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RESEARCH ARTICLE

Neuromuscular mechanisms of an elaborate wing display in the golden-collared manakin (*Manacus vitellinus*)

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ABSTRACT

Many species perform elaborate physical displays to court mates and compete with rivals, but the biomechanical mechanisms underlying such behavior are poorly understood. We address this issue by studying the neuromuscular origins of display behavior in a small tropical passerine bird, the golden-collared manakin (*Manacus vitellinus*). Males of this species court females by dancing around the forest floor and rapidly snapping their wings together above their back. Using radio-telemetry, we collected electromyographic (EMG) recordings from the three main muscles that control avian forelimb movement, and found how these different muscles are activated to generate various aspects of display behavior. The muscle that raises the wing (supracoracoideus, SC) and the primary muscle that retracts the wing (scapulohumeralis caudalis, SH) were activated during the wing-snap, whereas the pectoralis (PEC), the main wing depressor, was not. SC activation began before wing elevation commenced, with further activation occurring gradually. By contrast, SH activation was swift, starting soon after wing elevation and peaking shortly after the snap. The intensity of this SH activation was comparable to that which occurs during flapping, whereas the SC activation was much lower. Thus, light activation of the SC likely helps position the wings above the back, so that quick, robust SH activation can drive these appendages together to generate the firecracker-like snap sonation. This is one of the first looks at the neuromuscular mechanisms that underlie the actuation of a dynamic courtship display, and it demonstrates that even complex, whole-body display movements can be studied with transmitter-aided EMG techniques.

KEY WORDS: Skeletal muscle, Electromyography, Courtship behavior, Social signal, Biomechanics, Golden-collared manakin

INTRODUCTION

Spectacular acrobatics and dance are not exclusive to humans. Rather, decades of research show that numerous species incorporate extraordinary physicality into the social displays used for courtship and competition (Amézquita and Hödl, 2004; Frith and Beehler, 1998; Garrick and Lang, 1977; Girard et al., 2011; Grafe et al., 2012; Hogg and Forbes, 1997; Masonjones and Lewis, 1996; Miles et al., 2017; Ord et al., 2001, 2002; Pelletier et al., 2004; Prum, 1990; Voigt et al., 2001; Walls and Semlitsch, 1991). Underlying many of these athletic courtship displays are remarkable anatomical and physiological specializations that have evolved to support behavioral output (Bostwick et al., 2012; Clifton et al., 2015; Friscia et al., 2016; Fuxjager et al., 2016a; Lindsay et al., 2015; Mangiamele et al., 2016). However, one of the biggest challenges to studying adaptations for behavioral display centers around assessing the neuromuscular mechanisms that control complex and unusual physical movements. This is because most animals that perform such signals do so only in the wild during a certain time of year, or in remote geographic locations that are challenging to access. Additionally, experimental manipulations necessary to record meaningful physiological data from animals can cease their motivation to produce a display. These difficulties leave a fundamental gap in our understanding of precisely how the neuromotor system controls and facilitates elaborate display performance.

In recent decades, the golden-collared manakin [*Manacus vitellinus* (Gould 1843)] has emerged as a useful organism to study mechanisms of physical reproductive displays. These small tropical birds inhabit the lowland rainforests of Central and South America, and males court females by dancing around the forest floor and snapping their wings together above their back (Fusani et al., 2014; Schlinger et al., 2013). This latter maneuver, called the wing-snap, results in a loud mechanical sonation that echoes through the forest. Kinematic analyses of this signal indicate that it is produced when males first elevate their wings above their back, and then rapidly retract them. This causes the radii to collide medially above the axial mid-line (Bostwick and Prum, 2003; Fusani et al., 2007b; Fuxjager et al., 2013), generating a loud and characteristic 'snap' sound (Bodony et al., 2016). Females are thought to evaluate this display as a component of mate choice (Barske et al., 2011), which highlights its functional importance to reproduction and suggests that sexual selection for the display drives the evolution of supportive physiological mechanisms.

The wing-snap display is likely actuated by the main wing muscles, which otherwise raise and retract the wing for use in locomotion. These muscles include the supracoracoideus (SC) and the scapulohumeralis caudalis (SH). During normal flight, the SC plays a major role in elevating the humerus, whereas the SH rotates the wing forward and retracts the humerus back to the body (Dial, 1992; Dial et al., 1991; Tobalske, 1995). The biomechanical role of these muscles in forearm movement certainly implicate their involvement in the wing-snap, but other studies also highlight remarkable adaptations in these muscles that go beyond supporting everyday locomotion. For example, the SC and SH of male golden-collared manakins are both hypertrophied, compared with the SC and SH of females, which do not wing-snap (Schultz et al., 2001).

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Received 7 August 2017; Accepted 18 October 2017

Journal of Experimental Biology (2017) 220, 4681-4688 doi:10.1242/jeb.167270

Moreover, both of these tissues constitutively express high levels of genes, such as parvalbumin and IGF-I, which augment contractile speed and strength compared with what might otherwise be expected for such tissues (Fuxjager et al., 2012a,b). Finally, physiological evidence shows that the SH of the golden-collared manakin is specialized to produce contraction–relaxation cycling speeds of nearly 100 Hz, which is over two times faster than the SH speed of many other related birds that do not wing-snap (Fuxjager et al., 2016a, 2017). Despite all of this research highlighting male-specific specialization in these wing muscles, no study to date has tested whether and how they are activated during the wing-snap display.

