; Witelson, 1989; Highley *et al.*, 1999; Nopoulos *et al.*, 2000). Cell packing density, or number of neurons per unit volume, in the planum temporale was also greater in women than men (Witelson *et al.*, 1995).

Compared to women, men have been found to have larger volumes, relative to cerebrum size, or differences in neuronal densities in other limbic and paralimbic regions [i.e. amgydala (Giedd *et al.*, 1996), hypothalamus (Swaab and Fliers, 1985; Allen *et al.*, 1989; Zhou *et al.*, 1995) and paracingulate gyrus (Paus *et al.*, 1996)], larger genu of the corpus callosum (Witelson, 1989) and overall white matter volume (Passe *et al.*, 1997; Gur *et al.*, 1999), and greater cerebrospinal fluid [lateral ventricles (Agartz *et al.*, 1992; Kaye *et al.*, 1992) or sulcal volume (Gur *et al.*, 1999)]. Some have argued that men have more neurons across the entire cortex (Pakkenberg and Gundersen, 1997; Rabinowicz *et al.*, 1999) and women, more neuropil (Jacobs *et al.*, 1993; Rabinowicz *et al.*, 1999). However, these findings are inconsistent with others (Witelson *et al.*, 1995; Harasty *et al.*, 1997), and suggest that sex differences in neuronal characteristics depend on the brain region and/or cortical layer assessed (Witelson *et al.*, 1995). Thus, the consistency and etiology of sexual dimorphisms in the human brain remain unresolved.

One potential factor involved in human sexual dimorphisms may be the effects of sex steroid hormones on brain development. However, for the most part, this has been demonstrated only in animals (McEwen, 1983; Tobet *et al.*, 1993; Pilgrim and Hutchison, 1994; Park *et al.*, 1996; Gorski, 2000). Although

there are species-specific mechanisms, there may be some that are shared, given recent work demonstrating that the spatial organization of estrogen receptors in human adults in particular brain regions was similar to homologous regions in several other mammalian species (Donahue *et al.*, 2000).

Although the relative roles of testosterone and estrogen on the sexual differentiation of the human brain are as yet unclear, most likely both will contribute.

One mechanism well-studied in animals is the role of aromatization on sexual differentiation [reviewed by Kawata (Kawata, 1995)]. During critical periods of early development, testosterone is, in part, converted to estradiol by the enzyme aromatase. Estradiol has been found to enhance neuronal density and size, maturation and migration, neurite growth and synaptogenesis (McEwen, 1983; Miranda and Toran-Allerand, 1992), and masculinize the rat brain. During early brain development in rodents, ferrets and monkeys (MacLusky *et al.*, 1987; Clark *et al.*, 1988; Miranda and Toran-Allerand, 1992; Tobet *et al.*, 1993; Park *et al.*, 1996), aromatase activity has been found in the hypothalamus and amygdala, where there is the highest concentration of sex steroid receptors, and the hippocampus, thalamic nuclei, specific cortical regions, and the corpus callosum and optic tract (MacLusky *et al.*, 1987). In animals, cortical regions show high concentrations of these receptors only during fetal and early postnatal development, which then recede postnatally (MacLusky *et al.*, 1987; Miranda and Toran-Allerand, 1992), although not completely (Clark *et al.*, 1988). Animal studies have shown a significant association between the drop in cortical estrogen receptors postnatally and levels of messenger RNA, suggesting that estradiol may modulate cortical differentiation (Miranda and Toran-Allerand, 1992; Toran-Allerand, 1996). Further, animal studies have demonstrated the relationship between differential localization of androgen and estrogen receptors during critical periods of development and brain morphology and behavior (McEwen, 1983; Sandhu *et al.*, 1986), suggesting that specific neurons may be more affected than others at the local level where aromatization takes place (Roselli and Resko, 1986; MacLusky *et al.*, 1987; Clark *et al.*, 1988; Miranda and Toran-Allerand, 1992; Pilgrim and Hutchison, 1994).

In this study, we present a comprehensive examination of sexual dimorphisms in cortical and subcortical regions of the adult human brain using *in vivo* magnetic resonance imaging. We provide a preliminary step in indirectly examining the hypothesis that sex steroid hormones may be associated with sexual dimorphisms in the human brain. We tested the hypothesis that homologous brain regions in humans, identified in animal studies to have high levels of sex steroid receptors during early brain development, would be more likely to retain sexual dimorphisms in adulthood than brain regions that have not been so identified.

