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Click-Evoked Otoacoustic Emissions in Children and Adolescents with Gender Identity Disorder

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Abstract Click-evoked otoacoustic emissions (CEOAEs) are echo-like sounds that are produced by the inner ear in response to click-stimuli. CEOAEs generally have a higher amplitude in women compared to men and neonates already show a similar sex difference in CEOAEs. Weaker responses in males are proposed to originate from elevated levels of testosterone during perinatal sexual differentiation. Therefore, CEOAEs may be used as a retrospective indicator of someone's perinatal androgen environment. Individuals diagnosed with Gender Identity Disorder (GID), according to DSM-IV-TR, are characterized by a strong identification with the other gender and discomfort about their natal sex. Although the etiology of GID is far from established, it is hypothesized that atypical levels of sex steroids during a critical period of sexual differentiation of the brain might play a role. In the present study, we compared CEOAEs in treatment-naïve children and adolescents with early-onset GID (24 natal boys, 23 natal girls) and control subjects (65 boys, 62 girls). We replicated the sex difference in CEOAE response amplitude in the control group. This sex difference, however, was not present in the GID groups. Boys with GID showed stronger, more female-typical CEOAEs whereas girls with GID did not differ in emission strength compared to control girls. Based on the assumption that CEOAE amplitude can be seen as an index of relative androgen exposure, our results provide some evidence for the idea that boys with GID may have been exposed to lower amounts of androgen during early development in comparison to control boys.

Keywords Otoacoustic emissions · Gender Identity Disorder · Gender Dysphoria · Sex differences · Androgen · Sexual differentiation

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Introduction

Otoacoustic emissions (OAEs) are echo-like sound waves that originate from the inner ear. These emissions are a by-product of the cochlear amplification mechanism, produced by outer hair cells in the cochlea (Kemp, 2002; Morlet et al., 1996; Rodenburg & Hanssens, 1998). OAEs are classified on the basis of how they are evoked. When they occur without any external stimulus, they are called spontaneous OAEs. OAEs that are evoked in response to click stimuli are called click-evoked OAEs (CEOAEs) (Kemp, 2002). CEOAEs are echo-like sounds that persist in the ear canal for tens of milliseconds following a brief transient stimulus.

Interestingly, researchers have found sex differences in the frequency and emission strength of OAEs (Collet, Gartner, Veuillet, Moulin, & Morgon, 1993; McFadden, Loehlin, & Pasanen, 1996; Moulin, Collet, Veuillet, & Morgon, 1993; Strickland, Burns, & Tubis, 1985). Females appear to generate stronger and higher numbers of OAEs than males. This sex difference in emission strength and frequency is present directly after birth (Aidan, Lestang, Avan, & Bonfils, 1997; Berninger, 2007; Burns, Arehart, & Campbell, 1992; Cassidy & Ditty,

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2001; Driscoll et al., 1999; Kei, McPherson, Smyth, Latham, & Loscher, 1997; Saitoh et al., 2006; Strickland, Burns, & Tubis, 1985; Thornton, Marotta, & Kennedy, 2003). The outer hair cells of the cochlea have been reported to develop between the 9th and 22nd week of gestation (Lavigne-Rebillard & Pujol, 1986; Pujol & Lavigne-Rebillard, 1995), a time window that overlaps with the critical period for sexual differentiation, when testosterone levels in male fetuses are elevated (Finegan, Bartleman, & Wong, 1989). Therefore, it is assumed that the sex difference in OAE amplitude develops as part of the sexual differentiation of the fetus and thus is under the organizational influence of sex steroids.

