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Masculinized otoacoustic emissions in female spotted hyenas (*Crocuta crocuta*)

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Abstract

In humans and rhesus monkeys, click-evoked otoacoustic emissions (CEOAEs) are stronger in females than in males, and there is considerable circumstantial evidence that this sex difference is attributable to the greater exposure to androgens prenatally in males. Because female spotted hyenas are highly androgenized beginning early in prenatal development, we expected an absence of sexual dimorphism in the CEOAEs of this species. The CEOAEs obtained from 9 male and 7 female spotted hyenas confirmed that expectation. The implication is that the marked androgenization to which female spotted hyenas are exposed masculinizes the cochlear mechanism responsible for CEOAEs. The CEOAEs measured in 3 male and 3 female hyenas that had been treated with anti-androgenic agents during prenatal development were stronger than the CEOAEs of the untreated animals, in accord with the implied inverse relationship between prenatal androgen exposure and the strength of the cochlear mechanisms producing CEOAEs. The CEOAEs of three ovariectomized females and two castrated males were essentially the same as those for the untreated females and males, suggesting that there is little or no activational effect of hormones on CEOAE strength in spotted hyenas. Distortion product OAEs (DPOAEs) also were measured. Those sex differences also were generally small (as they are in humans), and the effects of the anti-androgen agents were inconsistent. Thus, prenatal androgen exposure apparently does affect OAEs, but the effects appear to be greater for the reflection-based cochlear mechanism that underlies CEOAEs than for the nonlinear cochlear mechanism underlying DPOAEs.
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The mammalian cochlea produces sounds known as otoacoustic emissions or OAEs (Kemp, 1978; Probst et al., 1991). In humans and rhesus monkeys, these emissions are sexually dimorphic, with females having stronger OAEs than males (see below for details). There is circumstantial evidence linking this sex difference to the actions of androgens secreted by the fetal testes of male mammals (McFadden, 1998, 2002). Described here are measurements of OAEs obtained from spotted hyenas (*Crocuta crocuta*), a species in which females are exposed to substantial concentrations of androgens in utero (Licht et al., 1992; Yalcinkaya et al., 1993). If it is correct that exposure to prenatal androgens reduces the strength of OAEs, then one might anticipate the sexual dimorphism observed in the OAEs of humans and rhesus monkeys to be reduced or eliminated in spotted hyenas.

The OAEs produced by the cochlea propagate back through the middle-ear system into the external ear canal where they can be measured using small microphone systems (e.g., Kemp, 1978; Probst et al., 1991). Only three of the several known forms of OAEs will be of interest here. Click-evoked OAEs (CEOAEs) are echo-like sounds that are produced by the cochlea in response to the presentation of brief acoustic stimuli (for more details, see McFadden et al., 2006). Spontaneous OAEs (SOAEs) are weak tonal sounds that are continuously emitted by the majority of normal-hearing human ears without

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the need for eliciting stimuli from the experimenter. Distortion product OAEs (DPOAEs) are tonal signals created by certain nonlinear mechanisms of the cochlea during the simultaneous presentation of two primary tones close in frequency (see Probst et al., 1991). The DPOAE of interest here appears at the frequency $2f_{lower}–f_{higher}$ (a frequency below the two primaries). DPOAEs are believed to be produced by a nonlinear cochlear mechanism that is fundamentally different from the linear, reflection-based mechanism that is responsible for CEOAEs and SOAEs (Shera and Guinan, 1999, 2003).

In humans, CEOAEs are stronger (McFadden et al., 1996; McFadden and Pasanen, 1998; McFadden and Shubel, 2003) and SOAEs are more numerous (Bilger et al., 1990; Talmadge et al., 1993; McFadden, 1993b; McFadden and Loehlin, 1995; McFadden and Pasanen, 1999; McFadden and Shubel, 2003) in females than in males, and these sex differences exist in newborns as well as in adults (Strickland et al., 1985; Burns et al., 1992; Morlet et al., 1995, 1996). In humans (Gaskill and Brown, 1990; Lonsbury-Martin et al., 1991; Moulin et al., 1993; Cacace et al., 1996; Dhar et al., 1998; Bowman et al., 2000) and in rhesus monkeys (Torre and Fowler, 2000; McFadden et al., 2006), the sex difference in DPOAEs appears to be much smaller than those for CEOAEs and SOAEs. A parsimonious interpretation of these facts is that the linear cochlear mechanism responsible for producing CEOAEs and SOAEs can be permanently weakened by exposure to high levels of androgens during prenatal development (McFadden, 1998, 2002)–an organizational effect of androgen exposure–and that the nonlinear mechanism responsible for DPOAEs is less susceptible to the prenatal effects of androgens.[1]