Here, we address this possibility using radio telemetry to record electromyography (EMG) in the SC and SH of actively displaying golden-collared manakins. This approach allows us to not only assess whether activation of these muscles is associated with the wing-snap, but also to describe how each tissue differentially contributes to each phase of signal production. Given the biomechanical role played by the axial wing muscles, we predict that SC activation is associated with the lifting of the wings into position, whereas the SH activation occurs during the movement that causes the rapid collision of the radii. At the same time, we also collected EMG recordings from another major wing muscle, the pectoralis (PEC). When this tissue contracts, it depresses the humerus and thus lowers the wing (Dial, 1992; Dial and Biewener, 1993; Dial et al., 1991). We studied this muscle because it may play a role in the actuation of the wing-snap, even though its wing depressing function does not suggest a prominent role; for example, it may mediate rapid wing elevation through a catapult-like mechanism (Astley, 2012; Olson and Marsh, 1998). In any case, we assessed how this muscle contributes to the display, allowing us to rule out such an alternative mechanism.

MATERIALS AND METHODS

Animals

We conducted this project in Gamboa, Panama, at the Smithsonian Tropical Research Institute (STRI). All birds in this study were males in pre-definitive plumage, which are distinguished from definitive (adult) males by their drab green color and occasional golden feathers in the collar. Such birds readily display in captivity after they are treated with testosterone (see below). Thus, we could stimulate display behavior in birds fitted with radio-transmitters and implanted with electrodes to record EMG.

We captured birds through passive mist-netting on active leks within the forests surrounding Gamboa. We quickly transported birds to a nearby laboratory, where they were fed papaya *ad libitum* as described previously (Day et al., 2007, 2006; Fusani et al., 2007a, 2012a; Fuxjager et al., 2012b). We completed this work during the height of the breeding season in March and April. All appropriate governmental authorities and Institutional Animal Care and Use Committees approved of the work outlined herein.

Hormone treatment

To stimulate wing-snapping behavior in our captured birds, we subcutaneously implanted each individual with a 12-mm piece of silicone tube (0.76 mm i.d., 1.65 mm o.d.) containing 10 mm of crystalline testosterone and sealed shut at both ends with 1 mm of silicone adhesive. Past studies show that such treatment increases frequencies of wing-snap, particularly in pre-definitive plumage males (Day et al., 2007, 2006). We placed the implant at the base of each bird's neck, ensuring that its presence under the skin did not interfere with the muscular control of the wing-snap (Day et al., 2007, 2006; Fusani et al., 2007a; Fuxjager et al., 2013). We then waited 14 days after this procedure to begin recording EMG from the different wing muscles, as previous studies show that this amount of time is optimal for testosterone to begin activating production of wing-snaps in captive animals (Day et al., 2007, 2006). Once a bird was observed snapping multiple times per day, we selected it for muscular recordings (see below).

Surgery and EMG

We collected EMG recordings in a single muscle from each of the birds that was used in this study. To implant birds with electrodes, we first anesthetized them using isoflurane (2–4% in O_2) and cut a small (1 cm) incision in the skin above the muscle of interest (either the SH or PEC; see below for SC). We implanted the muscle with an electrode made from two insulated stainless steel wires (California Fine Wire SS304, H-ML 0.025 mm), each of which had the insulation stripped off roughly 0.2 mm of their tips. We then bent both wires in a fishhook-like curl and fastened them together using a thin coat of epoxy. This ensured that we could use forceps to easily insert each electrode into the muscle. Once this was done, we fastened the electrode in place by applying a small drop of Vetbond™ tissue adhesive to the implantation site. We coiled excess electrode wire around this area, which provided sufficient slack to allow the bird to move freely.

We prepared the SC in a similar manner, although we made a few modifications to this surgery given that the SC lies deep to the PEC. Thus, we cut a small incision (1.5 cm) in the skin above the furcula and moved the fat-pad and inter-clavicular air sac aside. This exposed the SC, which sat above the keel and below the PEC; thus, we then implanted the muscle with the electrode, fixed it in position with Vetbond adhesive, coiled excess electrode wire around the implantation site and allowed the fat-pad and air sac to fall back into place.

In both preparations described above, we routed the remaining electrode wire subcutaneously to the lower back, where it was attached to a small custom-built radio-transmitter (total mass: ∼1 g; ∼5% body mass). The transmitter was stitched to a soft piece of fabric (2 cm^2), which was attached to the lower spinal feather track (as in Barske et al., 2014, 2011). We positioned the apparatus close to the bird's center of gravity, minimizing its effect on balance and ability to wing-snap. Transmitters were powered by a small battery that provided 2–5 days of life to the device. Once electrodes were hooked to the transmitter, we sutured the skin around the electrode insertion site using tissue adhesive. We then removed birds from the anesthesia machine and allowed them to recover alone in a clean cage. Later in the day, we moved implanted birds into the animal room. Muscle recordings occurred at lights-on (sunrise) on the following morning.