So far, several studies have suggested that CEOAEs are affected by androgens. Thus, lower amplitude CEOAEs, present in males, are proposed to originate from high prenatal exposure to androgens, which are suggested to diminish emission strength (McFadden, 1993, 1998; McFadden et al., 1996). The dampening effects of androgens on CEOAEs may not be restricted to the prenatal period, but rather extend to and coincide with the peri-/postnatal testosterone surge in male infants (Corbier, Edwards, & Roffi, 1992; Quigley, 2002). For instance, testosterone levels in male infants, assessed between the first 6 months post-natally, have recently been associated with later sex-typed play behavior in children (Lamminmäki et al., 2012). Results from several animal studies support the idea that higher concentrations of androgens, naturally present in males, exert inhibitory effects on CEOAEs. For instance, male and female rhesus monkeys (*Macaca mulatta*), treated with testosterone prenatally, showed weaker (i.e., masculinized) CEOAEs when 5–6 years old, whereas male monkeys that had received androgen receptor blockers during early development had stronger CEOAEs compared to untreated males (McFadden, Pasanen, Raper, Lange, & Wallen, 2006a). Similar hormonal manipulation studies have been conducted in other animal species such as the spotted hyena and sheep (McFadden, Pasanen, Valero, Roberts, & Lee, 2009; McFadden, Pasanen, Weldele, Glickman, & Place, 2006b). In both sexes of both species, prenatal treatment with testosterone had diminishing effects whereas treatment with androgen receptor blockers enhanced CEOAE amplitudes. Even though these animal studies are based on relatively small sample sizes, they suggest that androgens may have organizational and dampening effects on CEOAEs.

There is some indirect evidence from human studies supporting the explanation of prenatal androgen effects on sex differences in OAEs: women with a male co-twin showed a significant masculinization of their auditory system; that is, compared to women having a female co-twin, they exhibited a reduced prevalence of spontaneous OAEs (McFadden, 1993). Later studies confirmed that women with a male co-twin showed more male-typical numbers of spontaneous OAEs (McFadden & Loehlin, 1995) as well as weaker CEOAEs (McFadden et al., 1996); however, apparently due to a lack of statistical power, these effects failed to reach statistical significance. It has been proposed that females, sharing the womb with a male co-twin, are exposed to increased levels of androgen originating from the male fetus, a developmental occurrence observed in many mammalian and rodent species (Rohde Parfet et al., 1990; Ryan & Vandenbergh, 2002; vom Saal, 1989). McFadden (1993), McFadden and Loehlin (1995), and McFadden, Loehlin and Pasanen (1996) did not measure other purportedly masculinized characteristics or behaviors in their female subjects having a male co-twin, next to their relatively masculinized auditory system. However, several other studies found that women with a male co-twin, in contrast to same-sex female twins, showed significantly masculinized behavioral and cognitive traits, as well as more masculine personality traits (Boklage, 1985; Cohen-Bendahan, Buitelaar, van Goozen, & Cohen-Kettenis, 2004; Cohen-Bendahan, Buitelaar, van Goozen, Orlebeke, & Cohen-Kettenis, 2005; Resnick, Gottesman, & McGue, 1993; Slutske, Bascom, Meier, Medland, & Martin, 2011; Vuoksimaa et al., 2010).

Another study compared CEOAEs of men and women with a hetero-, homo- or bisexual orientation (McFadden & Pasanen, 1998). Sexual orientation is thought to be influenced by prenatal biological mechanisms, such as genes and sex steroid actions mediating and affecting the sexual differentiation of the brain (Balthazart, 2011; Dörner, 1988; Williams et al., 2000). McFadden and Pasanen (1998) found significantly weaker CEOAEs in homo- and bisexual women compared to heterosexual women. No effect, however, was found for sexual orientation on CEOAEs in men. When auditory evoked potentials (AEPs) were measured, the OAE differences in heterosexual and non-heterosexual females were replicated and the non-heterosexual males were hypermasculinized compared to heterosexual males (McFadden & Champlin, 2000). Based on the assumption that differences in perinatal androgen exposure underlie adult sexual orientation and other sexually differentiated characteristics, measuring CEOAEs might provide an insight into a person's perinatal sex hormone environment.

Next to the organizational effects sex hormones may exert on OAEs, a few studies addressed possible activational androgen effects on OAE production in humans. Snihur and Hampson (2012b) showed that CEOAE response amplitudes in adult men were negatively correlated to seasonal changes of testosterone levels. Other studies found small changes in OAEs during the menstrual cycle (Bell, 1992; Burns, 2009; Haggerty, Lusted, & Morton, 1993) and reported dampening effects of hormonal contraception on OAEs in women (McFadden, 2000; Snihur & Hampson, 2012a). Although these effects were small to moderate, they suggest, however, that OAE production might be modulated by sex steroid exposure beyond the perinatal period and that the cochlear-amplifier mechanism may be subject to plastic changes in structure and function later in life.

