1-28-2011 12:00 AM

Sexual Differentiation in the Auditory System: An Investigation into Prenatal and Adult Sex Steroid Influences on Otoacoustic Emissions

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Neuroscience

SEXUAL DIFFERENTIATION IN THE AUDITORY SYSTEM: AN INVESTIGATION INTO PRENATAL AND ADULT SEX STEROID INFLUENCES ON OTOACOUSTIC EMISSIONS

(Spine title: Endocrine Influences on Otoacoustic Emissions in Humans)

(Thesis format: Integrated Article)

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Graduate Program in Neuroscience

A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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School of Graduate and Postdoctoral Studies

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entitled:

Sexual Differentiation in the Auditory System: An Investigation into Prenatal and Adult Sex Steroids Influences on Otoacoustic Emissions

is accepted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Date

Chair of the Thesis Examination Board

Abstract

Otoacoustic emissions (OAEs) are a naturally occurring by-product of the outer hair cells in the cochlea of the inner ear. A sexual dimorphism in OAE production favouring females has been reported in both human and non-human species. The broad objective of the present set of studies is to explore how the sexual dimorphism originates and the degree to which it reflects the organizational and activational influences of sex steroid hormones.

Most previous studies of sex differences in OAEs have been based on neonatal, infant, or broad adult samples, Study 1 of the present work was done to verify the reported sex difference, both in spontaneously produced OAEs (spontaneous OAEs or SOAEs) and in OAEs produced in response to acoustic stimuli (click-evoked OAEs or CEOAEs), in a sample of non-hearing impaired young adults. Ear differences in OAE production also have been reported, and this study also investigated whether hand preference moderates the observed ear asymmetry in OAE production. Although a robust sex difference was documented in the numbers and powers of SOAEs produced, and in CEOAE response amplitude, there was no evidence to support a reduced ear asymmetry in left-handers.

The major theory purporting to explain the sex difference in OAE production proposes that prenatal androgen exposure in the male fetus dampens the cochlear mechanisms responsible for OAE production and is responsible for the observed sex difference in this trait. In order to test the proposed organizational influence on OAE production, the relationship between OAEs and the ratio of the lengths of the 2^{nd} to 4^{th} digits (the 2D:4D ratio), a marker of individual variations in prenatal androgen exposure, was examined in Study 2. A significant correlation between OAE production and 2D:4D

digit-ratios was not found. Fundamental differences in the prenatal development of these characteristics, however, may explain the lack of correlation and do not preclude a prenatal hormonal influence on OAE production.

Another source of variation that may contribute to the sex difference in OAE production is circulating levels of adult steroids. Evidence supporting this possibility is limited. Studies 3 and 4 provided a novel test of the hypothesized activational influence of sex steroids in women and men. Oral contraceptive use in women, which reliably decreases circulating sex steroids, was shown to reduce OAE production compared to normally-cycling women. In Study 4, a negative correlation was found between CEOAE response amplitude and circulating testosterone levels in men. Thus, it appears in men that elevations in circulating testosterone diminish OAE production in a manner similar to that hypothesized prenatally, whereas the results in women suggest that estradiol may influence OAE production in adulthood. These are the first systematic studies to support an activational effect of circulating steroids on OAE production in humans.

Keywords: otoacoustic emissions, auditory, prenatal, organizational, activational, hormones, sex steroids, testosterone, estrogens, sex difference

Co-Authorship

All experimental work in this thesis was carried out solely by Adrian W.K. Snihur. Dr. Elizabeth Hampson supervised and contributed to all aspects of the projects contained within this thesis dissertation (e.g., experimental design, data analysis, interpretation, writing of manuscripts). The written material in this thesis is my own work, but, as my advisory, Dr. Hampson provided assistance in editing and revising all of the material contained within this thesis.

Acknowledgements

I would like to take this opportunity to acknowledge all of the people who have contributed, in one form or another, to my graduate school experience. Firstly, I would like to thank my supervisor, Dr. Elizabeth Hampson, for her mentorship during the course of both my Ph.D. and Master's studies. Your constant words of encouragement, guidance, and invaluable contributions in all aspects of my thesis work were greatly appreciated, and I am extremely fortunate to have had the opportunity to work with you. I have learned a great deal from you, and would not be the researcher I am today if not for your outstanding supervision.

I would like to thank Dr. Dennis McFadden and Ed Pasanen from the University of Texas at Austin for introducing our lab to the epiphenomenon of otoacoustic emissions. If not for your dedicated pursuit of studying this auditory trait, I would not have had the opportunity to pursue these research interests. I would also like to express my sincerest gratitude to both of you for the time you took to train me in the delicate art of recording otoacoustic emissions, the use of your custom-written software, as well as your wonderful southern hospitality during my visit to Austin.

I would also like to acknowledge the members of my advisory committee, Dr. David Sherry and Dr. Martin Kavaliers, for providing useful feedback on my research projects and for overseeing my progress through graduate school.

I would like to acknowledge my family and friends for their constant support during my entire graduate school experience. I would like to thank my friends, both near and far, for always being there with words of encouragement when I needed them. To my mother, father, brother, and sister, I would like to thank you from the bottom of my heart for your unconditional love and support in all my endeavours. Your constant

encouragement and presence in my life has allowed me to achieve all that I have, as well as to strive to accomplish all that I desire. I love you!

Finally, I would like to acknowledge and thank my fiancée, Lesia Nestorowich, for accompanying me on this journey. It seems like yesterday that I met you and was explaining to you my Master's research as I was preparing for my defence, and now I am planning on closing another chapter of my academic life with you. You have been a wonderful companion throughout my entire Ph.D. experience, and have always been there with encouragement, reassurance, love, and support as I strived to reach my ultimate goal. I am extremely proud of you and what you have become, and I know that the feeling is reciprocated. I look forward to spending the rest of my life with you and am excited for the next chapter in our lives as husband and wife. I love you with all of my heart!

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List of Abbreviations

2D:3D: ratio of the length of the 2nd to 3rd digit
2D:4D: ratio of the length of the 2nd to 4th digit
2D:5D: ratio of the length of the 2nd to 5th digit
3D:4D: ratio of the length of the 3rd to 4th digit
3D:5D: ratio of the length of the 3rd to 5th digit
4D:5D: ratio of the length of the 4th to 5th digit
ANOVA: analysis of variance
ABR: auditory brainstem response
CAH: congenital adrenal hyperplasia
CAIS: complete androgen insensitivity syndrome
CEOAEs: click-evoked otoacoustic emissions
CNS: central nervous system
dB: decibels
DPOAEs: distortion-product otoacoustic emissions
ERα: estrogen-receptor alpha
ERβ: estrogen-receptor beta
hr: hour
Hz: hertz
kHZ: kilohertz
min: minute
mm: millimeter
mRNA: messenger ribonucleic acid
ms: millisecond
nmol/L: nanomoles per liter
NRH: non-right-handed
OAEs: otoacoustic emissions
OC: oral contraceptive
OS: operating system
p: significant values
peSPL: peak equivalent sound pressure level
pg/mL: picograms per millileter
r: correlation values
RH: right-handed
SD: standard deviation
sec: second
SEM: standard error of the mean
SHBG: sex hormone-binding globulin
SOAEs: spontaneous otoacoustic emissions
SPL: sound pressure level
X^2: chi-squared

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

The effects that hormones have on various physical, behavioural, and cognitive traits in human and non-human species are deeply rooted in history. As early as 350 B.C., Aristotle reported significant deviations from normal physical development and characteristics in adult male birds and humans whose testes were castrated or mutilated early in development (Aristotle, 1910). He also observed that the degree to which the physical characteristics in these males were affected, or altered, was dependent on the developmental period (i.e., either early development or adulthood) during which the endocrine system was disrupted. Although the physical changes resulting from castration were well-known and the practice applied to many different species for multiple purposes (e.g., castrating boys to maintain their high voices for opera singing), it was not until the 19th and 20th centuries that a physiological explanation for this phenomenon was provided and the field of behavioural endocrinology emerged.

Arnold Adolph Berthold's observations in the mid-1800s of the effects of castration and reimplantation of testis in cockerels on adult development represented the first formal study in endocrinology. Berthold showed that early castration of cockerels inhibited their normal male development, but that reimplantation of the testes into their abdominal cavity, either their own testes or those from another castrated cockerel, produced a normal male rooster (Berthold, 1849). His observation that the reimplanted testes formed vascular connections and functioned normally despite having their nerves severed suggested that a blood-borne product (i.e., hormones) can cause changes in various physical and behavioural characteristics. This idea was largely ignored and

repudiated in the scientific community until 1959, when Charles Phoenix and his colleagues conducted their classical study examining the role of hormones in guinea pig mating behaviour (Phoenix, Goy, Gerall, & Young, 1959). The results of their study not only offered support for the idea that hormones can cause changes in the probability that specific behaviours will be elicited in the appropriate behavioural or social setting, but also emphasized the importance of sex steroids in the manifestation of male-typical and female-typical behaviours, as well as their roles at different stages of development. Phoenix et al. (1959) observed that administration of testosterone to female guinea pigs during an early, critical period in prenatal development resulted in the suppression of female-typical mating behaviours in adulthood. This phenomenon, whereby exposure to sex steroids in early development can permanently alter the structural features of the brain and its behavioural characteristics in adulthood, is termed an *organizational* effect of the hormones (Arnold & Breedlove, 1985; Phoenix et al., 1959). In the case of sexual differentiation of behaviour, the presence or absence of high levels of testosterone during a finite time period during prenatal development (prenatal weeks 8-24 in humans; Forest, de Peretti, & Bertrand, 1976) results in the capacity to display male-typical or female-typical behaviours in adulthood, respectively. Phoenix et al. (1959) also observed that circulating sex steroids in adulthood are responsible for activating these neural substrates to produce specific behaviours. This *activational* effect of hormones was supported by the observation that male guinea pig mating behaviours were elicited in gonadectomised female guinea pigs treated with testosterone propionate prenatally only when a critical level of testosterone was present in their bloodstream in adulthood. Further, this

activational influence of testosterone was temporary and the effects on behaviour were reversible.

Sexually dimorphic behaviours in many species are believed to be organized during early development and are largely attributable to differential exposure of the two sexes to sex steroids during prenatal or perinatal differentiation. In humans, sexual differentiation occurs prenatally, and it is the differential exposure to sex steroids, namely testosterone derived from the fetal testes, that is responsible for organizing neural and peripheral substrates in a male-typical or female-typical manner. Under normal circumstances, males carry an X and Y chromosome, whereas females carry two X chromosomes. During normal human fetal development, the gonads, which are bipotential in the embryo, differentiate into testes in the presence of a gene on the Y chromosome known as SRY (sex-determining region of the Y chromosome; Berta, Hawkins, Sinclair, Taylor, Griffiths, & Goodfellow, 1990). In the absence of this gene, ovaries develop instead. Since the default sex in humans is female, the presence of the testes results in the secretion of both testosterone, which stimulates the development of the Wolffian duct system (i.e., male accessory sex organs) and the external genitalia (via conversion to dihydrotestosterone through the enzyme 5-α reductase), and Mullerian-inhibiting hormone, which inhibits the development of the Mullerian duct system (i.e., female accessory sex organs). If these two hormones are absent during this early period of development, the Mullerian duct system differentiates normally into female internal organs (e.g., fallopian tubes, uterus) and the Wolffian duct system regresses. Ovarian hormones are not required for normal female development.

In addition to differentiating the external genitalia, the development of testes in the male fetus and secretion of active androgenic hormones, specifically testosterone, are responsible for further sexual differentiation of neural and peripheral structures around prenatal weeks 8 to 24 (Forest, de Peretti, & Bertrand, 1976). Neural structures in the male fetus are masculinized via aromatization to estradiol in many species, but this conversion does not seem to be required for masculinisation to occur in humans or other primates, where direct actions of testosterone or dihydrotestosterone seem to be the dominant route by which sexual differentiation of the brain comes about (Breedlove & Hampson, 2002). As a result, it is this prenatal exposure to testosterone and other androgens from the fetal testes and, to a lesser extent, the adrenal glands, which provides the foundation for many of the sexually dimorphic physical, behavioural, and cognitive traits observed in humans.

There are numerous species-specific behaviours and traits that exhibit sexual dimorphism. Certain behaviours are under the influence of both organizational and activational effects of hormones, such as guinea pig mating behaviour (Phoenix et al., 1959), whereas other traits appear to be under the influence of one or the other (Goy & McEwen, 1980). Female zebra finches will not sing in adulthood even if injected with testosterone, suggesting that the mechanisms responsible for birdsong in this species are organized prenatally (Adkins-Regan & Ascenzi, 1987). Similarly, rough-and-tumble play in rhesus monkeys has been shown to be sexually dimorphic from birth and is organized by prenatal exposure to either testosterone or dihydrotestosterone (Goy, 1978). On the other hand, the pattern of electrical discharge in electric fish can be modified by

differential administration of sex steroids in adulthood, reflecting a purely activational basis for this sexually dimorphic characteristic (Bass, 1986).

Although the data from humans are controversial, a number of sexually dimorphic physical characteristics are thought to be organized during early prenatal development, including the size of the brain (Swaab & Hofman, 1984) and finger length ratios (Manning, Scutt, Wilson, & Lewis-Jones, 1998; Manning, Stewart, Bundred, & Trivers, 2004). Men and women also exhibit fundamental differences in performance on various cognitive tasks that may be indicative of early hormonal effects, such as visuospatial abilities (Hampson, Rovet, & Altman, 1998; Puts, McDaniel, Jordan, & Breedlove, 2008; Resnick, Berenbaum, Gottesman, & Bouchard, 1986). However, studies have also shown that fluctuations in the concentration of adult sex steroids can diminish or strengthen the magnitude of the observed sex difference and influence performance on spatial cognitive tasks (Kimura & Hampson, 1994; Hampson, 2008), emphasizing the importance of examining both the organizational and activational effects of hormones on brain and behaviour.

The main objective of this dissertation is to investigate the endocrine underpinnings, both organizational and activational, of an auditory trait called otoacoustic emissions (OAEs). Briefly, OAEs are faint sounds produced as a by-product of an amplification mechanism in a normally functioning cochlea that can be detected by a low-noise microphone inserted into the external ear canal (Kemp, 1978; Davis, 1983). This trait is sexually dimorphic in humans (at least in children) and in selected non-human species, and it has been hypothesized that the sex difference is mediated by differential exposure of males and females to androgens during prenatal development (McFadden &

Loehlin, 1995; McFadden, Pasanen, Valero, Roberts, & Lee, 2009; McFadden, Pasanen, Weldele, Glickman, & Place, 2006). However, to date the most definitive evidence in support of the proposed prenatal androgen hypothesis has been found in non-human species (rhesus monkeys and hyenas), and detailed information regarding the mechanism responsible for OAEs, the observed sex difference, and other OAE characteristics in humans is still missing. Furthermore, recent research has raised the possibility that circulating sex steroids in adulthood may influence OAE production, although, to date, research examining an activational influence of hormones on OAEs is extremely limited.

It is anticipated that the results of the experiments in this thesis will: 1) validate the sex difference in OAE production in normally-hearing young adults that has been previously shown to exist in infants and children; 2) provide a further test of the organizational hypothesis by examining whether or not a correlation exists between individual differences in OAE production and a known marker of individual variation in the level of prenatal androgen exposure; and 3) offer novel evidence to test the hypothesis of an activational influence of adult sex steroids on OAE production. Overall, these investigations will not only provide valuable insight into the underlying mechanisms and hormonal influences involved in this auditory trait, but may also offer further evidence of the dynamic modulatory effects that hormones can have on brain and behaviour.

1.2 Otoacoustic Emissions

The main roles of the auditory system are to deliver acoustic stimuli to receptors within the ear, to transduce stimuli from pressure changes (sound waves) into electric signals in the cochlea, and to effectively process these electric signals so that information

can be derived indicating the qualities of the sound source (Figure 1.1). Incoming sound waves initially are mechanically amplified by the middle ear system (the bony ossicles) prior to reaching the inner ear (the cochlea) to account for a mismatch between the low-impedance air medium in the ear canal and the high-impedance fluid medium in the cochlea. These sound waves reach the cochlea and displace the basilar membrane, resulting in the bending of the inner hair cells and transduction of the mechanical sound signal into electric signals via neurotransmitter release. In addition to the single row of approximately 3,500 inner hair cells arranged along the length of the Organ of Corti, approximately 12,000 outer hair cells are arranged nearby in three (or four) rows (Figure 1.2). The outer hair cells function as "active cochlear amplifiers" by providing additional energy to low-intensity sounds by increasing the vibration of selected regions of the basilar membrane, resulting in sharper tuning and greater frequency sensitivity. The distinct functions of the inner and outer hair cells are supported by the extensive afferent and efferent innervations, respectively, of these two types of hair cells. A natural by-product emitted by this active cochlear amplification by the outer hair cells is the phenomenon of otoacoustic emissions (OAEs; Davis, 1983;).

OAEs are inaudible to the person emitting them because of their faint nature but can be detected in the external auditory canal using a high-sensitivity microphone system (Kemp, 1978). OAEs were proposed to be highly dependent on normal cochlear and outer hair cell functioning (Davis, 1983), and numerous studies have offered support for both a cochlear origin of OAEs as well as a connection between normal hearing and OAE production. At high intensities, the cochlear amplification mechanism is protectively restrained by the outer hair cells to prevent acoustic trauma; Kim et al. (1980) and

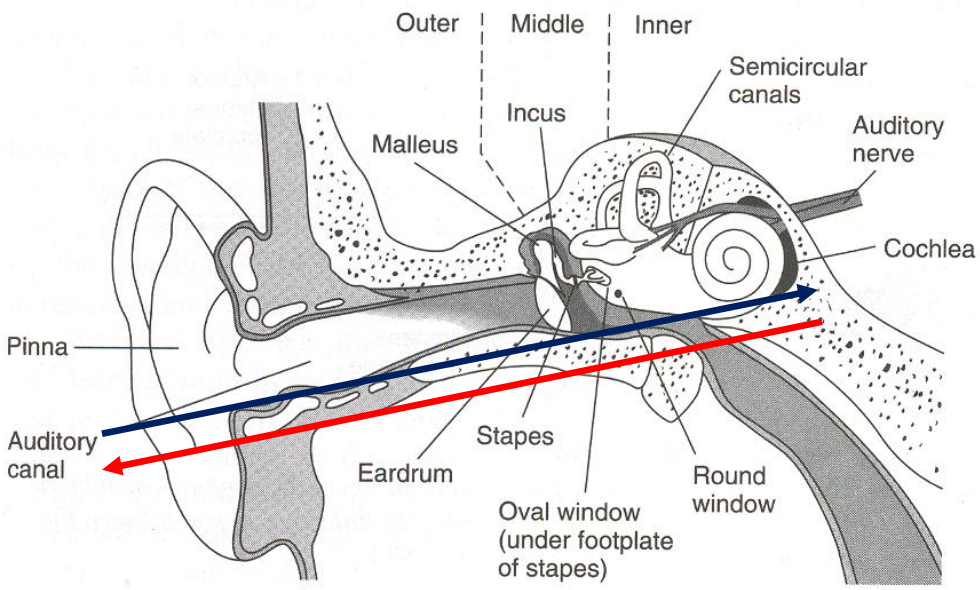

Figure 1.1. Schematic representation of the outer, middle, and inner ear components. The blue arrow represents the movement of sound from the external world to the cochlea, whereas the red arrow represents the opposite flow of OAEs. Adapted from *Principles of Neuroscience*, Kandel (2000).

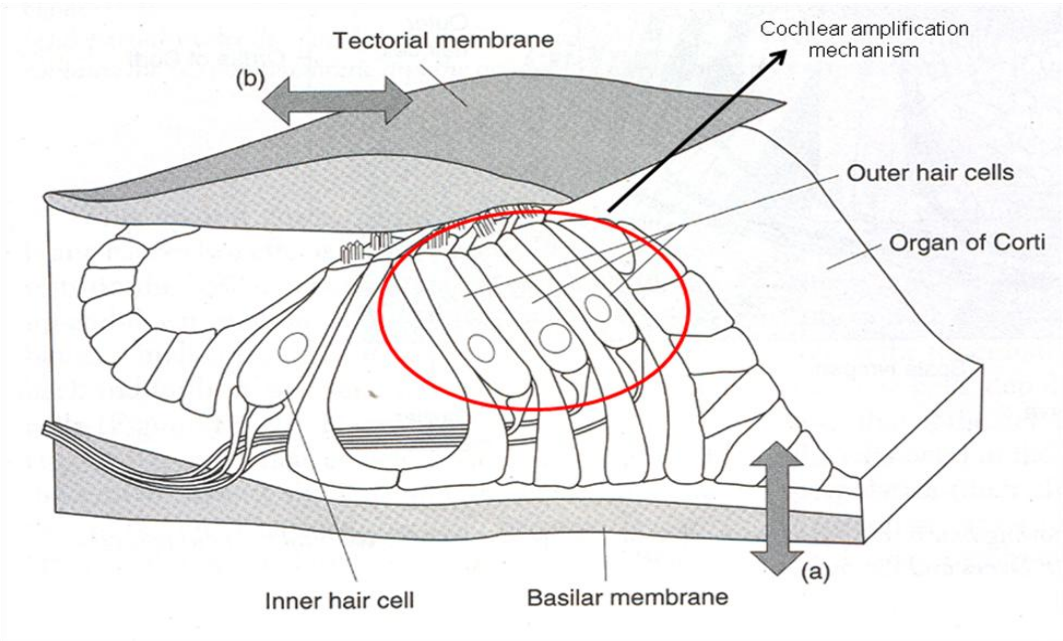

Figure 1.2. Schematic representation of the cross-section of the Organ of Corti and the arrangement of the inner and outer hair cells along the basilar membrane. Adapted from *Principles of Neuroscience*, Kandel (2000).

Lonsbury-Martin et al. (1987) showed in selected animals that excessive acoustic stimulation reduced OAE production. Evans, Wilson, and Borerwe (1981) recorded OAEs from guinea pigs administered paralyzing agents that abolished middle-ear muscle activity, suggesting that OAEs originate in the inner ear. Hypoxia has been shown to reduce both cochlear functioning and OAE production (Evans et al., 1981; Zwicker & Manley, 1981).

In humans, McFadden & Mishra (1993) observed that individuals producing greater than 4 SOAEs had better overall hearing compared to individuals with no detectable SOAEs, suggesting a positive relationship between hearing sensitivity and OAE production. OAEs also have been shown to be selectively absent in frequency regions where sensorineural hearing loss is greater than 30dB, but present in adjacent frequency regions where normal hearing persists (Probst, Lonsbury-Martin, Martin, & Coats, 1987). Further, exposure to ototoxic drugs that resulted in temporary hearing loss, such as aspirin or quinine sulphate, partially reduced or completely eliminated the detection of OAEs (McFadden & Pasanen, 1994; McFadden & Plattsmier, 1984; Weir, Pasanen, & McFadden, 1988). These studies offer substantial evidence that OAEs do in fact originate in the cochlea and are a by-product of the cochlear amplification mechanism involving the outer hair cells. Although OAEs are typically thought of as an epiphenomenon as opposed to a characteristic with an evolutionary purpose, OAE screening procedures are routinely used in clinical settings by audiologists to test for inner ear defects and possible hearing problems in newborn infants.

Three types of OAEs are commonly produced by normally-functioning cochleas: spontaneous, click-evoked, and distortion-product OAEs. Spontaneous OAE (SOAEs)

are emissions that are produced naturally in the ear without any deliberate external acoustic stimuli, and are produced by approximately 65% of the normal-hearing population (e.g., see Figure 1.3; Penner, Glotzbach, & Huang, 1993). Click-evoked (or transient-evoked) OAEs (CEOAEs), on the other hand, are echo-like waveforms produced in the ear in response to presentation of acoustic stimuli, either audible clicks or tone-burst stimuli. Nearly all normal-hearing individuals generate CEOAEs (Penner et al., 1993). Distortion-product OAEs (DPOAEs) are emissions that are produced as a product of two simultaneously-presented acoustic frequencies, with the new emissions consisting of frequencies that were not present in the eliciting stimuli. Since the measurement of DPOAEs is more traditionally used in animal research compared to human research (for review, see Probst, Lonsbury-Martin, & Martin, 1991), only SOAEs and CEOAEs are discussed in further detail.

SOAEs can be detected in preterm neonates as early as 30 weeks (Morlet et al., 1995), but appear to decrease slightly in prevalence and number throughout infancy and childhood (Lamprecht-Dinnessen et al., 1998), as well as into adulthood (Burns, Arehart, & Campbell, 1992). It appears that the SOAE frequencies that are lost with increasing age are typically those at higher frequency levels (Burns et al., 1992). That being said, SOAEs are fairly stable throughout life and new SOAEs are highly unlikely to appear (Burns, Campbell, & Arehart, 1994). Researchers have found decreases in CEOAE response amplitude with advancing age (Bonfils, Bertrand, & Uziel, 1988; Collet, Moulin, Gartner, & Morgan, 1990), and it has been suggested that these decreases may be attributable mostly to age-related hearing loss. A correlation of .76 between the number of SOAEs produced and CEOAE response amplitude has been reported (McFadden &

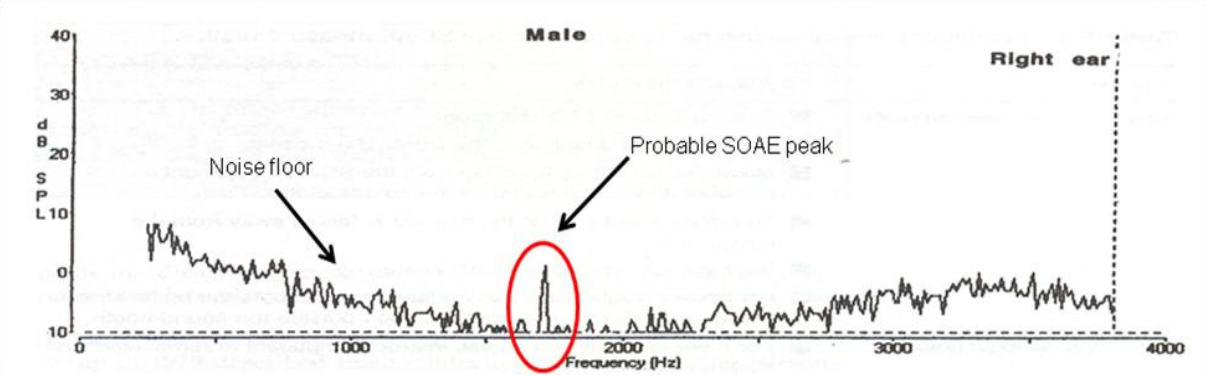

Figure 1.3. An example of a probable SOAE peak in a frequency spectrum from an adult male. Adapted from *Handbook of Otoacoustic Emission*, Hall (2000).

Pasanen, 1999), suggesting that the mechanisms underlying SOAE and CEOAE production are likely to be overlapping but not identical (Shera & Guinan, 1999).

SOAEs are often more pronounced and more frequent in the right ear than the left (Bilger, Matthies, Hammel, & DeMorest, 1990; Burns et al. 1992; Talmadge, Long, Murphy, & Tubis, 1993). A right ear advantage in hearing sensitivity has also been found in a large-scale audiometric study (Chung, Mason, Gannon, & Willson, 1983). Mechanistically, it has been proposed that this ear difference originates from differential efferent innervation of the outer hair cells of the cochlea by the medial olivocochlear system (McFadden, 1993a). Specifically, it is proposed that the medial olivocochlear efferent system that synapses with the right ear delivers *less* inhibition to those outer hair cells, resulting in greater hearing sensitivity and greater OAE production compared to the more highly inhibited left ear. This inverse relationship between efferent activation and OAE production has been supported by studies showing that electrical or mechanical stimulation of the medial olivocochlear system reduced or eliminated OAEs in the ipsilateral ear (Collet, Kemp, Veuillet, Duclaux, Moulin, & Morgan, 1990; Mountain, 1980). However, evidence opposing a right ear advantage in OAE production also exists (Collet, Gartner, Veuillet, Moulin, & Morgan, 1993), and the degree to which the proposed mechanism fully explains the ear asymmetry in OAE production remains unclear (Khalfa & Collet, 1996).

One of the specific aims of this thesis is to investigate the developmental origins of the observed ear difference in OAE production in a population of normally-hearing young adults. In addition to the aforementioned mechanism of differential efferent innervation between the ears, several other theories have been proposed to account for ear

differences in auditory properties in general, including peripheral lateralization in the auditory system (Previc, 1991) as well as the effects of differential androgen exposure on the development of lateralized systems (Geschwind & Galaburda, 1985a, 1985b, 1985c; Witelson, 1991; Witelson & Nowakowski, 1991; Lauter, 2007). A preliminary association between the medial olivocochlear efferent system and hand preference, a visible asymmetry in humans that acts as a marker of departures from standard patterns of lateralization, has been reported, albeit in a single study. Symmetrical activation of the medial efferent system was observed in the right and left ears of left-handed individuals, whereas greater activation was reported in the right ear of right-handed individuals (Khalfa & Collet, 1996; Khalfa, Veuillet, & Collet, 1998), a result consistent with greater lateralization of functioning in right-handed individuals and deviations from this pattern in non-right-handed individuals (Bryden, 1982). However, these results are only preliminary. Further investigation into the effects of differences in brain lateralization, as evident by differences in the direction and degree of hand preference, on SOAE and CEOAE production between the ears will shed greater light on the mechanisms responsible for the observed ear difference.

1.3 Organizational Influence on OAE Production

Sexual dimorphisms have been reported to exist in OAE production, with females, on average, producing greater numbers and strengths of SOAEs and CEOAEs with greater response amplitude compared to males (Bilger et al., 1990; Burns et al., 1992; Lamprecht-Dinnesen et al., 1998; Penner et al., 1993; Strickland, Burns, & Tubis, 1985). In addition, SOAEs have been reported to be more prevalent in females compared to

males (75%-85% in females vs. 45%-65% in males; Bilger et al., 1990; Talmadge, Long, Murphy, & Tubis, 1993). The sex difference has been found largely in neonates, infants, and children (Burns et al., 1992; Bonfils, Francois, Avan, Londero, Trotoux, & Narcy, 1992; Strickland et al., 1985), but also has been reported in specific adult populations (for review, see Bilger et al., 1990). The sex difference appears to be robust, although it does appear to be most prominent in the first year after birth (Lamprecht-Dinnesen et al., 1998).

The prevailing explanation for the sexual dimorphism in OAE production is the prenatal androgen hypothesis. This hypothesis states that higher levels of androgen exposure prenatally during the critical window for sexual differentiation *masculinizes* the auditory system, including the cochlear structures integral to OAE production (i.e., outer hair cells), resulting in diminished OAE production. Since the male fetus but not the female fetus is exposed to elevated androgens during prenatal development, it would be expected that OAE production would be diminished in males compared to females. Anatomical studies have shown that the onset of human cochlear functioning and maturation of cochlear structures overlaps with the period of elevated prenatal testosterone exposure in the developing male fetus (Lavigne-Rebillard & Pujol, 1986; Pujol & Lavigne-Rebillard, 1995). Structural observations of the anatomy of the human cochlea have shown that sex differences exist in several cochlear properties (Sato, Sando, & Takahashi, 1991), including the number of outer hair cells (Wright, Davis, Bredberry, Ulehlova, & Spencer, 1987). Given that many somatic sex differences are induced though the actions of prenatal testosterone, it is reasonable to postulate that differential

exposure to testosterone could explain the observed sexual dimorphism in OAE production.

Investigations of patterns of OAE production in several special populations of human and non-human subjects have offered support for the hypothesized prenatal masculinisation of OAEs. Female spotted hyenas (*Crocuta crocuta*), which are highly androgenised during prenatal development, exhibit CEOAE response amplitudes similar to those of male hyenas (McFadden, Pasanen, Weldele, Glickman, & Place, 2006). Prenatal administration of anti-androgenic drugs to developing male or female spotted hyenas resulted in the production of stronger CEOAE response amplitudes in adulthood compared to normally-developing hyenas, supporting an inverse relationship between prenatal androgen exposure and OAE production. A study in the domestic sheep showed a decrease in CEOAE response amplitude in female sheep who were treated with testosterone propionate during prenatal development, again offering evidence that exposure to high levels of testosterone prenatally *masculinises* the cochlear mechanisms responsible for OAEs, resulting in diminished OAE production.

In humans, support for the prenatal masculinisation of OAEs has been less direct and research has been limited by the inability to manipulate prenatal hormones in humans. Females with male co-twins (opposite-sex dizygotic twins) have been shown to have *masculinised* OAEs compared to females with female co-twins (same-sex dizygotic twins), monozygotic female twins, and singleton females (McFadden, 1993b; McFadden & Loehlin, 1995). It has been proposed that females with male co-twins are exposed to higher-than-normal levels of androgens from the male fetus during prenatal development, a developmental occurrence observed in many rodent species (vom Saal, 1989), resulting

in partially masculinised OAEs. However, whether or not appreciable amounts of testosterone diffuse from the male to the female co-twin in humans is still empirically unconfirmed. Another study found that homosexual and bisexual females produced SOAEs and CEOAEs that were intermediate in number and strength to heterosexual females and heterosexual males (McFadden & Pasanen, 1998, 1999). It was hypothesized that homosexual and bisexual females are exposed to elevated levels of androgens prenatally, thus resulting in both an altered sexual orientation and slightly masculinised OAEs. Although the evidence in humans alone is less direct, there is tentative support for the hypothesis that prenatal exposure to androgens influences SOAE and CEOAE production.

Consequently, another aim of this thesis is to provide a further test of the hypothesized organizational influence on OAE production in humans. This will be done by investigating the correlations between OAE production and an ostensible biological marker of prenatal androgen activity, the 2D:4D digit-ratio. It is known that the outer hair cells integral to OAE production develop during the critical period for brain and behavioural differentiation in humans (weeks 8-24 of gestation), a period when testosterone is elevated in the male fetus (Lavigne-Rebillard & Pujol, 1986; Pujol & Lavigne-Rebillard, 1995); however, this alone does not constitute evidence of a prenatal hormonal influence on OAE production. By investigating the relationship between OAEs and known marker of individual variation in prenatal androgen exposure, vital information can be gathered regarding the prenatal mechanisms that underlie OAE production.

The ratio of the lengths of the 2nd to 4th digit of the hand (2D:4D ratio) has been widely touted as a physiological marker of prenatal androgen exposure and offers the possibility of indirectly examining the hypothesized prenatal influence of androgens on OAEs. The 2D:4D digit-ratio exhibits a robust sex difference, with females having a higher ratio (closer to 1.0) compared to males (Manning, Scutt, Wilson, Lewis-Jones, 1998; Manning, Stewart, Bundred, & Trivers, 2004; McIntyre, Cohn, & Ellison, 2006; Peters, MacKenzie, & Bryden, 2002). Females exposed to excessive androgen prenatally, through endocrine disorders such as congenital adrenal hyperplasia, or females hypothesized to have been exposed to excessive androgen prenatally (i.e., females with a male co-twin) have been shown to exhibit male-typical 2D:4D digit-ratios (Brown, Hines, Fane, & Breedlove, 2002; Ciumas, Linden Hirschberg, & Savic, 2009; Okten, Kalyoncu, & Yaris, 2002; van Anders, Vernon, & Wilbur, 2006; Voracek & Dressler, 2007). Conversely, individuals possessing both X and Y chromosomes but who have no prenatal androgen exposure due to complete androgen insensitivity syndrome have female-typical 2D:4D digit-ratios (Berenbaum, Bryk, Nowak, Quigley, & Moffat, 2009). The sex difference in 2D:4D digit-ratios is observed as early as weeks 9-12 of gestation (Malas, Dogan, Evcil, & Desdicioglu, 2006), offering a timeline for the prenatal masculinization of finger lengths. Further still, 2D:4D digit-ratios in two-year old children have been found to be negatively correlated with their fetal testosterone:estradiol ratio, measured from amniotic fluid, supporting a continuum of digit development in relation to the concentrations of prenatal testosterone (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004). It is anticipated that an examination of the relationship between SOAE and CEOAE production and 2D:4D digit-ratios in men and women may provide

further evidence supporting an organizational hormonal influence on OAE production in humans.

