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Sex differences in subcortical auditory processing emerge across development

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ABSTRACT

Human subcortical auditory processing is sexually dimorphic. The prevailing view – that sex differences arise from cochlear differences – remains unproven, and the extent to which these differences reflect distinct auditory processes is unknown. To determine the origin of subcortical sex differences, we mapped their emergence onto the peripheral-to-central maturation of the auditory system in 516 participants (250 female) across three age groups: 3–5, 14–15, and 22–26 years. To examine whether these sex differences arise from distinct processes, we compared developmental trajectories of each evoked-response component and tested their ability to predict a participant's sex and age. We find that some subcortical sex differences emerge well after the cochlea is mature and that each measure uniquely contributes to predicting participant demographics, indicating that sex differences arise from multiple central auditory processes.

1. Introduction

A longstanding hypothesis in the field of auditory neuroscience is that sex differences observed in subcortical auditory processing reflect cochlear differences (Don et al., 1993; Sato et al., 1991). The male cochlea is longer and has a shallower stiffness gradient than the female cochlea (Don et al., 1993; Sato et al., 1991). This difference is presumed to result in the scalp-recorded subcortical evoked response being smaller and later in young adult males compared to young adult females (Jerger and Hall, 1980; Michalewski et al., 1980).

Many foundational studies supporting a cochlear origin for subcortical sex differences were carried out with short, simple sounds, such as a click or tone, on young adults. Because only the response to a sound's onset can be captured with these stimuli, the response evoked by these sounds does not reflect the intricate, complex processing the subcortical auditory system performs when listening to real-world sounds, like speech. Furthermore, because previous studies examined sex differences in the mature system of young adults, whether these differences result from central or peripheral (i.e., cochlear) processes cannot be determined. To do so, sex differences need to be examined at different timepoints across development (Moore and Linthicum, 2007).

To test whether sex differences in subcortical auditory processing manifest from a single, cochlear source, we collected evoked responses to a simple stimulus (i.e., click auditory brainstem response, or click-ABR) and a speech sound (i.e., frequency-following response, FFR, to 'da') in 266 males and 250 females at three points in development: 3–5 years, 14–15 years, and 22–26 years. By birth, the cochlea is structurally and functionally mature (Moore and Linthicum, 2007) and sex differences in cochlear length already exist (Sato et al., 1991), while central auditory-pathway relays continue to mature through adolescence into young adulthood (Moore and Linthicum, 2007; Skoe et al., 2015). Indeed, the inferior colliculus, a predominant generator of the later click-ABR peaks and FFR (Chandrasekaran and Kraus, 2010; Land et al., 2016; White-Schwoch et al., 2016b), continues to show functional maturation through young adulthood (Skoe et al., 2015).

The FFR lends itself to the study of subcortical developmental sex differences because the FFR is sensitive to continued central-auditory system maturation: with increasing age, there is a lengthening of peak timing and a reduction in response magnitude and spectral encoding (Skoe et al., 2015). Therefore, if the prevailing, cochlear hypothesis is true, then comparing males and females across early childhood, adolescence, and adulthood should yield

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sex differences of equal magnitude at all time points (i.e., main effects of sex and age). However, if the alternate hypothesis, that sex differences in subcortical auditory processing are due to differences in central auditory processes, is true, we should see sex differences emerge across development, with males and females becoming more distinct as they age (i.e., a sex by age interaction).

2. Methods

All procedures were approved by the Northwestern University Institutional Review Board. Adult participants gave their written informed consent to participate. For participants younger than 18 years of age, informed consent was obtained from the parent or guardian. Verbal assent was obtained from 3 to 5 years old, and written assent was collected from 14 to 15 years old using age-appropriate language. All participants were paid for their participation.

2.1. Participants

The dataset consists of 516 healthy participants (250 female) across three age groups: children aged 3–5 years, adolescents aged 14–15 years, and young adults aged 22–26 years. The data were pulled from past or ongoing studies in our laboratory. This is a retrospective reanalysis of data from our existing database; subsets of the data have been published previously to address other research questions (Hornickel et al., 2009; Johnson et al., 2008; Krizman et al., 2010, 2012a, 2015; Russo et al., 2008; Skoe et al., 2015; Tierney et al., 2015).

Exclusionary criteria were a history of learning disabilities or neurological dysfunction. All participants had normal audiometric profiles. Normal hearing was confirmed by air conduction thresholds (<20 dB HL for 500, 1000, 2000, 4000 Hz) for adolescent and young adult participants or an audiological screen (pass/fail based on distortion product otoacoustic emissions and/or behavioral response at 20 dB HL) for child participants.

2.2. Stimuli

Stimuli were a 100-μs square-wave click stimulus and a 40-ms speech syllable, 'da'. The 'da' is a five-formant synthesized speech sound (Klatt, 1980) consisting of an initial noise burst and a formant transition between the consonant and vowel. Over the 40 ms, the fundamental frequency (F0) and the first three formants (F1, F2, F3) change linearly (F0: 103-125, F1: 220-720, F2: 1700-1240, F3: 2580-2500 Hz) while F4 (3600 Hz) and F5 (4500 Hz) remain constant.

Stimuli were delivered and responses were collected with the Bio-logic Navigator Pro System (Natus Medical Incorporated, San Carlos, California). The click was presented in rarefaction at a rate of 31.25 Hz and the 'da' was presented in alternating polarity at a rate of 10.9 Hz. Both were presented monaurally through an insert earphone (Etymōtic Research, Elk Grove Village, IL) to the right ear at 80 dB SPL. During data collection, the participant sat in a comfortable chair and was instructed to relax or watch a movie of his or her choice.

Both responses were collected using Ag/AgCl electrodes applied in a vertical montage, with Cz referenced to the right ear lobe and forehead as ground. Sampling was at 24 kHz for the ABR and 12 kHz for the FFR. Evoked responses were averaged online. For the ABR, responses were filtered from 70 to 2000 Hz and epoched over a 10.66 ms window beginning at stimulus onset. FFRs were filtered from 100 to 2000 Hz and averaged over a 75 ms epoch that began 15.8 ms prior to stimulus onset. An artifact rejection criterion of ±23.8 μV was applied to both the ABR and FFR. Three blocks of 2000 artifact-free trials were collected in response to the click and two blocks of 3000 artifact-free trials were collected in response to 'da'; thus after combining blocks, there were 6000 artifact-free sweeps of each response.

