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# ORAL CONTRACEPTIVE USE IN WOMEN IS ASSOCIATED WITH DEFEMINIZATION OF OTOACOUSTIC EMISSION PATTERNS

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**Abstract—The production of otoacoustic emissions (OAEs) by the cochlea is a sexually dimorphic trait. Although often hypothesized to be influenced by testosterone *in utero*, little attention has been devoted to the possibility that levels of circulating sex steroids in adulthood might modulate the sex difference in OAE production. The purpose of the current study was to investigate whether oral contraceptive (OC) use affects OAE production in women, revisiting a question originally posed by McFadden [(2000) Hearing Research 142:23–33]. Forty-five males and 50 females were tested. The women were retrospectively classified based on whether or not they were using OCs at present. Two types of OAEs were quantified: those produced spontaneously (spontaneous otoacoustic emissions or SOAEs) and those produced in response to click stimuli (click-evoked otoacoustic emissions or CEOAEs). Women currently using OCs showed a defeminized pattern of OAE production: they produced fewer SOAEs, SOAEs with significantly less power, and smaller CEOAE response amplitudes compared with naturally cycling women who were tested irrespective of phase of the menstrual cycle. It is proposed that the observed group difference may be mediated by the interaction of circulating estradiol with estrogen receptor alpha (ER$\alpha$) or estrogen receptor beta (ER$\beta$) receptors in the cochlea.** 

**Key words: activational, auditory, cochlea, estrogens, testosterone.**

Otoacoustic emissions (OAEs) are faint sounds produced by a normally functioning cochlea, which can be detected in the external auditory canal using a highly sensitive microphone (Kemp, 1978). An association between the production of OAEs and normal hearing sensitivity has been found (Probst et al., 1987; McFadden and Mishra, 1993). OAEs are widely considered to be a natural by-product of an amplification mechanism in the cochlea designed to amplify low-intensity sounds, the "cochlear-amplifier system" (Davis, 1983), of which the outer hair cells are an integral component. Several types of OAEs have been identified. Of interest here will be two types: (1) those produced in the absence of external acoustic stimuli (spontaneous OAEs, SOAEs) and (2) those produced in response to the deliberate presentation of acoustic stimuli, either tonal bursts or clicks (click-evoked OAEs, CEOAEs).

A sex difference in OAE production has been found in humans. Females, on average, produce greater numbers of SOAEs, greater overall power of SOAEs, and higher CEOAE response amplitudes compared with males (Bilger et al., 1990; Burns et al., 1992; Penner et al., 1993). This robust sex difference has been observed in neonates, infants, and young children (Strickland et al., 1985; Burns et al., 1992; Morlet et al., 1995), as well as certain adult populations (for review, see McFadden, 2008, 2009), and is most obvious in the first year after birth (Lamprecht-Dinnesen et al., 1998). To explain the sexual dimorphism, it has been hypothesized that exposure to elevated levels of androgens, specifically testosterone, in the male fetus during the critical prenatal window for sexual differentiation masculinizes the auditory system, including the mechanisms responsible for OAE production (i.e., the cochlear-amplifier system), resulting in a diminished capacity to generate OAEs in males relative to females (McFadden, 1993b, 1998, 2002). A right-ear advantage in the production of both SOAEs and CEOAEs also has been reported (e.g., Bilger et al., 1990; Burns et al., 1992; Hall, 2000; McFadden, 2009; Talmadge et al., 1993, but see Collet et al., 1993).

Direct support for the prenatal androgen hypothesis remains limited, owing to the difficulty of studying prenatal effects in humans where the experimental manipulation of testosterone is not ethically permitted, but several lines of indirect evidence do exist. Female dizygotic twins who have male co-twins have been shown to produce male-typical patterns of OAEs, presumably due to exposure to higher-than-normal levels of androgens *in utero* from their male co-twin (McFadden, 1993a). Studies of sexual orientation and OAE production have shown that homosexual females lie intermediate to heterosexual females and heterosexual males with respect to the numbers and powers of SOAEs produced (McFadden and Pasanen, 1999) and CEOAE response amplitudes (McFadden and Pasanen, 1998). The latter finding is congruent with the possibility that homosexual women are exposed to elevated levels of androgens prenatally, resulting in partial masculinization of their brains, and subsequent behavior (see also Hall and Kimura, 1995; McFadden and Champlin, 2000 for partial masculinization of other traits).

A study of spotted hyenas (*Crocuta crocuta*) offers support from a non-human species for a prenatal hormonal effect on OAE production (McFadden et al., 2006). Both female and male hyenas are highly androgenized during prenatal development. Female hyenas not only produce

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*Abbreviations:* ANOVA, analysis of variance; CEOAE, click-evoked otoacoustic emission; dB, decibels; ER$\alpha$, estrogen receptor alpha; ER$\beta$, estrogen receptor beta; Hz, hertz; kHz, kilohertz; OAE, otoacoustic emission; OC, oral contraceptive; SOAE, spontaneous otoacoustic emission.

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CEOAE response amplitudes similar to those present in male hyenas, but also the prenatal treatment of both female and male hyenas with anti-androgens resulted in stronger CEOAE amplitudes in both sexes. Conversely, prenatal treatment with testosterone propionate has been found to reduce the amplitude of the CEOAE response in female sheep (McFadden et al., 2009). Thus, multiple lines of evidence suggest that prenatal androgens may act to weaken the cochlear amplifiers responsible for OAE production.

