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POSTNATAL EFFECTS OF SEX **HORMONES ON** CLICK-EVOKED OTOACOUSTIC EMISSIONS

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Abstract

Click-evoked otoacoustic emissions (CEOAEs) are echo-like sounds, generated by the inner ear in response to click-stimuli. Females generally show higher CEOAE amplitudes compared with males. Because this sex difference in emission strength is already observed in neonates, weaker responses in males were assumed to originate from elevated levels of testosterone during prenatal male sexual differentiation and to remain stable throughout life. However, recent studies suggested that sex hormones may exert activational, postnatal effects on variations in CEOAEs. Adolescents diagnosed with Gender Dysphoria (GD; DSM-5) may receive gonadotropin-releasing hormone analogs (GnRHa) in order to inhibit pubertal maturation, followed by cross-sex hormone (CSH) treatment to induce the development of secondary sex characteristics of their experienced gender. By means of cross-sectional comparisons, we examined whether hormonal interventions (GnRHa, CSH), in 63 natal girls and 45 natal boys (all diagnosed with GD) affected their CEOAEs. Across treatment groups, the normative sex difference in CEOAE amplitude was absent in boys and girls with GD, but not reversed. Groups receiving GnRHa showed weaker CEOAEs compared with the treatment-naïve groups, especially in natal females. In line with the assumed diminishing effects of androgens on CEOAEs, natal girls who received testosterone treatment showed significantly weaker right ear CEOAEs compared with treatment-naïve natal girls. Contrary to our expectations, left ear CEOAEs in natal boys receiving estradiol administrations were also weaker than those of their treatment-naïve peers. Our findings provide additional evidence for activational effects that testosterone and estradiol may exert on CEOAEs postnatally.

ECHO-LIKE SOUND WAVES THAT are produced by the outer hair cells in the cochlea are called *Otoacoustic emissions* (OAEs). OAEs can appear in absence of any external stimulus and are then called *spontaneous* OAEs. When OAEs occur in response to brief transient click-stimuli, they are called *click-evoked* OAEs (CEOAEs) (Rodenburg and Hanssens 1998; Kemp 2002).

In general, females were found to generate stronger and higher numbers of OAEs compared with males, and this sex difference in emission strength and frequency has frequently been observed in neonates (Strickland et al. 1985; Burns et al. 1992; Morlet et al. 1995). Therefore, it is assumed that the sex difference in OAE amplitude develops as part of the prenatal sexual differentiation of the fetus, and is thus under the organizational influence of sex steroids.

Several animal studies support the notion that lower amplitude CEOAEs, present in males, originate from relatively high prenatal exposure to androgens (for review see McFadden, 2008; McFadden et al., 2009). In addition, OAE studies in twins, showing that women who shared the uterus with a male co-twin had *masculinized* OAEs (McFadden 1993; McFadden et al. 1996), provided indirect evidence for the dampening, organizational effects of androgens on the auditory system.

Sex differences in OAEs have generally been assumed to remain stable throughout life (McFadden et al. 1996; Burns 2009). However, recently, Snihur and Hampson (2012 – a) showed that seasonal fluctuations in serum testosterone levels in adult men correlated negatively with their CEOAE amplitudes. A similar negative relationship between seasonal variations in testosterone and emission strengths had previously been reported in male adult monkeys by McFadden, Pasanen, Raper, et al. (2006). Thus, in addition to its prenatal effects on CEOAEs, testosterone may also exert activational dampening effects on emission amplitudes postnatally.

In another study, Snihur and Hampson (2012 – b) suggested that estradiol is also involved in regulating the production of OAEs, at least in the female cochlea. They showed that women, who used oral contraceptives (that suppress endogenous fluctuations in estradiol), had *defeminized* OAEs compared with a group of women undergoing a normal menstrual cycle. Since they were not able to find any association between the differences in OAEs and circulating testosterone levels between the two female groups, the authors concluded that it might reflect differences in estradiol exposure, with relatively higher levels of estradiol in normally cycling women resulting in more female-typical OAEs. In support of this, several studies suggested that OAEs fluctuate with the menstrual cycle, peaking around ovulation when levels of estradiol are high (Bell 1992; Haggerty et al. 1993; Penner 1995; Al-Mana et al. 2010).