1.4 Activational Influences on OAE Production

Although previous studies have addressed the potential role that prenatal masculinisation of auditory structures (i.e., the outer hair cells) may play in the production of OAEs, studies examining influences of circulating adult levels of hormones on OAE production are limited and inconsistent at best. Nearly all the evidence is indirect, and provides only circumstantial support for the idea that steroid hormones may be involved; no specific links to particular hormones have been identified, nor have hormonal measurements even been included in existing studies.

A few studies have been conducted examining the potential relationship between OAE production and various biological rhythms. Circadian changes in SOAE frequencies have been reported, with minimal decreases in SOAE frequency observed throughout the day in certain individuals, but not in others (Bell, 1992; Haggerty, Lusted, & Morton, 1993). It is unclear whether or not these circadian changes, should they prove to be reliable, are related to hormone levels; levels of several steroids including cortisol and testosterone do show a circadian rhythm in secretion or release (Nelson, 2005). Menstrual cycle effects on OAE production have been hypothesized, but not confirmed, based on single-case reports, with apparent decreases in SOAE frequencies observed around menstruation and increases in SOAE frequency near ovulation (Bell, 1992; Haggerty et al., 1993). For example, a case-study of a 21-year old female showed a pattern of cyclic fluctuations in her SOAE frequencies that appeared to approximate the

length of her menstrual cycle, as well as greater stability in SOAE frequencies during a period of amenorrhea and a period of oral contraceptive (OC) use. These data must be considered primarily speculative, in that endocrine verification of the menstrual cycle was not included. However, it is conceivable that circulating hormones (ovarian, in this case) may have an influence on OAE production in adulthood (Penner, 1995). It should be noted that all of these studies focused on the frequencies of the emitted SOAEs, not the numbers or amplitudes of the emissions. A potential effect of OC use on SOAE production and CEOAE response amplitude in women has been hypothesized (McFadden, 2000), but a significant relationship between OAEs and OC use has not been established.

Seasonal fluctuations in testosterone levels occur in male rhesus monkeys (Gordon, Rose, & Bernstein, 1976; McFadden et al., 2006), in the wild and in captivity. A recent study has documented parallel changes in their patterns of OAE production (McFadden et al., 2006). Male rhesus monkeys produced lower CEOAE response amplitudes during the breeding season (i.e., elevated testosterone levels) compared to the non-breeding season (i.e., basal testosterone levels), a pattern that is consistent with the hypothesized dampening effects of androgens on OAE production. A direct link between testosterone and the changes in CEOAE amplitude has not been established, however, and it must be acknowledged that many biological and environmental variables besides testosterone do show a seasonal change. A study examining the potential effects on OAEs of seasonal changes in testosterone in men has not been conducted, but humans too show seasonal variation in testosterone levels (Dabbs, 1990; Moffat & Hampson, 2000; Svartberg et al., 2003). In men, the only study to my knowledge examining the effects of

circulating levels of steroids on OAE production was a case-study that found that estradiol administration (and suppression of androgens) prior to sex-reversal surgery resulted in the appearance of SOAEs where there were previously none (McFadden, Pasanen, & Callaway, 1998).

In light of the minimal focus on the possibility of activational influences of sex steroids on OAE production, the final aim of this thesis is to examine the effects that circulating adult sex steroids may have on OAE production in men and women. The common use of OCs in the adult female population, which reliably reduce testosterone and estrogen levels, offers the opportunity of studying the effects of circulating sex steroids on OAE production in women. As mentioned above, one previous study has examined the effects of OC use on SOAE number, overall SOAE power, and CEOAE response amplitude but failed to find any significant effects, although slight non-significant decreases in all parameters were seen in females using OCs compared to females not using OCs (*p*s ~ 0.5-0.7; McFadden, 2000). Should significant effects of OC use be found, it would not only offer potential support for an activational influence of adult hormones on OAE production, but would also offer insight into which circulating sex steroid, either testosterone or estradiol, is most likely to mediate the observed effects.

In men, a study investigating the effects of circulating testosterone on OAE production has not yet been conducted. In light of the seasonal influences on CEOAE response amplitudes observed in males of another species (i.e., rhesus monkeys; McFadden et al., 2006), such a study would provide a valuable contribution to the literature examining postnatal effects on OAE production. Seasonal elevations in testosterone production are most often observed in men during the autumn months and a

nadir during the spring (Dabbs, 1990; Moffat & Hampson, 2000; van Anders, Hampson, & Watson, 2006). Thus, obtaining direct measures of circulating testosterone and OAE production in men at different times of the year would allow for an investigation of potential seasonal hormonal effects on this auditory trait.

1.5 The Current Study

In sum, the objective of this thesis is to examine the possibility of prenatal and postnatal hormonal influences on OAE production. Clinical audiometric screening guidelines and custom-written OAE software and recording equipment will be used to gather data on hearing sensitivity and SOAE and CEOAE production. Standardized methods of discerning hand preference and of measuring finger lengths will be used to examine organizational influences on OAE production, whereas bioavailable testosterone concentrations, measured in saliva, will be incorporated in the studies investigating the possibility of activational influences. Measuring testosterone in saliva is considered superior to blood serum or plasma, because it provides a more accurate picture of the amount of hormone that is available to tissue for metabolic purposes (Vittek, L'Hommedieu, Gordon, Rappaport, & Southren, 1985).

In the present thesis, four studies will be described. Study 1 was conducted to verify that the sexual dimorphism in OAE production that has previously been reported, mostly in young children, can also be identified in normally-hearing adults, and to test the hypothesis that left- and right-handed individuals may differ in the degree of asymmetry in OAE production between the two ears. As described in Study 1, handedness itself is potentially a marker of differences in prenatal androgen exposure. Study 2 will

investigate the hypothesis of an organizational effect of testosterone on OAE production by examining the association between OAEs and a putative marker of prenatal androgen exposure, the 2D:4D digit-ratio. This thesis also will investigate the possibility of an activational influence of hormones on OAE production in humans. Specifically Study 3 will investigate whether or not the use of OCs in women is associated with differences in OAE production compared to women with an unassisted menstrual cycle. Further, it is hypothesized, based on recent work in rhesus monkeys by McFadden et al. (2006), that seasonal fluctuations in testosterone production in men will affect CEOAE production, with dampened CEOAE response amplitudes observed during periods of elevated circulating testosterone, and vice versa (Study 4).

It is anticipated that the results of these studies will not only contribute to the growing body of literature examining the mechanisms involved in OAE production, but will also more globally aid in our understanding of the range of effects that prenatal and postnatal hormones have on the brain and body.

1.6 References

Adkins-Regan, E. & Ascenzi, M. (1987). Social and sexual behaviour of male and female zebra finches treated with oestradiol during the nestling period. *Animal Behaviour*, 35, 1100-1112.

Aristotle (1910). *Historia Animalium*. Translated by D.W. Thompson. Clarendon Press, Oxford.

Arnold, A.P., & Breedlove, S.M. (1985). Organizational and activational effects of sex steroid hormones on vertebrate brain and behaviour: A re-analysis. *Hormones and Behavior*, *19*, 469-498.

Bass, A. H. 1986. Electric organs revisited: Evolution of a vertebrate communication and orientation organ. In Bullock, T. H. and Heiligenberg, W. [eds.], 10, *Electroreception*, p 13-70. John Wiley and Sons, New York, NY.

Bell, A. (1992). Circadian and menstrual rhythms in frequency variations of spontaneous otoacoustic emissions from human ears. *Hearing Research, 51(1),* 91-100.

Berenbaum, S. A., Bryk, K. A., Nowak, N., Quigley, C. A., & Moffat, S. (2009). Fingers as a marker of prenatal androgen exposure. *Endocrinology, 150(11),* 5119-5124.

Berta, P., Hawkins, J.R., Sinclair, A.H., Taylor, A., Griffiths, B.L., & Goodfellow, P.N. (1990). Genetic evidence equating SRY and the testis-determining factor. *Nature, 348*, 448-450.

Berthold, A.A. (1849). Transplantation of testes. Translated by D.P. Quiring. *Bulletin of the History of Medicine* (1944), *16*, 399-401.

Bilger, R. C., Matthies, M. L., Hammel, D. R., & DeMorest, M. E. (1990). Genetic

implications of gender differences in the prevalence of spontaneous otoacoustic emissions. *Journal of Speech and Hearing Research, 33,* 418-432.

Bonfils, P., Bertrand, Y., & Uziel, A. (1988). Evoked otoacoustic emissions: Normative data and presbycusis. *Audiology*, *27*, 27-35.

Breedlove, S. M. & Hampson, E. (2002). Sexual differentiation of the brain and behavior. In: J. B. Becker, S. M. Breedlove, D. Crews, & M. M. McCarthy (Eds.), Behavioral endocrinology (2nd ed.)(pp. 75-114). Cambridge, MA: MIT Press.

Brown, W. M., Hines, M., Fane, B. A., & Breedlove, S. M. (2002). Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. *Hormones and Behavior*, *42(4),* 380-386.

Bryden, M. P. (1982). *Laterality: Functional asymmetry in the intact brain*. New York; Academic Press.

Burns, E. M., Arehart, K. H., & Campbell, S. L. (1992). Prevalence of spontaneous otoacoustic emissions in neonates. *Journal of the Acoustical Society of America, 91(3),* 1571-1575.

Burns, E.M., Campbell, S.L., & Arehart, K.H. (1994). Longitudinal measurements of spontanous otoacoustic emission in infants. *Journal of the Acoustical Society of America*, *95,* 385-394.

Chung, D., Mason, K., Gannon, R., & Willson, G. (1983). The ear effect as a function of age and hearing loss. *Journal of the Acoustical Society of America, 73,* 1277-1282.

Ciumas, C., Linden Hirschberg, A., & Savic, I. (2009). High fetal testosterone and sexually dimorphic cerebral networks in females. *Cerebral Cortex, 19(5),* 1167-1174.

Collet, L., Gartner, M., Veuillet, E., Moulin, A., & Morgon, A. (1993). Evoked and spontaneous otoacoustic emissions: A comparison of neonates and adults. *Brain Development, 15*, 249-252.

Collet, L., Kemp, D., Veuillet, E., Duclaux, R., Moulin, A., & Morgon, A. (1990). Effect of contralateral auditory stimuli on active cochlear micro-mechanical properties in human subjects. *Hearing Research, 43*, 251-262.

Collet, L., Moulin, A., Gartner, M., & Morgon, A. (1992). Age-ralted changes in evoked otoacoustic emissions. *Annals of Otology, Rhinology, and Laryngology, 99*, 993-997.

Dabbs, J.M.Jr. (1990). Age and seasonal variation in serum testosterone concentration among men. *Chronobiology International, 7(3)*, 245-249.

Davis, H. (1983). An active process in cochlear mechanics. *Hearing Research, 9(1)*, 79-90.

Evans, E.F., Wilson, J.P., & Borerwe, T.A. (1981). Animal models of tinnitus. *Ciba Foundation Symposium, 85*, 108-138.

Forest, M. G., de Peretti, E., & Bertrand, J. (1976). Hypothalamic-pituitary-gonadal relationships in man, from birth to puberty. *Clinical Endocrinology, 5*, 551-569.

Geschwind, N. &. Galaburda, A.M. (1985a). Cerebral lateralization. Biological mechanisms, associations, and pathology: I. A hypothesis and a program for research. *Archives of Neurology, 42*, 428-459.

Geschwind, N. &. Galaburda, A.M. (1985b). Cerebral lateralization. Biological mechanisms, associations, and pathology: II. A hypothesis and a program for research. *Archives of Neurology, 42*, 521-552.

Geschwind, N. &. Galaburda, A.M. (1985c). Cerebral lateralization. Biological mechanisms, associations, and pathology: III. A hypothesis and a program for research. *Archives of Neurology, 42*, 634-654.

Gordon, T., Rose, R., Bernstein, I., (1976). Seasonal rhythm in plasma testosterone levels in the rhesus monkey (*Macaca mulatta*): a three year study. *Hormones and Behavior*, *7*, 229–243.

Goy, R.W. (1978). Development of play and mounting behaviour in female rhesus virilised prenatally with esters of testosterone or dihydrotestosterone. In D.J. Chivers and J. Herbert (eds.), *Recent Advances in Primatology*, Vol. 1, 449-462. Academic Press, London.

Goy, R. W., & McEwen, B. S. (1980). *Sexual differentiation of the brain*. Cambridge, MA: MIT Press. Hall, J.W., III (1992). *Handbook of auditory evoked responses*. Allyn and Bacon, Boston, MA.

Haggerty, H.S., Lusted, H.S., & Morton, S.C. (1993). Statistical quantification of 24-hour and monthly variabilities of spontaneous otoacoustic emission frequency in humans. *Hearing Research, 70(1)*, 31-49.

Hampson, E. (2008). Endocrine contribution to sex differences in visuospatial perception and cognition. In: J. B. Becker, K. J. Berkley, N. Geary, E. Hampson, J. P. Herman, & E. A. Young (Eds), *Sex differences in the brain: From genes to behavior* (pp. 311-325) Oxford University Press.

Hampson, E., Rovet, J. F., & Altmann, D. (1998). Spatial reasoning in children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Developmental Neuropsychology, 14*, 299-320.

Kemp, D. T. (1978). Stimulated acoustic emissions from within the human auditory system. *Journal of the Acoustical Society of America, 64(5),* 1386-1391.

Khalfa, S., & Collet, L. (1996). Functional asymmetry of medial olivocochlear system in humans. Towards a peripheral auditory lateralization. *Neuroreport, 7,* 993-996.

Khalfa, S., Veuillet, E., & Collet, L. (1998). Influence of handedness on peripheral auditory asymmetry. *European Journal of Neuroscience, 10(8),* 2731-2737.

Kim, D.O., Molnar, C.E., & Matthews, J.W. (1980). Cochlear mechanics: nonlinear behaviour in two-tone responses as reflected in cochlear-nerve-fiber responses and in ear-canal sound pressure. *Journal of the Acoustical Society of America, 67,* 1704-1721.

Kimura D., & Hampson E. (1994). Cognitive partem in men and women is influenced by fluctuations in sex hormones. *Current Directions in Psychological Science, 3,* 57-61.

Lamprecht-Dinnesen, A., Pohl, M., Hartmann, S., Heinecke, A., Ahrens, S., Muller, E., & Riebandt, M. (1998). Effects of age, gender and ear side on SOAE parameters in infancy and childhood. *Audiology and Neuro-otology, 3(6),* 386-401.

Lauter, J.L. (2007). The EPIC model of functional asymmetries: implications for research on laterality in the auditory and other systems. *Frontiers in Biosciences, 12,* 3734-3756.

Lavigne-Rebillard, M., & Pujol, R. (1986). Development of the auditory hair cell surface in human fetuses. *Anatomical Embryology, 174,* 369-377.

Lonsbury-Martin, B.L., Martin, G.K., Probst, R., & Coats, A.C. (1987). Acoustic

distortion products in rabbit ear canal. I. Basic features and physiological vulnerability. *Hearing Research, 28(2-3),* 1730189.

Lutchmaya, S., Baron-Cohen, S., Raggatt, P., Knickmeyer, R., & Manning, J. (2004). 2nd to 4th digit ratios, fetal testosterone and estradiol. *Early Human Development, 77(1-2),* 23-28.

Malas, M. A., Dogan, S., Evcil, E. H., & Desdicioglu, K. (2006). Fetal development of the hand, digits and digit ratio (2D:4D). *Early Human Development, 82(7),* 469-475.

Manning, J., Scutt, D., Wilson, J., & Lewis-Jones, D. (1998). The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. *Human Reproduction, 13(11),* 3000-3004.

Manning, J., Stewart, A., Bundred, P., & Trivers, R. (2004). Sex and ethnic differences in 2nd to 4th digit ratio in children. *Early Human Development, 80(2),* 161-168.

McFadden, D. (1993a). A speculation about the parallel ear asymmetries and sex differences in hearing sensitivity and otoacoustic emissions. *Hearing Research, 68,* 143-151.

McFadden, D. (1993b). A masculinising effect on the auditory systems of human females having male co-twins. *Proceedings of the National Academy of Sciences, 90,* 11900-11904.

McFadden, D. (2000). Masculinizing effects on otoacoustic emissions and auditory evoked potentials in women using oral contraceptives. *Hearing Research, 142,* 23-33.

McFadden, D., & Loehlin, J. C. (1995). On the heritability of spontaneous otoacoustic

emissions: A twins study. *Hearing Research, 85,* 181-198.

McFadden, D., & Mishra, R. (1993). On the relation between hearing sensitivity and otoacoustic emissions. *Hearing Research, 71,* 208-213.

McFadden, D., & Pasanen (1994). Otoacoustic emissions and quinine sulphate. *Journal of the Acoustical Society of America, 95(6),* 3460-3474.

McFadden, D., & Pasanen, E. (1998). Comparison of the auditory systems of heterosexuals and homosexuals: Click-evoked otoacoustic emissions. *Proceedings of the National Academy of Science, 95,* 2709-2713.

McFadden, D., & Pasanen, E. (1999). Spontaneous otoacoustic emissions in heterosexuals, homosexuals, and bisexuals. *Journal of the Acoustical Society of America, 105(4),* 2403-2413.

McFadden, D., & Plattsmier, H. (1984). Aspirin abolishes spontaneous oto-acoustic emissions. *Journal of the Acoustical Society of America, 76(2),* 443-448.

McFadden, D., Pasanen, E.G., & Callaway, N.L. (1998). Changes in otoacoustic emissions in a transsexual male during treatment with estrogen. *Journal of the Acoustical Society of America, 104(3 Pt 1),* 1555-1558.

McFadden, D., Pasanen, E. G., Valero, M. D., Roberts, E. K., & Lee, T. M. (2009). Effect of prenatal androgens on click-evoked otoacoustic emissions in male and female sheep (Ovis aries). *Hormones and Behavior, 55,* 98-105.

McFadden, D., Pasanen, E. G., Raper, J., Lange, H. S., & Wallen, K. (2006). Sex differences in otoacoustic emissions measured in rhesus monkeys (Macaca mulatta). *Hormones and Behavior, 50,* 274-284.

McFadden, D., Pasanen, E., Weldele, M., Glickman, S., & Place, N. (2006). Masculinized

otoacoustic emissions in female spotted hyenas (Crocuta crocuta). *Hormones and Behaviour, 50(2),* 285-292.

McIntyre, M. H., Cohn, B. A., & Ellison, P T. (2006). Sex dimorphism in digital formulae in children. *American Journal of Physical Anthropology, 129(1),* 143-150.

Moffat, S.D., & Hampson, E. (2000). Salivary testosterone condcentration in left-handers: an association with cerebral language lateralization? *Neuropsychology, 14(1),* 71-81.

Morlet, T., Lapillonne, A., Ferber, C., Duclaux, R., Sann, L., Putet, G., et al. (1995). Spontaneous otoacoustic emissions in preterm neonates: prevalence and gender effects. *Hearing Research, 90(1-2),* 44-54.

Mountain, D.C. (1980). Changes in endolymphatic potential and crossed olivocochlear bundle stimulation alters cochlear mechanics. *Science, 210,* 71-72.

Nelson, R.J. (2005). An *Introduction to Behavioral Endocrinology (3rd Edition).* Sunderland, MA: Sinauer Associates, Inc.

Okten, A., Kalyoncu, M., & Yaris, N. (2002). The ratio of second and fourth digit lengths and congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Early Human Development, 70,* 47-54.

Penner, M.J. (1995). Frequency variation of spontaneous otoacoustic emissions during a naturally occurring menstrual cycle, amenorrhea, and oral contraception: a brief report. *Ear and Hearing, 16(4),* 428-432.

Penner, M. J., Glotzbach, L., & Huang, T. (1993). Spontaneous otoacoustic emissions: measurement and data. *Hearing Research, 68(2),* 229-237.

Peters, M., MacKenzie, K., & Bryden, P. (2002). Finger length and distal finger extent

patterns in humans. *American Journal of Physical Anthropology, 117(3),* 209-217.

Phoenix, C.H., Goy, R.W., Gerall, A.A., & Young, W.C. (1959). Organizing action of prenatally administered testosterone priopionate on the tissues mediating mating behaviour in the female guinea pig. *Endocrinology*, *65*, 369-382.

Previc, F. (1991). A general theory concerning the prenatal origins of cerebral lateralization in humans. *Psychological Review, 98,* 299-334.

Probst, R., Lonsbury-Martin, B., Martin, G., & Coats, A. (1987). Otoacoustic emissions in ears with hearing loss. *American Journal of Otolaryngology, 8(2),* 73-81.

Probst, R., Lonsbury-Martin, G., & Martin, G. (1991). A review of otoacoustic emissions. *Journal of the Acoustical Society of America, 89(5),* 2027-2067.

Pujol, R., & Lavigne-Rebillard, M. (1995). Sensory and neural structures in the developing human cochlea. *International Journal of Pediatric Otorhinolaryngolo*gy, *32*, S177-S182.

Puts, D. A., McDaniel, M. A., Jordan, C. L., & Breedlove, S. M. (2008). Spatial ability and prenatal androgens: meta-analyses of congenital adrenal hyperplasia and digit ratio (2D:4D) studies. *Archives of Sexual Behavior, 37(1),* 100-111.

Resnick, S. M., Berenbaum, S. A., Gottesman, I. J., & Bouchard, T. J. (1986). Early hormonal influences on cognitive functioning in congenital adrenal hyperplasia. *Developmental Psychology, 22,* 191-198.

Sato, H., Sando, I., & Takahashi, H. (1991). Sexual dimorphism and development of the human cochlea. Computer 3-D measurement. *Acta Otolaryngologica, 111(6),* 1037-1040.

Shera, C.A., & Guinan, J.J.Jr. (1999). Evoked otoacoustic emissions arise by two

fundamentally different mechanisms: A taxonomy for mammalian OAEs. *Journal of the Acoustical Society of America, 105,* 782-798.

Strickland, E., Burns, E., & Tubis, A. (1985). Incidence of spontaneous otoacoustic emissions in children and infants. *Journal of the Acoustical Society of America, 78(3),* 931-935.

Svartberg, J., Jorde, R., Sundsfjord, J., Bonaa, K.H., & Barrett-Connor, E. (2003). Seasonal variation of testosterone and waist to hip ratio in men: the Tromso study. *Journal of Clinical Endocrinology and Metabolism, 88(7),* 3099-3104.

Swaab, D.F, & Hofman, M.M. (1984). Sexual differentiation of the human brain: A historical perspective. *Progress in Brain Research, 61,* 361-374.

Talmadge, C.L., Long, G.R., Murphy, W.J., & Tubis, A. (1993). New off-line method for detecting spontaneous otoacoustic emissions in human subjects. *Hearing Research, 71,* 170-182.

van Anders, S. M., Vernon P. A., & Wilbur, C. J. (2006). Finger-length ratios show evidence of prenatal hormone-transfer between opposite-sex twins. *Hormones and Behavior, 49(3),* 315-319.

Vittek, J., L'Hommedieu, D.G., Gordon, G.G., Rappaport, S.C., & Southren, A.L. (1985). Direct radioimmunassay (RIA) of salivary testosterone: correlation with free and total serum testosterone. *Life Sciences, 37(8),* 711-716.

Voracek, M., & Dressler, S. G. (2007). Digit ratio (2D:4D) in twins: heritability estimates and evidence for a masculinized trait expression in women from opposite-sex pairs. *Psychological Reports, 100(1),* 115-126.

vom Saal, F.S. (1989) Sexual differentiation in litter-bearing mammals: influence of sex

of adjacent fetuses in utero. *Journal of Animal Science, 67(7)*, 1824-1840.

Wier, C.C., Pasanen, E. G., & McFadden, D. (1988). Partial dissociation of spontaneous otoacoustic emissions and distortion products during aspirin use in humans. *Journal of the Acoustical Society of America*, *84*, 24-30.

Witelson, S. F. (1991). Neural sexual mosaicism: Sexual differentiation of the human temporo-parietal region for functional asymmetry. *Psychoneuroendocrinology, 16*, 131-153.

Witelson, S. F. &. Nowakowski, R. S. (1991). Left out axons make men right: A hypothesis for the origin of handedness and functional asymmetry. *Neuropsychologia, 29(4),* 327-333.

Wright, A., Davis, A., Bredberg, G., Ulehlova, L., & Spencer, H. (1987). Hair cell distributions in the normal human cochlea. *Acta Otolaryngologica Supplemental*, *444*, 1-48.

Zwicker, E., & Manley, G. (1981). Acoustical responses and suppression-period patterns in guinea pigs. *Hearing Research*, *4(1)*, 43-52.

CHAPTER 2

SEX AND EAR DIFFERENCES IN SPONTANEOUS AND CLICK-EVOKED OTOACOUSTIC EMISSIONS IN YOUNG ADULTS

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A version of this manuscript has been submitted for publication in *Brain and Cognition*

2.1 Introduction

Prenatal exposure to testosterone or other androgens from the fetal testes and, to a lesser extent, the adrenal glands, results in the masculinisation of many physical, cognitive, and behavioural traits. Prenatal weeks 8 to 24 are believed to be critical to brain and behavioural differentiation in humans because of the testosterone surge at that time in the male fetus (Forest, de Peretti, & Bertrand, 1976). Differential prenatal exposure to androgens has been proposed to bring about a variety of sexual dimorphisms found in humans, including finger lengths (in particular the ratio of the 2^{nd} to 4^{th} digits; Manning, Scutt, Wilson, & Lewis-Jones, 1998; Manning, Stewart, Bundred, & Trivers, 2004; McIntyre, Cohn, & Ellison, 2006), childhood play preferences (Berenbaum & Hines, 1992; Collaer & Hines, 1995) and, potentially, spatial reasoning abilities (Resnick, Berenbaum, Gottesman, & Bouchard, 1986; Grimshaw, Sitarenios, & Finegan, 1995). Studies of the auditory system, which develops and matures during the hypothesized critical period for sexual differentiation (Lavigne-Rebillard & Pujol, 1986), have identified several physiological properties that are potentially influenced by prenatal androgens. For example, Chung, Mason, Gannon, and Willson (1983) found a small but significant sex difference in hearing acuity in humans, with females possessing better hearing than males across the frequency spectrum. Another example of a recently discovered sexually dimorphic auditory property, which forms the focus of the current study, is otoacoustic emissions (OAEs).

OAEs are faint sounds that are produced by the cochlea and propagated into the external auditory canal (Kemp, 1978). They are believed to be a natural by-product of a cochlear amplification mechanism, involving the outer hair cells of the inner ear, which

increases hearing sensitivity to low intensity sounds (Davis, 1983). OAEs can be detected in the external auditory canal using a low-noise microphone and quantified to provide information regarding the integrity of the auditory system and hearing sensitivity in general. In support of this origin, a positive correlation between hearing sensitivity and the number and strength of OAEs has been found (McFadden & Mishra, 1993). OAEs also have been shown to be absent in selective regions of the frequency spectrum where sensorineural hearing loss is greater than 30 dB (Probst, Lonsbury-Martin, Martin, & Coats, 1987). Further support for a common mechanistic origin regulating hearing sensitivity and OAEs comes from studies showing that OAEs are partially reduced or completely eliminated in subjects exposed to drugs that induce temporary hearing loss (McFadden & Plattsmier, 1984).

Three different types of OAEs have been identified, two of which were examined in the present study. Spontaneous OAEs (SOAEs) are emissions produced in most normally-hearing individuals without the deliberate presentation of external acoustic stimulation. Click-evoked OAEs (CEOAEs), on the other hand, are echo-like waveforms produced in response to the presentation of acoustic stimuli, either audible clicks or tone-bursts. Individual variability in OAE production exists, and a sex difference in OAEs has been reported in some studies. On average, females are reported to produce greater numbers and strengths of SOAEs and greater amplitudes of CEOAEs than males. This sexual dimorphism in OAE production has been found in preterm neonates, infants, and children (Burns, Arehart, & Campbell, 1992; Morlet et al., 1995; Strickland, Burns, & Tubis, 1985), as well as in certain adult populations (for review, see Bilger, Matthies, Hammel, & DeMorest, 1990), and appears to be relatively stable over time. Alterations

in OAE production also have been found in several special populations of human or nonhuman subjects. Female dizygotic twins who have male co-twins (opposite-sex dizygotic twins) exhibit masculinised OAE patterns compared to females who have female co-twins (same-sex dizygotic twins), monozygotic female twins, or singleton females, and this has been hypothesized to reflect exposure to higher-than-normal testosterone levels by diffusion from the male fetus during gestation (McFadden, 1993a). Female spotted hyenas, which are normally highly androgenised during prenatal development, exhibit CEOAEs similar to those of male hyenas (McFadden, Pasanen, Weldele, Glickman, & Place, 2006), offering further support for an effect of prenatal androgens on this auditory trait. Thus, it has been hypothesized from these studies and others that the observed sexual dimorphism in OAE production is a result of differential prenatal exposure to androgens between the sexes.

SOAEs also may be produced differentially between the right and left ears, with more pronounced and more frequent SOAEs in the right ear than the left (for review, see Bilger et al., 1990; Burns et al., 1992; Talmadge, Long, Murphy, & Tubis, 1993; for evidence contrary to a right ear advantage in SOAE production, see Collet, Gartner, Veuillet, Moulin, & Morgon, 1993). Right ear advantages in other auditory properties, such as hearing sensitivity (Chung et al., 1983) and the auditory brainstem response (Levine, Liederman, & Riley, 1988) also have been observed, though the presence and magnitude of right ear superiority is affected by a number of variables (for review, see McFadden, 1993b; McFadden, 1998). It has been proposed that a difference in the strength of the efferent influence by the medial olivocochlear system on the outer hair cells of the cochlea may be responsible for the observed ear asymmetries (McFadden,

1993b). Specifically, OAEs and hearing sensitivity may be greater in the right ear than the left because of less inhibition by the medial olivocochlear efferent system in the right ear. In support of such a mechanism, studies have shown that acoustical stimulation of the medial olivocochlear bundle, resulting in greater activation of this efferent inhibitory system, resulted in the reduced production of various types of OAEs (Collet, Kemp, Veuillet, Duclaux, Moulin, & Morgon, 1990; Puel & Rebillard, 1990). Support for lower inhibition in the right ear, however, has been equivocal (Khalfa & Collet, 1996). This theory of a differential efferent influence on the cochlea in the two ears also has been used in conjunction with the prenatal androgen hypothesis to help explain the female advantage in OAE production and hearing sensitivity.

An alternative explanation for the observed ear differences in auditory properties was proposed by Previc (1991), who viewed peripheral lateralization in the auditory system as the foundation for cerebral lateralization at the central level. According to Previc (1991), the origins of cerebral lateralization lie in the asymmetric prenatal development of vestibular organs, such as the ear and labyrinth. His theory claims that a right-ear advantage in monoaural sensitivity results from a smaller right craniofacial region during embryonic development, resulting in enhanced middle-ear conduction of sound. Other hypotheses have been put forth to account for the direction and degree of lateralization observed in various cortical functions, including language, but because they focus on lateralization in the forebrain, their applicability to OAEs is indirect. Nonetheless, several theories have explicitly proposed that androgen production by the male fetus can modify the development of lateralized systems (Geschwind & Galaburda, 1985a, 1985b, 1985c; Witelson, 1991; Witelson & Nowakowski, 1991; Lauter, 2007).

Prenatal androgen exposure is thus hypothesized to be an agent that is not only important for sexual differentiation, but also as an effector that can influence lateralized patterns of development.

The handedness of an individual, perhaps the most visible asymmetry in humans, has allowed researchers to investigate and provide evidence of a standard pattern of lateralization of various cerebral properties, specifically more lateralized functioning in right-handed individuals and deviations from this pattern in non-right-handed individuals (Bryden, 1982). Handedness thus acts as a visible marker of departures from the norm in lateralized patterns. A preliminary association between OAE production, handedness, and the medial efferent system mediating ear differences has been made. Greater activation in the efferent auditory system has been reported in the right ear compared to the left ear of right-handed individuals, with symmetrical activation observed in the two ears of left-handed individuals (Khalfa, Veuillet, & Collet, 1998). Further investigation is needed to establish a connection between the theories outlined above and the differential production of OAEs present between the right and left ear.

The purpose of the present study was to investigate sex and ear differences in hearing sensitivity, SOAE and CEOAE production in a sample of healthy young adults (ages 17-25). Young adults have been largely overlooked in this area of research but are of particular interest because 1) they are at their peak reproductive capacity, a period in the lifespan where many sex differences are at their most prominent, and 2) they are not yet vulnerable to the effects of degenerative hearing loss that accompanies aging. Most previous studies incorporating this age group have either focused on an excessively broad age range (Dallmayr, 1985), special populations (McFadden & Pasanen, 1998, 1999), or

clinical groups with identified hearing impairments. It was hypothesized that sex and ear differences in OAEs would be observed, with females and right ears producing greater numbers and powers of SOAEs and a greater amplitude of CEOAEs than males and left ears. In addition, the handedness of the participants, both direction and degree, was evaluated using a standardized instrument (Crovitz & Zener, 1962) in order to investigate whether hand preference is associated with discernible differences in OAE production. It was hypothesized, based on previous research (Khalfa et al., 1998), that right-handed individuals would show a right-ear advantage in OAE production, whereas non-right-handed individuals would exhibit a more symmetrical pattern of OAEs between the right and left ears.

2.2 Methods

2.2.1 Participants

Male (n = 45) and female (n = 48) volunteers were recruited from the University of Western Ontario. Participants ranged in age from 17 to 25 years, with a mean (SD) age of 20.8 (2.6) years for males and 19.9 (2.0) years for females, respectively. Any participant who had a hearing sensitivity worse than 25 dB hearing level at any frequency interval during the audiometric screening was excluded from the data analysis, as previous research has shown an association between OAE production and inner ear integrity as reflected in the hearing threshold (McFadden & Mishra, 1993). Participants thus were required to have a normal audiogram.

2.2.2 Equipment

Audiometric screening was performed using a GSI-17 pure-tone air conduction audiometer with Telephonics TDH-39P headphones. Visual inspection of the external ear canal for debris or potential interference with OAE recordings was accomplished using a Welch Allyn MacroView 23820 otoscope. For the OAE recordings, an Etymotic ER-10B low-noise microphone system, with an ER-2 earphone with a foam ear-tip attached, was used (Figure 2.1). This microphone system included 2 small diameter silicon tubes that protruded approximately 2 mm into the external auditory canal. The function of one tube was to detect OAEs during both the SOAE and CEOAE recordings, whereas the other tube served as a delivery conduit for click stimuli during CEOAE recording. Output from the low-noise microphone system passed through an ER10-72 pre-amplifier to a custom built low-noise amplifier/filter. The low-noise amplifier/filter system served two distinct functions: to amplify the output signal by 30 dB and to high-pass the output signal above 400 Hz in order to eliminate any extraneous bodily noises present at or below this frequency (e.g., blood flow, swallowing). The output from the amplifier/filter system was then sent to a spectrum analyzer and analog-to-digital converter (National Instruments, DAQ AI-16XE-50) and stored digitally on a Macintosh G4 Powerbook (OS 9.2) for later analysis (Figure 2.2). All collection and off-line analysis of the OAE data was accomplished using custom-written software in LabVIEW (National Instruments, Austin, Texas). The software programs were provided courtesy of the laboratory of Dr. Dennis McFadden (Department of Psychology, The University of Texas at Austin).