In this study, only one bird was used twice, in that we collected EMG recordings from two different muscles on two separate occasions. Otherwise, each bird was used in this study once. We obtained EMG recordings from (1) the SC for four different wing-snaps (collected from three separate individuals); (2) the SH for 10 different wing-snaps (collected from two separate individuals); and (3) the PEC for 10 different wing-snaps (collected in three separate individuals).

Data collection

The frequency-modulated signal broadcast from the bird's transmitter (transmission frequency=294.8 to 301 Hz) was collected using a communication receiver (AR8600, AOR, Torrance, CA, USA) that was connected to an antenna placed atop the bird's cage. We also positioned a Sennheiser microphone (K6 series, model ME66, Wedemark, Germany) outside the cage (within ∼20 cm of the bird),

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which allowed us to simultaneously record the wing-snap sonations from the individual in which we recorded EMG. We used the acoustic snap in the wing-snap display as a kinematic marker of behavior, as it occurs exactly when the wings collide (Bodony et al., 2016).

We digitized these signals from the transmitter and microphone through a four-channel AD converter (UR44 USB interface, Steinberg, Hamburg, Germany), using a custom multi-channel program (ASIO Rec, Markus Abels, MPIO Seewiesen) at a rate of 44.1 kHz. We then stored the data as uncompressed files (.wav) on a laptop computer, such that the EMG recordings were temporally aligned with the audio recordings. Note that because the microphone was positioned so close (within 20 cm) to each individual during wing-snap production, the time lag between the electrical impulse of the muscle measured through EMG and the sound waves produced by the snap event reaching the microphone was negligible (i.e. <1 ms).

While collecting these data, we also video-recorded each bird's behavior (24 frames s^{-1}) using a standard camcorder. This allowed us to validate that the bird in which we recorded EMG was the individual who produced the wing-snap heard in the audio recording. The camera was placed 1 m from the bird; we later synchronized this video file with the audio recordings collected alongside the EMG recordings using common acoustic cues present in both (i.e. manakin calls and other disturbances in the room). With this approach, there was a slight time discordance (<5 ms) between the audio file collected through the video and the audio file collected via the separate microphone. However, we did not need to account for this difference during our alignment, given that we only used the video to verify individual identity and the presence or absence of a wing-snap.

Analytic approach

We examined EMG recordings from the SC, SH and PEC that occurred during the production of a wing-snap. To better define this time parameter, we identified two kinematic phases of the wing-snap using previously published kinematic descriptions and high-speed video recordings of this behavior (Bodony et al., 2016; Fusani et al., 2007b; Fuxjager et al., 2013). The first phase is wing elevation, in which the bird extends its wings while moving them above the back from the rest position folded at the sides. The second phase, occurring after wing elevation, is wing retraction – the bird retracts its elevated wings such that the distal humeri continue to rise, causing the wrists to collide above the axial midline. Together, these phases are 80 ms in duration, with wing elevation occupying the first 60 ms and wing retraction occupying the last 20 ms (10 ms before and 10 ms after the snap).

Thus, by overlaying EMG data with these behavioral categories, we can begin to uncover muscular mechanisms by which the SC, SH and PEC contribute to the production of the wing-snap. All EMG data were visualized and analyzed in Praat (www.praat.org). Prior to any assessment, recordings were low-pass filtered at 200 Hz and high-pass filtered at 80 Hz, the latter of which removed EMG artifacts borne from physical movement. This upper bound corresponded to the upper boundary of the frequency bandwidth of the radio-transmitter, such that we were unable to visualize high-frequency components of the EMG signal.

EMG inspection and timing analysis

First, we visually inspected each EMG to determine whether the implanted muscle was activated during either phase of the wing-snap (representative EMGs are depicted in Fig. 1). Second, we explored the activation timing of each muscle both immediately before and during the wing-snap. We therefore plotted an amplitude (intensity) tier of the EMG signal (pitch range 80 to 200 Hz) from the period of no muscle activity immediately before a wing-snap to the moment of the snap event itself. We then measured the durations between: (1) the snap event and the point at which EMG amplitude doubled (i.e. when low levels of motor recruitment are first detectable); (2) the snap event and the point at which EMG amplitude quadrupled (i.e. when levels of motor recruitment are obvious and robust); and (3) the snap event and the maximum EMG amplitude (Moritani and Muro, 1987; Yao et al., 2000). Through this analysis, we could chart the onset of muscle activity preceding the wing-snap, while also characterizing when this activity increased and peaked.