In a previous study, we investigated the relationship between the (atypical) development of gender identity and prenatal androgen exposure, employing

CEOAE measurements (Burke et al. 2014). *Gender Dysphoria* (GD; DSM-5, American Psychiatric Association 2013) is the distress due to strong and persistent feelings of incongruence between one's natal sex and one's experienced/expressed gender. The etiology of GD is largely unknown. It is hypothesized that atypical levels of sex hormones, during a critical period of sexual differentiation, mediate a sex-atypical programming of the brain and thereby the development of a non-normative gender identity in individuals with GD. Several functional and structural brain imaging studies indeed provide some support for sex-atypical brain characteristics in individuals with GD (Berglund et al. 2008; Luders et al. 2009; Rametti, Carrillo, Gomez-Gil, Junque, Segovia, et al. 2011; Rametti, Carrillo, Gomez-Gil, Junque, Zubiaurre-Elorza, et al. 2011; Zubiaurre-Elorza et al. 2012).

In order to test the hypothesis of an atypical sexual brain differentiation in GD, by means of recording their CEOAEs, we estimated retrospectively the prenatal androgen exposure of a group of treatment-naïve children and adolescents diagnosed with GD (24 natal boys, 23 natal girls) and control subjects (65 boys and 62 girls). We replicated the normative sex difference in CEOAE response amplitude with significantly stronger emissions in the control girls compared with the control boys. However, this sex difference was absent in the gender dysphoric boys and girls. Boys with GD in particular showed stronger, more female-typical CEOAEs, whereas girls with GD did not differ in emission strength compared with control girls (Burke et al. 2014).

At the *Center of Expertise on Gender Dysphoria* in Amsterdam, eligible adolescents with GD may start using gonadotropin-releasing hormone analogs (GnRHa) in order to suppress pubertal maturation, and thus the irreversible development of the secondary sex-characteristics of their natal sex (Kreukels and Cohen-Kettenis 2011; de Vries and Cohen-Kettenis 2012). From the age of 16 years on, as a first step in the actual sex reassignment, adolescents with persisting GD may receive *cross-sex hormones* (CSH; testosterone for natal girls and estradiol for natal boys), in addition to their treatment with GnRHa, in order to develop secondary sex characteristics of their experienced gender (Delemarre-van de Waal and Cohen-Kettenis 2006; Hembree et al. 2009).

In the current study, based on the assumption that sex hormones may also exert activational effects on CEOAEs postnatally, we investigated whether hormonal interventions (pubertal suppression and CSH treatment) in individuals with GD affected their CEOAEs. We hypothesized that suppression of endo-

genous testosterone production (by means of GnRHa) and administration of estradiol in natal boys would result in stronger emissions, and thus in female-typical CEOAE response amplitudes in natal boys with GD. Conversely, suppressing endogenous estradiol (by means of GnRHa) and administration of testosterone in natal girls was assumed to result in diminished CEOAEs in natal girls with GD.

Material and Methods

PARTICIPANTS

A TOTAL OF 106 adolescents, all diagnosed with GD, were recruited at the *Center of Expertise on Gender Dysphoria* at the VU University Medical Center in Amsterdam. One participant was excluded due to invalid measurements in both ears. Out of the remaining 105 participants, 91 had valid CEOAE recordings in both ears, 9 had valid recordings in only their right ear, and 5 participants only in their left ear. All participants were inquired about any past and present hearing problems or ear traumas. Participants' age ranged from 10.3 to 20.3 years, with a mean age of 15.7 years (standard deviation (SD) = 2.3) (see Table 1).

Forty-three natal boys and 62 natal girls were divided into three groups according to their hormonal intervention: no hormonal intervention (treatment-naïve), pubertal suppression by means of GnRHa administration, or CSH treatment.

The treatment-naïve group consisted of 10 natal boys and 15 natal girls. The majority of these adolescents (7 natal boys and 15 natal girls) had also participated in our previous study (Burke et al. 2014). However, in the present study we chose to include only those (treatment-naïve) participants with GD who had already reached puberty (at least Tanner stage 2 (Marshall and Tanner 1969, 1970)) in order to match the three hormonal intervention groups with regard to their (previous) endogenous sex hormone exposure.

