SYMPOSIUM

Androgen Receptor Modulates Multimodal Displays in the Bornean Rock Frog (*Staurois parvus*)

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Synopsis Multimodal communication is common in the animal kingdom. It occurs when animals display by stimulating two or more receiver sensory systems, and often arises when selection favors multiple ways to send messages to conspecifics. Mechanisms of multimodal display behavior are poorly understood, particularly with respect to how animals coordinate the production of different signals. One important question is whether all components in a multimodal display share an underlying physiological basis, or whether different components are regulated independently. We investigated the influence of androgen receptors (ARs) on the production of both visual and vocal signal components in the multimodal display repertoire of the Bornean rock frog (*Staurois parvus*). To assess the role of AR in signal production, we treated reproductively active adult males with the antiandrogen flutamide (FLUT) and measured the performance of each component signal in the multimodal display. Our results show that blocking AR inhibited the production of multiple visual signals, including a conspicuous visual signal known as the "foot flag," which is produced by rotating the hind limb above the body. However, FLUT treatment caused no measurable change in vocal signaling behavior, or in the frequency or fine temporal properties of males' calls. Our study, therefore, suggests that activation of AR is not a physiological prerequisite to the coordination of multiple signals, in that it either does not regulate all signaling behaviors in a male's display repertoire or it does so only in a context-dependent manner.

Introduction

Multimodal displays, in which two or more sensory modalities are used to transmit information to receivers, have evolved for sexual communication in many species. Some of the more well-known examples include the intricate displays of male spiders, which incorporate a variety of visual and seismic signals (Hebets 2005; Taylor et al. 2005; ELIAS et al. 2012), as well as the elaborate courtship dances seen in many species of birds, which may combine both visual and acoustic signals along with acrobatic whole-body movements (Wilczynski et al. 2010; Cooper and Goller 2004; Fuxjager and Schlinger

2015). Typically, multimodal signaling evolves when the environment imposes signaling constraints, and multiple signals are necessary to improve communication efficacy (Hebets and Papaj 2005; Partan and Marler 2005; Preininger et al. 2009; Starnberger et al. 2014). Multimodal signaling may also arise when there is sexual selection for multiple ways by which males can advertise their quality to females (Elias et al. 2003; Price 2006; Wilson et al. 2013). Although many theoretical and empirical studies have addressed the questions of why multiple male signals have evolved and how they function from the perspective of the receiver (for review, Candolin 2003;

Hebets and Papaj 2005; Partan and Marler 2005; Starnberger et al. 2014; Patricelli and Hebets 2016), few studies address the equally important question of how signalers' physiology influences the production and coordination of multiple signals, which can also influence display function and evolution.

Unlike unimodal displays, the production of multimodal displays often involves the coordination of multiple body systems and motor patterns to produce signals with a variety of temporal relationships. In multimodal displays, two or more component signals may be "fixed" (Smith 1977), meaning they must always be performed together or in close succession, or they may be "free" (Smith 1977 ; Wickler 1978), meaning that they may be performed independently or flexibly combined with other display components. If two components of a multimodal display are fixed, they may be physiologically linked (Smith and Evans 2013). In particular, physiological factors may impose constraints on how and when multiple signals are deployed (Cooper and Goller 2004; Kime et al. 2013; Starnberger et al. 2014), and may therefore limit the degree to which signalers show flexibility in display performance. On the other hand, free display components can occur with a variety of temporal relationships and do not necessarily share production mechanisms (Partan and Marler 2005; Higham and Hebets 2013; Miles and Fuxjager 2018). Free components may share a common mechanism of regulation, or they may be regulated independently. Additional studies are needed to test these ideas.

One way that multimodal displays can be regulated is via the effects of androgenic hormones on the variety of signal production mechanisms that are integrated to form a whole display. Androgens, such as testosterone (T), play a central role in the development and expression of male reproductive traits (Crews and Moore 1986). The neuromuscular systems that underlie unimodal signal production in birds (Sartor et al. 2005), frogs (Zornik and Kelley 2011), and rodents (Pasch et al. 2011; Zheng et al. 2021) have high levels of androgen receptors (ARs) and signal production is androgen-dependent. In vertebrate species that use multimodal displays, emerging evidence suggests that androgens play a role in regulating multiple components of the display. For example, both the production of the incredibly rapid wing-snaps and vocalizations used in the golden collared manakin (*Manacus vitellinus*) courtship display are androgen-dependent (Fuxjager et al. 2013; Fuxjager et al. 2014), and comparative work with several manakin species shows that there is greater AR expression in muscles used to perform courtship displays compared to muscles that are not associated with courtship movements (Fuxjager et al. 2015). Thus, androgen action at different sites in the body could help to coordinate multiple motor pathways with the temporal precision needed for such elaborate multimodal displays. However, it is still unknown whether all signals that make up a multimodal display are necessarily androgen-dependent, or whether different signals may be modulated by different endocrine mechanisms. In fact, we know relatively little about the physiological mechanisms that regulate the performance of elaborate multimodal displays in general.