In a companion paper, we report that rhesus monkeys exhibit a substantial sex difference in CEOAEs and little or no sex difference in DPOAEs (McFadden et al., 2006), just as in humans (Gaskill and Brown, 1990; Lonsbury-Martin et al., 1991; Moulin et al., 1993; Cacace et al., 1996; Dhar et al., 1998; Bowman et al., 2000). This suggests that the cochlear mechanisms underlying OAEs in these two species may share some important similarities developmentally, a suggestion that currently is difficult to evaluate because so little is known about sex differences in OAEs in other species.

Female spotted hyenas are the only extant mammals that display a pseudoscrotum instead of an external vagina. They also have a hypertrophied penile clitoris, through which they urinate, mate, and give birth (Matthews, 1939). Current belief suggests that such "masculinization" of the external genitalia requires the action of androgens during fetal life (Jost, 1970). Additional findings supporting the hypothesis that female spotted hyenas have been "androgenized" in utero include: female spotted hyenas are somewhat larger than males and totally dominate adult immigrant males within clans in nature (Kruuk, 1972); the traditional sex differences in Onuf's nucleus in the spinal cord (Forger et al., 1996) and in a sexually dimorphic nucleus in the hypothalamus (Fenstemaker et al., 1999) are attenuated in spotted hyenas.

The ovaries of the pregnant spotted hyena secrete substantial quantities of androstenedione (Lindeque et al., 1986; Glickman et al., 1987), which is converted to testosterone and estradiol by the placenta and transferred to the developing fetus (Licht et al., 1992; Yalcinkaya et al., 1993). Although the essential development of masculine external genitalia in female (and male) spotted hyenas may be an androgen-*independent* phenomenon (Drea et al., 1999; Glickman et al., 2005), in utero treatment with anti-androgens does produce profound effects on genital morphology (Drea et al., 1998), reproduction (Drea et al., 2002), and endocrine function (Place et al., 2002) in both females and males (also see Glickman et al., 2005).

Given the implication that some OAEs appear to be affected by androgenic mechanisms operating early in development and the apparent blunting of certain androgen-dependent sexual dimorphisms in spotted hyenas, we predicted that the sexual dimorphism in OAEs, previously observed in humans and rhesus monkeys, would be attenuated, or eliminated, in *C. crocuta*. Furthermore, because the colony at the University of California at Berkeley contained some spotted hyenas that were treated with anti-androgens in utero and some that were gonadectomized after birth, we hoped to make a preliminary assessment of the organizational and activational effects of gonadal steroids on the cochleas and OAEs in this species. Because DPOAEs exhibit only small sex differences in humans (Gaskill and Brown, 1990; Lonsbury-Martin et al., 1991; Moulin et al., 1993; Cacace et al., 1996; Dhar et al., 1998; Bowman et al., 2000) and rhesus monkeys (Torre and Fowler, 2000; McFadden et al., 2006), we had little reason to expect a sex difference or a treatment effect on the DPOAEs of spotted hyenas.

Methods

Animals

Measurements were made on a total of 14 male and 13 female spotted hyenas. Of these, 9 males and 7 females were untreated; the others were treated as detailed below. These animals all were born and raised at the Field Station for the Study of Behavior, Ecology, and Reproduction at UC Berkeley, and all were housed singly or in small groups in outdoor enclosures. At the time of testing, all were adults, aged 4 to 16 years. The oldest animals, two 16-year-old females, were in the ovariectomized group. The mean ages (±SDs) of the untreated males and untreated females were 7.2 (±3.7) and 6.9 (±3.6) years, respectively, and all of the anti-androgen-treated animals were 9 years old, save for one 8-year-old female. Sexual maturity is typically achieved at about age 2 in males and about age 2.5–3 in females. In the wild, spotted hyenas typically live until age 15–20; in captivity, until age 25–30.