Assessing EMG strength

Finally, we contextualized the activation of each muscle during the wing-snap by comparing the relative EMG amplitude during the display to that of wing flapping. We therefore measured EMG amplitude [root mean squared (RMS) voltage] from each muscle during the wing elevation and wing retraction phases. Because the surgical preparation and electrode placement differed among the three muscles (see above), we could not directly compare RMS values across these tissues. Consequently, we conducted all comparisons within the same muscle, such that RMS values during the wing-snap were compared with (1) RMS values of baseline EMG recording and (2) RMS values of peak EMG activity during a wing flap. In these cases, we selected baseline recordings randomly, although we chose them from an area of the EMG trace that immediately preceded the wing-snap. We selected moments of peak activity in a similar manner; however, in this case, we first looked across the EMG recordings for instances of burst activity and then verified through the videos that such activity was associated with wing flapping (often when the bird was in flight). For these measurements, we set the peak activity to the middle of the time period for which we extracted RMS values. All comparisons were done on a within-phase basis, so that the durations in which RMS values were recorded matched each other (i.e. all RMS values were extracted from a 60 ms duration for comparisons of EMG strength during the wing elevation phase, whereas all RMS values were extracted from a 20 ms duration for comparisons of EMG strength during the wing retraction phase). For each bird, we collected an additional two baseline and three peak activity RMS measures for each wing-snap phase.

We statistically compared these values using a linear mixed-model ANOVA, in which behavior [baseline (at rest), snap or flap] was the fixed factor and number of wing-snaps nested within bird identity was the random variable. Given that individual models were run for each muscle and for each wing-snap phase, we reduced our α value to identify significance using methods outlined by Holm (1979). Significant effects were followed by *post hoc* pairwise comparisons, in which we used Bonferroni correction to adjust the significance level to account for multiple contrasts (Zar, 1999).

RESULTS

Neuromuscular activity and the manakin wing-snap

We successfully recorded EMG from wing muscles of male birds that produced wing-snaps in captivity (Fig. 1). By synchronizing these recordings with video and audio documentation of each snap event, we could overlay the activity of each muscle with the specific movement phases that comprise this behavior. These phases included the first prolonged period during which the wings were lifted above the back, as well as the subsequent period in which the elevated wings were retracted, i.e. snapped, together (Fig. 1). We

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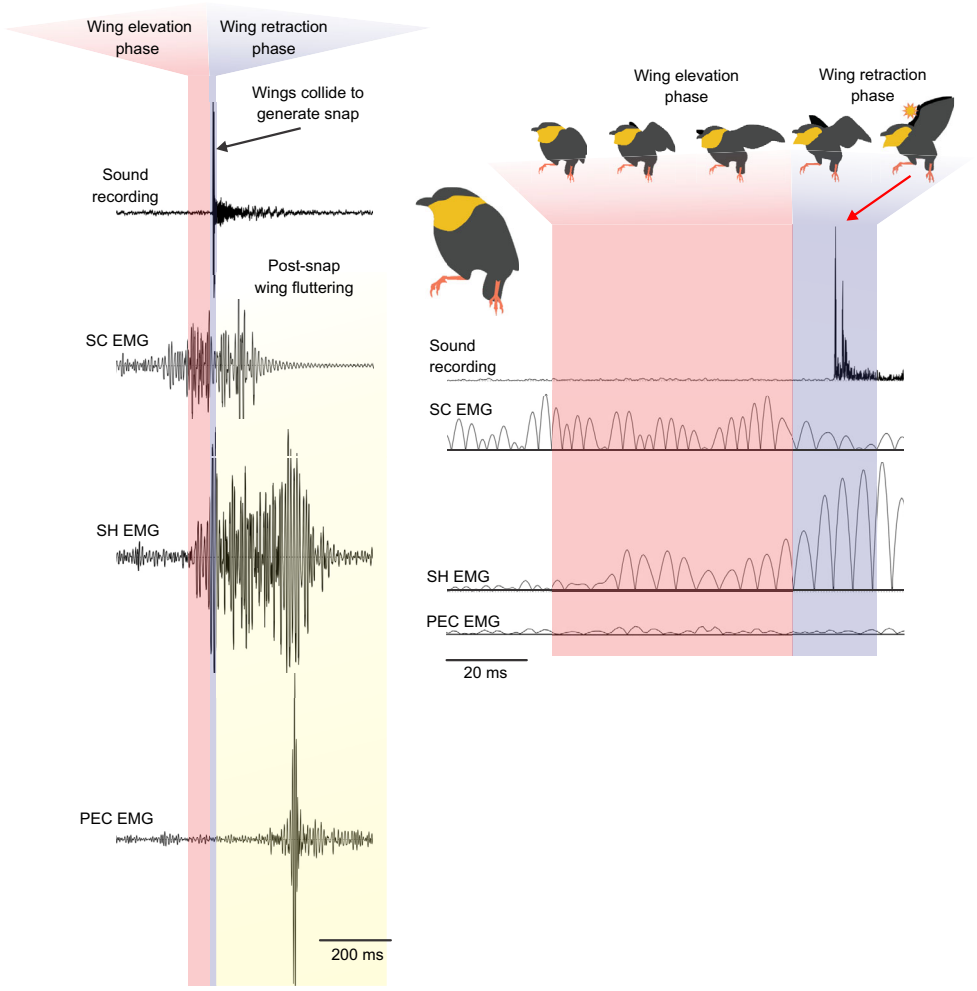