Materials and Methods

Sample

The sample for this study was recruited through advertisements in the Boston area, and consisted of males (n = 27) and females (n = 21) selected to be comparable based on age, ethnicity, parental socioeconomic status (SES), reading ability and handedness (all but four were right-handed). [These normal subjects were recruited as comparison subjects for two studies of psychosis [NIMH. MH56956 (J.M.G.); MH43518 and MH46318 (M.T.T.)]. Subjects were excluded if they had a current or lifetime history of any medical illness affecting central nervous system function, current psychopathology or lifetime history of major psychiatric disorders. Evidence of significant psychopathology was indicated by any T-Scale (except Masculinity-Femininity) elevations above 70 on the short form of the Minnesota Multiphasic Personality Inventory (MMPI) (Vincent *et al.*, 1984), evidence of substance abuse within the past 6 months, history of psychosis or psychiatric hospitalizations, and family history of psychosis. However, in order to avoid the risk of selecting a 'super-normal' group, subjects were not screened for a lifetime history of psychopathology or neuropsychological dysfunction. All psychiatric evaluations were conducted by masters-level clinical interviewers with extensive diagnostic interviewing experience. All clinical material was evaluated by diagnostic experts (J.M.G., L.J.S.) who assessed whether a subject should be considered a normal comparison subject. In five previous publications on the neuropsychological status of these subjects, subjects were shown to be in the higher end of the average range for cognitive functioning in normal populations (Faraone *et al.*, 1995).

Table 1
Sociodemographic characteristics of the male and female subjects

	Females (n = 21)	Males (n = 27)
Age (mean)	36.3 ± 10.5	39.1 ± 12
Ethnicity (% Caucasian)	95	92
Handedness (% right)	81	96
Parental SES	2.5 ± 0.8	2.9 ± 1.5
Parent education	12.2 ± 2.2	12.1 ± 2.5
Education	14.9 ± 2.3	14.7 ± 2.3
Range	11–18	9–18
WRAT reading[a]	105.8 ± 12	104.6 ± 11.7
IQ estimate[b]	111 ± 15	113 ± 12
Range	83–131	89–134

There were no statistically significant differences on any of these characteristics between men and women, based on t-tests or χ^2 tests.

[a]WRAT = Wide Range Achievement Test — Revised (Jastak and Jastak, 1985).

[b]IQ estimate derived from vocabulary and block design age-scaled scores (Brooker and Cyr, 1986).

Subjects had a mean age of 39.8 years, were 93% Caucasian, with 14.4 ± 2.3 years of education, had an average IQ of 106.9 ± 12.2, and were predominantly from middle SES backgrounds. [SES was assessed using the two-factor Hollingshead and Redlich scale (Hollingshead and Redlich, 1958), a well-established rating scale based on weighting parental education and occupation into social classes I–V.] There were no statistically significant or substantively meaningful differences between the characteristics of the men and the women (see Table 1) [see also Goldstein *et al.* (Goldstein *et al.*, 1999)]. All subjects gave informed consent and were paid for their participation. All procedures were approved by the Institutional Review Boards for Human Subjects at Harvard Medical School, Massachusetts Mental Health Center, and Massachusetts General Hospital.

MRI Acquisition Parameters and Segmentation Procedures

MRI scans were acquired at the NMR Center of the Massachusetts General Hospital (MGH) with a 1.5 T General Electric Signa scanner. Contiguous 3.1 mm coronal spoiled-gradient echo images of the entire brain were obtained using the parameters: T_R = 40 ms, T_E = 8 ms, flip angle = 50°, field of view = 30 cm, matrix = 256 × 256, and averages = 1. MR images were processed and analyzed at the MGH Center for Morphometric Analysis (CMA). Images were 'positionally normalized' by imposing a standard three-dimensional coordinate system on each three-dimensional MR scan using the midpoints of the decussations of the anterior and posterior commissures, and the midsagittal plane at the level of posterior commissure, as points of reference for rotation and (nondeformation) transformation (Filipek *et al.*, 1994; Caviness *et al.*, 1996b). Positional normalization overcomes potential problems caused by variation in head position across subjects during scanning. Scans were then resliced into the 3.1 mm coronal scans.

Each slice of the T_1-weighted, positionally normalized three-dimensional coronal scans was segmented into gray and white matter and ventricular structures using a semi-automated intensity contour mapping algorithm and signal intensity histogram distributions. This technique, described in detail elsewhere (Rademacher *et al.*, 1992; Filipek *et al.*, 1994; Caviness *et al.*, 1996b; Goldstein *et al.*, 1999) yields separate

compartments of neocortex, subcortical gray nuclei, white matter and ventricular system subdivisions, generally corresponding to the natural tissue boundaries distinguished by signal intensities in the T_1-weighted images. The neocortex, defined by the gray–white matter segmentation procedure, was subdivided or 'parcellated' into bilateral parcellation units, based on the system originally described by Caviness *et al.* (Caviness *et al.*, 1996b) and applied by Goldstein *et al.* (Goldstein *et al.*, 1999) on a subsample of the subjects reported here. This is a comprehensive system for neocortical subdivision, designed to approximate architectonic and functional subdivisions, and based on specific topographical anatomic landmarks present in virtually all brains [see original studies for details on the anatomic definitions (Rademacher *et al.*, 1992; Caviness *et al.*, 1996b)].