While pregnant, the mothers of 6 of the 27 animals were administered flutamide (two males) or flutamide plus finasteride (one male and three females). Flutamide blocks the androgen receptor, and finasteride blocks the enzymatic conversion of testosterone to 5α-dihydrotestosterone (DHT). Flutamide was administered daily during the final 75–89 days of pregnancy (the average gestation period is about 110 days), and finasteride was administered daily during the final 48–72 days (details of these administrations can be found in Place et al., 2002, Table 1.

[1] The reason for emphasizing prenatal androgen exposure is that, at birth and from about age 6 months until puberty, the circulating hormone levels in children are the same in both sexes. Beginning at birth, human males do exhibit a "second surge" of testosterone production that lasts about 6 months (Smail et al., 1981). Presumably, additional masculinization and defeminization is accomplished during this second surge, but details are still unknown. Male spotted hyenas also have substantially higher concentrations of testosterone than females during the immediate neonatal period. However, female spotted hyenas have high concentrations of androstenedione at birth, and that continues to be true for the first months of life while the male concentration of testosterone is declining (Frank et al., 1991; Glickman et al., 1992).

One male hyena was castrated on postnatal day 34, and one male was castrated at 10 years of age (well past puberty). One female was ovariectomized on postnatal day 30, and two others were ovariectomized at about 12 years of age (near the end of the reproductively active period of life). The two castrated males were administered androgens during adulthood on an intermittent, short-term basis, and, as adults, the ovariectomized females were administered estrogens on an intermittent, short-term basis. None of these animals had received sex steroids for at least 4 months prior to OAE testing.

Procedure and equipment

All OAE measurements were made in a room typically used as a surgery and located a short distance from the animal enclosures. During measurement sessions, the air-handling equipment and some nearby refrigerators were turned off to make the room as quiet as possible. Although the room was not optimal for recording OAEs, the custom-written data-acquisition software had various provisions for rejecting noisy samples, and the end result was acceptably quiet measurements.

Initially, each animal was darted with a combination of ketamine, xylazine, and atropine prior to transport to the surgery. Supplementary injections of ketamine alone were administered as needed to minimize jaw and head movements and muscle twitches. Ketamine is commonly used when recording OAEs in non-humans (Martin et al., 1999; Torre and Fowler, 2000). In a few instances, isofluorane vapors were administered via mask when the ketamine was inadequate to maintain the necessary level of immobilization. No changes in the strength of the OAEs were observed following the supplementary doses or gas anesthetic; the waveforms were simply more free of movement and noise artifacts. Administration of gas anesthetic produced no noticeable increase in the noise floor.

The geometry and size of the hyena ear canal allowed the entire microphone assembly to be located deep within the canal, making for a more stable preparation than is typical in humans and rhesus monkeys. However, to obtain a proper seal in the canal, larger-than-standard foam eartips (1.9 cm diameter) had to be obtained from Etymotic Research. The substantial diameter and length of the canal required that the seal be carefully evaluated at the beginning of the OAE session. When the eartip was correctly positioned, the seal was sufficiently tight that the ambient noise level of the test room was not measurable in the ear canal for the remainder of the testing of that ear. For most ear canals, cerumen ("earwax") was removed prior to insertion of the eartip using a cotton-tipped swab under direct otoscopic visualization.

Many details of stimulus presentation, data collection, and data analysis were similar to those described for rhesus monkeys (McFadden et al., in press). The microphone was different: an Etymotic Research ER-10B+. The data acquisition board was the same (National Instruments PCI-4451), but it was located in an external enclosure (Magma CB1F) connected to the cardbus of a laptop computer (Macintosh G3 Powerbook). The sampling rate for generating acoustic stimuli and digitizing the microphone output was the same (50 kHz; 16-bit precision). Timbuktu software again allowed a highly experienced OAE experimenter in Austin to work directly with the on-site experimenters during data collection (see Pasanen and McFadden, 2004).

CEOAEs and DPOAEs were obtained from both ears of each spotted hyena (an early attempt to find SOAEs failed). For CEOAEs, the click stimuli again were 100-μs electrical pulses presented in sets of 10 at a nominal rate of 10/s with 500 ms between sets. Data were collected for two click levels: 75 and 69 dB peak-equivalent sound-pressure level (dB SPL re 20 μPa). Prior to data collection from each ear, the strength of the click produced by the ER-2 earphone was individually adjusted to produce the target click levels. Thus, any individual or sex differences in the volume of the ear canal were largely nulled out, and the clicks were essentially equally strong at the ear drum in all ears tested. The higher click level was always tested before the lower level. Whether the left or right ear was tested first was determined pseudorandomly for each hyena.

