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# Brain functional connectivity patterns in children and adolescents with gender dysphoria: Sex-atypical or not?

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ABSTRACT

Various previous studies have reported that brains of people diagnosed with gender dysphoria (GD) show sex-atypical features. In addition, recent functional magnetic resonance imaging studies found that several brain resting-state networks (RSNs) in adults with GD show functional connectivity (FC) patterns that are not sex-atypical, but specific for GD. In the current study we examined whether FC patterns are also altered in prepubertal children and adolescents with GD in comparison with non-gender dysphoric peers. We investigated FC patterns within RSNs that were previously examined in adults: visual networks (VNs), sensorimotor networks (SMNs), default mode network (DMN) and salience network. Thirty-one children (18 birth assigned males; 13 birth assigned females) and 40 adolescents with GD (19 birth assigned males or transgirls; 21 birth assigned females or transboys), and 39 cisgender children (21 boys; 18 girls) and 41 cisgender adolescents (20 boys; 21 girls) participated. We used independent component analysis to obtain the network maps of interest and compared these across groups. Within one of the three VNs (VN-I), adolescent transgirls showed stronger FC in the right cerebellum compared with all other adolescent groups. Sex differences in FC between the cisgender adolescent groups were observed in the right supplementary motor area within one of the two SMNs (SMN-II; girls > boys) and the right posterior cingulate gyrus within the posterior DMN (boys > girls). Within these networks adolescent transgirls showed FC patterns similar to their experienced gender (female). Also adolescent transboys showed a FC pattern similar to their experienced gender (male), but within the SMN-II only. The prepubertal children did not show any group differences in FC, suggesting that these emerge with aging and during puberty. Our findings provide evidence for the existence of both GD-specific and sex-atypical FC patterns in adolescents with GD.

## 1. Introduction

Gender dysphoria (GD) refers to distress due to an incongruence between one's experienced gender and one's sex assigned at birth (American Psychiatric Association, 2013). The onset of GD can occur before, during, or after puberty (Nieder et al., 2011). However, in many prepubertal children GD remits after the first pubertal stages (Kreukels and Cohen-Kettenis, 2011). Children in whom GD is persistent often experience increasing distress at the onset of puberty, during which the development of secondary sex characteristics is initiated (Kreukels and Cohen-Kettenis, 2011). In the Netherlands, children diagnosed with GD can be treated with gonadotropin-releasing hormone analogues (GnRHas) when they are about 12 years or older and have entered the first stages of puberty (Tanner breast development stage two in girls and Tanner genital development stage two in boys), to prevent further maturation of sex characteristics (Hembree et al., 2009; Marshall and Tanner, 1970, 1969). Therefore, adolescents who fulfill these criteria are referred to a pediatric endocrinologist for the eventual initiation of GnRHa treatment shortly after the diagnosis GD is established. While global functioning and symptoms of depression usually improve after the initiation of puberty suppression, levels of GD (as measured by the Utrecht Gender Dysphoria Scale) do not change until the start of cross-

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sex hormone therapy, which is possible from the age of about 16 (De Vries et al., 2014; Hembree et al., 2009).

Although the number of neuroimaging studies in people diagnosed with GD is increasing, there is still little known about the neurobiology of GD. The most common hypothesis is that people with GD have experienced a sex-atypical differentiation of the brain during fetal development (Swaab, 2007). However, while various studies provide support for this hypothesis (Smith et al., 2015), others do not (Feusner et al., 2016; Manzouri et al., 2015; Savic and Arver, 2011; Smith et al., 2015). Recently, several resting-state functional magnetic resonance imaging (fMRI) studies which examined functionally integrated relationships among spatially distributed brain regions (also called functional connectivity (FC)) suggested some new explanations regarding the neurobiology of GD in adults (Burke et al., 2017; Feusner et al., 2016; Lin et al., 2014; Manzouri et al., 2015). These studies specifically focused on brain regions (medial prefrontal cortex, anterior insula, temporo-parietal junction, precuneus) and/or cerebral networks (visual network (VN), sensorimotor networks (SMNs), default mode network (DMN) and salience network (SN)) that were hypothesized to play a role in own-body perception and self-referential thinking. These previous studies found that FC in a group of transmen (female assigned at birth, male gender identity) (Burke et al., 2017; Feusner et al., 2016; Manzouri et al., 2015) or both transmen and transwomen (male assigned at birth, female gender identity) (Lin et al., 2014) differed from cisgender men as well as cisgender women, suggesting that the observed FC alterations in transgender individuals were specific for GD, and thus associated with the subjective experiences of incongruence between gender identity and sex assigned at birth.

One well-known method to analyze FC patterns is independent component analysis (ICA). ICA allows the identification of resting-state networks (RSNs), consistent groups of brain regions that are functionally highly connected to each other in rest (Lee et al., 2013). Virtually all identified RSNs are thought to be involved in multiple cognitive functions (Laird et al., 2011). Thus, the VNs, SMNs, DMN and SN which are hypothesized to play a role in own-body perception, are also involved in other functions such as visual perception of complex (emotional) stimuli (VNs), planning and execution of motor tasks (SMNs), social cognition (DMN), and cognitive-affective processing (SN).

