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Regional volumes and spatial volumetric distribution of gray matter in the gender dysphoric brain

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Abstract The sexual differentiation of the brain is primarily driven by gonadal hormones during fetal development. Leading theories on the etiology of gender dysphoria (GD) involve deviations herein. To examine whether there are signs of a sex-atypical brain development in GD, we quantified regional neural gray matter (GM) volumes in 55 female-to-male and 38 male-to-female adolescents, 44 boys and 52 girls without GD and applied both univariate and multivariate analyses. In girls, more GM volume was observed in the left superior medial frontal cortex, while boys had more volume in the bilateral superior posterior hemispheres of the cerebellum and the hypothalamus. Regarding the GD groups, at whole-brain level they differed only from individuals sharing their gender identity but not from their natal sex. Accordingly, using multivariate pattern recognition analyses, the GD groups could more accurately be automatically discriminated from individuals sharing their gender identity than those sharing their natal sex based on spatially distributed GM patterns. However, region of interest analyses indicated less GM volume in the right cerebellum and more volume in the medial frontal cortex in female-to-males in comparison to girls without GD, while male-to-females had less volume in

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the bilateral cerebellum and hypothalamus than natal boys. Deviations from the natal sex within sexually dimorphic structures were also observed in the untreated subsamples. Our findings thus indicate that GM distribution and regional volumes in GD adolescents are largely in accordance with their respective natal sex. However, there are subtle deviations from the natal sex in sexually dimorphic structures, which can represent signs of a partial sex-atypical differentiation of the brain.

1. Introduction

During a critical period of fetal and neonatal development, gonadal hormones are thought to primarily drive the sexual differentiation of the brain, sculpting brain morphology into a gender-typical configuration. In genetic males, the testes secrete high levels of testosterone during fetal life, rendering serum levels in the adult male range. Testosterone can be converted to 17-β-estradiol by the enzyme aromatase, and animal studies have demonstrated that pre- and perinatal estradiol acts as the principal regulator of the sexual differentiation of the rodent brain (Bakker and Baum, 2008; McCarthy, 2008). Cerebral masculinization is precluded in genetic females by the estrogen-binding activity of alpha-fetoprotein, a plasma protein secreted by the fetal liver in high concentrations (Bakker et al., 2006; Bakker and Baum, 2008). Manipulating the exposure to sex steroid hormones during a critical period of prenatal and early postnatal life substantially perturbs the sex-specific differentiation of the brain (Bakker et al., 2006; Bakker and Baum, 2008).

In humans, pre- and perinatal neuroendocrine factors are also thought to primarily regulate the sexual differentiation of the brain. Interestingly, individuals (46,XY) with complete androgen insensitivity syndrome, who have non-functional androgen receptor proteins due to a mutation in the AR gene, are born phenotypically female, are sexually attracted to men and have a female gender identity (Wisniewski et al., 2000; Hines et al., 2003), suggesting that in humans masculinization may be directed by testosterone rather than testosterone-derived estradiol. Although the sexual differentiation of the brain is believed to primarily take place during prenatal development, circulating hormones during adolescence exert activational effects and further potentiate neural sexual dimorphisms. In addition to gonadal hormone effects, direct effects of genes residing on the sex chromosomes may also contribute to the sexual differentiation of the brain (Arnold, 2004; Carruth et al., 2002).

In gender dysphoria (GD) there is a persistent incongruence between a person's natal sex and the experienced gender identity. Leading theories on the etiology of this condition involve a sex-atypical cerebral programming that diverges from the sexual differentiation of the rest of the body, postulated to reflect the organizational effects of altered levels of sex steroid hormones during a specific period of fetal development (Bao and Swaab, 2011; Cohen-Kettenis and Gooren, 1999; Savic et al., 2010; Swaab, 2007). Some twin studies suggest a role for genetic factors in the development of GD, potentially involving polymorphisms in genes encoding elements of the sex steroid signaling or metabolic pathways (Heylens et al., 2012).

Postmortem studies have observed sex-atypical volumes and neuronal numbers in hypothalamic nuclei in the brains of individuals with GD (Garcia-Falgueras and Swaab, 2008; Kruijver et al., 2000; Zhou et al., 1995). Brain structure in individuals with GD has also been investigated *in vivo*. MRI studies examining white matter tracts using diffusion tensor imaging have shown deviations from the natal sex in white matter microstructure in both female-to-males (FM) and male-to-females (MF) (Rametti et al., 2011a,b). A few neuroimaging studies have investigated gray matter (GM) in individuals with GD, observing both regional volumetric properties in line with the natal sex and others in line with the gender identity of the GD groups (Luders et al., 2009b, 2012; Savic and Arver, 2011; Zubiaurre-Elorza et al., 2012; Simon et al., 2013). A recent study by Zubiaurre-Elorza et al. (2012) was the first to examine GM in FM (Zubiaurre-Elorza et al., 2012). Interestingly, they observed signs of subcortical masculinization in female-to-male adults and of cortical feminization in male-to-female adults, providing the first insights into the developmental processes underlying GD in both sexes.

In our study, we wanted to examine brain anatomy in individuals with GD during adolescence, when puberty-related sex steroid hormones are driving the further 'activational' sexual differentiation of the brain rather than in adulthood, when both organizational and activational steroid hormone effects have already sculpted the brain into a sex-specific configuration. Therefore, we acquired high-resolution anatomical brain MRI scans of 55 female-to-male adolescents and 38 male-to-female (MF) adolescents with GD. In addition, to allow comparisons with individuals sharing either their gender identity or their natal sex, we also scanned 44 boys and 52 girls without GD going through adolescence.

First, we aimed to define sexually dimorphic structures in the adolescent brain by comparing GM volume in boys and girls without GD. As the principal aim of our study was to examine whether signs of sex-atypical cerebral programming are present in the brains of GD adolescents, we subsequently compared their regional GM volumes to individuals sharing either their gender identity or their natal sex. To ensure that the deviations from the natal sex did not result from hormone therapy, we repeated these analyses in the subsamples of GD participants who had never been exposed to any form of hormonal treatment.

Finally, considering the recent development of sophisticated machine learning algorithms for MRI analyses, we also wanted to explore the data beyond the classical mass-univariate statistical approach. Especially relevant for the study of individuals with GD is that multivariate pattern recognition approaches provide a tool to quantify the