Preface

Understanding sex differences in the brain, and their relation to human behavior, has a wide range of implications, from the most fundamental issues in human biology such as the mechanisms behind sexual identity and orientation, to the practical guidelines when diagnosing and treating patients suffering from brain disorders.

The debate about biological sex differences has historically been centered around reproduction, and mainly shaped by Darwin's sexual selection theory. However, simply reflecting on the definition of sex and considering that the chromosomal, gonadal, and own sex perception is not always congruent in humans make it clear that any scientific discussion about sex differences in humans must take the brain into account. Furthermore, with increased knowledge about the linkage between behavior and cerebral networks, and with the increased awareness of the disproportional sex distribution in major neuropsychiatric disorders, it is becoming increasingly obvious that studies of sex differences need to focus on the biology and sex differences of the brain. Collected epidemiological data suggest that men and women have different vulnerability for ADHD, obsessive-compulsive disorders, anxiety and depression, autism, Alzheimer's dementia, and several other behavioral disorders. The pathophysiology of these conditions is only partly recognized. A deep understanding of the mechanisms behind the observed sex difference offers opportunities to acquire entirely new information about the etiology, disease expression, and also the potential treatment of these disorders. Furthermore, it has been shown that behavioral and physiological responses to stress, a well-known trigger and enhancer of brain disorders, differ between men and women. Thus, even in the absence of sex differences in the pathophysiology of a brain disease, the response of incapacitated brain to the environmental stress stimuli may differ between men and women.

The aim of this book is to broadcast how the advances in molecular neuroscience, brain imaging methodology, and genetics coalesce to show that sex differences exist in the brain, and that these differences have important implications for our understanding of human physiology and disease processes. The first two parts focus on the major features of functional and structural dimorphism in the brain and provide information about the underlying mechanisms (the latter are also briefly mentioned in several separate chapters to facilitate the comprehension). The third part provides selected examples of the clinical implications of this dimorphism. Neuro-inflammatory diseases are not included, as a more profound understanding of sex differences in immunology requires detailed discussions about the basic immunological mechanisms, which is beyond the scope of the present initiative. Also, sex differences in psychiatric disorders are discussed more in general terms with focus on affective symptoms (Chapter 10 by Legato), as they constitute an extensive field requiring its own proceedings.

Sex differences in cerebral anatomy and function

Differences between men and women exist in structural volumes, partitions of gray and white matter, and cerebral organization, and have been documented by several converging lines of evidence. With the rapid

development of magnetic resonance (MR) methodology, it is now possible to acquire data for analyses of cortical thickness, tissue partitions, structural volumes, functional and structural connections, and regional concentrations of certain metabolites during only 120 min. This allows conclusions about important relationships between cerebral anatomy, neurochemistry, and function at both individual and group levels. Increase in strength of the magnetic field and advances in algorithms for tissue segmentation and brain normalization have improved the effective spatial resolution in MR measurements, permitting confident conclusions about sex differences to the level of small subcortical nuclei. Power problems, which hampered some of the earlier studies, are met by pooling scans from different centers, yielding validity to the published results. As illustrated in Chapter 1 by Luders and Toga in this book, reproducible morphometric studies show sex differences in several limbic structures, as well as in the superior temporal gyrus and the inferior parietal lobe. Emerging data suggest that sex differences may be even more pronounced in neuronal connections than in the structural anatomy, and Chapter 3 by Cahill shows that they have a functional relevance. Intriguing is also that although men and women perform equally well in certain tasks, they seem to engage entirely different neuronal networks. This has direct implications in the event of cerebral lesion, as the same lesion, consequently, may lead to different functional impairments in men and women and, thus, will require different training approaches. Cerebral injury is discussed in Chapter 12.

Important information from pooling databases shows that sex differences in cerebral anatomy vary with age (Chapter 2 by Paus). Of particular interest are findings from comparative analyses of pre-pubertal children and adults, which show sex-differentiated maturation patterns. Comparative investigations of children and adults, and longitudinal investigations of same cohorts may, thus, help identify the relative effect of post-natal exposures to gonadal hormones vis-à-vis a given sexual dimorphism. Such studies have a great conceptual impact for understanding of mechanisms behind sex differences in brain and behavior.

Hormonal versus genomic influence

The scientific literature about mechanisms underlying cerebral dimorphism in the brain has hitherto very much been dominated by the dogma that the prenatal exposure to androgens (testosterone and dihydrotestosterone) has the organizational influence on sexual differentiation of the brain, while both androgens and ovarian hormones (estrogen and progesterone) begin to exert their activational effects during puberty. This doctrine is based on early animal experiments by Phoenix et al., showing that injection of testosterone propionate in pregnant guinea pigs permanently differentiates the mating behavior of the offspring. Phoenix' organizational–activational theory survives as a central concept that explains many sex differences in phenotype. As discussed by Savic et al. (Chapter 4), it also fits well with the phenomenon gender dysphoria, as sexual differentiation of brain and gonads occurs during different periods of fetal development and may in some cases be disparate. The postulated testosterone effects on sexual differentiation of the brain are, however, difficult to test in humans, other than by investigations of so-called experiments of nature. While behavioral studies of congenital adrenal hyperplasia (CAH), (a CYP-21 hydroxylase gene mutation rendering female fetuses exposed to high concentrations of testosterone) support the fetal testosterone theory, emerging brain-imaging data suggest that CAH women, at least in the hitherto measured aspect/ADD/and investigated populations, have a sex typical cerebral anatomy and connections. Furthermore, over the last two decades sex differences have been found that are not explained by gonadal hormonal effects, but rather by the primary action of genes encoded on the sex chromosomes. As explained by Villain (Chapter 5) in this book, several genes show a sex-differentiated expression in the human brain before the development of gonads and production of androgenes. These

more recently discovered sex chromosome effects offer entirely new insights in the mechanisms of diseases with skewed sex distribution, as illustrated by Baron-Cohen in the discussion of autism (Chapter 11).

Brain plasticity and epigenetics

The view on adulthood as an extended period in which hormones act on a relatively unchanging, sexually differentiated neural substrate has traditionally evoked animated discussions and frequently been misinterpreted as deterministic and obstructive of the efforts to counteract social gender inequality. Findings from MRI studies, however, demonstrate experience-induced changes in the brain structure of healthy adults, induced by several weeks of specific sensory, motor, or cognitive stimulation. Although the underlying neurobiology of such experience-induced structural changes is unknown, these findings imply that the presence of a particular sexual dimorphism in the adult brain may not necessarily reflect "organizational" effects of gonadal hormones or genes, but may also be influenced by adult experiences, whether related to physiological (e.g., hormones) or psychosocial (e.g., cultural) factors. Furthermore, recent advances in epigenetics show that gene expression is sexually dimorphic during brain development, adult life, as well as aging, and that this dimorphism is orchestrated by the interplay between genetic, hormonal, and environmental influences. Chapter 6 by Qureshi and Mahler shows how epigenetic mechanisms such as DNA methylation, histone modifications, and chromatin remodeling, and non-coding RNAs (ncRNAs) are responsible for promoting sexual dimorphism in the brain. Epigenetic mechanisms also include the transgenerational programming that occurs in response to dietary influences and stress. These new and intriguing data may eventually shift the current paradigm about sexual differentiation of the brain. They will certainly also provide a more integrated view on the impact of sex chromosomes and hormones, as steroid hormones exert powerful effects on gene expression. Their action deserves special attention.

Common steriods and their derivatives are neuroactive (such as progesterone, dihydroprogesterone, and tetrahydroprogesterone, testosterone, dihydrotestosterone, and 5α-androstane-3α,17β-diol (3α-diol), dehydroepiandrosterone, and estrogen). They may be considered as neuroprotective agents in central and peripheral nervous system, as shown in experimental models of Alzheimer's disease, Parkinson's disease, multiple sclerosis, traumatic brain injury, stroke, autism, schizophrenia, and mood disorders. Furthermore, as described by Reddy in Chapter 8, some of them have interesting pharmacological effects (through action on the GABA receptor), which are of great clinical interest and need to be further explored.

In conclusion, the collective information from this book shows that although a concerted and multidisciplinary approach on sex and the brain is only in its beginning, there is undoubtedly a great potential to generate novel and better treatment strategies (see Chapter 9 by Mogil and Bailey), as well as an improved understanding of the fundaments of human biology.

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SECTION I

General Overview

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CHAPTER 1

Sex differences in brain anatomy

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Abstract: Over the past decades scientific studies have revealed a number of striking sex differences in the human brain. This chapter highlights some of the most important discoveries with particular emphasis on macro-anatomical observations based on magnetic resonance imaging (MRI) data. Cross-references to animal studies and to *post mortem* analyses, as well as an overview with respect to micro-anatomical findings, are provided. The chapter concludes with a discussion of possible determinants of sex differences in brain anatomy. The main goal of this chapter is to exemplify the variety of findings and to demonstrate how the presence, magnitude, and direction of observed sex differences strongly depend on a number of factors including (but not limited to) the following: the brain structure examined (cerebral cortex, corpus callosum, etc.), the specific brain feature assessed (cortical thickness, cortical convolution, etc.), the degree of regional specificity (global gray matter volume, voxel-wise gray matter volume, etc.), and whether measurements are adjusted for individual brain size or not.

Keywords: Brain; Cortex; Gender; MRI; Sex

Sex differences in brain macro-anatomy

The most consistent macroscopic observation is a larger brain volume and brain weight in men compared to women (Cosgrove et al., 2007), which is only partly accounted for by larger body dimensions in men (Ankney, 1992; Peters, 1991). Other sex differences have been observed with respect to the dimensions of cortical and sub-cortical regions. For example, the planum temporale and Sylvian fissure were found to be larger and longer in males compared to females (Harasty et al., 1997; Kulynych et al., 1994; Leonard et al., 2008; Witelson and Kigar, 1992). In contrast, the volumes of the superior temporal cortex, Broca's area, the hippocampus, and the caudate (expressed as a proportion of total brain volume) were significantly larger in females (Filipek et al., 1994; Harasty et al., 1997). The mid-sagittal areas and fiber numbers of the anterior commissure (connecting the temporal lobes) as well as the massa intermedia (connecting the thalami) were larger in women than in men, where the massa intermedia was also more often absent in males than in females (Allen and Gorski, 1991;

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Highley et al., 1999). Additional sex differences were reported with respect to the bifurcation patterns of the ascending and descending ramus of the Sylvian fissure: men showed more frequently a pattern where the ascending ramus is larger than the descending one, while in women both rami were of approximately equal size (Ide et al., 1996).

Some brain structures in particular have attracted considerable attention over the past decades. These structures include (1) the corpus callosum, (2) the brain tissue compartments, and (3) the cerebral cortex. Thus, they are subsequently discussed in more detail. The difference between the left and right hemispheres constitutes an additional feature of substantial interest and is commented on as (4) brain asymmetry.

The corpus callosum

The corpus callosum is the largest fiber tract in the human brain. It connects the two hemispheres through more than 200 million fibers and allows for an inter-hemispheric transfer of information (Aboitiz et al., 1992). Unprecedented findings in the early 1980s indicated a larger and more bulbous callosal splenium in female brains (DeLacoste-Utamsing and Holloway, 1982). These observations were followed by a considerable number of studies exploring possible sex effects on callosal size and shape. Although various observations suggest that sexual dimorphisms in callosal morphology exist, findings have not been consistently replicated across laboratories (Bishop and Wahlsten, 1997). For example, discrepancies exist concerning the affected callosal region, where studies reported sex differences for the callosal splenium (Clarke et al., 1989; Davatzikos and Resnick, 1998; DeLacoste-Utamsing and Holloway, 1982; Holloway and de Lacoste, 1986), for the callosal isthmus (Clarke and Zaidel, 1994; Steinmetz et al., 1992; Witelson, 1989); for the callosal genu (Giedd et al., 1999; Shin et al., 2005); or for the entire corpus callosum (Clarke et al., 1989; Holloway and de Lacoste, 1986; Leonard et al., 2008; Steinmetz et al., 1995; Westerhausen et al., 2004). Disagreement also exists with respect to the direction of the sex effect, with some studies detecting larger callosal regions in men and other studies detecting larger regions in women. Numerous studies failed to detect any significant sex differences (Giedd et al., 1999; Lee et al., 2003; Luders et al., 2003; Ng et al., 2005).

Study-specific criteria for callosal measurements (e.g., definitions of callosal subdivisions or adjustments for individual brain volumes) may account for some discrepancies in results. In fact, when applying a novel technique which circumvents pitfalls associated with traditional callosal parcellation by automatically estimating callosal thickness at 100 equidistant surface points, it was observed that sex differences were completely absent when data were adjusted for individual brain size (Luders et al., 2006). Without such adjustments, the same study revealed larger callosal dimensions in males, which may be attributable to larger dimensions in male brains. These recent findings based on state-of-the-art callosal measurements confirmed the outcomes of a meta-analysis of 49 studies published between 1980 and 1992 (Bishop and Wahlsten, 1997). The authors of the meta-analysis had proposed that unadjusted callosal size is slightly larger in males, with sex effects disappearing when statistically correcting for brain size.

The brain tissue compartments

There is extensive literature on sexual dimorphism of the major cranial tissue compartments, such as gray matter (predominantly consisting of neuronal bodies and dendrites) and white matter (consisting of axons that connect the neurons). It is well documented that larger volumes of gray matter and white matter exist in male brains if tissue measurements are not adjusted for individual brain size (Blatter et al., 1995; Good et al., 2001; Gur et al., 1999; Leonard et al., 2008; Luders et al., 2002). If, however, brain size is taken into consideration, some studies revealed higher percentages of gray matter in females (Gur et al., 1999; Luders et al., 2002),

while others failed to detect any sex differences (Nopoulos et al., 2000; Schlaepfer et al., 1995), or observed both higher gray matter (Good et al., 2001) and white matter proportions in males (Filipek et al., 1994; Goldstein et al., 2001; Gur et al., 1999; Passe et al., 1997). Another interesting aspect was pointed out by Allen et al. (2003): Overall, the sexual dimorphism appears to be greater for white matter than for gray matter. That is, while absolute gray matter and white matter volumes are smaller in women than in men, the white matter difference is more pronounced, with the result that women have a higher gray–white ratio than men.

In addition to such global sex differences, various analyses also revealed sex differences more locally based on pre-defined regions of interest (ROIs). For example, ROI studies indicated higher gray matter percentages or higher gray–white ratios in female brains in the dorsolateral prefrontal cortex (Schlaepfer et al., 1995), the superior temporal gyrus (Schlaepfer et al., 1995), and the parietal lobe (Koscik et al., 2008; Nopoulos et al., 2000). Additional sex differences were detected with respect to intra-sulcal gray matter, with larger volumes in the cingulate sulcus in females and larger volumes in the paracingulate sulcus in males (Paus et al., 1996).

In addition to global examinations and ROI-based analyses, automatic voxel-based whole-brain analyses exposed sex differences with an even higher regional specificity. For example, Good et al. (2001) observed that the *relative amount*[1] of gray matter was greater in female brains extensively and relatively symmetrically in the frontal, posterior temporal, and parietal cortices, in the parahippocampal gyri, as well as adjacent to the caudate heads, cingulate, and calcarine sulci. Luders et al. (2005) complemented and extended these gray matter findings by revealing their most significant sex differences bilaterally in the pre- and postcentral gyri (extending into the supramarginal gyri), as well as surrounding the temporal and occipital poles bilaterally expanding into posterior regions of the right inferior temporal gyrus. In addition, within the left hemisphere, they observed greater gray matter in the superior temporal gyrus (i.e., close to Broca's area) and in the inferior frontal gyrus (i.e., close to Wernicke's area).

Since men usually have larger brains than women, another recent whole-brain voxel-based study specifically examined a sample of men and women with similar brain size (Luders et al., 2009). The goal of the study was to determine whether greater gray matter is typical for female brains or just typical for small brains in general. Interestingly, comparing this set of matched male and female brains still revealed significantly greater gray matter in females, suggesting that (at least some) anatomical differences between male and female brains exist independently of brain size effects. While these outcomes appear to disagree with previous findings, indicating that brain size (rather than sex) is the main variable accounting for differences in proportional gray matter (Leonard et al., 2008; Luders et al., 2002), they are not contradicting but complementary if brain size effects account for global tissue volumes (and possibly the size of selected pre-defined structures), while sex effects account for regional gray matter. In strong agreement with this assumption, the above-mentioned study (Luders et al., 2009) did not detect any significant differences between matched male and female brains with respect to global gray matter and white matter ratios.

The cerebral cortex

The cerebral cortex contains approximately 80% of the neurons of the central nervous system. Over the course of evolution, the cerebral cortex has grown considerably in surface area. The cortex in humans is only 15% thicker than in macaque monkeys but has, at least, 10 times more surface

[1] When Good et al. compared the *relative amount* of gray matter, only female brains had regions of significantly greater gray matter. However, when comparing the *absolute amount* of gray matter, some brain regions contained more gray matter in female brains, while other regions contained more gray matter in male brains.

area. This enormous enlargement in surface area seems to be the result of a larger brain and, perhaps more importantly, of an increased folding of the brain's surface. Given that men usually have larger brains than women, researchers have suggested possible compensatory mechanisms in smaller female brains that might have occurred during human evolution (Luders et al., 2004). Sex differences in the anatomy of the cerebral cortex might constitute parts of such compensatory mechanisms and have been explored by focussing on three main features: (1) cortical thickness, (2) cortical convolution, and (3) cortical surface area.

Cortical thickness

When exploring sex differences with respect to "cortical depth" defined as approximately half the cortical thickness (Nopoulos et al., 2000), one study did not reveal any significant differences between men and women. However, albeit separating between "gyral cortical depth" and "sulcal cortical depth," this measurement was rather global in nature (i.e., the average depth across the entire cortex), and possibly existing sex differences might have been overseen. Newer methods comparing cortical thickness with a much higher precision at thousands of surface points revealed, for example, that women have significantly thicker cortices than men, after image scaling to take into account individual brain size (Luders et al., 2006). These sex effects were identified in all four lobes in each hemisphere, with temporal regions being least different. No regions with significantly thicker cortices were detected in males. When the actual brain sizes of men and women were preserved, the same pattern and general direction of the sex difference (females > males) were noticed, but the effect was considerably less pronounced. A small cortical region in the left lateral temporal lobe showed greater thickness in men (Luders et al., 2006). These findings were comparable to outcomes from another study where, after image scaling, female brains had a thicker cortex in numerous brain regions, with smaller effects in the temporal lobe (Im et al., 2006). Again, when brain sizes were preserved, the observed sex effects (females > males) were still present but considerably diminished. No cortical regions were thicker in males. Similarly, when analyzing brains in their native dimensions (i.e., without correcting for individual brain size), another study revealed thicker cortices in female brains in right inferior parietal, left ventral frontal, and posterior temporal regions. Thicker cortices in male brains were only detected in small clusters within right anterior temporal and orbitofrontal regions (Sowell et al., 2007).