The puberty suppressed group consisted of 14 natal boys and 26 natal girls who had been treated monthly with 3,75 mg Triptorelin (Decapeptyl-CR®, Ferring, Hoofddorp, the Netherlands) injections for, on average, 20.1 months (range 2 - 48 months), resulting in complete suppression of gonadal hormone production.

The CSH treatment group consisted of 19 natal boys who received 17β-estradiol (Progynova®, Bayer, Mijdrecht, the Netherlands or Cetura®, ACE Pharmaceuticals, Zeewolde, The Netherlands) on a daily basis for on average 22.7 months (range 5 - 47 months) and 21 natal girls who received an testosterone-ester mixture (Sustanon® 250 mg/ml, Merck Sharp & Dohme B.V., Oss, the Netherlands) every 2 to 4 weeks, for on average 11.8 months (range 2 - 28 months). CSH doses depended on the patient's weight (natal boys) or body surface area (natal girls) and the starting dosage varied with the subject's age. Until the age of 16.5 years, the starting dosage for Sustanon® was 25 mg/m² body surface area every two weeks and for estradiol 5 microgram/kg. When older than 16.5 years the dosage was 75 mg Sustanon® every two weeks or 1 mg Progynova®/Cetura®, respectively. All study participants who were treated with CSH also received monthly a Triptorelin injection in order to suppress endogenous gonadal sex hormone production.

As part of their regular visits to the pediatric endocrinologist (every 3 months), all participants were assessed with regard to their physical pubertal development according to the 5-point Tanner scales (Marshall and Tanner 1969, 1970). For the treatment-naïve participants, we used those Tanner stage assessments that were conducted at the nearest to the time of the CEOAE recordings. For both groups receiving GNRHa or CSH, we used the Tanner stage assessments from the day they started the GNRHa treatment. By doing so, we could determine whether the three hormonal intervention groups matched with regard to their previous exposure to endogenous sex hormones (see Table 1).

This study was approved by the Ethical Review Board of the VU University Medical Center Amsterdam, and all subjects and their legal guardians gave their written informed consent.

MATERIALS AND PROCEDURE

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 at five frequency bands (1000, 1414, 2000, 2828 and 4000 Hz) and in the Quick Screen (non-linear) mode with a time-window of 2.5 to 12.5 milliseconds. CEOAE responses were measured in terms of dB SPL (sound pressure level). Each ear was tested for a fixed number of 250 clicks; the average emission

response of the five frequency bands was used for further analyses. The click-stimulus input was set on approximately 80 (± 2.3) dB, which is in accordance with a clinical protocol for CEOAE recordings (Hall 2000). A probe with an appropriately-sized foam ear tip, thereby causing minimal discomfort for the participant, was placed in the external ear canal 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 would not reach a threshold of 6 mPa. Participants were seated in a comfortable chair and asked to relax their body and facial muscles during the recordings in order to ensure a low noise measurement. Besides external noise, test order of the left and right ear might also influence the CEOAE recordings (Thornton et al. 2003); therefore, the right ear was tested first for each participant.

ANALYSES

CEOAE RECORDINGS (the mean of the five frequency bands) with an amplitude of at least 0.99 dB SPL and a *whole-wave reproducibility* of more than 0.69 were used for analysis; whole-wave reproducibility was calculated as the correlation coefficient of interleaved non-linear responses (Berninger 2007). All recorded measurements were transferred to a database and analyzed using the Statistical Package for the Social Sciences, version 20 (SPSS Inc., Chicago, IL, USA). A mixed model analysis of variance (ANOVA) was used to analyze overall group differences in CEOAE amplitudes, with Ear tested (left; right) as within-subject factor and natal Sex (male; female) and Group (treatment-naïve; GNRHa; CSH) as between-subject factors.

Since CSH treatment differed between boys and girls, differences between the three (hormonal intervention) groups were investigated by means of one-way ANOVA, separately for each sex.

Finally, by means of Pearson's correlation analyses, we investigated whether CEOAE amplitudes varied linearly with a) treatment duration (in weeks), and b) the cumulative dosage of the CSH. Effects were considered statistically significant at $p \leq 0.05$ and Bonferroni correction was applied post hoc to control for multiple comparisons. Cohen's *d* was reported as an estimate of effect size for a mean difference between any two of the three groups, where *d* was calculated as the difference between two means divided by the pooled SDs of those two means (Cohen 1988).

