Sex differences in hearing: Probing the role of estrogen signaling

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Hearing loss is the most common form of sensory impairment in humans, with an anticipated rise in incidence as the result of recreational noise exposures. Hearing loss is also the second most common health issue afflicting military veterans. Currently, there are no approved therapeutics to treat sensorineural hearing loss in humans. While hearing loss affects both men and women, sexual dimorphism is documented with respect to peripheral and central auditory physiology, as well as susceptibility to age-related and noise-induced hearing loss. Physiological differences between the sexes are often hormone-driven, and an increasing body of literature demonstrates that the hormone estrogen and its related signaling pathways may in part, modulate the aforementioned differences in hearing. From a mechanistic perspective, understanding the underpinnings of the hormonal modulation of hearing may lead to the development of therapeutics for age related and noise induced hearing loss. Here the authors review a number of studies that range from human populations to animal models, which have begun to provide a framework for understanding the functional role of estrogen signaling in hearing, particularly in normal and aberrant peripheral auditory physiology.
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I. INTRODUCTION

According to the World Health Organization (March 2018), there are 466×10^6 individuals worldwide with debilitating hearing loss, and an estimated 1.1×10^9 young adults worldwide who are at risk for developing hearing loss due to recreational noise exposure (Deafness and hearing loss, 2018). According to the Center for Disease Control, between 2001 and 2008, an estimated 30×10^6 Americans over the age of 12 suffered from hearing loss in both ears, while an estimated 48×10^6 Americans suffered hearing loss in at least one ear (Lin *et al.*, 2011). Hearing loss is more than just an obstacle to communication, and its negative effects permeate and influence all aspects of the lives of those afflicted. According to the CDC, "Those who have hearing loss are more likely to have low employment rates, lower worker productivity, and high healthcare costs" (Themann *et al.*, 2013). Furthermore, hearing loss is the second most common health issue (following tinnitus) afflicting military veterans (Veterans Benefits Administration Reports Annual Benefits Report Fiscal Year 2017, 2017; Yankaskas, 2013). Hearing loss in the military is particularly concerning, since clear and efficient communication is absolutely critical to the success and safety of men and women on an often noisy and chaotic battlefield.

Hearing loss affects both men and women, but importantly, significant sex differences in hearing have been documented in a number of species and are particularly well-documented in humans. These differences in hearing physiology between the sexes have important implications not only for a complete understanding of hearing loss and hearing physiology, but also for the development of potential therapeutics to treat sensorineural hearing loss (SNHL), which encompasses both noise-induced hearing loss (NIHL) and age-related hearing loss (ARHL). Currently, there are no approved therapeutics to treat NIHL or ARHL, and it is quite reasonable to expect that the efficacy of any therapeutics may be influenced by differences in hearing physiology between the sexes. In fact, evidence already exists to suggest that this may be the case (Milon *et al.*, 2018). Unfortunately, a large sex bias still exists in many aspects of hearing research, and there is a real possibility that some of the conclusions reached in studies using only males, or where biological sex as an independent variable was not considered, may not apply similarly to both sexes (Lauer and Schrode, 2017; Villavisanis *et al.*, 2018). Indeed, the influences of biological sex and sex steroids, such as estrogens and androgens, on the molecular and cellular pathways underlying hearing loss represent a significant knowledge gap. Thus, there is value in investigating these sex differences, as a more complete understanding of the mechanisms underlying sex differences in hearing may benefit the development of therapeutics beneficial to both sexes.

II. SEX DIFFERENCES IN HEARING

Sex differences in hearing encompass both peripheral and central auditory processing, and range from cochlear function, to susceptibility to ARHL and NIHL, and even to binaural sound processing (Grinn *et al.*, 2017; McFadden *et al.*, 2009a; Pearson *et al.*, 1995; Szanto and Ionescu, 1983;

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Zündorf *et al.*, 2011). Since the majority of recent clinical trials targeting hearing loss primarily address the peripheral auditory system at the level of the cochlea and the auditory nerve (Crowson *et al.*, 2017), the focus of this review will be restricted to sex differences in peripheral auditory physiology, SNHL, and the possible mechanisms underlying these differences.