Many sexual dimorphisms that are initiated by androgen exposure during the prenatal or perinatal period are subject to further regulation by levels of circulating hormones in adults (Goy and McEwen, 1980). However, with respect to OAEs, research examining the possibility of a superimposed influence of adult steroids has been limited and has yet to firmly establish a role for circulating hormones in OAE production. Recent work by our laboratory confirmed a correlation between CEOAE response amplitudes and circulating levels of testosterone in adult men (Snihur and Hampson, 2012). McFadden et al. (2006) showed that male rhesus monkeys produce CEOAEs with lower response amplitude during the fall breeding season (i.e., elevated levels of sex steroids) compared with the summer non-breeding season (i.e., reproductively quiescent; lower levels of sex steroids). Androgens have been the focus of most existing research because of mounting evidence that they exert organizational effects on the development of the auditory system. However, other hormones might also play a role in the regulation of adult OAEs.

An estrogenic influence has not been demonstrated to date, but would be consistent with several indirect observations. In women, at least two case reports have described an infradian rhythm in the frequencies of emitted SOAEs that approximates the length of the menstrual cycle (changes in OAE numbers or amplitudes were not reported). Three of four women studied by Bell (1992) showed cyclic fluctuation of about 6–14 hertz (Hz) (0.4%) in the frequencies of the SOAEs they emitted and, in a single-case study, fluctuation in one woman's SOAE frequencies was reduced during periods of amenorrhea (Penner, 1995). Monthly fluctuations in SOAEs in females also were observed by Haggerty et al. (1993). Endocrine verification of the menstrual cycle was not provided. McFadden (2000) speculated that oral contraceptive (OC) use also might affect OAE production and tested this hypothesis in a retrospective analysis of SOAE and CEOAE data collected from young women. Modest differences in the means were observed on several OAE measures, but none of these differences were significant. Previously undetected SOAEs were found in a transsexual male while undergoing estrogen replacement (and androgen suppression) before sex-reassignment surgery (McFadden et al., 1998). Furthermore, hearing sensitivity, which shares physiological substrates with OAE production, exhibits variation over the menstrual cycle, with poorer auditory thresholds during menses when ovarian output is lowest (Swanson and Dengerink, 1988). Recent demonstrations of estrogen receptor expression in the mouse, rat, and adult human cochlea (Stenberg et al., 1999, 2001), notably the presence of ligand-dependent estrogen receptor beta (ER$\beta$, a subtype of the estrogen receptor) in the inner and outer hair cells (Meltser et al., 2008), afford a potential mechanism by which circulating estradiol, the dominant estrogen in women of reproductive age, could influence OAE production.

As a step toward defining the role of adult steroid concentrations, the goal of the current study was to investigate whether the use of OCs affects the production of SOAEs and CEOAEs in women as predicted and first tested by McFadden (2000). Although no significant difference between OC users and nonusers was found by McFadden (2000), OC formulations have changed appreciably over time. OCs reliably suppress the ovarian production of estradiol and the rise in progesterone that follows ovulation (Kafrissen and Adashi, 2003). If circulating estradiol levels are, indeed, an important regulator of OAE production, then we predict that the suppression of estradiol through OC use will influence the capacity to generate OAEs in women, as reflected in the number and overall power of SOAEs produced, and the response amplitude of CEOAEs elicited in response to acoustical stimulation.

## EXPERIMENTAL PROCEDURES

### Participants

Male ($n$=45) and female ($n$=50) undergraduates, aged 17–25 years, were recruited to participate in a study of sex differences in the auditory system. Ethnic differences in OAE production have been reported previously (Whitehead et al., 1993); 89% of the present sample was Caucasian, 1% Black, and 10% Asian (including one OC user and three non-OC users). In the present work, the OAE results were the same with and without all ethnic subgroups included, therefore the full data set is reported here.

All volunteers initially underwent standard clinical audiometric screening using a GSI-17 pure-tone air-conduction audiometer, at frequencies from 250 to 8000 Hz, to ensure inner-ear integrity. Individuals who did not pass the screening criterion (i.e., who had audiometric thresholds greater than 25 decibels (dB) hearing level at any of the tested frequencies) were not included.

Eligible participants were classified retrospectively into three groups based on their responses to a demographic and health questionnaire that was given following the OAE testing: males ($n$=39), females not using OCs at present (female non-OC users; $n$=26), and females who self-identified as using OCs at present (female OC users; $n$=20). Females in the OC group used standard low-dose OCs containing 20–30 $\mu$g/d of ethinyl estradiol; approximately 90% used OCs containing 25 $\mu$g or less (e.g., Alesse 21). About 60% used triphasic OCs that varied in their progestogen content over the 21-day contraceptive cycle but had a fixed dose of ethinyl estradiol (e.g., Tri-Cyclen Lo). The remainder took monophasic formulations. Sexually active females in the non-OC group used other methods of birth control that did not include any form of hormonal contraception (e.g., injections, patch). Because classification into groups took place retrospectively, no attempt was made to assess OAEs at any particular stage of the menstrual cycle. The three groups were well-matched on age: males ($M$=20.84 y±2.59 SD), female non-OC users (19.65 y±1.83 SD), female OC users (20.09 y±2.25 SD).

The demographics questionnaire also contained items that screened for health conditions previously shown to affect OAE production, such as use of certain prescription drugs and ear or cochlear damage or surgery (McFadden and Plattsmier, 1984;