Fig. 1. Representative electromyography (EMG) recordings from the supracoracoideus (SC), scapulohumeralis caudalis (SH) and pectoralis (PEC) during the wing elevation (pink shading) and wing retraction (blue shading) phases of the wing-snap in the golden-collared manakin. The left-hand side of the figure shows the three muscle recordings (EMG traces) in sync with the sound recording of the wing-snap event. Note that birds often fluttered and flapped their wings after each wing-snap, which is evident through the post-snap EMG activity in all three muscles (yellow shading). The three muscles were not collected from the same bird; rather, they represent different EMGs that have each been manually synchronized to a single snap event. The right-hand side of the figure shows rectified EMG traces magnified at the two behavioral phases. Above these signals is a schematic of the different wing movements manakins produce during the behavioral phases of the wing-snap [based on descriptions and high-speed video published by Bodony et al. (2016), Fusani et al. (2007b) and Fuxjager et al. (2013)]. Bird illustrations by Meredith Miles.

found within- and between-individual variation in EMG timing and strength during these phases of movement; nevertheless, both the SC and SH were active during the display (Fig. 1), implicating these two muscles as drivers of signal production. The PEC showed no appreciable activation in the fractions of a second before the wings snap (Fig. 1), implying that this muscle does not play a role in actuating wing-snap production. Note that in Fig. 1, we can see that the PEC is activated during post-snap fluttering, which indicates that our electrodes were properly implanted and able to record EMG during non-snap movements.

EMG timing

We first assessed signatures within EMG traces that shed light on the timing of muscle activation before and during the wing-snap (Fig. 2). For the SC (Fig. 2A), we found that the amplitude of the EMG signal doubled ∼127 ms prior to the wings hitting together. This activation level was present before wing movement occurred, which suggests this amplitude shift marks the onset of muscular activation leading up to behavioral actuation. The amplitude of the SC EMG signal quadrupled ∼70 ms prior to the wing snap, which corresponds to the moment when the birds begin to lift their wings. The EMG activity of the SC peaked ∼47 ms prior to the wing snap, which was well before the wings were pulled together above the back.

The timing of SH activation relative to the snap differed substantially from that of the SC (Fig. 2B). In this case, the amplitude of the EMG from the SH doubled ∼58 ms and quadrupled ∼36 ms prior to the snap. Both points are close together and occurred as the wings were raised. EMG amplitude of the SH peaked roughly 10 ms after the snap. Together, these data suggest that the nervous system begins to activate the SH as the wings are being lifted above the back, and that additional strong and rapid activation of this muscle occurs as the wrists are forced to collide.

Because the PEC was not activated in association with the wing-snap, we did not include it in this analysis (Fig. 2C).

EMG intensity

We first tested whether the EMG amplitude during wing elevation and wing retraction phases of the wing-snap differed from EMG baseline and peak activation during wing flapping (Fig. 3). Although visual examination of certain SC EMG traces suggested that this muscle was activated for wing elevation and wing retraction during production of snaps (e.g. Fig. 1), we found that this effect was not borne out through our statistical analyses. SC EMG strength was significantly different among groups during wing elevation ($F_{2,15}$=9.91, P=0.002; Fig. 3A) and wing retraction ($F_{2,15}$=13.25, P<0.001; Fig. 3B), but *post hoc* analyses showed that this effect was due to an increase in SC EMG strength during wing-flaps, compared with both wing-snaps (P=0.023) and baseline (P=0.003). In fact, these latter two groups were statistically indistinguishable from each other (P=0.99).

EMG amplitude of the SH differed markedly from that of the SC. Indeed, we found that it varied significantly across groups during wing elevation ($F_{2,17}$=22.50, P<0.001; Fig. 3C) and wing retraction

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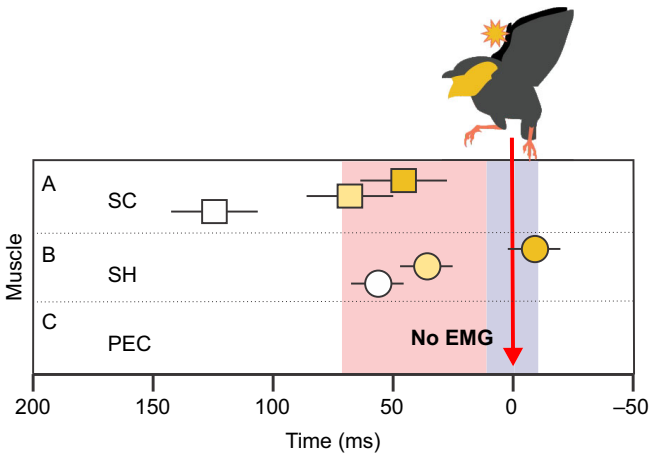

Fig. 2. Analysis of EMG timing from three muscles in the golden-collared manakin across the time periods before and after the wing snap (unshaded), and also during the wing elevation (pink shading) and wing retraction phases (blue shading). (A) Supracoracoideus (SC); (B) scapulohumeralis caudalis (SH); (C) pectoralis (PEC). The moment of the snap event is indicated by the red line at time zero, with positive times preceding the snap and negative times following it. For all muscles, white symbols represent the estimated marginal mean (EMM) of the time (error bars: ±1 s.e.m.) at which the EMG doubled, marking the onset of a low level of motor recruitment in the muscle. Light yellow symbols represent the EMM of the time (error bars: ±1 s.e.m.) at which the EMG quadrupled, marking the onset of a high level of motor recruitment in the muscle. Gold symbols represent the EMM of the time (error bars: ±1 s.e.m.) at which the EMG peaked, representing the maximum motor recruitment in the muscle. Note that, within each muscle, vertical offsetting of the symbols is intended to help better distinguish the error bars. Bird illustration by Meredith Miles.