Overall, it is widely accepted that the gross anatomy of the male is virtually indistinguishable from the gross anatomy of the female cochlea. Some studies have reported that the cochlear length in females is slightly shorter in comparison to males (~3%); however these findings are the subject of inconclusive reports and questionable statistical and biological significance (Miller, 2007; Sato *et al.*, 1991). When the physiology of the cochlea is examined more closely, however, intriguing differences between the sexes begin to emerge.

A. Otoacoustic emissions

In humans, a number of sex differences in outer hair cell (OHC) physiology have been documented. Otoacoustic emissions (OAEs) are sounds produced by vibrations that arise from the organ of Corti and are transmitted backward through the middle ear. These vibrations likely arise from the OHCs, and are due to their electromotive properties (Brownell, 1990). The presence and amplitudes of OAEs can be measured and used as a readout for general OHC function (although pathology of the middle ear can also influence the detection of OAEs) (Kemp, 2008). Spontaneous otoacoustic emissions (SOAEs) are likely produced by OHCs in the absence of an external stimulus. OHCs also contribute to the emission of more robust, reproducible sounds when the listener is presented with broadband clicks (click-evoked otoacoustic emissions, CEOAE) and when the listener is presented with two neighboring, simultaneous pure tones (distortion product otoacoustic emissions, DPOAEs) (Kemp, 2002).

While SOAEs are detectable in approximately 70% of all human listeners, (Abdala and Visser-Dumont, 2001) they are more likely to be detected in female listeners- approximately 80% compared to approximately 60% of male listeners (Penner and Zhang, 1996). In humans, females also tend to produce larger CEOAEs than males, and similar findings have been shown in rhesus monkeys (McFadden, 1998; McFadden *et al.*, 2006). Sex-differences in the amplitude of DPOAEs, however, are smaller in magnitude, variable between species, and the subject of equivocal reports (McFadden, 2009). While these phenomena are well-documented in human listeners, the exact mechanisms underlying sex-differences in OAEs are unknown, but may be linked to prenatal androgen (male hormone) exposure (McFadden *et al.*, 2006). In support of this hypothesis, studies of OAEs in humans have demonstrated that females with male co-twins, who are exposed to higher levels of prenatal androgens, display more masculinized OAEs in comparison to same-sex female twins or non-twin females (McFadden, 1993). Furthermore, female sheep exposed to testosterone prenatally develop weaker, more masculinized CEOAEs (McFadden *et al.*, 2009b).

B. Auditory brainstem response (ABR)

Neuronal activity as measured from the cochlea to the brainstem provides further evidence of sex differences in hearing. ABR wave-I amplitude is a measure of the synchronous neural firing at the level of the spiral ganglion in response to a sound stimulus. A recent publication using mice demonstrated that in adult mice, ABR wave-I amplitudes are larger in females compared to males (Milon *et al.*, 2018). Similar findings have been reported in humans when examining the effect of recreational noise exposure on cochlear nerve response amplitudes (Grinn *et al.*, 2017). While the mechanisms underlying the amplitude differences are not known, it has been postulated that a shorter cochlear length in females and often smaller head size may contribute to greater synchronous activity at the level of the spiral ganglion afferents, as well as a shorter afferent auditory pathway, both of which may lead to greater ABR wave amplitudes and shorter wave latencies (McFadden, 1998). In fact, analysis of speech-evoked ABR in humans demonstrates that females have shorter ABR onset response latencies, (Liu *et al.*, 2017) and it is well demonstrated that females have larger wave-V amplitudes, shorter wave-V latencies, and shorter wave I-V inter-peak latencies (Jerger and Hall, 1980; Lotfi and Zamiri Abdollahi, 2012). However, comparison of ABR latencies of males and females of similar head size suggests that differences in ABR latencies cannot be completely attributed to differences in skull or brainstem dimensions, suggesting underlying physiology also contributes to these sex differences (Sabo *et al.*, 1992).

C. Sensorineural hearing loss

According to a recent analysis of data obtained via two national surveys, approximately 6% of the adult population in the United States suffers from deafness or serious difficulty hearing (determined through analysis of self-reported answers to survey questions) (Li *et al.*, 2018). However, when the data are analyzed by sex, a higher prevalence of hearing loss is observed in males where the prevalence of deafness or serious difficulty hearing is 7.3% compared to only 4.8% in females (Li *et al.*, 2018). The higher prevalence of hearing loss in males as reported in these surveys has been confirmed by other cross-sectional and cross-sectional longitudinal cohort analyses, including a large meta-analysis of 42 studies conducted across 29 countries (Bishop *et al.*, 2019; Lin *et al.*, 2011; Stevens *et al.*, 2011).