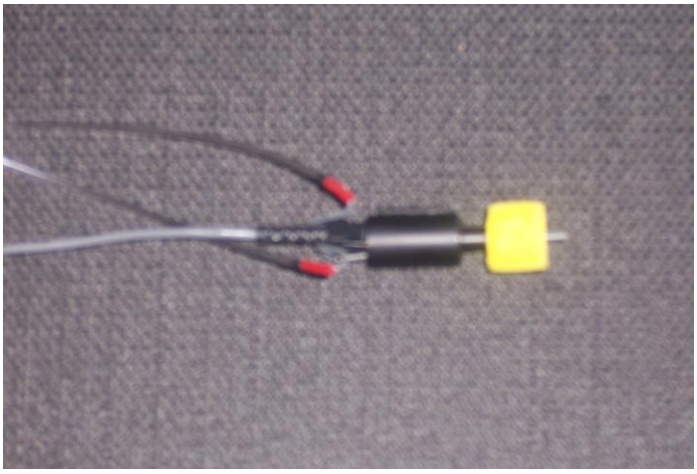

Figure 2.1. Photograph of the ER-2 earphone with foam ear-tip attached. This is inserted into the external ear canal and is used for OAE recording.

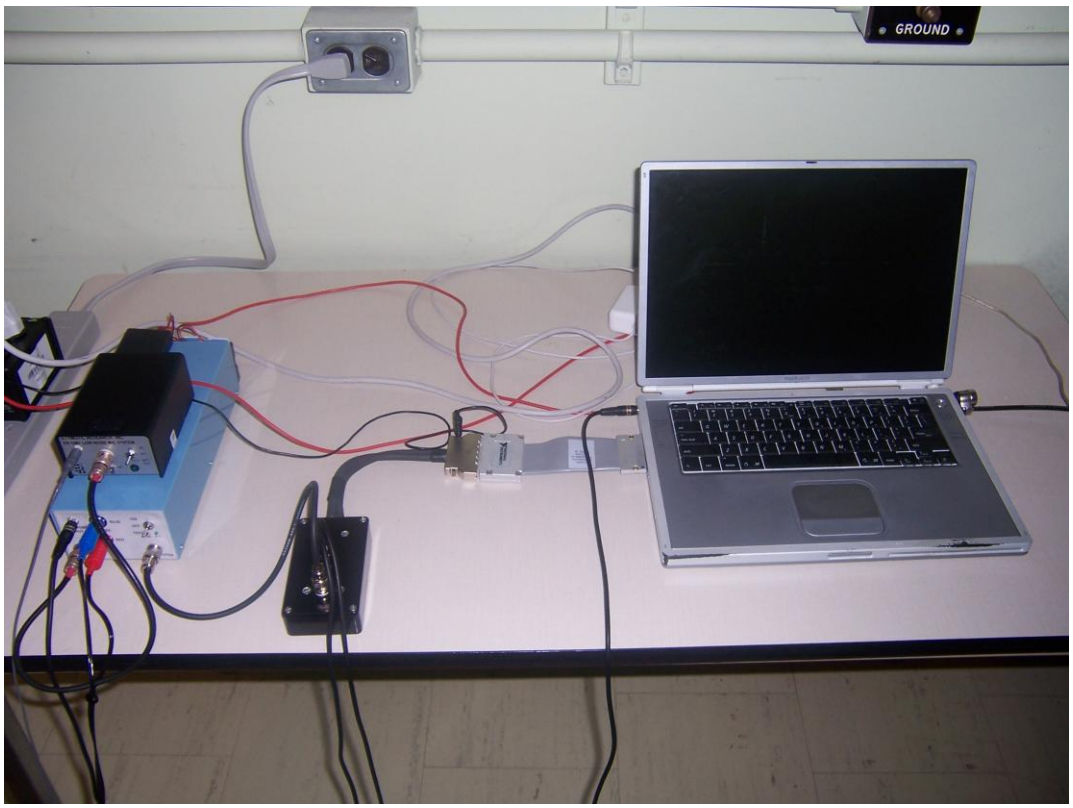

Figure 2.2. Photograph of the set-up used to record OAEs in participants.

2.2.3 Procedure

Participants were tested individually in a darkened quiet room. Audiometric screening was done first. Participants then filled out a demographic questionnaire and the Crovitz-Zener Handedness Inventory (Crovitz & Zener, 1962). Besides basic demographics, the questionnaire inquired about present and past experiences that are known to either temporarily or permanently alter hearing thresholds and OAE production (e.g., prescription drug use, ear damage or surgery; McFadden & Plattsmier, 1984; Probst et al., 1987). The Crovitz-Zener inventory was used to assess direction and degree of handedness. Participants rated which hand they would normally use to perform 14 common everyday tasks (e.g., "hold a drinking glass when drinking") using a five-point Likert scale (1 = right hand always, 2 = right hand most of the time, 3 = both hands equally often, 4 = left hand most of the time, and 5 = left hand always). A summed score was calculated, allowing handedness to be measured along a continuum (degree of right-handedness or non-right-handedness) and classified dichotomously (right-handed or non-right-handed) according to a previously established cutpoint (Crovitz & Zener, 1962). A participant was classified as right-handed if his/her cumulative score was less than or equal to 30 or non-right-handed if his/her cumulative score was greater than 30 (Crovitz & Zener, 1962).

After completing the questionnaires, participants sat in a reclined sofa chair in preparation for OAE recording. An otoscope was used to examine the external auditory canal for debris or blockage that might interfere with the recordings. The low-noise microphone system, with the foam ear-tip attached, was then inserted into the ear to be tested first. The ear-tip was inserted such that the foam was flush with the opening of the

ear canal. A habituation period of approximately 20 minutes ensued, during which the participant remained in a reclined position in order to acclimatize to the testing environment. This period and duration of relaxation prior to testing has been shown to be important for reliable OAE measurement and is a commonly used practice in OAE experiments (Whitehead, 1991; Zurek, 1981). Once the acclimatization period passed, SOAEs and CEOAEs were recorded separately from each ear. The recording of OAEs was counterbalanced within each sex for ear tested first (right or left) and type of OAE tested first (SOAE or CEOAE).

2.2.4 Audiometric Screening

Audiometric screening was done to assess inner ear integrity, to determine that participants met the hearing thresholds for inclusion in the study, and to measure hearing sensitivity. Standard clinical audiometric screening guidelines were followed, with participants tested for hearing sensitivity at the following frequency intervals, in order: 1000, 2000, 3000, 4000, 6000, 8000, 250, 500, 750, and 1500 Hz. The ear tested first (right or left) was counterbalanced within each sex. Pure tones were presented at the designated frequencies in 5 dB steps, and participants responded using a button press whenever a stimulus was perceived. Only data from participants with normal hearing thresholds of 25 dB or less at each frequency were analyzed (see *Participants*).

2.2.5 SOAE Recording

Participants were instructed to remain completely still and quiet during each recording interval, and were given notification by the experimenter as to the start and

finish of each interval. Raw SOAE data was obtained by taking four 30-second recordings (2 min in total), typically separated by 5 to 20 second rest periods, from each ear. Waveforms extracted from each raw SOAE measurement were digitized with 16-bit resolution at a sampling rate of 25 kHz and stored on a Macintosh computer. Using an established automated algorithm used and recommended by other labs (Pasanen & McFadden, 2000), the 2 min recordings were scanned offline in 655 ms segments (resulting in 16375 points with 75% overlap with other segments) and the quietest 150 time segments were saved. Fast-fourier transforms for each of the 150 quietest time segments were computed and averaged in the frequency domain to create a singular frequency spectrum. This averaged spectrum was then passed offline to the automated computer program designed to detect and analyze SOAEs. A spectral peak was identified as an SOAE if it met all of the following criteria: 1) the peak was 5.0 or more standard deviation units above the averaged spectral baseline, 2) the frequency of the peak was between 1000 and 9000 Hz (1000 Hz was used as the lower cut-off point to further eliminate extraneous noises present in the quiet testing room), and 3) the peak was not closer than 0.1 octaves to a stronger peak already accepted as an SOAE. The magnitude of each peak was then converted to sound-pressure level (SPL) units and stored. Two measures were obtained for each ear, the *number of SOAEs* detected and the *total power* for that ear in SPL.

2.2.6 CEOAE Recording

Screening for CEAOEs involved three phases: click calibration, determination of the noise floor threshold, and click presentation/CEOAE detection. Rarefaction DC

pulses, approximately 100 ms in duration, were generated by the sound output system of the laptop at a sampling rate of 44.1 kHz and served as the clicks for the CEAOE recordings. Two distinct click levels were used, 75 peSPL and 69 peSPL. These click levels correspond to the peak amplitude of a 1000 Hz tone at the desired intensities. Data were separately obtained and recorded for both click levels for each participant. The ambient noise within each ear, in the absence of any acoustic stimuli, was sampled to establish individual noise thresholds to be used during the recording procedure. This noise threshold was then used during click presentation and CEOAE detection to ensure that the ambient noise (e.g., environmental, physiological) did not exceed the established level; if it did, a delay in click presentation ensued until the ambient noise decreased to an acceptable level. After presentation of the acoustic clicks for CEOAE recording, a 4 ms delay was applied before recording commenced to avoid any acoustical ringing in the auditory canal. After the delay, acoustic activity was recorded for 40 ms, identified as the click-response for that stimulus, and analyzed. Output from the microphone was digitally sampled at 48 kHz and synchronized to the click stimulus as recorded directly from the sound output of the computer. Responses to 250 clicks were averaged to obtain a mean click-evoked response. After further eliminating another 2 ms from the averaged waveform, a 20.48 ms segment judged to be artefact-free was bandpass filtered at 1.0 to 8.0 kHz in preparation for final off-line analysis. The root-mean-square output of the filter was converted to SPL and recorded as the click-evoked response for that ear at that particular click level. Thus the amplitude of the evoked response constituted the dependent measure.

2.3 Results

2.3.1 Hearing Sensitivity

A mixed-effects ANOVA, with ear and frequency as the repeated factors and sex as the between-subjects factor, was used to investigate differences in hearing sensitivity. The 6000 Hz frequency interval had to be excluded because of a technical problem that affected the data of a large number of participants at that frequency. As shown in Figure 2.3, hearing sensitivity in the right and left ears of both sexes showed the characteristic U-shaped function that is representative of the audiometric threshold observed in humans (Chung et al., 1983).

In agreement with other literature, a significant sex difference was observed. Females showed significantly greater overall hearing sensitivity, or lower audiometric thresholds, than males, $F(1,90) = 12.79$, $p = .001$; see Figure 2.3. As expected, the threshold differed significantly depending on the frequency tested, $F(4,355) = 190.67$, $p < .001$. No overall ear difference in sensitivity was found, $F(1,90) = 0.62$, $p = .434$. The two-way interaction between sex and ear, $F(1,90) = 0.62$, $p = .434$, and the three-way interaction among sex, ear, and frequency, $F(6,498) = 0.396$, $p = .869$, were non-significant. The interactions between frequency and sex, $F(4,499) = 7.07$, $p < .001$, and between frequency and ear, $F(6,499) = 13.75$, $p < .001$ were found to be significant. Since the purpose of the study was to investigate sex and ear differences in hearing sensitivity, post-hoc tests were conducted in order to determine which individual frequencies in the right and left ear differed between the sexes. In the right ear, females had significantly lower auditory thresholds than males at 3000Hz ($p < .05$), 4000Hz ($p < .01$), and 8000Hz ($p < .05$). In the left ear, females had significantly lower auditory

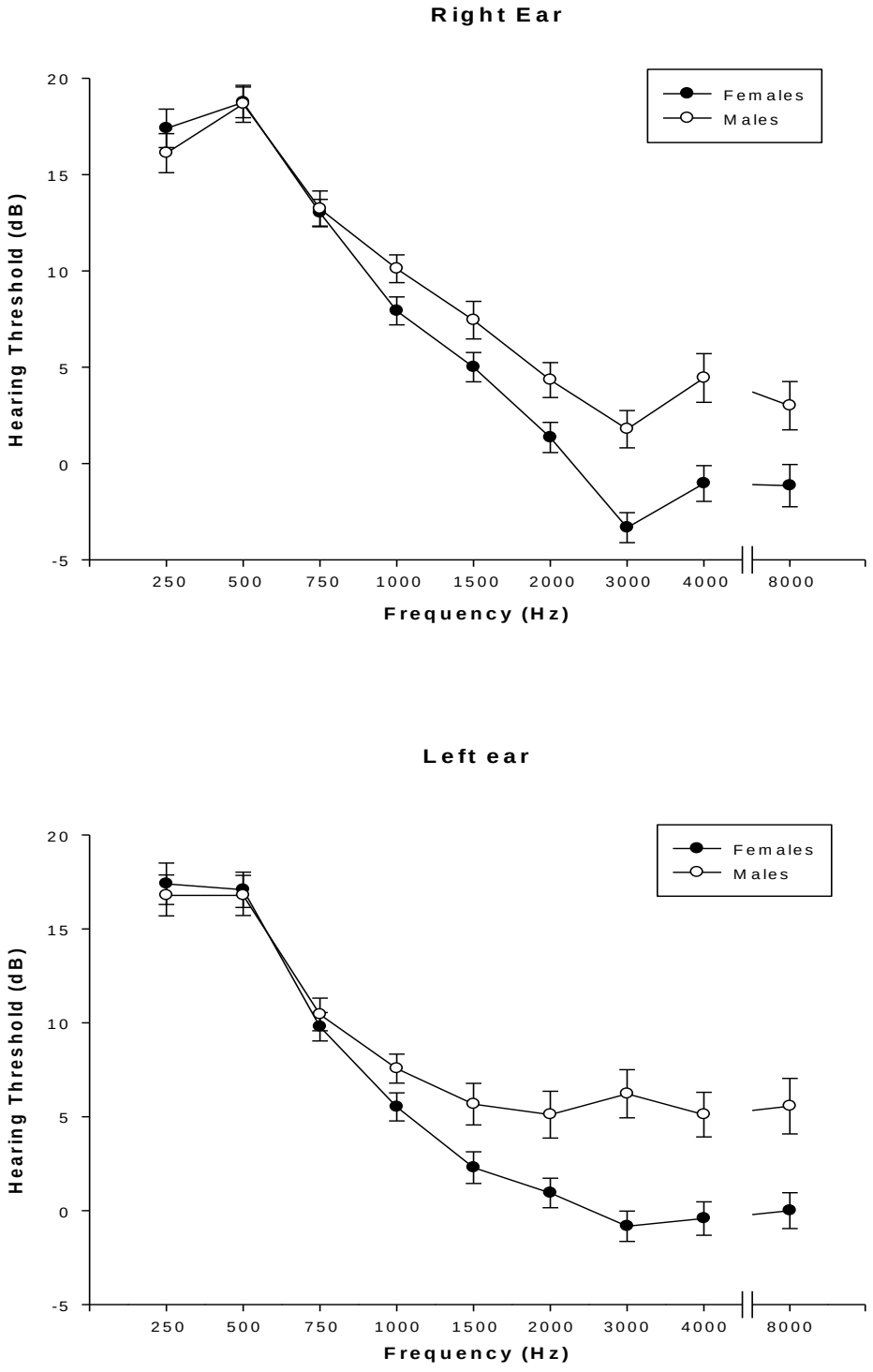

Figure 2.3. Audiometric thresholds for the right (upper panel) and left (lower panel) ears of male and female participants for frequencies between 250 and 8000Hz. Note the omission of the 6000Hz frequency due to a technical problem that affected the data of a large number of participants at that frequency. Error bars represent standard error of the means (SEM).

thresholds than males at 2000Hz ($p < .05$), 3000Hz ($p < .001$), 4000Hz ($p < .01$), and 8000Hz ($p < .01$).

2.3.2 OAEs

Analyses of the OAE data focused on the following dependent variables: the number and prevalence of SOAEs, total power of the SOAEs produced, and CEOAE response amplitudes. Unless otherwise stated, all SOAE and CEOAE analyses employed mixed-effects ANOVA, with sex as a between-subjects factor, and ear (and for CEOAEs, dB click level) as a repeated factor.

2.3.3 SOAEs

Figure 2.4 shows the breakdown of SOAE production for females and males in the right and left ears. Females produced significantly greater numbers of SOAEs compared to males, $F(1,83) = 6.04$, $p = .016$. SOAE production was greater in the right ear than the left ear, $F(1,83) = 11.21$, $p = .001$. However, the interaction between sex and ear was not significant. Cohen's *d* statistic, calculated as the difference between the sample means divided by the sample standard deviation, was used to express the absolute magnitude of the effect of sex on SOAE production (Cohen, 1977). The calculated effect size for the sex difference in SOAE production was $d = 0.54$, indicating a medium effect.

The prevalence of SOAEs has been found to differ by sex or by ear in some studies (e.g., Bilger et al., 1990; Penner & Zhang, 1997). Accordingly, chi-square analyses (χ^2) were conducted to determine whether the distribution of SOAEs differed between females and males or between the right and left ears in the present study. A 2x2

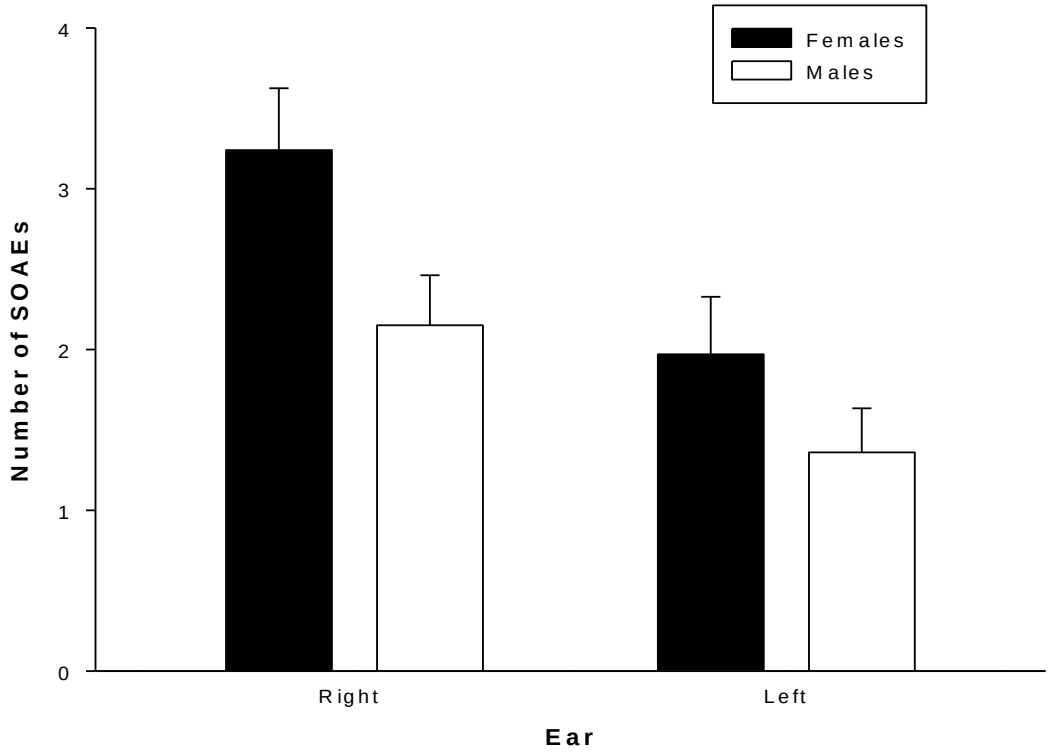

Figure 2.4. Number of SOAEs produced by the right and left ears of male and female participants. Error bars represent SEM.

chi-square test showed that the distribution of SOAEs did not differ significantly between females and males, $\chi^2 = 1.16$, $p > .05$, with 78.3% of females and 87.2% of males showing the presence of at least one detectable SOAE. A second 2x2 chi-square was used to test for a difference in prevalence between the two ears. The distribution of SOAEs between the right and left ears did not differ significantly, $\chi^2 = 3.60$, $p < .10$, with 74.4% of right ears and 60.9% of left ears showing at least one detectable SOAE.

Total power of the SOAEs produced in females and males was analyzed using one-way ANOVA to determine whether a sex difference was also present with respect to the strength of the SOAEs. For this analysis, a single value was obtained for each participant reflecting the total (or overall) power of SOAEs summed across both ears. Thus, data used for the power analysis were from participants producing SOAEs in *both* ears, and excluded those participants who did not produce an emission in either one or both of their ears. As shown in Figure 2.5, females produced SOAEs with significantly greater power than males, $F(1,70) = 5.01$, $p = .028$. Total power of the SOAEs produced in the right and left ears was also analyzed to determine whether an ear difference was present. Mixed-effects ANOVA, with ear as a repeated factor and sex as a between-subjects factor, showed that the power of the SOAEs produced did not differ significantly between the two ears, $F(1,70) = 1.87$, $p = .175$.

2.3.4 CEOAEs

Females were found to produce CEOAEs with significantly greater response amplitudes than males, $F(1,76) = 13.91$, $p < .001$ (Figure 2.6). A significant main effect of click level also was found, such that CEOAE response amplitude was greater for the 75

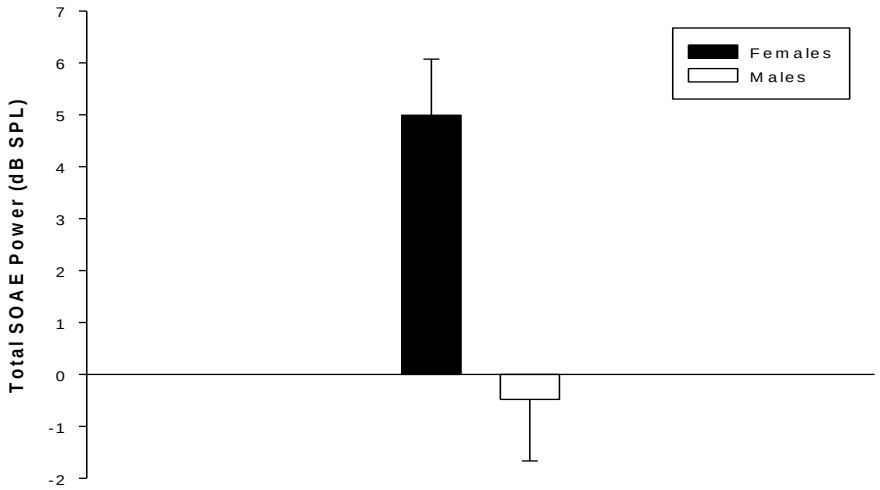

Figure 2.5. Total power of SOAEs produced by males and females. A single value was obtained for each participant reflecting the total (or overall) power of SOAEs summed across both ears; thus, this graph reflects data from participants who produced SOAEs in *both* ears only. Error bars represent standard error of the means (SEM).

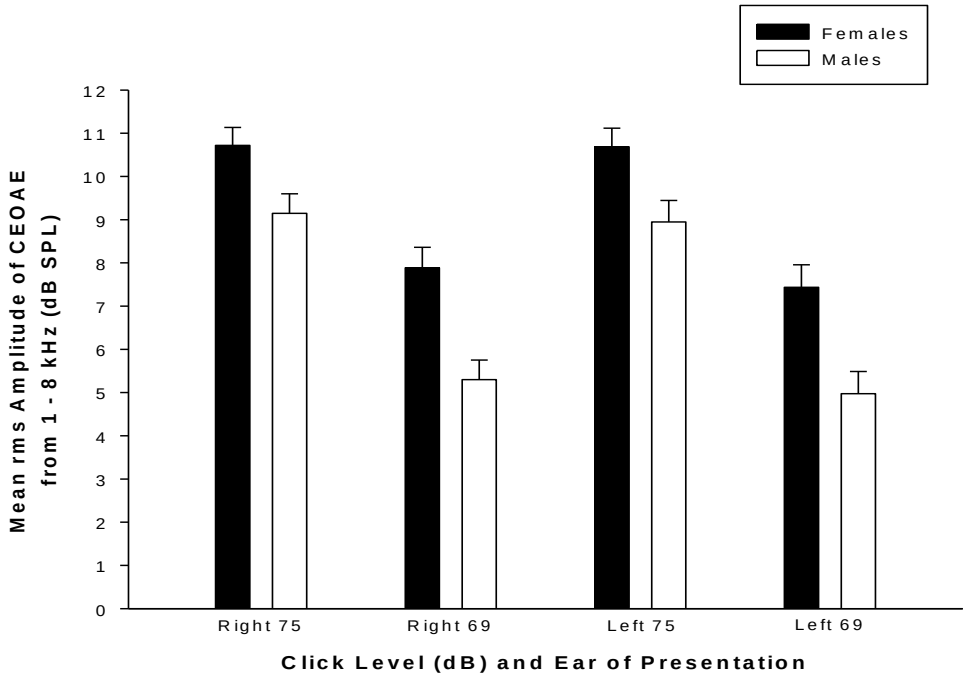

Figure 2.6. CEOAE response amplitude in the right and left ears of male and female participants at two distinct click levels (75dB and 69dB). Error bars represent standard error of the means (SEM).

dB click level than for the 69 dB click level, $F(1,76) = 746.49$, $p < .001$. No significant main effect of ear was found, $F(1,76) = .571$, $p = .452$. A significant two-way interaction between click level and sex, $F(1,76) = 14.66$, $p = .001$, indicated that the sex difference was slightly larger for the 69 dB than the 75 dB stimuli. Effect size was calculated using Cohen's *d* to quantify the magnitude of the observed difference between females and males, across ear and click level, with respect to CEOAE response amplitude. The observed effect size was $d = 0.85$, indicating a large effect (Cohen, 1977).

2.3.5 Influence of Handedness

Based on their total scores on the Crovitz-Zener Handedness Inventory (1962) participants were divided into the following handedness groups: right-handed females ($n = 43$), non-right-handed females ($n = 5$), right-handed males ($n = 33$), and non-right-handed males ($n = 12$). The non-right-handed female group was omitted from further analyses because of its small sample size. To determine whether handedness classification affected the magnitude of the ear difference in OAE production, the other three groups were entered into ANOVAs which included handedness group and ear (and for the CEOAE data, click level) as factors. The dependent variables analyzed were the number of SOAEs produced, SOAE power, and the CEOAE response amplitude. The ANOVAs revealed no significant interaction between ear and handedness on any dependent measure [Number of SOAEs: $F(2,77) = 0.34$, $p = .715$; SOAE power: $F(2,65) = 0.44$, $p = .644$; CEOAE response amplitude: $F(2,70) = 0.42$, $p = .657$]. Thus, contrary to our hypothesis, there was no evidence that handedness influenced the pattern of ear differences.

Although the predicted interaction was not found, the non-RH males produced the lowest numbers of SOAEs, both in the right ear [M = 1.64, *vs.* M = 2.11 and M = 3.27 for RH males and RH females respectively] and the left [M = 1.00, *vs.* M = 1.50 and 2.22 respectively], $F(1,77) = 3.35$, $p = .04$. Given the small number of non-RH, however, this difference between the RH and non-RH male groups was not significant by a post-hoc test. To further explore the influence of handedness, Pearson correlations were computed between the OAE variables and self-reported variation in strength of handedness as revealed by the Crovitz scores. Among those classified as RH, scores ranged from strongly right dominant to scores close to the non-RH range. Among the RH males, higher Crovitz scores, representing weaker right hand preference, were associated with lower CEOAE amplitudes, a pattern that was significant in the right ear (75 dB: r = -.416, p = .035; 69 dB: r = -.358, p = .067; Table 2.1). For non-RH males, a comparable correlation of r = -.403 between stronger left hand preference and lower SOAE numbers was found but was non-significant.

2.4 Discussion

The current study provided a comprehensive investigation of sex and ear differences in hearing sensitivity and OAEs in a population of healthy young adults with intact hearing. A standardized handedness inventory (Crovitz & Zener, 1962) was utilized to assess whether a relationship is present between handedness, a conspicuous marker of CNS lateralization, and ear asymmetry in OAE production. The results showed a significant sex difference in nearly all auditory measures taken, with females displaying lower audiometric thresholds, greater numbers of SOAEs, stronger SOAEs, and CEOAEs

Table 2.1. Correlations between Crovitz-Zener Handedness Scores and OAE Variables

		SOAEs				CEOAEs			
		R Number	L Number	R Power	L Power	R 75dB	R 69dB	L 75dB	L 69dB
Females	RH	-.24	-.16	-.34*	-.09	-.11	-.14	-.28*	-.12
Males	RH	.07	.21	-.05	-.01	-.42**	-.36*	-.26	-.26
	NRH	-.40	-.28	.24	-.32	.08	.05	.28	.17

RH = right-handed; NRH = non-right-handed
* $p < .10$, ** $p < .05$

with greater response amplitude than males. Only SOAE prevalence did not significantly differentiate the two sexes. A right ear advantage in the total number of SOAEs produced also was found. Although we hypothesized, based on data from Khalfa et al. (1998), that the production of OAEs between the ears would differ depending on the handedness of our participants, this hypothesis was not supported. Exploratory analyses, however, revealed that departures from strong right hand dominance were associated, within our sample, with reduced numbers or strengths of OAEs irrespective of ear.

Sex and ear differences have been found in a number of different auditory measures, including the production of greater wave-V amplitude and shorter wave-V latency in females compared to males (Mitchell, Phillips, & Trune, 1989); the production of larger amplitude and shorter latency auditory brainstem responses in the right ear compared to the left ear (Levine et al., 1988); as well as better hearing sensitivity, or lower audiometric thresholds, in both females and the right ear (Axelsson et al., 1981; Chung et al., 1983; McFadden & Mishra, 1993). In particular, the sex difference in hearing sensitivity, though evident throughout the entire frequency spectrum, has been shown to appear maximally at higher frequencies (Chung et al., 1983). As expected, females in the present study exhibited not only greater overall hearing sensitivity, in general, compared to males, but these differences also were most pronounced at higher frequency levels. Specifically, frequencies at or above 2000 Hz showed the greatest female advantage (approximately 4-5 dB), whereas the sex difference diminished significantly or completely disappeared for frequency levels below 2000 Hz. It should be emphasized that the magnitude of the difference in hearing sensitivity between females and males represents a sizeable difference, given that the decibel scale is a logarithmic

scale. The difference obtained in the current study is comparable to the large-scale audiometric study conducted by Chung et al. (1983) and other studies discussed in the review by McFadden (1993b).

The present study failed to find a significant ear difference in hearing sensitivity, either between or within the sexes. There are previous reports of better hearing sensitivity in the right ear compared to the left ear, with the difference being more pronounced in males than in females (Chung et al., 1983; Emmerich, Harris, Brown, & Springer, 1988). This reported difference, however, was only on the magnitude of 1-2.5 dB and was found in a sample substantially larger than ours. Thus, the lack of an observed ear difference in hearing sensitivity in the current study may in fact reflect a true property of this auditory trait in the present sample, or may simply be due to the lack of a comparably large sample size.

The current results showed a robust sexual dimorphism in the number and power of SOAEs and in CEOAE response amplitude, but not in the prevalence of SOAEs produced. In preterm and full-term neonates (Morlet et al., 1995a, 1995b) as well as infants and children (Burns et al., 1992; Strickland et al, 1985), a sex difference in SOAE numbers is well-established, with females typically showing greater numbers of SOAEs than males, especially in the right ear. However, the prevalence, numbers, and amplitudes of SOAEs have been shown to decrease from neonates to older children (Burns, Campbell, & Arehart, 1994; Lamprech-Dinnesen et al., 1998), resulting in a decrease in the magnitude of the observed sex difference in SOAE, as well as CEOAE, production (Burns, 2009; Kok, van Zanten, & Brocaar, 1993). In adults, a sex difference in SOAE number has been documented (Bilger et al., 1990), though less evidence is

available, particularly from young adult samples. Similarly, support for a sex difference in the power of SOAEs produced in an adult population is quite limited (for support, see McFadden & Pasanen, 1999), possibly due to the fact that individual SOAEs can vary hourly with respect to their amplitudes despite maintaining stable frequencies on the auditory spectrum (Dallmayr, 1985). The current study found a sex difference in both SOAE numbers and powers, with females producing greater numbers of SOAEs and SOAEs with greater power compared to males. The values obtained were comparable to those previously reported for samples in the age range of that used in the current study (Kok et al., 1993). Our data thus offer further support for a sex difference in SOAE production in normally-hearing young adults.

The current study also found a significant sex difference in CEOAE response amplitude. Females produced CEOAEs with greater response amplitude than males at both the 75 and 69 dB click levels. CEOAEs can be elicited in essentially all normally-hearing ears (Kemp, 1978; Probst, Lonsbury-Martin, & Martin, 1991). Although a sex difference in CEOAE response amplitude has not been well characterized in children, a number of studies have shown that CEOAEs are significantly higher in adult females than males (McFadden, 1998; McFadden & Pasanen, 1998). We observed a significant overall sex difference in CEOAE response amplitude, as well as a significant interaction between click level and sex, whereby the sex difference in response amplitude was slightly greater at the 69 dB level compared to the 75 dB level. More importantly, the current study yielded a significant sex difference in response amplitude at both click levels, offering both support for a sex difference from our population of young adults as well as

reproducibility of the difference at multiple click levels, as has been reported previously by other labs (McFadden & Pasanen, 1998).

Some studies have shown that SOAEs are more prevalent in females than males, with approximately 75-85% of females and 45-65% of males exhibiting at least one emission (Bilger et al., 1990; Talmadge, Long, Murphy, & Tubis, 1993); however, in various infant/children and young adult samples, similar prevalence rates in males and females also have been observed (Bonfils et al., 1992; Burns et al., 1992). Further, it has been shown that the sex difference in SOAE prevalence is most evident in the first year after birth, and that a decrease occurs throughout infancy and into childhood (Lamprecht-Dinnesen et al., 1998). The current study found no significant difference between females and males in the prevalence of SOAE production. In fact, in absolute terms, it was males not females who showed greater prevalence. Because SOAE power was calculated in our data for participants exhibiting SOAEs in *both* ears only, a sex difference in prevalence rates, should one be present, would have had no effect on our measure of SOAE power. Conversely, the lack of a significant difference in SOAE prevalence between the sexes adds strength to our observation of a sexual dimorphism in SOAE *number*. The fact that females and males did not differ significantly in whether or not they produced SOAEs suggests that the female advantage in SOAE number is in fact a genuine difference in the rate or numbers of SOAEs produced by individuals, and not merely a statistical artefact of a sex difference in prevalence rates.

The sex difference in OAE production has been identified in infants (Burns et al., 1994) and preterm neonates (weeks 30-40 of gestational age; Burns et al., 1992; Morlet et al., 1995). Somatic sex differences that are already apparent at birth can be caused by

either of two major classes of mechanisms: either direct effects of genes carried on the X or Y chromosome (sex-linked genes) or by the organizational actions of testosterone or its metabolites on some type of neutral physiological substrate (Eckel, Arnold, Hampson, Becker, Blaustein, & Herman, 2008). In the case of OAE production, evidence from specialized populations offers support for the latter explanation. Female twins with male co-twins produce masculinised OAEs later in life, presumably due to elevated exposure to testosterone from the male fetus during prenatal development (McFadden, 1993a; McFadden & Loehlin, 1995). Female hyenas, which are naturally exposed to high concentrations of androgens prenatally, produce male-typical OAEs, further substantiating the claim for a prenatal hormonal action (McFadden et al., 2006). The prenatal androgen hypothesis proposes that exposure of the male fetus to elevated testosterone during the critical window for differentiation dampens the cochlear amplifiers (i.e., outer hair cells) responsible for OAE production, thereby decreasing the prevalence, frequency, and amplitude of OAEs in males compared to females. The results of the current study are consistent with the possibility of an organizational influence, mediated by hormonal differences between the sexes, on the inner ear structures responsible for OAE production.