Cortical convolution

One *post mortem* study investigated cortical convolution based on a two-dimensional "gyrification index" as the ratio between the total (deep) and superficial cortex in coronal brain slices (Zilles et al., 1988). Another *in vivo* study defined whole-brain "surface complexity" as the ratio of the total cortical surface area to the overall brain volume, raised to the 2/3 power (Nopoulos et al., 2000). None of these two studies detected any significant sex differences with respect to cortical convolution. However, other *in vivo* analyses seemed to indicate that sex possibly modulates the degree of cortical convolution by interacting with other variables. For example, one study (Blanton et al., 2001) estimated "cortical complexity" by modeling the cortical surface with different spatial resolutions and regressing the resulting surface areas against the respective spatial resolutions (Thompson et al., 1996a,b). That study examined cortical complexity for four lobar regions in each hemisphere (i.e., superior frontal, inferior frontal, temporal, parieto-occipital) and detected a significant sex-by-age interaction in children and adolescents for frontal brain regions with cortical complexity only increasing with age in females. Another study used surface-to-volume ratios

to calculate a "fissurization index" for the hemispheres and cingulate cortices (Yucel et al., 2001). That study observed a hemisphere-by-sex interaction reflecting a more asymmetric fissurization in male brains. Finally, two newer studies provided direct evidence for significant sex differences (Luders et al., 2004, 2006). In the first study researchers estimated "cortical complexity" (as described above) for five different lobar regions (i.e., superior frontal, inferior frontal, temporal, parietal, occipital). That study revealed a greater cortical complexity in female brains in the frontal and parietal lobes (Luders et al., 2004). The second study was based on the measurement of *mean curvature* (Do Carmo, 1976) to estimate "cortical convolution" across the entire cortex at thousands of surface points (Luders et al., 2006). This latter analysis confirmed the previous outcomes of greater cortical complexity in frontal and parietal regions in female brains. In addition, more pronounced female convolutions were detected in temporal and occipital cortices.

Cortical surface area

Sex differences with respect to the overall area of the cortical surface have been reported, where the direction of the sex effect seems to depend on whether measurements are adjusted for individual brain size. For example, a number of studies reported that male brains have larger surface areas than female brains when measured in their native dimensions (Luders et al., 2006; Nopoulos et al., 2000; Pakkenberg and Gundersen, 1997; Salat et al., 2004). However, when area measurements were adjusted by co-varying for brain tissue volumes, Nopoulos et al. (2000) observed that women tend to have somewhat greater surface area measures, although this did not reach statistical significance. Similarly, after image scaling, Luders et al. (2006) reported that the surface areas of the cortices were larger in females compared to males, where sex differences were highly significant.

Brain asymmetry

A number of studies also looked at possible sex effects (sometimes in association with handedness effects) when comparing the anatomy of the left and right hemispheres. For example, one study demonstrated a stronger lateralization of right frontal petalia (in right-handers and left-handers) and occipital petalia (in left-handers only) in men compared to women (Zilles et al., 1996). These outcomes partly resembled prior findings of greater frontal and occipital asymmetries in men and reversals of the typical asymmetries in women (Bear et al., 1986). Other analyses revealed a more pronounced rightward asymmetry of the planum parietale in right-handed men compared to right-handed women, while left-handed subjects demonstrated the opposite pattern (Jancke et al., 1994). It was also reported that male right-handers have a significantly deeper central sulcus on the left hemisphere than on the right hemisphere, whereas no inter-hemispheric asymmetry was found in female right-handers (Amunts et al., 2000). Moreover, it was observed that there is a larger leftward asymmetry of gray matter concentration posteriorly to the central sulcus in men than in women (Luders et al., 2004). Additional sex differences were detected with respect to parasagittal callosal measures, with larger rightward asymmetries in the anterior callosal midbody in men than in women (Luders et al., 2005). Finally, studies also revealed sex-dependent asymmetries of the inferior parietal lobe and planum temporale, with males having significantly larger leftward asymmetries and females showing either reversed, diminished, or no asymmetries (Frederikse et al., 1999; Good et al., 2001; Kulynych et al., 1994). Altogether, the majority of studies seem to indicate increased asymmetries in male brains. However, various analyses also failed to detect any significant sex effects with respect to hemispheric differences (Foundas et al., 1999; Lyttelton et al., 2009; Paus et al., 1996; Watkins et al., 2001) or revealed even more pronounced asymmetries in female brains (Rabinowicz et al., 2002).

Sex differences in brain micro-anatomy

Important insights with respect to microscopic sex differences mostly come from animal studies and concern the micro-topography of synaptic relations among neurons, steroid receptor mechanisms in neurons, and the metabolism of neurotransmitters (Arnold and Gorski, 1984). A few studies, however, were conducted directly on humans. These *post mortem* analyses revealed pronounced sex differences associated with the distribution of hormone receptors and with the levels of certain neurotransmitters (Fernandez-Guasti et al., 2000; Konradi et al., 1992). They also demonstrated that specific nuclei or cell groups were larger in men than in women (Allen et al., 1989; Swaab and Fliers, 1985; Zhou et al., 1995) or had different shapes such as elongated in women and more spherical in men (Swaab et al., 1985). Moreover, these analyses revealed that regional neuropil and dendritic arborization were larger in female brains (Jacobs et al., 1993; Rabinowicz et al., 1999, 2002,), while neuronal densities were larger in male brains (de Courten-Myers, 1999; Pakkenberg and Gundersen, 1997; Rabinowicz et al., 1999, 2002). The latter finding, however, is in contrast to a report of larger neuronal densities in female brains depending on the ROI examined (Witelson et al., 1995).

Possible determinants of sex differences in brain anatomy

Clearly, sex differences that arise before birth must be a consequence of prenatal or perinatal sex-specific hormonal action and genetic determination rather than a result of differential social stimulation. In contrast, morphological sex differences first arising after birth could be the result of either prenatal, perinatal, or postnatal influences (Breedlove, 1994). However, except for the larger brain weight (Dekaban, 1978) and the larger brain and tissue volumes (Gilmore et al., 2007) in males compared to females, it is not known conclusively whether any of the sexual dimorphisms in the human brain are present at birth or not. Thus, the exact underlying mechanisms and determinants remain to be established in future work, where interplay between genetic determination, hormonal exposure, and environment is very likely. Below is a brief overview of potential mechanisms and factors that might cause, sustain, accentuate, or attenuate the sexual dimorphism in the brain.

Non-environmental determinants

It was proposed that genes on the sex chromosomes determine the sexually dimorphic phenotype of the brain both by directly acting in the brain cells themselves and by regulating the action of sex hormones (sex steroids), such as androgens and estrogens (Arnold, 2004, 2009). Intriguingly, the organizational effects of sex steroids on brain anatomy are much better understood than the direct actions of sex chromosomes (Arnold, 2004). Steroid-induced alterations in gene expression can stimulate the neuron to generate new synapses, to discard old synapses, to remain alive, or to die (Breedlove, 1994). Thus, sex steroids can have growth-promoting, growth-inhibiting, neuro-protective, and deleterious effects (Kawata, 1995). Importantly, rather than being limited to a certain time frame in early stages of neurodevelopment (i.e., around birth), steroid effects may constitute lifelong influences on aspects of brain architecture and function (Forget and Cohen, 1994). An indirect link between the action of sex steroids and observable sex differences in brain anatomy was established in a study which compared parcellated volumes of the cortex between men and women (Goldstein et al., 2001). Interestingly, the regions of the cortex with greater sexual dimorphism corresponded closely to those identified in animal studies showing greater levels of androgen and estrogen receptors. Considering this conglomerate of scientific findings, sex hormones are likely to have contributed to observed sex differences in the anatomy of the brain.

Environmental determinants

Over the past centuries, the demands for traditional gender roles have significantly changed with environmental and social influences being increasingly similar for men and women. Nevertheless, differences still exist, with gender-specific environments being established as early as in infancy (e.g., through toys, social interactions, behavioral expectations). It is known not only from environmental enrichment studies in animals that the mammalian brain changes as a consequence of experience (Diamond, 2001; Juraska et al., 1985; Kempermann et al., 2002; Trachtenberg et al., 2002), but also from studies in humans. For example, a *post mortem* study revealed that dendritic lengths and branching altered in dependence of education levels, such that dendritic measures increased as educational levels increased (Jacobs et al., 1993). In addition, a number of cross-sectional *in vivo* studies (Gaser and Schlaug, 2003; Luders et al., 2009; Maguire et al., 2000; Mechelli et al., 2004) detected regionally greater gray matter in individuals pursuing activities that required a high level of training or practice in certain cognitive, sensory, and motor domains (e.g., in taxi drivers, bilingual speakers, meditation practitioners, piano players). Moreover, recent longitudinal *in vivo* studies (Boyke et al., 2008; Draganski et al., 2004, 2006; Driemeyer et al., 2008; May et al., 2007) observed gray matter changes in the brain as a direct consequence of intense cognitive and motor practices (e.g., due to learning for a medical exam, due to learning how to juggle). Thus, given that research has provided clear evidence for such experience-, stimulus-, and practice-induced alterations, similar mechanisms are likely to have caused the observed differences between male and female brains.

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CHAPTER 2

Sex differences in the human brain: a developmental perspective

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Abstract: At a population level, women and men differ in a wide variety of behavioral traits and in the probabilities of developing certain mental disorders. Some of these sex differences may be related to sexual dimorphism in brain structure, as it emerges during prenatal and post-natal development. Here, I provide a brief overview of the sex-chromosome–specific pathways that underlie sexual dimorphisms in general, describe the most common brain phenotypes derived *in vivo* with magnetic resonance imaging, discuss the challenges in interpreting these phenotypes vis-à-vis the underlying neurobiology, and, finally, review the known sex differences in brain structure from birth, through adolescence, to adulthood.

Keywords: Pregnancy; Adolescence; Brain; MRI; Gonadal hormones

Introduction

One of the practical motivations for studying sex differences in the human brain is the possibility of uncovering sex-specific pathways and mechanisms underlying mental disorders that show variations in their prevalence, symptoms, course of development, or treatment efficacy as a function of a patient's sex. There are a number of examples. Externalizing disorders (i.e., attention-deficit hyperactivity disorder and conduct disorder [Merikangas et al., 2010] and autism [Wing, 1981]) are more common in boys; schizophrenia begins earlier (by ~5 years; Hafner et al., 1998) and is often more drug-resistant in men (Vanelle, 1995); suicide also occurs more frequently in men (Thomas and Gunnell, 2010). On the other hand, depression and eating disorders are more common in adolescent girls (Hankin et al., 1998; Merikangas et al., 2010; Nolen-Hoeksema and Girgus, 1994) and pre-menopausal women (Kessler et al., 2005; Weissman et al., 1993).

Given the complexity of mental health and its dependence on the interplay of genetic and environmental influences throughout the life span

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(e.g., Robertson and Poulton, 2008; Sleiman and Grant, 2010), it is, of course, unlikely that a single sex-specific mechanism would account for any of the above-mentioned differences. On the other hand, it is possible that some of the basic biological processes that underlie sexual dimorphism in reproductive behavior also induce sex differences in the way we process and evaluate information—and use it to formulate our actions—in other contexts. These sex differences in non-reproductive behavior then moderate how we interact with physical and social environments and, in turn, may bring about the differential vulnerability to—and expression of—certain mental disorders in men and women. When searching for the neural systems mediating such sex differences in behavior, it is helpful to identify structural features of the brain, whether global or regional, that show sexual dimorphism and to evaluate whether or not the degree of its expression in a given neural system affects the probability of developing a disease. Let us begin by reviewing briefly two topics relevant for this discussion: (1) the biology of sexual dimorphism and (2) *in vivo* measurements of brain structure and their interpretations.

The biology of sexual dimorphism

Sex differences in a phenotype arise from genes located on the X and Y chromosomes. This occurs in three principal ways (reviewed in Arnold, 2009). First and foremost, Y chromosome genes are present only in males; the most important of these is the testis-determining gene (*Sry*) that drives the differentiation of gonads into testes, with the subsequent secretion of testosterone. Second, females have two copies of some of the X chromosome genes. Although this effect is largely eliminated by inactivation of X chromosome genes, the exact pattern of this inactivation may vary across individuals (Carrel and Willard, 2005). Third, given that this inactivation may silence X chromosome genes inherited from either the mother or the father, female tissues are a mosaic containing maternal and paternal imprints of the X chromosome; the paternal imprint is missing in males. Finally, recent work also suggests that maternal or paternal alleles are preferentially expressed in the mouse brain for a large number (over 1000) of autosomal genes (Gregg et al., 2010). Although the dominating factor vis-à-vis the expression of sexual dimorphisms is *Sry* expression in the gonads, with the subsequent secretion of testosterone during prenatal and post-natal life in males and the absence of it in females, other X- and Y-linked genes may influence sex differences in the brain directly, that is, in a manner independent of sex differences at the level of gonadal hormones. Whether such direct effects act in synergy with hormonal effects or may counteract each other is largely unknown (discussed in Arnold, 2009). The following text does not make a distinction between such direct "hormone-independent" effects on the brain and the common (traditional) effects induced via gonadal hormones.

Gonadal hormones represent a powerful environment shaping various target organs, including the brain. During the prenatal period, the dominating influence is that of testosterone produced by fetal testes; in humans, fetal levels of testosterone are assumed to peak between 14 and 18 weeks of pregnancy (Prince, 2001; Reyes et al., 1974; but see Sarkar et al. [2007] for no fluctuations in testosterone levels in the amniotic fluid between 15 and 37 weeks of pregnancy). Both male and female fetuses also produce androgens (e.g., dehydroepiandrosterone [DHEA]) in their adrenal glands (Rainey et al., 2004). Finally, placenta also plays an important role in the synthesis and conversion of sex steroids (e.g., Loganath et al., 2002; Matt et al., 1986). During post-natal life, production of gonadal hormones begins in puberty. In males, the main gonadal hormone—testosterone—is also converted (by 5 alpha reductase) into a more potent dihydrotestosterone (DHT) or (by aromatase) into estradiol. In females, ovaries produce estrogen and progesterone; their production declines rapidly after menopause. All gonadal hormones influence target tissues mainly through (1) their receptors,

which belong to the nuclear family of intracellular receptors and (2) receptor binding to steroid response elements of a given gene, thereby influencing its expression. In addition, some of the (fast) non-genomic effects of gonadal hormones are mediated by less specific membrane receptors (e.g., PKC, MAPK; Foradori et al., 2008).

As described initially by Phoenix et al. (1959), the effects of gonadal hormones during the fetal period are considered to be of a permanent (long-lasting) "organizational" nature, whereas gonadal hormones produced during puberty and onward have transient (short-lasting) "activational" effects. As pointed out above, the prenatal period is dominated by the organizational influence of androgens (testosterone and DHT), while both androgens and ovarian hormones (estrogen and progesterone) begin to exert their activational effects during puberty. Note that the distinction between "organizational" and "activational" effects is somewhat arbitrary; for example, it is likely that gonadal hormones exert "organizational" effects also during puberty (Sisk and Zehr, 2005). Overall, the organizational effects of androgens during the prenatal period are powerful: the administration of testosterone to pregnant rhesus monkeys has both "masculinizing" and "defeminizing" effects on female offspring, involving both reproductive and non-reproductive behaviors. In the case of the latter, the frequency of sexually dimorphic behaviors, such as rough-and-tumble play (more common in males) and certain vocalizations (e.g., separation–rejection calls and agonistic [social] calls), changes as early as in the juvenile period in female offspring exposed prenatally to androgens. When evaluated during puberty, prenatal exposures to androgens appear to decrease the sensitivity of exposed female offspring to the activational effects of estradiol on female-typical behaviors (reviewed in Thornton et al., 2009). In humans, a handful of reports suggest that there are long-term effects of prenatal exposure to androgens on a variety of morphological, physiological, and behavioral parameters. These include findings on the association between maternal testosterone during pregnancy and follicular development during adolescence (Hart et al., 2010), the levels of testosterone in the amniotic fluid and the ratio of the length of the second and fourth fingers (2D:4D ratio; Lutchmaya et al., 2004), as well as exposure to testosterone from a male co-twin and 2D:4D ratio (Voracek and Dressler, 2007) and brain size (Peper et al., 2009) in the female co-twin (see Whitehouse et al. [2010] for findings opposite to those of Peper et al. when using head circumference as a proxy of brain size). Fetal testosterone is correlated with behaviors that, in the extreme, would count as diagnostic symptoms for autism, including decreased eye contact and social functioning, delayed development of vocabulary, and narrow interests (Chapman et al., 2006; Knickmeyer et al., 2005, 2006; Lutchmaya et al., 2004).

In summary, sexual dimorphism in the human brain can arise from direct hormone-independent effects of X and Y chromosome genes or through different levels of gonadal hormones during both prenatal and post-natal periods. The former pathway/genes are largely unknown. The latter, and arguably more powerful, pathway begins with the differentiation of gonads into testes under the control of the Y-linked *Sry* gene, with the subsequent production of testosterone during the prenatal period in males, and re-emerges during puberty with the renewed production of testosterone in males and the first production of the ovarian hormones (estrogen and progesterone) in females.

Before examining the current knowledge of sexual dimorphism in the human brain in this context, we will review first the basics of magnetic resonance imaging (MRI)-based measurements of brain structure and some of the challenges in interpreting these phenotypes.

MRI-based assessment of brain structure: measures and their meaning

MRI provides unprecedented opportunities for quantifying *in vivo* a wide variety of structural properties of the human brain throughout the life

span. Here, I will focus on those measures that can be obtained readily on standard MR scanners available in most clinical or research settings (1–3 T magnets), in a relatively short scanning session (<1 h), and throughout the brain. As such, many of these measures have been acquired in reasonably large numbers of individuals and, therefore, will be the basis for reviewing existing findings of sexual dimorphism in the next section.

T1-weighted (T1W) images represent the most common acquisition sequence used to visualize brain structure; typically, a T1W image can be acquired in about 10 min to cover the whole brain with a resolution of $1 \times 1 \times 1$ mm. As there is excellent contrast between gray matter (GM) and white matter (WM), as well as cerebrospinal fluid (CSF), on such T1W images, these are used in most automatic image-processing "pipelines." These pipelines enable one to extract in a fully automatic fashion a large number of global and local morphological features of the human brain. The two basic steps employed in most pipelines are those of registering (or warping) each 3D T1W image to a template image and classifying the brain tissue into three classes, namely GM, WM, and CSF. There are two general classes of features that can be extracted from T1W images: (1) volumetric (or surface) measures; and (2) voxel- or vertex-wise features derived for each X, Y, and Z location (Fig. 1). The latter approach is enabled by registering all brains into the common stereotaxic space of a template brain. The volumetric/surface measures include global volumes, such as brain size, total cortical surface, or total volume of GM and WM, as well as regional

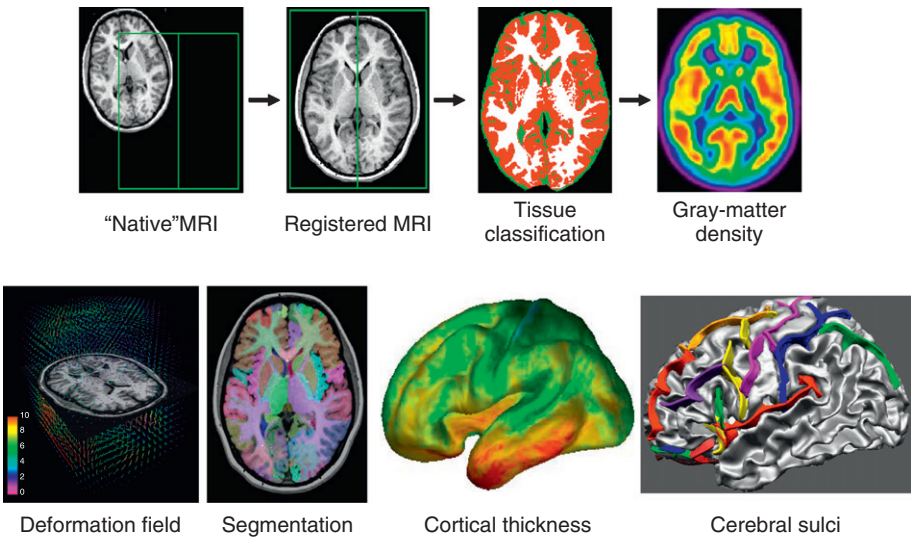

Fig. 1. Image-processing pipeline. **Top row**: A typical image-processing pipeline begins with the transformation of a MR image from the acquisition ("native") to standardized stereotaxic space; this process generates an image "registered" with the template brain. The next step involves voxel-wise classification of brain tissue into three main classes: gray matter (in dark grey), white matter (in white), and cerebrospinal fluid (in light grey). Each of such binary images (0, tissue absent; 1, tissue present) is then filtered (or smoothed) to generate "density" images; the image of white-matter density shown here indicates, at each voxel, local concentration of WM on a continuous scale from 0 to 1 (the hotter the color, the higher the value of WM density). **Bottom row**: Non-linear registration of the sample image to the template brain allows one to characterize local shape differences; the deformation field quantifies such sample–template differences throughout the brain. By combining non-linear registration with tissue classification, one can segment automatically various brain structures, such as the frontal lobe or the amygdala. Other techniques produce maps of cortical thickness or identify sulci in the subject's cerebral cortex. Reprinted with permission from Paus (2005).

measures, such as the size of the amygdala or corpus callosum, or the cortical thickness of the frontal lobe. The voxel/vertex-wise measures characterize local features at each 3D location in the brain; depending on the extent of the "blurring" of an image during its processing, the local measure reflects an average of values over a smaller (less blurring) or larger (more blurring) region. The following measures are often derived in this fashion: GM/WM "density," cortical thickness, cortical folding, or local differences in the shape and position of small structures.

