

Neuromuscular and Endocrine Control of an Avian Courtship Behavior

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In many species of birds, males perform complex visual and acoustic courtship displays to attract and stimulate females. Some of these displays involve considerable use of the wings and legs, suggesting that they may be controlled by sexually dimorphic spinal motoneurons and their target muscles. Sex steroid hormones are known to organize and activate many sexually dimorphic phenotypes, so these neuromuscular systems may also be steroid sensitive. To test these ideas, we have begun studies of wild golden-collared manakins (*Manacus vitellinus*) in Central America. Males of this species establish a courtship arena in the forest, where they perform an elaborate dance that includes use of their wings to generate loud snapping sounds. Here we describe male golden-collared manakin courtship behavior, including the various "wingsnaps." We also review our studies, and those of others, showing sexually dimorphic properties of manakin wings, the wing musculature, and sex steroid accumulation in the spinal cord. These data suggest that manakins are useful models for evaluating steroid control of complex peripheral neuromuscular systems. © 2001 Academic Press

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Among the vertebrates, birds demonstrate especially pronounced sexually dimorphic phenotypes and behaviors, suggesting that sexual selection operates intensively in avian species (Darwin, 1871). Male birds sometimes utilize extraordinary behaviors to signal both visually and acoustically toward members of the same sex, in defense of territories or courtship

arenas, or to attract and stimulate members of the opposite sex. Acoustic signaling evolved to an especially high degree in the oscine songbirds in the form of songs produced by the muscular syrinx (Catchpole and Slater, 1995). In other species, males perform spectacular courtship displays that can involve intense and prolonged acrobatic movements on the ground, on perches, or in midair (Johnsgard, 1994; Prum, 1998). These displays are often enhanced by the presence of conspicuous feathers or skin ornaments. In some cases, these displays can include acoustic signals produced by the wings, the tail, or by airsacs and not by airflow through the syrinx.

Sex steroid hormones have considerable influence on the organization and activation of central and peripheral neuromuscular systems controlling vertebrate reproductive behaviors (Nelson, 1995). Sex steroids also act on the brain and on syringeal muscles to control oscine birdsong (Schlinger, 1997). However, we know little about the hormonal control of other visual and acoustic signals produced by birds. Because elaborate courtship displays are often performed by males, and not by females, and because they are performed in reproductive contexts, we might expect that the motoneurons and muscles controlling these behaviors would be sensitive to sex steroid hormones and would be anatomically and physiologically sexually dimorphic.

Motivated by these ideas, we have begun studies of the neuromuscular and hormonal control of male behavior in the golden-collared manakin (*Manacus vitellinus*), a species in which only the males perform a striking visual and acoustic courtship display. Manakins are a family (Pipridae) of approximately 42 species of small, suboscine passeriform birds. They are found almost exclusively in wet, tropical areas of the New World and many are strikingly sexually dimor-

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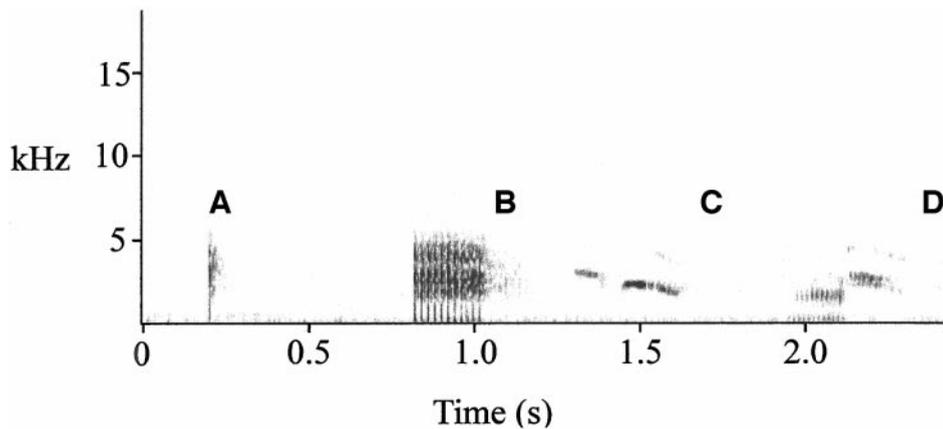


FIG. 1. Representative spectrogram (generated using Canary 1.2.4) of manakin wingsnaps and a common vocalization. (A) A single wingsnap; (B) a rolling wingsnap; (C) a *chee-poo* vocalization; and (D) a “reedy whirr” wingsnap, immediately followed by a higher frequency *chee* vocalization (as in C).

phic in physical characteristics and in behavior. Sexual selection appears to have driven many manakins to develop complex reproductive strategies that can include highly polygynous mating systems and elaborate courtship displays (Snow, 1962; Parsons, Olson, and Braun, 1993; Johnsgard, 1994; Prum, 1998).