Individuals, diagnosed with Gender Identity Disorder (GID) according to the *Diagnostic and Statistical Manual of Mental Disorders* (American Psychiatric Association, 2000) are characterized by the conviction of being born in the body of the

opposite sex and show a strong discomfort about their natal sex. We will use the term GID instead of Gender Dysphoria, because our study was conducted when DSM-5 (American Psychiatric Association, 2013) was not yet published. It has been hypothesized that atypical levels of perinatal sex steroids during a critical period of sexual differentiation of the brain may be involved in the development of GID (Swaab, 2007; Van Goozen, Slabbekoorn, Gooren, Sanders, & Cohen-Kettenis, 2002). Adults diagnosed with GID often exhibited "early-onset" GID in childhood, which reveals that feelings of gender dysphoria as well as the persistent and profound cross-gender interests and behaviors are present well before puberty. The etiology and biological underpinnings of GID are still largely unknown and may be different for males and females (Cohen-Kettenis, Van Goozen, Doorn, & Gooren, 1998; Schagen, Delemarre-van de Waal, Blanchard, & Cohen-Kettenis, 2012). Female-to-male transsexualism has been linked to the *CYP17* gene (Bentz et al., 2008) whereas male-to-female transsexualism to a polymorphism of the *CAG* repeat length in the androgen receptor (Hare et al., 2009). Although these associations between certain genes and transsexualism have not yet been replicated (Ujike et al., 2009) and may not be applicable to all subtypes of transsexualism (Lawrence, 2010), there is some suggestion that genes regulating sex steroid signaling and steroid receptor functioning are implicated in the development of GID. Based on case reports of twins with GID (for a recent review, see Heylens et al., 2012), it is argued that GID may indeed have a genetic component. However, the significant numbers of monozygotic twins who are discordant for GID support the notion that other factors, such as pre- and postnatal environmental effects, also may play a role in the development of GID. Thus, it is probable that GID is caused by interactions between genetic and environmental events.

The current working hypothesis is therefore that alterations in exposure to sex steroids, potentially due to genetic factors, during a critical period of sexual differentiation of the brain may underlie the strong sense of incongruence of one's gender identity and natal sex. In the attempt to retrospectively evaluate their perinatal hormonal environment, in particular the relative extent of androgen exposure, we measured CEOAEs in a group of children and adolescents diagnosed with GID and compared their emission strengths to those of boys and girls without GID.

Method

Participants

The initial sample consisted of 187 subjects, of whom 13 had to be excluded due to invalid measurements or errors during data collection in both ears. All other 174 subjects had at least one (left or right ear) valid CEOAE measurement. Twenty-four boys and 23 girls, all meeting the DSM-IV-TR criteria for early onset GID, were recruited via the Center of Expertise on Gender Dysphoria at the VU University Medical Center in Amsterdam. Sixty-five boys and 62 girls served as control subjects, who were recruited via several primary and secondary schools in the Netherlands and by inviting friends and relatives of the participants with GID.

At the time of measurement, none of the individuals with GID had undergone any medical intervention (i.e., pubertal suppression with a gonadotropin-releasing hormone analogue or cross-sex hormone treatment) (Cohen-Kettenis, Steensma, & de Vries, 2011; Kreukels & Cohen-Kettenis, 2011). Age of participants ranged from 5.0 to 16.9 years; therefore, several adolescents were already pubertal at the time of measurement. Female adolescents were tested randomly according to their menstrual cycle. By means of a short questionnaire, all participants were screened for current hearing problems, prior ear infections, or other past adverse events that might have compromised current hearing. All children received a small gift as reimbursement for participation in the study. The Ethical Review Board of the VU University Medical Center Amsterdam approved the study and written informed consent was provided by all subjects and their legal guardians.