Results

SUBJECTS' AGE, PUBERTAL STAGE, and mean CEOAE amplitudes for each ear in boys and girls are presented in Table 1. The Kolmogorov-Smirnov test and Levene's test confirmed normality of the CEOAE data and that homogeneity of variance between groups could be assumed.

Within each sex, Tanner stages were not significantly different between the three hormonal intervention groups (see Table 1). However, between the sexes, irrespective of treatment group, Tanner stages were significantly different for pubic hair growth, $F(5, 104) = 6.5$, $p < .001$ and breast/genital development, $F(3, 104) = 4.1$, $p = .009$. Natal girls were more physically mature compared to natal boys at the moment of the CEOAE recordings (treatment-naïve groups) or when GNRHa administration was started (GNRHa and CSH groups). We therefore conducted additional analyses by using Tanner stage as a covariate in those models including sex as an independent variable.

MIXED MODEL ANOVA

THE 2 (EAR) × 2 (SEX) × 3 (GROUP) mixed model ANOVA revealed a significant main effect for Ear, $F(1, 85) = 7.1$, $p = .009$, with overall stronger right than left ear emissions. Between-subject comparisons revealed no main effect for Sex, but a borderline significant main effect for Group, $F(2, 85) = 3.1$, $p = .052$, which reflected weaker CEOAEs in those individuals receiving CSH compared with the treatment-naïve groups. There were no significant interactions between Ear or Sex, and Group. CEOAE amplitudes as a function of Ear, Sex and Group are plotted in Figure 1. Since left and right ear emissions differed significantly, which is in line with numerous previous studies (Aidan et al. 1997; Driscoll et al. 2000b; Ismail and Thornton 2003; Saitoh et al. 2006), separate 2 (Sex) × 2 (Group) ANOVAs were conducted for each ear's CEOAEs.

The 2 (Ear) × 2 (Sex) × 3 (Group) mixed model ANOVA with Tanner stages as covariate in order to correct for differences in pubertal development between the natal girls and the natal boys, revealed no within-subject effect of Ear (ear asymmetry in CEOAE amplitude) anymore. Again, no main effect of Sex was observed, and the main effect of Group remained borderline significant, $F(2, 84) = 2.8$, $p = .070$, when we co-varied for breast/genital development. When

Table 1 Subject characteristics and mean CEOAE response amplitudes 1-4 kHz (in dB SPL) as a function of sex, group, and ear

		Total sample								Left ear CEOAEa			Right ear CEOAEa			Left and right ear CEOAEb				
		Age (years)				Tanner stages (M (SD))														
		M	(SD)	RANGE	N	P	F	B/G*	F	M	(SD)	N	M	(SD)	N	M	(SD)	M	(SD)	N
Natal girls	treatment-naïve	13.7	(2.4)	10.3-17.3	15	4.1 (1.3)		4.3 (1.0)		13.8	(4.0)	14	15.6	(3.9)	13	13.8	(3.8)	15.4	(4.0)	12
	GnRHa	15.0	(1.6)	12.3-18.0	26	4.2 (1.2)	1.3	3.9 (1.1)	1.1	12.2	(5.5)	25	12.4	(5.6)	26	12.2	(5.5)	12.8	(5.3)	25
	CSH	17.8	(1.1)	16.8-20.0	21	4.7 (1.0)		4.6 (1.0)		11.5	(4.0)	20	10.8	(4.9)	21	11.5	(4.0)	10.7	(5.0)	20
		M	(SD)	RANGE	N	P	F	B/G*	F	M	(SD)	N	M	(SD)	N	M	(SD)	M	(SD)	N
Natal boys	treatment-naïve	12.6	(0.9)	11.0-14.0	10	2.8 (1.7)		2.9 (1.2)		11.9	(4.4)	6	13.1	(5.0)	10	11.9	(4.4)	14.5	(4.6)	6
	GnRHa	15.2	(1.0)	13.3-17.1	14	3.5 (1.2)	1.0	3.6 (1.2)	1.5	11.8	(4.0)	14	12.4	(4.1)	13	12.2	(3.9)	12.4	(4.0)	13
	CSH	18.1	(0.9)	16.3-20.3	19	3.6 (1.4)		3.8 (1.2)		9.3	(3.9)	17	11.4	(2.8)	17	9.7	(3.8)	11.6	(2.9)	15