Segmentation and cortical parcellation are conducted by extensively trained, BA-level MR technicians who have had some college-level background in neuroanatomy or behavioral neuroscience. They are trained and supervised on these procedures on an ongoing basis by our neuroanatomist (N.M.). MR technicians are blind to any sociodemographic or clinical characteristics of the subjects, including their sex. Very good reliability of the cortical and subcortical regions has been established in several previous studies, including for the sample presented in this study (Caviness *et al.*, 1996b; Goldstein *et al.*, 1999; Seidman *et al.*, 1999). Volumes, measured in cm^3, were calculated for each brain region by multiplying the slice thickness by the area measurement of the region on each slice, and then summing over all slices on which the region appeared.

Data Analytic Approach

Sex differences in volumes of brain regions were tested using proportional volumes, relative to cerebrum size. This approach is consistent with methods used by other imaging studies (Filipek *et al.*, 1994), and is necessary to compare men and women, given that men tend to have larger cerebrums than women. Total volumes of brain regions were analyzed for the hypothesis presented here. Effect sizes were calculated based on the adjusted mean female brain volume minus the adjusted mean male brain volume, divided by the pooled standard deviation of male and female volumes. (Analyses of covariance, controlling for cerebrum size, were also conducted to ensure that results were consistent across methods.)

We hypothesized that one potential reason for sexual dimorphisms across brain regions may be related to the impact of sex steroid hormones during brain development. In an indirect test of this association, we *a priori* divided the 45 brain regions into two groups. One group consisted of 30 homologous brain regions in humans, that were identified in rat, ferret and monkey studies to have a high density of estrogen and androgen receptors during early development (Pfaff and Keiner, 1973; MacLusky *et al.*, 1987; Clark *et al.*, 1988; Sibug *et al.*, 1991). The other group consisted of 15 other brain regions, for which a developmentally high concentration of sex steroid receptors has not been identified in the animal literature. The groups were independently created by an expert neuroanatomist (N.M.) and the principal author (J.M.G.) to ensure reliability. The 'high receptor-density' group included: superior and middle frontal gyri, frontomedial and frontoorbital cortices (MacLusky *et al.*, 1987; Clark *et al.*, 1988; Simerly *et al.*, 1990; Kolb and Stewart, 1991); basal forebrain (Pfaff and Keiner, 1973); primary motor cortex (MacLusky *et al.*, 1987; Simerly *et al.*, 1990) (precentral gyrus); supplementary motor cortex (Clark *et al.*, 1988); anterior, posterior and paracingulate gyri (Pfaff and Keiner, 1973; Clark *et al.*, 1988; Shughrue *et al.*, 1990; Kolb and Stewart, 1991; Sibug *et al.*, 1991); agranular insular cortex (Kolb and Stewart, 1991; Sibug *et al.*, 1991) (insula); parahippocampal gyrus (Clark *et al.*, 1988; Sibug *et al.*, 1991); posterior parietal cortex (MacLusky *et al.*, 1987; Clark *et al.*, 1988) (angular and supramarginal gyri); primary somatosensory (MacLusky *et al.*, 1987; Clark *et al.*, 1988; Simerly *et al.*, 1990) (postcentral gyrus); primary visual cortex (MacLusky *et al.*, 1987; Clark *et al.*, 1988) (lingual gyrus, occipital pole and superior calcarine sulcus); primary auditory cortex (Simerly *et al.*, 1990; Yokosuka *et al.*, 1995) (Heschl's gyrus); and subcortical regions: amygdala (Clark *et al.*, 1988; Simerly *et al.*, 1990), hypothalamus (Pfaff and Keiner, 1973; MacLusky *et al.*, 1987; Clark *et al.*, 1988; Simerly *et al.*, 1990; Tobet *et al.*, 1993; Park *et al.*, 1996); hippocampus (MacLusky *et al.*, 1987); thalamic nuclei (Pfaff and Keiner, 1973; Simerly *et al.*, 1990), the nucleus accumbens (Pfaff and Keiner, 1973), and the caudate, putamen, globus pallidum (Sibug *et al.*, 1991).

In order to conduct a two-group comparison across multiple brain regions, a normalized summary measure, the absolute value of the *t*-statistic, was calculated for each brain area, estimating the mean magnitude of difference in proportions between female and male subjects (see *t*-statistics in Table 2). For each of the areas, the critical values for the *t*-statistics were 2.01 at the $\alpha = 0.05$ level and 1.68 at the $\alpha = 0.10$ level. A permutation test (Good, 1994) was conducted to examine whether the distribution of these 45 standardized scores (*t*-statistics) significantly differed based on the dichotomous grouping by developmental level of estrogen and androgen receptor-concentration. Specifically, a difference in the means of the absolute values of the *t*-statistics was calculated using the observed 45 scores. The magnitude of this difference was compared to 20 000 iterations in which the brain regions were randomly regrouped. Under the null hypothesis that there is no relation between sexual brain dimorphism and developmental estrogen and androgen receptor-concentration level, the observed difference in the means of the absolute values of the *t*-statistics is not expected to be extreme when compared to the permutation distribution.