Procedure and Measures

CEOAE recordings were performed with EZ-screen software and with an Otodynamics echo-port system ILO288, in combination with a laptop computer. The apparatus was calibrated each time it was put online for use. CEOAEs were recorded in five different frequency bands: 1,000, 1,414, 2,000, 2,828, and 4,000 Hz. CEOAEs were recorded in the nonlinear QuickScreen mode with a time window of 2.5 to 12.5 ms. CEOAE responses were measured in terms of decibels sound-pressure level (dB SPL). Each ear was tested with a fixed number of 250 clicks. The average emission of the five frequency bands was used for further analysis and the click-stimulus input was set on approximately 80 dB SPL (range of 77.1–85.0 dB SPL), which is in accordance with clinical protocols for CEOAE recordings (Hall, 2000; Maico Diagnostics, 2009). A probe with an appropriately-sized foam ear tip was placed in the external ear canal, causing minimal discomfort for the participant. The ear tip was carefully placed in the ear canal so as to seal the cavity completely. The probe fit was evaluated by the noise-level rejection meter; CEOAE data were regarded useful when environmental-noise levels did not reach a threshold of 6 mPa. Participants were seated in a comfortable chair and asked to relax their body and face muscles during the recordings in order to ensure a low noise measurement. The testing rooms in the hospital and school buildings used for CEOAE recordings were not fully soundproof. However, environmental noise had no effect on the measurements as long as the participants were relaxed enough to avoid noise production. Because CEOAE responses have been reported to be influenced by the effect of which ear was tested first (Thornton, Marotta, & Kennedy, 2003), we tested all participants' right ears first.

Data Analyses

Only CEOAE measurements with an amplitude of at least 0.99 dB SPL and a reproducibility of more than .70 were considered valid. Whole-wave reproducibility was calculated as the correlation coefficient of two interleaved non-linear response amplitudes (Berninger, 2007); a perfect recording would have a reproducibility of 1.0. All recorded CEOAE measurements were stored in a database and analyzed using the Statistical Package for the Social Sciences, version 20 (SPSS Inc., Chicago, IL). A repeated measures ANOVA was conducted to assess overall group differences in CEOAE amplitude, with Ear tested (left; right) as a within-subject factor and Sex and Group (GID, control) as between-subject factors. Next, sex differences and ear asymmetries in CEOAE data were analyzed separately for both groups (GID; controls). Finally, by means of one-way ANOVA, treating gender as one factor with four levels (control boys; control girls; girls with GID; boys with GID), differences in mean CEOAE amplitude between groups were compared, separately for the left and right ear CEOAE data. Duncan's homogeneous subsets test was applied post hoc, controlling for multiple comparisons between unequal sample sizes. Effects were considered statistically significant at $p < .05$. Cohen's d was reported as an estimate of effect size for a gender difference between means of any two of the four groups, where d was calculated as the difference between two means divided by the pooled SDs of those two means (Cohen, 1977).

Results

Subject characteristics and mean CEOAE amplitudes are shown in Table 1. From the sample of 174 participants tested, we were able to collect a total of 166 valid right ear and 141 valid left ear CEOAE measurements. The groups did not differ significantly with regard to age, although boys with GID were slightly younger compared to the other groups. We therefore explored possible effects of age, by including age (in years) as a covariate in the analyses. CEOAE data were inspected for normality (Kolmogorov–Smirnov test) and for homogeneity of variances (Levene's test). Despite the much smaller sample sizes of the participants diagnosed with GID, data in each group were normally distributed and variances between the groups did not differ significantly from each other. Therefore, normality and homogeneity of variances were assumed.

CEOAE Ear Asymmetries

For CEOAE, a 2 (Sex) × 2 (Group) × 2 (Ear) mixed-model ANOVA revealed a significant main effect for Ear, $F(1, 129) = 15.9$, $p < .001$, and a borderline Group x Ear interaction, $F(1, 129) = 3.5$, $p = .065$. We found no significant main or interaction effects for Sex. t tests showed that CEOAEs obtained from the left and right ears differed significantly in terms of emission strength in these groups: control girls: $t(51) = 3.1$, $p = .003$; girls with GID: $t(17) = 2.4$, $p = .031$; boys with GID: $t(18) = 2.4$, $p = .026$, but not the control boys: $t(43) < 1$.