CEOAEs = click-evoked otoacoustic emissions; a CEOAE data used in one-way ANOVAs; b CEOAE data used in mixed model ANOVA; GnRHa = gonadotropin releasing hormone analogue, puberty suppression; CSH = cross-sex hormone treatment; P = pubic hair growth; B = breast development; G = genital development; *B applies for natal girls and G for natal boys; pubertal stages were assessed by means of the five-point (1 = prepubertal, 5 = postpubertal) Tanner Maturation Scale.

co-varied for pubic hair growth, the main effect of Group became just significant, $F(2, 84) = 3.1$, $p = .050$.

RIGHT EAR CEOAES

THE SEX BY GROUP ANOVA in right ear CEOAEs revealed no main effect of Sex, but a significant main effect for Group was observed, $F(2, 94) = 3.6$, $p = .033$. No Sex by Group interaction was revealed. Post-hoc comparisons indicated that CEOAEs in the groups receiving CSH treatment were significantly weaker than CEOAEs in the treatment-naïve groups, $p = .017$ (see Figure 3.1). These results remained unchanged after co-varying breast/genital development, $F(2, 93) = 3.6$, $p = .033$ or pubic hair growth, $F(2, 93) = 3.8$, $p = .025$.

One-way ANOVA, investigating group differences in natal girls, yielded significant differences in CEOAE amplitude between the three hormonal intervention groups, $F(2, 57) = 3.7$, $p = .031$. Natal girls receiving testosterone administrations had significantly weaker CEOAE amplitudes compared with the treatment-naïve girls, $p = .027$, $d = 1.09$. The girls receiving GNRHa also showed weaker CEOAE amplitudes than the treatment-naïve group, $d = .64$, but this group difference did not reach statistical significance (see Figure 3.1 and Table 2).

One-way ANOVA for right ear CEOAEs in natal boys revealed no significant differences between the three hormonal intervention groups.

Correlation analyses for the right ear CEOAEs yielded neither a significant relationship between treatment duration and emission strengths, nor between cumulative CSH dosage administered and CEOAE amplitude for either sex.

Table 2 Cohen's *d* effect sizes for mean differences in CEOAEs between the hormonal intervention groups, separately for each sex

	COMPARISON	LEFT EAR CEOAE	RIGHT EAR CEOAE
Natal girls	treatment-naïve vs. CSH	0.36	**1.09**
	treatment-naïve vs. GnRHa	0.33	**0.64**
	GnRHa vs. CSH	0.15	0.31
Natal boys	treatment-naïve vs. CSH	**0.68**	0.47
	treatment-naïve vs. GnRHa	0.03	0.16
	GnRHa vs. CSH	**0.66**	0.30

CEOAEs = click-evoked otoacoustic emissions; GnRHa = gonadotropin releasing hormone analogue, puberty suppression; CSH = cross-sex hormone treatment.