Diffusion tensor imaging (DTI) is another increasingly common MR modality, which is used mainly for studies of fiber tracts and their morphological properties; typically, a DTI image can be acquired in about 20 min to cover the whole brain in 32 directions with a resolution of $2 \times 2 \times 2$ mm. This imaging technique allows one to estimate several parameters of water diffusion in live tissue, such as mean diffusivity (MD) and fractional anisotropy (FA). The latter parameter reflects the degree of directionality of water diffusion; voxels containing water moving predominantly along a single direction have higher FA. In WM, FA is believed to depend on the microstructural features of fiber tracts, including the relative alignment of individual axons, their packing "density" (which affects the amount of interstitial water), axonal caliber, and myelin content. Data acquired with DTI can be analyzed using global and regional methods, the former providing, for example, mean values of FA/MD in all WM, and the latter providing average FA/MD values in specific fiber tracts (e.g., corpus callosum) or comparisons of different groups (e.g., males and females) at each 3D location (voxel) after all images have been registered with the common template brain.

The above examples illustrate the richness of quantitative brain phenotypes, both global and local, derived from just two types of MR acquisition sequences (i.e., T1W and DTI). The next paragraph addresses some of the challenges associated with the interpretation of these MR-derived measures vis-à-vis the underlying neurobiology.

First of all, the complexity of brain tissues clearly precludes one from attributing, for example, a sex difference in cortical thickness to a single cellular compartment. As shown by Braitenberg and Schüz (1998), the volumetric contribution of different cellular compartments in a sample of mouse cortex is as follows: axons, 29%; dendrites, 30%; dendritic spines, 12%; glia, 10%; cell bodies and vessels, 14%; and extracellular space, 5%. Thus, any observed sex differences in cortical thickness or regional volumes of GM could be influenced by changes in any of these compartments; clearly, relative sex differences (e.g., 2%) in the largest compartments, such as those occupied by axons and dendrites, will translate to a larger absolute difference and will be therefore more readily detectable with MRI. In this context, it is of interest to note that the volume of dendrites in the (left) posterodorsal subnucleus of the rat medial amygdala is larger in (pre-pubertal) males than females, thus accounting for the known sex differences in the volume of this structure (Cooke et al., 2007). Similarly, sex differences in WM, whether volumetric (based on T1W scans) or local (FA based on DTI), cannot be simply attributed to differences in the degree of myelination, as it is often assumed. We have suggested, for example, that age (and testosterone)-related changes in the volume of WM during male adolescence are likely due to the increase in axonal caliber (Herve et al., 2009; Perrin et al., 2008); in general, sex differences in WM might reflect differences in the ratio between axonal caliber and fiber diameter (axonal caliber + myelin thickness), the so-called *g* ratio (Paus and Toro, 2009).

The second important issue involves the dynamic nature of brain structure. It is generally accepted that most of the structural features measured with MRI continue to mature during childhood and adolescence (e.g., Paus, 2010; Paus et al., 2008). But it is often assumed that, in the adult brain, the same morphological features are static and not amenable to further modifications on a short timescale (days or weeks). Therefore, one can reason that an individual's more recent experiences, including his/her

current levels of gonadal hormones, may not be relevant when evaluating (sex) differences in brain structure. This is not necessarily correct. A number of MRI studies have demonstrated experience-induced changes in the brain structure of healthy adults (young and old), induced by several weeks of specific sensory, motor, or cognitive stimulation including juggling (GM: Boyke et al., 2008; Draganski et al., 2004; Driemeyer et al., 2008; WM: Scholz et al., 2009), memory training (Enving et al., 2010) and mirror reading (Ilg et al., 2008). Although the underlying neurobiology of such experience-induced structural changes is unknown (see above), these findings suggest that the presence of a particular sexual dimorphism in the healthy adult brain may not necessarily reflect "organizational" effects of gonadal hormones but may be also influenced by recent (adult) experiences, whether related to physiological (e.g., hormones) or psychosocial (e.g., cultural biases) factors.

In summary, MRI provides unprecedented opportunities for *in vivo* quantification of sex differences in the human brain; a 30-min MR session provides a wealth of image-based data from which one can extract multiple structural features, from global (brain size, cortical surface, WM volume) to local (volume of amygdala, thickness of a cortical region, FA of a fiber tract) levels. Interpretation of these measurements and related sexual dimorphisms is complicated by the lack of specificity vis-à-vis the underlying neurobiology and, to some extent, by the remarkable short-term (weeks) plasticity of many of these structural features in the adult brain.

Sexual dimorphism in the human brain: findings

In this section, I will review some of the existing MR literature on the presence of sexual dimorphisms in various global and local measures of brain structure in healthy individuals. Given the importance of gonadal hormones in shaping the brain, I will take a developmental perspective and, when possible, ask whether a given dimorphism is present at birth, in pre-pubertal children (0–9 years), adolescents (10–19 years), pre-menopausal adults (20–49 years), and post-menopausal adults (50+ years). Although the above age groups are somewhat arbitrary, they may help us in understanding the relative importance of prenatal and post-natal exposures to gonadal hormones vis-à-vis the emergence and/or maintenance of a given sexual dimorphism. The age of 10 years has been chosen as the beginning of puberty based on the onset of genital growth (boys) and breast development (girls) ascertained in the participants of the National Health and Nutrition Examination Survey III carried out in the United States between 1988 and 1994 (Herman-Giddens, 2006). The age of 50 years is based on the mean age of menopause as determined in US and Puerto Rican women in the Sister Study carried out between 2003 and 2009 (Steiner et al., 2010).

At a global level, the most robust sexual dimorphism observed in the adult brain is that of its size; on average, the male brain is about 11% larger than the female brain. This difference remains significant after co-varying body height or weight (Ankney, 1992; Peters et al., 1998; Skullerud, 1985). As shown in Table 1a and Fig. 2 (top), which are based on data obtained across a number of MR studies carried out in a total of 1263 males and 1159 females, sex differences in brain size are present at birth (7.8% difference), are about the same in pre-pubertal children (~11%) and adolescents (~11%), and reach their highest value in pre-menopausal adults (~14%). The size of this effect (Cohen's *d*) varies between 0.65 (birth) and 1.6 (pre-menopausal adults). These values should be compared with caution, however, due to the differences in the number of individuals included in these studies, the exact type of MR acquisition and segmentation, as well as differences in the definition of "brain volume." Nonetheless, given that very similar numbers of males and females were included in each study, at least the main effect of these methodological factors is minimized. Which of the two tissues, namely GM and WM, drive these sex differences in brain size?

Table 1. Sex differences in brain size (A) and the volumes of grey (B) and white (C) matter

A. Brain size

Group	Males: n	Males: Mean (ccm)	Males: SD	Females: n	Females: Mean (ccm)	Females: SD	% Diff ([M-F]/F)	Cohen's d (Male-Female/ MaleSD	Reference
Prenatal/Birth	40.00	525.52	58.64	34.00	487.38	41.85	7.83	0.65	Gilmore et al. (2007)
Children	124.00	1205.00	115.40	112.00	1102.00	84.30	9.35	0.89	Lenroot et al. (2007)
Children (MZ twins)	44.00	1418.00	94.90	46.00	1268.00	96.50	11.83	1.58	Peper et al. (2009)
Children (DZ same-sex twins)	43.00	1441.00	96.90	41.00	1290.00	82.30	11.71	1.56	Peper et al. (2009)
Adolescents	315.00	1233.00	99.90	208.00	1104.00	98.80	11.68	1.29	Lenroot et al. (2007)
Adolescents	204.00	1446.00	117.00	215.00	1299.00	97.00	11.32	1.26	Paus et al. (2009)
PreMP Adults	145.00	1417.00	86.20	145.00	1304.00	87.50	8.67	1.31	Kruggel (2006)
PreMP Adults	50.00	1.51	0.40	50.00	1.32	0.10	14.39	0.48	Luders et al. (2002)
PreMP Adults	57.00	1365.00	106.20	59.00	1195.00	99.50	14.23	1.60	Gur et al. (2002)
PostMP Adults	241.00	1201.00	97.00	249.00	1057.00	84.00	13.62	1.48	Ikram et al. (2008)

B. Grey Matter

Group	Males: n	Males: Mean (ccm)	Males: SD	Females: n	Females: Mean (ccm)	Females: SD	% Diff ([M-F]/F)	Cohen's d (Male-Female/ MaleSD	Reference
Prenatal/Birth (1)	40.00	218.20	28.70	34.00	197.90	197.90	10.26	0.71	Gilmore et al. (2007)
Children	124.00	770.80	73.10	112.00	708.40	57.20	8.81	0.85	Lenroot et al. (2007)
Children (MZ twins)	44.00	748.60	50.20	46.00	668.70	53.40	11.95	1.59	Peper et al. (2009)
Children (DZ same-sex twins)	43.00	758.40	55.10	41.00	678.90	52.20	11.71	1.44	Peper et al. (2009)
Adolescents	315.00	753.90	64.60	208.00	680.40	62.40	10.80	1.14	Lenroot et al. (2007)
Adolescents	204.00	629.00	54.00	215.00	577.00	46.00	9.01	0.96	Paus et al. (2009)
PreMP Adults	145.00	714.30	47.50	145.00	668.00	46.70	6.93	0.97	Kruggel (2006)
PreMP Adults	50.00	820.00	60.00	50.00	740.00	60.00	10.81	1.33	Luders et al. (2002)
PreMP Adults	57.00	699.60	66.70	59.00	642.70	50.20	8.85	0.85	Gur et al. (2002)
PostMP Adults		N/A	N/A		N/A	N/A	N/A	N/A	Ikram et al. (2008)

(*Continued*)

Table 1 (*Continued*)

C. White Matter

Group	Males: n	Males: Mean (ccm)	Males: SD	Females: n	Females: Mean (ccm)	Females: SD	% Diff ([M-F]/F)	Cohen's d (Male-Female/ MaleSD	Reference
Prenatal/Birth (1)	40.00	163.90	18.40	34.00	154.20	15.70	6.29	0.53	Gilmore et al. (2007)
Children	124.00	425.70	51.90	112.00	383.30	36.40	11.06	0.82	Lenroot et al. (2007)
Children (MZ twins)	44.00	499.00	44.70	46.00	448.90	45.50	11.16	1.12	Peper et al. (2009)
Children (DZ same-sex twins)	43.00	509.70	49.30	41.00	455.30	40.90	11.95	1.10	Peper et al. (2009)
Adolescents	315.00	466.50	47.20	208.00	412.80	45.60	13.01	1.14	Lenroot et al. (2007)
Adolescents	204.00	431.00	51.00	215.00	361.00	37.00	19.39	1.37	Paus et al. (2009)
PreMP Adults	145.00	703.00	69.00	145.00	636.40	69.90	10.47	0.97	Kruggel (2006)
PreMP Adults	50.00	420.00	60.00	50.00	360.00	40.00	16.67	1.00	Luders et al. (2002)
PreMP Adults	57.00	551.20	71.10	59.00	452.10	51.60	21.92	1.39	Gur et al. (2002)
PostMP Adults		N/A	N/A		N/A	N/A	N/A	N/A	Ikram et al. (2008)

Notes: (1) Hemipsheric volumes MZ, monozygotic; DZ, dizygotic; M, male; F, female; PreMP Adults, pre-menopausal adults; PostMP Adults, post-menopausal adults;

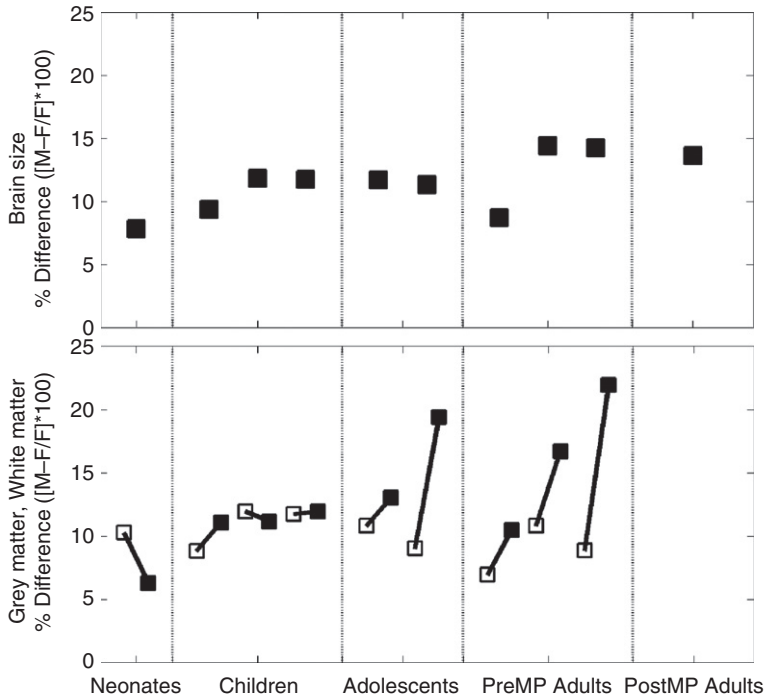

Fig. 2. Sex differences in global brain volumes. **Top row**: Sex differences in brain size across 10 MR studies (see Table 1 for sample details and effect sizes). **Bottom row**: Sex differences in the volume of gray matter (empty symbols) and white matter (filled symbols) obtained in 9 of the 10 studies included above. A line connects gray and matter volumes obtained in the same study. M, male participants; F, female participants; PreMP Adults, pre-menopausal adults (20–49 years of age); PostMP Adults, post-menopausal adults (50+ years of age).

Tables 1B (GM) and 1C (WM) summarize the relevant findings obtained in the same set of studies. Overall, it appears that sex differences in the absolute volume of GM are very similar across all age groups while those in the absolute volume of WM are the lowest at birth and increase significantly during adolescence and adulthood (Fig. 2, bottom). The latter observation is consistent with testosterone-mediated increases in WM volume during male adolescence, as discussed previously (e.g., Paus and Toro, 2009; Perrin et al., 2008).

Overall, sex differences in brain size, as estimated *in vivo* from MR images, appear to be present at birth and continue to increase post-natally to reach an approximate maximum of 14% in adulthood (Fig. 2, top). The relative contribution of GM and WM to these sex differences changes during development, with a smaller sex difference in the case of WM vs. GM at birth and *vice versa* during adolescence and adulthood (Fig. 2, bottom). Thus, androgens (and other sex-chromosome-related processes) appear to influence brain growth both prenatally and post-natally, perhaps through distinct effects on GM (e.g., cell proliferation, apoptosis, dendritic branching [e.g., Isgor and Sengelaub, 2003; Nunez et al., 2000; Reid and Juraska, 1992]) and WM (e.g., axonal caliber [e.g., Paus and Toro, 2009]).

Could similar trends be observed vis-à-vis regional volumes? Unfortunately, a thorough evaluation of sex differences in the five age groups employed in the above "global" comparisons

(i.e., birth, children, adolescents, pre-menopausal adults, and post-menopausal adults) is not possible at this time. This is mainly due to the differences across published reports in the methods used to estimate volumes of different brain structures, and the fact that different structures are often investigated in separate reports. To circumvent these difficulties, we will focus here on data obtained with the same suite of segmentation algorithms, namely FreeSurfer (Fischl et al., 2002), in two datasets: an adolescent dataset (Saguenay Youth Study; $n = 579$, 12–18 years of age) and an adult dataset (Fjell et al., 2009; pooled data from seven studies of healthy adults; $n = 1143$, 18–94 years of age). As expected, absolute volumes of the eight structures considered here, namely the hippocampus, amygdala, putamen, caudate nucleus, pallidum, nucleus accumbens, thalamus, and brainstem, are higher in male than female adolescents (Table 2; Fig. 3, top), as well as in adult men than women (Fig. 4, top; Fjell et al., 2009). After correcting for intracranial volume (ICV), a slightly different pattern of (residual) sex differences is observed in the two samples. In adolescents (Fig. 3, bottom), the caudate nucleus is slightly larger in girls than boys while the following structures are larger in boys than girls: the putamen, pallidum, amygdala, and thalamus. In adults (Fig. 4, bottom), none of the ICV-adjusted volumes is larger in women compared with men while the following structures are larger in men compared with women: the putamen, pallidum, amygdala, hippocampus, thalamus, and brainstem. Note that the adult sample consists of individuals falling into both the "pre-menopausal" and "post-menopausal" groups; as such, this sample is highly heterogeneous vis-à-vis the levels of gonadal hormones. Overall, sex differences in absolute volumes observed at a global level are, not surprisingly, found also at a local level for all

Table 2. Absolute (A) and relative (B) volumes of subcortical brain structures obtained in an adolescent sample

A. Absolute volumes

Structure	Males: n	Males: Mean (ccm)	Males: SD	Females: n	Females: Mean (ccm)	Females: SD	% Diff ([M–F]/F)	Cohen's d (Male–Female/MaleSD	p (sex effect)
Hippocampus	281.00	4.40	0.35	298.00	4.10	0.36	7.32	0.86	<.0001
Amygdala	281.00	3.20	0.32	298.00	2.89	0.27	10.73	0.97	<.0001
Putamen	281.00	12.60	1.10	298.00	11.40	1.10	10.53	1.09	<.0001
Caudate	281.00	7.90	1.00	298.00	7.30	0.97	8.22	0.60	<.0001
Pallidum	281.00	4.20	0.45	298.00	3.70	0.40	13.51	1.11	<.0001
Accumbens	281.00	1.30	0.18	298.00	1.18	0.16	10.17	0.67	<.0001
Thalamus	281.00	17.10	1.68	298.00	15.20	1.40	12.50	1.13	<.0001
Brainstem	281.00	22.60	2.20	298.00	20.50	1.95	10.24	0.95	<.0001

B. ICV-adjusted volumes

Structure	Males: n	Males: Mean (ccm)	Males: SD	Females: n	Females: Mean (ccm)	Females: SD	% Diff ([M–F]/F)	Cohen's d (Male–Female/MaleSD	p (sex effect)
Hippocampus	281.00	4.30	0.29	298.00	4.30	0.31	0.00	0.00	n.s.
Amygdala	281.00	3.08	0.26	298.00	3.02	0.23	1.99	0.23	0.002
Putamen	281.00	12.10	0.97	298.00	11.90	0.99	1.68	0.21	0.004
Caudate	281.00	7.55	0.85	298.00	7.69	0.86	–1.82	–0.16	0.010
Pallidum	281.00	4.00	0.38	298.00	3.90	0.36	2.56	0.26	<.0001
Accumbens	281.00	1.26	0.16	298.00	1.25	0.14	0.80	0.06	n.s.
Thalamus	281.00	16.20	1.24	298.00	16.00	1.17	1.25	0.16	0.010
Brainstem	281.00	21.60	1.76	298.00	21.40	1.61	0.93	0.11	n.s.