When within-subject effects of Ear were inspected for the controls and participants with GID separately, a 2 (Sex) × 2 (Ear) ANOVA in the control groups revealed a significant main effect for Ear, $F(1, 94) = 4.1$, $p = .045$, and a borderline Sex by Ear interaction, $F(1, 94) = 3.6$, $p = .060$. The main effect for Sex was not significant, $F(1, 94) = 2.8$, $p = .101$. Thus, we observed a sex difference in ear asymmetry of medium effect size ($d = .39$), with control girls showing stronger right than left ear CEOAEs compared to control boys.

In the subjects with GID, a significant main effect for Ear was found, $F(1, 35) = 11.4$, $p = .002$, whereas the main effect for Sex was not significant, $F(1, 35) < 1$, nor was the Ear by Sex interaction, $F(1, 35) < 1$. Thus, boys and girls with GID showed no significant sex differences in CEOAE amplitude, but both showed significant ear asymmetries, with stronger mean CEOAEs in their right compared to their left ears.

When adding age as a covariate to these ANOVAs for within-subject effects of Ear, the results for the ear asymmetries changed. The 2 (Sex) × 2 (Group) × 2 (Ear) mixed-model ANOVA also showed a borderline Ear by Group interaction, $F(1, 128) = 3.4$, $p = .066$, but no main effect for Ear, $F(1, 128) = 1.0$. Thus, age modulated the CEOAE ear asymmetry across sex and between groups, but showed no significant main effect in itself, $F(1, 128) < 1$.

The 2 (Sex) × 2 (Ear) ANOVA including only the control group, when co-varying age, revealed a borderline main effect of Sex, $F(1, 93) = 2.9$, $p = .090$, and for the Ear by Sex interaction, $F(1, 93) = 3.7$, $p = .058$. Thus, dependent on the ear tested, when co-varying age, control boys and girls show sex differences in CEOAE amplitude.

The 2 (Sex) × 2 (Ear) ANOVA including only participants with GID now revealed a borderline main effect of age, $F(1, 34) = 3.3$, $p = .079$, but again, co-varying for age diminished the former effects of Ear, $F(1, 34) = 1.9$. Also, no main effect for Sex was observed, $F(1, 34) < 1$, in the GID group.

Gender Differences in Right Ear Emissions

In order to investigate sex differences in CEOAE amplitude between groups, one-way ANOVAs were conducted, separately for the right ear and left ear CEOAE data.

Mean CEOAE amplitudes of the right ear data differed significantly between the four groups, $F(3, 165) = 5.6$, $p = .001$ (see Fig. 1). Duncan's post hoc test showed that the boys with GID neither differed significantly from the control boys nor from the control girls and girls with GID. In contrast, the control boys had significantly lower response amplitudes than the two natal female groups. Effect size calculations confirmed the significant sex difference in right ear CEOAE

Table 1 Mean CEOAE response amplitudes 1–4 kHz (in dB SPL) as a function of gender, group, and ear

	Total sample		Left ear CEOAE[a]		Right ear CEOAE[a]		Left and right ear CEOAE[b]		
	Age (in years) *M* (*SD*), range	*N*	*M* (*SD*)	*N*	*M* (*SD*)	*N*	*M* (*SD*)	*M* (*SD*)	*N*
Ctrl boys	12.0 (3.0), 6.5–16.9	65	12.8 (3.8)	48	12.2 (3.8)	61	13.4 (3.3)	13.4 (3.5)	44
Ctrl girls	11.3 (2.9), 5.0–16.4	62	13.9 (3.7)	53	14.8 (4.2)	61	14.0 (3.7)	15.1 (3.9)	52
Girls with GID	12.1 (2.5), 7.6–16.6	23	13.1 (4.3)	21	15.2 (4.5)	20	13.2 (4.4)	14.9 (4.6)	18
Boys with GID	11.0 (1.8), 6.7–14.0	24	13.1 (4.3)	19	14.0 (3.7)	24	13.1 (4.3)	14.5 (3.8)	19