GOLDEN-COLLARED MANAKIN BEHAVIOR

The earliest description of golden-collared manakin reproductive behavior was that of Chapman (1935). Males are territorial and court females on small (~30-in.-long) elliptical arenas on the forest floor within their territory. Territories of several males lie adjacent to each other; apparently the synchronous activity of several males in close proximity intensifies the stimulation and attraction of females. Masculine courtship behavior includes visual and acoustic elements. When actively courting on his territory, a male extends his bright yellow throat feathers fully beyond the tip of his bill, much like a lizard extends his dewlap. He may also jump up and down the length of his perch. If a female approaches the arena, the male descends to the saplings that border his “court.” He flits from branch to branch, or from branch to the forest floor, continuing as long as he holds the female’s attention. Occasionally, his performance results in a successful copulation.

The male courtship dance is punctuated by loud acoustic signals, known generally as “wingsnaps,” that are produced by upward flips of the bird’s wings

(Chapman, 1935). The single wingsnap is typically performed in tight synchrony with a jump, occasionally while the bird remains perched (Fig. 1A). Perched males also produce a rolling wingsnap that consists of a series of 5–15 individual snaps repeated at great speed (~50 Hz; Fig. 1B). Males infrequently produce a reedy whirr, sounding somewhat like a muffled rolling wingsnap (Fig. 1D). Males also produce relatively weak vocalizations (Fig. 1C). Figure 2 illustrates a typical sequence of jumps and wingsnaps by a courting male golden-collared manakin.

MANAKIN MORPHOLOGY

Although the exact anatomical source of these wingsnaps is unknown, they are probably produced by specialized primary and secondary wing feathers. Chapman (1935) showed that some primaries and secondaries of males differed from those of females by having either curved, heavy, stiffened vanes that were unusually asymmetric especially narrow. He hypothesized that these feathers are whipped against each other resulting in the snapping sounds. Because the proper movement of the wings and their sexually dimorphic feathers seem crucial to sound production, it is likely that the muscles controlling wing movements and/or feather position are sexually dimorphic as well.

Lowe (1942) described several unique characteristics of manakin musculature that might be associated with the generation of wingsnaps. First, he identified several muscle groups whose size in manakins ap-

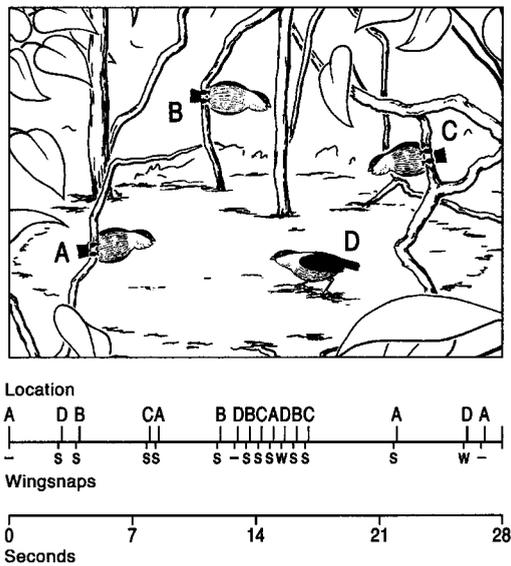


FIG. 2. Schematic of a male manakin courtship sequence shown spatially around an arena on the forest floor (top) and temporally along a timeline (bottom). The letter above the timeline refers to the location (A, B, C, or D) to which the bird jumps at a given time. The occurrence of wingsnaps with jumps are shown below the timeline; S, a single snap; W, a reedy whirr; (-), no snap.

peared to be hypertrophied relative to nonmanakin bird species. These muscles include those involved in lifting and retracting the wing (the supracoracoideus, SC, and the scapulohumeralis dorsalis, SD), in pronation of the distal wing (the pronators), and in the jump often associated with a snap (the gluteal or iliotibialis). In particular, the SD, which originates on the scapula and inserts on the head of the humerus, was described as “enormously hypertrophied” to the point that it displaced other muscles of the region, such as the subscapularis. The SD assists in bringing the humerus upward and backward, motions consistent with those seen during wingsnaps. Lowe also described specializations of the triosseal canal, thought to be important in supporting the forceful contractions required for the generation of the wingsnap, and of the tendinous attachments from forewing muscles onto flight feathers, thought to position these feathers during the wingsnap. Unfortunately, Lowe did not examine females.