2.3. Data analyses

2.3.1. Timing

Neural response timing was quantified by identifying stereotyped peaks in the ABR and FFR. Peak picking was performed using previously published criteria (Krizman et al., 2012a). Briefly, for each participant, timing of peaks I, III, and V of the click-ABR and peaks V, A, C, D, E, F, and O of the FFR were visually identified (see Fig. 1). Click peaks originate at progressively more central structures of the auditory system, with peak I reflecting firing predominately in the distal 8th nerve and peak V reflecting activity from the lateral lemniscus and inferior colliculus, an auditory midbrain structure (Hall, 2006; Hood, 1998). The FFR peaks reflect processing in central subcortical structures, including the lateral lemniscus and inferior colliculus (Bidelman, 2018; White-Schwoch et al., 2016a) and each peak reflects processing of different stimulus features. Peaks V and A are in response to the stimulus onset, O is the response to the stimulus offset, the transition between the onset burst and formant transition is encoded at peak C, and periodicity corresponding to the F0 over the formant transition is encoded by peaks D, E, and F (Skoe and Kraus, 2010).

If an individual did not have a reliable peak, the participant's data was excluded only for that peak. All click peaks and many FFR peaks were identifiable in all individuals; other FFR peaks had at least 90% detectability in each of the six groups and the groups did not differ in their rates of detectability on any measure (Table 1). To provide an objective measure of timing, the stimulus-to-response lag was calculated for each participant as the time difference between stimulus and response that resulted in a maximal correlation between the two waveforms.

2.3.2. Magnitude

For the FFR, non-stimulus activity and broadband response magnitude were calculated as root mean square (RMS) amplitude over the 15.8 ms prestimulus interval and 19.5–44.2 ms of the response, respectively.

2.3.3. Spectral encoding

For the FFR, spectral encoding was analyzed using a fast Fourier analysis of the formant transition of 'da' (19.5–44.2 ms), a region of the response that includes peaks D, E, and F. Spectral encoding was compared for the fundamental frequency (F0) from 75 to 175 Hz, a neural correlate of pitch perception and for harmonics of the F0. The harmonics were divided into lower and higher harmonic bins. The lower harmonics comprise the first formant (F1) from 175 to 750 Hz of the 'da' and contribute to perception of phonetic content (i.e., what distinguishes the 'da' from a 'ga'). The higher harmonics are referred to as high frequency (HF), and correspond to frequencies above the first formant that are still within midbrain phase-locking limits (up to 1050 Hz).

2.3.4. Statistical analyses

A 2 (Sex: female, male) by 3 (Age: child, adolescent, adult) multivariate analysis of variance (MANOVA) was performed for the 16 measures of subcortical auditory processing. To characterize the age by sex interaction, a MANOVA was performed to look at differences between the three age groups separately for each sex. Planned comparisons of males and females at each age were performed using independent-samples *t*-test.

Click-ABR and FFR measures were entered together in a discriminant function analysis to determine if these measures