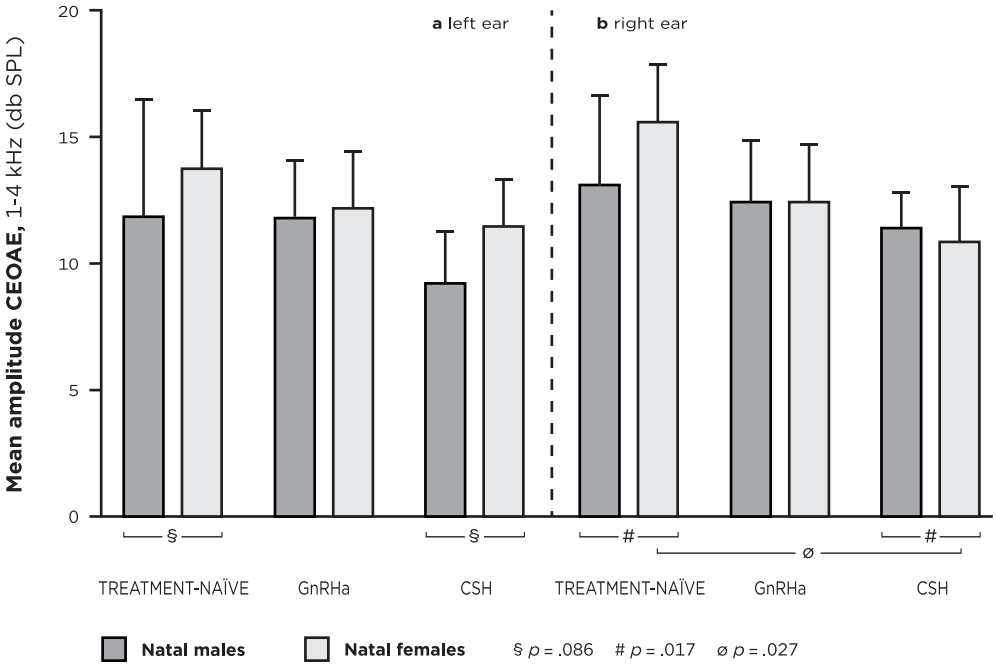

Figure 3.1 CEOAE response amplitude as a function of natal sex and hormonal intervention, for the left **a)** and right **b)** ears. Error bars represent the 95% confidence interval. CEOAEs = click-evoked otoacoustic emissions; GnRHa = gonadotropin releasing hormone analoge, puberty suppression; CSH = cross-sex hormone treatment.

LEFT EAR CEOAES

THE SEX BY GROUP ANOVA revealed no main effects for Sex or Group in left ear CEOAEs, also not after correction for individual differences in Tanner stage. Visual inspection of the data (see Figure 3.1) suggested that CEOAEs in those groups receiving CSH were weaker than CEOAEs in the treatment-naïve groups. Exploratory group comparisons indeed revealed a borderline significant difference between the treatment-naïve groups and those receiving CSH, $p = .086$.

One-way ANOVA, investigating treatment group-specific effects on CEOAEs, separately for each sex, revealed no significant group differences for either sex. However, moderate to large effect sizes indicated that natal boys receiving estradiol administrations had lower CEOAEs than the treatment-naïve group ($d = .68$) and that they also had lower emissions compared to the natal boys receiving GNRHa, $d = .66$ (see Figure 3.1 and Table 2).

As for the right ear measurements, treatment duration or the cumulative CSH dosage did not correlate with CEOAE responses in either sex.

Discussion

IN THE PRESENT STUDY, we examined whether CEOAE response amplitudes in adolescents diagnosed with GD, differed as a function of their hormonal intervention, and thus whether CEOAEs were affected postnatally by circulating levels of sex hormones. In accordance with the assumed diminishing effects of androgens on CEOAEs, natal girls who received testosterone treatment showed significantly weaker right ear CEOAEs compared with treatment-naïve natal girls. Thus, the testosterone administration seemed to dampen, or *masculinize* CEOAE response amplitudes. In addition, our results suggest dampening effects of pubertal suppression on CEOAEs. Contrary to our expectations that estradiol might exert enhancing effects on emission amplitudes, left ear CEOAEs in natal boys receiving estradiol administrations were also weaker than those of their treatment-naïve peers. In line with several previous findings reported for neonates, children as well as adults (Aidan et al. 1997; Kei et al. 1997; Driscoll et al. 2000; Ismail and Thornton 2003; Saitoh et al. 2006), CEOAEs obtained from left ears were overall significantly lower compared with right ear recordings. Asymmetric processing at the cochlear level has been suggested to precede and underlie the development of hemispheric specialization for auditory and language processing of the brain (Sininger and Cone-Wesson 2004; Markevych et al. 2011). However, when Tanner stages were added as a covariate to the model, the differences between left and right ear CEOAEs disappeared. This would suggest that asymmetric cochlear processing is associated with pubertal development, but exploratory correlation analyses between Tanner stages and the difference between left and right ear CEOAEs (as an index of asymmetry) revealed no significant associations. Nevertheless, it is possible that such a relationship may only be revealed in participants without GD diagnosis and thus that the GD status of our participants might have confounded our findings. Alternatively, the absence of an ear asymmetry effect in our participants with GD, corrected for differences in pubertal development, may reflect the assumed sex-atypical brain development in GD. Therefore, future studies should investigate pubertal development in larger control groups in association with the sex differences in CEOAEs and the underlying asymmetric cochlea processing.