CEOAEs click-evoked otoacoustic emissions, *GID* gender identity disorder, *Ctrl* control

[a] CEOAE data used in the one-way ANOVAs

[b] CEOAE data used in the mixed model ANOVAs

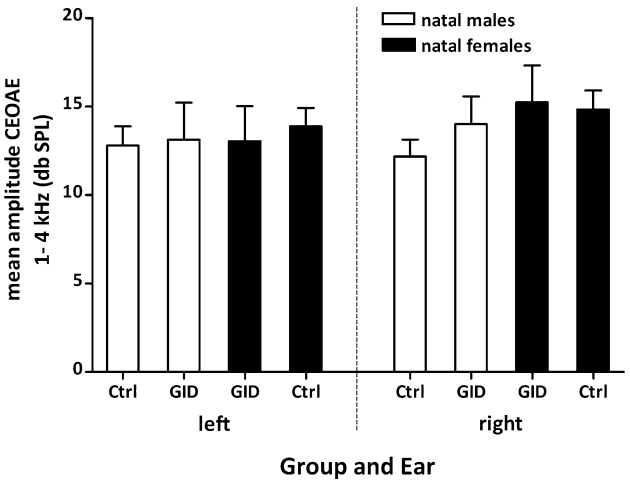

Fig. 1 CEOAE response amplitude in the left and right ears of male and female control (Ctrl) groups and participants diagnosed with gender identity disorder (GID). *Error bars* represent the 95 % confidence interval

amplitude in the control group ($d = .65$) and revealed a similar effect size for the difference between the boys with GID and the control boys ($d = .48$). The girls with GID had similar mean right CEOAEs compared with control girls ($d = .09$) and the sex difference in CEOAE amplitude observed between the groups with GID showed a small to medium ($d = .30$) effect size.

Gender Differences in Left Ear Emissions

The one-way ANOVA for the left ear CEOAE data was not significant, $F(3, 140) < 1$ (see Fig. 1; Table 1). Accordingly, Cohen's *d* effect sizes revealed that the sex difference in the control group was less strong ($d = .30$), but in the same direction as in the right ear CEOAE data, with the control girls showing higher amplitudes than the control boys. Both groups with GID showed emission amplitudes more in the range of the control boys ($d = .08$ and $d = -.08$ for boys with GID minus control boys and girls with GID minus control boys, respectively). Effect sizes for each the boys and girls with GID compared to the control girls were $d = .21$ and $d = -.21$, respectively.

Discussion

In the present study, we retrospectively investigated possible organizational effects of prenatal androgens on CEOAEs in relation to gender identity. We found that boys with GID had sex-atypical (hypomasculinized) emissions. Their mean response amplitudes, though, were not significantly different from either the male or female controls. Thus, boys with GID had an intermediate position between the sexes in terms of CEOAE response amplitudes. By contrast, girls with GID showed emissions in the same range as female controls.

Consistent with several earlier studies (Collet et al., 1993; McFadden, 1998; McFadden et al., 2009; Moulin et al., 1993; Strickland et al., 1985), sex differences in emission strengths were observed in the control group, with girls having significantly stronger emission amplitudes than boys. Our finding that boys with GID showed stronger, more female-typical emissions compared to control boys suggests that boys with GID might have been exposed to relatively lower amounts of androgens during early development. The effect sizes for the comparison boys with GID versus control boys were similar to those for control girls versus control boys, supporting the notion of a hypomasculinized early sexual differentiation in boys with GID. However, considering the lack of statistically significant differences between the control boys and the boys with GID and the relatively small sample size of subjects with GID, this conclusion may still be premature and our results therefore need to be interpreted with caution. Furthermore, our findings did not support the hypothesis of an increased exposure to androgens in girls with GID during prenatal development. Though speculative, this might reflect that GID in girls does not develop under the influence of prenatal androgens or at least not during the same critical time window as when androgens exert influences over OAEs.