While answers to these two surveys were self-reported, and the data were not analyzed by etiology of the hearing loss, well documented sex differences exist with regard to specific etiologies of hearing loss. In particular, sex differences in the prevalence and progression of age-related hearing loss are well documented. A number of longitudinal and cross-sectional studies in humans demonstrate that pure-tone hearing thresholds decline more rapidly in males compared to females, especially at higher frequencies (Allen and Eddins, 2010; Cruickshanks *et al.*, 1998; Pearson *et al.*, 1995). Hearing loss (as measured in frequencies ranging from 0.5 to 8 kHz) is detectable in males as early as age 30, while the onset and frequency range of the hearing loss in females is both later in life and more variable (Pearson *et al.*, 1995). Furthermore, a

cross-sectional study by Allen and Eddins (2010) demonstrated that a decline in DPOAE amplitude begins in 30 year old males, while a similar decrease in DPOAE amplitude begins a decade later in females (Allen and Eddins, 2010). In a Swedish cohort study of adult males and females ages 70 and 75, the 4 kHz pure-tone thresholds were approximately 20 dB worse in the male subjects (Jönsson *et al.*, 1998). In a mouse model of ARHL, hearing thresholds at higher frequencies, as measured via ABR, declined more rapidly in males than females (Henry, 2004). Additional mouse studies have demonstrated that the magnitude of DPOAEs declines more rapidly in males than females, which may partially underpin the more rapid deterioration of ABR thresholds in males (Guimaraes *et al.*, 2004).

Evidence also suggests that females are protected from NIHL in comparison to males. One retrospective study of industrial factory workers concluded that females experience less severe deterioration of hearing thresholds as a result of occupational noise exposure in comparison to males when occupational sound intensities are approximately 98 dB (Szanto and Ionescu, 1983). The evidence is even more pronounced in animal models. Sound-conditioned female chinchillas display reduced permanent threshold shifts (PTS) compared to males at most frequencies (except 16 kHz) after exposure to simulated rifle fire at 150 dB peak sound pressure level (SPL) (McFadden *et al.*, 2000). Work from our group shows that female mice are relatively protected from a PTS-inducing noise exposure in comparison to males (Milon *et al.*, 2018). In addition, wave-I amplitudes in female mice, following the noise trauma, were larger than amplitudes in males, indicating greater synchronous activity at the level of the auditory nerve. The same study investigated the therapeutic efficacy of suberoylanilide hydroxamic acid (SAHA), a potential treatment for NIHL that had previously been tested only in male mice. When treated with SAHA, males were protected (a significant reduction in the PTS) at 16 kHz while females were protected at 24 kHz, further highlighting differential response to acoustic injury and the need to fully understand the mechanisms underlying these differences prior to the development of oto-therapeutics and targeted therapies (Milon *et al.*, 2018).

III. ESTROGEN AND HEARING

Differences in the sex steroid milieu often underlie sex differences in physiology. Indeed, an increasing body of literature demonstrates that the aforementioned sex differences in hearing are modulated, at least in part, by estrogens acting via their classical steroid receptor signaling pathways.

A. Hearing, the menstrual cycle, and menopause

Substantial evidence exists linking serum levels of estrogens to hearing thresholds in a variety of human populations. Estrogens are primarily produced by the ovaries in pre-menopausal women but are also synthesized in smaller amounts by the brain and adipose tissue. Of the estrogens produced by the ovaries, estradiol—or more specifically 17β- estradiol—is the most potent (Blair *et al.*, 2000). Pure-tone hearing thresholds have been shown to fluctuate during the different stages of the menstrual cycle in adult pre-menopausal women. In one study of pre-menopausal women ages 18–39, lowest hearing thresholds occurred during the late-follicular phase of the menstrual cycle, which corresponds to the highest levels of serum estrogens. Hearing thresholds subsequently increased during the late-luteal phase and early follicular phases, which correspond to low levels of serum estrogen and increases in serum progesterone levels (Da Silva Souza *et al.*, 2017). A relationship between changes in hearing to changes in circulating levels of estrogens is also seen in animal models. In post-partum female mice, changes in hearing and the response to pup vocalizations correspond to fluctuations in estrogen levels (Frisina, 2012).