PARTICIPANTS RECEIVING GNRHa (which suppressed any endogenous gonadal hormone production) had somewhat lower, though not statistically significant, CEOAE response amplitudes compared with the treatment-naïve groups. This is in line with the assumed enhancing effects of endogenous estradiol on CEOAEs in natal females (e.g. during the menstrual cycle) (Bell 1992; Haggerty et al. 1993; Penner 1995).

In contrast, GNRHa administration, which should have suppressed the assumed dampening effects of endogenous testosterone, did not significantly elevate CEOAEs in natal boys. However, we previously (Burke et al. 2014) showed that treatment-naïve natal boys diagnosed with GD already exhibit stronger, more female-typical emission amplitudes, compared with control boys. As a result, potential enhancing effects of suppressing endogenous testosterone production on CEOAE amplitudes might be relatively small in natal boys with GD, because emission amplitudes in their treatment-naïve peers were increased a priori.

Gonadal hormone production is regulated by the *hypothalamic-pituitary-gonadal* (HPG) axis. The HPG-axis is active during fetal development and continues to function in infancy. During childhood the HPG-axis is virtually quiescent until it is reactivated at the onset of puberty (Grumbach 2002; Nathan and Palmert 2005). In male fetal development, by the end of the first trimester of pregnancy (Grumbach 2002), the hypothalamus starts to produce GNRH, which stimulates the production of gonadotropins and gonadal sex steroids. During the first few weeks after birth, GNRH secretion again increases significantly in males, resulting in a second, postnatal testosterone surge between 1–3 months of life, followed by a gradual decrease to pre-pubertal levels by 4–6 months (Waldhauser et al. 1981; Finegan 1989). In females, the ovaries are relatively quiescent prenatally, but female infants show a similar, though somewhat later postnatal activation of the HPG-axis as boys. High levels of estradiol are secreted by the ovaries during 6–12 months after birth, which start to decline by 12 months of age, but continues until the age of 24 months (Waldhauser et al. 1981; Grumbach 2002; Quigley 2002).

Interestingly, relatively weaker CEOAEs in girls and, therefore, less distinct sex differences in emission strengths, have been observed in pediatric (2–6 years of age) populations (Lamprecht-Dinnesen et al. 1998; Kapoor and Panda 2006), thus, during childhood quiescence of the HPG-axis. Neonates and in-

fants during the first year of age, in contrast, have been reported to show significant sex differences in OAEs, similar to adult populations (Collet et al. 1993; Lamprecht-Dinnesen et al. 1998; Kapoor and Panda 2006). Therefore, sex differences in OAE frequency and amplitude are most distinct during periods of GnRH secretion, and thus during gonadal hormone action. In the current study, we showed that suppressing gonadal hormones by means of GnRHa, particularly in girls, indeed resulted in weaker CEOAEs.

EFFECTS OF TESTOSTERONE ON CEOAES

IN NATAL GIRLS DIAGNOSED with GD testosterone treatment had diminishing effects on their CEOAE response amplitudes. To the best of our knowledge, this is the first study that investigated the effects of postnatal androgen administration on CEOAEs in natal females. Postnatal androgen effects on CEOAEs in males have previously been suggested by two studies in rhesus monkeys (McFadden, Pasanen, Raper, et al. 2006) and men (Snihur and Hampson 2012). Associated with seasonal androgen fluctuations, CEOAEs in rhesus monkeys appeared to be weaker in winter time, when circulating testosterone levels were high (McFadden, Pasanen, Raper, et al. 2006). Similarly, CEOAEs in men correlated negatively with monthly fluctuations in blood testosterone levels (Snihur and Hampson 2012). Our findings thus provide additional evidence for the dampening influences of postnatal androgens on CEOAEs.