An overall right ear advantage was observed in the present study in the number of SOAEs produced, but not for the power of SOAEs or CEOAE response amplitude. Although a right ear advantage in SOAE production has been observed previously (Bilger et al., 1990; Burns et al., 1992; Talmadge et al., 1993) and a mechanism mediating this ear difference has been proposed (McFadden, 1993b), the robustness of such a difference has been questioned in studies that have reported contrary results (Collet et al., 1993).

The present study confirmed a right ear advantage in the number of SOAEs produced. Failures to find a significant right ear advantage are not surprising, and are to be expected if ear effects are under the control of other moderator factors as discussed below (e.g., lateralization). SOAE amplitudes, as noted earlier, do exhibit temporal variability (Dallmayr, 1985) and this variability, plus the reduced sample size that was used to analyze SOAE power, may have mitigated against finding a significant ear difference in the current study.

A right ear advantage in the number of SOAEs produced was found in the context of no significant ear advantage in hearing sensitivity. Although previous research has offered support for an association between hearing sensitivity and OAE production (McFadden & Mishra, 1993; McFadden & Plattsmeir, 1984; Probst et al., 1987), this relationship has been demonstrated in special populations exhibiting either hearing loss or selected production of SOAEs. Thus, the relationship between hearing sensitivity and SOAEs is a global one. The unencumbered production of OAEs apparently requires an intact cochlea, but among normally-hearing ears, an ear difference in sensitivity is not necessary in order for a right ear advantage in SOAEs to be expressed.

It has been hypothesized that ear asymmetries in OAEs may be due to a difference in the strength of the efferent influence by the medial olivocochlear system in the two ears (McFadden, 1993b). Khalfa and Collet (1996) experimentally confirmed that asymmetrical activation was present, though inhibition was found to be stronger in the right ear, not the left ear as anticipated by McFadden (1993b). In a subsequent study, symmetrical activation between the two ears was reported to be present in a group of non-right-handers (Khalfa et al., 1998). To our knowledge this finding has not been

replicated. If asymmetry in medial olivocochlear inhibition is the basis for ear differences in OAEs (McFadden, 1993b), and if the asymmetry found in right-handers is absent or reduced in non-right-handers, then handedness would be expected to affect the ear differences observed in OAEs. The present study is the first direct test of this hypothesis (*cf.*, Khalfa et al., 1998). We predicted that OAEs would be differentially produced between the ears depending upon hand preference (i.e., a significant interaction between ear and handedness). The results of the current study showed no significant interactions between ear and handedness, either for the number of SOAEs produced, SOAE power, or CEOAE response amplitude. Thus the hypothesis was not supported.

Several possibilities exist for why a handedness effect was not found. It has been shown that only a minority of left-handed (i.e., non-right-handed) individuals differ from right-handed individuals in brain lateralization, at least with respect to language (Bryden, 1982; Milner, Branch, & Rasmussen, 1966). If this is true for other lateralized differences too, then a much larger sample size may be needed in order to detect a difference in the asymmetry of OAEs between non-right-handed and right-handed groups. In addition, finding a handedness effect might depend on the sex stratification of the sample. There may be more scope for identifying a handedness difference in females because males produce only low levels of SOAEs to begin with. Thus, a large sample, including non-right-handed females, may be needed in order to observe a handedness effect on OAE production. The potential of the current study to detect a handedness difference also was reduced by the fact that we found a significant ear difference only in the number of SOAEs produced, not in SOAE power or CEOAE response amplitude;

thus, it was really only for the number of SOAEs that we had the capacity to see an attenuated ear difference in the non-right-handed group.

Although we did not find the hypothesized interaction between ear and hand preference, we did find evidence that handedness, at least within the present sample, was associated with the absolute numbers and powers of SOAEs and CEOAE response amplitude. This pattern reached significance in right-handed males, where weak right hand preference was associated with lower CEOAE values. Consistent with this finding, Khalfa et al. (1998) found a tendency for increased left hand dependence to be associated with increased MOC inhibition. This type of relationship would be consistent with a theory proposed by Geschwind & Galaburda (1985a,b,c), which states that elevated levels of prenatal testosterone predispose an individual towards non-right-handedness, either left-handedness or ambidexterity. If this is true, and if increased androgen exposure is also the basis for the lower numbers and amplitudes of OAEs that are found in men *vs.* women, then an association between weak right hand preference in males (i.e., higher Crovitz-Zener scores) and *lower* OAE values might be expected, as seen in the current study. Thus a common mechanism could explain both the handedness and sex effects. Further investigation is needed to explore the relationship between handedness, a product of cerebral lateralization, and capacity for OAE production.

2.5 References

Axelsson, A., Jerson, T., Lindberg, U., & Lindgren, F. (1981). Early noise-induced hearing loss in teenage boys. *Scandinavian Journal of Audiology, 10,* 91-96.

Berenbaum, S. A., & Hines, M. (1992). Early androgens are related to sex-typed toy preferences. *Psychological Science, 3*, 203-206.

Bilger, R., Matthies, M., Hammel, D., & DeMorest, M. (1990). Genetic implications of gender differences in the prevalence of spontaneous otoacoustic emissions. *Journal of Speech and Hearing Research, 33,* 418-432.

Bonfils, P., Francois, M., Avan, P., Londero, A., Trotoux, J., & Narcy, P. (1992). Spontaneous and evoked otoacoustic emissions in preterm neonates. *Laryngoscope, 102(2),* 182-186.

Bryden, M. P. (1982). *Laterality: Functional asymmetry in the intact brain*. New York; Academic Press.

Burns, E. (2009). Long-term stability of spontaneous otoacoustic emissions. *Journal of the Acoustical Society of America, 125(5),* 3166-3176.

Burns, E.M., Arehart, K., & Campbell, S. (1992). Prevalence of spontaneous otoacoustic emissions in neonates. *Journal of the Acoustical Society of America, 91(3),* 1571-1575.

Burns, E., Campbell, S., & Arehart, K. (1994). Longitudinal measurements of spontaneous otoacoustic emissions in infants. *Journal of the Acoustical Society of America, 95,* 385-394.

Chung, D., Mason, K., Gannon, R., & Willson, G. (1983). The ear effect as a function of age and hearing loss. *Journal of the Acoustical Society of America, 73,* 1277-1282.

Cohen, J. (1977). *Statistical power analysis for the behavioral sciences* (revised edition). New York: Academic Press.

Collaer, M. L., & Hines, M. (1995). Human behavioral sex differences: A role for gonadal hormones during early development? *Psychological Bulletin, 118*, 55-107.

Collet, L., Gartner, M., Veuillet, E., Moulin, A., & Morgon, A. (1993). Evoked and spontaneous otoacoustic emissions: A comparison of neonates and adults. *Brain Development, 15*, 249-252.

Collet, L., Kemp, D., Veuillet, E., Duclaux, R., Moulin, A., & Morgon, A. (1990). Effect of contralateral auditory stimuli on active cochlear micro-mechanical properties in human subjects. *Hearing Research, 43*, 251-262.

Crovitz, H., & Zener, K. (1962). A group-test for assessing hand- and eye-dominance. *American Journal of Psychology, 75*, 271.276.

Dallmayr, C. (1985). Spontane oto-akustiche emissionen: Statistik und reakiton auf akustiche storone. *Acustica, 59*, 67-75.

Davis, H. (1983). An active process in cochlear mechanics. *Hearing Research, 9(1)*, 79-90.

Eckel, L. A., Arnold, A. P., Hampson, E., Becker, J. B., Blaustein, J. D., & Herman, J. P. (2008). Research and methodological issues in the study of sex differences and hormone-behavior relations. In: J. B. Becker, K. J. Berkley, N. Geary, E. Hampson, J. P. Herman, & E. A. Young (Eds), *Sex differences in the brain: From genes to behavior* (pp. 35-61) Oxford University Press.

Emmerich, D.S., Harris, J., Brown, W.S., & Springer, S.P. (1988). The relationship

auditory sensitivity and ear asymmetry on a dichotic listening task. *Neuropsychologia, 26(1),* 133-143.

Forest, M. G., de Peretti, E., & Bertrand, J. (1976). Hypothalamic-pituitary-gonadal relationships in man, from birth to puberty. *Clinical Endocrinology, 5,* 551-569.

Geschwind, N. &. Galaburda, A.M. (1985a). Cerebral lateralization. Biological mechanisms, associations, and pathology: I. A hypothesis and a program for research. *Archives of Neurology, 42,* 428-459.

Geschwind, N. &. Galaburda, A.M. (1985b). Cerebral lateralization. Biological mechanisms, associations, and pathology: II. A hypothesis and a program for research. *Archives of Neurology, 42,* 521-552.

Geschwind, N. &. Galaburda, A.M. (1985c). Cerebral lateralization. Biological mechanisms, associations, and pathology: III. A hypothesis and a program for research. *Archives of Neurology, 42,* 634-654.

Grimshaw, G. M., Sitarenios, G., & Finegan, J. K. (1995). Mental rotation at 7 years: Relations with prenatal testosterone levels and spatial play experiences. *Brain and Cognition, 29,* 85-100.

Kemp, D. (1978). Stimulated acoustic emissions from within the human auditory system. *Journal of the Acoustical Society of America, 64(5),* 1386-1391.

Khalfa, S., & Collet, L. (1996). Functional asymmetry of medial olivocochlear system in humans. Towards a peripheral auditory lateralization. *Neuroreport, 7,* 993-996.

Khalfa, S., Veuillet, E., & Collet, L. (1998). Influence of handedness on peripheral auditory asymmetry. *European Journal of Neuroscience, 10(8),* 2731-2737.

Kok, M., van Zanten, G., & Brocaar, M. (1993). Aspects of spontaneous otoacoustic

emissions in healthy newborns. *Hearing Research, 69,* 115-123.

Lamprecht-Dinnesen, A., Pohl, M., Hartmann, S., Heinecke, A., Ahrens, S., Muller, E., et al. (1998). Effects of age, gender, and ear side on SOAE parameters in infancy and childhood. *Audiology and Neuro Otology, 3,* 386-401.

Lauter, J.L. (2007). The EPIC model of functional asymmetries: implications for research on laterality in the auditory and other systems. *Frontiers in Biosciences, 12,* 3734-3756.

Lavigne-Rebillard, M., & Pujol, R. (1986). Development of the auditory hair cell surface in human fetuses. *Anatomical Embryology, 174,* 369-377.

Levine, R., Liederman, J., & Riley, P. (1988). The brainstem auditory evoked potential asymmetry is replicable and reliable. *Neuropsychologia, 26,* 603-614.

Manning, J., Scutt, D., Wilson, J., & Lewis-Jones, D. (1998). The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. *Human Reproduction, 13(11),* 3000-3004.

Manning, J., Stewart, A., Bundred, P., & Trivers, R. (2004). Sex and ethnic differences in 2nd to 4th digit ratio in children. *Early Human Development, 80(2),* 161-168.

McFadden, D. (1993a). A masculinising effect on the auditory systems of human females having male co-twins. *Proceedings of the National Academy of Sciences, 90,* 11900-11904.

McFadden, D. (1993b). A speculation about the parallel ear asymmetries and sex differences in hearing sensitivity and otoacoustic emissions. *Hearing Research, 68,* 143-151.

McFadden, D. (1998). Sex differences in the auditory system. *Developmental*

Neuropsychology, 14(2/3), 261-298.

McFadden, D., & Loehlin, J. (1995). On the heritability of spontaneous otoacoustic emissions: A twins study. *Hearing Research, 85,* 181-198.

McFadden, D., & Mishra, R. (1993). On the relation between hearing sensitivity and otoacoustic emissions. *Hearing Research, 71,* 208-213.

McFadden, D., & Pasanen, E. (1998). Comparison of the auditory systems of heterosexuals and homosexuals: Click-evoked otoacoustic emissions. *Proceedings of the National Academy of Science, 95,* 2709-2713.

McFadden, D., & Pasanen, E. (1999). Spontaneous otoacoustic emissions in heterosexuals, homosexuals, and bisexuals. *Journal of the Acoustical Society of America, 105(4),* 2403-2413.

McFadden, D., & Plattsmier, H. (1984). Aspirin abolishes spontaneous oto-acoustic emissions. *Journal of the Acoustical Society of America, 76(2),* 443-448.

McFadden, D., Pasanen, E., Weldele, M., Glickman, S., & Place, N. (2006). Masculinized otoacoustic emissions in female spotted hyenas (Crocuta crocuta). *Hormones and Behaviour, 50(2),* 285-292.

McIntyre, M., Cohn, B., & Ellison, P. (2006). Sex dimorphism in digital formulae in children. *American Journal of Physical Anthropology, 129(1),* 143-150.

Milner, B., Branch, C., & Rasmussen, T. (1966). Evidence for bilateral speech representation in some non-right-handers. *Transactions of the American Neurological Association, 91,* 306-308.

Mitchell, C., Phillips, D., & Trune, D. (1989). Variables affecting the auditory brainstem response: Audiogram, age, gender and head size. *Hearing Research, 40,* 75-86.

Morlet, T., Collet, L. Duclaus, R., Lapillonne, A., Salle, B., Putet, G., & Morgon, A. (1995a). Spontaneous and evoked otoacoustic emissions in pre-term and full-term neonates: is there a clinical application? *International Journal of Pediatric Otorhinolaryngology, 33(3),* 207-211.

Morlet, T., Lapillonne, A., Ferber, C., Duclaux, R., Sann, L., Putet, G., et al. (1995). Spontaneous otoacoustic emissions in preterm neonates: prevalence and gender effects. *Hearing Research, 90(1-2),* 44-54.

Pasanen, E. G., & McFadden, D. (2000). An automated procedure for identifying spontaneous otoacoustic emissions. *Journal of the Acoustical Society of America,* 108(3), 1105-1116.

Penner, M.J., & Zhang, T. (1997). Prevalence of spontaneous otoacoustic emissions in adults revisited. *Hearing Research, 103(1-2),* 28-34.

Previc, F. (1991). A general theory concerning the prenatal origins of cerebral lateralization in humans. *Psychological Review, 98,* 299-334.

Probst, R., Lonsbury-Martin, B., Martin, G., & Coats, A. (1987). Otoacoustic emissions in ears with hearing loss. *American Journal of Otolaryngology, 8(2),* 73-81.

Probst, R., Lonsbury-Martin, G., & Martin, G. (1991). A review of otoacoustic emissions. *Journal of the Acoustical Society of America, 89(5),* 2027-2067.

Puel, J.-L., & Rebillard, G. (1990). Effect of contralateral sound stimulation on the distortion product 2F1-F2: Evidence that the medial efferent system is involved. *Journal of the Acoustical Society of America, 87,* 1630-1635.

Resnick, S. M., Berenbaum, S. A., Gottesman, I. J., & Bouchard, T. J. (1986). Early

hormonal influences on cognitive functioning in congenital adrenal hyperplasia. *Developmental Psychology, 22*, 191-198.

Strickland, E., Burns, E., & Tubis, A. (1985). Incidence of spontaneous otoacoustic emissions in children and infants. *Journal of the Acoustical Society of America, 78(3)*, 931-935.

Talmadge, C.L., Long, G.R., Murphy, W.J., & Tubis, A. (1993). New off-line method for detecting spontaneous otoacoustic emissions in human subjects. *Hearing Research, 71*, 170-182.

Whitehead, M.L. (1991). Slow variations of the amplitude and frequency of spontaneous emissions. *Hearing Research, 53*, 269-280.

Witelson, S. F. (1991). Neural sexual mosaicism: Sexual differentiation of the human temporo-parietal region for functional asymmetry. *Psychoneuroendocrinology, 16*, 131-153.

Witelson, S. F. &. Nowakowski, R. S. (1991). Left out axons make men right: A hypothesis for the origin of handedness and functional asymmetry. *Neuropsychologia, 29(4)*, 327-333.

Zurek, P. (1981). Spontaneous narrowband acoustic signals emitted by human ears. *Journal of the Acoustical Society of America, 69(2)*, 514-523.

CHAPTER 3

INDIVIDUAL DIFFERENCES IN 2D:4D DIGIT-RATIOS AND OTOACOUSTIC EMISSIONS: DO THEY SHARE A COMMON DEVELOPMENTAL ORIGIN?

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A version of this manuscript has been accepted for publication in *Personality and Individual Differences*

3.1 Introduction

Differences between men and women have been shown to exist in a variety of behavioral, physical, and cognitive traits. Some of these sexual dimorphisms are believed to be organized during fetal development, whereas others originate postnatally and may reflect the actions of environmental as well as biological factors. A well-known physical characteristic that shows a sex difference during prenatal development (Galis, Ten Broek, Van Dongen, & Wignaendts, 2010; Malas, Dogan, Evcil, & Desdicioglu, 2006) and remains robust throughout life (Manning, Scutt, Wilson, & Lewis-Jones, 1998) is the ratio between the lengths of the 2nd and 4th digits (2D:4D). Studies have shown that the 2D:4D ratio is significantly higher (closer to 1.0) in women compared to men (Manning et al., 1998; Manning, Stewart, Bundred, & Trivers, 2004; McFadden & Shubel, 2002; McIntyre, Cohn, & Ellison, 2006; Peters, MacKenzie, & Bryden, 2002), and this difference has been observed as early as the end of the first trimester of fetal development (Malas et al., 2006).

It has been proposed that the observed difference in 2D:4D ratios is due to the differential exposure of males and females to androgens prenatally, during the sensitive period for brain and behavioral differentiation (Manning et al., 1998). Masculinized 2D:4D ratios in women with congenital adrenal hyperplasia (CAH), a disorder characterized by excessive androgen production during prenatal development, offers one line of evidence supporting an influence of prenatal androgens on digit development (Brown, Hines, Fane, & Breedlove, 2002; Ciumas, Linden Hirschberg, & Savic, 2009; Okten, Kalyoncu, & Yaris, 2002; but see Buck, Williams, Hughes, & Acerini, 2003). Further support has been provided in a study by Lutchmaya, Baron-Cohen, Raggat,

Knickmeyer, and Manning (2004) that showed a negative association between infant digit ratios and the ratio of fetal testosterone to fetal estradiol sampled during the second trimester of gestation. Partial masculinisation of the 2D:4D ratio also has been found in female dizygotic twins who gestated in the presence of a male co-twin (van Anders, Vernon, & Wilbur, 2006; Voracek & Dressler, 2007). Previous studies on digit ratio support its use as a biomarker of individual differences in androgen exposure for studies of sex differences that originate during prenatal development, presumably due to different endocrine environments.

A recently discovered auditory trait that also is established during prenatal development is called otoacoustic emissions (OAEs). OAEs are faint sounds produced naturally by the cochlea as a by-product of an amplification mechanism for higher hearing sensitivity and are propagated into the external auditory canal (Davis, 1983; Kemp, 1978). OAEs have been shown to be a robust sexually dimorphic trait, with females producing greater numbers, strengths, and amplitudes of OAEs compared to males (Bilger, Matthies, Hammel, & DeMorest, 1990; Burns, Arehart & Campbell, 1992; Lamprecht-Dinnesen et al., 1998; Penner, Glotzbach, & Huang, 1993; Strickland, Burns, & Tubis, 1985). The sex difference can be detected in ear recordings from newborn infants (Burns et al., 1992). Because the development of the cochlear structures (i.e., outer hair calls) responsible for OAE production takes place during a developmental window (Lavigne-Rebillard & Pujol, 1986) that overlaps the timing of the critical period for sexual differentiation when the testes are active in the male fetus, it has been hypothesized that prenatal exposure to androgens gives rise to the sex difference by *diminishing* the capacity for OAEs. Indirect evidence supporting this hypothesis was

provided by findings that female twins who had a male co-twin *in utero* produced fewer OAEs than other female twin or non-twin groups (McFadden, 1993; McFadden & Loehlin, 1995), presumably due to exposure to small amounts of androgens from the male fetus. Further, bisexual and homosexual women have been shown to produce OAEs that are intermediate to heterosexual women and heterosexual men (McFadden & Pasanen, 1998; McFadden & Pasanen, 1999). It is believed that atypical exposure to androgens prenatally could be responsible for the masculinisation of OAEs in these women (for reviews, see McFadden, 2002; 2009).

Previous studies have attempted to establish a relationship between 2D:4D ratios, as a marker of prenatal androgen exposure, and other sexually dimorphic physical and behavioral traits, albeit with inconsistent results. The logic underlying such studies is that if two sexually dimorphic traits are both organized prenatally by exposure to androgens, then observed individual variations in the two traits should be correlated as a reflection of their common origin. Spatial abilities, for example, have been studied in relation to variations in the 2D:4D ratio, but a reliable correlation has not been found (Manning & Taylor, 2001; McFadden & Shubel, 2003; *cf.* Puts, McDaniel, Jordan, & Breedlove, 2008). Relationships between digit ratios and personality traits have been reported, but the directionality and magnitude of the association depends on the specific personality trait examined and the sex of the individual (Austin, Manning, McInroy, & Mathews, 2002). Androgen-dependent indices of body shape, such as waist-to-hip ratio or body mass index, have been found to correlate with digit ratios in some studies (e.g., Fink, Neave, & Manning, 2003).

The purpose of the current study was to investigate the relationship between individual differences in 2D:4D ratio and OAEs in a sample of young men and women. Only one previous study has attempted to address this theoretical question. McFadden and Shubel (2003) found no significant correlations between digit ratios and OAEs, but differed from the present study in the methods used to obtain finger length measurements, including the hand position used to visualize the anatomical markers. This study forms a backdrop for the present work. In the present study, two types of OAEs were recorded and compared with digit ratios: 1) emissions that are produced naturally, without any deliberate external stimuli (spontaneous OAEs or SOAEs) and 2) echo-like waveforms produced in response to the presentation of clicks or tones (click-evoked OAEs or CEOAEs). Both types of OAEs show robust sex differences, and the development of both digits and cochlear structures responsible for OAEs have been proposed to be influenced prenatally by exposure to androgens (McFadden, 2002). From a theoretical point of view, if variation in androgen levels is responsible for individual variation in these two characteristics, then it is predicted that observed differences across individuals in 2D:4D digit ratios and OAEs will be positively correlated. Specifically, it is hypothesized that individuals who have larger (more female-typical) 2D:4D ratios will have greater SOAE production and CEOAE response amplitude, whereas individuals who have smaller (more male-typical) 2D:4D ratios will have fewer SOAEs and CEOAEs with a smaller response amplitude. If, on the other hand, the findings of McFadden and Shubel (2003) are replicated, the lack of a significant relationship between digit ratios and OAEs would suggest that other prenatal and/or postnatal factors may be influencing the development of one or both of these traits.

3.2 Methods

3.2.1 Participants

Undergraduate male and female participants (n = 153) between the ages of 17 and 26 were recruited from The University of Western Ontario. Testing took place between 2pm and 8pm in a darkened, quiet testing room. The ethnic composition of the sample was predominantly Caucasian; 2% of the sample was black and 14% Asian. Ethnic differences in 2D:4D (Manning, Stewart, Bundred, & Trivers, 2004) and OAEs (Whitehead, Kamal, Lonsbury-Martin, & Martin, 1993) have been documented, but in the present data, analyses limited to the Caucasian group produced results similar to those obtained with the entire population. Thus, the full dataset is reported here. To evaluate inner-ear integrity, participants were screened for hearing sensitivity separately in both ears at frequencies between 250 and 8000 Hz using standard clinical audiometric screening guidelines and equipment (GSI-17 pure-tone, air-conduction audiometer). Any participant who exhibited a hearing threshold greater than 25 dB at any test frequency was excluded from the analysis. In total, data from 22 participants were discarded due to hearing impairments or technical difficulties during the OAE recording (e.g., elevated environmental or participant noise). As a result, data from 67 men and 64 women (total n = 131) were included in this study. The mean age and standard deviation were 20.0 (2.5) and 19.5 (2.0), respectively.

3.2.2 OAE recording

Participants first were asked to sit in a reclined sofa chair, and their external ear canals were examined using a clinical otoscope (Welch Allyn MacroView 23280) to

detect any type of blockage or debris that could interfere with OAE detection. Next, a foam ear-tip attached to an ER-2 earphone was securely placed into the ear canal to be tested first, such that the base of the foam ear-tip was flush with the opening of the ear canal. A low-noise microphone system (Etymotic ER-10B) was used to detect output generated by the ear (spontaneous or click-evoked), as well as delivering trains of clicks during CEOAE testing. Before commencement of the SOAE and CEOAE recording procedures, participants were instructed to relax and remain completely still for a period of 20 minutes in order to acclimatize to the environment and the inserted ear-tip (Whitehead, 1991).

Acoustic output detected during SOAE and CEOAE testing first was passed through a pre-amplification device (Etymotic ER10-72) and then on to a custom-built low-noise amplifier and filter system, where the raw output was both amplified by 30 dB and high-pass filtered above 400 Hz. The output then was passed on to a spectrum analyzer and analog-to-digital converter installed in the cardbus slot of the laptop computer (National Instruments, DAQ AI-16XE-50) and stored in digital form on a Macintosh G4 Powerbook (OS 9.2). The SOAE and CEOAE data were analyzed off-line using custom-written software in LabVIEW (National Instruments, Austin, Texas).

Raw SOAE output was collected in four 30-second segments at a sampling rate of 25 kHz and stored in digital form. Participants were informed by the experimenter as to the start and finish of each recording session, and were given a small amount of time between sessions to relax. Off-line analysis of each participant's two-minute SOAE recording was performed to isolate the quietest 150 time segments (each segment was 655 ms in length and consisted of 75% overlap with other segments). Fast-fourier transforms

were computed for each isolated time segment and were averaged to produce a singular frequency spectrum, which then was analyzed using the custom software to detect the presence of SOAEs according to established criteria (Pasanen & McFadden, 2000). Only spectral peaks appearing between 1000 and 9000 Hz in the frequency domain were considered during SOAE identification, to avoid artifact attributable to low-frequency physiological noise. In order to be classified as an SOAE, each initially flagged spectral peak was required to be more than five standard deviations above the averaged spectral baseline flanking the peak in question, and not closer than 0.1 octaves to a stronger peak already accepted as an SOAE. The magnitude of each peak was then converted to sound-pressure level (SPL) and stored.

CEOAE recording was performed individually for each ear using two separate computer-generated trains of clicks (75 peSPL and 69 peSPL) whose maximal amplitudes corresponded to the peak amplitudes of a continuous 1000-Hz tone presented at the desired intensity. The acoustic clicks were generated using the built-in sound output of the Macintosh computer as rarefaction DC pulses at a sampling rate of 44.1 kHz. The clicks first were calibrated for 20 seconds in the absence of any recording to set a nominal presentation rate of 10 clicks per second for the CEOAE recording procedure. Next, a 20-second sample of the ambient noise present within the ear canal (not involving the presentation of clicks) was taken in order to determine the maximum noise threshold and click-response artifact rejection level. If, during CEOAE testing, the ambient noise exceeded the acceptable level, the presentation of the clicks was delayed until the noise level returned to the established baseline (or below) and the response to the click was recorded. Clicks were presented through the low-noise microphone system and the click-

response was recorded and averaged for the first 250 clicks that were judged to be artifact-free. Each presented click (approximately every 100 ms) was followed by a 4-ms delay in the recording of a response to avoid any acoustical ringing that may have resulted from the presentation of the click. The next 40 ms then were recorded, identified as the click-response, and analyzed for inclusion as an acceptable response. The averaged waveform obtained from the 250 clicks was further analyzed off-line. After eliminating the first 2 ms of the waveform to further ensure the absence of any acoustical ringing in the ear, a 20.48-ms segment was band-pass filtered between 1.0 to 5.0 kHz and the root-mean-square output of the filter was converted to SPL and recorded as the click-evoked response for that particular ear at that particular click level (for further details of the CEOAE procedure see McFadden & Pasanen, 1998).

3.2.3 Finger-length measurement

Precise scanned images (using a digital photocopier) of the underside of both the right and left hands were taken with fingers extended in a splayed position. A white cloth was placed over each hand in order to increase the clarity and visibility of the landmarks. Two distinct landmarks were used for finger-length measurement: a) the lower landmark was the most basal crease on each digit adjoining the palm and b) the upper landmark was the most distal point on the finger tip. This method and the utilization of these landmarks has been used in our lab previously and by other labs studying digit ratios (Brown et al., 2002; Manning et al., 2004; van Anders & Hampson, 2005; Figure 3.1). A high precision digital calliper (Digital Measurement Metrology, Inc., Model ABS) with a resolution of 0.005 mm was used to measure the finger lengths. Finger lengths were independently

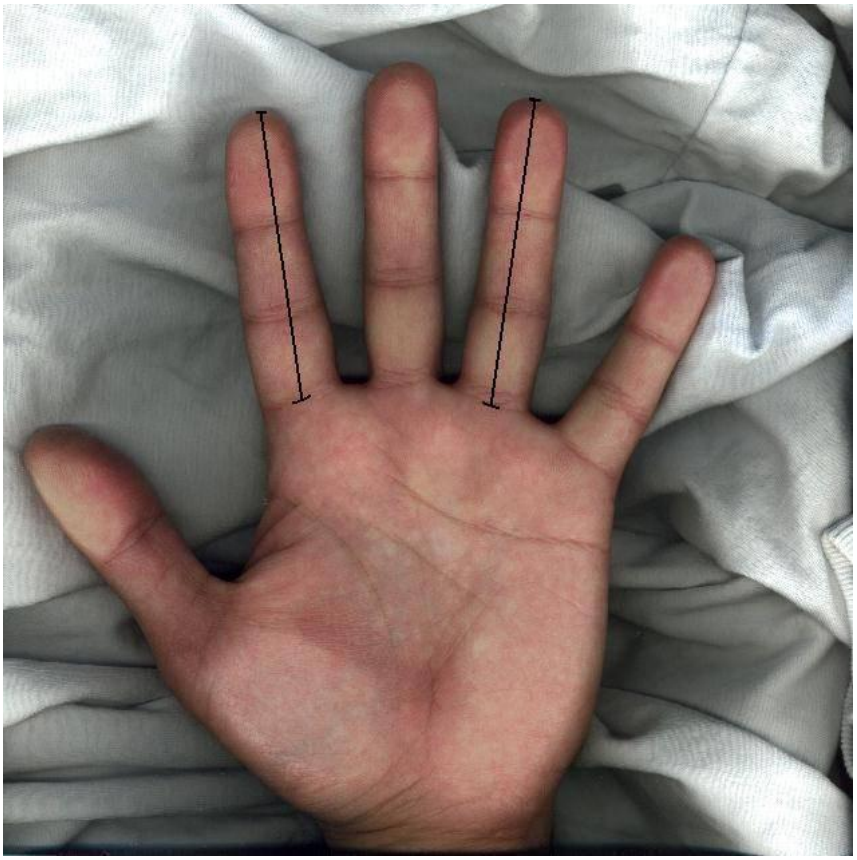

Figure 3.1. Photograph of the landmarks used to measure the lengths of the 2nd and 4th digits. Similar landmarks were used to measure the lengths of the 3rd and 5th digits as well.

measured by a second rater and the inter-rater reliabilities of the ratios computed using intraclass correlation. For both left and right hand ratios, the inter-rater reliability was ICC = 0.99. Although the digit ratio of primary interest in this study was the 2nd to 4th digit ratio, all four finger lengths (thumb excluded) were measured and all digit ratio combinations were calculated (2D:3D, 2D:4D, 2D:5D, 3D:4D, 3D:5D, and 4D:5D). Although it is the 2D:4D ratio that has been hypothesized to be influenced by fetal androgens, several other digit ratios, notably 2D:5D and 3D:4D, also exhibit sexual dimorphism (McFadden & Shubel, 2002). Thus, examining ratios beyond 2D:4D might increase the potential to detect significant associations.

3.3 Results

Table 1 shows the means and standard deviations for all of the variables of interest. To confirm the presence of a sex difference in the highly sexually dimorphic 2D:4D ratio, the 2D:4D data were entered into a mixed-effects ANOVA with sex and hand as factors. A main effect of sex was found, $F(1,129) = 11.05$, $p = 0.001$, with women, as expected, exhibiting greater digit ratios (closer to 1.0) compared to men. A main effect of hand also was found, $F(1,129) = 7.01$, $p = 0.009$; however, the interaction between sex and hand was not significant. Expressed as Cohen's *d*, the effect size for the sex difference in the 2D:4D ratio was $d = 0.54$, suggesting a medium effect. This is in agreement with the average effect size found in other work (Voracek, Manning, & Dressler, 2007).

As reported elsewhere (McFadden & Shubel, 2002), sex differences in several other digit ratios also were significant, including 3D:5D: $F(1,129) = 7.31$, $p = 0.008$;

2D:3D: $F(1,129) = 6.49$, $p = 0.012$; 2D:5D: $F(1,129) = 16.27$, $p < 0.001$; and 3D:4D: $F(1,129) = 5.08$, $p = 0.026$. One other variable, the directional asymmetry in 2D:4D (i.e., right 2D:4D minus left 2D:4D) was analyzed. Though sexual dimorphism has been found in some studies, there was no evidence of a sex difference in directional asymmetry in the present data [$M = 0.006$, $SD = 0.02$ *versus* $M = 0.007$, $SD = 0.04$ for men and women respectively; $F(1,129) = 0.12$, $p = 0.725$]. Thus, directional asymmetry was not analyzed further.

Sex differences in the OAE data in this sample have been reported in detail elsewhere (Snihur & Hampson, 2008b). In brief, mixed-effects ANOVAs were used to analyze the sex difference in SOAE production and CEOAE response amplitude. A significant sex difference was found for both types of OAE parameters. As summarized in Table 3.1, women produced a significantly greater number of SOAEs [$F(1,119) = 9.97$, $p = 0.002$] and produced CEOAEs with significantly greater response amplitude [$F(1,111) = 10.76$, $p = 0.001$] compared to men. The effect sizes were $d = 0.54$ and $d = 0.85$ for the differences in SOAEs and CEOAEs, respectively (*cf.* McFadden & Shubel, 2003).