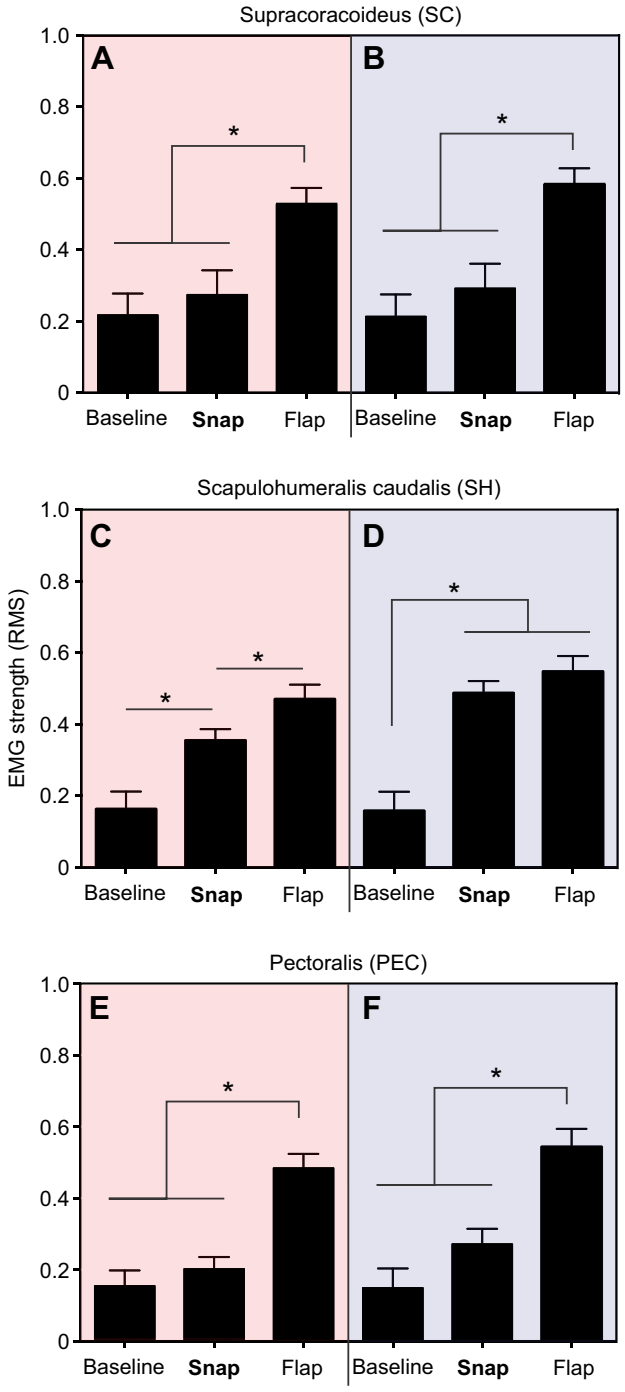

Fig. 3. Analysis of EMG intensity (root mean squared, RMS) from three muscles in the golden-collared manakin. (A,B) Supracoracoideus (SC); (C,D) scapulohumeralis caudalis (SH); (E,F) pectoralis (PEC). Comparisons are made within each phase of the wing snap, either the wing elevation phase (pink shading) or the wing retraction phase (blue shading), but different phases are never compared with each other because they represent different durations of EMG activity (see Materials and methods). 'Baseline' refers to a section of EMG activity preceding the wing-snap in which the bird was not observed moving, whereas 'flap' refers to a section of EMG activity in which the muscle in question was maximally engaged. 'Snap' (highlighted in bold) refers to EMG intensity associated with the specific phase of the wing-snap. All models are significantly different (P<0.002), with significant *post hoc* contrast depicted by asterisks (*P<0.05 after Bonferroni correction). Data represent estimated marginal means obtained from linear mixed models, with error bars denoting ±1 s.e.m.

($F_{2,17}$=30.10, P<0.001; Fig. 3D) phases. Compared with baseline values, EMG amplitude in both phases was significantly greater during wing-snap behavior (P<0.002). During the wing elevation phase, EMG amplitude during the wing-snap was still lower than during wing-flaps (P=0.007), but reached this level during the wing retraction phase. At this time point, SH EMG amplitude was statistically indistinguishable between these two behaviors (P=0.22), suggesting that similar levels of motor recruitment are required to retract the wings together for the wing-snap and to flap the wings for flight.

For the PEC, visual inspection of the EMG traces largely agreed with our analysis of activation strength, in that both implied that this muscle is significantly involved in the actuation of the wing-snap itself. As such, we found that PEC EMG strength differed significantly among groups during wing elevation ($F_{2,13.8}$=22.10, P<0.001; Fig. 3E) and wing retraction ($F_{2,20}$=15.00, P<0.001; Fig. 3E) phases. *Post hoc* analyses showed that this effect was the result of increased EMG activity during wing-flaps, compared with such activity during the wing-snap (P<0.002) and baseline (P<0.001).