Results

Consistent with many previous studies (Filipek *et al.*, 1994; Witelson *et al.*, 1995; Passe *et al.*, 1997; Rabinowicz *et al.*, 1999), adult men had significantly larger cerebrums than women, unadjusted for whole brain size ($P = 0.001$; see Table 2). Men also had larger cerebrums relative to whole brain size, but this was not significantly different from the relative size of the cerebrum in women. Men had significantly larger lateral ($P = 0.03$) and third ($P = 0.01$) ventricular volumes, relative to cerebrum size, than women, and a larger proportion of overall white matter volume, relative to cerebrum size ($P = 0.08$). Women had a significantly larger proportion of overall cortical volume, relative to cerebrum size, than men ($P = 0.04$; see Table 2).

The permutation test (Good, 1994), which compares regions that, according to the animal literature, have developmentally high levels of estrogen and androgen receptors with other regions that do not, showed that only 120 of 20 000 iterations yielded a more extreme value than the observed data ($P = 0.006$, SE $P = 0.00055$, 95% CI = 0.0049, 0.0071). Thus, there was a significantly greater magnitude of adult sexual dimorphism among the group of brain areas with developmentally high levels of sex steroid hormone receptors than among the other regions. We investigated whether size of area accounted for the results by plotting the *t*-values and absolute *t*-values by size of region. There was no evidence that size of region accounted for the association between level of receptor density and magnitude of sexual dimorphism.

Region-specific sex differences can be seen in Table 2, in which brain regions were rank-ordered by effect size (ES), i.e. from the largest positive ES, which represented women with larger relative volumes than men, to the largest negative ES, which represented men with larger relative volumes than women. As seen in Table 2, represented as positive ESs, women had larger cortical volumes, relative to cerebrum size, than men in the majority of the frontal and medial paralimbic brain regions. Significantly larger volumes in women than men ($P < 0.05$) were seen in the precentral gyrus, frontoorbital cortex, superior frontal and lingual gyri. Significance levels for sexual dimorphisms ($P \leq 0.10$) were in the middle frontal, cingulate and posterior supramarginal gyri. Represented in Table 2 as large negative ESs, men had larger volumes, relative to cerebrum size, in frontomedial cortex and the hypothalamus ($P = 0.11$) and the amygdala and angular gyrus. Figure 1 illustrates that the brain regions with the largest positive and negative sexual dimorphism

Table 2
Brain volumes (in cm^3), unadjusted and adjusted for cerebrum size, in normal men and women: effect sizes comparing volumes in women versus men

