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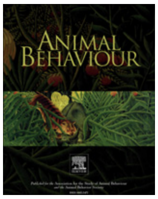

Articles

Androgen-dependent male vocal performance influences female preference in Neotropical singing mice

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Vocalizations used in aggressive and mating contexts often convey reliable information about signaller condition when physical or physiological limitations constrain signal expression. In vertebrates, androgens modulate the expression of vocal signals and provide a proximate link between male condition and signal form. In many songbirds, assessment of males is based on production of trills that are constrained by a performance trade-off between how fast notes are repeated and the frequency bandwidth of each note. In this study, we first recorded trills of male Neotropical singing mice (*Scotinomys*) to examine whether they show a similar performance trade-off, and then manipulated androgen levels to assess their role in modulating vocal performance. Lastly, we broadcast experimentally manipulated trills to females to determine whether they preferred versions resembling those of androgen-treated males. Singing mice showed a vocal performance trade-off similar to that of birds. Males treated with androgens maintained vocal performance, but castrated mice that were administered empty implants produced trills with lower performance. Females approached high-performance trills more rapidly and spent more time near corresponding speakers. Together, our results demonstrate that androgens modulate the production of physically challenging vocalizations, and the resulting signal variation influences female receiver response.

Vocalizations used in aggressive and mating contexts are among the most diverse and elaborate displays in the animal kingdom. Physical or physiological constraints can result in signals that convey information about signaller condition (Maynard Smith & Harper 1995; Vehrencamp 2000; Fitch & Hauser 2003; Hurd & Enquist 2005; Taylor & Reby 2010), and receiver responses to such signals can generate substantial intra- and intersexual selection (Andersson & Simmons 2006). In birds, anurans and mammals, conspicuous features of vocal signals (e.g. duration and fundamental frequency) provide accurate indicators of male quality and size that are attractive to females and threatening to rival males (Clayton & Prove 1989; Welch et al. 1998; Reby et al. 2005). Such displays are thought to transmit honest information because only individuals in better condition are able to afford costs associated with signal production.

In vertebrates, vocal displays associated with reproduction are often mediated by androgens released from the testes (Floody 1981; Ball et al. 2003; Moore et al. 2005; Bass & Remage-Healey 2008). Androgens can influence vocalizations through actions on motivational centres and vocal motor pathways in the central nervous system (reviewed in Yamaguchi & Kelley 2002; Bass & Remage-Healey 2008) or via modulation of peripheral structures involved in signal production. For example, changes in fundamental frequency result from anabolic effects of androgens on the larynx and syrinx, the 'source' of sound production in mammals and birds (Beckford et al. 1985; Beani et al. 1995; Cynx et al. 2005; Evans et al. 2008; Taylor & Reby 2010; Pasch et al. 2011). By modulating signal duration, rate or fundamental frequency, androgens can provide a mechanistic link between male condition and signal form (Remage-Healey & Bass 2007; Charlton et al. 2010).

While androgens influence conspicuous features of vocal signals, little is known about their effects on vocal attributes that reflect male performance. For example, many avian species produce a series of rapidly repeated notes of similar structure, termed trills. Trill production is constrained by a mechanical trade-off between how fast notes are repeated (trill rate) and the frequency bandwidth of each note, resulting in a performance limit (Podos 1997). Males with high vocal performance scores are considered greater threats by rival males and are more attractive to females in a variety of species (Ballentine et al. 2004; Illes et al. 2006). Vocal performance is also correlated with age, social status and reproductive

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success (Janicke et al. 2008; Ballentine 2009; Botero et al. 2009; de Kort et al. 2009). Despite the association between circulating testosterone and these same variables (age: Morton et al. 1990; Vleck & Brown 1999; social status: Wingfield 1985; Wingfield & Moore 1987; reproductive success: Reed et al. 2006), the role of androgens in mediating variation in vocal performance is unclear.

In birds, vocal performance trade-offs are thought to arise via ‘filter’ constraints whereby sound frequencies track beak gape, with wide gapes corresponding to high frequencies and small gapes corresponding to low frequencies (Westneat et al. 1993; Hoese et al. 2000). However, other aspects of respiratory and syringeal motor patterns are important in modulating aspects of note rate and frequency modulation (Goller & Suthers 1996; Nelson et al. 2005; Riede et al. 2006). In particular, ‘minibreaths’ that birds take between each note suggest a potential role for respiratory musculature in limiting temporal aspects of note production (Calder 1970; Hartley & Suthers 1989; Lambrechts 1996). While the influence of androgens on avian respiratory musculature has not been well characterized, the mammalian diaphragm is highly sensitive to androgens that can influence the velocity and force of respiration (Prezant et al. 1997). Similarly, the craniomandibular musculature of rodents is responsive to androgens (Lyons et al. 1986; Eason et al. 2000), which suggests a mechanism by which androgens could influence the rate of gape and frequency modulation. These data suggest that androgens could provide an important yet underappreciated mechanism modulating vocal performance, and highlight the potential for an appropriate mammalian model to inform our understanding of vocal signals.