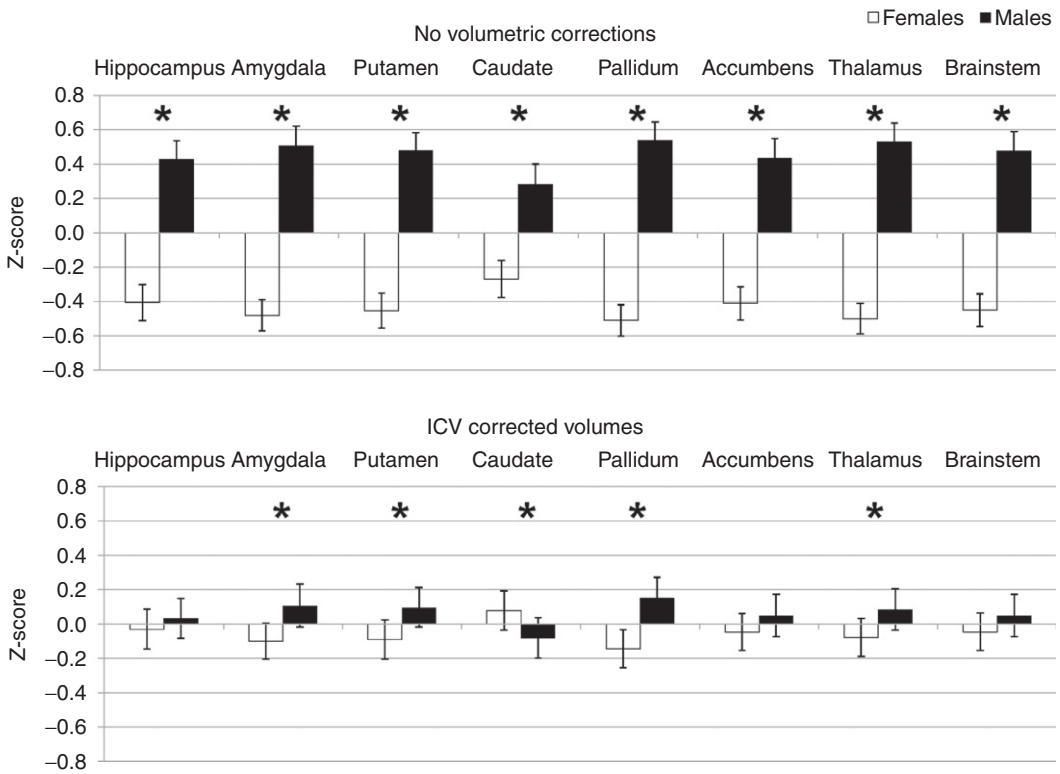

Fig. 3. Sex differences in regional brain volumes: adolescents. **Top row**: Sex differences in the absolute volume of eight brain structures as estimated by Freesurfer in a sample of typically developing adolescents ($n = 579$; see Table 2 for sample details, effect sizes, and statistical significance). **Bottom row**: Sex differences in the volumes of the same structures after adjustment for the intracranial volume (see Table 2 for effect sizes and statistical significance). ICV, intracranial volume.

brain structures considered here; in the case of adolescents, they range between 7.3% (hippocampus) and 13.5% (pallidum). Once the overall brain size (or ICV) is factored out, very little difference remains; in the case of adolescents, these residual sex differences vary between 0 (hippocampus) and 2.6% (pallidum).

Conclusions

Sexual dimorphism in brain structure, as assessed *in vivo* with MRI, is expressed most robustly in brain size. This sex difference is present at birth and increases through childhood and adolescence into adulthood. It indicates the presence of sex-specific factors facilitating brain growth of the male offspring; whether these are the same or different from those affecting growth in general is unknown. Although height predicts brain size ($r^2 = 0.26$ in our adolescent sample), the inclusion of height in the statistical model does not eliminate the finding of sex differences. A comparison of sex differences in the absolute volumes of GM and WM suggests that factors acting early (prenatal and early post-natal period) are more likely to affect the former while those acting later (adolescence) affect the latter. Given the rapid growth of the brain during pregnancy and the first two years of life, it is likely that a large portion of the variation

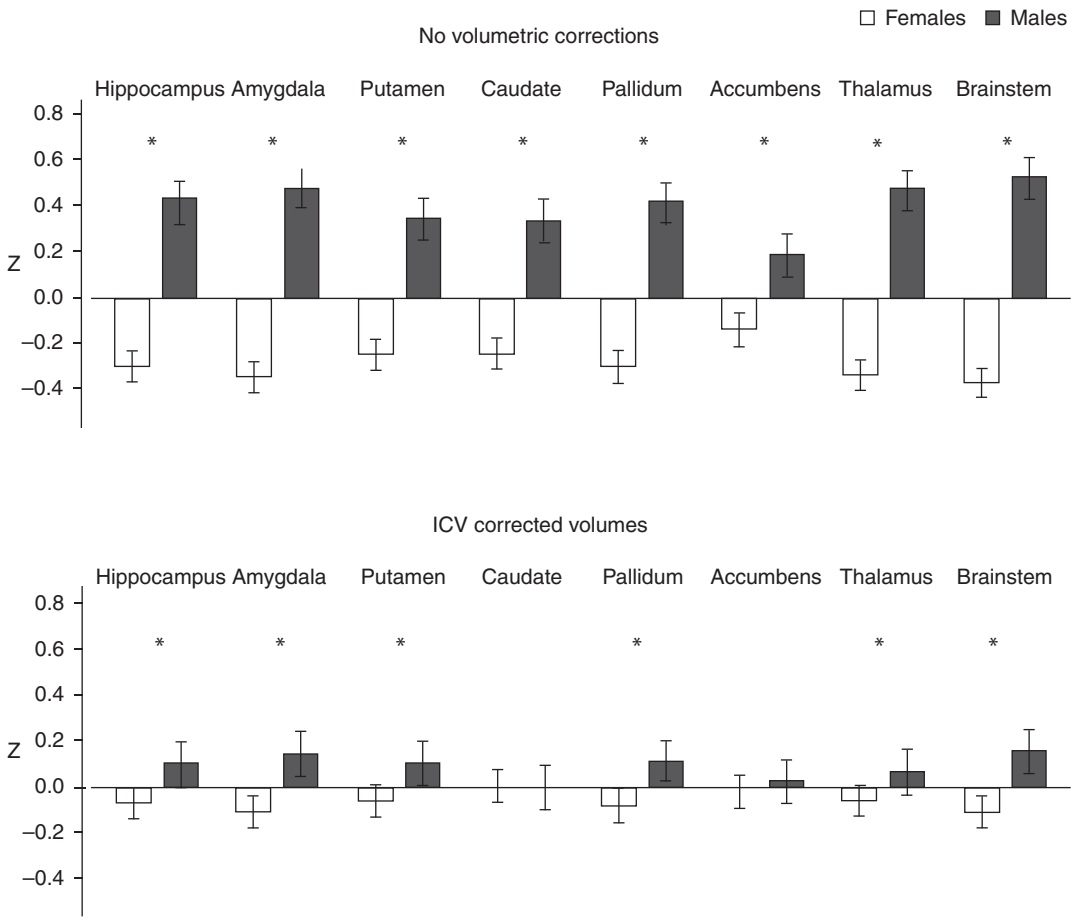

Fig. 4. Sex differences in regional brain volumes: adults. **Top row**: Sex differences in the absolute volume of eight brain structures as estimated by Freesurfer in a sample of adults ($n = 1143$; Fjell et al., 2009). **Bottom row**: Sex differences in the volumes of the same structures after adjustment for the intracranial volume. ICV, intracranial volume. Reprinted with permission from Fjell et al. (2009).

in brain size and GM volume is determined in these developmental periods; cellular processes related to cell proliferation (neurons and neuropil) and cell death will play significant roles in this respect. X-linked and Y-linked genes, such as those coding histone demethylases (*Jardic1c, Jardic1d, Utx*, and *Uty*) may be potential candidates, given their possible role in brain development (Xu and Disteche, 2006; Xu et al., 2008a,b). The global nature of these sex differences suggests that the underlying developmental mechanisms may be of interest for those disorders that are characterized by both a higher prevalence in males and differences, between affected and unaffected individuals, in overall brain size. This is the case, for example, for ADHD (attention deficit hyperactivity disorder) (e.g., Batty et al., 2010; Castellanos et al., 2002; Narr et al., 2009). On the other hand, the observed accentuation of sex differences in the volume of WM during adolescence suggests a different set of mechanisms, possibly related to the effect of androgens on axonal caliber. As I discussed in greater detail

elsewhere (Paus, 2010), the axon and its cytoskeleton provide the necessary "infrastructure" for an unhindered transport of various cargo between the cell body and the synapse. In this way, cytoskeleton and motor proteins are essential contributors to a large number of cellular processes, such as cell metabolism (e.g., transport of mitochondria and glycolytic enzymes) and neurotransmission (e.g., transport of synaptic vesicle precursors). Any disturbances related, for example, to short-term alterations in the cytoskeleton, due to the fluctuating levels of testosterone during puberty, may affect these processes and, in turn, modulate (amplify or dampen) the transfer of information throughout the brain. It is possible that such a process increases vulnerability to schizophrenia and contributes to its earlier onset in men, as compared with women (Hafner et al., 1998).

Sex differences in the volume of specific brain regions, such as the amygdala, are rather small once the global differences in brain size are removed. Whether such subtle differences in size are meaningful in functional terms remains to be seen. It is true, however, that even a 2% difference (~60 mm^3 in the case of the amygdala) translates likely into a substantial difference in the number of neurons and/or their dendrites. For example, the (right) posterodorsal subnucleus (MeApd) of the rat amygdala (at the 26th postnatal day) is larger in males than females by about 16% (~0.042 mm^3; total volume of 0.258 mm^3 in juvenile males); this difference is largely due to a higher (by ~23%) number of neurons—a sex difference of 5471 neurons (Cooke et al., 2007). Given the structural and functional heterogeneity of the (human) amygdala, one should also keep in mind that a small difference detected when the entire amygdala is segmented might, in theory, reflect a large difference in one of its subnuclei. The same reasoning applies to other subtle differences in regional volumes and other structural features, such as cortical thickness or characteristics of the WM in specific fiber tracts.

Overall, sex differences in brain structure, as quantified with MRI at a gross morphological level, may provide a useful springboard for investigating developmental mechanisms that underlie sex-specific vulnerabilities in mental-health problems. This brief review touched only on the most simple of possible structural "phenotypes." Multi-modal MR acquisitions carried out at higher sub-millimeter resolution will undoubtedly increase the power, fidelity, and specificity of detecting sexual dimorphism in the human brain at different developmental periods. The developmental strategy, whether applied in the context of longitudinal or trans-generational studies, will add an important dimension not explored here, namely that of sex-specific changes in developmental trajectories (e.g., Giedd et al., 2008). The use of other techniques, such as positron emission tomography (in adult participants) and functional MRI (at any age), will allow investigators to evaluate sexual dimorphism in the neurochemistry (e.g., Munro et al., 2006) and functional engagement of specific neural circuits (e.g., Dickie and Armony, 2008) as well as their connectivity (e.g., Savic and Lindstrom, 2008). Altogether, this work will allow us to understand how sex chromosomes shape our brains.

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CHAPTER 3

Sex influences on brain and emotional memory: the burden of proof has shifted

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Abstract: Sex influences are ubiquitous on brain function, including the human brain. This chapter addresses the issue of sex influences on human brain function first as it pertains to studies of emotional memory, then as it pertains to the field of neuroscience more generally. The striking quantity and diversity of sex-related influences on nervous system function argue that the burden of proof regarding the issue has shifted from those examining the issue in their investigations generally having to justify why, to those not doing so having to justify why not.

Keywords: Emotion; Memory; Sex difference; Amygdala

Introduction

Emotionally arousing events tend to be better retained than do relatively neutral events. On this point overwhelming evidence concurs. A long-standing question is: why? Multiple factors are, of course, likely involved. But extensive research involving both animals and humans over the past 50 years has identified what appears to be the core of an endogenous "memory modulating" system—an interaction between stress hormones and brain structure (most notably the amygdala) that amplifies memory storage when it would be adaptive to amplify memory storage, namely, after emotionally arousing events (McGaugh, 2004). One goal of this chapter is to briefly summarize this work.

The past decade witnessed a striking, and I think largely unanticipated, development: the rapid accumulation of evidence for significant sex influences on the brain's stress-hormone based memory modulating system. It was unanticipated because investigators of emotional memory for the most part possess the same conceptual blinders as do most investigators in our field regarding sex influences on the brain. A second goal of this chapter is therefore to describe how these new developments are impacting not only the study of emotional memory, but also neuroscience in general.

The chapter will progress in three phases. The first will briefly summarize evidence for a neural "memory modulating" system whereby emotion

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influences memory storage. The second will discuss developments in the past decade revealing evermore sex influences on this modulatory mechanism. The final section will consider the sex influence issue more generally and argue that essentially no domain of neuroscience may safely ignore the issue any longer. It will also argue that the burden of proof as regards sex influences on brain function has shifted, from those now addressing the issue in their research to those not yet doing so.

Modulation of memory storage

Abundant evidence now points to the existence of an endogenous memory storage modulating mechanism in both men and women (discussed in great detail elsewhere, e.g., McGaugh, 2004), and is briefly outlined here. Extensive evidence from animal research identifies two key "players" that, working together, influence the strength of memory storage for emotional events. These players are (1) endogenous stress hormones (both the andrenomedullary and the adrenocortical) known to be released during and after emotionally stressful events and (2) the amygdala, in particular its basolateral region. McGaugh and colleagues first showed in the 1970s that endogenous stress hormones could modulate (enhance or impair, depending upon many factors such as dose) memory consolidation processes in animals (McGaugh, 2004). Stress hormones, in turn, appear to act through the basolateral amygdala, which has proven remarkably critical to the memory-enhancing and memory-impairing effects of all drugs and hormones to date. The basolateral amygdala appears to be a "door" through which all drugs and hormones must go to influence memory. The basolateral amygdala, in turn, appears to modulate memory storage elsewhere in the brain, such as in the hippocampus, striatum, and neocortex (McGaugh, 2004). This view fits very well with the known anatomical connectivity of the primate amygdala. A meta-analysis of cortico-cortical connectivity in the monkey by Young and Scannell (1994) revealed that the amygdala is remarkably, and uniquely, well suited to widely modulate mnemonic functions in the rest of the brain (see Fig. 1).

More recent evidence strongly suggests that the same "memory modulation" system exists in humans. For example, we showed that a post-learning stressor (cold pressor stress, or CPS, induced by arm immersion in ice water) both elevated cortisol levels in healthy subjects and enhanced memory for emotional information learned just before the stressor (Cahill et al., 2003). These results, and others like them, led us to propose a new concept, namely that endogenous stress hormones do not enhance memory for all recently acquired information; rather, that they somehow interact with the degree of arousal associated with initial encoding of information to modulate memory storage only for that information.

Amygdala activity and emotional memory in humans–emergence of sex effects

Cahill et al. (1996) first established a relationship between activity of the amygdala at encoding of emotional material and subsequent memory success, a finding predicted by "memory modulation" concept of amygdala function derived from animal research. Male subjects received PET scans for regional cerebral glucose while viewing either some emotionally arousing films or some closely matched, but essentially neutral films. Memory for the films was tested 3 weeks later, allowing the investigators to relate amygdala activity at encoding to subsequent memory success (in fact, the first "subsequent memory" study in the human imaging literature). The results showed that amygdala activity related significantly and selectively to long-term recall of the emotional material. Although subsequent studies from several laboratories confirmed the general finding vis-à-vis the amygdala, I noted that those studies reporting effects predominantly or exclusively in the right hemisphere amygdala utilized only male subjects, whereas those studies reporting left hemisphere amygdala utilized only female subjects.

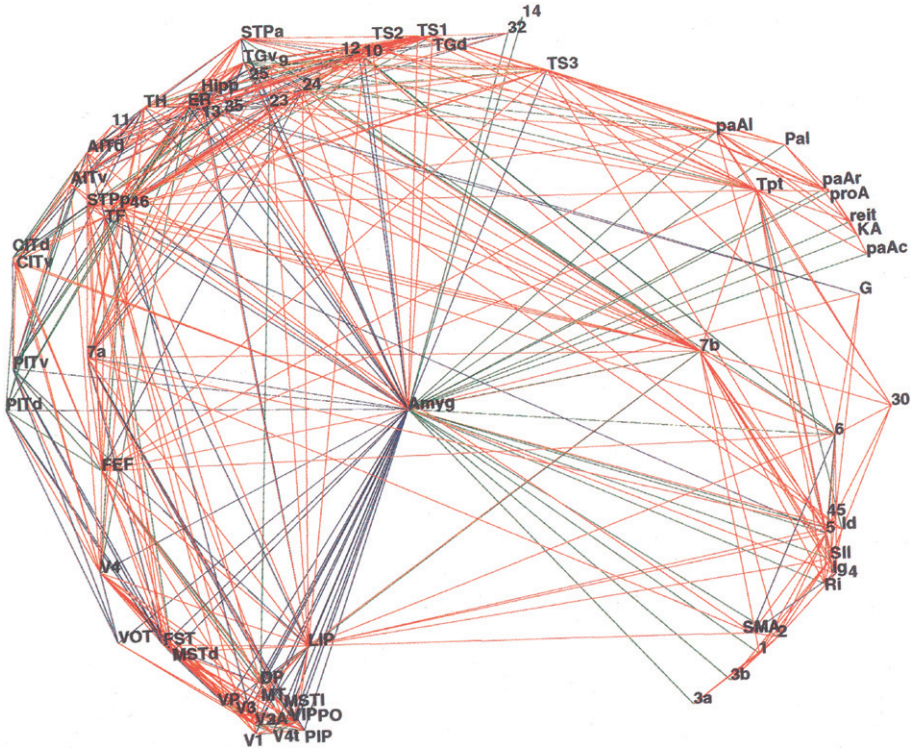

Fig. 1. Amygdala is built to widely modulate. Results of a meta-analysis of cortico-cortical connectivity by Young and Scannell (1994). Note that the vast majority of the amygdala connections are amygdalofugal.

To determine whether subject sex was influencing lateralization of the amygdala relationship to long-term memory for emotional material, we scanned men and women for regional cerebral glucose while they watched a series of emotionally arousing films, and again while they watched a series of more emotionally neutral films (Cahill et al., 2001) and assessed memory 3 weeks later. The results indicated that right hemisphere amygdala activity significantly related to enhanced memory for the emotional film clips in men, whereas left hemisphere amygdala activity related to enhanced memory for the emotional films in women. Shortly thereafter, Canli and colleagues (2002) confirmed this sex-related lateralization in an fMRI study of amygdala function.

Cahill et al. (2004) provided the single strongest demonstration of the effect to date. Using fMRI, they found that right hemisphere amygdala related significantly to subsequent memory for emotional pictures more in men than in women, but left hemisphere amygdala activity related significantly to subsequent memory for the emotional pictures more in women than in men (see Fig. 2). These investigators also reported a significant interaction between the variables of sex and hemisphere in the amygdala relationship to long-term memory for emotional material.