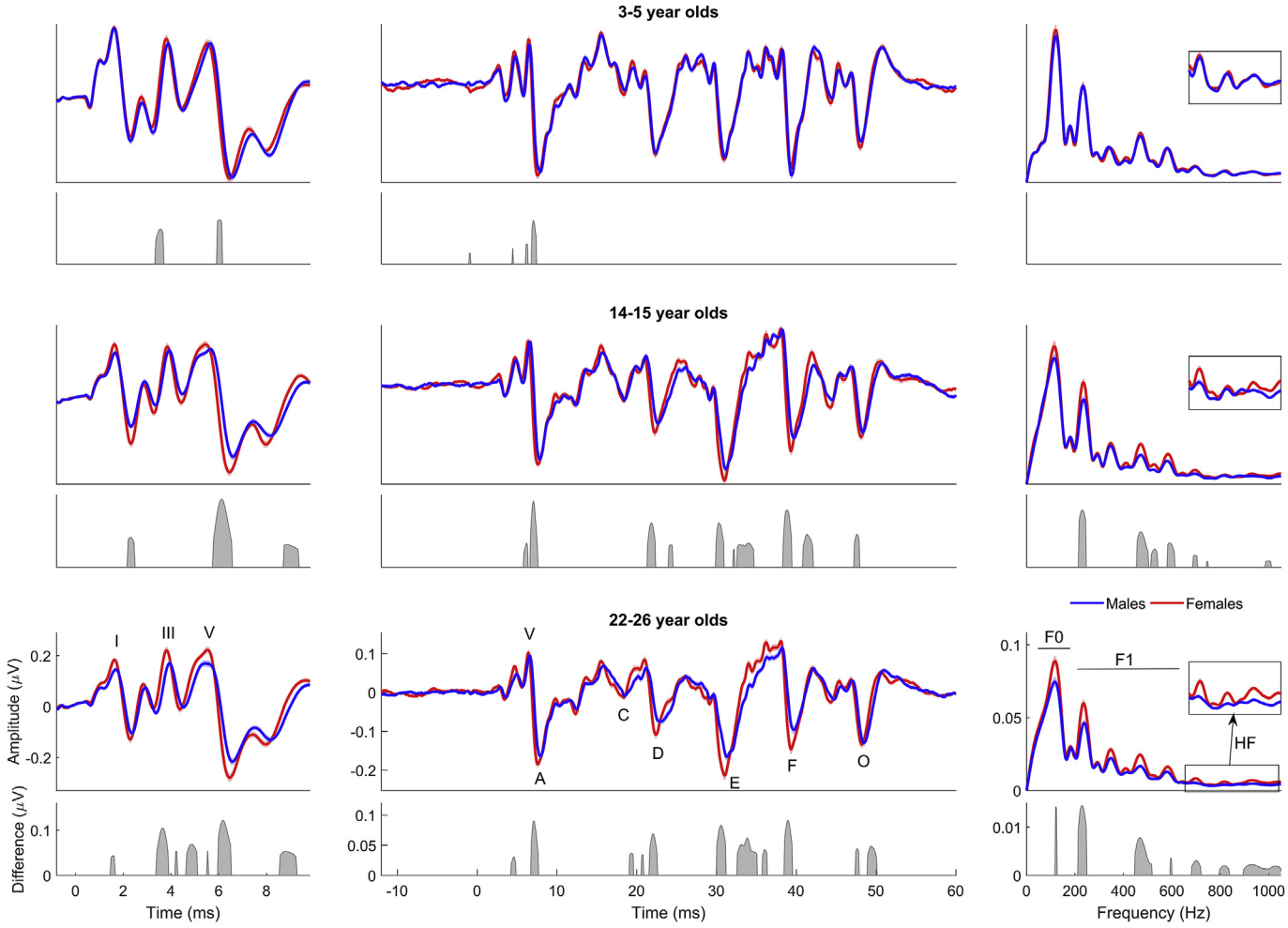

Fig. 1. Comparison of evoked responses between males and females at each age. Grand averages for each sex at each age group for males (blue) and females (red) in the time domain in response to the click (left panel), and the time (center panel) and frequency (right panel) domain in response to 'da'. Peaks and frequency regions are labeled on the adult responses (bottom). Below each grand average is a rectified difference plot showing differences between males and females that exceed an effect size d of 0.45, which corresponds to a p-value ~0.005. As evidenced by the lack of differences greater than the effect size cutoff, the 3–5 yr old responses are most similar between the sexes (top). Male and female responses are more distinct in adolescents (14–15 yrs, middle), and adults (22–26 yrs of age, bottom). The inset of each frequency plot highlights the emergence of sex differences in high harmonic encoding (750–1050 Hz). Prestimulus magnitude corresponds to the amplitude of the FFRs prior to stimulus onset (middle column, activity to the left of 0 ms). Response magnitude and encoding of the fundamental frequency (F0) and harmonics (F1 and HF) are measured over 19.5–44.2 ms, the time region encompassing formant transition peaks D, E, and F. With increasing age, males show a greater lengthening of peak timing and reduction in harmonic encoding than females. (see Fig. 2, Table 3).

Table 1
Detectability of click-ABR and FFR peaks for each age and sex group.

		Click-ABR			FFR to d						
		I	III	V	V	A	C	D	E	F	O
3–5 years	F	100%	100%	100%	100%	100%	98.75%	100%	100%	98.75%	100%
	M	100%	100%	100%	100%	100%	94.50%	100%	100%	100%	97.80%
14–15 years	F	100%	100%	100%	100%	100%	91.60%	97.60%	100%	100%	96.40%
	M	100%	100%	100%	100%	100%	96.50%	100%	100%	100%	97.70%
22–26 yrs	F	100%	100%	100%	100%	100%	97.70%	98.90%	100%	100%	97.70%
	M	100%	100%	100%	100%	100%	94.40%	95.50%	100%	98.90%	98.90%
	$\chi 2$ p =	1	1	1	1	1	.248	.056	1	.531	.658

could accurately classify individuals into their age and sex groups (out of 6 possible groups). We included 12 out of the 16 variables considered in our previous analysis. Peak C timing was excluded because it was not replicable for 23 participants across the dataset, and as part of the formant-transition region we included only the middle peak E, as the three peaks (i.e., D-E-F) were highly correlated and E was the only peak of those three with 100% detectability across participants (Table 1). Stimulus-to-response lag was also omitted as it overlapped with other timing measures. Data processing was performed using custom routines coded in Matlab (2015a) (The MathWorks, Inc., Natick, MA) and statistical analyses were performed in SPSS (SPSS Inc., Chicago, IL).

3. Results

Only the timing of peaks in response to a sound's onset (i.e., click-evoked peaks and peaks V and A of the FFR to 'da') differed at all ages, in support of the cochlear hypothesis, while the remaining sex differences emerged across development, supporting the alternate, central-differences hypothesis (Figs. 1 and 2, Table 2). Males and females were most similar during early childhood and became increasingly different with age (Fig. 1, Tables 2 and 3), due to greater developmental changes in the male response (Fig. 2, Table 3). Across development, response timing became later and magnitude declined for males, while females maintained robust, earlier responses (Tables 3 and 4). Neural noise showed no sex differences at any point in development (Fig. 1, Table 2).

3.1. Group comparison

Across all measures, a multivariate analysis of variance revealed an effect of age ($F_{(32,\ 914)} = 7.019$, $p < .0005$, $\eta p^2 = 0.197$), and sex ($F_{(16,\ 457)} = 5.281$, $p < .0005$, $\eta p^2 = 0.156$) as well as an interaction of the two ($F_{(32,\ 914)} = 1.509$, $p = .036$, $\eta p^2 = 0.050$). With respect to the individual measures, the onset peaks V and A of the FFR and peaks III and V of the click-ABR, showed an effect of sex (all $p < .005$, Figs. 1 and 2, Table 2) but no interaction with age (all $p > .16$). Timing of the FFR formant transition peaks D and F and peak I of the click-ABR became more distinct between males and female with increasing age (all $p < .04$, Figs. 1 and 2, Table 2); this effect was also trending for transition peak E and offset peak O of the FFR (all $p < .065$). FFR stimulus-to-response lag, a composite measure of timing, and onset-to-formant transition peak C showed an effect of sex, similar to the onset peaks ($p < .0005$).

In the FFR, encoding of the fundamental frequency, a sound feature contributing to pitch perception, was lower in males than females across development ($p = .018$), while sex differences in encoding of harmonics, which convey phonetic information, emerged later in development (i.e., F1 and HF, all $p < .035$, Figs. 1 and 2, Tables 2 and 3). Males showed greater declines in encoding of harmonic information with increasing age (Figs. 1 and 2, Table 3). Response magnitude in the time domain (i.e., RMS) aligned with fundamental frequency sex differences ($p < .0005$) while prestimulus magnitude, a measure of neural noise, did not differ between the sexes ($p = .913$), but did become smaller over development ($p < .0005$). Overall, males showed a greater maturational decline (i.e., later timing, smaller magnitude), declining on 87.5% of measures. Females only declined on 37.5% of measures (Table 4).