In order to determine whether there was an association between digit ratios and OAEs, bivariate correlations were computed using Pearson's *r* coefficient. Because the incidence of SOAEs was low, the total number of SOAEs summed over the two ears was used to compute the correlations. Correlations with 2D:4D ratios were of primary interest because of the significance of this particular digit ratio in the literature. However, the correlations for all six digit ratios are shown in Tables 3.2 and 3.3. There was an absence of significant associations between the 2D:4D ratio and any of the OAE variables. This

Table 3.1. Means and standard deviations for 2D:4D digit-ratios and OAE variables

		Men		**Women**	
Ear/Hand	**Variable**	Mean	SD	Mean	SD
Right	2D:4D	0.96	0.03	0.98	0.04
	# SOAEs	1.83	1.98	3.15	2.75
	CEOAE amplitude 75 dB	9.82	2.96	11.23	2.98
	CEOAE amplitude 69 dB	6.10	3.24	8.37	3.47
Left	2D:4D	0.95	0.03	0.97	0.04
	# SOAEs	1.44	1.68	2.27	2.12
	CEOAE amplitude 75 dB	9.66	2.88	10.85	2.97
	CEOAE amplitude 69 dB	5.69	3.18	7.69	3.59

Table 3.2. Correlations between digit-ratios and OAE measures in men

				CEOAEs			
			Total SOAEs	Right 75	Right 69	Left 75	Left 69
MEN	Right Hand	2D:3D	**.143** *.271*	**-.087** *.520*	**-.086** *.518*	**.042** *.748*	**.049** *.703*
		2D:4D	**.058** *.652*	**.029** *.832*	**.082** .539	**.087** *.499*	**.095** *.462*
		2D:5D	**-.094** *.470*	**.014** *.918*	**.114** *.391*	**.332** *.008*	**.302** *.017*
		3D:4D	**-.144** *.269*	**.142** *.293*	**.211** *.109*	**.067** *.607*	**.073** *.573*
		3D:5D	**-.173** *.184*	**.059** *.661*	**.163** *.217*	**.316** *.012*	**.288** *.023*
		4D:5D	**-.120** *.358*	**-.003** *.984*	**.077** *.562*	**.329** *.009*	**.287** *.024*
	Left Hand	2D:3D	**.058** *.658*	**.003** *.983*	**.095** *.474*	**.121** *.349*	**.210** *.101*
		2D:4D	**.111** *.384*	**.110** *.416*	**.176** *.182*	**.175** *.173*	**.245** *.055*
		2D:5D	**-.139** *.287*	**.116** *.217*	**.235** *.073*	**.352 **** *.005*	**.355 **** *.005*
		3D:4D	**.050** *.701*	**.156** *.247*	**.147** *.267*	**.119** *.358*	**.124** *.337*
		3D:5D	**-.173** *.182*	**.178** *.185*	**.200** *.129*	**.304** *.016*	**.262** *.040*
		4D:5D	**-.221** *.088*	**.118** *.381*	**.147** *.266*	**.283** *.026*	**.230** *.072*
		D(r-l)	**-.099** *.449*	**-.124** *.356*	**-.149** *.261*	**-.142** *.272*	**-.237** *.063*

Bolded values represent **correlations (r)** and italicized values represent *probabilities (p)*
****** *significant using Bonferroni correction.*

Table 3.3. Correlations between digit-ratios and OAE measures in women

				CEOAEs			
			Total SOAEs	Right 75	Right 69	Left 75	Left 69
WOMEN	Right Hand	2D:3D	**-.129** *.31*	**-.057** *.669*	**.004** *.978*	**.113** *.396*	**.097** *.463*
		2D:4D	**-.179** *.163*	**.004** *.978*	**.034** *.803*	**.197** *.136*	**.157** *.235*
		2D:5D	**-.140** *.279*	**.061** *.649*	**.117** *.383*	**.147** *.265*	**.180** *.172*
		3D:4D	**-.168** *.193*	**.087** *.518*	**.054** *.688*	**.219** *.095*	**.163** *.218*
		3D:5D	**-.069** *.597*	**.124** *.352*	**.140** *.294*	**.085** *.523*	**.138** *.297*
		4D:5D	**.034** *.793*	**.082** *.538*	**.119** *.374*	**-.048** *.720*	**.042** *.754*
	Left Hand	2D:3D	**-.119** *.357*	**-.055** *.684*	**-.041** *.763*	**-.011** *.933*	**-.085** *.521*
		2D:4D	**-.099** *.443*	**-.055** *.684*	**-.008** *.952*	**-.015** *.913*	**-.087** *.512*
		2D:5D	**-.100** *.440*	**.033** *.807*	**.097** *.467*	**.034** *.797*	**.018** *.890*
		3D:4D	**-.021** *.871*	**-.023** *.862*	**.042** *.753*	**-.009** *.948*	**-.039** *.770*
		3D:5D	**-.021** *.870*	**.069** *.607*	**.122** *.360*	**.035** *.790*	**.073** *.583*
		4D:5D	**-.004** *.978*	**.082** *.542*	**.104** *.436*	**.041** *.759*	**.096** *.470*
		D(r-l)	**-.110** *.397*	**.064** *.632*	**.049** *.743*	**.257** *.049*	**.286** *.028*

Bolded values represent **correlations (r)** and italicized values represent *probabilities (p)*
****** *significant using Bonferroni correction*

was true in both females and males and for both hands. In most cases, the observed correlations were between .1 and -.1. The correlations observed for other digit ratios were of a similar magnitude, with the exception of correlations with CEOAE response amplitudes for the left ear in the male sample, where several correlation coefficients in the .30 range were seen. Only two of these, for left hand 2D:5D, survived Bonferroni correction whereby the criterion for significance was set at $\alpha = .005$ to correct for multiple statistical tests.

3.4 Discussion

The goal of the current study was to investigate whether a relationship exists between 2D:4D digit ratios and OAEs, two characteristics that exhibit a robust sex difference and are hypothesized to be organized by testosterone during prenatal development. A significant correlation between individual differences in the two traits was predicted based on the notion of a shared developmental origin. If found, a correlation would warrant further investigation into similarities in the mechanisms responsible for these traits, such as differential exposure to androgens between fetuses. Further, establishing an association between digit ratios and OAEs would strengthen the empirical basis for using OAEs as a biological marker of differences in prenatal hormone activity. Although two significant correlations were identified between digit-ratios and OAE variables, the overall results of the current study, and especially the lack of associations with 2D:4D, do not support an association between digit-ratios and OAEs despite the presence of significant sex differences in both traits. A previous study by McFadden and Shubel (2003), using different methods of digit ascertainment, likewise

failed to find significant correlations between digit-ratios and OAE production (see also McFadden et al., 2005). A number of basic differences do exist between digit formation and development of the auditory system that may help to explain the lack of an observed association between these two characteristics.

Digit formation and the maturation of the auditory system differ in terms of their developmental trajectory *in utero*, and this fundamental difference may be a contributing reason for the absence of an association between the variables. Garn, Burdi, and Babler (1975) showed that adult bone-to-bone ratios are attained in human fetuses by week 13 of gestation, offering support for early prenatal completion of digit development. This developmental timeline is in accordance with the observation that a sex difference in digit ratios is present in human fetuses early in prenatal development (Malas et al., 2006). However, the development of the cochlear structures integral to OAE production, such as the outer hair cells, as well as the functionality of the cochlea are not completed until later in the gestational period (Nemzek et al., 1996). Specifically, it has been observed from anatomical studies that the onset of human cochlear functioning occurs around weeks 18 – 20 of gestation and that maturation of the cochlear structures is not completed until week 30 of gestation and beyond (Pujol & Lavigne-Rebillard, 1995). The fact that there is a difference in the developmental timeframe highlights the separate genetic control of these two traits. With respect to the prenatal androgen hypothesis, the testes are active in the male fetus from weeks 8 – 24 of gestation (Forest, de Peretti, & Bertrand, 1976) and during this temporal window, various brain and behavioral systems are believed to pass through narrower windows (‘sensitive periods’) when they transiently become receptive to testosterone or its metabolites. Despite both characteristics overlapping the period of

testicular activity, the precise timing of the onset and completion of digit versus cochlear formation and development during this period is vastly different, increasing the likelihood that the applicable sensitive periods, during which hormones exert their influence on the basic genetic programs that control these processes, may not coincide in the two systems. Thus, taking into account the differences in developmental timing, it may not be surprising that a relationship between 2D:4D ratios and OAEs was not found.

Differences in the timing and length of the maturational processes underlying digit and cochlear development could conceivably result in differential exposure to androgens. Instead of a single sustained surge, androgen production in the fetus varies, within limits, in response to external and internal stimuli (e.g., stressors, maturational stage of the testes) during the critical period and, in humans, there is an additional testosterone surge that occurs *postnatally* (for review, see Cohen-Bendahan, van de Beek, & Berenbaum, 2005; Smail, Reyes, Winter, & Faiman, 1981). Differential exposure of the digits and auditory structures to testosterone, due to differences in either the duration of exposure and/or hormonal concentration during the sensitive period, could account for our failure to observe any relationship between these characteristics. Though less likely, an unrecognized role for the postnatal testosterone surge in male infants cannot be ruled out. In principle, postnatal testosterone exposure could act to mask any positive correlations between digit-ratios and OAEs that existed at birth by either further dampening the cochlear processes responsible for OAE production or via further effects on digit development and enlargement of the 2D:4D sexual dimorphism postnatally. Galis et al. (2010) have recently suggested that the sexual dimorphism in the 2D:4D ratio may be initiated *in utero* but further refined by postnatal developmental processes.

The *type* of hormonal influence experienced during development also could account for the absence of a direct correlation between digit ratios and OAEs. Dihydrotestosterone and estradiol, both metabolites of testosterone, have been shown to have individual masculinising effects on specific physical and behavioral traits (for review, see Cohen-Bendahan et al., 2005). Evidence suggests that either testosterone or its androgenic metabolite dihydrotestosterone is the hormone responsible for sexual differentiation of 2D:4D (Berenbaum, Bryk, Nowak, Quigley, & Moffat, 2009; Manning, Bundred, Newton, & Flanagan, 2003), but the hormone responsible for sex differences in OAEs has not been identified.

In addition to the organizational effects that hormones have during the developmental period, a number of behavioral and physiological characteristics are reversibly affected by hormones in adulthood. Though not previously believed to apply to OAEs, recent research has offered support for a superimposed influence of *adult* steroid levels on OAE production, in addition to the underlying sex difference. In women, slight fluctuations in SOAE frequency have been reported across the menstrual cycle (Bell, 1992) and significant departures from typical female SOAE and CEOAE patterns recently were found in women using oral contraceptives (Snihur & Hampson, 2008a; for an earlier report of a non-significant contraceptive effect on OAE production, see McFadden, 2000). Furthermore, a case report of a transsexual male undergoing hormone replacement therapy prior to sex re-assignment surgery showed evidence of SOAEs where previously there were none (McFadden et al., 1998). In men, we recently found that concentrations of circulating testosterone are associated with CEOAE response amplitudes (Snihur & Hampson, 2009). Similar results supporting a role for *current*

testosterone in OAE production have been found in rhesus monkeys through the effects that seasonal hormonal fluctuations have on CEOAEs (McFadden, Pasanen, Raper, Lange, & Wallen, 2006). On the other hand, no known evidence exists for an effect of adult hormones on digit ratios. Consequently, failure to observe a significant relationship between digit ratios and OAEs could simply be the result of uncontrolled postnatal hormonal influences that affect the stability of OAE patterns. This explanation leads to a logical future experiment investigating the relationship between 2D:4D ratios and OAEs in young children under the age of six, so as to eliminate any potential *postnatal* hormonal influences that could differentially affect these two traits.

Though correlations with 2D:4D were of primary interest in the present study, other digit ratios also were investigated. Consistent with McFadden and Shubel (2002), sex differences in 2D:5D and 3D:4D, among others, were observed. Moreover, two significant correlations were found between CEOAE response amplitudes and other digit ratios, despite a stringent criterion for significance of $p = .005$ that was adopted. These correlations were of sufficient magnitude to be theoretically meaningful, were in the expected positive direction, and occurred for the digit ratio that showed the largest sex difference in our data, 2D:5D. On the other hand, there are several reasons to believe these correlations could be spurious: the associations were restricted to the left ear, with no indication of a similar correlation in the right ear, which is typically the stronger ear with respect to the magnitude of the CEOAE response, and no convergent evidence from the 2D:4D ratio. Furthermore, a correlation of nearly the same size was found for right 4D:5D, a ratio that was not sexually dimorphic in either the present work or in other literature (McFadden & Shubel, 2002). Little data is available regarding ratios other than

2D:4D, and their association, if any, with prenatal androgens has not been established. To the extent that the sexual dimorphism in 2D:4D is attributable to androgens, however, one might expect other digit ratios that are sexually differentiated to have the same origins. These correlations therefore bear following up in future research.

Finally, it is possible that we failed to observe a correlation between OAEs and 2D:4D because of limitations of digit ratios themselves as an acceptable index of prenatal testosterone exposure. A recent study of women with complete androgen insensitivity syndrome (CAIS), a condition characterized by XY sex chromosomes but absent or dysfunctional androgen receptors (i.e., they are unable to respond to endogenous or exogenous androgens), offered compelling support for alternative influences on digit development (Berenbaum et al., 2009). This study found that although women with CAIS showed feminized digit ratios that resembled those of typical female controls, all three groups under investigation (women with CAIS, typical women, and typical men) varied greatly in their 2D:4D ratios. If individual differences in the digit ratio were under the sole guidance of prenatal androgens, then it would be expected that women with CAIS, in particular, would not vary to any appreciable extent because they cannot respond effectively to androgens. However, the fact that the digit ratios in this experimental group did vary offers support for a mechanism other than prenatal androgens influencing digit development. To the extent that this is true, the 2D:4D ratio may be an imprecise marker of prenatal androgen exposure and thus the failure of individual differences in 2D:4D, within each sex, to correlate with other traits hypothesized to be under prenatal control by androgens, would not be entirely surprising.

Future research is needed to clarify possible genetic components, and other potential factors, that regulate the development and expression of both digit ratios and OAEs.

3.5 References

Austin, E. J., Manning, J. T., McInroy, K., & Mathews, E. (2002). A preliminary investigation of associations between personality, cognitive ability and digit ratio. *Personality and Individual Differences, 33*, 1115-1124.

Bell, A. (1992). Circadian and menstrual rhythms in frequency variations of spontaneous otoacoustic emissions from human ears. *Hearing Research, 58*, 91-100.

Berenbaum, S. A., Bryk, K. A., Nowak, N., Quigley, C. A., & Moffat, S. (2009). Fingers as a marker of prenatal androgen exposure. *Endocrinology, 150(11)*, 5119-5124.

Bilger, R. C., Matthies, M. L., Hammel, D. R., & DeMorest, M. E. (1990). Genetic implications of gender differences in the prevalence of spontaneous otoacoustic emissions. *Journal of Speech and Hearing Research, 33*, 418-432.

Brown, W. M., Hines, M., Fane, B. A., & Breedlove, S. M. (2002). Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. *Hormones and Behavior, 42(4)*, 380-386.

Buck, J.J., Williams, R.M., Hughes, I.A., & Acerini, C.L. (2003). In-utero androgen exposure and 2^{nd} to 4^{th} digit length ratio – comparison between health controls and females with classical congenital adrenal hyperplasia. *Human Reproduction, 18(5)*, 967-979.

Burns, E. M., Arehart, K. H., & Campbell, S. L. (1992). Prevalence of spontaneous otoacoustic emissions in neonates. *Journal of the Acoustical Society of America, 91(3)*, 1571-1575.

Ciumas, C., Linden Hirschberg, A., & Savic, I. (2009). High fetal testosterone and

sexually dimorphic cerebral networks in females. *Cerebral Cortex, 19(5),* 1167-1174.

Cohen-Bendahan, C. C. C., van de Beek, C., & Berenbaum, S. A. (2005). Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings. *Neuroscience and Biobehavioral Reviews, 29*, 353-384.

Davis, H. (1983). An active process in cochlear mechanics. *Hearing Research, 9(1),* 79-90.

Fink, B., Neave, N., & Manning, J. T. (2003). Second to fourth digit ratio, body mass index, waist-to-hip ratio, and waist-to-chest ratio: their relationships in heterosexual men and women. *Annals of Human Biology, 30*, 728-738.

Forest, M. G., de Peretti, E., & Bertrand, J. (1976). Hypothalamic-pituitary-gonadal relationships in man, from birth to puberty. *Clinical Endocrinology, 5,* 551-569.

Galis, F., Ten Broek, C.M., Van Dongen, S., & Wignaendts, L.C. (2010). Sexual dimorphism in the prenatal digit ratio (2D:4D). *Archives of Sexual Behavior, 39(1),* 57-62.

Garn, S. M., Burdi, A. R., & Babler, W. J. (1975). Early prenatal attainment of adult metacarpal-phalangeal rankings and proportions. *American Journal of Physical Anthropology, 43,* 327-332.

Kemp, D. T. (1978). Stimulated acoustic emissions from within the human auditory system. *Journal of the Acoustical Society of America, 64(5),* 1386-1391.

Lamprecht-Dinnesen, A., Pohl, M., Hartmann, S., Heinecke, A., Ahrens, S., Muller, E., & Riebandt, M. (1998). Effects of age, gender and ear side on SOAE parameters in infancy and childhood. *Audiology and Neuro-otology, 3(6),* 386-401.

Lavigne-Rebillard, M., & Pujol, R. (1986). Development of the auditory hair cell surface in human fetuses. *Anatomical Embryology, 174*, 369-377.

Lutchmaya, S., Baron-Cohen, S., Raggatt, P., Knickmeyer, R., & Manning, J. (2004). 2nd to 4th digit ratios, fetal testosterone and estradiol. *Early Human Development, 77(1-2)*, 23-28.

Malas, M. A., Dogan, S., Evcil, E. H., & Desdicioglu, K. (2006). Fetal development of the hand, digits and digit ratio (2D:4D). *Early Human Development, 82(7)*, 469-475.

Manning, J. T., Bundred, P. E., Newton, D. J., & Flanagan, B. F. (2003). The second to fourth digit ratio and variation in the androgen receptor gene. *Evolution and Human Behavior, 24*, 399-405.

Manning, J. T., Scutt, D., Wilson, J., & Lewis-Jones, D. I. (1998). The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. *Human Reproduction, 13(11)*, 3000-3004.

Manning, J. T., Stewart, A., Bundred, P. E., & Trivers, R. L. (2004). Sex and ethnic differences in 2nd to 4th digit ratio of children. *Early Human Development, 80*, 161-168.

Manning, J. T., & Taylor, R. P. (2001). 2nd to 4th digit ratio and male ability in sport: implications for sexual selection in humans. *Evolution and Human Behavior, 22*, 61-69.

McFadden, D. (1993). A masculinizing effect on the auditory systems of human females having male co-twins. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 11900-11904.

McFadden, D. (2000). Masculinizing effects on otoacoustic emissions and auditory evoked potentials in women using oral contraceptives. *Hearing Research, 142(1-2),* 23-33.

McFadden, D. (2002). Masculinization effects in the auditory system. *Archives of Sexual Behavior, 31,* 93-105.

McFadden, D. (2009). Masculinization of the mammalian cochlea. *Hearing Research, 252*, 37-48.

McFadden, D., & Loehlin, J. C. (1995). On the heritability of spontaneous otoacoustic emissions: A twins study. *Hearing Research, 85,* 181-198.

McFadden, D., & Pasanen, E. (1998). Comparison of the auditory systems of heterosexuals and homosexuals: Click-evoked otoacoustic emissions. *Proceedings of the National Academy of Science, 95*, 2709-2713.

McFadden, D., & Pasanen, E. (1999). Spontaneous otoacoustic emissions in heterosexuals, homosexuals, and bisexuals. *Journal of the Acoustical Society of America, 105(4),* 2403-2413.

McFadden, D., Pasanen, E.G., & Callaway, N.L. (1998). Changes in otoacoustic emissions in a transsexual male during treatment with estrogen. *Journal of the Acoustical Society of America, 104(3 Pt 1),* 1555-1558.

McFadden, D., Pasanen, E. G., Raper, J., Lange, H. S., & Wallen, K. (2006). Sex differences in otoacoustic emissions measured in rhesus monkeys (Macaca mulatta). *Hormones and Behavior, 50*, 274-284.

McFadden, D., & Shubel, E. (2002). Relative lengths of fingers and toes in human males and females. *Hormones & Behavior, 42*, 492-500.

McFadden, D., & Shubel, E. (2003). The relationships between otoacoustic emissions and relative lengths of fingers and toes in humans. *Hormones and Behavior, 43(3),* 421-429.

McFadden, D., Westhafer, J.G., Pasanen, E.G., Carlson, C.L., & Tucker, D.M. (2005). Physiological evidence of hypermasculinization in boys with the inattentive subtype of attention-deficit/hyperactivity disorder (ADHD). *Clinical Neuroscience Research, 5,* 233-245.

McIntyre, M. H., Cohn, B. A., & Ellison, P T. (2006). Sex dimorphism in digital formulae in children. *American Journal of Physical Anthropology, 129(1),* 143-150.

Nemzek, W. R., Brodie, H. A., Chong, B. W., Babcook, C. J., Hecht, S. T., Salamat, S., et al. (1996). Imaging findings of the developing temporal bone in fetal specimens. *American Journal of Neuroradiology, 17(8),* 1467-1477.

Okten, A., Kalyoncu, M., & Yaris, N. (2002). The ratio of second and fourth digit lengths and congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Early Human Development, 70,* 47-54.

Pasanen, E.G., & McFadden, D. (2000). An automated procedure for identifying spontaneous otoacoustic emissions. *Journal of the Acoustical Society of America, 108,* 1105-1116.

Penner, M. J., Glotzbach, L., & Huang, T. (1993). Spontaneous otoacoustic emissions: measurement and data. *Hearing Research, 68(2),* 229-237.

Peters, M., MacKenzie, K., & Bryden, P. (2002). Finger length and distal finger extent patterns in humans. *American Journal of Physical Anthropology, 117(3),* 209-217.

Pujol, R., & Lavigne-Rebillard, M. (1995). Sensory and neural structures in the

developing human cochlea. *International Journal of Pediatric Otorhinolaryngolo*gy, *32,* S177-S182.

Puts, D. A., McDaniel, M. A., Jordan, C. L., & Breedlove, S. M. (2008). Spatial ability and prenatal androgens: meta-analyses of congenital adrenal hyperplasia and digit ratio (2D:4D) studies. *Archives of Sexual Behavior, 37(1),* 100-111.

Smail, P. J., Reyes, F. I., Winter, J. S. D., & Faiman, C. (1981). The fetal hormonal environment and its effects on the morphogenesis of the genital system. In Kogan, S. J., & Hafez, E. S. (Eds.), *Pediatric Andrology* (pp. 9 - 19). The Hague, Netherlands: Martinus Nijhoff.

Snihur, A. W. K., & Hampson, E. (2008a). Defeminization of otoacoustic emission patterns associated with oral contraceptive use in women [Abstract]. *Canadian Journal of Experimental Psychology, 62(4),* 294.

Snihur, A. W. K., & Hampson, E. (2008b, June). *Spontaneous and click-evoked otoacoustic emissions: confirmation of a sex difference in young adults*. Poster session presented at the annual meeting of Theoretical and Experimental Neuropsychology (TENNET), Waterloo, ON, Canada.

Snihur, A. W. K., & Hampson, E. (2009). Click-evoked otoacoustic emissions are associated with circulating testosterone levels in men [Abstract - online]. *Society for Neuroscience Abstracts,* 865.7.

Strickland, E. A., Burns, E. M., & Tubis, A. (1985). Incidence of spontaneous otoacoustic emissions in children and infants. *Journal of the Acoustical Society of America, 78(3),* 931-935.

van Anders, S. M., & Hampson, E. (2005). Waist-to-hip ratio is positively associated with

bioavailable testosterone but not sexual desire in healthy premenopausal women. *Psychosomatic Medicine, 67(2),* 246-250.

van Anders, S. M., Vernon P. A., & Wilbur, C. J. (2006). Finger-length ratios show evidence of prenatal hormone-transfer between opposite-sex twins. *Hormones and Behavior, 49(3),* 315-319.

Voracek, M., & Dressler, S. G. (2007). Digit ratio (2D:4D) in twins: heritability estimates and evidence for a masculinized trait expression in women from opposite-sex pairs. *Psychological Reports, 100(1),* 115-126.

Voracek, M., Manning, J. T., & Dressler, S. G. (2007). Repeatability and interobserver error of digit ratio (2D:4D) measurements made by experts. *American Journal of Human Biology, 19,* 142-146.

Whitehead, M. L. (1991). Slow variations of the amplitude and frequency of spontaneous otoacoustic emissions. *Hearing Research, 53,* 269-280.

Whitehead, M.L., Kamal, N., Lonsbury-Martin, B.L., & Martin, G.K. (1993). Spontaneous otoacoustic emissions in different racial group. *Scandinavian Audiology, 22(1),* 3-10.

CHAPTER 4

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

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A version of this manuscript has been submitted for publication in *Hormones & Behavior*

4.1 Introduction

Otoacoustic emissions (OAEs) are faint sounds produced by the outer hair cells of a normally functioning cochlea that 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 (McFadden & Mishra, 1993; Probst, Lonsbury-Martin, Martin, & Coats, 1987), and these emissions are widely considered to be a natural by-product of an amplification mechanism in the cochlea designed to amplify low-intensity sounds (Davis, 1983). Three types of OAEs have been identified: 1) those produced in the absence of external acoustic stimuli (spontaneous OAEs, SOAEs); 2) those produced in response to the deliberate presentation of acoustic stimuli, either tonal bursts or clicks (click-evoked OAEs, CEOAEs); and 3) those produced as a product of two simultaneously presented acoustic frequencies (distortion-product OAEs, DPOAEs).

A sex difference in OAE production has been found in humans, with females, on average, producing greater numbers of SOAEs, greater overall power of SOAEs, and higher CEOAE response amplitudes compared to males (Burns, Arehart, & Campbell, 1992; Penner, Glotzbach, & Huang, 1993; Snihur & Hampson, 2010a; for review, see Bilger, Matthies, Hammel, & DeMorest, 1990). This robust sex difference has been observed in neonates, infants, and young children (Burns et al., 1992; Morlet et al., 1995; Strickland, Burns, & Tubis, 1985), as well as certain adult populations (Burns et al., 1992; Snihur & Hampson, 2010a; for review, see Bilger et al., 1990), 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 androgens, specifically testosterone, in the male fetus during the critical window for sexual differentiation

masculinises the auditory system, including the structures responsible for OAE production (i.e., outer hair cells), 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 (Bilger et al., 1990; Burns et al., 1992; Talmadge, Long, Murphy, & Tubis, 1993), but evidence to the contrary also exists (Collet, Gartner, Veuillet, Moulin, & Morgon, 1993).

Support for the prenatal androgen hypothesis remains limited, due to the difficulty of studying prenatal effects in humans where the experimental manipulation of testosterone is not ethically permitted. Female dizygotic twins who have male co-twins, however, 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 & Pasanen, 1999), as well as CEOAE response amplitudes (McFadden & Pasanen, 1998). The latter finding is congruent with the prospect that homosexual women are exposed to elevated levels of androgens prenatally, resulting in partial masculinisation of their brains, and subsequent behaviour (see also McFadden & Champlin, 2000; Hall & Kimura, 1995 for partial masculinisation of other traits).

A recent study of spotted hyenas (*Crocuta crocuta*) offers support from a non-human species for a prenatal hormonal effect on OAE production. Both female and male hyenas are highly androgenised during prenatal development. Female hyenas not only produce 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 (McFadden, Pasanen, Weldele, Glickman, & Place, 2006). Conversely, prenatal treatment with testosterone propionate has been found to reduce the amplitude of the CEOAE response in female sheep (McFadden, Pasanen, Valero, Roberts, & Lee, 2009). These results support an effect of prenatal androgens on the production of OAEs and are consistent with the hypothesized dampening effect of testosterone exposure.

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 & McEwen, 1980). However, little empirical attention has been devoted to the possibility of a superimposed influence of *adult* steroids on OAE production. McFadden et al. (2006) recently showed that male rhesus monkeys produce CEOAEs with lower response amplitude during the fall breeding season (i.e., elevated levels of sex steroids) compared to the summer non-breeding season (i.e., reproductively quiescent; lower levels of sex steroids). Seasonal changes in levels of circulating testosterone might underlie the observed variation in response amplitude, though a connection to testosterone has yet to be established. Androgens have been the focus of most existing research because of the mounting evidence that they exert organizational effects on the development of the auditory system, but 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 4 women studied by Bell (1992) showed cyclic fluctuation of about 6-14 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). Endocrine verification of the menstrual cycle was not provided. McFadden (2000) speculated that oral contraceptive (OC) use, too, might affect OAE production in women. This hypothesis has yet to be tested in a formal investigation. Previously undetected SOAEs were exhibited by a transsexual male while undergoing estrogen replacement (and androgen suppression) prior to sex-reassignment surgery (McFadden, Pasanen, & Callaway, 1998). 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 (e.g., Swanson & Dengerink, 1988). Recent demonstrations of estrogen receptor expression in the mouse, rat, and adult human cochlea (Stenberg, Wang, Fish, Schrott-Fischer, Sahlin, & Hultcrantz, 2001; Stenberg, Want, Sahlin, & Hultcrantz, 1999), notably the presence of ligand-dependent ERβ (a subtype of the estrogen receptor) in the inner and outer hair cells (Meltser et al., 2008), affords a potential mechanism by which circulating estradiol, the dominant estrogen in women of reproductive age, could influence OAE production.

As a first 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 by McFadden (2000). Oral contraceptives reliably suppress the ovarian production of estradiol and the rise in progesterone that follows ovulation (Kafrissen & Adashi, 2003). If circulating estradiol levels are 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.

4.2 Methods

4.2.1 Participants

Male (n = 45) and female (n = 50) undergraduates, ranging in age from 17 to 25 years, were recruited from The University of Western Ontario to participate in a study of sex differences in the auditory system. All volunteers initially underwent standard clinical audiometric screening using a GSI-17 pure-tone air-conduction audiometer, at frequencies from 250 Hz 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 dB hearing level at any of the tested frequencies) were not included. Eligible participants were classified retrospectively into 3 groups based on their responses to a demographic and health questionnaire that was given following the OAE testing: males (n = 39), females not using oral contraceptives at present (female non-OC users; n = 26), and females who self-identified as using oral contraceptives at present (female OC users; n = 20). Females in the OC group were taking standard low-dose OCs containing 20 to 30 ug/day of ethinyl estradiol. Sexually active females in the non-OC group used other methods of birth control that did not include any alternative form of hormonal contraception (e.g., injections, patch). The demographics questionnaire also contained items that screened for health conditions previously shown to affect OAE production,

either temporarily or permanently, such as use of certain prescription drugs and ear or cochlear damage or surgery (McFadden & Plattsmier, 1984; Probst et al., 1987), which served as exclusionary criteria.

The groups were well-matched on age: males (M = 20.84 years $\pm$ 2.59 SD), female non-OC users (19.65 $\pm$ 1.83), and female OC users (20.09 $\pm$ 2.25).

4.2.2 General procedure and equipment.

All testing took place in a darkened, quiet testing room between 1400h and 2000h. As classification into groups took place retrospectively, no attempt was made to assess OAEs at any particular stage of the menstrual cycle. Retrospective assignment to groups ensured the experimenter was blind to participants' OC status during the OAE recording, identification, and scoring procedures.

Participants reclined in a sofa chair during OAE detection. The external auditory canal was examined for any debris or blockage using an otoscope (Welch Allyn MacroView 23820) to ensure the ear was not obstructed. A foam ear-tip attached to an Etymotic ER-10B low-noise microphone system then was tightly fitted into the external auditory canal of the ear to be tested first. The ear tested first, during both the audiometric and OAE procedures, and type of OAE tested first (SOAE or CEOAE) was counterbalanced. The microphone system consisted of 2 small diameter coupling tubes protruding approximately 2 mm into the external auditory canal, connected to an Etymotic ER-2 miniature insert earphone, which served two functions: 1) to act as a conduit for the delivery of acoustic stimuli to the inner ear during the CEOAE recordings and 2) to allow the detection of emissions during both the SOAE and CEOAE testing.

Since previous research has shown that a period of habituation of approximately 15-20 min with the ear-tip inserted into the auditory canal allows for better OAE detection (Whitehead, 1991; Zurek, 1981), participants remained still during a 15 min habituation period prior to commencing the SOAE and CEOAE testing.

Emissions detected by the low-noise microphone system during the SOAE and CEOAE testing were amplified and filtered then stored digitally on a laptop computer for offline analysis and identification. An ER 10-72 pre-amplifier received output from the microphone system and passed it along to a custom-built amplifier/filter. Output responses were amplified by 30 dB to compensate for the loss in emission intensity from inner to outer ear and high-passed above 400 Hz to eliminate any extraneous bodily or environmental noises present during the recording. The output was then digitized using a spectrum analyzer and analog-to-digital converter (National Instruments, DAQ AI-I6XE-50) before being stored on a Macintosh G4 Powerbook (OS 9.2). All data collection and offline analysis of the OAE data was accomplished using custom-written software in LabVIEW (National Instruments, Austin, Texas). Programs were obtained courtesy of the laboratory of Dr. D. McFadden at the University of Texas at Austin.

4.2.3 SOAE detection and identification

For both SOAE and CEOAE recordings, participants were instructed to remain completely still throughout the procedure and were signalled by the experimenter as to the start and completion of each recording interval. During SOAE testing, four 30-sec recordings of spontaneous activity were taken from each ear. These raw SOAE recordings were then digitized with 16-bit resolution at a sampling rate of 25 kHz and

stored on the computer hard drive. All further transformations, detection, and analysis of SOAEs were conducted offline. The quietest 150 time-segments (655 ms in length; approximately 75% overlap with other time-segments) from the entire 2 min recording from each participant were selected, and fast fourier transforms of these segments were computed and averaged in the frequency domain. This averaged spectrum corresponded to approximately 25% of the original 2 min sample. Using an automated computer algorithm (for details, see Pasanen & McFadden, 2000), identification of true SOAE peaks was then determined. To be defined as an SOAE, all of the following criteria had to be met: 1) the frequency of the peak resided between 1000 Hz and 9000 Hz; 2) the peak was at least 5 standard deviations above the averaged spectral baseline; and 3) the peak was not within 0.1 octaves of a stronger SOAE, as it has been previously suggested that true SOAEs cannot exist closer than 0.1 octaves of one another (Zwicker, 1990). Once identified as an SOAE, the peak was converted to sound-pressure level units (SPL) and stored for statistical analysis. The dependent variables computed were the number of SOAEs produced, overall SOAE power summed across all the SOAEs identified in each ear (or across both ears), and the power per SOAE.

4.2.4 CEOAE detection

CEOAE detection was performed for two distinct click intensities (75 peSPL and 69 peSPL) in each ear. These click levels corresponded to the peak amplitude of a 1000 Hz tone at the specified intensities and were generated as rarefaction DC pulses (97.7 ms in duration) by the laptop sound output system at a sampling rate of 44.1 kHz. Each click intensity was calibrated prior to each CEOAE recording procedure. Similarly, the level

of ambient noise present in the ear being tested was sampled and averaged to establish individual noise thresholds. Once click calibration and noise floor threshold determination were completed, clicks were presented at a nominal rate of 10 per sec through the microphone system. Evoked responses to the presentation of clicks were recorded unless the ambient noise during click presentation exceeded the pre-determined noise threshold by 0.25 standard deviations or more; if this occurred, presentation of subsequent clicks was delayed until the ambient noise returned to an acceptable level. Cochlear output was digitally sampled at 48 kHz, synchronized to the click stimulus as recorded directly from the sound output of the computer, and bandpass filtered at 1 to 5 kHz. In order to avoid interference from any acoustical ringing that resulted from the click presentation, a 6 ms delay was applied at the beginning of each response. This corresponded to a 2 ms delay in the physical recording after presentation of the click, as well as a 4 ms delay during the off-line analysis of the click-evoked response. The click-evoked response used for statistical analysis consisted of an averaged response from 250 of the quietest clicks (20.48 ms in duration) with the 4 ms delay applied. The click-evoked response was then converted from the root-mean-square output to SPL and stored for statistical analysis. The dependent variable was therefore the amplitude of the click-evoked response.

4.2.5 Saliva collection and hormonal quantification

The primary mechanism of OC action is to inhibit pituitary gonadotropins (Kafrissen & Adashi, 2003). Thus endogenous production of estradiol by the ovaries is inhibited. Estradiol concentrations are suppressed to levels typical of menses or below

(e.g., Gaspard, Romus, Gillain, Duvivier, Demey-Ponsart, & Franchimont, 1983). Bioavailable estradiol, the fraction of the hormone unbound to sex-hormone binding globulin (SHBG), is even lower and challenges the technical limits of detection by conventional assays in serum or saliva. Most OCs also reduce bioavailable testosterone levels (Wiegratz et al., 2003) but effects are more variable. Therefore, we quantified bioavailable testosterone using saliva in order to evaluate whether any changes in OAEs that result from OC use could be explained by testosterone rather than by the suppression of estradiol levels.