Sex difference in human amygdala functional connectivity at rest

To begin addressing the functional implications of the sex-related amygdala lateralization, we sought

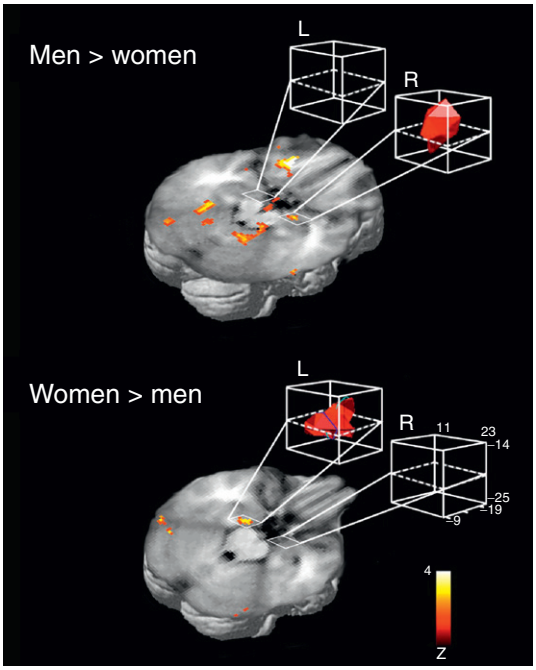

Fig. 2. Sex-related hemispheric lateralization of amygdala function in long-term memory for emotionally arousing films. Activity of the right hemisphere amygdala in males while viewing emotionally arousing films related significantly to memory for the films 2 weeks later. Left hemisphere amygdala in women related significantly to memory for the same films. From Cahill et al. (2004).

to determine whether sex differences exist in the functional connectivity of the human amygdala at rest, before any emotional stimulation is given. If that were the case, we could conclude that the sex difference in response to emotional stimulation likely results, at least in part, from a baseline already differentially "tilted" in the two sexes at rest. To ask this question, we examined the patterns of functional covariance between the left and right hemisphere amygdalae and the rest of the brain in a large sample of men and women given blood-flow PET scans while resting with their eyes closed (Kilpatrick et al., 2006). The results of this analysis revealed that activity of the right hemisphere amygdala covaried to a much larger extent with other brain regions in men than in women;

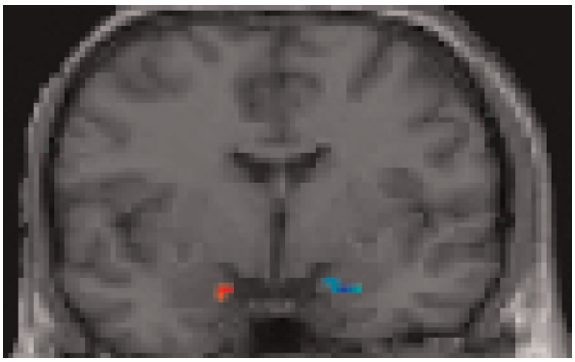

Fig. 3. Amygdala seed voxels displaying significant sex-related differences in amygdala functional connectivity during resting conditions (original figure in color). Red areas, which appeared exclusively in the left hemisphere amygdala, indicate greater functional connectivity with other brain regions in women than in men. Blue areas, which appeared exclusively in the right hemisphere amygdala, indicate greater functional connectivity with other brain regions in men than in women. From Kilpatrick et al. (2006). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this book.)

conversely, activity of the left hemisphere amygdala covaried far more with other brain regions in women than in men. The key result is shown in Fig. 3. Consistent with findings from several earlier investigations, no difference existed between the sexes in the overall levels of amygdala activity; rather, the sexes differed in the pattern of amygdala connectivity with the rest of the brain. The essential findings have been strongly confirmed in heterosexuals by Ivanka Savic and colleagues (e.g., Savic and Lindstrom, 2008).

The issue of sex differences in human brain function at rest has been brought strongly to the forefront of the field by a recent analysis of the functional connectivity of the human brain at rest in a sample of over 1400 people (Biswal et al., 2010). The results of this exceptionally powerful study indicated that subject sex robustly altered the patterns of functional connectivity between many brain regions at rest. Just as the findings of Kilpatrick et al. (2006) and Savic and Lindstrom (2008) indicate that sex may no longer be safely

ignored by investigators of human amygdala function, the findings of Biswal et al. (2010) indicate that the sex can no longer be safely ignored by essentially anyone in the field of human brain imaging.

Relationship of the sex-related amygdala hemispheric specialization to hemispheric global/local processing bias

What might the sex-related hemispheric lateralization of amygdala function in relation to memory mean? One possibility we have pursued concerns memory for the "gist" versus details of an emotional event.

Evidence suggests that the two cerebral hemispheres differentially process global and local aspects of a situation, in particular that the right hemisphere preferentially processes more global, holistic aspects of a situation, but the left hemisphere preferentially processes local, finer detail processing of the same situation (Beeman and Bowden, 2000; Fink et al., 1996, 1999). We integrated the evidence of a sex-related hemispheric laterality of amygdala function in memory for emotional material ("males/right, females/left") with the global/local view of cerebral hemisphere function ("holistic/right, detail/left") to create a specific, testable theory of how an amygdala-based modulatory system may differentially influence emotional memory in men and women. Given that (1) the ability of the amygdala to modulate memory depends on β-adrenergic function (McGaugh, 2004) and (2) each amygdala overwhelming is connected with its own hemisphere (Young and Scannell, 1994), we hypothesized that a β-adrenergic receptor antagonist given to men before they view an emotional story should impair the presumed modulatory effect of the right hemisphere amygdala on the more global processing of the right hemisphere, and thus impair men's memory for the more global (central) aspects of an emotional story. Similarly, we hypothesized that the same antagonist should block the modulatory effect of the left hemisphere amygdala on the more local processing of the left hemisphere in women, and thus reduce women's memory for the details of the same emotional story. We tested this hypothesis by re-analyzing data from two studies demonstrating an impairing effect of β-adrenergic antagonist (propranolol) on memory for an emotionally arousing story (Cahill and van Stegeren, 2003; see Fig. 4). The results revealed a double dissociation of gender and type of to-be-remembered information (central versus peripheral) on propranolol's impairing effect on memory: Propranolol significantly impaired memory of central information in men but not in women, yet impaired memory of peripheral detail in women but not in men (this effect is seen in what is labeled "P2," the story phase containing the emotional elements).

These results are consistent with the view that, under emotionally arousing conditions, activation of right amygdala/hemisphere function disproportionately enhances memory for central (gist) information in males, whereas activation of left amygdala/hemisphere function enhances memory for peripheral details in females. In very recent (as yet unpublished work) from my laboratory, we have confirmed an enhancement of memory for details of this emotional story in naturally cycling women, and of memory for gist in men. Intriguingly, the effect in women is reversed in women taking oral contraception (i.e., their pattern of retention looks like that of men). This finding strongly suggests that sex hormones are crucial to the enhanced detail memory for emotional events in women, and in ways that remain almost wholly unexplored.

The Blinders come off: uncovering influences of sex on mechanisms of emotional memory

Experiments like those described above forced our laboratory to examine—then permanently remove—the conceptual blinders permitting the

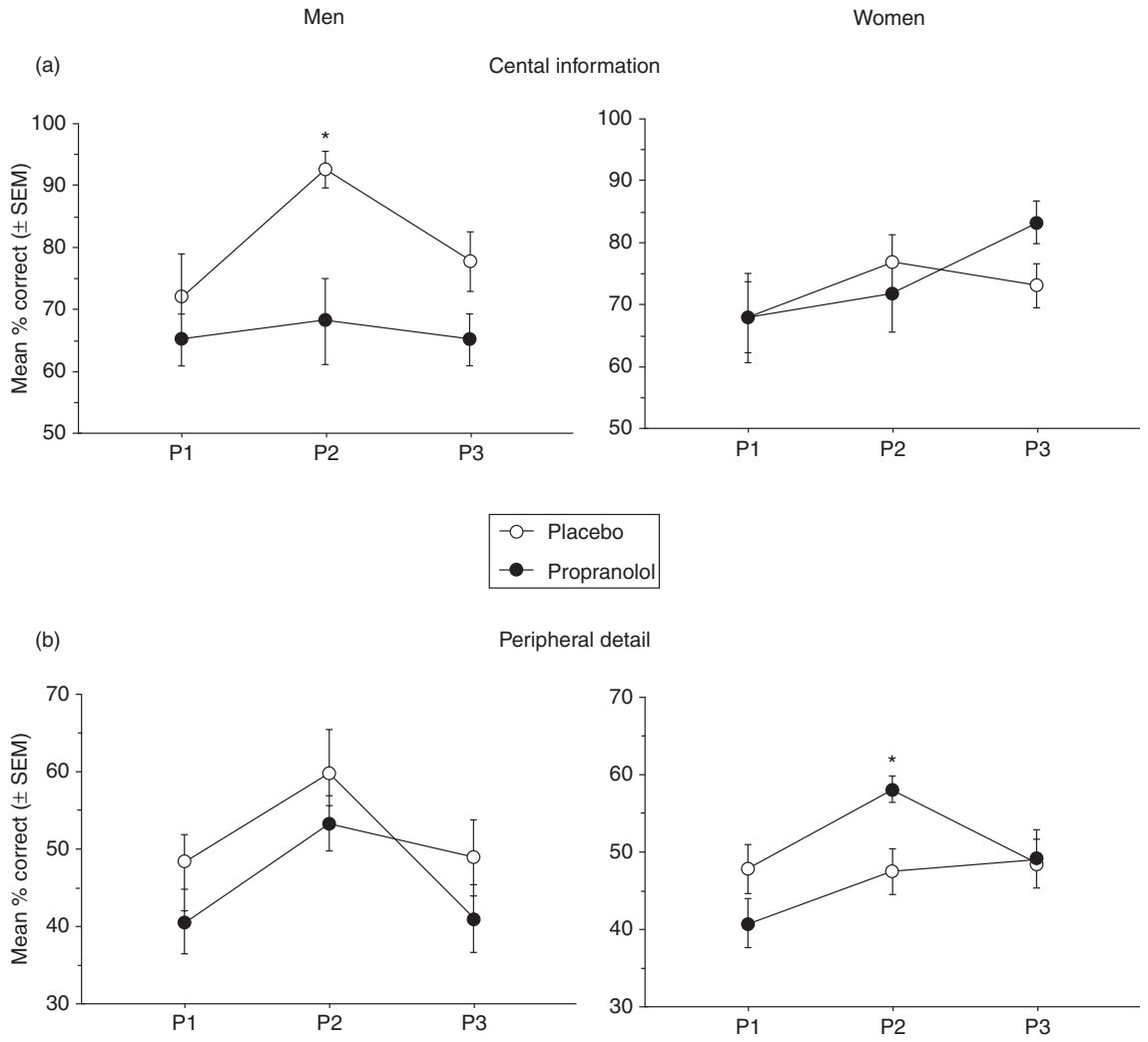

Fig. 4. Recognition test scores for the three-phase emotional story phase. (a) Values for questions defined as pertaining to central information. (b) Values for questions defined as pertaining to peripheral detail. Values represent mean percent correct (±SEM) on the recognition test in each experimental group. P1, P2, and P3 indicate story phases 1, 2, and 3 respectively. Emotional story elements were introduced in P2. The symbol * indicates $p < 0.01$ placebo compared with corresponding P2 propranolol group (post hoc, two-tailed, unpaired *t*-test comparison). From Cahill and van Stegeren (2003).

view that sex matters little, if at all in our investigations, and can thus be safely ignored. As the blinders have fallen, we have uncovered many new, often surprising sex effects (such as that involving oral contraception mentioned above).

As one example, with Antonella Gasbarri and colleagues (2007) we examined EEG responses to emotional and neutral stimuli in healthy men and women. The P300 response was assessed from electrodes located over the left and right

hemispheres as men and women viewed emotional images. For the negative arousing slides, the P300 was greater when recorded over the left hemisphere in women than it was in men. Conversely, it was greater when recorded over the right hemisphere in men than it was in women. Note that the pattern ("women/left, men/right") is similar to that observed in earlier studies regarding the amygdala. Strikingly, despite an enormous P300 literature, no prior investigation to our knowledge had examined P300 responses to emotional stimuli while simultaneously controlling for both sex and hemisphere. Thus no prior study had uncovered this sex by hemisphere lateralization evident within 300 ms of the onset of emotional stimuli processing.

As a second example, we examined whether a post-learning CPS would differentially affect memory consolidation in men and women (Andreano and Cahill, 2006). Healthy men and women received CPS or a control procedure immediately after hearing a short story. Memory for the story was assessed 1 week later. As shown in Fig. 5, CPS enhanced memory in men as compared to controls, but not in women, despite having produced a similar cortisol response in both sexes. Additionally, the effect in men exhibited a classic "inverted-U" relationship between cortisol release by CPS and memory, constituting the first demonstration to our knowledge of an "inverted-U" relationship between endogenous stress hormone release and memory in humans since Yerkes and Dodson first conceived of the "inverted-U" concept in 1908.

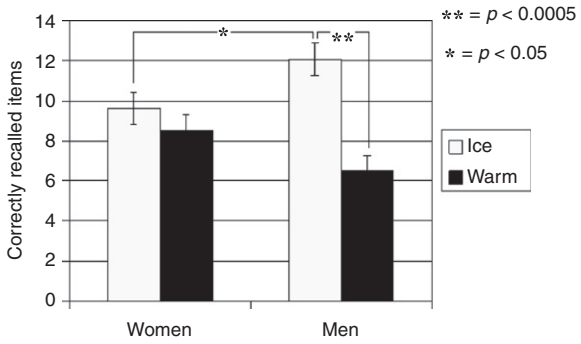

Fig. 5. Effect of post-learning cold pressor stress (CPS) on memory consolidation in men and women. The treatment significantly elevated memory in men as compared to controls, but not in women. From Andreano and Cahill (2006).

Why did CPS fail to enhance memory in women? There are numerous reports of menstrual cycle influences on cognition, including learning (e.g., Milad et al., 2010). Given such evidence, we sought to determine whether influences of menstrual cycle hormones may help explain the overall lack of an enhancing effect of CPS on consolidation in women in our previous study (Andreano and Cahill, 2008). Naturally cycling women listened to the same story used in our previous study, but this time in one of three hormonally defined menstrual cycle phases: (1) early follicular (low estrogen and progesterone); (2) late follicular (significantly elevated estrogen); (3) mid-luteal (significantly elevated progesterone). All subjects received CPS immediately after hearing a short story, and their memory for the story was tested 1 week later. The most critical result concerned the relationship between cortisol release and memory in the different phases. Specifically, cortisol levels in response to CPS did not correlate at all with memory in early-follicular women (low estrogen and progesterone), correlated negatively in late-follicular women (high estrogen, though this relationship only approached significance), and correlated strongly positively in mid-luteal women (high progesterone). Thus, these findings help explain why no overall relationship between cortisol release and memory was detected in our earlier study (Andreano and Cahill, 2006), as that study failed to account for menstrual effects. Perhaps more importantly, the findings are the first to indicate that the well-established effects of stress hormones on memory storage depend crucially upon the levels of circulating sex hormones, a possibility again almost completely unexplored at present by the field. The findings regarding mid-luteal (high

progesterone) women also converge with a recent study, suggesting that progesterone increases amygdala reactivity to emotional stimuli in women (van Wingen et al., 2008).

Sex influences on human brain function generally considered

I turn now to the general issue of sex influences on brain function, including the human brain. Simply put, *sex influences on brain function are ubiquitous*. They have been reported in studies ranging from human behavior literally down to the structure of ion channels, and everywhere in between (Cahill, 2006; Jazin and Cahill, 2010). Even the molecular mechanisms of neuronal apoptosis differ in important ways between the sexes (Li et al., 2005). That sex influences are ubiquitous is of course equally true for non-human mammalian brains, despite the fact that the issue has been grossly understudied by animal investigators, who still overwhelmingly study only males (Beery and Zucker, 2010). Thus the available evidence eliminates the view that sex influences on the human brain somehow arise only from human cultural influences.

Sex differences exist in every brain lobe, including in "cognitive" brain regions such as the neocortex and hippocampus. In human subject work, these discoveries happened in large measure, thanks to the widespread advent of modern imaging techniques, for which both males and females are typically used (in contrast to the vast majority of animal research), and which continue to reveal sex-related differences in brain correlates of many brain functions (Cahill, 2006; Jazin and Cahill, 2010). Interestingly, sex influences are also increasingly reported by those making genetic manipulations in mice. These studies are an exception among animal investigations in that they too often involve both sexes (Jazin and Cahill, 2010).

Some sex differences in the human brain are relatively global in nature, involving widespread brain regions (Luders et al., 2005). Many, however, are local in nature. Collectively, these many sex influences suggest a "mosaic" concept of sex differences in the brain. Many sex influences of many different sizes exist at many different levels of brain function, down to the level of ion channels, all of which undoubtedly interact (Cahill, 2006; Jazin and Cahill, 2010). The inherent complexity belies attempts to create simplistic, all-explaining dichotomies of function between the sexes.

A specific example of a localized sex differences in human brain comes from an MRI study of cortical complexity. Luders et al. (2004) reported that the degree of cortical gyrification was significantly larger in parts of the frontal and parietal cortex in women than in men, but not in other cortical regions. Interestingly, the authors also suggest that the differences may result in part from differential developmental trajectories in the sexes. And in fact, differences in developmental trajectories are among the most striking of sex differences in the human brain (Giedd et al., 1996). Sex differences have even been reported in the effects of both prenatal and postnatal environmental factors on the subsequent size of particular brain regions in adulthood (Buss et al., 2007).

A large challenge for the domain of sex influences on human brain function, as for all of behavioral neuroscience, is to find the behavioral meaning—if any—in neurobiological sex differences. Some progress in this effort is being made. For example, Gur and colleagues (1999) correlated brain gray and white matter to cognitive performance in healthy adults. They confirmed previous findings that women have a higher percentage of gray matter, whereas men have a higher percentage of white matter. They found that both gray and white matter volumes correlated positively with a global index of cognitive ability in both men and women, but that the relationship was much steeper in women, leading the authors to suggest that there exists a more efficient use of white matter in the female brain. No matter the validity of this particular

conclusion, the study represents an area in which far more work is needed, namely, relating sex differences in structure/function in the human brain to behavior.

More rapid progress toward this goal might be achieved if investigators of sex differences also addressed potential influences of cerebral hemisphere in their studies. Indeed, this point was made as long ago as 1964, when Lansdell discovered apparent sex differences in hemispheric asymmetries of mylenization in the human brain, commenting that "the sex of patients is a factor which should be heeded in investigations of the laterality of cerebral function." For example, consider the results of Frings et al. (2006), who examined sex-related differences in activation of the hippocampus during memory processing. Men and women received fMRI scans while performing a virtual spatial memory task. Men and women performed the task equally well, yet the left hemisphere hippocampus was activated in women performing the task, whereas the right hemisphere amygdala was activated in men. This finding may reflect a fundamental difference in brain organization between the sexes, a difference in the use of cognitive strategies, or both. In any case, it illustrates the need to attend to potential influences of sex and hemisphere in imaging studies of human memory.

Are sex influences in the human brain small and unreliable?

Unfortunately, the issue of sex influences on brain function remains subject to widespread, inaccurate biases held by many neuroscientists (Cahill, 2006). For example, many investigators believe that sex differences are small and unreliable. Overwhelmingly, in my experience, they cite two issues they believe to support their view: (1) sex differences in the size/shape of the corpus callosum and (2) sex differences in the functional organization or language. Let us consider each.