3.2. Discriminant function analysis

To determine if the click-ABR and FFR measures independently contribute to predicting participant demographics, we ran a discriminant function analysis, which identified five functions for classifying participants by age and sex. In the discriminant analysis, of the five functions, the first explained 67.2% of the variance (canonical $R^2 = 0.35$), the second explained 21.9% of the variance (canonical $R^2 = 0.15$), the third explained 6.4% of the variance

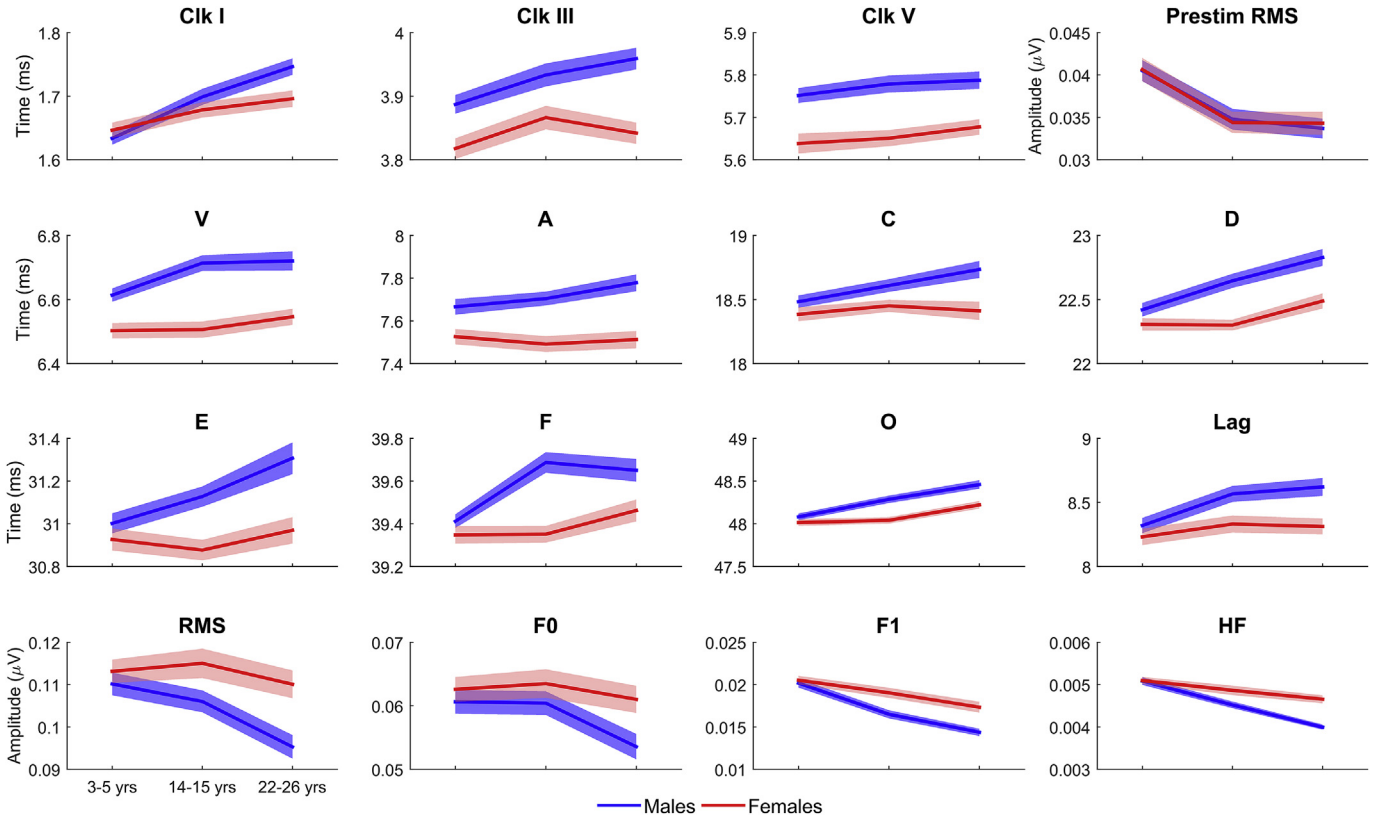

Fig. 2. Line plots showing changes in click-ABR and FFR measures across age in males (blue) and females (red). Solid lines represent means and shaded bars represent ±1 standard error. Consistent with previous reports (Skoe et al., 2015), responses became later and smaller during development. The emergence of sex differences for the later FFR peaks (D,E,F, and O), peak I of the click-ABR, and harmonic encoding (F1 and HF) resulted from a greater change in males from early childhood through young adulthood (Tables 2 and 3). By comparing males and females at each age group, we find that sex differences are minimal in early childhood: only timing of peaks III and V of the click-ABR and peaks V and A of the FFR differ. By young adulthood, the sexes differ on all measures except neural noise (i.e., Prestim RMS, Tables 3 and 4).

Table 2
MANOVA results for individual measures. Significant effects are bolded.