	Brain regions (approximate Brodmann's areas)	Unadjusted volumes (cm^3)				Adjusted volumes (cm^3)				*t*	Effect size
		Female ($n = 21$)		Male ($n = 27$)		Female ($n = 21$)		Male ($n = 27$)			
		Mean	SD	Mean	SD	Mean	SD	Mean	SD		
	Total cerebrum volume[a]	1021.8	89.5	1113.1	92.5	86.8	0.9	87.0	0.8	–3.4	–0.9
	Total cortex volume	548.8	54.8	580.1	52.5	53.7	2.4	52.2	2.6	2.2	0.6
	Total cerebral white matter	405.4	44.5	456.6	52.4	39.7	2.4	41.0	2.6	–1.8	–0.5
	Lateral ventricles	13.3	4.2	17.9	6.0	1.3	0.4	1.6	0.6	–2.3	–0.6
	Third ventricle	0.7	0.2	1.0	0.3	0.1	0.02	0.1	0.03	–3.0	–0.8
	Fourth ventricle	1.7	0.7	1.8	0.5	0.1	0.1	0.1	0.1	0.1	–0.04
☐	precentral g. (6, 4)	35.6	4.5	35.2	4.4	3.5	0.4	3.2	0.4	3.2	0.9
☐	frontoorbital cortex (47)	13.8	2.4	13.4	2.1	1.4	0.2	1.2	0.2	2.6	0.7
☐	sup. frontal g. (6, 8, 9)	25.7	3.3	25.7	4.2	2.5	0.3	2.3	0.3	2.2	0.6
☐	lingual g. (17, 18)	14.3	2.8	13.7	2.7	1.4	0.3	1.2	0.3	2.1	0.6
☐	a. cingulate g. (33, 24)	12.4	3.0	12.1	2.1	1.2	0.3	1.1	0.2	1.9	0.5
☐	p. cingulate g. (23, 31, 26, 29, 30)	11.2	2.0	11.2	1.9	1.1	0.2	1.0	0.2	1.8	0.5
☐	p. supramarginal g. (p. 40)	11.1	3.8	10.2	3.3	1.1	0.4	0.9	0.3	1.8	0.5
☐	mid. frontal g. (6, 8, 9, 46)	23.8	4.5	24.4	4.3	2.3	0.4	2.2	0.3	1.6	0.5
☐	frontal operculum (45, 44)	3.1	0.4	3.1	0.7	0.3	0.1	0.3	0.1	1.5	0.4
☐	caudate	6.4	0.9	6.6	1.2	0.6	0.1	0.6	0.1	1.5	0.4
●	sup. occipital lateral gyri (18, 19)	36.5	7.4	36.6	8.0	3.6	0.5	3.3	0.7	1.3	0.4
☐	sup. calcarine sulcus	2.8	1.1	2.7	0.9	0.3	0.1	0.2	0.1	1.2	0.3
●	planum polare (a. 22)	3.2	0.6	3.3	0.9	0.3	0.1	0.3	0.1	1.2	0.3
☐	hippocampus	7.9	0.6	8.3	0.9	0.8	0.1	0.8	0.1	1.1	0.3
☐	putamen	9.4	1.3	9.9	1.2	0.9	0.1	0.9	0.1	0.9	0.3
☐	basal forebrain	2.8	0.7	2.8	0.9	0.3	0.1	0.3	0.1	0.9	0.3
☐	insula (13, 14, 15, 16)	14.1	1.8	15.1	1.7	1.4	0.1	1.4	0.1	0.8	0.2
●	central operculum (43)	7.7	1.1	8.1	1.4	0.8	0.1	0.7	0.1	0.8	0.2
☐	p. parahippocampal g. (27, 35)	3.1	0.8	3.2	0.7	0.3	0.1	0.3	0.1	0.8	0.2
☐	nucleus accumbens	1.2	0.2	1.2	0.2	0.1	0.02	0.1	0.02	0.7	0.2
☐	a. parahippocampal g. (28, 34)	5.5	1.1	5.8	1.3	0.5	0.1	0.5	0.1	0.6	0.2
☐	thalamus	13.5	1.3	14.7	1.7	1.3	0.1	1.3	0.1	0.4	0.1
☐	Heschl's gyrus	2.8	0.7	3.0	0.7	0.3	0.1	0.3	0.1	0.3	0.1
☐	suppl. motor cortex (Medial 6)	5.8	1.7	6.2	1.6	0.6	0.2	0.6	0.1	0.2	0.1
●	sup. parietal lobule (5, 7)	11.0	2.0	11.9	3.1	1.1	0.2	1.1	0.3	0.2	0.1
☐	paracingulate cortex (32)	11.8	1.8	12.8	2.0	1.2	0.1	1.2	0.1	0.2	0.1
●	inf. occipital lateral gyri (18, 19)	17.1	4.3	18.2	4.0	1.7	0.3	1.6	0.4	0.2	0.01
●	temporal pole (38)	18.0	3.0	19.5	3.0	1.8	0.3	1.8	0.3	0.02	0.01
●	temporooccipital fusiform g. (37)	6.3	1.6	6.9	1.4	0.6	0.2	0.6	0.1	–0.04	–0.01
●	frontal pole (10, 11)	54.8	9.7	59.7	10.4	5.4	0.7	5.4	0.8	–0.1	–0.01
☐	pallidum	3.4	0.4	3.7	0.6	0.3	0.03	0.3	0.04	–0.1	–0.04
●	mid. temporal g. (22)	14.5	2.5	15.9	2.6	1.4	0.2	1.4	0.2	–0.3	–0.1
☐	a. supramarginal g. (a. 40)	7.5	3.2	8.4	2.4	0.7	0.3	0.8	0.2	–0.3	–0.1
☐	postcentral g. (3a, 3b, 1, 2, (5))	25.9	3.1	28.4	3.6	2.5	0.3	2.6	0.4	–0.4	–0.1
●	precuneus (medial 7)	20.7	3.5	22.9	3.4	2.0	0.3	2.1	0.3	–0.4	–0.1
●	sup. temporal g. (22)	10.6	1.6	11.8	1.8	1.0	0.1	1.1	0.1	–0.7	–0.2
☐	occipital pole (17, 18)	18.0	6.0	21.3	6.3	1.8	0.6	1.9	0.5	–0.8	–0.2
☐	amygdala	3.8	0.5	4.3	0.7	0.4	0.1	0.4	0.1	–0.9	–0.3
●	temporal fusiform g. (36, 20)	10.0	1.6	11.3	2.1	1.0	0.1	1.0	0.2	–0.9	–0.3
●	inf. temporal g. (20)	11.0	2.7	12.5	2.5	1.1	0.2	1.1	0.2	–0.9	–0.3
●	subcallosal cortex (25, p. 32)	4.2	1.0	4.9	0.8	0.4	0.1	0.4	0.1	–1.0	–0.3
☐	angular g. (39)	10.3	3.5	12.9	4.4	1.0	0.4	1.2	0.4	–1.4	–0.4
☐	hypothalamus	0.8	0.2	0.9	0.1	0.1	0.02	0.1	0.01	–1.5	–0.4
☐	frontomedial cortex (11, 12)	4.2	1.1	5.0	1.0	0.4	0.1	0.5	0.1	–1.5	–0.4

Permutation test (20 000 iterations): $p = 0.006$, SE (P) = 0.00055 (95% CI 0.0049, 0.0071).