STEROID ACCUMULATION IN THE SPINAL CORD

To begin to understand whether sex steroids influence male manakin anatomy and courtship behavior,

we first asked if motoneurons in the manakin spinal cord were sensitive to sex steroids. We collected male and female manakins in central Panama, injected them with drugs to block androgen synthesis, and 24 h later injected them with [3 H]testosterone (T; Schultz and Schlinger, 1999). Ninety minutes later the birds were sacrificed and the spinal cords collected, frozen on dry ice, and later returned to our laboratory at UCLA. The cords were sectioned longitudinally onto slides dipped in photographic emulsion and stored in the dark for 3 to 12 months. After the slides were developed, we quantified silver grains over nuclei or somas. Cells that demonstrated a grain density greater than $5\times$ background were considered to have accumulated [3 H]T or metabolites of [3 H]T. Many such sex steroid accumulating cells were found and after preparing a map of their distribution in six male and five female manakins, two results became apparent. First, we found most of these cells in the ventral and middle parts of the cervical and lumbosacral enlargements of the spinal cord (Fig. 3). Additionally, many of these cells were very large and multipolar, consistent with the morphology of motoneurons. Based on their size and position in the spinal cord, it is likely that some of these steroid-accumulating motoneurons innervate the musculature of the peripheral appendages. Second, although we found considerable variability in the numbers of sex-steroid-accumulating cells across birds, in general we found a larger number of these cells in the spinal cords of males than of females. Whereas accumulation of sex steroid was found in the spinal cords of all six males, only one female showed a significant number of these cells (about half as many cells as was found in the typical male), and the remaining females had few if any. These results suggest that muscles involved in male-specific behavioral displays involving the wings and legs may be innervated by motoneurons whose morphology and physiology may be regulated by sex steroids.

CURRENT AND FUTURE STUDIES

Based on descriptions of hypertrophied muscles by Lowe (1942), we have begun also to evaluate the possibility that some manakin muscles are sexually dimorphic in overall size or in the sizes of individual fibers or their expression of myosin isoforms and metabolic enzymes. Our data suggest that the SC and SD are indeed significantly larger in males than in females and/or exhibit other physiological differences. By contrast the gluteal muscles lack these sex differences

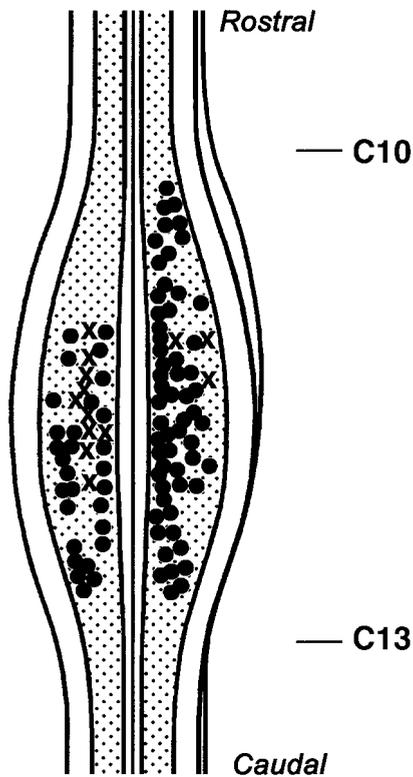


FIG. 3. Sex-steroid-accumulating cells in the ventral cervical enlargement of the manakin spinal cord. Solid dots represent cells ($n = 3/\text{dot}$) found in male spinal cords; X's represent cells ($n = 3/\text{X}$) found in female spinal cords. Cervical segments 10 and 13 are represented by C10 and C13 respectively. (Taken from Schultz and Schlinger, 1999).

(Schultz *et al.*, 2000). As described above, the SC and SD are the two principal muscles that lift and retract the wings, and it is just these movements that are thought to produce wingsnaps. Thus, it is not surprising that these muscles are more highly developed in males than in females. At this point, we do not know the mechanisms producing these sex differences in muscle anatomy and physiology. Androgens are known to regulate properties of adult vertebrate muscles (e.g., Sassoon *et al.*, 1987; Brantley *et al.*, 1993; Regnier and Herrera, 1993), suggesting that the SD and SC may be androgen sensitive.

The identification of sexually dimorphic peripheral muscles in birds is not unexpected (Breedlove, 1992). Acoustic signals that are produced by males and not females can be accompanied by sex differences in the neuromuscular systems controlling the production of these sounds. In birds, sex differences in singing are associated with sex differences in the muscular syrinx

(Luine *et al.*, 1980). In the amphibian *Xenopus laevis*, the male performs a courtship call and the larynx in males is correspondingly larger than that in females (Kelley, 1986). In plainfin midshipman fish (*Porichthys notatus*), one type of male courts females with a loud "hum" produced by the muscular swim bladder. The neuromuscular system controlling the hum is more developed in these males than that in females (Brantley *et al.*, 1993). However, outside of the forearm muscles that control copulatory "clasping" by male *Xenopus* (Regnier and Herrera, 1993), the perineal muscles in rodents that controls penile reflexes (Breedlove, 1992) and the foam gland of quail (Seiwert and Adkins-Regan, 1998), there are few other well studied sexually dimorphic peripheral neuromuscular systems in vertebrates.