DISCUSSION

To uncover the neuromuscular origins of an athletic courtship display, we used radio-telemetry to record EMG from the main wing muscles – SC, SH and PEC – of male golden-collared manakins that actively performed wing-snap displays. As expected, our data suggest that both the SC, the main wing elevator, and the SH, which normally retracts and rotates the wings, play a role in the actuation of the wing-snap. Additional analyses of the timing and strength of EMG from these muscles imply that contraction of the SH actuates

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the movement involved in snap generation itself. Activation of this muscle immediately before the wings are swiftly snapped together above the back is similar to that seen during normal wing flapping for bursts of flight. Together, these findings offer a glimpse at the neuromuscular mechanisms underlying this complex physical display, thus also offering a methodological approach toward successful study of sophisticated and unusually physical display movements.

Timing of muscular contraction during the wing-snap

Overall, our data support the hypothesis that both the SC and SH play a role in the actuation of the wing-snap. EMG recordings show that both muscles are activated when a bird produces this signal, indicating a tight correlation between the contraction of these tissues and the production of the behavior. The PEC, however, showed no clear signs of activation when the bird produced a wing-snap. This is also consistent with our hypothesis that this muscle plays little or no role in driving this behavior, given that it is the main wing depressor (Biewener et al., 1992; Dial, 1992; Dial and Biewener, 1993; Dial et al., 1991).

With regard to the specific functions of the SC and SH in the wing-snap, the timing of the respective activation patterns suggests that the former effects the initial elevation of the wings before they are retracted together above the back. Indeed, we show that SC EMG amplitude begins to increase (i.e. it doubles) roughly 60 ms before the wings begin to rise. This activity becomes more robust (i.e. it quadruples) once the bird unfurls its wings from the body and raises them upward. Thus, the SC is activated at the onset of the wing-snap and contributes to moving the wings in the elevated position so that they can be snapped together. This movement is entirely consistent with known biomechanical role of this muscle in forelimb mobility, as it elevates the humerus and thus actuates the up-stroke of the wings during flight (Biewener, 2011; Dial, 1992; Dial et al., 1991). Also interesting is that the activation of the SC peaks halfway through the wing elevation phase (and declines thereafter), even though the wings continue to elevate. This is likely the point at which the initial inertia of wings is overcome and wing elevation continues with slightly decreasing muscle activation. A similar effect is observed during flight, where SC activation ends in the middle of the up-stroke despite the fact the wings continue to lift until they experience antagonistic effects of the PEC (Biewener, 2011; Dial, 1992; Dial et al., 1991).

The SH performs a different role in the actuation of the wing-snap. This muscle is activated (i.e. EMG amplitude doubles) approximately 15 ms after the wings initially are elevated. The intensity of the EMG quickly increases, quadrupling within another 20 ms and peaking shortly (roughly 10 ms) after the wings are hit together. Thus, the SH appears to be more rapidly activated, relative to the SC, with much of this activation centering around the phase of the behavior in which the humeri are retracted to cause the wrists to collide. This result is also consistent with our understanding of the biomechanical role of the SH, as it is believed to act as the primary wing rotator and retractor during flight (Dial, 1992; Dial et al., 1991).

Muscular activation during wing-snap behavior

We also evaluated the relative EMG amplitude of each muscle during the behavioral phases of the wing-snap. One of the most interesting results from our study is the high EMG amplitude of the SH during the wing snap. In the wing elevation phase, EMG amplitude in this muscle was greater than baseline, but still less than that observed during wing-flapping. However, in the wing retraction phase of the snap, the EMG amplitude of the SH was just as high as that during flapping. This suggests that the muscle experiences especially robust temporal synchrony in afferent activation and/or significant motor unit recruitment precisely when the wings are being forced together to produce the loud snap (Moritani and Muro, 1987; Solomonow et al., 1990; Yao et al., 2000). Thus, the timing and amplitude pattern of the SH EMG implicate this muscle in the production of the movement that generates the snap.

For the SC, we were surprised to see not only that the EMG amplitude both before and during the wing snap was significantly lower than that observed when this muscle was engaged in flapping during short bursts of flight, but also that mean activation levels were not significantly different from baseline levels while the bird was perched and not visibly moving its wings. This result should not be interpreted as a lack of involvement of the SC in the actuation of the wing-snap; indeed, we find clear patterns of SC activity while this behavior is being produced (see Fig. 1). We suspect that there are several, possibly interacting, explanations for the lack of a significant difference. The first possibility is that the activation required of the SC to lift the wings for a wing-snap is simply very low, and thus indistinguishable from 'noise' in the EMG signal that otherwise occurs. We recorded EMG from birds that produced wing-snaps while perched. This means that the SC needed to lift the wings from their resting position, which likely requires less activation than when the SC contributes to wing-flapping. In the latter case, the SC presumably must generate enough strength to not only slow downward movement driven by the PEC, but also reverse the wings' trajectory upward (Dial, 1992; Dial et al., 1991). Another possibility is that SC plays a large role in guiding small forelimb movements needed to help maintain balance while perched. Studies show that wing movements are used in this manner (Necker et al., 2000), and thus the SC may be activated – albeit minutely – to help maintain perching behavior. If so, baseline measures of EMG from the SC may be clouded by small bursts of activity, even though the wings were not observed to dramatically move.