Effect size = (adjusted mean female brain volume – adjusted mean male brain volume)/pooled SD of male and female volumes; brain volumes in cm^3, adjusted for total cerebral volume.

a. = anterior; p.= posterior; inf. = inferior; mid. = middle; sup. = superior; suppl. = supplementary; g. = gyrus.

☐ = Developmentally high density estrogen and androgen receptor region; ● = developmentally low estrogen and androgen receptor region.

[a]Effect size for total cerebrum is based on volumes, adjusted for whole brain volume.

effect sizes, seen in Table 2, fell into the group of regions designated as having developmentally high levels of sex steroid receptors.

Discussion

Findings from this study replicate that normal men have larger cerebrums than women, but also show that there are region-specific sex differences in adult brain volumes, relative to cerebrum size, particularly in the cortex. That is, sexual dimorphisms of adult brain volumes are not diffusely spread across the brain. We raised the hypothesis that these region-specific sex differences in the adult brain may be related to factors affecting *in utero* and early postnatal sexual differentiation of the brain (McEwen, 1983; MacLusky *et al.*, 1987; Pilgrim and Hutchison, 1994). Independent of our work, this was recently suggested in

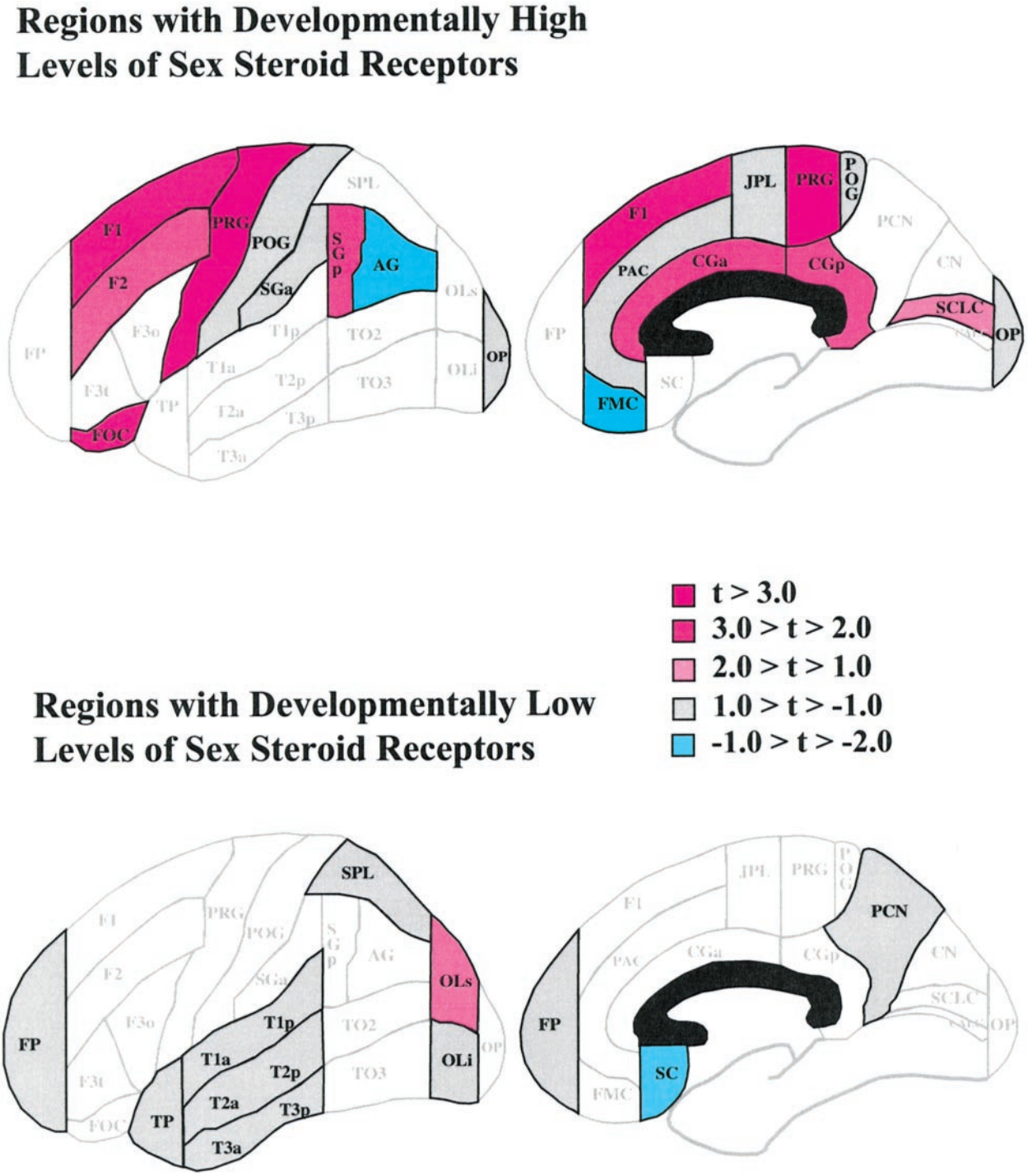

Figure 1. Cortical regions of sexually dimorphic volumes. The figure represents the cortical parcellation units hypothesized to have developmentally high and low estrogen receptor expression in the upper and lower portions of the figure respectively. Each shaded region is a member of the respective set of corresponding regions identified from the animal literature as having either a high or low density of estrogen receptors. Within each of these sets of regions, those shaded gray demonstrated minimal volumetric dimorphism between the genders. Regions shaded red demonstrated some evidence for female greater than male volumetric dimorphism; and regions shaded blue demonstrated some evidence for male greater than female volumetric dimorphism. The degree of color shading is scaled by the *t*-value of the between group analyses. As can be seen, the predominant regions that were sexually dimorphic were found in the regions with high developmental estrogen receptor density. Of the dimorphic regions in the cerebrum, the majority demonstrated female greater than male volumetric dimorphism, and are seen to be concentrated in frontal areas, except for frontomedial cortex. (See Table 2 for the complete list of volumetric comparisons.) Subcortical regions of interest in Table 2 are not shown in this figure. The greatest effect size among the subcortical regions was male greater than female volume of the hypothalamus and female greater than male volume of the caudate.

a study that found sex differences in the distribution of androgen receptors in the adult human hypothalamus (Fernández-Guasti *et al.*, 2000). In a preliminary attempt to indirectly examine this hypothesis, we found that cerebral regions implicated in early sexual differentiation, in several mammalian species, were significantly more likely to retain sexual dimorphisms of adult human cerebral volumes than brain regions that, according to the animal literature, do not have a high density of sex steroid receptors early in development.