It is indeed the case that sex differences in the size/shape of various aspects of the corpus callosum have been much debated, with replication failures being a clear issue, although there exists consensus that some small sex differences in the corpus callosum exist, particularly in its anterior and posterior regions (Luders et al., 2003). Why, however, should investigators invariably cite this example as evidence for a general unreliability of sex differences in brain structure? Why should not investigators instead cite extremely large ($p < 0.000001$) sex differences in the "texture" of white matter (an MRI-based assessment of the orderliness of fibers within white matter, see Kovalev and Kruggel, 2007) as evidence that sex differences in brain anatomy are quite large? Obviously, an excessive focus on any individual sex influence cannot give an accurate overall picture. Crucially, there exists no evidence of which I am aware that the average effect size in the domain of sex differences in brain anatomy/function differs from that seen in other domain of neuroscience, the bias that sex differences are "small" notwithstanding.

A second, supposed example of the unreliability of sex influences on human brain function concerns language. Since a report by Shaywitz and colleagues (1995), considerable attention focused on whether language function, as determined by imaging techniques, is more left-hemisphere dependent in men than it is in women. Although several investigations replicated the essential Shaywitz findings, a few did not. From these failures, it appears, came the view that the key findings were unreliable. However, as convincingly argued by Clements and colleagues (2006), the apparent failures to replicate actually stem from methodological differences between studies. Furthermore, Clements et al. provide their own evidence, as well as a clear literature summary, both strongly pointing to the validity of the conclusion that language is more left lateralized in males. This crucial study was essentially ignored in a recent review whose author apparently preferred to argue that there is no solid evidence for a

sex influence on the degree of language lateralization (Wallentin, 2009).

To reiterate, there is simply no evidence to support the view that sex effects on brain function are, in general, any smaller than are typical effects seen in other domains of brain science. This simple fact merits repeated emphasis, since the best way to counteract the deeply entrenched, harmful, and generally implicit biases against sex influence research among neuroscientists is to make these biases explicit.

What Darwin actually said

One's general conviction about the importance of sex influences for understanding brain function increases the more one accepts what Charles Darwin actually argued about evolution. He explicitly argued that evolution by natural selection alone would fail. Too many facts (most famously the male peacock's tail) could not be explained by the concept. He therefore developed a second concept, originally described in the first edition of *Origin of Species*, which he called "sexual selection." Whereas natural selection, he argued, acted in relation to the fitness of an individual for surviving, sexual selection acted in relation to the fitness of the animal for reproducing. A fascinating history exists concerning the fate of the sexual selection concept (see Cronin, 1991, for an excellent summary), which has been enjoying a resurgence among evolutionary biologists since approximately the 1980s. The relevant point here is that sexual selection, by definition, often acts exclusively or predominantly on one sex or the other. Thus evolution as described by Darwin, involving a complex mix of natural and sexual selection forces, must produce brains of males and females that are a complex mosaic of similarities and differences, big and small. In other words, evolution as described by Darwin should produce exactly what we are finding in the brains of males and females.

Summary

The issue of sex influences on human brain function is rapidly achieving overdue respect from neuroscientists. Sex differences in nervous system function so great that they can negate or even reverse conclusions about brain function depending on which sex is considered. Sex influences exist at essentially all levels of nervous system function. They cannot simply be dismissed as trivial, nor as attributable solely to human culture. These conclusions are equally apparent in the domain of emotional memory, where a number of discoveries in the past decade show that while a basic "memory modulating" system (composed at minimum of stress hormones and the amygdala) exists in both sexes, it does so with some very important, and as yet still vastly understudied, differences.

When one in addition considers the abundant evidence from animal research, it becomes clear that investigators may no longer safely assume that sex influences may be ignored in virtually any study of human brain function. To make progress, the field must challenge the still strong, widespread, and often implicit biases against the issue found in many neuroscientists. Understanding sex influences on brain function will also, of course, be mandatory for fully understanding the many disorders of brain function with established sex differences in their incidence and/or nature.

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CHAPTER 4

Sexual differentiation of the human brain in relation to gender identity and sexual orientation

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Abstract: It is believed that during the intrauterine period the fetal brain develops in the male direction through a direct action of testosterone on the developing nerve cells, or in the female direction through the absence of this hormone surge. According to this concept, our gender identity (the conviction of belonging to the male or female gender) and sexual orientation should be programmed into our brain structures when we are still in the womb. However, since sexual differentiation of the genitals takes place in the first two months of pregnancy and sexual differentiation of the brain starts in the second half of pregnancy, these two processes can be influenced independently, which may result in transsexuality. This also means that in the event of ambiguous sex at birth, the degree of masculinization of the genitals may not reflect the degree of masculinization of the brain.

There is no proof that social environment after birth has an effect on gender identity or sexual orientation. Data on genetic and hormone independent influence on gender identity are presently divergent and do not provide convincing information about the underlying etiology. To what extent fetal programming may determine sexual orientation is also a matter of discussion. A number of studies show patterns of sex atypical cerebral dimorphism in homosexual subjects. Although the crucial question, namely how such complex functions as sexual orientation and identity are processed in the brain remains unanswered, emerging data point at a key role of specific neuronal circuits involving the hypothalamus.

Keywords: Gender identity; Homosexuality; Human brain; Sexual orientation; Sexual differentiation; Transsexuality

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General concepts

Gender identity and sexual orientation represent two fundamental functions in human neurobiology. These functions have hitherto mainly been discussed in relation to the specific signs of sexual dimorphism in the brain and the potential mechanisms thereof. By mapping differences between men and women in cerebral anatomy, function, and neurochemistry, neuroscientists are trying to identify sex typical and sex atypical actors in transsexual and homosexual individuals. This has been done in postmortem analyses of the brain, and investigations of neuronal anatomy, connectivity, and function by means of positron emission tomography (PET) and magnetic resonance imaging (MRI). The extracted networks are then mapped onto those known to be related to sexual behavior in animals to formulate biological underpinnings of homo- and transsexuality in humans. This widely used approach has several difficulties with this approach: (1) gender identity cannot be investigated in animals; (2) sexual behavior in animals is reflex-like and cannot simply be translated to sexual orientation and attraction in humans; (3) reliable sex differences in the human brain require investigations of large populations and have only recently been demonstrated reliably; (4) the majority of studies on sex differences do not account for sexual orientation of the investigated participants; (5) studies of homo- and transsexual persons are very limited, and only few comparisons have hitherto been presented between homo- and transsexual subjects.

An alternative and parallel approach is pinpointing the specific neuronal networks related to gender identity and sexual orientation, analyzing the factors programming these networks and possible differences between control, homo-, and transsexual subjects. Emerging fMRI and PET studies suggest that sexual arousal is mediated by specific core neuronal networks, which may be also involved in sexual orientation.

Sexual organization and activation of the human brain

The process of sexual differentiation of the brain brings about permanent changes in brain structures and functions via interactions of the developing neurons with the environment, understood in its widest sense. The environment of a developing neuron is formed by the surrounding nerve cells and the child's circulating hormones, as well as the hormones, nutrients, medication, and other chemical substances from the mother and the environment that enter the fetal circulation via the placenta. Along with the genetic code, all these factors may have a lasting effect on the sexual differentiation of the brain.

The testicles and ovaries develop in the sixth week of pregnancy. This occurs under the influence of a cascade of genes, starting with the sex-determining gene on the Y chromosome (*SRY*). The production of testosterone by a boy's testes is necessary for sexual differentiation of the sexual organs between weeks 6 and 12 of pregnancy. The peripheral conversion of testosterone into dihydrotestosterone is essential for the formation of a boy's penis, prostate, and scrotum. Instead, the development of the female sexual organs in the womb is based primarily on the absence of androgens (Swaab et al., 2003).

Once the differentiation of the sexual organs into male or female is settled, the next thing that is differentiated is the brain, under the influence, mainly, of sex hormones on the developing brain cells. The changes (permanent) brought about in this stage have organizing effects; later, during puberty, the brain circuits that developed in the womb are activated by sex hormones. This paradigm of sexual differentiation of the brain was coined by Phoenix et al. (1959) and has dominated the view on cerebral sex dimorphism during the last decades.

The fetal brain is protected against the effect of circulating estrogens from the mother by the protein α-fetoprotein, which is produced by the fetus

and binds strongly to estrogens but not to testosterone (Bakker et al., 2006, 2008). However, not only estrogens reach the brain via circulation, but the brain itself is capable of producing estrogens. In human beings testosterone may thus not only have a direct effect on a masculine brain, but, once converted into estrogens by aromatase, may also act on developing neurons. In addition, there are sex differences in brain steroid receptor distribution not only in adulthood (Ishunina and Swaab, 2008; Kruijver and Swaab, 2002; Kruijver et al., 2001; Swaab et al., 2001) but also during development (Chung, 2003), which may be genetically determined. In addition, in rat hormone receptor genes a sex difference in methylation pattern occurs during development (Schwarz et al., 2010). In rats, the formation of estradiol in the brain by aromatization of circulating testosterone is the most important mechanism for virilization of the brain (Gorski, 1984), but, as seen below, it does not determine human gender identity or sexual orientation.

There may also be direct genetic effects that affect the sexual differentiation of the brain without involving the sex hormone receptors.

Sex hormones and human brain development

During fetal development, the brain is influenced by sex hormones such as testosterone, estrogens, and progesterone (Swaab, 2004). From the earliest stages of fetal brain development, many neurons throughout the entire nervous system already have receptors for these hormones (Chung, 2003). The early development of boys shows two periods during which testosterone levels are known to be high. The first surge occurs during mid-pregnancy: testosterone levels peak in the fetal serum between weeks 12 and 18 of pregnancy (Finegan et al., 1989) and in weeks 34–41 of pregnancy the testosterone levels of boys are ten times higher than those of girls (De Zegher et al., 1992; Van de Beek et al., 2009). The second surge takes place in the first three months after birth. At the end of pregnancy, when the α-fetoprotein level declines, the fetus is more exposed to estrogens from the placenta, this exposure inhibiting the hypothalamus–hypophyseal–gonadal axis of the developing child. Loss of this inhibition once the child is born causes a peak in testosterone in boys and a peak in estrogens in girls (Quigley, 2002). The testosterone level in boys at this time is as high as it will be in adulthood, although a large part of the hormone circulates bound. Also at this time the testosterone level is higher in boys than in girls. During these two periods, therefore, girls do not show high levels of testosterone. These fetal and neonatal peaks of testosterone, together with the functional steroid receptor activity, are, according to the current dogma, thought to fix the development of structures and circuits in the brain for the rest of a boy's life (producing "programming" or "organizing" effects). Later, the rising hormone levels that occur during puberty "activate" circuits and behavioral patterns that were built during development, in a masculinized and de-feminized direction for male brains or in a feminized and de-masculinized direction for female brains.

The brain structure differences that result from the interaction between hormones and developing brain cells are thought to be the major basis of sex differences in a wide spectrum of behaviors, such as gender role (behaving as a man or a woman in society), gender identity (the conviction of belonging to the male or female gender), sexual orientation (heterosexuality, homosexuality, or bisexuality), and sex differences regarding cognition, aggressive behavior, and language organization. Factors that interfere with the interactions between hormones and the developing brain systems during development in the womb may permanently influence later behavior.

As sexual differentiation of the genitals takes places much earlier in development (i.e., in the first two months of pregnancy) than sexual differentiation of the brain, which starts in the second half of pregnancy and becomes overt upon

reaching adulthood, these two processes may be influenced independently of each other. In rare cases, these two processes may be incongruent, providing one possible mechanism for transsexuality, that is, people with male sexual organs who feel female or vice versa. It also means that in the event of an ambiguous sex at birth, the degree of masculinization of the genitals may not always reflect the degree of masculinization of the brain (Hughes et al., 2006; Swaab, 2004, 2008). In addition, gender identity may be determined by prenatal hormonal influences, even though the prenatal hormonal milieu might be inadequate for full genital differentiation (Reiner, 1999).

Programmed gender identity is irreversible

The irreversibility of programmed gender identity is clearly illustrated by the sad story of the John–Joan–John case (i.e., the case of David Reimer). In the 1960s and 1970s, in the context of the concept of behaviorism, it was postulated that a child is born as a *tabula rasa* and is subsequently forced in the male or female direction by society's conventions. Although it is true that, in humans, self-face recognition appears to emerge at around 18 months of age (Keenan et al., 2000) and that by the age of 2–3 years children are able to correctly label themselves and others according to gender (Ahmed et al., 2004), there is no evidence that external or social events might modify these processes. However, J. Money argued that "Gender identity is sufficiently incompletely differentiated at birth as to permit successful assignment of a genetic male as a girl. Gender identity then differentiates in keeping with the experiences of rearing" (Money, 1975). This view had devastating results in the John–Joan–John case (Colapinto, 2001). Money maintained that gender imprinting does not start until the age of 1 year, and that its development is well advanced by the age of 3–4 years (Money and Erhardt, 1972). This was, indeed, the basis for the decision to make a girl out of an 8-month-old boy who lost his penis due to a mistake during minor surgery (i.e., an operation to correct phimosis). The testicles of this child were removed before he reached the age of 17 months in order to facilitate feminization. The child was dressed in girls' clothes, received psychological counseling, and was given estrogens in puberty. According to Money, this child developed as a normal female. However, Milton Diamond later made it clear that this had not been the case at all. In adulthood, this child changed back to male, married, and adopted several children (Diamond and Sigmundson, 1997). Unfortunately, he had a troubled life and committed suicide in 2004. This story illustrates the enormous programming influence of the intrauterine period on gender. Other cases have been described in the literature (Bradley et al., 1998), due to enzymatic disorders (al-Attia, 1996; Cohen-Kettenis, 2005; Praveen et al., 2008) or to cloacal exstrophy (Reiner, 2005), that support the existence of early permanent programming of brain sex by biological factors and androgen exposure, rather than by social environment and learning (Jürgensen et al., 2007; Swaab, 2004).

The mechanism of sexual differentiation of the brain: neurobiological factors

In male rats, testosterone is turned into estrogens by local aromatization in the brain, and these estrogens then masculinize certain brain areas. This finding agrees with the observation that, in partially androgen insensitive (testosterone feminized—Tfm) male rats, no reversion of the sex difference was present in the preoptic area (Gorski, 1984) and the bed nucleus of the stria terminalis (Garcia-Falgueras et al., 2005). These animals retained a male neuroanatomy. Other brain nuclei, such as the posteromedial amygdala, the ventromedial hypothalamus, and the locus coeruleus were, however, feminized in Tfm male rats (Morris et al., 2005; Zuloaga et al., 2008).

In humans, however, the main mechanism appears to involve a direct effect of testosterone

on the developing brain. Complete androgen insensitivity syndrome is caused by mutations in the receptor gene for androgens. Despite their genetic (XY) masculinity, affected individuals develop as phenotypical women and experience "heterosexual" sexual orientation, fantasies, and experiences, without gender problems (Wisniewski et al., 2000). On the other hand, when a male fetus has a 5α-reductase-2 or 17β-hydroxy-steroid-dehydrogenase-3 deficiency preventing peripheral testosterone from being transformed into dihydrotestosterone, a "girl" with a large clitoris is born. These children are generally raised as girls. However, when testosterone production increases in these XY children during puberty, this "clitoris" grows to penis size, the testicles descend, and the child's build begins to masculinize and become muscular. Despite the fact that these children are initially raised as girls, the majority (60%) change into heterosexual males (Cohen-Kettenis, 2005; Hughes et al., 2006; Imperato-McGinley et al., 1979; Praveen et al., 2008; Wilson et al., 1993), apparently due to the organizing effect of testosterone on early brain development. Boys who are born with a cloacal exstrophy—that is, with bladder exstrophy and a partly or wholly absent penis—are usually changed into girls immediately after birth. A survey showed that in adulthood only 65% of these children who were changed into girls continued to live as girls, and when individuals with gender dysphoria were excluded, the figure dropped to 47% (Meyer-Bahlburg, 2005; Reiner and Gearhart, 2004). From these examples, it appears that the direct action of testosterone on the developing brain in boys and the lack of it in the developing brain in girls are crucial factors in the development of male and female gender identity and sexual orientation, although other sexually dimorphic functions still need to be investigated in these people. Conversely, studies on cloacal exstrophy suggest that the postnatal testosterone peak is not crucial for gender identity development, given that these children generally undergo operation shortly after birth.

Recent data show that environmental compounds during early development may interfere with sexual differentiation of the human brain. Plastic softeners, that is, phthalate esters, are pervasive environmental chemicals with anti-androgenic effects. Exposure to these compounds is accompanied by reduced masculine play in boys (Swan et al., 2010). Higher prenatal polychlorinated biphenyls (PCB) levels were related with less masculine play in boys, while higher prenatal dioxin levels were associated with more feminized play in boys as well as in girls (Vreugdenhil et al., 2002). The effect of such environmental endocrine disruptors on sexual differentiation of brain systems should be further studied in future.

Sex differences in the human brain

A sex difference in brain weight is already present in children from the age of 2 years (Swaab and Hofman, 1984) and sex differences can thus be expected throughout the brain from early in development. In the adult human brain structural sex differences can be found from the macroscopic level (Goldstein et al., 2001) down to the ultramicroscopic level (Alonso-Nanclares et al., 2008). Functionally, too, a large number of sex differences in different brain regions have recently been described (Allen et al., 2003; Amunts et al., 1999, 2007; Savic, 2005; Savic and Lindstroom, 2008). Sexual differentiation of the human brain is also expressed in behavioral differences, including sexual orientation (homo-, bi-, and heterosexuality) and gender identity (Allen and Gorski, 1992; Hines, 2003; LeVay, 1991; Swaab, 2003), and in differences at the level of brain physiology and in the prevalence of neurological and psychiatric disorders (Bao and Swaab, 2007; Savic and Engel, 1998; Swaab, 2003). In the current review we focus on the sex differences in the human hypothalamus and adjacent areas.

When observed by Swaab's group, the structural difference in the intermediate nucleus of the human hypothalamus (InM) (Braak and

Braak, 1987; Brockhaus, 1942; Koutcherov et al., 2007) was found to be 2.5 times larger in men than in women and to contain 2.2 times as many cells (Swaab and Fliers, 1985). This InM nucleus was at first termed "the sexually dimorphic nucleus of the preoptic area (SDN-POA)" (Swaab and Fliers, 1985). In the preoptic area, Allen et al. (1989) described four interstitial nuclei of the anterior hypothalamus (INAH-1 to 4, while INAH-1 is identical to the InM/SDN-POA) and found a larger volume of the INAH-3 and INAH-2 subdivisions in men compared to women (respectively 2.8 and 2 times greater). The fact that they could not find a sex difference in INAH-1 (InM), as found by Swaab's group (Swaab and Fliers, 1985), could be fully explained by the strong age effect on the sex differences of this nucleus (Swaab, 2003; Swaab and Hofman, 1988). In fact, the sex difference develops only after the age of 5 years and disappears temporarily after the age of 50 years (Swaab and Fliers, 1985; Swaab et al., 1992). Further analysis of INAH-1 galanin cell population in the transsexual people and controls is ongoing and confirms the presence of a clear sex difference in adult controls up to 45 years of age.