Although we have made some progress in identifying sexually dimorphic and steroid-sensitive neural and muscular attributes in manakins, we are a long way from understanding the mechanics of the wingsnap and the hormonal mechanisms underlying performance of these behaviors. For example, we need to castrate adult males, or treat them with androgen receptor antagonists to see if we can diminish or eliminate this courtship display. We do not know yet which muscles are innervated by the sex-steroid-accumulating motoneurons. However, we have preliminary evidence (based on combined androgen-receptor *in situ* hybridization and retrograde labeling of motoneurons by injection of muscles with fluorescent tracers) that at least one muscle, the SD, is innervated by androgen-receptor-expressing motoneurons (Schultz and Schlinger, unpublished observation). This suggests that androgens influence the neuromuscular system controlling production of the wingsnap. We do not know if sex steroids influence the morphology or physiology of specific motoneurons or whether the peripheral muscles themselves are sex steroid sensitive. Our current studies are exploring these various questions.

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REFERENCES

- Brantley, R. K., Marchaterre, M. A., and Bass, A. H. (1993). Androgen effects on vocal muscle structure in a teleost fish with inter and intrasexual dimorphisms. *J. Morphol.* **215**, 305–318.
- Breedlove, S. M. (1992). Sexual dimorphism in the vertebrate nervous system. *J. Neurosci.* **12**, 4133–4142.
- Catchpole, C. K., and Slater, P. J. B. (1995). *Bird Song: Biological Themes and Variations*. Cambridge Univ. Press, Cambridge, UK.
- Chapman, F. M. (1935). The courtship of Gould's Manakin (*Manacus manacus vitellinus*) on Barro Colorado Island, Canal Zone. *Bull. Am. Mus. Nat. Hist.* **68**, 471–525.
- Darwin, C. (1871). *The Descent of Man and Selection in Relation to Sex*. J. Murray, London.
- Johnsgard, P. A. (1994). *Arena Birds: Sexual Selection and Behavior*. Smithsonian Institution Press, Washington DC.
- Kelley, D. B. (1986). Neuroeffectors for vocalization in *Xenopus laevis*: Hormonal regulation of sexual dimorphism. *J. Neurobiol.* **17**, 231–248.
- Luine, V., Nottebohm, F., Harding, C., and McEwen, B. S. (1980). Androgen affects cholinergic enzymes in syringeal motor neurons and muscle. *Brain Res.* **192**, 89–107.
- Nelson, R. J. (1995). *An Introduction to Behavioral Endocrinology*. Sinauer, Sunderland, MA.
- Lowe, P. (1942). The anatomy of Gould's Manakin (*Manacus vitellinus*) in relation to its display. *Ibis* **6**, 50–83.
- Parsons, T. J., Olson, S. L., and Braun, M. J. (1993). Unidirectional spread of secondary sexual plumage traits across an avian hybrid zone. *Science* **260**, 1643–1646.
- Prum, R. O. (1998). Sexual selection and the evolution of mechanical sound production in manakins (Aves: Pipridae). *Anim. Behav.* **55**(4), 977–994.
- Regnier, M., and Herrera, A. A. (1993). Differential sensitivity to androgens within a sexually dimorphic muscle of male frogs (*Xenopus laevis*). *J. Neurobiol.* **24**, 1215–1228.
- Sassoon, D. A., Gray, G. E., and Kelley, D. B. (1987). Androgen regulation of muscle fiber type in the sexually dimorphic larynx of *Xenopus laevis*. *J. Neurosci.* **7**, 3198–3206.
- Schlinger, B. A. (1997). Sex-steroids and their actions on the bird song system. *J. Neurobiol.* **35**, 619–631.
- Schultz, D., and Schlinger, B. A. (1999). Widespread accumulation of ³H-testosterone in the spinal cord of a wild bird with an elaborate courtship display. *Proc. Natl. Acad. Sci. USA* **96**, 10428–10432.
- Schultz, J. D., Phelps, P. E., and Schlinger, B. A. (2000). Spinal cord androgen receptors and sexually dimorphic wing muscles in a wingsnapping bird. *Soc. Neurosci. Abs.* **26**, 1269.
- Seiwert, C. M., and Adkins-Regan, E. (1998). The foam production system of the male Japanese quail: Characterization of structure and function. *Brain Behav. Evol.* **52**, 61–80.
- Snow, D. W. (1962). A field study of the Black and White Manakin, *Manacus Manacus*, in Trinidad. *Zoologica* **47**(8), 65–104.