Although in our study, the locations of sex steroid receptors were extrapolated from animal studies, there are only a few studies in humans, of which we are aware, that have reported mapping estrogen and androgen receptors in the brain (Rance *et al.*, 1990; Sarrieau *et al.*, 1990; Puy *et al.*, 1995; Donahue *et al.*, 2000; Fernández-Guasti *et al.*, 2000). The studies were in human adults, i.e. not during development, although there was one study of brain tissue from five adolescent epileptic patients (Puy *et al.*, 1995). Further, the only cortical regions examined in these studies were temporal cortex (Sarrieau *et al.*, 1990; Puy *et al.*, 1995) and the basal forebrain (Donahue *et al.*, 2000). Finally, the most recent study (Donahue *et al.*, 2000) demonstrated that the spatial organization of estrogen receptors in human adults in the hypothalamus, basal forebrain, basal ganglia and amygdala were similar to homologous regions in several other mammalian species (Donahue *et al.*, 2000). This suggested that there are some similarities across mammalian species in the location of sex steroid receptors, even though animal studies have reported differences across species as well.

The interpretation that our findings implicate fetal and early postnatal factors is underscored by the significant specific cortical sexual dimorphisms, since the density of cortical gonadal receptors recedes dramatically after early postnatal development, as demonstrated in rats and monkeys (McEwen, 1983; MacLusky *et al.*, 1987; Clark *et al.*, 1988; Toran-Allerand, 1996). Further, early postnatal effects in rats and monkeys are analogous to fetal timing in humans. Finally, we know from previous animal studies that during early critical periods of brain development, the effects of sex steroid hormones can potentially be irreversible (Pilgrim and Hutchison, 1994; Gorski, 2000). This was suggested in a recent study of Turner's syndrome women, i.e. women with chromosomal (XO) and hormonal abnormalities that affect early brain development (Murphy *et al.*, 1993). *In vivo* brain imaging studies of these women in adulthood demonstrated genetic and early hormonal effects on adult temporo-parietal and hippocampal volumes respectively (Murphy *et al.*, 1993). Although our study presents a more comprehensive examination of the cerebrum than previously reported, the validity of our findings is underscored by their consistency, in part, with previous *in vivo* human imaging studies that found similar normal sexual dimorphisms using different methods to assess brain volumes [e.g. cingulate gyrus (Paus *et al.*, 1996) middle frontal gyrus (Schlaepfer *et al.*, 1995), caudate (Filipek *et al.*, 1994) and overall cortical gray matter (Gur *et al.*, 1999)]. Further, sexual dimorphisms found in this study did not appear to be explained by size of region, functional type of cortical tissue (e.g. unimodal–heteromodal) or cortical–subcortical divisions.