Saliva was collected from each participant immediately prior to the SOAE and CEOAE recordings. Participants refrained from eating, drinking (except water), smoking, or brushing their teeth for 1 hr prior to the experiment. Before providing a sample of saliva, the mouth was rinsed with water to eliminate residual debris. An inert sugarless gum (Trident™ peppermint) was used to stimulate saliva flow. This stimulant is known to be inert in the assay employed here (*cf.*, van Anders, 2010). The saliva was collected into a polystyrene culture tube that had been pre-treated with sodium azide to prevent bacterial degradation. The samples were covered with parafilm and allowed to settle at room temperature for 18-24 hr, after which they were stored at -20ºC until assay.

Assays were performed in a single batch by an experienced lab technician. Testosterone was measured by radioimmunoassay using an ^{125}I Coat-a-Count kit for total testosterone (Diagnostic Products Corporation, Los Angeles, CA) modified for saliva according to an established protocol (Moffat & Hampson, 1996; Puts, Cardenas, Bailey, Burriss, Jordan, & Breedlove, 2010). The saliva was centrifuged and a double ether extraction was carried out prior to the assay. All samples were analyzed in duplicate.

The lower limit of detection for the assay was equal to 2.5 pg/mL and the average intra-assay coefficient of variation was 5.2 %. Concentrations are expressed in picograms per milliliter of saliva (pg/mL).

4.3 Results

Mixed-effects ANOVAs with ear and, where applicable, click level as repeated measures were used to analyze group differences in SOAE production and CEOAE response amplitude. One-way ANOVA was used to analyze group differences in SOAE power and testosterone concentrations. Fisher's Least Significant Difference test was used to perform post-hoc pairwise comparisons. Effect sizes were expressed using Cohen's *d* (Cohen, 1977). By convention an effect size of $d = .50$ is considered a medium effect and .80 or above is considered large (Cohen, 1977).

4.3.1 SOAEs

The hypothesis predicting an effect of OC use on SOAE production in females was supported, as female OC users produced significantly less numerous and weaker SOAEs compared to female non-OC users.

With respect to the total number of SOAEs produced, a significant main effect of group was found [$F(2,82) = 7.47$, $p = 0.001$; see Figure 4.1], with female non-OC users producing a greater number of SOAEs summed across both ears compared to female OC users ($p = 0.005$) and compared to males ($p < 0.001$). The difference between non-OC females and males confirms the sex difference in SOAE production that has been reported in previous studies (e.g., Burns et al., 1992; Strickland et al., 1985). No significant

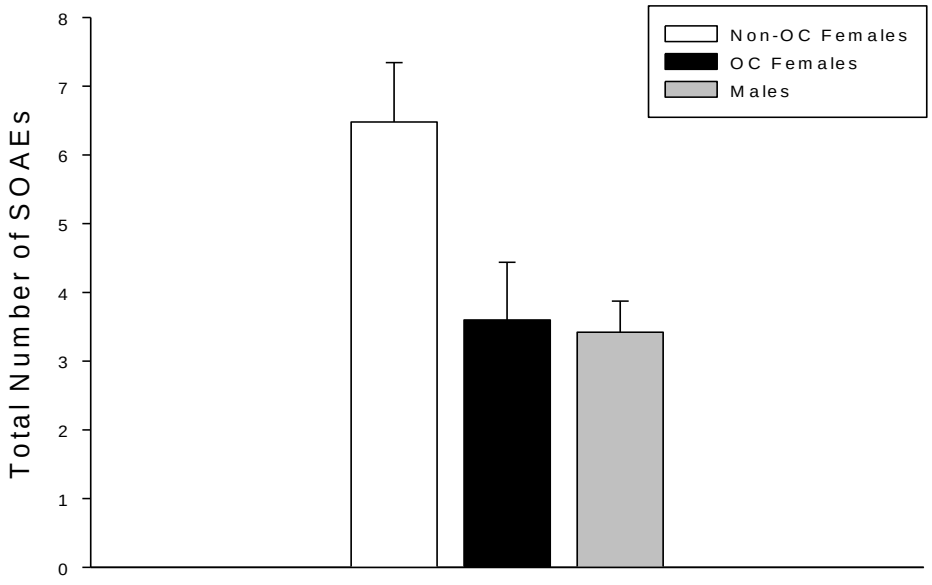

Figure 4.1. Total number of SOAEs produced in both ears. Female non-OC users produced significantly greater numbers of SOAEs than either female OC users or males. Error bars represent SEM.

difference was found between the female OC users and males. A right-ear advantage in SOAE production was evident, with a greater overall number of SOAEs produced in the right ear than the left ear [$F(1,82) = 11.34$, $p = 0.001$; see Figure 4.2]. The ear advantage was seen most clearly among the female non-OC users, though the interaction between group and ear was only marginally significant [$F(2,82) = 2.53$, $p = 0.086$]. Effect sizes for the differences between the non-OC females and OC females and the non-OC females and males were 0.95 and 0.93, respectively.

Participants who did not produce any SOAEs were not included in the analyses of SOAE power. A significant difference in overall SOAE power, summed across both ears, was found among the three groups as shown in Figure 4.3, $F(2,69) = 8.62$, $p < 0.001$. Post-hoc comparisons revealed that female non-OC users produced SOAEs with greater power than males ($p < 0.001$) and female OC users ($p = 0.03$), whereas the mean for OC users was shifted in the male direction and not significantly different from the male group. Effect sizes for the group differences between non-OC females and OC females and between non-OC females and males were 0.83 and 1.11, respectively. This pattern was mainly attributable to power in the right ear, $F(2,61) = 4.87$, $p = 0.011$. Female non-OC users showed greater overall power in the right ear than either males ($p = 0.004$) or female OC users ($p = 0.033$). There was no significant difference between OC users and males ($p = 0.746$). Group differences in the left ear were not significant, $F(2,50) = 0.94$, $p = 0.397$. To disambiguate whether the difference in overall power more likely resulted from larger numbers of SOAEs or larger amplitudes of the individual OAEs produced, ANOVA was performed using the power per SOAE as the dependent variable. Though

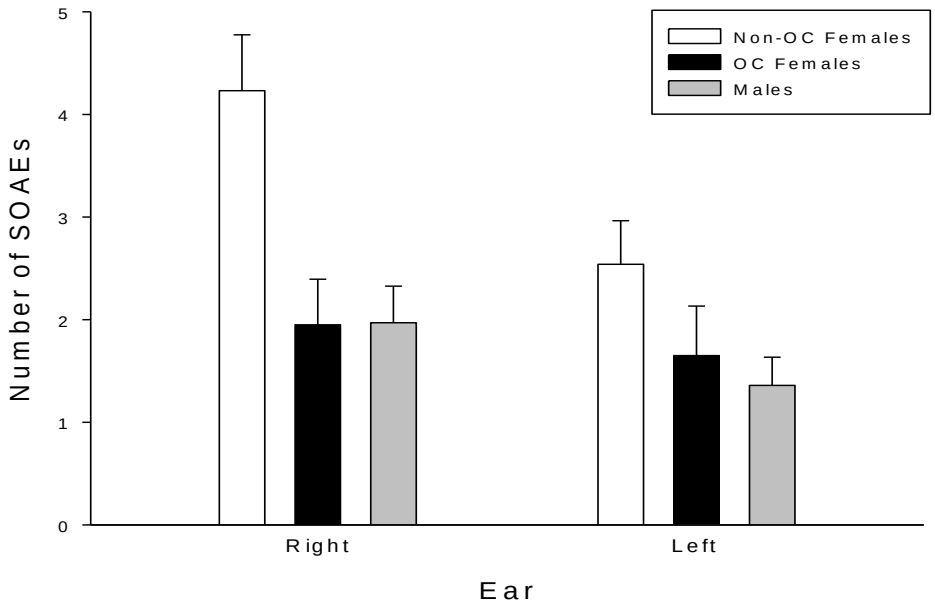

Figure 4.2. Number of SOAEs produced in the right and left ears. An overall right ear advantage was observed, most prominently among female non-OC users. Error bars represent SEM.

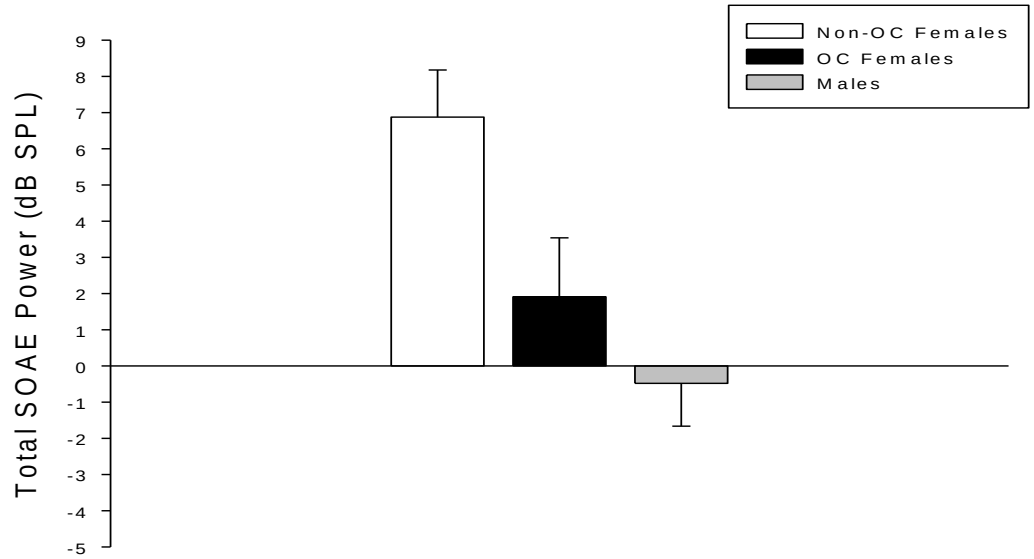

Figure 4.3. Total power of all SOAEs produced in both ears. Female non-OC users produced SOAEs with greater power than female OC users and males. Error bars represent SEM.

the rank ordering of the group means was the same, power per SOAE considered on its own did not significantly differentiate the 3 groups, $F(2,60) = 0.79$, $p = 0.458$.

4.3.2 CEOAEs

It was hypothesized that females using OCs would differ in the amplitude of their click-evoked responses compared to females not currently using OCs. Figure 4.4 shows the average CEOAE response amplitude in female non-OC users, female OC users, and males for all ear and click level combinations. A significant main effect of group [$F(2,75) = 8.89$, $p < 0.001$] and main effect of click level [$F(1,75) = 621.73$, $p < 0.001$] was found. Female non-OC users produced the greatest overall CEOAE response amplitudes, whereas males produced the lowest amplitudes ($p < 0.001$ by post-hoc test). There was no significant difference between the ears. Significant interactions also were found between click level and group [$F(2,75) = 5.97$, $p = 0.004$] and between click level and ear [$F(1,75) = 5.30$, $p = 0.024$]. Tests of simple main effects were used to break down the interaction between click level and group. At 69dB, the non-OC females showed significantly greater response amplitudes than either OC females ($p = 0.035$) or males ($p < 0.001$) with effect sizes of 0.54 and 1.13, respectively, whereas at 75dB the difference between non-OC females and OC females was marginally significant ($p = 0.076$; $d = 0.55$). OC females did not differ significantly from males at either intensity ($p = 0.099$ and $p = 0.194$ for the two click levels, respectively).

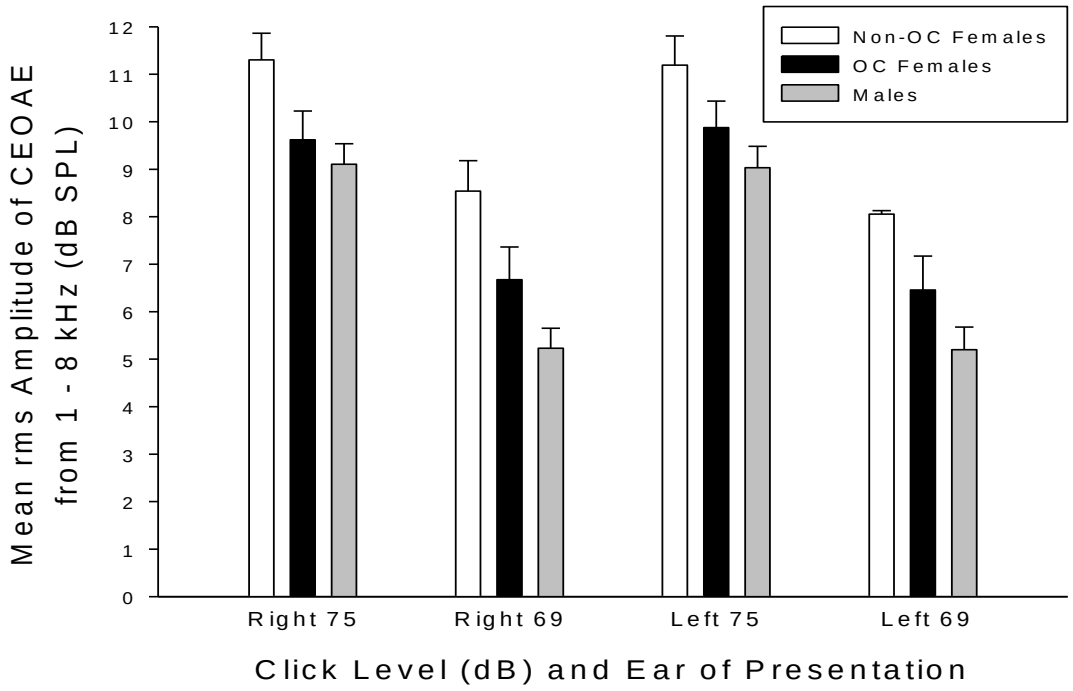

Figure 4.4. Mean CEOAE response amplitude in the right and left ears at two click levels (75dB and 69dB). At 69dB, female non-OC users showed significantly greater response amplitudes than either female OC users or males. At 75dB, the difference between non-OC users and OC users was marginally significant. Error bars represent SEM.

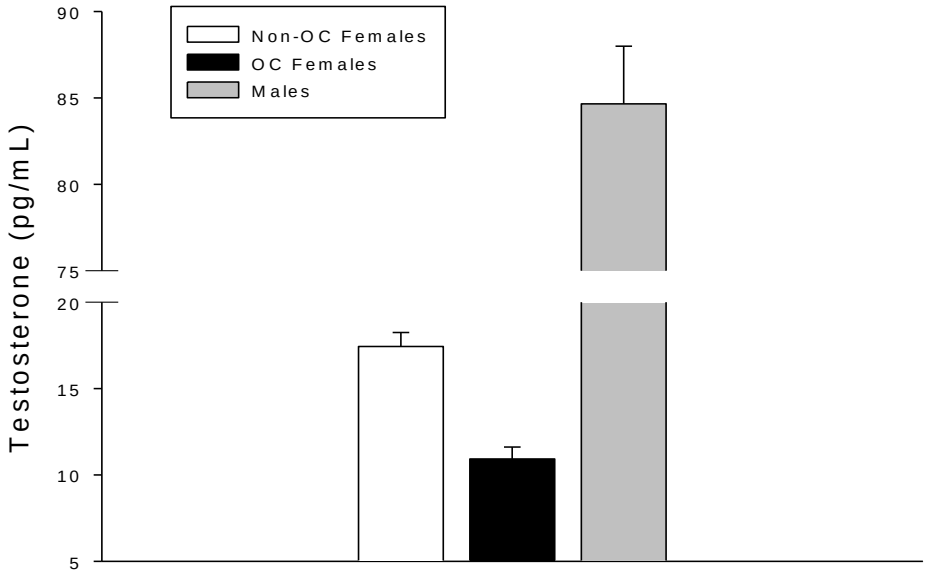

Figure 4.5. Mean salivary testosterone concentrations in the three groups. Female non-OC users had significantly higher circulating testosterone than OC users. Error bars represent SEM.

4.3.3 Testosterone

As shown in Figure 4.5, there was a significant group difference in salivary testosterone concentration in the current study, $F(2,90) = 227.56$, $p < 0.001$. Males, as expected, had significantly higher testosterone levels compared to female non-OC users and OC users (both ps < 0.001). A post-hoc *t*-test was run to compare the two female groups to determine whether OC use had the expected suppressant effect on testosterone (Bancroft, Sherwin, Alexander, Davidson, & Walker, 1991). OC users were confirmed to have significantly lower salivary testosterone levels compared to female non-OC users, $t(46) = 34.50$, $p < 0.001$.

4.4 Discussion

The present study is among the first to investigate the effects of reproductive steroids on OAE production in adults. As predicted, significant differences between OC users and non-users were found. Female OC users produced significantly lower numbers of SOAEs, SOAEs with less total power and less power in the right ear particularly, and had significantly lower CEOAE response amplitudes than female non-OC users. The lowered response amplitude was significant at the 69dB click level and approached significance at 75dB. For each of the OAE variables, the OC users showed a pattern that was shifted in a direction away from the pattern typically seen in non-OC females. That is, they were muted or diminished in their OAE output and thus, may be considered defeminised with respect to this particular trait. The term ‘defeminization’ is used in the neuroendocrine literature to denote the reduction in a female-typical characteristic (Breedlove & Hampson, 2002).

The present results confirm the hypothesis put forward by McFadden (2000) that OC use in women may alter patterns of OAE production. Despite retrospectively combining data from two earlier published studies, McFadden (2000) was unable to confirm a difference between OC users and non-users on four different measures of OAE strength. Thus, the present study is the first to find statistical support for this proposition. The difference between the current results and those of McFadden (2000) may be due to changes in the formulations of OCs that have occurred over the past 20 years. McFadden (2000) found no significant differences between OC users and non-users, but current OC formulations are exceedingly low in estrogen activity, as indicated by reports of decreased bone density in girls who have been using OCs for an extended period of time compared to non-users (Teegarden, Legowski, Gunther, McCage, Peacock, & Lyle, 2005). In the current study, female OC users were defeminised with respect to the number of SOAEs produced, total SOAE power, and CEOAE response amplitude, offering empirical support for an effect of OC use, as well as support for the broader idea that circulating levels of *adult* hormones may influence OAE production. The present data suggest that OC use in women diminishes, or dampens, the cochlear mechanisms responsible for SOAE and CEOAE production.

Female OC users in the present study did not demonstrate significant sex differences and resembled males in each measured element of OAE production. In contrast, sexual dimorphism was confirmed when the normally-cycling females (i.e., OC non-users) were compared to males. Previous studies have found similar sex differences, both in SOAEs and CEOAEs (e.g., Burns et al., 1992; Morlet et al., 1995; Strickland et al., 1985) in samples of participants that were either not using OCs (e.g., neonates,

infants, and children) or that contained a mix of OC users and non-users (Penner et al., 1993). Sex differences in OAE production generally have been attributed to androgen exposure *in utero*. The prenatal androgen hypothesis posits that exposure of the cochlea to elevated levels of testosterone during a critical period in development dampens the capacity of the outer hair cells to produce OAEs in the male fetus. This hypothesis has been invoked to explain the sexual dimorphism, as well as reductions in SOAE and CEOAE production seen in females with male co-twins (McFadden, 1993a) and in females of differing sexual orientation (McFadden & Pasanen, 1998, 1999). The sex difference and, by implication, the androgen effect is often found to be more pronounced in the right ear than the left (Bilger et al., 1990; Burns et al., 1992; Talmadge et al., 1993), which is reminiscent of the ear differences found in the present study. Though an organizational effect of prenatal androgens on the basilar membrane might exist, and can explain the existence of sex differences in OAE production in prepubertal children (Burns et al., 1992; Morlet et al., 1995; Strickland et al., 1985), it cannot explain the effects of OC use observed here. The fact that the sex difference was attenuated so markedly among women choosing to use OCs suggests that the adult hormonal milieu is at least as important, if not more important, than are prenatal influences in determining the adult pattern of OAE production.

OCs alter circulating hormone concentrations in several ways. The primary mechanism of contraceptive action is the suppression of circulating estradiol and, secondarily, progesterone. Alterations in ovarian hormones are perhaps the most likely to underlie the present effects, but testosterone levels also are altered by OC use, as confirmed in the present data. We found that bioavailable testosterone levels were

substantially decreased in the OC users. Thus, one question that arises is whether decreased testosterone can explain the observed effects of OCs on OAE production. This seems unlikely for several reasons. Studies of the prenatal effects of testosterone, including animal studies where testosterone levels were manipulated experimentally (e.g., McFadden et al., 2009), have shown that the direction of testosterone's effects is opposite to what was found in the present study (i.e., higher not lower levels of testosterone were associated with diminished OAE production). Little work is available on the effects of adult testosterone, but recent studies suggest that the effect of adult testosterone, too, is to diminish OAE production. In male rhesus monkeys, for example, decreased CEOAE response amplitudes were found during the breeding season when testosterone is elevated, compared to the non-breeding season when testosterone is low (McFadden et al., 2006). Further evidence that circulating testosterone may dynamically regulate OAE production has been offered by our lab. Snihur and Hampson (2010b) reported a negative correlation between the level of testosterone in the circulation and CEOAE response amplitude in adult men. In the current study, OC users showed the reverse pattern extremely low testosterone accompanied by reduced OAE production a result that is inconsistent with all previously observed associations between testosterone and OAEs. If testosterone were the functional hormone involved, we would expect to find lower OAE production among *non-OC* users, who exhibited higher testosterone, not among OC-users. It seems unlikely that elevated testosterone would be associated with diminished OAEs in numerous prior studies yet exert the opposite effect in the current study. Therefore, another mechanism influencing OAEs must exist.

A possible explanation for the observed results is that estradiol, the primary form of estrogen that is present in females, is actively involved in regulating OAE production in the female cochlea. Recent studies in fact support a potential role for estradiol in normal cochlear functioning. Meltser et al. (2008), for example, showed that ERβ (an estrogen receptor subtype) in the mouse cochlea is involved in auditory sensitivity and protection from acoustic trauma, suggesting that estradiol may exert prophylactic effects on hearing. Further research in mice has shown that estradiol protects against age-related hearing loss (Simonoska et al., 2009). Aging women receiving hormone replacement therapy tend to have better hearing than women not on therapy (Hultcrantz, Simonoska, & Stenberg, 2006). ERα and ERβ expression have been described in segments of the mouse, rat, and adult human cochlea, including the outer hair cells, raising the probability that estradiol actively affects cochlear function (Stenberg et al., 1999, 2001). Because previous work has established a relationship between hearing sensitivity and OAE production in humans (McFadden & Mishra, 1993; Probst et al., 1987), it is conceivable that the group differences in OAE production observed among women in the present study are due to a difference in estradiol availability to bind to ligand-dependent receptors in the inner ear.

Previous studies of humans or other primates have implicated estrogen indirectly in OAE production. But the possibility that estrogen modulates cochlear function has not received dedicated research attention and, as a result, most existing evidence is anecdotal. The acoustic frequencies of emitted SOAEs have been reported to fluctuate with the menstrual cycle (Bell, 1992; Penner, 1995), though studies are limited to case-reports of a small number of individual women. SOAE frequencies peaked near the suspected time of

ovulation with some evidence of a second maximum between ovulation and menses (Penner, 1995), a pattern that would support an estradiol-driven effect. The prospect of an estrogen-dependent mechanism is further supported by a case-study of a transsexual male undergoing estrogen replacement therapy prior to sex-reassignment surgery, in whom SOAEs appeared at frequencies where there previously were none (McFadden et al., 1998). In a recent study by McFadden et al. (2006), a group of female rhesus monkeys showed greater CEOAE response amplitudes during the fall breeding season when estradiol levels are elevated (Walker, Wilson, & Gordon, 1984), although this pattern did not reach statistical significance given the small sample size available. In the current study, normally-cycling females who did not use OCs produced more female-typical OAEs than females whose ovarian hormones were suppressed by their use of OCs. Thus the data from the current investigation, as well as previous studies, are consistent with the possibility that elevated levels of circulating estradiol in women are associated with enhanced OAE production, whereas lower levels are associated with diminished OAEs. The motility of the outer hair cells is controlled by acetylcholine (Frolenkov, 2006), a transmitter known to be modulated by estradiol levels.

An effect of estradiol on OAE production would complement studies documenting a sex difference in the auditory brainstem response (ABR) and the ability of estradiol administration in ovariectomized rats to modify ABR latencies reflecting changes in both cochlear and brainstem processing (Coleman, Campbell, Cooper, Welsh, & Moyer, 1994). Shortened latencies in the ABR have been found in postmenopausal women taking hormone replacement, especially with estrogen-only replacement (Khaliq, Tandon, & Goel, 2005). Shortened latencies also have been found during the periovulatory phase

of the menstrual cycle in naturally-cycling women when estradiol but not progesterone levels are elevated (Serra, Maiolino, Agnello, Messina, & Caruso, 2003).

Although we favour an explanation in terms of estradiol, it should be noted that the use of OCs also reduces progesterone production due to the prevention of ovulation in women on OCs. As a result, the increase in progesterone that normally occurs during the luteal phase of the cycle is absent. To our knowledge, progesterone receptors in the cochlear structures integral to OAE production have not been found. Though no current data are available that speak to the issue of progesterone modifying OAEs, support for a potential effect of progesterone on auditory evoked potentials in humans has been provided (Elkind-Hirsch, Wallace, Malilnak, & Jerger, 1994) with progesterone, given in the form of medroxyprogesterone acetate, attenuating the effects of estradiol It should be emphasized that the higher-order mechanisms regulating these neural responses in the brainstem differ greatly from those responsible for OAE production in the inner ear. Thus, at present, there is little reason to think that progesterone may influence OAE production.

We have assumed that if ovarian hormones play a role in influencing OAE production, they do so via direct interaction with the outer hair cells in the cochlea through a receptor mechanism. However, the possibility that an *indirect* effect of the hormonal changes induced by OCs could be responsible for the changes in OAE production exists. For example, differences in body temperature exist between normally-cycling women and OC users. In normally-cycling women, there is an increase in basal metabolic rate after ovulation, reflecting the thermogenic effects of progesterone. This will be absent in OC users, where ovulation is suppressed. These differences in body

temperature have been previously reported (Kattapong, Fogg, & Eastman, 1995). Hall (1992) described an influence of body temperature changes on certain auditory properties, including auditory brainstem responses, although no such evidence exists for the effects of body temperature on OAE production. Indeed clinical studies have shown no changes in OAEs except under extreme departures from normo-thermia. In this respect, it appears unlikely that body temperature differences due to hormonal changes induced by OC use could indirectly influence SOAEs and CEOAEs, although other secondary mechanisms with effects on OAEs may as of yet be identified.

The current study offers novel support for an effect of *adult* reproductive steroid levels on OAE production. Relative to a matched group of female controls who were not currently using oral contraception, OC users showed a defeminised pattern of OAEs, characterized by fewer numbers of SOAEs, SOAEs with less total power, and smaller CEOAE response amplitudes in response to acoustical stimulation. A comparison group of males showed the lowest numbers of SOAEs, lower total SOAE power, and lower CEOAE response amplitudes, consistent with previously established sex differences. OC users did not differ significantly from males in any of the measured OAE parameters. Defeminisation of SOAEs and CEOAEs in women using OCs is likely to be mediated through an ovarian steroid-dependent mechanism.

4.5 References

Bancroft, J., Sherwin, B.B., Alexander, G.M., Davidson, D.W., & Walker, A. (1991). Oral contraceptives, androgens, and the sexuality of young women: I. A comparison of sexual experience, sexual attitudes, and gender role in oral contraceptive users and nonusers. *Archives of Sexual Behavior, 20(2),* 105-120.

Bell, A. (1992). Circadian and menstrual rhythms in frequency variations of spontaneous otoacoustic emissions from human ears. *Hearing Research, 51(1),* 91-100.

Bilger, R., Matthies, M., Hammel, D., & DeMorest, M. (1990). Genetic implications of gender differences in the prevalence of spontaneous otoacoustic emissions. *Journal of Speech and Hearing Research, 33,* 418-432.

Breedlove, S. M. & Hampson, E. (2002). Sexual differentiation of the brain and behavior. In: J. B. Becker, S. M. Breedlove, D. Crews, & M. M. McCarthy (Eds.), Behavioral endocrinology (2nd ed.)(pp. 75-114). Cambridge, MA: MIT Press.

Burns, E.M., Arehart, K., & Campbell, S. (1992). Prevalence of spontaneous otoacoustic emissions in neonates. *Journal of the Acoustical Society of America, 91(3),* 1571-1575.

Cohen, J. (1977). *Statistical power analysis for the behavioral sciences* (revised edition). New York: Academic Press.

Coleman, J.R., Campbell, D., Cooper, W.A., Welsh, M.G., & Moyer, J. (1994). Auditory brainstem responses after ovariectomy and estrogen replacement in the rat. *Hearing Research, 80,* 209-215.

Collet, L., Gartner, M., Veuillet, E., Moulin, A., & Morgon, A. (1993). Evoked and

spontaneous otoacoustic emissions: A comparison of neonates and adults. *Brain Development, 15*, 249-252.

Davis, H. (1983). An active process in cochlear mechanics. *Hearing Research, 9(1)*, 79-90.

Elkind-Hirsch, K.E., Wallace, E., Malilnak, L.R., & Jerger, J.F. (1994). Sex hormones regulate ABR latency. *Otolaryngology – Head and Neck Surgery, 110*, 46-52.

Frolenkov, G. I. (2006). Regulation of electromotility in the cochlear outer hair cell. *Journal of Physiology, 576*, 43-48.

Gaspard, U. J., Romus, M. A., Gillain, D., Duvivier, J., Demey-Ponsart, E., & Franchimont, P. (1983). Plasma hormone levels in women receiving new oral contraceptives containing ethinyl estradiol plus levonorgestrel or desogestrel. *Contraception, 27*, 577-590.

Goy, R. W., & McEwen, B. S. (1980). *Sexual differentiation of the brain*. Cambridge, MA: MIT Press. Hall, J.W., III (1992). *Handbook of auditory evoked responses*. Allyn and Bacon, Boston, MA.

Hall, J.W., III (1992). *Handbook of auditory evoked responses*. Needham Heights, MA: Allyn & Bacon.

Hall, J.A. & Kimura, D. (1995). Sexual orientation and performance on sexually dimorphic motor tasks. *Archives of Sexual Behavior, 24(4)*, 395-407.

Hultcrantz, M., Simonoska, R., & Stenberg, A.E. (2006). Estrogen and hearing: A summary of recent investigations. *Acta Oto-Laryngologica, 126*, 10-14

Kafrissen, M., & Adashi, E. (2003). Fertility control: Current approaches and global

aspects. In: P. R. Larsen, H. M. Kronenberg, S. Melmed, K. S. Polonsky (Eds.), *Williams textbook of endocrinology* (10th ed.), pp. 665-708. Philadelphia: Saunders.

Kattapong, K.R., Fogg, R., & Eastman, L.F. (1995). Effect of sex, menstrual cycle phase, and oral contraceptive use on circadian temperature rhythms. *Chronobiology International, 12*, 257-266.

Kemp, D. (1978). Stimulated acoustic emissions from within the human auditory system. *Journal of the Acoustical Society of America, 64(5)*, 1386-1391.

Khaliq, F., Tandon, O.P., & Goel, N. (2005). Differential effects of exogenous estrogen versus a estrogen-progesterone combination on auditory evoked potentials in menopausal women. *Indian Journal of Physiology and Pharmacology, 49(3)*, 345-352.

Lamprecht-Dinnesen, A., Pohl, M., Hartmann, S., Heinecke, A., Ahrens, S., Muller, E., et al. (1998). Effects of age, gender, and ear side on SOAE parameters in infancy and childhood. *Audiology and Neuro Otology, 3*, 386-401.

McFadden, D. (1993a). A masculinising effect on the auditory systems of human females having male co-twins. *Proceedings of the National Academy of Sciences, 90*, 11900-11904.

McFadden, D. (1993b). A speculation about the parallel ear asymmetries and sex differences in hearing sensitivity and otoacoustic emissions. *Hearing Research, 68*, 143-151.

McFadden, D. (1998). Sex differences in the auditory system. *Developmental Neuropsychology, 14(2/3)*, 261-298.

McFadden, D. (2000). Masculinizing effects on otoacoustic emissions and auditory evoked potentials in women using oral contraceptives. *Hearing Research, 142*, 23-33.

McFadden, D. (2002). Masculinization effects in the auditory system. *Archives of Sexual Behavior, 31(1)*, 99-111.

McFadden, D., & Champlin, C.A. (2000). Comparison of auditory evoked potentials in heterosexual, homosexual, and bisexual males and females. *Journal for the Association of Research in Otolaryngology*, *1(1)*, 89-99.

McFadden, D., & Mishra, R. (1993). On the relation between hearing sensitivity and otoacoustic emissions. *Hearing Research, 71*, 208-213.

McFadden, D., & Pasanen, E. (1998). Comparison of the auditory systems of heterosexuals and homosexuals: Click-evoked otoacoustic emissions. *Proceedings of the National Academy of Science*, *95*, 2709-2713.

McFadden, D., & Pasanen, E. (1999). Spontaneous otoacoustic emissions in heterosexuals, homosexuals, and bisexuals. *Journal of the Acoustical Society of America, 105(4)*, 2403-2413.

McFadden, D., Pasanen, E.G., & Callaway, N.L. (1998). Changes in otoacoustic emissions in a transsexual male during treatment with estrogen. *Journal of the Acoustical Society of America*, *104(3 Pt 1)*, 1555-1558.

McFadden, D., Pasanen, E., Weldele, M., Glickman, S., & Place, N. (2006). Masculinized otoacoustic emissions in female spotted hyenas (Crocuta crocuta). *Hormones and Behaviour, 50(2),* 285-292.

McFadden, D., Pasanen, E. G., Valero, M. D., Roberts, E. K., & Lee, T. M. (2009).

Effect of prenatal androgens on click-evoked otoacoustic emissions in male and female sheep (Ovis aries). *Hormones and Behavior, 55,* 98-105.

McFadden, D., & Plattsmier, H. (1984). Aspirin abolishes spontaneous oto-acoustic emissions. *Journal of the Acoustical Society of America, 76(2),* 443-448.

Meltser, I., Tahera, Y., Simpson, E., Hultcrantz, M., Charitidi, K., Gustafsson, J.A., et al. (2008). Estrogen receptor beta protects against acoustic trauma in mice. *Journal of Clinical Investigation, 118(4),* 1563-1570.

Moffat, S.D. & Hampson, E. (1996). Salivary testosterone levels in left- and right-handed adults. *Neuropsychologia, 34(3),* 225-233.

Morlet, T., Lapillonne, A., Ferber, C., Duclaux, R., Sann, L., Putet, G., et al. (1995). Spontaneous otoacoustic emissions in preterm neonates: prevalence and gender effects. *Hearing Research, 90(1-2),* 44-54.

Pasanen, E. G., & McFadden, D. (2000). An automated procedure for identifying spontaneous otoacoustic emissions. *Journal of the Acoustical Society of America,* 108(3), 1105-1116.

Penner, M.J., Glotzbach, L., & Huang, T. (1993). Spontaneous otoacoustic emissions: measurement and data. *Hearing Research, 68(2),* 229-237.

Penner, M.J. (1995). Frequency variation of spontaneous otoacoustic emissions during a naturally occurring menstrual cycle, amenorrhea, and oral contraception: a brief report. *Ear and Hearing, 16(4),* 428-432.

Probst, R., Lonsbury-Martin, B., Martin, G., & Coats, A. (1987). Otoacoustic emissions in ears with hearing loss. *American Journal of Otolaryngology, 8(2),* 73-81.

Puts, D.A., Cardenas, R.A., Bailey, D.H., Burriss, R.P., Jordan, C.L., and Breedlove, S.M. (2010). Salivary testosterone does not predict mental rotation performance in men or women. *Hormones and Behavior, 58*, 282-289.