In post-menopausal women, the ovaries no longer produce estrogens, and serum levels drop significantly. A cross-sectional study analyzing hearing thresholds in 1830 post-menopausal women found a significant association between serum estradiol levels and hearing thresholds, and concluded that lower levels of serum estradiol are associated with decreased hearing sensitivity (Kim *et al.*, 2002). A natural follow up question would be whether hormone replacement therapy (HRT) could prevent or ameliorate changes in hearing in post-menopausal women. In one study using low dose estrogen treatment in women who had undergone surgically induced menopause for benign diseases, ABR wave latencies were shortened, indicative of improved sensitivity/neuronal reactivity in the presence of estrogen (Caruso *et al.*, 2003). Another study of post-menopausal women demonstrated that women taking estrogen therapy displayed better low frequency mean air conduction thresholds (250–2000 Hz) compared to the control subjects not taking any form of HRT (Kilicdag *et al.*, 2004). However, not all studies show that HRT results in otoprotection from ARHL. Admittedly, studies on the effect of HRT are more challenging to compare and interpret, as HRT regimens vary in dosage, composition, duration, and initiation with regards to the onset of menopause (Caruso *et al.*, 2003; Curhan *et al.*, 2017; Guimaraes *et al.*, 2006; Hederstierna *et al.*, 2007; Kilicdag *et al.*, 2004). Natural progesterone or synthetic progestins are common components in some HRT formulations, and have been associated with adverse effects on hearing (Guimaraes *et al.*, 2006). In a study comparing post-menopausal women receiving either estrogen, an estrogen and progestin combination therapy, or no HRT, the women receiving the combination treatment showed worse hearing thresholds and DPOAEs in comparison to the untreated group (Guimaraes *et al.*, 2006). Similar results were obtained also in studies using mice. A follow-up study in middle-aged female mice demonstrated that mice receiving both estrogen and progestin exhibited accelerated hearing loss in comparison to mice receiving estrogen only (Price *et al.*, 2009). A more recently published study by Williamson *et al.* (2019) recapitulated the finding that a combination HRT including estrogen and progestin in middle-aged female mice is detrimental to hearing (Williamson *et al.*, 2019). Interestingly, a study analyzing progesterone receptor localization found no nuclear localization in the stria vascularis, organ of Corti, or spiral

ganglion neurons (SGN) in either the rat or human cochlea, suggesting any negative effects of progesterone and progestin on hearing are likely indirect (Bonnard *et al.*, 2013). Conversely, a recently published prospective cohort study of approximately 80 000 women from the Nurses' Health Study II found that a longer duration of HRT—estrogen alone or as a combination therapy—was associated with an increased self-perception of hearing loss (Curhan *et al.*, 2017). The same study also correlated older age at menopause with an increase in self-reported hearing loss. On the other hand, a recently published manuscript associated early ovarian failure with increased high frequency hearing thresholds (10–16 kHz), as well as improved hearing at these high frequencies, only in the right ear, in women treated with HRT (here, the treatment group was heterogenic) (Zhang *et al.*, 2018). Taken together, these studies demonstrate the challenge in studying the effect of HRT on hearing and demonstrate the need for a well stratified approach in researching this topic.

B. Hearing and Turner syndrome

Early-onset sensorineural hearing loss and presbycusis are hallmark sequelae of Turner syndrome (45, X) (TS), in which dysfunction of the ovaries results in a reduction of serum estrogen levels. Analysis of Swedish women with TS showed that only 13% of women over the age of 40 displayed normal hearing—defined as a four frequency average less than 20 dB hearing level (HL) (0.5, 1, 2, and 4 kHz)—while the expected proportion of women in the normal Swedish population with normal hearing over the age of 40 is 66% (Hederstierna *et al.*, 2009). The authors concluded in the study that the pure-tone threshold elevations in women with TS were likely of cochlear origin (Hederstierna *et al.*, 2009). Interestingly, the incidence of sensorineural hearing loss can be correlated with karyotype. Patients with a complete loss of an X chromosome were more likely to suffer from sensorineural hearing loss compared to women with a mosaic pattern of X chromosome loss (Hultcrantz and Sylven, 1996). Similar findings of early presbycusis and sensorineural hearing loss were demonstrated in a mouse model of TS. One year old "Turner mice" displayed increased threshold shifts at the higher frequencies compared to littermate controls. This high frequency hearing loss was further exacerbated in comparison to littermate controls when ABRs were conducted at 19 months of age (Hultcrantz *et al.*, 2000).