In accord with numerous past findings (Aidan, Lestang, Avan, & Bonfils, 1997; Driscoll, Kei, & McPherson, 2000; Ismail & Thornton, 2003; Kei, McPherson, Smyth, Latham, & Loscher, 1997; Keogh, Kei, Driscoll, & Smyth, 2001; Saitoh et al., 2006), CEOAEs obtained from left ears showed consistently lower mean response amplitudes compared to right ear measurements in each group, except for the control boys, who showed similar mean CEOAEs in both ears. Of note, all three female groups (female natal sex and/or female gender identity) showed significantly stronger right than left ear CEOAEs compared to the control males, which is in line with previous studies (Ismail & Thornton, 2003; Markevych, Asbjørnsen, Lind, Plante, & Cone, 2011), reporting that women had greater (right > left) ear asymmetries than men. Other studies, however, found stronger asymmetries in males (Newmark, Merlob, Bresloff, Olsha, & Attias, 1997; Saitoh et al., 2006) or observed no significant differences in ear asymmetry between males and females (Thornton et al., 2003). It has been suggested that the asymmetric processing at the cochlear level may precede and underlie hemispheric specialization for auditory and language processing of the brain (Markevych et al. 2011; Sininger & Cone-Wesson, 2004).

In those analyses involving only the control subjects, the ear asymmetry effects masked the sex differences in mean CEOAE amplitude, which for both the right and left ear CEOAE data were revealed when age was added as a covariate to the design. Thus, the sex difference in CEOAE amplitude in both ears was significantly modulated by the participants' age. CEOAEs are used as a screening instrument for cochlea dysfunction and hearing impairment in newborns. Thus, much data about sex differences in OAEs are available in this age group. However, whether these sex differences in OAEs are still present at later stages of development, i.e., around puberty, has been less investigated. It is assumed that the sex difference in OAEs, probably organized under the influence of prenatal sex hormones, remains stable throughout life (Burns, 2009; McFadden et al., 1996). However, activational effects of circulating sex hormones in adult populations have been suggested to affect OAEs (Snihur & Hampson, 2012a, b). In the current study, control participants' age ranged from 5.0 to 16.9 years; thus, several boys and girls were pubertal at the time of the measurement. Therefore, our finding that age modulated the sex difference in CEOAE amplitude responses may reflect variability in pubertal status and associated circulating sex hormone levels in our control group samples, exerting activational effects on the sex difference in OAEs. In the current study, however, no attempt was made to assess pubertal stages (Marshall & Tanner, 1969, 1970) or to determine participants' actual sex hormone levels. Therefore, we cannot provide a direct link between sex steroid levels and differences in emission strength between girls and boys. Future studies are required, in which Tanner stages and/or circulating gonadal hormone levels, as more direct indicators of puberty, are related to CEOAE strength.

To our knowledge, no other study has investigated the relationship between the development of gender identity and prenatal androgen exposure, using OAE measurements. Another supposed retrospective indicator of relative androgen exposure during early development is the ratio between the length of the index finger and the ring finger, the 2D:4D ratio (Grimbos, Dawood, Burriss, Zucker, & Puts, 2010; Hönekopp & Watson, 2010; McFadden & Shubel, 2002; Peters et al., 2002; Williams et al., 2000). Although OAEs and digit ratios, when directly compared, showed no correlation (McFadden & Shubel, 2003; Snihur & Hampson, 2011), these two measures may provide complementary evidence for early androgenic effects on neuro-
biological sexual differentiation. Men generally have lower 2D:4D ratios compared to women. This sex difference has been shown to be already present in young children (Manning et al., 1998; McIntyre et al., 2006) and even prenatally (Galis, Ten Broek, Van Dongen, & Wijnaendts, 2010; Malas et al., 2006). Furthermore, 2D:4D ratios in children at 2 years of age were significantly predicted by the ratio of testosterone to estradiol levels in amniotic fluid (Lutchmaya et al., 2004; McIntyre, 2006) and digit ratios were associated with circulating sex hormone levels in adults (Manning et al., 1998).