Serra, A., Maiolino, L., Agnello, C., Messina, A., & Caruso, S. (2003). Auditory brain stem response throughout the menstrual cycle. *Annals of Otology, Rhinology, and Laryngology, 112*, 549-553.

Simonoska, R., Stenberg, A.E., Duan, M., Yakimchuk, K., Fridberger, A., Sahlin, L., et al. (2009). Inner ear pathology and loss of hearing in estrogen receptor-beta deficient mice. *Journal of Endocrinology, 201(3)*, 397-406.

Snihur, A.W.K. & Hampson, E. (2010a). Sex and ear differences in spontaneous and click-evoked otoacoustic emissions in young adults. Submitted to *Brain and Cognition*.

Snihur, A.W.K. & Hampson, E. (2010b). Circulating testosterone levels are associated with fluctuations in CEOAE response amplitude in men. Submitted to *Hormones and Behavior*.

Stenberg, A. E., Wang, H., Fish III, J. Schrott-Fischer, A., Sahlin, L., & Hultcrantz, M. (2001). Estrogen receptors in the normal adult and developing human inner ear and in Turner's syndrome. *Hearing Research, 157*, 87-92.

Stenberg, A.E., Wang, H., Sahlin, L., & Hultcrantz, M. (1999). Mapping of estrogen receptors alpha and beta in the inner ear of the mouse and rat. *Hearing Research, 136(1-2)*, 29-34.

Strickland, E., Burns, E., & Tubis, A. (1985). Incidence of spontaneous otoacoustic

emissions in children and infants. *Journal of the Acoustical Society of America, 78(3),* 931-935.

Swanson, S. J., & Dengerink, H. A. (1988). Changes in pure-tone thresholds and temporary threshold shifts as a function of menstrual cycle and oral contraceptive use. *Journal of Speech and Hearing Research*, *31*, 569-574.

Talmadge, C.L., Long, G.R., Murphy, W.J., & Tubis, A. (1993). New off-line method for detecting spontaneous otoacoustic emissions in human subjects. *Hearing Research, 71*, 170-182.

Teegarden, D., Legowski, P., Gunther, C.W., McCage, G.P., Peacock, M., & Lyle, R.M. (2005). Dietary calcium intake protects women consuming oral contraceptives from spine and hip bone loss. *Journal of Clinical Endocrinology & Metabolism, 90(9),* 5127-5133.

van Anders, S.M. (2010). Chewing gum has large effects on salivary testosterone, estradiol, and secretory immunoglobulin A assays in women and men *Psychoneuroendocrinology, 35(2),* 305-309.

Walker, M.L., Wilson, M.E., & Gordon, T.P. (1984). Endocrine control of the seasonal occurrence of ovulation in rhesus monkeys housed outdoors. *Endocrinology, 114*, 1074-1081.

Whitehead, M.L. (1991). Slow variations of the amplitude and frequency of spontaneous emissions. *Hearing Research, 53*, 269-280.

Wiegratz, I., Kutschera, E., Lee, J. H., Moore, C., Mellinger, U., Winkler, U. H., & Kuhl, H. (2003). Effect of four different oral contraceptives on various sex hormones and serum-binding globulins. *Contraception, 67*, 25-32.

Zurek, P. (1981). Spontaneous narrowband acoustic signals emitted by human ears. *Journal of the Acoustical Society of America, 69(2),* 514-523.

Zwicker, E. (1990). On the frequency separation of simultaneously evoked otoacoustic emissions' consecutive extrema and its relation to cochlear traveling waves. *Journal of the Acoustical Society of America, 88(3),* 1639-1641.

CHAPTER 5

CLICK-EVOKED OTOACOUSTIC EMISSIONS ARE ASSOCIATED WITH CIRCULATING TESTOSTERONE LEVELS IN MEN

Adrian W.K. Snihur & Elizabeth Hampson

5.1 Introduction

Otoacoustic emissions (OAEs) are a natural by-product of the cochlea of the inner ear that are propagated into the external ear canal and can be detected using a sensitive microphone system (Davis, 1983; Kemp, 1978). OAEs can be produced either spontaneously (SOAEs), or in response to acoustic stimuli (clicked-evoked; CEOAEs), or else as a by-product of two simultaneously presented frequencies (distortion-product; DPOAEs). The production of OAEs is highly dependent on normal cochlear functioning, as shown by previous research supporting a relationship between hearing sensitivity and OAE production (McFadden & Mishra, 1993) and by evidence that OAEs are absent in regions of the frequency spectrum with sensorineural hearing deficits greater than 30 dB (Probst, Lonsbury-Martin, Martin, & Coats, 1987). OAEs tend to be differentially produced between the right and left ears, with the right ear producing more frequent SOAEs and stronger CEOAEs in response to acoustic stimuli than the left (Burns, Arehart, & Campbell, 1992; Talmadge, Long, Murphy, & Tubis; but see Collet, Gartner, Veuillet, Moulin, & Morgon, 1993 for an exception). Further, sexual dimorphism in OAE production has been reported in some studies, with females producing larger and more numerous SOAEs and stronger CEOAEs than males (Bilger, Matthies, Hammel, & DeMorest, 1990; Burns et al., 1992; Lamprecht-Dinnesen et al., 1998; Penner, Glotzbach, & Huang, 1993; Strickland, Burns, & Tubis, 1985). Because the sexual dimorphism is present in newborns and as early as 30 weeks of gestational age (Morlet et al., 1995), it has been proposed that prenatal androgen exposure in the male fetus *dampens* the capacity for OAE production by affecting the development of cochlear structures,

specifically the outer hair cells, which are integral to the production of OAEs (see McFadden, 2002, 2009).

Support for the prenatal androgen hypothesis has come from studies of specialized human and non-human populations. Masculinised OAE patterns have been found in female dizygotic twins who have male co-twins, presumably due to diffusion of androgens from the male fetus during prenatal development (McFadden, 1993a). Partial masculinization of SOAEs and CEOAEs has been observed in bisexual and homosexual females (i.e., SOAEs and CEOAEs that were intermediate to heterosexual females and heterosexual males) (McFadden & Pasanen, 1998, 1999). These data remain controversial, because a role for androgens in the establishment of sexual orientation in humans is uncertain. The lack of a sex difference in CEOAE response amplitude in male and female spotted hyenas, a species in which both chromosomal sexes are highly androgenised during prenatal development, offers further support for the masculinisation of OAEs by androgens prenatally (McFadden, Pasanen, Weldele, Glickman, & Place, 2006). The effect of prenatal testosterone on OAE production also has been demonstrated in sheep, as substantially weaker, or masculinised, CEOAEs are evident in female sheep exposed prenatally to testosterone propionate compared to female sheep exposed to a normal prenatal environment (McFadden, Pasanen, Valero, Roberts, & Lee, 2009).

In addition to the proposed effect of prenatal androgens, recent evidence has raised the prospect of a superimposed influence of adult steroid levels on OAE production. Though highly preliminary, a study in rhesus monkeys has offered novel support for an effect of seasonal changes in circulating testosterone concentrations on CEOAE production (McFadden, Pasanen, Raper, Lange, & Wallen, 2006). It was

reported that male rhesus monkeys produced CEOAEs of lower amplitude during the breeding season when testosterone levels are elevated compared to the non-breeding season when testosterone levels are low, a pattern that is consistent with the hypothesized dampening effects of prenatal androgens on OAE production. In humans, seasonal fluctuations in men's testosterone levels have been associated with variations in other anatomical or physiological traits such as waist-to-hip ratio (Svartberg, Jorde, Sundsfjord, Bonaa, & Barrett-Conner, 2003), but to our knowledge, an association between circulating testosterone and OAE production in men has not been investigated. The demonstration of seasonal variation in human OAEs, in association with testosterone levels, would provide convergent support for the rhesus monkey data and constitute further evidence in favour of an activational, not just organizational, effect of testosterone on the cochlear mechanisms that underlie the production of OAEs. If the auditory system is dynamically regulated by testosterone levels in men, it would significantly expand our theoretical understanding of the OAE model and its neuroendocrine basis.

Seasonal variation in testosterone production has been reported in humans, but patterns are not as clear and reliable as they are in non-human species. Typically, seasonal elevations in testosterone production have been observed in men during the autumn months (Dabbs, 1990; Moffat & Hampson, 2000; Svartberg et al., 2003; van Anders, Hampson, & Watson, 2006), although seasonal peaks have also been reported during winter (Perry, Miller, Patrick, & Morley, 2000; Svartberg, Jorde, Sundsfjord, Bonaa, & Barrett-Connor, 2003) and even in the summer months, specifically June (Merrigiola, Noonan, Paulsen, & Bremner, 1996).

The current study investigated the association between circulating testosterone and CEOAEs in adult men. Specifically, we examined whether expected seasonal differences in human testosterone production were associated with discernible differences in CEOAE response amplitude. Click-evoked responses at two different intensities were recorded, in both the right and left ears. Saliva was collected and analyzed to quantify the bioavailable testosterone levels present during the auditory recording. It was hypothesized that men would exhibit seasonal differences in testosterone production, which in turn would differentially affect their CEOAE response amplitudes. Specifically, based on the findings in rhesus macaques (McFadden et al., 2006), periods of elevated seasonal testosterone production were expected to result in lower, or more male-typical, CEOAE response amplitudes, and periods of reduced seasonal testosterone production were expected to result in greater, or less male-typical, CEOAE response amplitudes. It was also hypothesized that an overall negative correlation would be present between individual differences in testosterone levels and CEOAE production, offering further support for a postnatal dampening effect of testosterone on OAE production.

5.2 Materials & Methods

5.2.1 Participants

Male (n = 67) and female (n = 37; not using oral contraceptives or OCs) undergraduates between the ages of 17 and 25 were recruited from the University of Western Ontario. All were part of a larger study of sex differences in the auditory system. Women using hormonal contraceptives were excluded as oral contraceptives suppress bioavailable testosterone levels (Bancroft, Davidson, Warner, & Tyrer, 1980).

Participants initially underwent an inspection of the external ear canals using an otoscope (Welch Allyn MacroView 23830), to ensure there was no debris/blockage that might interfere with the auditory measures being taken. This was followed by standard clinical audiometric screening (GSI-17 pure-tone air conduction audiometer with Telephonics TDH-39P headphones) to verify normal hearing thresholds. Because previous research has shown a relationship between normal hearing and the capacity for OAE production (McFadden & Mishra, 1993; Probst et al., 1987), any participant exhibiting a hearing threshold greater than 25 dB at any of the tested frequency levels between 250 and 8000 Hz was excluded. A total of four participants failed to meet the audiometric hearing criterion and were excluded on this basis.

5.2.2 General Procedure

CEOAE data were collected, using an observational design, during all months of the year except two. To control for time of day, the testing was conducted in a darkened, quiet testing room between 1400h and 2000h, a period in the diurnal cycle during which changes in circulating testosterone levels are at a minimum (Rose, Kreuz, Holaday, Sulak, & Johnson, 1972).

5.2.3 *Saliva Collection and Radioimmunoassay*

On the test day, prior to CEOAE recording, participants provided a saliva sample for analysis of *current* levels of testosterone and cortisol. Cortisol was included as a control hormone which, like testosterone, is a steroid and exhibits a diurnal rhythm in its pattern of basal release similar to testosterone. No known relationship between cortisol

and OAE production exists. To optimize the quality of the saliva, participants were asked to refrain from eating, drinking (except water), smoking, and brushing their teeth for 1 hr prior to the beginning of the experiment. Saliva was collected into polystyrene test tubes using an inert sugarless gum (Trident™) to stimulate saliva flow. Gum can alter apparent steroid concentrations in some types of assays (van Anders, 2010) but is known to be inert in the techniques used here (see below). The tubes for saliva collection were pre-treated with sodium azide to prevent bacterial growth in the sample. The tubes were stored at -20 °C until analysis.

The saliva was assayed in a single batch by an experienced lab technician. After ether extraction, a ^{125}I Coat-a-Count kit for total testosterone (Diagnostic Products Corporation, Los Angeles, CA) was used to quantify testosterone concentrations. The Coat-A-Count method was modified for saliva according to an established protocol (Moffat & Hampson, 1996). Samples were analyzed in duplicate. The obtained sensitivity was 2.5 pg/mL and the intra-assay coefficient of variation was 4.4%. Testosterone was expressed in picograms per milliliter of saliva (pg/mL). For cortisol, the samples were analyzed directly, in duplicate, using a Coat-A-Count ^{125}I cortisol kit (Diagnostic Products Corporation, Los Angeles, CA) following the manufacturer's protocol for saliva. Cortisol was analyzed in two separate assay. The sensitivity of both of the assays was 0.69 nmol/L, and intra -assay coefficients of variation for the assays were 4% and 4.3%, respectively. Cortisol concentrations were expressed in nanomoles per liter (nmol/L).

5.2.4 COAE Recording

Participants were asked to sit in a reclined sofa chair for the duration of the CEOAE recording process. A foam ear-tip was placed onto an ER-2 earphone attached to a low-noise microphone system (Etymotic ER-10B) and inserted into the auditory canal. This microphone system consisted of two silicon tubes that protruded approximately 2 mm into the auditory canal. One tube functioned as a delivery conduit for click stimuli generated by the computer system, whereas the other tube served as an opening for the detection of evoked OAEs. In accordance with previously reported initializing effects on OAE production, participants were asked to relax and remain still for approximately 15 min in order to acclimatize to the testing environment (Whitehead, 1991). The ear tested first was counterbalanced within each sex.

Rarefaction DC pulses, approximately 100 ms in duration and sampled at a rate of 44.1 kHz, were produced by the built-in sound output of the laptop (MacIntosh G4 Powerbook OS 9.2) and served as the medium for generating click-evoked responses. Two separate computer generated click levels, whose maximal amplitudes corresponded to the peak amplitude of a continuous 1000 Hz tone presented at the desired intensity, were used for CEOAE screening (75 peSPL and 69 peSPL). Data were individually obtained and recorded for both click levels in both ears of each participant. Initially, presentation of acoustic clicks was calibrated at the desired intensity to obtain a nominal presentation of approximately 10 clicks per second that would be used during the final detection phase of the CEOAE screening process. Next, a 20 ms sample of the ambient noise within the auditory canal, in the absence of any acoustic clicks, was recorded to establish a baseline noise threshold and click-response artifact rejection level. During

detection of click-evoked responses (the final phase of CEOAE screening), elevated levels of noise in the auditory canal above the established rejection level resulted in a delay in the presentation of subsequent clicks until the ambient noise decreased to an acceptable level.

During the final phase of CEOAE screening, trains of acoustic clicks were presented through the low-noise microphone system and evoked-responses were recorded. A delay of 4 ms after the presentation of each individual click was applied to avoid any potential acoustical ringing in the ear canal, after which a 40 ms response was detected and recorded. The raw acoustic output was then passed first through a pre-amplification device (Etymotic ER10-72) and then on to a custom-built low-noise amplifier and filter system. As a result, the acoustic output was further amplified by 30 dB and high-pass filtered above 400 Hz. The raw output was then passed on to a spectrum analyzer and analog-to-digital converter (National Instruments, DAQ AI-16XE-50) before being stored in digital form on the laptop.

Following previously established procedures (McFadden, 1998), evoked responses to the first 250 presented acoustic clicks judged to be artifact-free were recorded, averaged, and analyzed off-line using custom-written software in LabVIEW (National Instruments, Austin, Texas). During off-line analysis, the first 2 ms of the averaged waveform was eliminated to further avoid any potential effects of ringing in the ear canal from the acoustic clicks. The subsequent 20.48 ms segment of the waveform was bandpass filtered at 1.0 to 8.0 kHz, and the root-mean-square output of the filter was converted to SPL. This was then recorded as the click-evoked response for the tested ear at the specific click level used.

5.3 Results

To confirm that a sexual dimorphism in CEOAE response amplitude was present, a mixed-effects ANOVA was performed, with ear tested and click level as repeated factors and sex as the between-subjects factor. Data for eight participants were unavailable due to technical difficulties during the CEOAE recording (e.g., elevated environmental background noise). As reported by other labs (e.g., McFadden, 1993a; McFadden & Pasanen, 1998), men in the present sample produced CEOAEs with significantly smaller response amplitudes compared to women [$F(1,89) = 12.60$, $p = 0.001$; see Figure 1]. In agreement with prior work, the ANOVA showed that the amplitude of the evoked response to the 75dB click level was significantly greater than the amplitude of the response to 69dB, $F(1,89) = 853.39$, $p < 0.001$. Two-way interactions between sex and click level [$F(1,89) = 12.37$, $p = 0.001$] and between ear tested and click level [$F(1,89) = 5.26$, $p = 0.024$] were found; the sex difference was slightly larger in magnitude at 69dB than at 75dB.

An overall sex difference in bioavailable testosterone was confirmed. As expected, men ($M = 79.8$ pg/mL, $SD = 22.9$) had significantly higher circulating testosterone than women [$M = 16.5$ pg/mL, $SD = 4.2$; $F(1,89) = 260.98$, $p < 0.001$]. The values for both sexes fell within the normal range for time of day (Dabbs et al., 1995). Cortisol concentrations did not exhibit a sex difference, $F(1,89) = 2.89$, $p = 0.93$.

One-way ANOVA was performed to test whether the expected seasonal variation in testosterone production was present among the male participants. One male outlier was removed from the analysis (testosterone greater than 3 SD above the mean). An observed seasonal pattern would allow for a parallel analysis to determine if seasonality

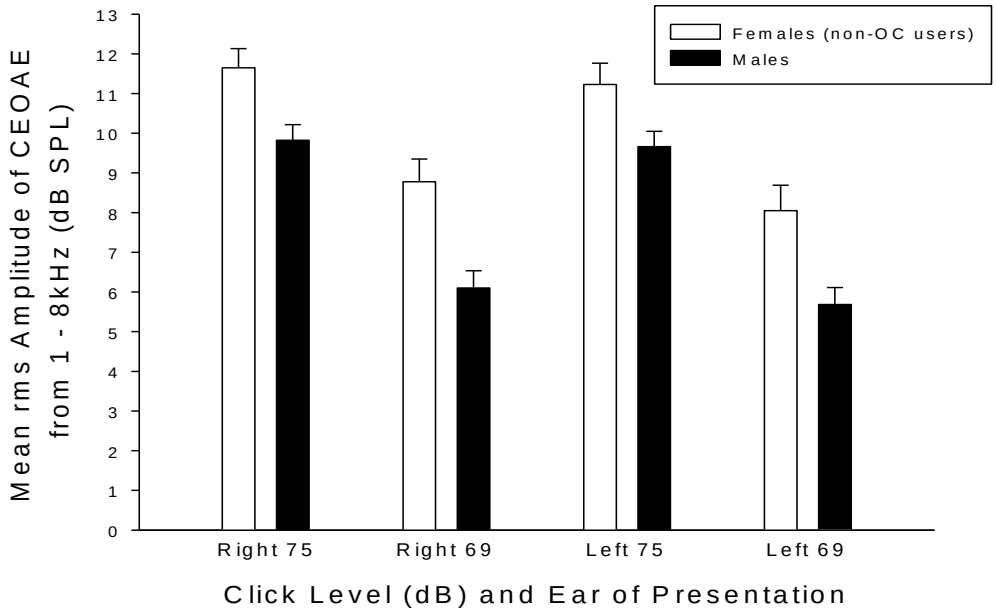

Figure 5.1. Mean CEOAE response amplitude for the right and left ears at two click levels (75dB and 69dB). Error bars represent SEM.

in testosterone was accompanied by seasonality in CEOAE production. Seasonality was evaluated by using the month during which participants were tested and creating four groups according to the timing of the solstices as follows: Fall (October to December), Winter (January to March), Spring (April to June), and Summer (July to September). Although mean testosterone levels appeared highest in winter and spring (Figure 2), seasonal differences in testosterone production were not statistically significant [$F(3,52) = 0.852$, $p = 0.47$] and individual variability in the level of circulating testosterone was substantial. Despite the lack of significance in the testosterone ANOVA, a mixed-effects ANOVA of the CEOAE data was carried out. Seasonal variation in CEOAE response amplitude was significant, $F(3,52) = 3.53$, $p = 0.021$, with amplitudes tending to be higher in summer and fall, the seasons having the lowest mean testosterone levels.

To help clarify whether the seasonal variation in CEOAE amplitude was associated with testosterone or with other seasonally dependent factors, a median-split of the 10 months was performed based exclusively on the mean testosterone concentration for each month, ignoring season. The median split yielded a "high testosterone" group composed of the months of March, April, May, July, and December, and a "low testosterone" group that included June, August, September, October, and November. A confirmatory t-test verified that the resulting two groups differed significantly in mean testosterone concentration, $F(1,54) = 8.17$, $p = 0.006$. Mixed-effects ANOVA, with ear tested and click level as repeated factors, then was performed to determine if CEOAE response amplitude differed between the months with high or low testosterone production. The main effect of month approached significance, $F(1,54) = 3.58$, $p = .064$, and there was a significant interaction between ear tested and month (high *vs.* low

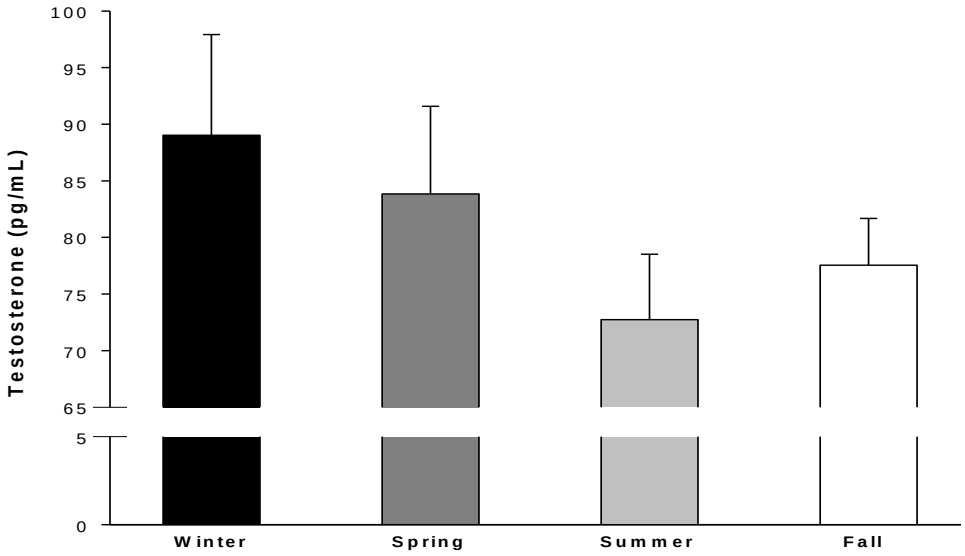

Figure 5.2. Salivary testosterone concentrations in men across four seasons. No significant differences were found. Errors bars represent SEM.

testosterone months), $F(1,54) = 6.02$, $p = .017$. As shown in Figure 3, for the right ear but not the left, CEOAE response amplitude was significantly lower in the high testosterone months. This was significant for both the Right 75dB ($p < .001$) and Right 69dB ($p < .01$) ear and click level combinations by post-hoc test. The effect size was $d = 1.04$ at 75dB and $d = 0.79$ at 69dB, based on Cohen's *d*-statistic (Cohen, 1977).

Given that circulating testosterone levels varied considerably from one male to another, bivariate correlations were performed to assess the association between individual differences in current levels of the hormones, testosterone and cortisol, and CEOAE response amplitude. If circulating testosterone levels influence CEOAE amplitude, we might expect to find a significant correlation between a male's testosterone level at the time of his CEOAE recording and the size of his evoked response amplitude. Table 1 shows the correlations found for all four CEOAE ear and click level combinations and current testosterone and cortisol levels. A significant negative correlation between testosterone concentration and CEOAE response amplitude was found for the right ear at both the 75dB ($r = -.308$, $p = .020$) and 69dB click levels ($r = -.305$, $p = .019$). Correlations in the left ear were smaller and did not achieve significance. There was no evidence of an association between CEOAE response amplitude and cortisol levels.

5.4 Discussion

Recent work has offered support for a seasonal influence of circulating testosterone on CEOAE production in male rhesus monkeys (McFadden et al., 2006). The present work is the first to investigate whether seasonal fluctuations in testosterone

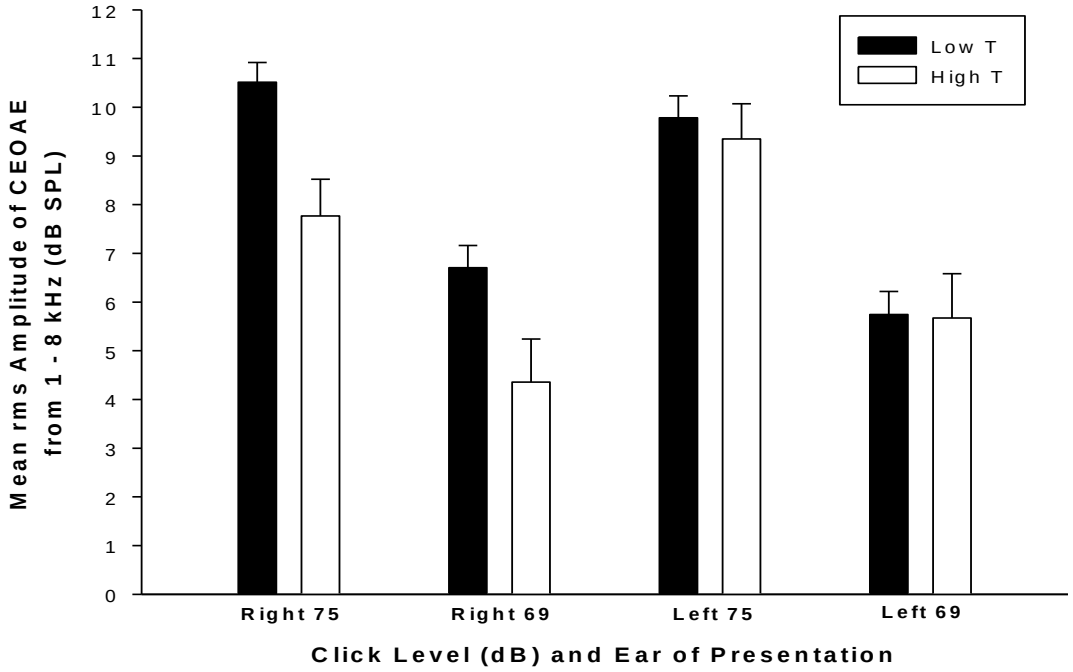

Figure 5.3. Average CEOAE response amplitude for right and left ears at two click levels (75dB and 69dB) during high and low testosterone months. Error bars represent SEM.

Table 5.1. Correlations between testosterone (or cortisol) levels and CEOAE response amplitudes

	CEOAE response amplitude			
	Right 75dB	Right 69dB	Left 75dB	Left 69dB
Testosterone (pg/mL)	**-.308** *.020*	**-.305** *.019*	**-.233** *.068*	**-.204** *.112*
Cortisol (nmol/L)	**-.061** *.653*	**-.015** *.909*	**-.026** *.842*	**.095** *.461*

Bold values represent Pearson (r) correlations; *italicized* values represent probabilities (p-values).

can influence CEOAE response amplitudes in men. Both studies address the possibility of *activational* influences of steroids on OAE production. It generally has been assumed that sex differences in SOAEs and CEOAEs arise from the organizational effects of androgens prenatally, thus studies of adult hormones, and especially testosterone, are very limited. Unexpectedly, the anticipated seasonal differences in testosterone production were not found in the current study. Nevertheless, a significant difference in CEOAE response amplitude was identified when comparing months of the year characterized by high vs. low testosterone production. The CEOAE response amplitudes of men tested in high testosterone months were lower than those tested in low testosterone months, though only for the right ear. A significant negative correlation between circulating testosterone levels and CEOAE response amplitude also was observed on an individual basis. Overall, these results offer novel support for an influence of *current* testosterone on CEOAEs in men, in a manner consistent with the dampening effects of testosterone on OAE production proposed to occur during prenatal development.

With respect to the observed sexual dimorphism in OAE production, it has been proposed that exposure to elevated androgens prenatally during the critical window for differentiation in the male fetus *masculinises* the auditory system, including cochlear structures integral to OAE production, resulting in diminished OAEs in males. Support for a prenatal mechanism of action has been shown in studies demonstrating that a sex difference in the number and strength of OAEs can be identified in newborn infants or as early as 30 weeks of gestational age (Burns et al.., 1992; Morlet et al., 1995). While early expression of a sex difference could alternatively be explained by cell-autonomous gene effects (Arnold, 2004), support for an androgen-dependent mechanism has been derived

from studies of specialized human and non-human populations (McFadden, 1993a; McFadden & Pasanen, 1998, 1999; McFadden et al., 2006; McFadden et al., 2009). In addition to providing confirmatory evidence for a sex difference in CEOAE response amplitude, the current study offers novel support in humans for further down-regulation of OAE production in men due to elevations in *postnatal* testosterone in a manner consistent with the prenatal androgen hypothesis. A negative association was found in the present study between testosterone and CEOAE response amplitude, such that men with elevated levels of circulating testosterone during the CEOAE recording produced CEOAEs with lower, or diminished, response amplitude. The direction of the observed relationship is consistent with the fact that elevations in prenatal testosterone have been shown to have dampening effects on OAE production, suggesting that similar effects may exist in the postnatal environment as well.

In order to provide evidence for the specificity of the relationship between circulating testosterone and CEOAEs, current levels of cortisol were also analyzed in the saliva samples of men in the present study and correlations with all four CEOAE measures examined. Cortisol and testosterone exhibit very similar circadian rhythms in humans (see Nelson, 2005; Rose et al., 1972), but cortisol has no reported or hypothesized influence on OAE production. As expected, no significant association between circulating cortisol levels and CEOAE response amplitude was found, for either ear or click level. The lack of any apparent association substantiates the validity of the observed negative relationship between circulating testosterone and CEOAEs and demonstrates that an association is not evident for a control steroid.

The present study did not find differences in seasonal production of testosterone in men, as has been reported previously in a number of studies (e.g., Dabbs, 1990; Perry et al., 2000; van Anders et al., 2006). The lack of a significant seasonal change in testosterone was somewhat surprising, as previous research has reported seasonal variation, albeit inconsistently. It should be acknowledged that seasonal differences are not always found (e.g. Brambilla, O'Donnell, Matsumoto, & McKinlay, 2007a; Svartberg & Barrett-Connor, 2004; Wisniewski & Nelson, 2000). In the current study, the smaller sample size obtained during certain months due to sampling constraints, combined with large individual differences in men's testosterone levels, may have reduced the probability of obtaining a seasonal pattern. Inconsistency across studies in the observation of a seasonal effect may reflect the lack of a distinct breeding period in humans, geographical variation (e.g., Ellison et al., 2002), the multiple dietary, health, and lifestyle factors that can affect testosterone production (e.g., Svartberg & Barrett-Connor, 2004), and the substantial individual variation that exists in average testosterone levels in humans (Brambilla, O'Donnell, Matsumoto, & McKinlay, 2007b).

Despite the absence of significant seasonal variation in testosterone levels, the current study nonetheless found a seasonal difference in CEOAE response amplitude, which became even sharper and clearer when season was disregarded in favour of classifying months as 'high' or 'low' based on monthly average testosterone. The resulting high testosterone group included months spanning all four seasons, and a similar mixture of seasons was evident in the low testosterone group. The fact that the statistical association between testosterone and CEOAE response amplitude was strengthened and clarified by the re-classification supports the likelihood that testosterone is the active

agent that underlies the association, rather than some other variable that ordinarily covaries with season, such as temperature or photoperiod. Thus, the lack of the expected seasonal pattern in testosterone levels in the present work paradoxically may help to build a case for testosterone as the operative variable responsible for the changes in CEOAE amplitudes.

It was initially hypothesized, based on recent research in rhesus monkeys (McFadden et al., 2006), that seasonal differences in testosterone levels in men would result in differential production of CEOAEs. In male rhesus monkeys, diminished CEOAE response amplitude was observed during the breeding season when testosterone production is elevated, compared to the non-breeding season when circulating testosterone is appreciably reduced, with a calculated effect size of 0.79. Although the current study failed to find the anticipated seasonal pattern in testosterone production, a median-split comparing CEOAE response amplitudes in months with high versus low testosterone production yielded the hypothesized differences in CEOAEs. It was found that the group of men with high circulating testosterone produced CEOAEs with smaller response amplitudes compared to the group of men with lower circulating testosterone, a result directly analogous to the fluctuations in CEOAEs observed between high and low testosterone seasons in male rhesus monkeys. Further, the effect size for this difference at the comparable intensity level (75dB) in the right ear is 1.03, suggesting that the magnitude of the observed difference in humans is greater than that observed in rhesus monkeys. In both the current study and McFadden et al. (2006), elevations in *current* levels of testosterone served to further dampen, or masculinise, CEOAE response amplitude. This comparable effect of circulating testosterone on CEOAEs in men in the

current study to that previously shown in rhesus monkeys offers further evidence for a postnatal influence of testosterone on OAE production, whereby natural elevations and reductions in the *circulating* testosterone production result in transient decreases and increases in CEOAE response amplitude, respectively.

The current study also found significant correlations between circulating testosterone levels and CEOAE response amplitude on an individual basis. Negative correlations were found in the right ear at both the 75dB and 69dB click levels only, although the correlations in the left ear were also in the anticipated negative direction. This result, coupled with the differential production of CEOAEs observed in the median-split, enhances the probability that testosterone is the active agent influencing CEOAEs in adulthood. Individual variations in circulating testosterone were shown to influence CEOAE response amplitude, although interestingly for both the group and individual analyses, significant results were only obtained for the right ear. Previous research has offered support for greater CEOAE response amplitudes in the right ear of adults (McFadden, Loehlin, & Pasanen, 1996), and mechanisms mediating a right ear advantage in OAE production have been proposed (McFadden, 1993b). Thus, the presence of significant differences in the right ear only in the current study suggests that the two ears are not equally susceptible to testosterone's effects, and that the mechanisms regulating these effects may differ slightly between the ears.

For circulating testosterone to have an influence on CEOAE response amplitude in men, it needs to act on appropriate receptors in the auditory structures integral to OAE production (i.e., cochlea, outer hair cells). Although, to date, androgen receptors have not been found in the human cochlea, evidence for androgen receptors in species other than

humans exists. Maruska and Fernald (2010) found expression of androgen receptor mRNA in the main peripheral hearing organ of the African cichlid fish (*Astatotilapia burtoni*). An abundance of androgen receptor mRNA also was found in the inner ear of the teleost fish (Forlano, Marchaterre, Deitcher, & Bass, 2010). Thus, the presence of androgen receptors in the peripheral auditory system of these species and other vertebrates suggests that circulating androgens may play a role in hearing. If similar receptors are present in the human cochlea, then a viable mechanism exists whereby fluctuations in circulating testosterone levels in adulthood can influence OAE production. If androgen receptors are not localized in the human cochlea, then an alternative mechanism may mediate the effects observed in the current study. Noirot et al. (2009) used immuncytochemistry to localize ER-α (an estrogen receptor subtype) and aromatase, the enzyme responsible for converting testosterone into estradiol, in the hair cells of both male and female zebra finches. Previously, estrogen receptor α and estrogen receptor β expression has been demonstrated in various parts of the adult human cochlea, but only in females (Stenberg, Wang, Fish, Schrott-Fischer, Sahlin, & Hultcrantz, 2001). Thus, if estrogen, and not androgen, receptors are present in the adult male cochlea, it is plausible that conversion of testosterone into estradiol (via aromatase) and binding of estradiol to appropriate estrogen receptors may mediate the observed activational effect of circulating male sex steroids on OAEs.