EFFECTS OF ESTRADIOL ON CEOAES

WE FOUND THAT ESTRADIOL administrations had dampening effects on left ear CEOAEs in natal boys diagnosed with GD. Although we were unable to find statistically significant group differences, which may be related to the relatively small sample sizes, the effect sizes were moderate to large. This negative association between estradiol and emission amplitudes was contrary to our hypothesis. It seemed counterintuitive at first glance, because several studies suggested that relatively high levels of estradiol, such as during ovulation, correlated positively with OAE fluctuations in frequency and amplitude during the menstrual cycle (Haggerty et al. 1993; Penner 1995; Al-Mana et al. 2010). However, recently Snihur and Hampson (2012-b) showed that women using oral contraceptives had significantly weaker and less frequent OAEs compared with normally

cycling women. Likewise, natal boys with GD receiving daily estradiol administrations as part of their CSH treatment showed diminished CEOAEs. These *masculinizing* effects of oral contraceptives on the auditory system have previously been suggested by Elkind-Hirsch et al. (1992) and McFadden (2000), who found that OAEs and several auditory brainstem response measures were *defeminized* in women using oral contraceptives in comparison with female non-users. Therefore, as has previously been suggested by Haggerty (1993) and Penner (1995), female-typical fluctuations in OAE amplitude and frequency seem to be dependent on a cyclical, pulsatile pattern of estradiol secretion. Accordingly, our results are in line with the notion that a continuous administration of estradiol (like in oral contraceptive use and CSH administration) results in more male-typical, thus weaker and less frequent OAEs.

Taking these findings together, both testosterone and estradiol seem to be actively implicated in facilitating or inhibiting the cochlear amplification mechanism, and may thus actively *feminize* or *masculinize* OAEs. It should be noted though, that both the testosterone-mediated dampening effects on CEOAEs in our natal female participants and also the weaker emissions in natal boys receiving estradiol administration may be explained by an estradiol-driven effect.

Dependent on the target tissue, testosterone may either have a direct effect by binding to the androgen receptor, or, alternatively, may be locally converted to estradiol by the enzyme aromatase, and consequently bind to *estrogen receptors* (ER) to exert its effect. Animal studies showed that in the male brain, high levels of testosterone are locally aromatized to estradiol, which then actively defeminizes brain structures, and thus promotes male-typical sexual differentiation (Jost 1983; Bakker et al. 2006). Similarly, in humans, the influences of sex hormones on bone maturation and epiphyseal closure have been shown to be an estradiol-mediated mechanism (MacGillivray et al. 1998). Therefore, more indirectly in males, testosterone first needs to be aromatized into estradiol, whereas in females estrogens are directly produced by the ovaries, in order to regulate the growth spurts and bone maturation during puberty.

Testosterone and estradiol may exert their effects on OAEs during different time windows. The outer hair cells in the rodent as well as the human cochlea have been shown to contain the receptor types ERα and ERβ (Stenberg et al. 1999, 2001; Hultcrantz et al. 2006; Motohashi et al. 2010). In rats, both receptor types were reported to be up- or down-regulated dependent on different post-

natal developmental stages, whereas no ER expression was observed during fetal development (Simonoska et al. 2009), suggesting that any estrogen-sensitive mechanisms associated with auditory functioning may occur during postnatal life.

Taken together, the dampening effects of the testosterone treatment on CEOAEs in our natal girls may reflect a physiological estradiol-mediated mechanism, whereas the weaker emissions following estradiol treatment in the natal boys may be explained by a pharmacological effect of continuously administered (in contrast to cyclic) non-physiological levels of estradiol.

Our results should be viewed in light of some limitations that may be addressed by future studies. Since we conducted cross-sectional comparisons, no inferences regarding developmental changes in CEOAEs associated with the hormonal interventions in individuals with GD could be made. Therefore, prospective studies following adolescents with GD during the different phases of hormonal intervention (prior to any intervention, during pubertal suppression, and CSH treatment) should provide more direct evidence for the hypothesized relationship between gonadal hormone action and CEOAE amplitudes. Furthermore, our findings should be compared to control groups without GD, matched for age and pubertal status.

In conclusion, the present study provides additional evidence for the hypothesis that sex hormones exert significant postnatal effects on CEOAEs. We propose that postnatal variations in CEOAE amplitude are mediated by estradiol-regulated mechanisms.

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