With these considerations in mind, it is interesting to note that manakins displaying in the wild only produce wing-snaps in mid-air when they jump among saplings as part of their courtship dance (i.e. the jump-snap display) (Fusani et al., 2007b). In these cases, the SC may be activated more than it needs to be when birds produce a snap while perched. Again, in the wild, individuals only snap their wings together while perched when they generate multiple snaps in rapid succession (i.e. a roll-snap). We did not record EMG from individuals who produced roll-snaps, but this behavioral nuance, combined with our physiological data, suggests the possibility that the neural control mechanisms for generating these two 'types of snapping' differ.

Finally, regarding the SC, we cannot rule out the possibility that we recorded from a region of the muscle in which motor recruitment is weak during the actuation of the wing-snap. The SC itself is a large muscle and many regions are difficult to access for EMG recording, given that it lies deep to the PEC. We could only implant electrodes along the medial surface of this muscle between the keel and PEC. The targeted region may only experience greater activation if additional power to lift the wings is required, as occurs during flight.

A model for the neuromuscular control of the wing-snap

Together, our results establish a likely model for the neuromuscular mechanisms that generate the wing-snap. First, to unfurl and raise the wing up from the body, the SC is weakly activated. As activation of the SC increases, the wings are lifted above the back. Then, the nervous system elicits a rapid, strong contraction of the SH muscle. The result is a swift retraction of the wing, causing the wrists to move

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medially and collide. As such, we hypothesize that the SC helps position the wings for this behavior, whereas the SH acts as the main muscle that generates the species' characteristic 'snapping' sound.

This model for wing-snap generation is corroborated by our understanding of the anatomical and physiological adaptations of the neuromuscular system of the manakin wing that are believed to support its unique courtship display. Recent work shows that this animal's SH – but not its SC – has evolved extremely rapid contraction–relaxation cycling kinetics, and thus can generate distinct wing oscillations that approach 100 Hz (Fuxjager et al., 2016a, 2017). This highlights the degree to which the SH specifically is modified to accommodate wing-snap production, given that it appears to be the major muscle to effect the fast snapping movements. Other work similarly finds that these two muscles are hypertrophied (Schultz et al., 2001) and that they abundantly express genes that encode proteins to enhance muscle contraction speeds (Fuxjager et al., 2012a, 2016b). In this way, the forelimb muscular system of this bird is highly specialized to accommodate not only locomotion, but also this remarkable sexual display that is acrobatic and athletic in nature.

Although our study suggests that the SC and SH are largely involved in the production of the wing-snap, we do not rule out the involvement of the many other wing muscles that help fine-tune forearm movements in birds. It is difficult to predict exactly how these tissues would contribute to the kinematics of the display we are investigating, considering that their biomechanical roles in locomotion are poorly understood. Nevertheless, preliminary examinations of certain wing muscles offer interesting hints about how they may be involved. For example, the extensor carpi radialis, which controls wrist movement and forearm retraction (George and Berger, 1966), is also hypertrophied in the golden-collared manakin, compared with other passerine bird species (A. Friscia and M.J.F., unpublished observations). Thus, this and potentially other muscles may play important roles in the regulation of the wing-snap, a complex behavior that undoubtedly requires skill and coordination to execute.

Conclusions

We used radio telemetry to investigate the neuromuscular mechanisms of the highly physical courtship display in the golden-collared manakin, in which an individual rapidly snaps its wings together to generate a loud mechanical sound. Two wing muscles – the SC and SH – are likely involved in the production of this behavior, with the latter muscle bearing the primary role of actuating the display. We therefore establish a firm connection between an elaborate courtship behavior and the muscle performance that produces it. Identifying the key neuromuscular components of a display allows us to better understand the physiological and evolutionary mechanisms that underlie it, which to date has been a challenging and elusive feat.

Acknowledgements

We thank STRI and its administrative staff for their assistance with this project, as well as the Autoridad Nacional del Ambiente and the Autoridad del Canal de Panama for permission to conduct this work. We thank Willow Lindsay and Michele Rensel for logistical support, as well as Meredith Miles for assistance with the illustrations. We thank Hannes Sagunsky for engineering and building the radio transmitters that made this work possible.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.J.F., B.A.S.; Methodology: M.J.F., L.F., F.G., L.T., A.T.M., M.G., I.C., J.C.; Software: L.T., A.T.M., M.G.; Validation: M.J.F.; Formal analysis: M.J.F., F.G., R.M.L.; Investigation: M.J.F., L.F.; Resources: F.G., L.T., A.T.M., M.G.; Data curation: M.J.F., R.M.L.; Writing - original draft: M.J.F., F.G.; Writing - review & editing: L.F., L.T., A.T.M., M.G., I.C., R.M.L., J.C.; Project administration: M.J.F., F.G., B.A.S.; Funding acquisition: M.J.F., B.A.S.

Funding

This research was supported by National Science Foundation grants IOS-1655730 (to M.J.F.) and IOS-0646459 (to B.A.S.).

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