C. Estrogen receptors α and β and hearing

The actions of estrogens are complex and varied, and are mediated both via genomic and non-genomic pathways (Björnström and Sjöberg, 2005; Vrtačnik *et al.*, 2014). The genomic actions of estrogen are mediated through interaction with the two canonical estrogen receptors (ER)—ERα and ERβ (also known as ESR1 and ESR2)—which are part of the nuclear receptor family of ligand-activated transcription factors (Charitidi *et al.*, 2009). Ligand-bound ERα and ERβ modulate transcription by binding to estrogen response elements (ERE), sequences of DNA that are recognized by the receptor. 17β-estradiol, which is the most potent form of estrogen produced in the body, shares a similar affinity for ERα and ERβ, which both bind to the same ERE (Blair *et al.*, 2000; Gruber *et al.*, 2002; Heldring *et al.*, 2007).

ERα and ERβ are both detected in the human, mouse, and rat inner ears in partially overlapping patterns of expression. A human study focused on the localization of estrogen receptors in females detected protein expression of only ERα between gestational weeks 14–20, which localized to the SGN. In contrast, both ERα and ERβ were expressed in the mature inner ear, localizing to the SGN and stria vascularis, respectively (Stenberg *et al.*, 2001). In the mouse inner ear, the reported expression patterns were broader (Motohashi *et al.*, 2010; Stenberg *et al.*, 1999). Stenberg *et al.* (1999) showed that ERα localizes to both the inner hair cells (IHC) and OHCs, type-1 and type-2 SGNs, Reissner's membrane, the stria vascularis, and the spiral ligament. However, ERβ localizes to the IHCs but not OHCs, Reissner's membrane, and the stria vascularis, with a lower expression in type-1 and type-2 SGNs (Stenberg *et al.*, 1999). Motohashi *et al.* (2010) reported similar expression patterns of ERα and ERβ in the mouse inner ear with the exception that they also found expression of ERβ in the OHCs (Motohashi *et al.*, 2010). Interestingly, while the expression patterns of ERα and ERβ did not vary by age or sex, the immunoreactivity of ERα was stronger in young female compared to young male mice and decreased for both receptors with age, in both sexes (Motohashi *et al.*, 2010). Stenberg *et al.* (1999) demonstrated similar expression patterns of the ERs in the rat inner ear, but also demonstrated ERβ expression in the OHC and pillar cells. Furthermore, the immunoreactivity of the antibody for ERβ in the SGN was more intense in the rat inner ear compared to the mouse (Stenberg *et al.*, 1999).

The mRNA expression of ERs in the cochlea may be modulated by hormone levels, and has been shown to fluctuate during the normal menstrual cycle and after ovariectomy (Charitidi *et al.*, 2012). In female CBA/Ca mice, mRNA levels of ERα but not ERβ decreased significantly in the cochlea during the proestrous phase when compared to the metestrous and estrous phases. Chronic 17β-estradiol treatment in ovariectomized CBA/Ca mice leads to a downregulation of ERα mRNA levels in a similar fashion when compared to ovariectomized animals not receiving treatment. No effect was seen on ERβ mRNA levels after ovariectomy and chronic 17β-estradiol treatment (Charitidi *et al.*, 2012).

Analysis of mice with targeted deletions of ERα (ERKO mice), ERβ (BERKO mice), and aromatase (ARKO mice)—the enzyme responsible for the conversion of testosterone to estrogen—demonstrates that ERβ is crucial for maintenance of hearing, and further illuminates the role of estrogen signaling in hearing. A study of hearing thresholds in BERKO mice determined that while there were no differences in ABR thresholds of BERKO mice compared to wild-type (WT) controls at 3 months of age, 12 month-old WT mice displayed significantly lower thresholds at all frequencies tested (12 month-old BERKO mice were deaf) (Simonoska *et al.*, 2009). BERKO mice also experienced more severe OHC and IHC loss along the entire length of the cochlea, especially in the basal turn. Additionally, BERKO mice also displayed increased spiral ganglion atrophy along the entire length of the cochlea in comparison to WT mice. In support

of the role of ERβ in the maintenance of hearing, suppressing estrogen signaling with tamoxifen—which demonstrates ERβ-mediated antagonistic effects—has been shown to negatively impact contralateral suppression of DPOAEs in mice (Barkhem *et al.*, 1998; Thompson *et al.*, 2006). Contralateral suppression of DPOAEs is thought to be a protective mechanism that enhances cochlear function, and it has been demonstrated that a decrease in contralateral suppression often precedes an age-related decline in DPOAEs (Thompson *et al.*, 2006).