While resting-state fMRI studies in adults with GD have received increasing attention, (f)MRI studies in children with GD are rare, and resting-state studies that highlight the neuro-developmental aspects of GD are currently lacking. Therefore, in the current cross-sectional study, we examined FC patterns in youth with GD and compared these patterns to those of age-matched cisgender boys and girls. Because puberty influences brain maturation and may also affect FC patterns (Rubia, 2013; Solé-Padullés et al., 2016) we included a group of prepubertal children, as well as a group of adolescents. Based on previous studies on FC in adults with GD (Feusner et al., 2016; Lin et al., 2014; Manzouri et al., 2015), we selected the VNs, SMNs, DMN and the SN as our RSNs of interest and hypothesized that prepubertal and adolescent boys and girls with GD show GD-specific FC patterns. We further expected that (cisgender) sex differences in FC patterns would be more prominent or only apparent in the adolescent groups.

## 2. Material and methods

### 2.1. Study design and participants

In this cross-sectional resting-state fMRI study, which was approved by our local ethical committee (trial number: NL31283.029.10), we included prepubertal children and adolescents diagnosed with GD according to the fourth revised edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2000), and age-matched cisgender boys and girls. A written informed consent was obtained from all participants and their legal guardians. Participants with GD were recruited at the Center of Expertise on GD of the VU University Medical Center in Amsterdam. Cisgender participants were recruited at several primary schools in the Netherlands and by inviting friends of the participants with GD. In all participants, except the cisgender adolescent controls, the (pre)pubertal stage was determined by a pediatric endocrinologist according to the Tanner classification (Marshall and Tanner, 1970, 1969). In the cisgender adolescents, the pubertal stage was assessed by means of the five-point Tanner Maturation Scale self-report questionnaire which correlates highly with physician assessments (Morris and Udry, 1980). The prepubertal children with GD received psychological counseling, but no hormone treatment at time of inclusion. The adolescents with GD were included while receiving GnRHa: triptorelin 3.75 mg/four weeks (mean: 23 months; standard deviation: 14 months).

At the day of fMRI scanning, the sexual orientation of each participant was assessed by asking whether they had ever been in love with somebody and whether that person was a boy or a girl. Furthermore, urinary estradiol levels and salivary free testosterone levels were determined in the cisgender adolescents (not in the adolescents with GD, because sex steroids were suppressed due to GnRHa treatment). Cisgender adolescents were asked to collect urine and saliva samples at home, directly after waking up. Samples were brought to the clinic and stored at $-80^{\circ}$ C until analysis. During fMRI scanning, which was already practiced in a mock MR scanner (De Bie et al., 2010), participants were instructed to close their eyes, to lie as still as possible and try not to fall asleep.

Of the initial 81 included prepubertal children, 11 were excluded from analysis: nine because of excessive head motions during scanning (translation of $> 3$ mm or rotation of $> 3^{\circ}$ between fMRI volumes), one because of technical errors during data collection and one because the child no longer fulfilled criteria for a GD diagnosis at time of scanning. Finally, data for analysis were available from 70 prepubertal children: 18 boys with GD, 13 girls with GD, 21 cisgender boys and 18 cisgender girls. Of the initial 83 included adolescents, two were excluded from analysis: one because of a cerebral venous anomaly and one because of excessive head motions. Therefore we used the data of 81 adolescents: 19 boys with GD (transgirls), 21 girls with GD (transboys), 20 cisgender boys and 21 cisgender girls.

For further details with regard to participants and study design see also Burke et al. (2014).

### 2.2. Hormonal assays

After hydrolysis with helix pomatia juice (Pall Biosepra, Cergy-Saint-Christophe, France) and extraction with diethyl ether, urinary estradiol concentration was measured by a competitive immunoassay (Architect, Abbott Laboratories Diagnostics Division, Abbott Park, Illinois, USA). Intra-assay coefficients of variation are 9%, 3% and 4% at levels of 150, 1400 and 9000 pmol/L, respectively and the inter-assay coefficient of variation is 10% for the entire range. Estradiol levels were corrected for creatinine concentration, which was measured by the Jaffé method (Modular, Roche Diagnostics, Mannheim, Germany) with inter-assay coefficients of variation of 2.2% at 5.9 mmol/L and 1.7% at 12.5 mmol/L. Salivary free testosterone levels were determined with an isotope dilution-liquid chromatography-tandem mass spectronomy method. For further details about the analysis see Bui et al. (2013).

### 2.3. Image acquisition

Scans were performed on a whole-body 3.0 T MR scanner (Signa HDXt, General Electric, Milwaukee, WI, USA). T1-weighted images were acquired prior to the resting-state fMRI (repetition time = 7.8 ms, echo time = 3.0 ms, matrix size = 256 × 256, voxel size = 1 × 1 × 1 mm, number of slices = 176). A gradient-echo-planar imaging sequence was used to obtain the T2*-weighted resting-state images (repetition time = 1800 ms, echo time = 35 ms, matrix