Component		F(df)	p	ηp^2
Click-ABR				
I peak timing (Clk I)	Age	**23.077 (2, 476)**	**<.0005**	**.089**
	Sex	2.641 (1, 476)	.105	.006
	Age x Sex	**3.561 (2,476)**	**.029**	**.015**
III peak timing (Clk III)	Age	**5.460**	**.005**	**.023**
	Sex	**29.932**	**<.0005**	**.060**
	Age x Sex	0.771	.463	.003
V peak timing (Clk V)	Age	2.222	.110	.009
	Sex	**46.331**	**<.0005**	**.090**
	Age x Sex	0.162	.850	.001
FFR to da				
Prestimulus magnitude (prestim RMS)	Age	**18.878**	**<.0005**	**.074**
	Sex	0.004	.950	0
	Age x Sex	0.112	.894	0
V peak timing (V)	Age	**4.676**	**.010**	**.019**
	Sex	**57.063**	**<.0005**	**.108**
	Age x Sex	1.836	.161	.008
A peak timing (A)	Age	1.173	.310	.005
	Sex	**36.592**	**<.0005**	**.072**
	Age x Sex	1.497	.225	.006
C peak timing (C)	Age	2.923	.055	.012
	Sex	**14.400**	**<.0005**	**.030**
	Age x Sex	1.497	.225	.006
D peak timing (D)	Age	**14.252**	**<.0005**	**.057**
	Sex	**30.682**	**<.0005**	**.061**
	Age x Sex	**3.305**	**.038**	**.014**
E peak timing (E)	Age	**4.186**	**.016**	**.017**
	Sex	**21.725**	**<.0005**	**.044**
	Age x Sex	2.917	.055	.012
F peak timing (F)	Age	**9.905**	**<.0005**	**.040**
	Sex	**26.814**	**<.0005**	**.054**
	Age x Sex	**4.674**	**.010**	**.019**
O peak timing (O)	Age	**20.981**	**<.0005**	**.082**
	Sex	**24.887**	**<.0005**	**.050**
	Age x Sex	2.916	.055	.012
Stimulus-to-response lag	Age	**6.215**	**.002**	**.026**
	Sex	**14.906**	**<.0005**	**.031**
	Age x Sex	1.885	.153	.008
Response magnitude (RMS)	Age	**4.929**	**.008**	**.021**
	Sex	**11.965**	**.001**	**.025**
	Age x Sex	1.912	.149	.008
Fundamental frequency amplitude (F0)	Age	2.579	.077	.011
	Sex	**5.657**	**.018**	**.012**
	Age x Sex	0.592	.554	.003
First formant amplitude (F1)	Age	**34.760**	**<.0005**	**.129**
	Sex	**17.257**	**<.0005**	**.035**
	Age x Sex	**3.467**	**.032**	**.015**
High frequency amplitude (HF)	Age	**38.208**	**<.0005**	**.140**
	Sex	**19.926**	**<.0005**	**.041**
	Age x Sex	**7.264**	**.001**	**.030**

(canonical $R^2 = 0.05$), the fourth explained 3.2% of the variance (canonical $R^2 = 0.04$), and the fifth explained only 2.4% of the variance (canonical $R^2 = 0.02$).

In combination, these five discriminant functions (1 through 5) significantly differentiated the groups, $\Lambda = 0.505$, $\chi^2(60) = 339.143$, $p < .0005$. After removing the first function, the remaining functions (2 through 5) still significantly differentiated the groups, $\Lambda = 0.779$, $\chi^2(44) = 123.847$, $p < .0005$. However, removing the first two functions indicated that the remaining functions did not significantly differentiate the groups: 3 through 5 $\Lambda = 0.917$, $\chi^2(30) = 42.879$, $p = .060$; 4 through 5 $\Lambda = 0.965$, $\chi^2(18) = 17.855$, $p = .465$; 5 $\Lambda = 0.989$, $\chi^2(8) = 5.247$, $p = .731$.

Four discriminating variables loaded onto function 1, six discriminating variables loaded onto function 2, and the two remaining variables independently loaded onto functions 4 and 5 (Table 5). The two discriminant functions that significantly differentiated the groups accounted for 89.1% of the variance and are defined as:

Function 1 = 40.247−2.530*Clk I - 1.512*Clk III +.805*Clk V - .339*V + 1.539*A + .200*E + 48.708*F0 + 185.984*F1 + 291.447*HF + 28.934*prestim RMS - 55.056*RMS - 1.107*O

Function 2 = −41.565−3.854*Clk I - 0.209*Clk III +1.668*Clk V + 2.486*V + .527*A + .080*E + 40.976*F0 + 94.686*F1 - 93.649*HF + 24.193*prestim RMS - 44.673*RMS +.347*O

The functions that did not significantly contribute to age and sex classification are:

Function 3 = −39.663 + 2.868*Clk I −1.217*Clk III -1.885*Clk V −3.575*V + 1.430*A + 1.016*E + 32.225*F0 + 142.927*F1 -188.627*HF + 29.345* prestimRMS −46.664*RMS +.664*O

Table 3
Age effects for males and females. When looking at development separately for males and females, males showed declines on more measures than females, as evidenced by greater lengthening of peak timing and reduction of the response in the time and frequency domain. Significant differences are bolded.

Component		F(df)	p	ηp^2
Click				
Clk I	Female	**4.361 (2, 232)**	**.014**	**.037**
	Male	**23.073 (2, 245)**	**<.0005**	**.160**
Clk III	Female	2.122	.122	.018
	Male	**4.133**	**.017**	**.033**
Clk V	Female	1.045	.353	.009
	Male	1.368	.257	.011
Da				
Prestim RMS	Female	**10.573**	**<.0005**	**.085**
	Male	**8.408**	**<.0005**	**.065**
V	Female	0.867	.422	.008
	Male	**5.768**	**.004**	**.046**
A	Female	0.112	.894	.001
	Male	2.678	.071	.022
C	Female	0.495	.610	.004
	Male	**3.978**	**.020**	**.032**
D	Female	**3.078**	**.048**	**.026**
	Male	**13.412**	**<.0005**	**.100**
E	Female	0.573	.565	.005
	Male	**6.309**	**.002**	**.050**
F	Female	1.687	.187	.015
	Male	**12.859**	**<.0005**	**.096**
O	Female	**7.631**	**.001**	**.062**
	Male	**15.712**	**<.0005**	**.115**
Stimulus-to-response lag	Female	1.004	.368	.009
	Male	**7.076**	**.001**	**.055**
Response RMS	Female	0.336	.715	.003
	Male	**7.393**	**.001**	**.058**
F0	Female	0.331	.719	.003
	Male	**3.050**	**.049**	**.025**
F1	Female	**7.285**	**.001**	**.060**
	Male	**35.676**	**<.0005**	**.228**
HF	Female	**5.048**	**.007**	**.042**
	Male	**48.682**	**<.0005**	**.287**

Function 4 = −51.881 -.040*Clk I −7.356*Clk III +3.000*Clk V +1.955*V −1.290*A −.074*E −34.876*F0 -65.365*F1 +127.227*HF + 15.722* prestimRMS +28.372*RMS +1.277*O