The present results offer convergent support, from another species, for the possibility of an effect of postnatal testosterone on OAE production. It was found that elevations in circulating testosterone in men were associated with dampened, or more male-typical, CEOAE response amplitudes, whereas reductions in circulating testosterone

were associated with greater, or less male-typical, CEOAE response amplitudes. More research is needed to corroborate this effect. To date, exploration of postnatal hormonal influences on OAE production in humans has been exceedingly limited, but such effects may have both theoretical and applied implications given that OAEs are used in clinical auditory assessment. Ideally future work can employ a repeated measures design with active manipulation of circulating testosterone to substantiate an effect on OAE production. While it is not ethically permissible to manipulate testosterone in humans for research purposes, such a design may be possible where testosterone is used medically.

5.5 References

Arnold, A.P. (2004). Sex chromosomes and brain gender. *Nature Reviews Neuroscience, 5(9),* 701-708.

Bancroft, J., Davidson, D.W., Warner, P., & Tyrer, G. (1980). Androgens and sexual behaviours in women using oral contraceptives. *Clinical Endocrinology (Oxford), 12(4),* 327-340.

Bilger, R., Matthies, M., Hammel, D., & DeMorest, M. (1990). Genetic implications of gender differences in the prevalence of spontaneous otoacoustic emissions. *Journal of Speech and Hearing Research, 33,* 418-432.

Brambilla, D.J., O'Donnell, A.B., Matsumoto, A.M., & McKinlay, J.B. (2007a). Lack of seasonal variation in serum sex hormone levels in middle-aged to older men in the Boston area. *Journal of Clinical Endocrinology and Metabolism, 92(11),* 4224-4229.

Brambilla, D.J., O'Donnell, A.B., Matsumoto, A.M., & McKinlay, J.B. (2007b). Intraindividual variation in levels of serum testosterone and other reproductive and adrenal hormones in men. *Clinical Endocrinology (Oxford), 67(6),* 853-862.

Burns, E.M., Arehart, K., & Campbell, S. (1992). Prevalence of spontaneous otoacoustic emissions in neonates. *Journal of the Acoustical Society of America, 91(3),* 1571-1575.

Cohen, J. (1977). *Statistical power analysis for the behavioral sciences* (revised edition). New York: Academic Press.

Collet, L., Gartner, M., Veuillet, E., Moulin, A., & Morgon, A. (1993). Evoked and

spontaneous otoacoustic emissions: A comparison of neonates and adults. *Brain Development, 15*, 249-252.

Dabbs, J.M.Jr. (1990). Age and seasonal variation in serum testosterone concentration among men. *Chronobiology International, 7(3)*, 245-249.

Dabbs, J. M., Campbell, B. C., Gladue, B. A., Midgley, A. R., Navarro, M. A., Read, G. F., et al. (1995). Reliability of salivary testosterone measurements: A multicenter evaluation. *Clinical Chemistry, 41*, 1581-1584.

Davis, H. (1983). An active process in cochlear mechanics. *Hearing Research, 9(1)*, 79-90.

Ellison, P.T., Bribescas, R.G., Bentley, G.R., Campbell, B.C., Lipson, S.F., Panter-Brick, C., et al. (2002). Population variation in age-related decline in male salivary testosterone. *Human Reproduction, 17(12)*, 3251-3253.

Forlano, P.M., Marchaterre, M. Deitcher, D.L., & Bass, A.H. (2010). Distribution of androgen receptor mRNA expression in vocal, auditory, and neuroendocrine circuits in teleost fish. *Journal of Comparable Neurology, 518(4)*, 439-512.

Kemp, D. (1978). Stimulated acoustic emissions from within the human auditory system. *Journal of the Acoustical Society of America, 64(5)*, 1386-1391.

Lamprecht-Dinnesen, A., Pohl, M., Hartmann, S., Heinecke, A., Ahrens, S., Muller, E., et al. (1998). Effects of age, gender, and ear side on SOAE parameters in infancy and childhood. *Audiology and Neuro Otology, 3*, 386-401.

Maruska, K.P., & Fernald, R.D. (2010). Steroid receptor expression in the fish inner ear varies with sex, social status, and reproductive state. *BMC Neuroscience, 30*, 11-58.

McFadden, D. (1993a). A masculinising effect on the auditory systems of human females having male co-twins. *Proceedings of the National Academy of Sciences, 90,* 11900-11904.

McFadden, D. (1993b). A speculation about the parallel ear asymmetries and sex differences in hearing sensitivity and otoacoustic emissions. *Hearing Research, 68,* 143-151.

McFadden, D. (2002). Masculinization effects in the auditory system. *Archives of Sexual Behavior, 31(1),* 99-111.

McFadden, D. (2009). Masculinization of the mammalian cochlea. *Hearing Research, 252(1-2),* 37-48.

McFadden, D., & Mishra, R. (1993). On the relation between hearing sensitivity and otoacoustic emissions. *Hearing Research, 71,* 208-213.

McFadden, D., & Pasanen, E. (1998). Comparison of the auditory systems of heterosexuals and homosexuals: Click-evoked otoacoustic emissions. *Proceedings of the National Academy of Science, 95,* 2709-2713.

McFadden, D., & Pasanen, E. (1999). Spontaneous otoacoustic emissions in heterosexuals, homosexuals, and bisexuals. *Journal of the Acoustical Society of America, 105(4),* 2403-2413.

McFadden, D., Loehlin, J.C., & Pasanen. E.G. (1996). Additional findings on heritability and prenatal masculinization of cochlear mechanics: click-evoked otoacoustic emissions. *Hearing Research, 97(1-2),* 102-119.

McFadden, D., Pasanen, E. G., Raper, J., Lange, H. S., & Wallen, K. (2006). Sex

differences in otoacoustic emissions measured in rhesus monkeys (Macaca mulatta). *Hormones and Behavior, 50,* 274-284.

McFadden, D., Pasanen, E. G., Valero, M. D., Roberts, E. K., & Lee, T. M. (2009). Effect of prenatal androgens on click-evoked otoacoustic emissions in male and female sheep (Ovis aries). *Hormones and Behavior*, *55,* 98-105.

McFadden, D., Pasanen, E., Weldele, M., Glickman, S., & Place, N. (2006). Masculinized otoacoustic emissions in female spotted hyenas (Crocuta crocuta). *Hormones and Behaviour, 50(2),* 285-292.

Merrigiola, M.C., Noonan, E.A., Paulsen, C.A., & Bremner, W.J. (1996). Annual patterns of luteinizing hormone, follicle stimulating hormone, testosterone and inhibin in normal men. *Human Reproduction, 11(1),* 248-252.

Moffat, S.D. & Hampson, E. (1996). Salivary testosterone levels in left- and right-handed adults. *Neuropsychologia, 34(3),* 225-233.

Moffat, S.D., & Hampson, E. (2000). Salivary testosterone condcentration in left-handers: an association with cerebral language lateralization? *Neuropsychology, 14(1),* 71-81.

Morlet, T., Lapillonne, A., Ferber, C., Duclaux, R., Sann, L., Putet, G., et al. (1995). Spontaneous otoacoustic emissions in preterm neonates: prevalence and gender effects. *Hearing Research, 90(1-2),* 44-54.

Nelson, R.J. (2005). *An Introduction to Behavioral Endocrinology (3rd Edition).* Sunderland, MA: Sinauer Associates, Inc.

Noirot, I.C., Adler, H.J., Cornil, C.A., Harada, N., Dooling, R.J., Balthazart, J., et al.

(2009). Presence of aromatase and estrogen receptor alpha in the inner ear of zebra finches. *Hearing Research, 252(1-2),* 49-55.

Penner, M.J., Glotzbach, L., & Huang, T. (1993). Spontaneous otoacoustic emissions: measurement and data. *Hearing Research, 68(2),* 229-237.

Perry, H.M. 3rd, Miller, D.K., Patrick, P., & Morley, J.E. (2000). Testosterone and leptin in older African-American men: relationship to age, strength, function, and season. *Metabolism, 49(8),* 1085-1091.

Probst, R., Lonsbury-Martin, B., Martin, G., & Coats, A. (1987). Otoacoustic emissions in ears with hearing loss. *American Journal of Otolaryngology, 8(2),* 73-81.

Rose, R.M., Kreuz, L.E., Holaday, J.W., Sulak, K.J., & Johnson, C.E. (1972). Diurnal variation of plasma testosterone and cortisol. *Journal of Endocrinology, 54(1),* 177-178.

Stenberg, A. E., Wang, H., Fish III, J. Schrott-Fischer, A., Sahlin, L., & Hultcrantz, M. (2001). Estrogen receptors in the normal adult and developing human inner ear and in Turner's syndrome. *Hearing Research, 157,* 87-92.

Strickland, E., Burns, E., & Tubis, A. (1985). Incidence of spontaneous otoacoustic emissions in children and infants. *Journal of the Acoustical Society of America, 78(3),* 931-935.

Svartberg, J., & Barrett-Connor, E. (2004). Could seasonal variation in testosterone levels in men be related to sleep? *Aging Male, 7(3),* 205-210.

Svartberg, J., Jorde, R., Sundsfjord, J., Bonaa, K.H., & Barrett-Connor, E. (2003). Seasonal variation of testosterone and waist to hip ratio in men: the Tromso study. *Journal of Clinical Endocrinology and Metabolism, 88(7),* 3099-3104.

Talmadge, C.L., Long, G.R., Murphy, W.J., & Tubis, A. (1993). New off-line method for detecting spontaneous otoacoustic emissions in human subjects. *Hearing Research, 71,* 170-182.

van Anders, S.M. (2010). Chewing gum has large effects on salivary testosterone, estradiol, and secretory immunoglobulin A assays in women and men *Psychoneuroendocrinology, 35(2),* 305-309.

van Anders, S. M., Vernon P. A., & Wilbur, C. J. (2006). Finger-length ratios show evidence of prenatal hormone-transfer between opposite-sex twins. *Hormones and Behavior, 49(3),* 315-319.

Whitehead, M.L. (1991). Slow variations of the amplitude and frequency of spontaneous emissions. *Hearing Research, 53,* 269-280.

Wisniewski, A.B., & Nelson, R.J. (2000). Seasonal variation in human functional cerebral lateralization and free testosterone concentrations. *Brain and Cognition*, *43(1-3),* 429-438.

CHAPTER 6

GENERAL DISCUSSION

6.1 Discussion

The data presented in this dissertation significantly expand our current understanding of otoacoustic emissions (OAEs) and the endocrine mechanisms that are involved in and influence their production. A comprehensive investigation validated female superiority in several different measures of spontaneous OAE (SOAE) and click-evoked OAE (CEOAE) production in Study 1. Unlike many previous studies, the female superiority was observed in a population of normally-hearing young adults. Differential production of emissions between the ears was observed for the number of SOAEs produced, but an attempt to support the recent suggestion of an influence of handedness on the magnitude of the asymmetry in OAE production between the right and left ears yielded inconclusive results. The objective of Study 2 was to further test the hypothesis of an organizational influence of prenatal androgens on the sexual dimorphism in OAE production. A statistical correlation between a biological marker of individual variations in prenatal androgen exposure, the 2D:4D digit-ratio, and OAEs was not found, although fundamental differences in the timing of prenatal development of these two traits as well as other influences do not exclude the possibility of a prenatal contribution to OAE production in humans. A highlight of the thesis was the final two experiments, which provide new evidence that circulating levels of adult sex steroids, not merely organizational influences, may be capable of modulating OAE production in humans. Specifically, in women, oral contraceptive (OC) use was found to result in dampened SOAE and CEOAE production compared to non-users and in young men, an association between circulating testosterone levels and CEOAE response amplitude was found.

Combined, these two results offer some of the first support for an influence of adult sex steroids on OAE production in humans. In turn, this suggests that the observed sex difference may be a dynamic function of both organizational and activational influences of sex steroids.

The sex difference in SOAE and CEOAE production previously observed in neonates and children (Burns et al., 1992; Strickland et al., 1985), as well as certain broad adult populations (see Bilger et al., 1990) was demonstrated in a population of non-hearing-impaired young adults in the current thesis. This not only corroborated the basic sex difference in a distinct population that has been previously ignored, but also validated the use of a new technique in our laboratory and a platform on which to further investigate the endocrine influences on OAE production. Sex differences in a number of distinct OAE parameters were confirmed, including differences in the numbers of SOAEs produced, overall SOAE power, and the response amplitudes of the CEOAEs elicited in response to deliberate acoustical stimulation. The sex difference in number of SOAEs was detected in the absence of a significant difference in SOAE prevalence in the current study, thus confirming that the sex difference in SOAE number reflects a genuine sexual dimorphism in OAE production, and not an artefact of a sex difference in prevalence. This has not always been clear from previous literature. This is further supported by the observed sex difference in SOAE power and CEOAE response amplitudes, two OAE variables that are independent of differences in prevalence rates.

This dissertation was unable to offer support for a link between individual variations in 2D:4D digit-ratios and OAE production. A significant correlation would have provided support from a novel paradigm for an organizational effect of early

androgen exposure. Currently the idea that early androgens can alter the capacity for OAE production is supported mostly by work in other species. Data from human work has been difficult to obtain and has been limited by the inability to manipulate androgen levels in humans in order to investigate the resulting effects on OAEs. Recently, it has been suggested that the 2D:4D digit-ratio may be a valid and easily accessible proxy for prenatal androgen exposure at the end of the 1st trimester (Breedlove, 2010), which falls near the beginning of the period of active testosterone secretion in the male fetus (Forest, de Peretti, & Bertrand, 1976). Cochlear structures, however, continue to develop and mature until 30 weeks of gestation and beyond (Pujol & Lavigne-Rebillard, 1995), and it is reasonable to speculate that the hypothesized prenatal influence of androgens on the auditory structures responsible for OAEs occurs *later* in gestation. If correct, then the absence of a detectable relationship between individual variations in 2D:4D digit-ratios and OAE production in the current thesis may not be surprising, particularly if androgen levels do not remain stable and constant over the entire developmental period. In spite of the lack of significant results in the present thesis, an organizational component influencing this auditory trait cannot be ruled out.

Evidence for an effect of circulating adult sex steroids on OAE production was provided in this dissertation. However, the existence and magnitude of these effects was neither controlled for nor anticipated while conducting Study 2. These effects could not be anticipated because, to date, the research literature has been centered almost entirely on the possibility of an organizational effect of prenatal androgens. It is plausible that the relationship between 2D:4D digit-ratios and OAEs was masked in Study 2 because of the influence of circulating hormones on OAE production in adulthood; no evidence for an

effect of circulating sex steroids on 2D:4D exists. As a result, a study utilizing a pre-pubertal population to investigate the hypothesized relationship between digit-ratios and OAEs, thereby eliminating the effects of circulating adult sex steroids, would be of great benefit in testing the hypothesized organizational influence on the auditory structures responsible for OAEs in humans.

Future research investigating the hypothesized influence of prenatal androgens on OAE production could alternatively focus on specialized populations exposed to abnormal prenatal hormonal environments. Examples of such endocrine disorders include congenital adrenal hyperplasia (CAH), a condition characterized by excessive prenatal production of adrenal androgens, and complete androgen insensitivity syndrome (CAIS), a condition characterized by XY chromosomes but absent or dysfunctional androgen receptors. If exposure to androgens influences OAE production, then it would be hypothesized that females with CAH would exhibit male-typical OAEs (due to exposure to elevated androgens prenatally) and genetic males with CAIS would exhibit female-typical OAEs (because prenatal androgens are unable to bind to the required receptors in order to exert their physiological influences). Similar studies to those proposed have provided support for a prenatal hormonal influence on other traits (e.g., Brown et al., 2002; Ciumas et al, 2009; Berenbaum et al., 2009; Hampson, Rovet, & Altman, 1998), and would provide the most direct evidence in humans that OAEs are in fact influenced by prenatal androgens.

The current thesis is the first to provide compelling evidence supporting an activational influence of sex steroids on OAE production in humans. In Study 3 and Study 4, both men and women showed differences in OAE production that were

associated with differences in their current levels of testosterone. However, together these data also revealed a paradox. In men, elevations in circulating testosterone levels were associated with diminished CEOAE response amplitudes, a result consistent with the hypothesized dampening effects of prenatal testosterone on OAE production (McFadden, 1998, 2002). In women, those who had higher levels of current testosterone (i.e., normally-cycling women) had *greater* SOAE and CEOAE production than women with lower levels (i.e., those using OCs). Because it is highly unlikely that testosterone exerts different effects on OAEs within each sex (i.e., diminishing OAE production in men yet enhancing OAE production in women), it is proposed that another sex steroid is involved in modulating OAE production in adult women.

A negative association between current levels of circulating testosterone and CEOAE response amplitude was found in adult men, suggesting that testosterone may exert a similar dampening effect on OAE production in adulthood as has been proposed to occur prenatally (see McFadden, 2002; 2009). Although, to our knowledge, comparable evidence does not exist in humans to date, androgen receptor mRNA has been localized in various inner ear structures of other species (Forlano et al., 2010; Maruska & Fernald, 2010). If androgen receptors are similarly present in the human cochlea, then a mechanism exists whereby testosterone can exert its effects on the auditory structures responsible for OAE production in adulthood. In light of the novel association found in the present work between testosterone and OAEs in adult men, future research should focus first and foremost on confirming a causal relationship. For example, by examining the effects of actively manipulating testosterone levels in men, through injections or medication, on OAE production, a causal relationship between testosterone and OAEs in

adults could be more firmly established. Further studies attempting to localize androgen receptor mRNA in the human cochlea would shed light on the proposed hormonal mechanism involved in differential OAE production in adulthood.

In women, on the other hand, the evidence reported in this dissertation suggests that a hormone other than testosterone, possibly estradiol, may be involved in mediating the observed differences in OAE production between OC and non-OC users. Women using OCs were found to produce more male-typical SOAEs and CEOAEs, despite possessing levels of circulating testosterone that were suppressed to nearly undetectable levels, whereas women not currently using OCs produced female-typical SOAEs and CEOAEs. This result contradicts the observed relationship between testosterone and OAEs found in men in the current thesis as well as other studies (e.g., McFadden et al., 2006). Because OC use in women also reliably suppresses estradiol (as well as progesterone) levels, and because evidence for both a beneficial effect of estradiol on hearing and a mechanism through which it can exert its actions exists, it is reasonable to propose that estradiol levels in women may influence OAE production. Estradiol has been shown to have a positive effect on hearing in mice (Meltser et al., 2008; Simonoska et al., 2009), as well as an unconfirmed influence on OAE production (e.g., Bell, 1992; McFadden et al., 1998). Estrogen receptor mRNA has been localized in the mouse, rat, and adult human cochlea (Stenberg et al., 1999, 2001), supporting a direct mechanism whereby estradiol could influence OAE production. Thus, it is proposed that a reduction in the amount of circulating estradiol available to interact with estrogen receptors in the cochlea is responsible for diminished production of OAEs in women using OCs, and that estradiol, not testosterone, is responsible for influencing OAE production in adult women.

Future research examining the effects of estradiol on OAE production could include a systematic and in-depth study into the differential production of OAEs across the menstrual cycle (including precise hormonal quantification), as well as an investigation into the effect that manipulation of estradiol levels has on OAE production (e.g., in postmenopausal women undergoing estrogen replacement therapy).

As the future unfolds, it is imperative that researchers now consider the effects that circulating sex steroids have on OAE production when further examining this auditory trait in humans and, potentially, other species. Despite the presence of hormonal influences on OAE production, OAE screening in newborn babies should remain a valid method of assessing inner ear integrity. Although much research is still required, the current thesis has greatly enhanced our understanding of endocrine influences on OAE production in humans, and in doing so, has provided a further example of the dynamic effects that sex steroids can have on various cognitive, behavioural, and physical traits.

6.2 References

Bell, A. (1992). Circadian and menstrual rhythms in frequency variations of spontaneous otoacoustic emissions from human ears. *Hearing Research, 51(1)*, 91-100.

Berenbaum, S. A., Bryk, K. A., Nowak, N., Quigley, C. A., & Moffat, S. (2009). Fingers as a marker of prenatal androgen exposure. *Endocrinology, 150(11)*, 5119-5124.

Bilger, R. C., Matthies, M. L., Hammel, D. R., & DeMorest, M. E. (1990). Genetic implications of gender differences in the prevalence of spontaneous otoacoustic emissions. *Journal of Speech and Hearing Research, 33*, 418-432.

Breedlove, S.M. (2010). Minireview: organizational hypothesis: instances of the fingerpost. *Endocrinology, 151(9)*, 4116-4122.

Brown, W. M., Hines, M., Fane, B. A., & Breedlove, S. M. (2002). Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. *Hormones and Behavior*, *42(4)*, 380-386.

Burns, E. M., Arehart, K. H., & Campbell, S. L. (1992). Prevalence of spontaneous otoacoustic emissions in neonates. *Journal of the Acoustical Society of America, 91(3)*, 1571-1575.

Ciumas, C., Linden Hirschberg, A., & Savic, I. (2009). High fetal testosterone and sexually dimorphic cerebral networks in females. *Cerebral Cortex, 19(5)*, 1167-1174.

Forest, M. G., de Peretti, E., & Bertrand, J. (1976). Hypothalamic-pituitary-gonadal relationships in man, from birth to puberty. *Clinical Endocrinology, 5*, 551-569.

Forlano, P.M., Marchaterre, M. Deitcher, D.L., & Bass, A.H. (2010). Distribution of

androgen receptor mRNA expression in vocal, auditory, and neuroendocrine circuits in teleost fish. *Journal of Comparable Neurology, 518(4),* 439-512.

Hampson, E., Rovet, J. F., & Altmann, D. (1998). Spatial reasoning in children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Developmental Neuropsychology, 14,* 299-320.

Maruska, K.P., & Fernald, R.D. (2010). Steroid receptor expression in the fish inner ear varies with sex, social status, and reproductive state. *BMC Neuroscience, 30,* 11-58.

McFadden, D. (1998). Sex differences in the auditory system. *Developmental Neuropsychology, 14(2/3),* 261-298.

McFadden, D. (2002). Masculinization effects in the auditory system. *Archives of Sexual Behavior, 31(1),* 99-111.

McFadden, D. (2009). Masculinization of the mammalian cochlea. *Hearing Research, 252(1-2),* 37-48.

McFadden, D., Pasanen, E.G., & Callaway, N.L. (1998). Changes in otoacoustic emissions in a transsexual male during treatment with estrogen. *Journal of the Acoustical Society of America, 104(3 Pt 1),* 1555-1558.

McFadden, D., Pasanen, E. G., Raper, J., Lange, H. S., & Wallen, K. (2006). Sex differences in otoacoustic emissions measured in rhesus monkeys (Macaca mulatta). *Hormones and Behavior, 50,* 274-284.

Meltser, I., Tahera, Y., Simpson, E., Hultcrantz, M., Charitidi, K., Gustafsson, J.A., et al. (2008). Estrogen receptor beta protects against acoustic trauma in mice. *Journal of Clinical Investigation, 118(4),* 1563-1570.

Pujol, R., & Lavigne-Rebillard, M. (1995). Sensory and neural structures in the developing human cochlea. *International Journal of Pediatric Otorhinolaryngolo*gy, *32*, S177-S182.

Simonoska, R., Stenberg, A.E., Duan, M., Yakimchuk, K., Fridberger, A., Sahlin, L., et al. (2009). Inner ear pathology and loss of hearing in estrogen receptor-beta deficient mice. *Journal of Endocrinology, 201(3),* 397-406.

Stenberg, A. E., Wang, H., Fish III, J. Schrott-Fischer, A., Sahlin, L., & Hultcrantz, M. (2001). Estrogen receptors in the normal adult and developing human inner ear and in Turner's syndrome. *Hearing Research*, *157*, 87-92.

Stenberg, A.E., Wang, H., Sahlin, L., & Hultcrantz, M. (1999). Mapping of estrogen receptors alpha and beta in the inner ear of the mouse and rat. *Hearing Research*, *136(1-2)*, 29-34.

Strickland, E., Burns, E., & Tubis, A. (1985). Incidence of spontaneous otoacoustic emissions in children and infants. *Journal of the Acoustical Society of America, 78(3)*, 931-935.

Appendix A

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Use of Human Subjects - Ethics Approval Notice

Review Number	**07 02 09**	**Approval Date**	**07 02 19**
Principal Investigator	**Elizabeth Hampson/Adrian Snihur**	**End Date**	**07 12 31**
Protocol Title	**Sex differences in the auditory system and finger lengths**		
Sponsor	**n/a**		

This is to notify you that The University of Western Ontario Department of Psychology Research Ethics Board (PREB) has granted expedited ethics approval to the above named research study on the date noted above.

The PREB is a sub-REB of The University of Western Ontario's Research Ethics Board for Non-Medical Research Involving Human Subjects (NMREB) which is organized and operates according to the Tri-Council Policy Statement and the applicable laws and regulations of Ontario. (See Office of Research Ethics web site: http://www.uwo.ca/research/ethics/)

This approval shall remain valid until end date noted above assuming timely and acceptable responses to the University's periodic requests for surveillance and monitoring information.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the PREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of research assistant, telephone number etc). Subjects must receive a copy of the information/consent documentation.

Investigators must promptly also report to the PREB:
a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to the PREB for approval.

Members of the PREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the PREB.

Clive Seligman Ph.D.

Chair, Psychology Expedited Research Ethics Board (PREB)

The other members of the 2006-2007 PREB are: Mike Atkinson, Bertram Gawronski, Rick Goffin, and Jim Olson

CC: UWO Office of Research Ethics

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Curriculum Vitae

Adrian W.K. Snihur
Neuroscience Program
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Education

2005 – present	Ph.D. Candidate Graduate Program in Neuroscience Department of Psychology The University of Western Ontario London, Ontario, Canada Thesis title: "Sexual differentiation in the auditory system: An investigation into prenatal and adult sex steroid influences on otoacoustic emissions" Advisor: Dr. Elizabeth Hampson
2003 – 2005	Master of Science Graduate Program in Neuroscience Department of Psychology London, Ontario, Canada Thesis title: "The role of ovarian and adrenal hormones in spatial learning and memory in rats" Advisor: Dr. Peter Cain Collaborator: Dr. Elizabeth Hampson
1999 – 2003	Honours Bachelor of Science, Biology & Psychology McMaster University Hamilton, Ontario, Canada Thesis title: "An investigation of the behavioural manifestations of the Bruce effect in mice" Advisor: Dr. Denys deCatanzaro

Awards, Scholarship, and Honours

Donald O. Hebb Best Poster Award Finalist, 2008
Nominated for best poster at the 18th meeting of the Canadian Society for Brain, Behaviour, and Cognitive Science, London, Ontario, Canada

Western Graduate Research Scholarship, 2005 – 2009
Awarded for research proficiency and academic excellence

Graduate Special University Scholarship, 2003 – 2005
Awarded for research proficiency and academic excellence

Ukrainian Canadian P's and B's Award, 2001
Awarded for academic excellence and leadership in the community

Miller Thomson Foundation Scholarship, 1999
Awarded for academic excellence and leadership in the community

Ukrainian Credit Union Scholarship, 1999
Awarded for academic excellence and leadership in the community

McMaster Science Incentive Award, 1999
Awarded for academic excellence

McMaster Governor General's Scholarship, 1999 – 2000
Awarded for academic excellence

University of Toronto Book Award, 1999
Awarded for academic excellence and leadership in the community

Teaching and Research Experience

Course Instructor, Brescia University College

Psychology 2800E: Research Methods in Psychology (2009 – 2010)
Primary lecturer for a second year undergraduate course (enrolment of 50). Developed and prepared all course material, including the course outline, PowerPoint lectures, assignments, and exams for both lecture and laboratory sections.

Honour's Thesis Supervision, University of Western Ontario

Psychology 485 Senior Thesis course: Carly Goodman (2009 – 2010)
Psychology 485 Senior Thesis course: Jacqueline Corlett (2008 – 2009)
Psychology 485 Senior Thesis course: Erin Yong Ping (2007 – 2008)
Designed and planned experiment, supervised data collection, and assisted in data analysis, thesis write-up, and poster presentation. I was the primary contact for these undergraduate students with respect to all aspects of their project from start to finish, as well as the primary reader of their thesis and poster.

Teaching Assistant, University of Western Ontario

Neuroscience 9500: Behavioural Neuroscience component (2009)

Planned tutorial meetings, led classroom discussions, gave tutorial lectures and revised previous material, answered student questions.

Psychology 2210A: Animal Cognition (2008)

Attended classes on a regular basis, proctored and marked quizzes and exams, held office hours and answered student questions. Guest lecturer for the topic of Reinforcement and Punishment.

Neuroscience 500: Behavioural Neuroscience component (2008)

Planned tutorial meetings, led classroom discussions, gave tutorial lectures and revised previous material, answered student questions.

Psychology 428F: Behavioural Pharmacology (2007)

Assisted students with essay writing.

Psychology 325a: Sex Differences in Human Brain and Behaviour (2007)

Proctored and marked exams, held office hours, and answered student questions.

Psychology 324b: Neuropsychology and Cognitive Neuroscience (2007)

Proctored and marked quizzes and exams, held office hours, and answered student questions.

Psychology 023: Biological Basis of Psychology (2007)

Attended classes regularly, proctored and marked exams, held office hours, and answered student questions. Guest lecturer for the topic of Social Development.

Psychology 325a: Sex Differences in Human Brain and Behaviour (2006)

Proctored and marked exams, held office hours, and answered student questions.

Psychology 326a: Hormones and Behaviour (2006)

Attended classes regularly, proctored and marked exams, held office hours, and answered student questions.

Psychology 020: Introductory Psychology (2005 – 2006)

Attended classes regularly, proctored exams, held office hours, and answered student questions.

Psychology 280: Research Methods in Psychology (2004 – 2005)

Planned and taught lab component (worth 50% of final grade) to approximately 20 students, including research design, data collection/analysis, and manuscript preparation.

Psychology 020: Introductory Psychology (2003 – 2004)

Attended classes regularly, proctored exams, held office hours, and answered student questions.

Membership in Learned Societies

Society for Neuroscience
Society for Behavioral Neuroendocrinology
Canadian Society for Brain, Behaviour, and Cognitive Science

Departmental and University Service

Elected Neuroscience Student Representative on the Graduate Affairs Commitee (2008 – 2009)

This committee was composed of graduate student representatives and graduate chairs from various programs, and met monthly to discuss relevant graduate issues such as scholarships/funding, graduate recruitment (internal/external), and graduate research promotion.

Elected Occupational Health and Safety Officer, Graduate Teaching Assistants Union (2007 – 2009)

Served as a liaison for health and safety issues involving graduate teaching assistants at the university. I was a member of the university-wide Joint Occupational Health and Safety Committee and was involved in campus-wide health and safety inspections.

Elected Student Representative on the Neuroscience Program Committee (2004 – 2009)

The committee dealt with issues pertinent to the advancement of the Graduate Program in Neuroscience. My duties included providing a student perspective at meetings as requested.

President of the Western Ukrainian Students' Club (2007 – 2008)

My duties included holding regular meetings, planning educational and social events, dealing with finances, and holding fundraising events.

Elected Student Representative on the Psychology Graduate Affairs Committee (2006 – 2007)

This committee was comprised of faculty from the Department of Psychology and 5 graduate student representatives who met monthly to discuss relevant graduate issues, including enrolment, admission requirements, and funding/scholarships.

Elected Student Representative on the Society for Graduate Students (2004 – 2007)

Served as a liaison between the SOGS committee and graduate students in Neuroscience, and attended monthly meetings discussing relevant graduate student issues and concerns.

Executive Member on the Western Psychology Graduate Students' Association (2003 – 2004)

This student-run organization was responsible for organizing social functions and fundraisers for Psychology graduate students.

President of the McMaster Ukrainian Students' Association (2002 – 2003)

My duties included holding regular meetings, planning educational and social events, dealing with finances, and holding fundraising events (included planning a province-wide volleyball tournament).

Vice-President of Academics of the McMaster Biology & Psychology Society (2001 – 2003)

My duties included planning and supervising various academic functions (e.g., professor meet-and-greet events, graduate school information sessions).

Vice-President of the McMaster Ukrainian Students' Association (2001 – 2002)

My duties included holding regular meetings, planning educational and social events, dealing with finances, and holding fundraising events.

Publications

Refereed Journal Articles

Snihur, A.W.K., Hampson, E., & Cain, D.P. (2008). Estradiol and corticosterone independently impair spatial navigation in the Morris water maze in adult rats. *Behavioral Brain Research, 187(1)*, 55-66.

Articles submitted and in preparation

Snihur, A.W.K., & Hampson, E. (submitted). Individual differences in 2D:4D digit-ratios and otoacoustic emissions: Do they share a common developmental origin? *Personality and Individual Differences*.

Snihur, A.W.K., & Hampson, E. (submitted). Sex and ear difference in spontaneous and click-evoked otoacoustic emissions in young adults. *Brain and Cognition*.

Snihur, A.W.K., & Hampson, E. (in preparation). Defeminization of otoacoustic emission patterns associated with oral contraceptive use in women. To be submitted to *Hormones and Behavior*.

Snihur, A.W.K., & Hampson, E. (in preparation). Click-evoked otoacoustic emissions are associated with circulating testosterone levels in men. To be submitted to *Hormones and Behavior*.

Refereed Abstracts

Snihur, A.W.K., & Hampson, E. (2009). Click-evoked otoacoustic emissions are associated with circulating testosterone levels in men. *Society for Neuroscience Abstracts*.

Snihur, A.W.K., Corlett, J., & Hampson, E. (2009). Effects of observed aggression on testosterone release and affective mood state in men. *Society for Behavioral Neuroendocrinology Abstracts*.

Snihur, A., & Hampson, E. (2008). Defeminization of otoacoustic emission patterns associated with oral contraceptive use in women. *Canadian Journal of Experiment Psychology, 62(4)*, 294.

Snihur, A., Hampson, E., & Cain, D.P. (2005). The role of ovarian hormones in spatial navigation behaviour in rats in the absence of a corticosterone response. *Southern Ontario Neuroscience Association Abstracts*, 81.

Snihur, A., Hampson, E., & Cain, D.P. (2004). The role of estrogen in spatial navigation behaviour in rats in the absence of a corticosterone response. *Society for Neuroscience Abstracts*, 897.1.

Paper and Poster Presentations at Conferences

Snihur, A.W.K., & Hampson, E. (2009). Click-evoked otoacoustic emissions are associated with circulating testosterone levels in men. Presented at the 39th annual meeting of the Society for Neuroscience, Chicago, Illinois, USA (October).

Snihur, A.W.K., Corlett, J., & Hampson, E. (2009). Effects of viewing vicarious aggression on testosterone levels and affective mood state in males. Presented at the 13th annual meeting of the Society for Behavioural Neuroendocrinology, East Lansing, Michigan, USA (June).

Snihur, A., & Hampson, E. (2008). Defeminization of otoacoustic emission patterns associated with oral contraceptive use in women. Presented at the 18th meeting of the Canadian Society for Brain, Behaviour, and Cognitive Science, London, Ontario, Canada (June).

Snihur, A., & Hampson, E. (2008). Spontaneous and click-evoked otoacoustic emissions: Confirmation of a sex difference in young adults. Presented at the annual meeting of Theoretical and Experimental Neuropsychology (TENNET), Waterloo, Ontario, Canada (June).

Snihur, A., Hampson, E., & Cain, D.P. (2005). The role of ovarian hormones in spatial navigation behaviour in rats in the absence of a corticosterone response. Presented at the annual meeting of the Southern Ontario Neuroscience Association, Hamilton, Ontario, Canada (June).

Snihur, A., Hampson, E., & Cain, D.P. (2004). The role of estrogen in spatial navigation behaviour in rats in the absence of a corticosterone response. Presented at the 34th annual meeting of the Society for Neuroscience, San Diego, California, USA (October).