At present, three studies have analyzed 2D:4D ratios in individuals with GID. In accordance with the present results, Schneider, Pickel, and Stalla (2006) as well as Kraemer et al. (2009) found evidence for more female-typical 2D:4D ratios in a group of 63 adult males diagnosed with GID, especially when restricting the analysis to right-handed subjects. This finding supports the hypothesis that men with GID might have been exposed to lower levels of androgen during early development. In line with the present findings, both studies failed to demonstrate any differences in digit ratios of women with GID compared to the female control groups, thereby contradicting the hypothesis of an androgen-induced masculinization of gender identity in women with GID. Wallien, Zucker, Steensma, and Cohen-Kettenis (2008) determined 2D:4D ratios in a group of 95 children and 75 adults, all diagnosed with GID. They found altered, more male-typical 2D:4D ratios in the female participants diagnosed with GID, but only in the adult sample. By contrast, 2D:4D ratios in boys and girls with GID, as well as in adult men with GID were sex-typical, thus not significantly different from their respective control groups. However, in the present study we found more female-typical CEOAEs only in the boys with GID, but no male-typical emission strengths in the girls with GID. This discrepancy might suggest that digit ratios and OAEs are two distinct measures and may be differentially affected by circulating hormone levels.

Some limitations of the present study should be addressed. It should be noted that we could not control for two important factors, possibly influencing our results. One factor is sexual orientation, simply because most of the participants were too young to report about their sexual preferences. Prospective follow-up studies have shown that most, but not all of the children and young adolescents diagnosed with GID, will develop a

homosexual orientation (in relation to natal sex) later in life (Drummond, Bradley, Badali-Peterson, & Zucker, 2008; Green, 1987; Singh, 2012; Wallien & Cohen-Kettenis, 2008). Another factor is whether the feelings of gender dysphoria in children, diagnosed with GID at the time of measurement, will persist into adulthood. Only about 15.8 % of the childhood GID cases will eventually lead to adult GID (Steensma, Biemond, de Boer, & Cohen-Kettenis, 2011). At present, we cannot determine whether the stronger response amplitudes of OAEs in boys with GID may be related to their different gender identity, may be related to current but possibly passing feelings of gender dysphoria and gender variant behaviors, or could be the disposition for a future homosexual orientation. Interestingly, when OAEs were measured as a function of sexual orientation, it was found that lesbians showed more male-typical, i.e., less frequent and lower response amplitude OAEs, compared to heterosexual females (Loehlin & McFadden, 2003; McFadden & Pasanen, 1999, McFadden & Pasanen, 1998) whereas gay men did not differ from heterosexual men in OAEs. These findings suggest that any association between sexual orientation and OAEs only applies for females and may further emphasize the different mechanisms underlying sexual orientation and gender identity. Future comparative studies in homosexual and non-homosexual adult transsexual populations should address the question how OAEs vary as a function of sexual orientation and gender identity.

Although there is some evidence (Balthazart, 2011; Breedlove, 2010; Gooren, 2006; McFadden et al., 1996) that prenatal androgens play a role in the development of gender identity, sexual orientation, and the production of OAEs, we still do not know much about their possibly causal relationship and certainly the present findings need replication. Probably, fluctuations in levels of androgen during early development are not the only explanation for the observed sex differences in OAEs. Other biological factors that most certainly play an important role in the prenatal masculinization process are androgen receptor availability, critical time windows for androgen action, androgen metabolism, and genes involved in androgen and other sex hormone actions during the sexual differentiation of the brain. Unfortunately, our current understanding of these early developmental mechanisms is very limited. For obvious ethical reasons, only correlational studies and no experiments applying hormonal manipulations are possible to conduct in humans. However, two medical conditions, congenital adrenal hyperplasia (CAH) and complete androgen insensitivity syndrome (CAIS), naturally occurring in humans, might help to understand the effects of prenatal hormonal events, in particular those of androgens. In CAH, the fetal adrenal gland produces above normal levels of androgens whereas in CAIS individuals, due to a defective androgen receptor, androgens are ineffective in masculinizing their body, brain and behavior. Therefore, girls with CAH should have diminished OAEs after birth. Likewise, in individuals with CAIS, OAEs would be expected to be feminized, i.e., high response amplitudes. Future research, possibly in the form of a clinical OAE screening program for infants with a disorder of sex development, as suggested by McFadden (1999), may help to unravel early androgen effects on the sexual differentiation of the body and the brain.

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