Function 5 = 15.902 + 1.382*Clk I −.228*Clk III -4.920*Clk V +4.561*V −1.090*A + .118*E + 65.903*F0 -2.188*F1 +53.709*HF + 23.696*prestimRMS −37.742*RMS -.326*O

Using the first two functions to classify individuals by age and sex, overall, 40.9% of cases were correctly classified (Fig. 3). For all groups, the highest percentage to which individuals were classified was to their appropriate group, very few misclassifications fell above chance (i.e., greater than 16.7% of individuals from one group misclassified to another group). Of the 80 3–5 year old females, 33 were classified correctly (41.3%) and of their 91 male peers, 45 were classified correctly (49.5%). When children were misclassified, the highest likelihood was that they were correctly classified by age, but not by sex: 26.3% of the female children were misclassified as male children, and 20.9% of the male children were misclassified as female children. For the adolescents, of the 83 females, 31 were classified correctly (37.3%) while 26 of the 86 males (30.2%) were correctly classified. Misclassifications were more likely by age, but not sex. Females tended to be misclassified as either female child (15.7%) or female young adult (14.5%), while males tended to be misclassified as male young adult (22.1%). Of the 87 female young adults, 25 were correctly classified (28.7%), and 51 of the 89 (57.3%) young adult males were assigned to their correct sex and age group. Adult males showed no specific pattern of misclassification, while females tended to be misclassified by sex but not age: 23% were categorized as young adult males.

4. Discussion

From our findings, we draw four conclusions: (1) sex differences over development are driven by an accelerated lengthening of peak timing and reduction in response magnitude and frequency encoding in males, while females maintain earlier, larger responses; (2) sex differences are not monolithic but emerge through multiple processes reflected by distinct sex differences across evoked response components; (3) cochlear differences cannot account for all observed sex differences in subcortical auditory processing, thus some of these differences are central in origin; and, (4) the differences are not driven by changes in neural noise, as this measure did not differ between the sexes.

Only onset timing showed an effect consistent with a cochlear origin of sex differences. Therefore, we conclude that subcortical sex differences are more than a mere reflection of cochlear differences. Even though the cochlea is structurally and functionally mature, and sex differences in basilar membrane length and thickness are present before the earliest age group tested (Moore and Linthicum, 2007; Sato et al., 1991), we find that sex differences are continuously and cumulatively forged across development. The 3–5 year old males and females only differed on peak timing of click III and V and FFR V and A, while adult males and females differed on everything except neural noise (Fig. 2, Tables 3 and 4). In line with a continued emergence of sex differences, the types of errors made in group classification in the discriminant analysis changed with increasing age of the participant. The analysis was successful in classifying ~70% of the 3–5 year old children into their appropriate age, but showed a high error rate in grouping by sex. In contrast, most adolescents were successfully classified by sex, not age, with ~68% of female adolescents correctly classified as female, and ~65% of male adolescents correctly classified as male.

If not cochlear differences, then what causes male and female subcortical auditory processing to diverge over development? Two other factors have been proposed to account for subcortical sex differences: differences between males and females in head size and in hormones (Dehan and Jerger, 1990).

Head size is presumed to be a proxy for brain size (Dempsey et al., 1986). Thus the larger head size, on average, for men results in longer neural tracts for the signal to traverse to reach the subcortical nuclei as well as greater brain volume separating these subcortical generators and the recording electrode (Chambers et al., 1989). These factors together would result in a delayed or smaller response as measured at the scalp (Yamaguchi et al., 1991).

Our results do not support this interpretation, however. In early childhood and adolescence, relative head size between the sexes is fairly constant, with male head size being, on average, ~0.8 cm larger than female head size at both time points (Nellhaus, 1968). Even though relative head size between males and females is constant, we find that auditory processing sex differences not present in early childhood, specifically, timing of later FFR peaks and harmonic encoding, are evident in adolescence. Furthermore, for both males and females, head size increases ~5 cm in circumference from early childhood into young adulthood (Nellhaus, 1968). Despite similarity in head growth, females do not change on a number of measures as function of age, while males decline on almost all measures of subcortical auditory processing. This aligns with other studies showing that, even when comparing males and females of equal head size, sexual dimorphism of ABR latency and magnitude persist (Trune et al., 1988).

Table 4
Group comparisons across development. Within-age sex effects for individual measures. Mean ± 1 standard deviation for each group and independent-samples t-tests for those groups are reported. Significant group differences, as determined by a Bonferroni correction value of .0005, are in bold.