Beginning at the onset of each click, 50 ms of the echo-like response was collected. Noisy time segments in the CEOAE response were rejected as in the rhesus monkey study (see McFadden and Shubel, 2003, for details), and the cochlear responses from at least 250 clicks were averaged for each test condition. The initial 6 ms of the averaged waveform was discarded in order to eliminate the click stimulus itself and most of the ringing in the ear canal and middle-ear system, the next 20.48 ms of the averaged waveform was bandpass-filtered between 1.0 and 5.0 kHz, and the rms level of the resulting waveform was calculated and transformed into dB SPL (re 20 μPa).

As a further precaution against the inclusion of noisy data, the 20.5-ms segment of the click-evoked waveform obtained with the 75-dB click (beginning 6 ms post-click) was cross-correlated with the corresponding segment obtained with the 69-dB click from that same ear, and when the maximum value of the cross-correlation was lower than about 0.6 and/or when the maximum value occurred for a time lag other than zero, the waveforms were examined carefully. This led to the exclusion of the CEOAE data for the left ears of two untreated females. A lack of similarity in these responses suggests that one or both responses were excessively noisy, possibly originating from a poor seal of the eartip in the ear canal.

As described for rhesus monkeys (McFadden et al., 2006), DPOAEs were collected with equal-level primary tones having a frequency ratio (f_{higher}/f_{lower}) of approximately 1.21 and a duration of 4 s. Functions relating DPOAE level to primary-tone level (input/output functions) were obtained for each of six pairs of primary frequencies generating DPOAEs in each of three frequency regions (2.0–2.5 kHz, 3.3–3.8 kHz, and 5.0–5.5 kHz) that were tested in a random order. Again, the final dependent variable was the level of the primary tones necessary to produce a DPOAE of 0 dB SPL. Data for the 2-kHz region were deleted for one untreated male hyena and two untreated females because the noise floors associated with those measurements were larger than our normative values.

CEOAEs always were tested first in each ear because the calibration procedure for CEOAEs was especially revealing about the tightness of the seal of the probe tip. Test sessions took between 60 and 90 min. This study was approved as being in compliance with the NIH *Guide for the Care and Use of Laboratory Animals* by animal care committees at both universities.

In part because the Ns here are small, we will emphasize effect sizes over statistical significance. Effect sizes were calculated by dividing the difference between the two means being compared by the square root of the weighted mean of the two variances. For comparisons of this sort, Cohen (1992) has suggested that effect sizes of 0.2, 0.5, and 0.8 be regarded as small, medium, and large, respectively.

Results

Untreated animals

Click-evoked OAEs

The evoked OAEs of untreated female spotted hyenas were not stronger than those of the males (see Fig. 1). This is in accord with our predictions and unlike the facts for humans (McFadden et al., 1996; McFadden and Pasanen, 1998) and rhesus monkeys (McFadden et al., 2006). The average CEOAEs were actually slightly weaker for females than for males, and that was true for both click levels and both ears. However, no comparison between the two sexes even approached statistical significance. The effect sizes for the sex differences in the various CEOAE measures are shown in the top half of Table 1.

The CEOAE data were discarded for the left ears of two untreated females because they failed the cross-correlation test (see above). Both animals were retested, with the same result. The CEOAE data for the right ear of another untreated female were lost for technical reasons.

Distortion product OAEs

As noted, the strength of the 2f1−f2 distortion product was measured for six pairs of primaries in each of three frequency regions (called the 2000-Hz, the 3500-Hz, and the 5000-Hz regions). The dependent variable was the level of the primary tones necessary to produce a DPOAE of 0 dB SPL. Thus, weak levels correspond to a strong DPOAE. In accord with the findings for CEOAEs, the DPOAEs produced by female ears were either equal to or weaker than those produced by male ears, not stronger.