The uncinate nucleus (Un) was localized and delineated using three different stainings, that is, thionin, neuropeptide-Y, and synaptophysin. We found sex differences in volume and neuron number in the INAH-3 subdivision while no differences were found for INAH-4 (Fig. 1; Garcia-Falgueras and Swaab, 2008). The presence of a sex difference in INAH-3 volume fully agreed with previously reported data (Allen et al., 1989; Byne et al., 2000, 2001; LeVay, 1991), as did the

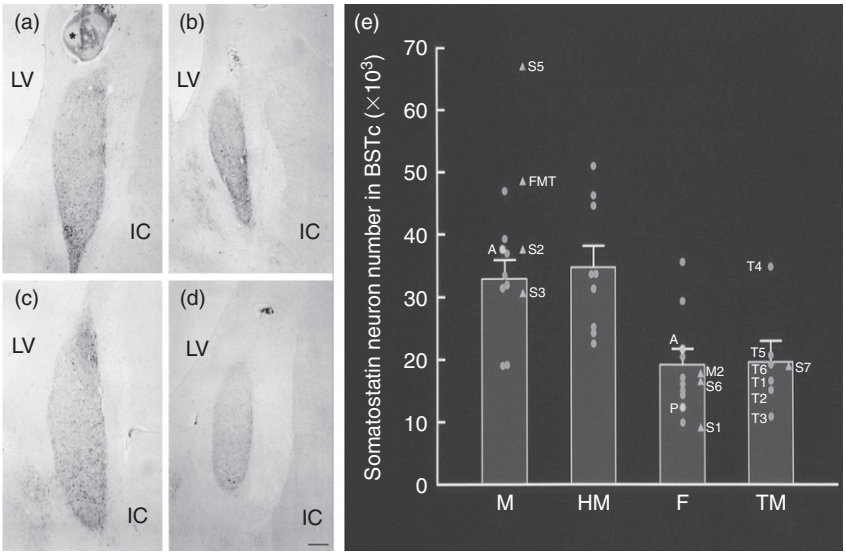

Fig.1. Representative immunocytochemical staining of the somatostatin neurons and fibers in the bed nucleus of the stria terminalis, central subdivision (BSTc) of a reference man (a), a reference woman (b), a homosexual man (c), and a male-to-female transsexual (d). *, Blood vessels; LV, lateral ventricle; IC, internal capsule. Bar represent 0.35 mm. (e) Graph of BSTc number of neurons in different groups according to sexual orientation and gender identity (M, heterosexual male reference group; HM, homosexual male group; F, female reference group; TM, male-to-female transsexual people; T1-T6, transsexual subjects; A, AIDS patient; P, postmenopausal woman; S7, Gender Identity Disorder subject). The sex hormone disorder patients S1, S2, S3, S5, S6, and M2 indicate that changes in sex hormone levels in adulthood do not change the neuron numbers of the BSTc. There is a statistical difference between the M and the TM group ($p < 0.04$) while no difference was between the heterosexual male reference group and the homosexual group. The female to male transsexual (FMT) subject is in the male range. From Kruijver et al. (2000) with permission.

sex difference for the number of neurons in INAH-3. A number of different names have been used to refer to the two Un subnuclei (Garcia-Falgueras and Swaab, 2008): (1) periventricular and uncinate nucleus (the former closer to the third ventricle than the latter) (Braak and Braak, 1987); (2) INAH-4 (closer to the third ventricle than the INAH-3) (Allen et al., 1989); and, most recently, (3) lateral and medial subdivisions of the Un (Koutcherov et al., 2007). In view of the evidence provided by neurochemical markers such as neuropeptide-Y and synaptophysin and the fact that they appear as one structure in some subjects, there are indeed arguments in favor of considering these two subdivisions a single structure called the Un. It has been suggested the INAH-3 was the homologue of the rat central nucleus of the medial preoptic area (Koutcherov et al., 2007) that, in this animal, is clearly related to the brain network for input and output of male sexual behavior (Schober and Pfaff, 2001; Swaab, 2004). On the other hand, the INAH-1 (InM) may be a candidate for that homology. Further research with specific markers is required to solve this issue.

Moreover, similar to the BSTc, the INAH-3 was found in male-to-female (MtF) transsexual people to be small (of female size and cell number), while the INAH-4 subdivision did not show gender-related differences, or any morphological sex difference between men and women (Fig. 1; Garcia-Falgueras and Swaab, 2008). Other sex differences have been found in the human anterior commissure, the interthalamic adhesion and in the corpora mammillaria (Allen and Gorski, 1991; Swaab, 2003).

Sex hormone receptors and neurosteroids

Sex hormone receptors, too, are expressed in a sexually dimorphic way in the human hypothalamus and adjacent areas.

In most hypothalamic areas that show androgen receptor staining, nuclear staining, in particular, is less intense in women than in men. The strongest sex difference was found in the lateral and the medial mammillary nucleus (MMN; Fernandez-Guasti et al., 2000). The mammillary body complex is known to be involved in several aspects of sexual behavior, such as arousal of sexual interest and penile erection (Fernandez-Guasti et al., 2000; MacLean and Ploog, 1962; Swaab, 2003). In addition, a sex difference in androgen receptor staining was present in the horizontal diagonal band of Broca, SDN-POA, medial preoptic area (mPOA), dorsal and ventral zone of the periventricular nucleus (PVN), supraoptic nucleus (SON), ventromedial hypothalamic nucleus, and infundibular nucleus (INF). However, no sex differences were observed in androgen receptor staining in the adult bed nucleus of the stria terminalis (BSTc), the nucleus basalis of Meynert, and the islands of Calleja (Fernandez-Guasti et al. 2000).

No differences related to male sexual orientation were found in nuclear androgen receptor activity in the mammillary complex, this activity not being found to differ in heterosexual men compared with homosexual men, but it was significantly stronger in men than in women. A female-like pattern was found in 26- and 53-year-old castrated men and in intact old men. These data indicate that the amount of nuclear receptor staining in the adult mammillary complex is dependent on the circulating levels of androgens rather than on gender identity or sexual orientation. This idea is supported by the findings that a male-like pattern of androgen receptor staining was found in a 36-years-old bisexual noncastrated MtF transsexual (T6) and a heterosexual virilized woman aged 46 (Kruijver et al., 2001), while a female-like pattern for INAH-3 volume and number of cells was found in the former patient (T6) (Garcia-Falgueras and Swaab, 2008).

Various sex differences have been observed for estrogen receptor α (ERα) staining in the hypothalamus and adjacent areas of young adult human subjects. More intense nuclear ERα immunoreactivity was found in young men compared with young women, for example, in the SDN-POA, the SON, and the PVN. Women showed a stronger nuclear ERα immunoreactivity in the suprachiasmatic nucleus (SCN) and MMN. No sex

differences in nuclear ERα staining were found in, for example, the bed nucleus of the stria terminalis (BSTc), the islands of Calleja (Cal), or the INF. More intense nuclear ERβ staining was found in men in, for example, the neurons of the BSTc, the islands of Calleja, and the InM/SDN-POA. Women showed more nuclear ERβ staining in the SCN, the SON, the PVN, the INF, and the MMN (Ishunina et al., 2007). Observations in subjects with abnormal hormone levels showed, in most areas, ERβ immunoreactivity distribution patterns that were consistent with the level of circulating estrogens, suggesting that the majority of the reported sex differences in ERβ immunoreactivity are "activational" rather than "organizational" in nature (Kruijver et al., 2002, 2003).

In the BSTc, differences in sex hormone receptors such as ERα, ERβ, androgen receptor (AR), and progesterone receptor (PR) are present from fetal age onward. More nuclear ERβ was observed in females than in males during the fetal/neonatal ages, whereas there were no overt sex differences in the other three sex hormone receptors detected. In adult men, ERα and PR immunoreactivity was more pronounced in the BSTc of men than in women (Chung, 2003). Hence, the sensitivity of the BSTc for the different sex hormones depends strongly on sex and age.

Transsexuality

There is a vast array of factors that may lead to gender problems (Table 1). Twin and family research has shown that genetic factors play a part (Coolidge et al., 2002; Gómez-Gil et al., 2010a; Hare et al., 2009; van Beijsterveldt et al., 2006). Rare chromosomal abnormalities may lead to transsexuality (Hengstschläger et al., 2003) and it was found that polymorphisms of the genes for ERα and ERβ, AR repeat length polymorphism and polymorphisms in the aromatase or CYP17 gene also produced an increased risk (Bentz et al., 2008; Hare et al., 2009; Henningsson et al., 2005).

Abnormal hormone levels during early development may play a role, as suggested by the high frequency of polycystic ovaries, oligomenorrhea and amenorrhea in female-to-male (FtM) transsexuals. This observation suggests early intrauterine exposure of the female fetus to abnormally

Table 1. Prenatal factors that influence gender identity (the conviction of being a man or a woman) and that may result in transsexuality

Genetic factors	Rare chromosomal disorders (Hengstschläger et al., 2003)
	Twin studies (van Beijsterveldt et al., 2006; Coolidge et al., 2002; Gómez-Gil et al., 2010a; Hare et al., 2009)
	Polymorphisms in ERβ, androgen receptor, and aromatase genes (Bentz et al., 2008; Hare et al., 2009; Henningsson et al., 2005)
Hormones	Phenobarbital/diphantoin taken by pregnant mother (Dessens et al., 1999)
	Hormones, cloacal exstrophy (Meyer-Bahlburg, 2005; Reiner and Gearhart, 2004)
	5α-reductase-2 or 17β-hydroxy-steroid-dehydrogenase-3 deficiency (Cohen-Kettenis, 2005; Hughes et al., 2006; Imperato-McGinley et al., 1979; Praveen et al., 2008; Wilson et al., 1993)
	Girls with CAH (Dessens et al., 2005; Meyer-Bahlburg et al., 1995, 1996; Zucker et al., 1996)
	Complete androgen insensitivity syndrome results in XY heterosexual females with feminine identity (Wisniewski et al., 2000)
	DES sons: 25% gender problems (http://des-sons.grouply.com/login/)
Immune response	Fraternal birth order (Gómez-Gil et al., 2010b)
Social factors	Postnatally no evidence (Cohen-Kettenis and Gooren, 1999; Colapinto, 2001; Diamond and Sigmundson, 1997; Swaab, 2004)

Abbreviations: CAH, congenital adrenal hyperplasia; DES, diethylstilbestrol.

high levels of testosterone (Padmanabhan et al., 2005). A recent study did not confirm a significantly increased prevalence of polycystic ovary syndrome. However, there was a significantly higher prevalence of hyperandrogynism in FtM transsexuals, also indicating the possible involvement of high testosterone levels in transsexuality (Mueller et al., 2008). A girl with congenital adrenal hyperplasia (CAH), who has been exposed to extreme levels of testosterone in utero, will also have an increased chance of becoming transsexual. Although the likelihood of transsexuality developing in such cases is 300–1000 higher than normal, the risk for transsexuality in CAH is still only 1–3% (Zucker et al., 1996), whereas the probability of serious gender problems is 5.2% (Dessens et al., 2005). The consensus is, therefore, that girls with CAH should be raised as girls, even when they are masculinized (Hughes et al., 2006).

Epileptic women who were given phenobarbital or diphantoin during pregnancy also have an increased risk of giving birth to a transsexual child. Both these substances change the metabolism of the sex hormones and can act on the sexual differentiation of the child's brain. In a group of 243 women who had been exposed to such substances during pregnancy, Dessens et al. (1999) found three transsexual children and a few others with less radical gender problems; these are relatively high rates for such a rare condition. On the "DES" (diethylstilbestrol, an estrogen-like substance—see later) children's website they claimed that transsexuality occurs in 35.5% and a gender problem in 14% of the DES cases (links GIRES and DES SONS webpages). This is alarming, but needs, of course, to be confirmed in a formal study. There are no indications that postnatal social factors could be responsible for the occurrence of transsexuality (Cohen-Kettenis et al., 1998).

In addition, homosexual MtF transsexual people were found to have a later birth order and more brothers than sisters (Gómez-Gil et al., 2010b), suggesting the presence of immunological processes during pregnancy directed toward products of the Y chromosome.

It should be noted that only in 23% of cases does a childhood gender problem lead to transsexuality in adulthood. With regard to sexual orientation, the most likely outcome of childhood gender identity disorder is homosexuality or bisexuality (Cohen-Kettenis and Gooren, 1999; Coolidge et al., 2002; Wallien and Cohen-Kettenis, 2008). Moreover for the diagnosis of transsexuality other disorders inducing temporal transsexual desires—such as bipolar psychosis, schizophrenia, and personality disorders—should be excluded (à Campo et al. 2003; Habermeyer et al., 2003; Mouaffak et al., 2007).

Transsexuality and the brain

The theory on the origins of transsexuality is based on the fact that the differentiation of sexual organs takes place during the first couple of months of pregnancy, before the sexual differentiation of the brain. As these two processes have different timetables, it is possible, in principle, that they take different routes under the influence of different factors. If this is the case, one might expect to find, in transsexuals, female structures in a male brain and vice versa, and indeed, we did find such reversals in the central nucleus of the BSTc and in the INAH-3 (Figs. 1 and 2), two brain structures that, in rats, are involved in many aspects of sexual behavior. However, a gender identity test for rats does not exist, and this hypothesis can therefore be studied only in humans.

We found a clear sex difference in the human BSTc and INAH-3. In men, the BSTc area was twice that found in women and contained twice as many somatostatin neurons (Garcia-Falgueras and Swaab, 2008; Kruijver et al., 2000; Zhou et al., 1995). The same was true of the INAH-3, which was found to be 1.9 times larger in men than in women and to contain 2.3 as many neurons (Fig. 2; Garcia-Falgueras and Swaab, 2008). In relation to sexual orientation, no difference was found in the size or number of neurons in the BSTc area, while for the INAH-3 the volume has previously been found to be related to sexual

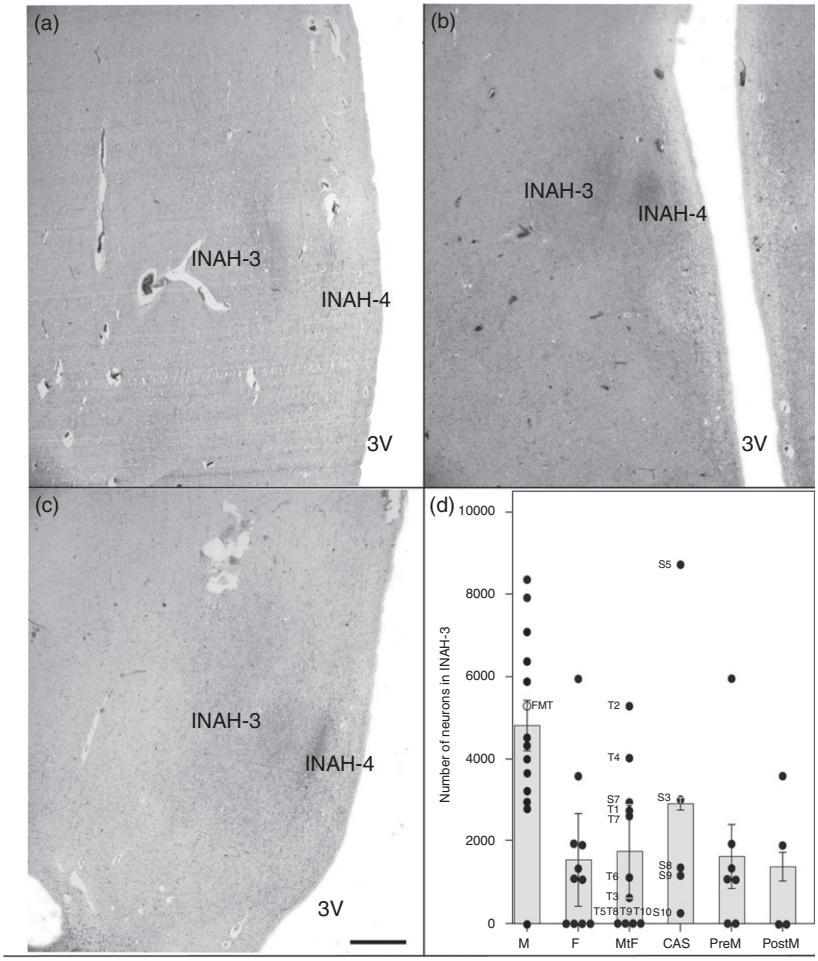

Fig. 2. Representative immunocytochemical staining of the NPY innervation of the uncinate nucleus (INAH-3 and INAH-4) of a reference man (a), a reference woman (b), and a male-to-female transsexual (c). Note that the size is larger in the male group (a) than in the other two groups (b and c). Bar represent 500 mm. (d) Distribution of the INAH-3 number of neurons among different groups according to their gender identity and hormonal changes in adulthood. M, control male group; F, control female group; MtF, male-to-female transsexual group; CAS, castrated male group; PreM, premenopausal women; PostM, postmenopausal women T1-T10, transsexual subjects; S3, S5, S8, S9, S10, castrated subjects because of prostate cancer. Bars represent means and standard errors of the mean. Statistically significant differences were found between men (M) and women (F) ($p < 0.029$) and between men (M) and male-to-female transsexual MtF groups ($p < 0.002$). The female to male transsexual subject (FTM), in the male group, had a masculine INAH-3 number of neurons and the untreated S7 subject, in the MtF group, had a similar number of neurons to the other transsexuals examined. (a, b, c and d) Adapted from Garcia-Falgueras and Swaab (2008) with permission.

orientation, being larger in heterosexual than in homosexual men (Byne et al., 2001; LeVay, 1991). In MtF transsexuals, we found a completely female BSTc and INAH-3. Until now, we have only been able to obtain material from one FtM transsexual, and his BSTc and INAH-3 indeed turned out to have all the male characteristics. We were able to exclude the possibility that the reversal of sex differences in the BSTc and INAH-3 were caused by changing hormone levels in adulthood

(Garcia-Falgueras and Swaab, 2008; Kruijver et al., 2000; Zhou et al., 1995), and it therefore seems that we are dealing with a developmental effect. Our observations thus support the above-mentioned neurobiological theory about the origin of transsexuality. The size of the BSTc and the INAH-3 and their number of neurons match the gender that transsexual people feel they belong to, and not the sex of their sexual organs, birth certificate or passport. Unfortunately, the sex difference in the BSTc volume does not become apparent until early adulthood (Chung et al., 2002), meaning that this nucleus cannot be used for early diagnosis of transsexualism.

One person we studied had untreated male gender dysphoria (S7), took no hormones and kept his transsexual feelings under wraps. He appeared to have a large INAH-3 volume—in the male range—but a female INAH-3 number of neurons (Garcia-Falgueras and Swaab, 2008; Fig. 2d) and a female BSTc somatostatin neuron number (Kruijver et al., 2000). Hence, this individual's hypothalamic characteristics were mid-way between male and female values.