Neotropical singing mice (genus *Scotinomys*) are diurnal insectivorous rodents that inhabit montane cloud forests throughout Central America (Hooper & Carleton 1976; Wilson & Reeder 2005). Adult males commonly produce stereotyped trills (see Supplementary Material, Audio S1) that are androgen dependent and used in male–male aggression and mate attraction (Pasch et al. 2011; this study). Presentation and subsequent removal of females increases male song rate, and males countersing in response to broadcast songs of intruding males in the field and laboratory (Pasch et al. 2011; B. Pasch & S. M. Phelps, unpublished data). When singing mice trill, they take ‘minibreaths’ between each note and appear to modulate the fundamental frequency (10–43 kHz) with their mouth gape (Miller & Engstrom 2007; see Supplementary Material, Video S1) in a manner analogous to some bird song (Suthers et al. 1999).

In this study, we first asked whether Alston’s singing mouse (*Scotinomys teguina*) shows a vocal performance trade-off between trill rate and frequency bandwidth. We then manipulated androgens in laboratory-reared males to investigate whether they play a role in modulating vocal performance. Finally, we experimentally manipulated trills to determine whether females would respond to versions resembling those of androgen-treated males. We predicted that androgen removal would negatively impact vocal performance, and that females would prefer male mouse trills with higher performance scores over those with lower scores.

METHODS

Generating the Population Performance Limit

To assess variation in male trills, we live-captured animals in Sherman traps baited with peanut butter and oats in four localities throughout Costa Rica and Panamá from 2006 to 2009 (see Campbell et al. 2010). We measured their mass and hindfoot length prior to transferring males to PVC-coated wire-mesh cages (28 × 28 × 28 cm). Cages were placed inside anechoic recording chambers (42 × 42 × 39 cm) made from expanded PVC. Details on song recording can be found in Campbell et al. (2010). We recorded spontaneous trills from 102 males ($N = 3.73 \pm 1.57$ trills/male, range 1–12). Animals were uniquely marked and released at the site of capture. All capture and recording procedures in the field were conducted with approval from the Institutional Animal Care and Use Committee (IACUC) at the University of Florida (No. E436), the Costa Rican Ministerio del Ambiente y Energia and the Panamanian Autoridad Nacional del Ambiente.

We used an automated code in Matlab (available upon request) to quantify the average trill rate and frequency bandwidth for the entire song. Frequency bandwidths were calculated by a threshold routine that identified frequencies at the onset and offset of notes that were eight standard deviations above the average background noise (sampling rate: 96 kHz; fast Fourier transform size: 256; Hann window, time resolution = 50 ms). We plotted an average of each male’s songs (see Fig. 2a, filled circles) and calculated vocal performance limits as described in detail elsewhere (Podos 1997; Ballentine et al. 2004). Briefly, we first pooled each male’s average trill into bins of one note/s. For each bin, we determined the male with the largest frequency bandwidth (see Fig. 2a, open circles) and calculated a linear regression through these maxima (after Blackburn et al. 1992). This statistical technique attempts to describe biological boundaries by defining theoretical limits to bivariate plots. In this context, the upper-bound regression operationally defines the population performance limit on trill production, with trills closer to the limit being more difficult to produce (Podos 1997).

Androgen Manipulation

We experimentally manipulated androgen levels of laboratory-reared offspring (F1) of wild-captured mice from Cerros de la Carpintera, Costa Rica. Sexually experienced adult males were assigned randomly to one of three treatment groups and housed individually in cages for 1 month prior to experimentation. Animals were given cat chow and water ad libitum and maintained on a 12:12 h light:dark cycle at 20 ± 3 °C. Mice were anaesthetized with isoflurane (SurgiVet Isotec T^3 Classic Isoflurane Vaporizer) and castrated (Cast) bilaterally. We placed a 10 mm silastic implant (1.47 mm inner diameter × 1.96 mm outer diameter, Dow Corning Corporation, Midland, MI, U.S.A.) subcutaneously along the dorsal midline. Implants were filled with either 1 mm (15 mg) of testosterone (T; Sigma T1500), 1 mm of dihydrotestosterone (DHT; Sigma A8380), or left empty ($N = 9$ per treatment). The DHT treatment group was included to identify whether androgens were sufficient to cause acoustic changes in the absence of aromatization (Nyby et al. 1992). Implants were sealed with silicon adhesive, sterilized with ethylene oxide, and soaked in 0.1% saline solution at 37 °C for 12 h prior to implantation. Implant concentrations approximated physiological doses of plasma T of field-captured animals (see Pasch et al. 2011).

Fourteen days pre- and postsurgery, we placed mouse cages inside an anechoic recording chamber to record trills ($N = 3$/male) as described above. We then plotted trill rate and frequency bandwidth for all experimental animals pre- and post-treatment and calculated performance scores as the orthogonal deviation (i.e. vocal deviation in Podos 2001) from the performance limit generated from field-captured males. Researchers were blind to animal treatment. All laboratory procedures were conducted with permits from IACUC at the University of Florida (No. 200801939).

Female Phonotaxis

To assess female response to male trills, we live-captured females at Cerros de la Carpintera, Costa Rica in February 2010.