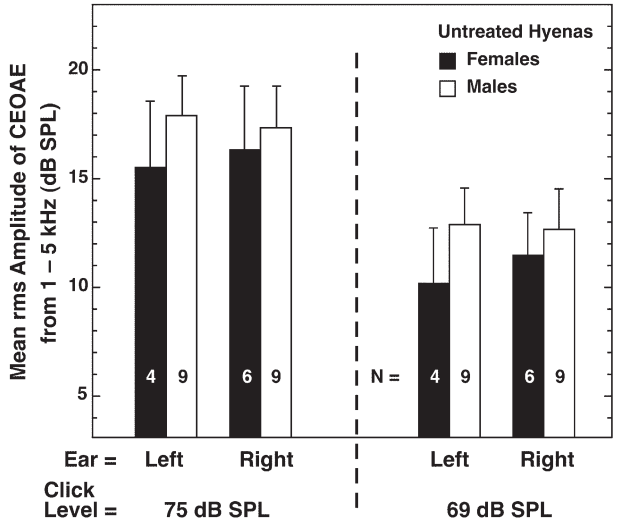

Fig. 1. Mean rms amplitude of the averaged echo-like response to a click stimulus shown for the untreated hyenas only. Analyzed were 20.5-ms samples of the response (filtered between 1 and 5 kHz) beginning 6 ms after click presentation. Data for the 75-dB and 69-dB clicks are shown in the left and right panels, respectively. In this and all subsequent figures, the flags designate one standard error of the mean.

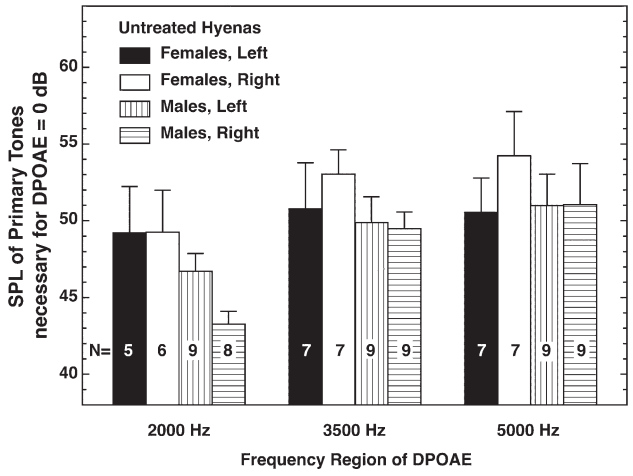

Fig. 2. The average magnitude of the primary tones necessary to produce a median cubic distortion product of 0 dB in each of the three frequency regions studied. Data shown are for the untreated hyenas only. (Stronger primary tones are required when a DPOAE is weaker.) The two primary tones were equal in level, and the ratio of f_{higher}/f_{lower} was 1.21. Six DPOAEs were measured in each frequency region for each of several levels of the primary tones. The noise floor for these measurements was typically about −20 dB SPL in the two higher frequency regions.

The results are shown in Fig. 2, and the effect sizes are shown in the bottom half of Table 1. (Note that the signs of the effect sizes for DPOAEs were reversed to make them conceptually similar to those for CEOAEs.) The largest effect sizes for the between-sex comparison were for the right ears in both the 2000-Hz and 3500-Hz regions: 1.27 and 0.97, respectively. Note that both of these differences were in the direction of weaker, not stronger, DPOAEs in females than in males.

Treated animals

If greater prenatal exposure to androgen is responsible for weaker OAEs (McFadden, 1998, 2002), then the spotted hyenas administered anti-androgenic compounds during prenatal development should have *stronger* OAEs than untreated hyenas. Although the Ns in the treated groups were quite small, they do constitute a census of all known treated animals, and there is no realistic possibility of anyone else reporting on larger Ns in the foreseeable future. Apart from rhesus monkeys (McFadden et al., 2006), we are not aware of any other reports about OAEs obtained from animals treated with anti-androgenic agents.

Table 1
Effect sizes for sex differences in OAEs

Condition	Left ear	Right ear	2-ear average
CEOAEs			
75-dB clicks	−0.42	−0.16	−0.45
69-dB clicks	−0.53	−0.23	−0.50
DPOAEs			
2-kHz region	−0.51	−1.27**	−1.28**
3.5-kHz region	−0.14	−0.97*	−0.50
5.0-kHz region	0.08	−0.40	−0.23
Mean	−0.19	−0.40	−0.37

All entries calculated as females minus males, but signs for DPOAEs were reversed for conceptual consistency with CEOAEs.
Negative entries correspond to weaker OAEs in the females.
* Significantly different, two-tailed *t* test: $P<0.05$.
** Significantly different, two-tailed *t* test: $P<0.01$.