There are a number of study limitations that raise questions about the interpretation of our results. First, we are making inferences about associations between early developmental factors and adult brain outcomes 40 years later. There are many changes that affect the emergence of adult sexual dimorphisms that are unaccounted for here. These include circulating androgens in adulthood, as indicated by recent work demonstrating morphometric changes in specific amygdaloid nuclei in the adult rat that were wholly controlled by circulating androgens (Cooke *et al.*, 1999; McEwen, 1999), and hormonal actions affecting structural plasticity of the adult brain during life experience (McEwen, 1999). Second, morphometric analyses of MR images are only an approximation of the architectonically defined brain regions evaluated in animal studies. Thus, further work is required to demonstrate the translation from animal to human brain areas. Third, this study was not a study of developmental mechanisms, which would be necessary in order to actually test the hypothesis that hormonal activity early in development is associated with adult human brain volumes. Nevertheless, we do find region-specific, volumetric sexual dimorphisms of the adult human brain. Further, we suggest, in a preliminary step, that they may be, in part, associated with sex steroid activity early in development.

There is precedence for this idea suggested by the animal literature. For example, animal studies have demonstrated that aromatase activity is one of the primary causes of sexual differentiation (Shughrue *et al.*, 1990; Pilgrim and Hutchison, 1994; Kawata, 1995). Aromatase activity is due to epigenetic hormonal factors, e.g. secretion of testicular testosterone, and sex-specific genetic programs affecting early brain development (Beyer *et al.*, 1993, 1994) [reviewed by Kawata (Kawata, 1995)], that are enhanced or modified by gonadal steroids later in development. These latter studies, as well as others (Tobet *et al.*, 1993; Park *et al.*, 1996), suggest that dimorphic determination may, in part, begin during neurogenesis and/or migration, which may have important implications for understanding the determinants of the postmigratory neuronal effects of sex steroid receptor activity (Tobet *et al.*, 1993; Park *et al.*, 1996). In addition, other developmental mechanisms responsible for sexual differentiation may include direct effects of testosterone, differential apoptotic cell death – which has been found to be, in part, regulated by androgens – and 'activational effects' of circulating hormones, occurring later in development, e.g. during puberty, which can potentiate neural circuits laid down during early development (Pilgrim and Hutchison, 1994; Kawata, 1995).

Animal studies have demonstrated a sex difference in the density of estrogen or androgen receptors in different brain regions [reviewed by Kawata (Kawata, 1995)]. However, sex differences in morphology may not be accounted for by differential densities of receptors, since others have shown similar availability of these receptors (Simerly *et al.*, 1990; Sibug *et al.*, 1991), but sex differences in the level of aromatase enzymes, the structure of the aromatase-containing neurons, or the level of proteins such as α-fetoprotein that may 'protect' the female brain from the masculinizing effects of aromatization (Shughrue *et al.*, 1990; Shinoda *et al.*, 1993, 1994; Pilgrim and Hutchison, 1994; Kawata, 1995). The latter studies suggest that the potential for sexual dimorphisms may be the same in males and females, and determined more by factors affecting enzymatic activity. In addition, the co-localization of gonadal receptors with neurotransmitters, such as the monoamines (Canick *et al.*, 1987; Reisert *et al.*, 1990; Beyer *et al.*, 1991; Stewart *et al.*, 1991) and γ-aminobutyric acid (GABA) (O'Connor *et al.*, 1988; Tobet *et al.*, 1999), and growth factors, such as insulin and nerve growth factor (Kawata, 1995; Toran-Allerand, 1996), may mediate the relationship between receptor density and dimorphism. These findings in animals raise hypotheses about potential mechanisms to test in human studies.

Although our findings do not provide a test of a developmental

mechanism, they have implications for testing hypotheses about the timing of sexual dimorphisms in human brain development, which can lead to hypotheses about developmental mechanisms. The findings may also have implications for understanding sex differences in particular cognitive domains (Goldman *et al.*, 1974; Collaer and Hines, 1995), since studies have demonstrated that early exposure to gonadal hormones affects brain morphology and cognition (Murphy *et al.*, 1993; Collaer and Hines, 1995; Wilson, 1999). Normal population studies have identified small, but significant, sex differences in aspects of verbal fluency, perceptual speed, olfaction and visual–spatial skills (Collaer and Hines, 1995; Toomey and Goldstein, 2000). Our findings regarding sexual dimorphisms in prefrontal regions (e.g. middle, inferior and orbital prefrontal), and posterior parietal and occipital cortices may contribute to explaining some of these effects. In fact, human and animal studies have demonstrated significant associations between sex differences in brain morphology and specific cognitive domains, such as verbal and visual–spatial skills (Goldman *et al.*, 1974; Andreasen *et al.*, 1993; Raz *et al.*, 1998; Gur *et al.*, 1999). Thus, our findings may have important implications for understanding sex differences in brain and behavioral abnormalities in neurodevelopmental disorders with fetal origins.

Notes

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