Importantly, from a translational perspective, ERβ but not ERα also mediates neuroprotection following acoustic trauma (Meltser *et al.*, 2008). BERKO mice experience more severe temporary threshold shift (TTS) after acoustic injury in comparison to ERKO or WT mice, whereas ERKO male and female mice experience a TTS similar to WT. This suggests that ERβ and not ERα can protect against acoustic injury. Interestingly, no sex-differences in threshold shifts were detected between male and female BERKO mice. The same study demonstrated that the ERβ-selective agonist DPN (2, 3-bis (4-hydroxyphenyl)-propionitrile) protects against the same acoustic injury at some of the frequencies tested when administered to female WT mice, providing further evidence of the protective effects of ERβ.

D. Estrogen related receptors and hearing

In addition to the two canonical ERs ERα and ERβ, a family of estrogen related receptors (ESRR or ERR)—ERRα, ERRβ, and ERRγ—may also play a role in hearing physiology. Although the ERRs share sequence and structural homology with the canonical ERs, estrogens are not an endogenous ligand. In fact, ERRs are orphan receptors and can be constitutively active and regulate transcription without ligand binding (Saito and Cui, 2018). The search for endogenous ligands of ERRs thus far, has largely proven unfruitful, but a recently published study identified cholesterol as an endogenous ligand of ERRα (Wei *et al.*, 2016). Regardless, even in the absence of ligand, ERRs can bind to ERE in addition to a set of estrogen-related response elements (ERRE). Interestingly, ERα but not ERβ has also demonstrated the ability to bind to ERRE (Saito and Cui, 2018).

Numerous studies have clearly demonstrated that ERRβ is particularly important for hearing and normal cochlear physiology (Bhatt *et al.*, 2016; Chen and Nathans, 2007; Collin *et al.*, 2008; Lee *et al.*, 2011; Saïd *et al.*, 2011). In the early postnatal cochlea ERRβ is expressed in the endolymph-secreting marginal cells of the stria vascularis, but not in the sensory cells of the cochlea, partially regulating the expression of ion channels and ion transporters in these cells (Chen and Nathans, 2007). Conditional knockout (cKO) of ERRβ from the lateral wall using *Sox2*-Cre results in a concomitant loss of mRNA transcripts encoding potassium channels (KCNQ1 and KCNE1) and a subunit of the Na/K ATPase (Chen and Nathans, 2007). These cKO mice also display severe auditory impairment, with ABR thresholds greater than 100 dB SPL. Furthermore, mutations of ERRβ underlie DFNB35 (autosomal-recessive, nonsyndromic hearing impairment) and a single nucleotide polymorphism (SNP) in the gene encoding ERRβ in humans is associated with increased TTS after exposure to a 10 min narrow-band noise centered at 2 kHz (Bhatt *et al.*, 2016; Collin *et al.*, 2008; Lee *et al.*, 2011; Saïd *et al.*, 2011).

Although less is known about ERRγ—which is expressed in a variety of cell types in the female mouse inner ear including IHCs and OHCs—a British cohort study correlated a SNP in the gene encoding the ERRγ receptor with increased risk of ARHL, but only in females (Nolan *et al.*, 2013). Furthermore, the same authors demonstrated that ERRγ KO mice have elevated thresholds compared to WT and heterozygous mice, and that female KO have worse hearing than males at 12 weeks of age (Nolan *et al.*, 2013). In addition, a recent clinical report implicated disruption of the gene encoding ERRγ on chromosome 1 in the case of a female born with moderate bilateral SNHL with an accompanying developmental delay (Schilit *et al.*, 2016).

IV. CONCLUSIONS

There is abundant and robust evidence to support the conclusion that estrogen not only modulates hearing and hearing physiology, but also that estrogen and its signaling pathways are protective and required for normal hearing and maintenance of hearing (e.g., the amelioration of age-related hearing loss and noise-induced hearing loss) in both sexes.