CEOAE results

The spotted hyenas treated with anti-androgenic drugs did, in fact, have slightly stronger CEOAEs than the untreated animals, and this was true for both sexes (see Fig. 3). The effect size for the difference between untreated and anti-androgen females was −0.35, and the effect size for the difference between untreated and anti-androgen males was −0.54. Neither difference was

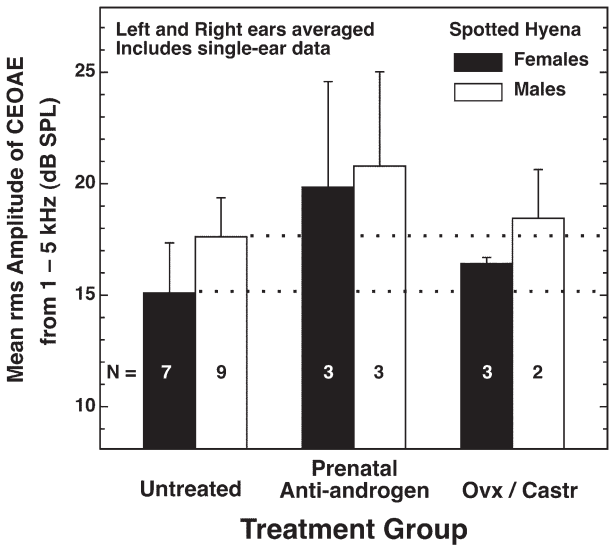

Fig. 3. CEOAE strength for the treated and untreated males and females obtained with the 75-dB SPL click. When data were available for both ears from an individual hyena, those values were averaged for this analysis; if data for only one ear were available, they were used in lieu of a two-ear mean.

statistically significant, but there were only three hyenas in the anti-androgen group for each sex. When the data for untreated males and females were combined and then compared with the combined data for treated males and females, the effect size for two-ear averages was −0.64 ($P=0.10$, one-tailed test).

The three ovariectomized females and the two castrated males had CEOAEs that were quite similar to those of the untreated hyenas. Those effect sizes were −0.26 and −0.17 for the females and males, respectively. Because these animals were not receiving androgen supplements, the androgen levels in these adult males were presumably lower than those in the untreated males (Glickman et al., 1992). Thus, the data from the treated hyenas are not conclusive but do suggest that androgens have an organizational effect on CEOAEs and not an activational effect.

DPOAE results

Because DPOAEs show only a small sex difference in humans (Gaskill and Brown, 1990; Lonsbury-Martin et al., 1991; Moulin et al., 1993; Cacace et al., 1996; Dhar et al., 1998; Bowman et al., 2000) and rhesus monkeys (Torre and Fowler, 2000; McFadden et al., 2006), the expectation is that anti-androgen treatment would have less effect on DPOAEs than on CEOAEs. Unfortunately, the results were not consistent. The data are not shown, but the effect sizes are shown in the bottom half of Table 2. As in Table 1, the signs for the DPOAE effect sizes have been reversed, meaning that a positive entry in Table 2 corresponds to a stronger mean DPOAE in the untreated hyenas than in the relevant treated group. The mean effect sizes at the bottom of Table 2 reveal that the treated hyenas typically had slightly weaker DPOAEs than the untreated hyenas, but examination of the values going into those means shows considerable variation in magnitude and sign, both for the anti-androgen groups and the gonadectomized groups. Furthermore, when the data for untreated males and females were combined and compared with the combined data for the six males and females treated with anti-androgens, the mean effect size across frequency regions fell to 0.21. Accordingly, we expect that DPOAEs eventually will prove to be less affected by anti-androgen treatment than are CEOAEs.

Table 2
Effect sizes for prenatal treatment with androgen or anti-androgen compounds on CEOAEs and DPOAEs

	Females		Males	
Condition	Anti-androgen	Ovx	Anti-androgen	Castrate
CEOAEs				
75-dB clicks	−0.71	−0.26	−0.54	−0.17
69-dB clicks	−0.66	−0.01	−0.45	−0.10
DPOAEs				
2-kHz region	−0.49	0.04	1.98 **	−0.38
3.5-kHz region	−1.00	0.43	1.24 *	0.42
5.0-kHz region	−1.04	−0.95	−1.48 *	−1.13
Mean	−0.84	−0.16	0.58	−0.36

All entries calculated as untreated group minus treated group, but signs for DPOAEs were reversed for conceptual consistency with CEOAEs.
Negative entries correspond to weaker OAEs in the untreated group.
Two-ear averages used when available; when not, value from single ear used.
* Significantly different, two-tailed t test: $P<0.05$.
** Significantly different, two-tailed t test: $P<0.01$.