Component	Age Group	Males	Females	t (df)	p	d
Click						
Clk I	3–5 yrs	1.63 (0.09)	1.65 (0.11)	0.847 (169)	.398	0.2
	14–15 yrs	1.7 (0.12)	1.68 (0.11)	1.174 (167)	.242	0.17
	22–26 yrs	1.75 (0.12)	1.7 (0.12)	**2.785 (174)**	.006	0.42
Clk III	3–5 yrs	3.89 (0.14)	3.82 (0.14)	**3.207 (169)**	.002	0.5
	14–15 yrs	3.93 (0.17)	3.87 (0.17)	**2.592 (167)**	.010	0.35
	22–26 yrs	3.96 (0.16)	3.84 (0.15)	**4.968 (174)**	**<.0005**	**0.77**
Clk V	3–5 yrs	5.75 (0.17)	5.64 (0.21)	**3.919 (169)**	**<.0005**	**0.58**
	14–15 yrs	5.78 (0.19)	5.65 (0.17)	**4.638 (167)**	**<.0005**	**0.72**
	22–26 yrs	5.79 (0.19)	5.68 (0.17)	**4.027 (174)**	**<.0005**	**0.61**
Da						
Prestim RMS	3–5 yrs	0.04 (0.01)	0.04 (0.01)	0.068 (169)	.946	0
	14–15 yrs	0.03 (0.01)	0.03 (0.01)	0.223 (167)	.824	0
	22–26 yrs	0.03 (0.01)	0.03 (0.01)	0.338 (174)	.736	0
V	3–5 yrs	6.61 (0.19)	6.5 (0.21)	**3.571 (169)**	**<.0005**	**0.55**
	14–15 yrs	6.71 (0.23)	6.51 (0.23)	**5.906 (167)**	**<.0005**	**0.87**
	22–26 yrs	6.72 (0.28)	6.55 (0.23)	**4.493 (174)**	**<.0005**	**0.66**
A	3–5 yrs	7.67 (0.35)	7.53 (0.32)	2.715 (169)	.007	0.42
	14–15 yrs	7.7 (0.3)	7.49 (0.34)	**4.362 (167)**	**<.0005**	**0.65**
	22–26 yrs	7.78 (0.36)	7.51 (0.37)	**4.775 (174)**	**<.0005**	**0.74**
C	3–5 yrs	18.48 (0.47)	18.39 (0.48)	1.336 (163)	.184	0.19
	14–15 yrs	18.61 (0.45)	18.45 (0.42)	2.287 (157)	.024	0.37
	22–26 yrs	18.73 (0.62)	18.41 (0.65)	3.279 (167)	.001	0.50
D	3–5 yrs	22.42 (0.5)	22.31 (0.44)	1.593 (169)	.113	0.23
	14–15 yrs	22.65 (0.5)	22.3 (0.39)	**4.938 (165)**	**<.0005**	**0.78**
	22–26 yrs	22.83 (0.62)	22.49 (0.55)	**3.806 (169)**	**<.0005**	**0.58**
E	3–5 yrs	31 (0.45)	30.93 (0.46)	1.088 (169)	.278	0.15
	14–15 yrs	31.13 (0.44)	30.88 (0.43)	**3.732 (167)**	**<.0005**	**0.57**
	22–26 yrs	31.31 (0.7)	30.97 (0.58)	3.498 (174)	.001	0.53
F	3–5 yrs	39.41 (0.3)	39.35 (0.37)	1.244 (168)	.215	0.18
	14–15 yrs	39.69 (0.45)	39.35 (0.35)	**5.435 (167)**	**<.0005**	**0.84**
	22–26 yrs	39.65 (0.5)	39.46 (0.48)	2.547 (173)	.012	0.39
O	3–5 yrs	48.08 (0.37)	48.01 (0.34)	1.215 (167)	.226	0.2
	14–15 yrs	48.29 (0.4)	48.04 (0.35)	**4.222 (162)**	**<.0005**	**0.67**
	22–26 yrs	48.46 (0.46)	48.22 (0.41)	**3.572 (171)**	**<.0005**	**0.55**
Stimulus-to-response lag	3–5 yrs	8.32 (0.56)	8.23 (0.56)	1.025 (169)	.307	0.16
	14–15 yrs	8.57 (0.57)	8.33 (0.61)	2.580 (167)	.011	0.41
	22–26 yrs	8.62 (0.65)	8.31 (0.57)	3.341 (174)	.001	0.51
Response RMS	3–5 yrs	0.11 (0.025)	0.113 (0.024)	0.777 (169)	.438	0.12
	14–15 yrs	0.106 (0.024)	0.115 (0.031)	2.095 (167)	.038	0.32
	22–26 yrs	0.095 (0.026)	0.11 (0.031)	3.444 (174)	.012	0.52
F0	3–5 yrs	0.061 (0.018)	0.063 (0.017)	0.729 (169)	.467	0.11
	14–15 yrs	0.06 (0.017)	0.063 (0.02)	1.055 (167)	.293	0.16
	22–26 yrs	0.054 (0.019)	0.061 (0.02)	2.552 (174)	.012	0.36
F1	3–5 yrs	0.02 (0.005)	0.021 (0.004)	0.482 (169)	.630	0.22
	14–15 yrs	0.016 (0.004)	0.019 (0.005)	3.350 (167)	.001	0.66
	22–26 yrs	0.014 (0.004)	0.017 (0.006)	**3.888 (174)**	**<.0005**	**0.59**
HF	3–5 yrs	0.006 (0.002)	0.006 (0.001)	0.109 (169)	.913	0
	14–15 yrs	0.005 (0.001)	0.006 (0.002)	2.572 (167)	.011	0.63
	22–26 yrs	0.004 (0.001)	0.005 (0.002)	**5.960 (174)**	**<.0005**	**0.63**

Hormone differences between males and females are more likely to account for sexual dimorphism of subcortical auditory processing during adolescence and young adulthood. In young adult females, fluctuations in estrogen level are known to affect timing of the click-ABR (Caruso et al., 2003; Elkind-Hirsch et al., 1992) and FFR peaks (Liu et al., 2017). These fluctuations result in later peaks and smaller amplitudes at certain points in the ovarian cycle (Elkind-Hirsch et al., 1994), more consistent with an adult male response, which could account for the poorer classification of some young adult females in the discriminant analysis. However, the literature lacks consensus on these effects (Al-Mana et al., 2008).

One possible explanation for the inconsistency across studies is that hormonal effects are not linear, but interact with environment and experience. Findings in both animals and humans support this idea. For example, over the course of a year, response properties of the bat midbrain vary in a sex-specific manner to adapt to the vocalizations specific to the behaviors of a given season (e.g., mating versus infant rearing) and this is believed to result from an interaction of hormones and environment (Miller et al., 2016). In female mice, the interaction of post-partum hormone changes together with exposure to pup calls leads to greater temporal precision and earlier peaks in the ABRs of mother mice compared to virgin or non-mother caregiver mice (Miranda et al., 2014). Likewise, in humans, experience is known to influence subcortical auditory processing. Lifelong experiences sharpen aspects of auditory processing specific to that experience, such as enhanced processing of

Table 5
Absolute correlation between each variable and any discriminant function. A higher number indicates a greater weighting of that dependent variable for that function, and variables with positive and negative coefficients are contributing to the function in opposite ways.

	Function				
	1	2	3	4	5
HF	**.610**	-.355	-.343	.188	-.005
F1	**.573**	-.334	-.092	-.016	-.087
Clk I	**-.449**	.087	.192	-.127	.138
Prestim RMS	**.358**	.181	.285	.171	.183
V	-.275	**.772**	-.243	-.056	.227
A	-.158	**.686**	.076	-.184	-.083
Clk V	-.175	**.679**	-.274	-.012	-.403
E	-.254	**.474**	.288	-.080	.092
O	-.469	**.471**	.222	.310	-.142
RMS	.228	**-.359**	-.318	.053	.170
Clk III	-.255	.513	-.207	**-.597**	-.174
F0	.148	-.243	-.296	.012	**.359**

the fundamental frequency for bilinguals (Krizman et al., 2012b) or faster timing and stronger harmonic encoding in musicians (Kraus and Chandrasekaran, 2010). Therefore, experiential and hormonal effects on subcortical auditory processing may work in tandem to influence an individual's response profile and may also account for the poorer discriminant analysis classification in the older age groups.