In transsexual MtF patients who receive hormonal treatment, some intermediate values, between those typical for men and women, have been found for lateralization and cognitive performance (Cohen-Kettenis et al., 1998). Recently, functional reversals have been reported in the brains of transsexual people. A PET study in non-homosexual MtF transsexual people (i.e., erotically attracted to women), who were not treated hormonally, showed that a number of brain areas in the transsexual hypothalamus were activated by pheromones in a sex-atypical way. Although the functional reactions in the hypothalamus to an estrogen-derived pheromone were predominantly female, MtF transsexual people also showed some characteristics of a male activation pattern (Berglund et al., 2008). Also studies of mental rotation task, in which men typically outperform women, showed an "in-between" pattern in MtF transsexuals. Compared to control males, the activation in MtF transsexuals during the task was, like in female controls, lower in the superior parietal lobe. MtF transsexuals differed, however, also from the females, and showed higher activation in orbital and right dorsolateral prefrontal regions and lower activation in the left prefrontal gyrus. Interestingly, the reduced parietal activation in MtF transsexuals was correlated with years of estrogen treatment (Carrillo et al., 2010), suggesting that a major reason for the observed "female feature" could have been the hormone supplement treatment. When viewing erotic stimuli, MtF transsexuals before treatment tended to display female-like cerebral processing on functional magnetic resonance imaging (fMRI). The core network consisting of the occipitotemporal cortex, anterior cingulate cortex, medial prefrontal cortex, pre- and postcentral cortex, thalamus, hypothalamus, and bilateral amygdala was activated in males, females, and MtF transsexuals. The three latter regions, however, were more activated in male controls than in female controls and MtF transsexuals (Gizewski et al., 2009). One possible explanation could be that both females and MtF transsexuals reported a lower degree of sexual arousal, and particularly the hypothalamus activation is reported to arousal-dependent. Transsexual persons have recently been investigated with diffusion tensor imaging (DTI), which measures fractional anisotropy (FA) and provides information about neuronal fiber tracts. The study showed significantly higher FA values in the medial and posterior parts of the right superior longitudinal fasciculus (SLF), the forceps minor, and the corticospinal tract in male controls and FtM transsexuals compared to control females (Rametti et al., 2010). In contrast to these two studies, which suggested sex atypical parietal activations and fronto-parietal neuronal connections, no difference from sex matched controls were detected in a comparative study of regional gray and white matter volumes, with exception for an increase in gray matter volume in the left putamen in MtF transsexuals compared to both male and female controls (Luders et al., 2009). Recently, Savic and coworkers combined voxel-based

morphometry and structural volumetry to find that MtF transsexuals have reduced structural volumes of the putamen and thalamus compared to both male and female controls. In addition, their gray matter fraction in the right insular cortex, and the right temporo-parietal junction was larger than in both control groups. Together, these anatomical findings question the dogma that transsexual persons simply have an inverted sex dimorphism of the brain in relation to their biological sex. The findings also raise question as to whether transsexuality may be associated with changes in the cerebral networks involved in self-perception—the temporo-parietal junction, the thalamus, and the insular-inferior frontal cortex (Northoff et al., 2006).

Sexual orientation: heterosexuality, homosexuality, and bisexuality

Sexual orientation in humans is also determined during early development, under the influence of our genetic background and factors that influence the interactions between the sex hormones and the developing brain (Table 2).

The apparent impossibility of getting someone to change their sexual orientation is a major argument against the importance of the social environment in the emergence of homosexuality, as well as against the idea that homosexuality is a lifestyle choice. The mind boggles at the methods used in the attempt to bring about changes in sexual orientation: hormonal treatments such as castration, administration of testosterone or estrogens (treatments that appeared to affect libido but not sexual orientation); psychoanalysis; apomorphine administered as an emetic in combination with homoerotic pictures; psychosurgery (lesions in the hypothalamus); electroshock treatment; chemical induction of epileptic insults and imprisonment. As none of these interventions has led to a well-documented change in sexual orientation (LeVay, 1996), there can be little doubt that our sexual orientation is fixed by the time we reach adulthood and is beyond further influence. Changes in sexual orientation in adulthood have been described—for example, from heterosexual to pedophile—but only in cases of brain tumors in the hypothalamus and prefrontal cortex (Burns and Swerdlow, 2003; Miller et al., 1986). However, these devastating changes in the hypothalamus are too large to interpret them in terms of functional changes in particular neuronal circuits. There are also claims that pedophiles and homosexual men have switched to heterosexual behavior as a result of stereotactical psychosurgery (lesions in the nucleus ventromedialis) (Dieckmann and Hassler, 1977), but these interventions are not only ethically questionable, they also do not meet any

Table 2. Prenatal factors that may influence sexual orientation (homosexuality, heterosexuality, bisexuality)

Genetic factors	Twin studies (Bailey and Bell, 1993; Bockalandt and Vilain, 2007; LeVay and Hamer, 1994) Molecular genetics (Swaab, 2004)
Hormones	Girls with CAH (Meyer-Bahlburg et al., 1995, 1996; Swaab, 2004; Zucker et al., 1996) DES (Cohen-Kettenis et al., 1998; Ehrhardt et al., 1985; Swaab, 2004)
Chemical factors	Prenatal exposure to nicotine, amphetamines, or thyroid medication (Ellis and Cole-Hardin, 2001; Ellis and Hellberg, 2005)
Immune response?	Homosexual orientation in men is most likely to occur in men with a large number of older brothers (Blanchard, 2001; Bogaert, 2003)
Social factors?	Stress in the mother during pregnancy (Bailey et al., 1991; Bogaert, 2003; Ellis et al., 1988) Being raised by transsexual or homosexual parents does not affect sexual orientation (Green, 1978)

Abbreviations: CAH, congenital adrenal hyperplasia; DES, diethylstilbestrol.

scientific standards. There are also some recent reports postulating that the sexual orientation of homosexual women, more than that of homosexual men, may sometimes change, either spontaneously or under the influence of psychotherapy (Spitzer, 2003). The effectiveness of therapy and the absence of bisexuality has, however, never been convincingly demonstrated in these cases.

The presence of a substantial genetic component in the development of sexual orientation is apparent from family and twin studies (Bailey and Bell, 1993; Bocklandt and Vilain, 2007). However, exactly which genes play a role is not yet clear. According to LeVay and Hamer (1994), the size of the genetic component in homosexuality for both sexes is over 50%. A number of genetic studies have suggested maternal transmission, indicating X-linked inheritance. The X chromosome has accumulated genes involved in sex, reproduction, and cognition. A meta-analysis of four linkage studies suggested that Xq28 plays an important role in male homosexuality (Hamer et al., 1993). However, 16 years after the initial findings the exact genes involved have not yet been identified (Bocklandt and Vilain, 2007). A different technique also indicated a role for the X chromosome in male sexual orientation. Women with gay sons appeared to have an extreme skewing of X-inactivation when they are compared to mothers without gay sons (Bocklandt et al., 2006). Although this unusual methylation pattern supports a possible role of the X chromosome in male homosexuality, its mechanism of action is far from clear. Given the complexity of the development of sexual orientation, it is likely to involve many genes. A genome-wide linkage screening indeed identified several chromosomal regions and candidate genes for further exploration (Mustanski et al., 2005).

Whatever the exact nature of the genetic factor, it is interesting that such a factor has stayed present in the population throughout human history, given that homosexuals do not tend to procreate as much as the rest of the population. A good explanation could be that the genetic factors that are responsible for homosexuality also have a beneficial effect on the procreation of the population. Indeed, Camperio Ciani et al. (2004) have found that women on a homosexual male's mother's side tend to be more fertile. This antagonistic inheritance that promotes fecundity in females and a homosexual orientation in males is partly linked to the X chromosome (Iemmola and Camperio Ciani, 2009).

Abnormal hormone levels originating from the child itself during intrauterine development may influence sexual orientation, as is apparent from the large percentage of bisexual and homosexual girls with CAH (Meyer-Bahlburg et al., 1995, 1996; Zucker et al., 1996). Between 1939 and 1960, some two million pregnant women in the United States and Europe were prescribed diethylstilbestrol (DES) in order to prevent miscarriage. DES is an estrogen-like substance that actually turned out not to prevent miscarriage; furthermore, it also found, in small dosages, not only to give a slightly elevated risk of cervical cancer but also to increase the chance of bisexuality or lesbianism in adult woman (Ehrhardt et al., 1985; Meyer-Bahlburg et al., 1996; Titus-Ernstoff et al., 2003) although this was not confirmed in an other study (Ellis et al., 1988).

The chance that a boy will be homosexual increases with the number of older brothers he has. This phenomenon is known as the fraternal birth order effect and is putatively explained by an immunological response by the mother to a product of the Y chromosome of her sons. The chance of such an immune response to male factors would increase with every pregnancy resulting in the birth of a son (Blanchard, 2001; Bogaert, 2003). Prenatal exposure to nicotine, amphetamine, or thyroid-gland hormones increases the chances of giving birth to lesbian daughters (Ellis and Cole-Harding, 2001; Ellis and Hellberg, 2005). A stressed pregnant woman has a greater chance of giving birth to a homosexual son (Ellis and Cole-Harding, 2001; Ellis et al., 1988) or a lesbian daughter (Bailey et al., 1991) (Table 2).

Although it has often been postulated that postnatal development is also important for the

direction of sexual orientation, there is no solid proof for this. On the contrary, children who were born after artificial insemination with donor sperm and who were raised by a lesbian couple are heterosexually oriented (Green, 1978). There is also no proof for the idea that homosexuality is the result of a deficient upbringing, or that it is a "lifestyle choice" or an effect of social learning (LeVay, 1996). It is curious, therefore, that some children are still forbidden to play with homosexual friends, an unthinkable attitude left over from the idea that homosexuality is "contagious" or can be learned.

Sexual orientation and the brain

Clinical observations have shown the involvement of a number of brain structures in sexual orientation. It has been reported that in some patients with Klüver-Bucy syndrome, which involves lesions of the temporal lobe, orientation changed from heterosexual to homosexual. Shifts in sexual orientation (to homosexual and pedophile) have also been reported in connection with tumors in the temporal lobe and hypothalamus. Lesions in the preoptic area of the hypothalamus in male rodents, such as ferrets and rats, produce shifts in sexual orientation (Swaab, 2003). Lesions of the same structure in their female conspecifics do not change sexual behavior. Instead, female rats become aggressive toward male intruders and start approaching their female conspecifics upon lesion of the ventromedial hypothalamic nuclei (Kindon et al., 1996; Leedy, 1984; Paredes and Baum, 1995).

Of interest is also that male rat knockouts lacking Ca-TRP channels (TRPC2), which are necessary for pheromone signal transduction, do not approach to fertile females, but do mount male rats (Zufall, 2005). These data have two implications: first, intact pheromone signal detection, as well as an intact hypothalamic transduction seems necessary for heterosexual behavior. Second, the hypothalamic nuclei mediating sexual behavior seem, at least in some rodents, to differ between males and females. The exact function of the these nuclei is not well known, but it seems to be crucial for the approach to a sexual partner, since it is implicated in the recognition and integration of sensory stimuli such as sexual clues, in arousal mechanisms and in copulatory behavior and its motor expression (Schober and Pfaff, 2007; Swaab, 2003).

Several structural and functional differences in the brain have been described in relation to sexual orientation (for a review see Swaab, 2008). Swaab's group found the first difference in the SCN, or brain clock, which turned out to be twice as large in homosexual compared with heterosexual men (Swaab and Hofman, 1990). In an experiment with rats a similar difference could be induced, by pharmacologically disturbing the interaction between testosterone and the developing brain around the time of birth, using the aromatase inhibitor 1,4,6-androstatrien-3,17-dione (ATD) in the neonatal period. This experiment yielded bisexual adult rats, which had larger numbers of cells in their SCN (Swaab et al., 1995). The difference in the SCN was therefore not caused by a change in sexual behavior, as was suggested at the time, but by a disturbed interaction between sex hormones and the developing brain. In 1991, LeVay reported that homosexual men, just like heterosexual women, have a smaller volume of hypothalamic nucleus (INAH-3) (LeVay, 1991). No differences were found in the BSTc volume or number of somatostatine neurons in homosexual men compared to heterosexual men (Kruijver et al., 2000; Zhou et al., 1995). In 1992, Allen and Gorski reported that the anterior commissure of homosexual men is larger than that of heterosexual men (Allen and Gorski, 1992). This structure, which is larger in women than in men, takes care of left–right connections within the temporal cortex and is thus involved in sex differences in cognitive abilities and language. The difference in its size may possibly be related to the sex-atypical hemispheric asymmetries observed in homosexual men and homosexual women by Savic and Lindström (2008). Witelson et al. (2008) recently reported that the isthmal

area of corpus callosum was larger in the homosexual compared to heterosexual men, which also could contribute to the observed differences in hemispheric asymmetry.

Emerging studies with functional imaging show differences in the hypothalamus activation in relation to sexual orientation. The first brain imaging paper to point out differences in the hypothalamus in relation to sexual orientation by means of fluorodeoxy glucose (FDG)—PET, by Kinnunen et al. (2004), did not receive much scientific or public attention, although it may have clinical consequences. The hypothalamus of homosexual men turned out not to be as responsive to a classic antidepressant (fluoxetine) as that of heterosexual men, which suggests a different kind of activity of the serotonergic system. Savic et al. (2001) used androstadienone, a pheromone-like compound derived from progesterone and excreted in perspiration in concentrations. Smelling of this compound activated the hypothalamus of heterosexual women and homosexual men in the same way, but did not elicit any hypothalamus response in heterosexual men. Apparently in heterosexual men the hypothalamic pathway is not stimulated by a male body-scent, which suggests that pheromone-like compounds in humans may contribute to determining our behavior in relation to our sexual orientation (Savic et al., 2005). In a follow-up study (Berglund et al., 2006), lesbian women, as compared to heterosexual women, reacted in a sex-atypical, almost reciprocal way (Fig. 3). These observations, too, show that there are hypothalamic circuits that function in a way that depends on our sexual orientation. The hypothalamic circuits are incorporated in the core network system for sexual arousal (Karama et al., 2002). Interestingly, when balancing for the degree of sexual arousal, this network seems similar in homo- and heterosexual subjects. Just like the pheromone responding core network, the triggering stimulus is reciprocal in homosexual compared to heterosexual subjects. Indeed, viewing erotic videos of heterosexual or homosexual content produced activation in the hypothalamus, detectable by fMRI, but only when subjects were

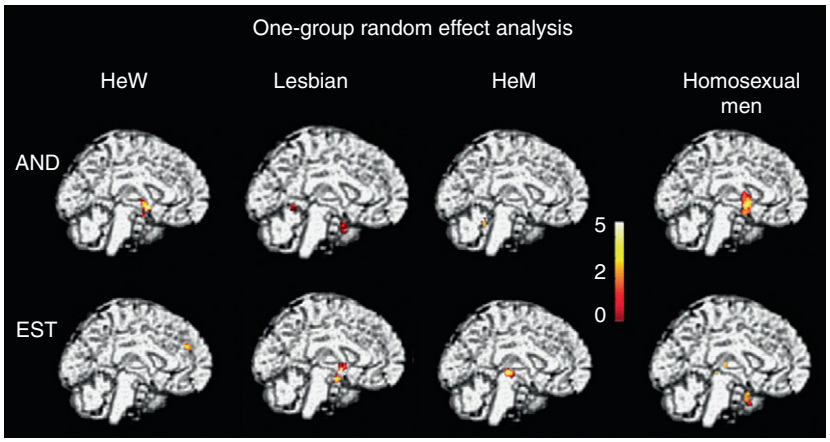

Fig. 3. Illustration of group-specific activations with the two putative pheromones (AND and EST). AND, androstadienone. EST, estratetraenol, is derivative of estrogene. The Sokoloff color scale illustrates *Z*-values reflecting the degree of activation (0.0–5.0). Because the same brain section is chosen, the figures do not always illustrate maximal activation for each condition (*Upper*). Cerebral activation during smelling of AND and EST. Clusters of activated regions are superimposed on the standard MRI brain (midsagittal plane). HeW, heterosexual women; HeM, heterosexual men. Note that there are hypothalamic circuits that function in a way that depends on our sexual orientation. From Berglund et al. (2006) with permission. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this book.)

viewing videos of their respective sexual orientation (Paul et al., 2008). Accordingly Ponseti et al. (2006, 2009) found that neuronal response of the ventral striatum and the centromedian thalamus was stronger to prefer relative to non-preferred stimuli. Using fMRI, Kranz and Ishai found that face perception is modulated by sexual preference. Looking at a female face made the thalamus and medial prefrontal cortex of heterosexual men and homosexual women react more strongly, whereas in homosexual men and heterosexual women these structures reacted more strongly to the face of a man (Kranz and Ishai, 2006). A sexual-orientation-related difference in processing neuronal networks was suggested only by Hu et al. (2008). However, their subjects viewed erotic film involving mixed and same sex couples, evoking different levels of sexual arousal and disgust in homo- and heterosexual subjects, which may account for the detected differences. While being compelling in pinpointing the neuronal circuits for sexual attraction and arousal, these data cannot explain why the object of arousal differs.

Savic's previous studies raised the question of whether certain sexually dimorphic features in the brain, which are unlikely to be directly involved in reproduction, may differ between homosexual and heterosexual individuals. This issue was explored by studying hemispheric asymmetry, using volumetric MRI, and functional connectivity of the amygdala, using PET measurements of cerebral blood flow (Savic and Lindström, 2008). Volumetric measurements in heterosexual men and homosexual women showed a rightward cerebral asymmetry, whereas the volumes of the cerebral hemispheres were symmetrical in homosexual men and heterosexual women (Savic and Lindström, 2008). Moreover, homosexual subjects also showed sex-atypical amygdala connections. In homosexual men, as in heterosexual women, the connections were more widespread from the left amygdala. In homosexual women and heterosexual men, on the other hand, they were more widespread from the right amygdala. Furthermore, in homosexual men and heterosexual women the connections displayed were primarily with the contralateral amygdala and the anterior cingulate, while in heterosexual men and homosexual women the connections displayed were primarily displayed with the caudate, putamen, and the prefrontal cortex (Savic and Lindström, 2008). In verbal fluency and other verbal skills a lesbian group presented different values from the other three groups (heterosexual woman, heterosexual man, and homosexual man) (Rahman et al., 2003). Moreover dichotic listening performance has also been found to show a greater right ear advantage in heterosexual men as compared to heterosexual women, while lesbian women were somewhat masculinized in their functional cerebral asymmetry (Rahman and Koerting, 2008). Interestingly, lesbian women were recently found to have less gray matter bilaterally in the temporo-basal cortex, ventral cerebellum, and left ventral premotor cortex in relation to heterosexual women (Ponseti et al., 2009).

Together, these later studies suggest a linkage between sexual orientation and neurobiological entities that cannot be primarily linked to reproduction.

Conclusions

The human fetal brain becomes sex differentiated through direct hormone-independent effects of X and Y chromosome genes or through different levels of gonadal hormones during both prenatal and postnatal periods. The latter pathway is more powerful. By a direct action of testosterone the fetal brain develops into the male direction, and in absence of this hormone into the female direction. During the intrauterine period, gender identity (the conviction of belonging to the male or female gender), sexual orientation, cognition, aggression, and other behaviors are programmed in the brain in a sexually differentiated way. Sexual differentiation of the genitals takes place in the first two months of pregnancy, whereas sexual differentiation of the brain starts in the second half of

pregnancy. This means that in the event of an ambiguous sex at birth, the degree of masculinization of the genitals may not reflect the degree of masculinization of the brain.

Our observations on reversed sex differences in the brains of transsexual people support the idea that transsexuality, at least to some extent, is based on an opposite sexual differentiation of (1) sexual organs during the first couple of months of pregnancy and (2) the brain in the second half of pregnancy. There is no proof that the social environment after birth has an effect on the development of gender or sexual orientation, while the possible effects on sexual differentiation of the brain by endocrine disrupters in the environment and in medicines given to the pregnant mother should be investigated.

The differences observed in the INAH-3 in relation to sexual orientation and gender identity and this structure's possible connection with the BSTc suggest that these two nuclei and the two earlier described nuclei that were found to be related to gender and sexual orientation, that is, the SDN-POA (= InM = INAH-1) and SCN, are all part of a complex network involved in various aspects of sexual behavior. Neurobiological research on sexual orientation and gender identity in humans is only just gathering momentum, but the evidence shows that humans have a vast array of brain differences, related not only to gender, but also to sexual orientation. There is a need for further multidisciplinary research on the putative influence of testosterone in development, for example, in individuals with complete androgen insensitivity syndrome.

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