Ear differences

In humans, CEOAEs are generally stronger in right ears than in left (e.g., McFadden, 1993a, 1998, 2002; McFadden et al., 1996; McFadden and Pasanen, 1998). No consistent laterality was seen in the OAEs of spotted hyenas, and this was true for both the untreated and treated animals. No consistent laterality was evident in the OAEs of rhesus monkeys either (McFadden et al., 2006).

Discussion

This study reveals that the CEOAEs of female spotted hyenas are not stronger than those in males (Fig. 1, Table 1), unlike the situation in humans (McFadden et al., 1996; McFadden and Pasanen, 1998; McFadden and Shubel, 2003) and rhesus monkeys (McFadden et al., 2006). If anything, the OAEs of females were slightly weaker than those of males. The absence (or possible reversal) of a sex difference in the CEOAEs of spotted hyenas is in accord with the assumption that the typical sex difference in human OAEs is attributable to the difference in exposure to androgens that occurs during prenatal development. Female spotted hyenas exhibit numerous characteristics reflecting the fact that they, as well as males, are exposed to androgens during prenatal development (e.g., Forger et al., 1996; Drea et al., 2002; Place et al., 2002; Cunha et al., 2005). The implication is that androgen exposure has led to a diminution in the strength of the linear, reflection-based cochlear mechanisms responsible for CEOAEs in female spotted hyenas, rendering their CEOAEs more similar to those in males than is true in humans or rhesus monkeys. Although the basic outcome here is an absence of effect, the results do provide supporting evidence for the suggestion (McFadden, 1998, 2002) that exposure to high levels of androgens prenatally diminishes the strength of CEOAEs and SOAEs (not measured here)—the prenatal androgen exposure explanation.

A measure of caution is needed when considering the above interpretation because an alternative possibility does exist. Namely, it may be that certain species or genera do not exhibit sex differences in their OAEs for reasons other than androgen exposure and that *Crocuta* is an example. Specifically, it may be that other species of hyena, in which the females are not exposed to high levels of androgens prenatally and are not masculinized in body and behavior, also will exhibit no sex difference in their OAEs once they are measured. At this time, all we know about sex differences in OAEs is from three species. Humans and rhesus monkeys both exhibit stronger CEOAEs in females than in males (e.g., McFadden and Shubel, 2003; McFadden et al., 2006), and both show small or no sex difference in their DPOAEs (e.g., Gaskill and Brown, 1990; Torre and Fowler, 2000; McFadden et al., 2006). Furthermore, one report suggests that mice exhibit little or no sex difference in their DPOAEs (Guimaraes et al., 2004). These findings suggest that CEOAEs are superior to DPOAEs for investigating sex differences across species, but unfortunately

CEOAEs have been difficult to measure in the small species typically used for auditory physiology, presumably because their cochleas are so small that the CEOAEs emerge rapidly and are masked by the ringing of the external and middle ear. Furthermore, SOAEs have proved to be extraordinarily rare in non-humans (see Lonsbury-Martin and Martin, 1988; Probst et al., 1991). Accordingly, the opportunity to observe sex differences in other species has been limited. Perhaps the current interest in stimulus-frequency OAEs (SFOAEs), which can be measured in small species (e.g., Guinan et al., 2003), will lead to more information about sex differences in non-humans. Whatever the future reveals about sex differences in the OAEs of non-human species, for now, it is necessary for readers to keep in mind the alternative explanation that perhaps all species of hyena exhibit no sex difference in OAEs.

The existence of an inverse relationship between prenatal androgen exposure and the strength of the linear, reflection-based mechanisms in the cochlea is bolstered by the finding that CEOAE magnitude was greater in spotted hyenas treated with anti-androgenic agents during prenatal development than in controls (Fig. 3, Table 2). The Ns for both sexes were low, but if this outcome can be confirmed, then a logical interpretation would be that the reduction in androgen exposure led to reduced masculinization of the reflection-based cochlear mechanism and thus to stronger CEOAEs.