The magnitude and timing of the scalp-recorded FFR are affected by the synchrony of firing across a population of neurons. Thus, the effects of hormones and experience may occur through microstructural changes affecting communication among neurons, such as changes in synaptic density, efficiency or axon myelination. These FFR differences may reflect differences in connectivity in the ascending auditory pathway or differences in the corticofugal tuning of the descending pathway. The inferior colliculus, the primary generator of the scalp-recorded FFR, is a central hub of auditory processing, receiving and sending connections to subcortical and cortical regions of the brain, both auditory and non-auditory (Ito and Malmierca, 2018; Malmierca, 2015).

The interplay between the ascending auditory pathway and cortiofugal modulation of it is important to consider, as sex differences are not limited to subcortical structures. It is well known that there is sexual dimorphism of the cortex, likely including the auditory cortex (Giedd et al., 1997; Lenroot and Giedd, 2010; Sisk and Zehr, 2005). Indeed, activation patterns of auditory cortex in response to language (Phillips, 2000; Wallentin, 2009) show sexual dimorphism, albeit not consistently (Etchell et al., 2018; Sommer et al., 2004). These cortical differences may lead to differences in the corticofugal tuning of subcortical structures, manifesting as sex differences in the scalp-recorded FFR. Methods that bias cortical generators such as MEG, are likely to be useful in revealing sex differences in the cortical FFR.

A number of language disorders have a higher prevalence in males compared to females (Felix et al., 2018), including autism spectrum disorder and reading or language disabilities. Converging evidence points to biological sex as an underlying factor for the difference in incidence or severity of these disorders (Cosgrove et al., 2007; Shors et al., 2001; Tabatadze et al., 2015). Yet, the neural foundations for these differences have remained poorly understood. Given that regardless of the sex of the child, individuals diagnosed with a language disorder demonstrate impairments in the very FFR measures that males are lower on compared to females (Banai et al., 2009; Russo et al., 2008), these findings may provide insight into the otherwise limited knowledge that currently exists on this topic. This similarity raises the possibility that baseline auditory processing for males is lower than females, thereby increasing their susceptibility to or risk for communication disorders. More broadly, these findings may be important for injury, stress or other impairments to auditory function and may underscore the need for different therapeutic or intervention strategies for males and females.

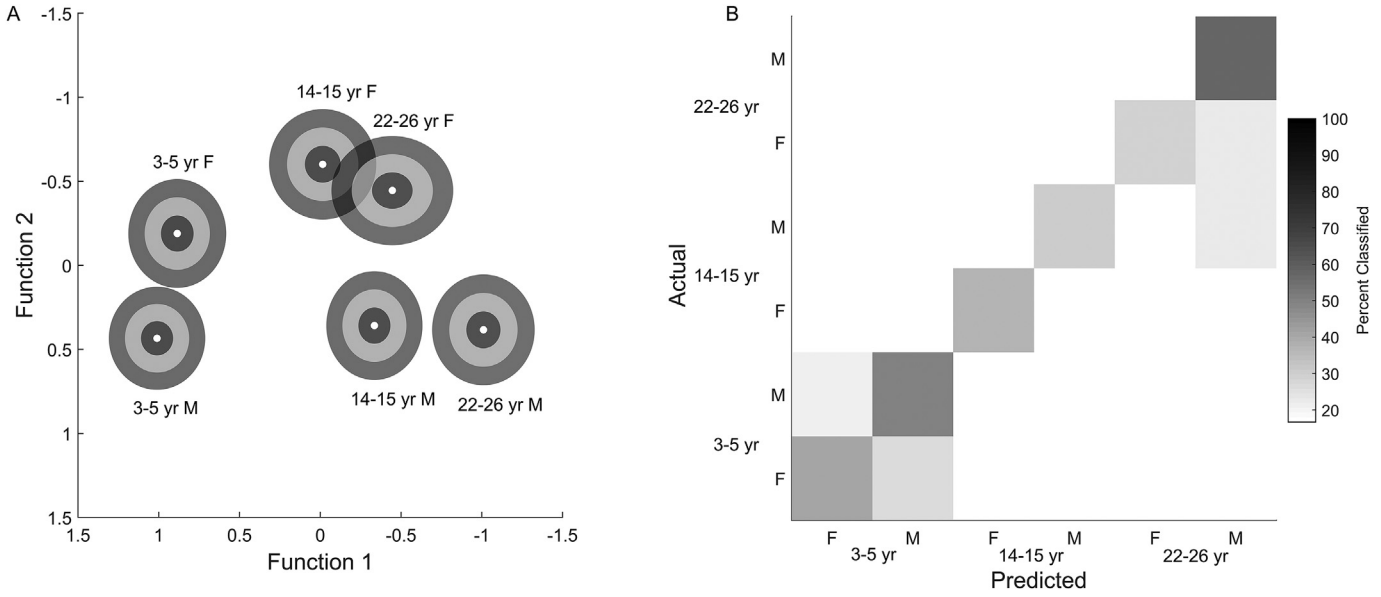

Fig. 3. Discriminant function (A) and classification (B) plots from the discriminant analyses. For the discriminant function plot, the center of each bullseye represents the mean of the two functions for the six age by sex groups. Each ring represents +1 standard error. Function 1 predominately separates by age while function 2 predominately separates by sex. The classification plot is zeroed (white) at chance classification (16.7%). For all groups, the predicted group membership was highest for actual membership (i.e., the diagonal). When errors were made for the 3–5 year olds, they were misclassified by sex but not age. For the 14–15 year olds, males were misclassified by age, but not sex. There was also a subset of the 22–26 year old females who were misclassified by sex but not age.

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Appendix A. Supplementary data

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