Estrogen levels correlate with hearing thresholds during the menstrual cycle, and hearing thresholds decline rapidly in post-menopausal women when levels of serum estrogen decline drastically. Women with Turner syndrome, a disease characterized by reduced serum estrogen levels, are more likely to suffer from hearing loss, and begin to suffer from hearing loss earlier in life. Females are protected from ARHL and NIHL in comparison to males, and female mice display decreased ABR threshold shifts following a noise exposure.

Evidence of the modulatory and protective role of estrogen is also abundant at the molecular level. Estrogen receptors and estrogen-related receptors are expressed in the inner ears of mice and humans in a variety of cells types. Of significance, the expression patterns of ERα and ERβ appear to be more widespread in mice than in humans, highlighting the need for additional studies to localize these proteins in non-human primates. From a functional perspective, most of the otoprotective effects of estrogen are thought to be mediated by ERβ, as mice with a deletion of ERβ are more susceptible to noise trauma and suffer from accelerated age-related hair cell loss and spiral ganglion deterioration. In support of ERβ's role in hearing protection, WT female mice administered DPN, a selective ERβ agonist, displayed reduced threshold shifts following noise exposure.

Studies of the family of estrogen-related receptors demonstrate disruption of ERRβ and ERRγ, both of which localize to the inner ear of mice, negatively impact hearing health and physiology. Although estrogens are not endogenous ligands of the ERRs, ERRs can modulate gene expression by binding to the same ERE that are also bound by ERs. Evidence also exists to suggest that some ERRs may interact

directly with ERs and modulate the classical estrogen pathways (Tanida *et al.*, 2015). Additionally, estrogen—a known regulator of calcium mobilization—may contribute to more rapid, genomic and non-genomic changes in cellular physiology via a G-protein coupled receptor, GPR30 (also called GPER1). While rapid activation of GPR30 by estrogen influences gene expression resulting in both short-term and long-term effects on transcription, its actions in the inner ear are unknown (Prossnitz, 2009).

How estrogen confers protection, and how estrogen contributes to normal hearing physiology and its maintenance is far from being completely understood. Despite this, natural estrogen signaling is particularly potent, and the therapeutic potential is large and untapped. Fortuitously, signaling through ERβ—rather than signaling via ERα, which may more heavily underly estrogen's carcinogenic potential—appears to be more important for hearing preservation in protection (Clemons and Goss, 2001). In order to harness the full potential of the estrogen signaling pathway on hearing preservation, sex differences in hearing including estrogen's role in the modulation and protection of hearing must continue to be investigated.

As the investigation into the effects of estrogen signaling—and potentially other hormones—on hearing and hearing physiology continues, experimental methods will need to be refined. Studies using full knockout animal models are valuable for understanding the roles of hormones and their signaling pathways, but care must be taken not to overdraw conclusions about effects on the physiologic system (in this case the auditory system) without due consideration of the indirect and unknown effects caused by a systemic loss of a particular hormone or hormone receptor (McCarthy and Arnold, 2011). Here, the generation of conditional knockouts will prove absolutely critical for the elucidation of a precise and confident understanding of hormonal modulation of hearing. The use of conditional knockouts will also provide more translationally relevant results applicable to therapeutic development.

In addition to the use of conditional knockouts to study the effects of estrogen and other hormones on the auditory system, prenatal masculinization and feminization studies that experimentally alter the hormonal milieu during development may fill in additional knowledge gaps and provide a clearer picture of sex differences in auditory physiology. In fact, studies that have utilized prenatal masculinization and feminization as an experimental tool have begun to demonstrate that sex differences in auditory physiology, are, in part, shaped by prenatal hormone exposure (McFadden *et al.*, 2009b). For example, there is evidence that the previously mentioned sex difference in the prevalence of SOAE exists at birth in humans (Qi *et al.*, 2014). Differences in prenatal 'molding' of auditory physiology may explain why exogenous estrogen delivery to gonadectomized males does not confer protection from a PTS-inducing noise exposure (manuscript in preparation). Perhaps the male auditory system has lost the ability, through prenatal androgen exposure, to respond to estrogen. This is all speculative, of course, but highlights the need for an understanding of the role of hormones in the early developmental events of the auditory system and its physiology.

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