Other evidence indicates that the anti-androgen treatments were effective. Treated hyenas from the present study had "feminized" external genitalia, which was evident by 3 months of age (Drea et al., 1998) and persisted throughout adult life (Drea et al., 2002). These same animals exhibited altered patterns of luteinizing hormone and testosterone secretion following gonadotropin-releasing hormone challenge (Place et al., 2002). Fetal specimens taken from other treated pregnancies showed that the "feminized" external genitalia developed prenatally (Cunha et al., 2005). Similarly, Forger et al. (1996) found that stillborn males treated prenatally with anti-androgen drugs had fewer motoneurons in Onuf's nucleus in the spinal cord and smaller, laterally displaced bulbocavernosus muscles; that is, they were "feminized" compared to untreated males. (Note: animals from the present study are extant, and studies of their spinal cord motoneurons and perineal muscles have not been completed.)

In humans, both hearing sensitivity and OAEs are stronger in females than in males (e.g., McFadden and Mishra, 1993; McFadden, 2002). The weak CEOAEs in female spotted hyenas suggest that their hearing sensitivity also should not be better than that in males, but to our knowledge, behavioral measures of hearing sensitivity have not been obtained in spotted hyenas.

DPOAEs exhibited little or no sex difference in spotted hyenas (Fig. 2, Table 1), and that is the same outcome as in humans (Gaskill and Brown, 1990; Lonsbury-Martin et al., 1991; Moulin et al., 1993; Cacace et al., 1996; Dhar et al., 1998; Bowman et al., 2000) and rhesus monkeys (Torre and Fowler, 2000; McFadden et al., 2006). Obtaining the same basic outcome in humans and spotted hyenas, a species whose females are highly androgenized, strongly supports the implication that the nonlinear cochlear mechanism responsible for the production of DPOAEs is, for whatever reasons, much less susceptible to prenatal androgen exposure than is the linear cochlear mechanism responsible for CEOAEs and SOAEs. Otherwise, one might expect to see weaker DPOAEs in female than male spotted hyenas. The inconsistent effects of the anti-androgen agents on DPOAEs also suggest less effect of androgens on the nonlinear cochlear mechanism (Table 2).

Next to nothing is known about the exact mechanisms through which sex differences in OAEs are produced. Our speculations include, but are not limited to: sex differences in the amount of electromotility (Brownell et al., 1985) in the cochlear outer hair cells, possibly as a result of a sex difference in the number or alignment of the prestin motor molecules (Liberman et al., 2002) or a sex difference in the stereocilia (Martin and Hudspeth, 2001) in the outer hair cells; sex differences in the strength of the reflection-based cochlear mechanism (Shera and Guinan, 1999, 2003), possibly as a result of the 13% shorter length of female cochleas (Don et al., 1993; Sato et al., 1991; Kimberley et al., 1993); sex differences in the magnitude of the endocochlear potential; and sex differences in the number or density of androgen (or estrogen) receptors in some cochlear cells at some point(s) early in development (e.g., Stenberg et al., 1999; Nathan et al., 1999). Another logical possibility is that the sex (or ear) differences existing in OAEs stem at least in part from differences in the external- and middle-ear spaces that lie between the cochlea and those earphones and microphones involved in evoking and recording OAEs. For example, if the ear-canal volumes in humans were smaller in females and right ears than in males and left ears, then the sex and ear differences observed in human SOAEs and CEOAEs might be due simply to the higher SPL in the smaller volumes. In contradiction to this logical possibility, Johansson and Arlinger (2003) did not observe sex differences either in middle-ear compliance or middle-ear pressure when they pooled their data across the nearly 500 subjects and seven age groups they studied, yet they did obtain the standard outcome of more SOAEs and stronger TEOAEs (transient-evoked OAEs) in their female subjects than in their male subjects. Furthermore, Keefe et al. (2005) have demonstrated that various middle-ear differences in humans do not explain the ear differences in OAEs. Nothing is known about sex differences in middle-ear compliance or middle-ear pressure in the spotted hyena.

We do not believe that OAEs themselves provide any particular evolutionary advantage to an individual animal. It is more likely that they are epiphenomena accompanying characteristic(s) that do provide an advantage—such as good hearing sensitivity (McFadden and Mishra, 1993; McFadden, 1993a). What is interesting here is that CEOAEs and SOAEs do appear to serve as sensitive markers to androgenic events which occur during prenatal development (McFadden, 1998, 2002), and the present evidence from spotted hyenas appears to strengthen that implication.

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