Here, we examine how activation of ARs influences multiple components of the multimodal display in the "foot-flagging" frog, *Staurois parvus*. The foot flag is a conspicuous visual signal in which frogs extend their rear limb, rotate it backward in an arc while displaying their white foot webbing, then retract the limb back toward their body (Grafe and Wanger 2007; Preininger et al. 2009; Grafe et al. 2012; see Supplementary video). It is a signal used in both an intersexual and an intrasexual context. Foot flagging emerged after vocal signaling in the anuran lineage, primarily in stream-breeding species that live in noisy environments (Hödl and Amezquita 2001), and it is used as the dominant component of a signaling repertoire that also includes other visual signals, such as an inflated vocal sac and upright posturing, as well as vocalizations. In *S. parvus*, multiple signals are highly coordinated into a complex multimodal display with a predictable temporal sequencing of signal components (Grafe et al. 2012). However, *S. parvus* also maintains some degree of flexibility in the use of visual and vocal signals, using foot flagging more frequently and vocalizations less frequently when environmental background noise is high (Grafe and Tony 2017).

In previous studies, we have found that the evolution of multimodal signaling in foot-flagging frogs is associated with high androgen sensitivity in those tissues that are used to produce the frog's signals. Compared to other frog species that are unimodal signalers, *S. parvus* males have over twice the AR expression in neuromuscular tissues that produce the different components of their multimodal display (Mangiamele and Fuxjager 2018). When we consider species differences in tissue-specific expression of AR, we observe large and selective increases in AR in hindlimb muscle tissue in foot-flagging species, and frogs that foot-flag have a higher ratio of AR expression in their hindlimb muscle compared to their larynx muscle, whereas it is the opposite in

non-foot flagging species (Mangiamele et al. 2016). Thus, multimodal signalers have higher AR expression overall, and specifically in those muscles that drive the output of visual signals compared to vocal signals. In anurans, androgens are well known to have activating effects on male vocal signaling and many studies have demonstrated that advertisement calls are androgen-dependent (Wada et al. 1976; Wada and Gorbman 1977; Wetzel and Kelley 1983; Solís and Penna 1997; Burmeister and Wilczynski 2001). However, in foot-flagging frogs, T increases the frequency of foot-flagging behavior in *S. parvus* within a few hours post-injection, but it does not influence calling (Mangiamele et al. 2016). These results suggest that different hormonal mechanisms or different thresholds in hormone sensitivity may underlie the regulation of the derived and ancestral signals in *S. parvus*.

To test whether blocking AR would influence all components of *S. parvus*' multimodal displays or only some components, we treated reproductively active adult males with T and experimentally assessed whether the AR-antagonist flutamide (FLUT) would block the effects of T. We then measured how frequently males produced vocalizations and displayed each of the four visual signals in their repertoire: foot flag, foot flash, vocal sac inflation without audible sound, and upright posturing. Because the production of vocalizations is obligately linked to the visual cue of an inflating vocal sac, we also assessed the influence of AR on the acoustic features of males' vocalizations. We focused specifically on dominant frequency, call duration, and number of notes in males' calls because these features can be influenced by androgen's effects on the sound-generating larynx in frogs (Zornik and Kelley 2011).

Methods

Animals

Adult Bornean rock frogs (*S. parvus*) were captive-raised at the Vienna Zoo. Frogs were maintained in a large terrarium ($150 \times 120 \times 100$ cm), which houses over 100 individuals of all life stages at a temperature (23–25°C), relative humidity (70–90%), and day length (12 h of light : 12 h of dark) that are similar to conditions in their native habitat in Borneo. The signaling behavior of these captive males does not differ from the behavior of *S. parvus* found in the wild in that males can be observed both vocalizing and foot flagging in terraria in which other males and females are present (Preininger et al. 2012). This work was conducted at the Vienna Zoo,

Vienna, Austria. Procedures were approved by the Institutional Animal Care and Use Committee at Smith College, the European Union Directive, and the University of Vienna.

Experimental design

We conducted experiments to elicit and measure multimodal signaling behavior in *S. parvus* using a previously established behavioral testing paradigm (Mangiamele et al. 2016). Behavioral testing occurred over a period of 10 days in June and July 2017 between 730 and 1700 h. Reproductively active adult male *S. parvus* ($n = 34$) were identified through observation of their foot-flagging behavior, vocal sac coloration, and size (mean snout–vent length [SVL] $= 19.7$ mm, range $= 16.1$–22.0 mm. Body weight not measured). Males were captured 24–48 h prior to behavioral tests and placed in medium-sized terraria ($50 \times 60 \times 70$ cm) in small groups of 5–10 frogs. Females were also captured 24–48 h prior to behavioral testing, but were housed separately from the males.

To assess whether AR activation is necessary for expression of vocalizations, foot flagging, and other visual signaling behaviors, males received either a single $25\,\mu$L subcutaneous injection of T plus FLUT dissolved in 4% ethanol solution (dose: $10\,\mu$g T and $50\,\mu$g FLUT per frog) or T plus ethanol vehicle (Veh) in an equivalent injection volume. FLUT is a ubiquitous AR-antagonist that acts throughout the entire body, blocks both T and the non-aromatizable androgen 5α-dihydrotestosterone (DHT) from binding to AR, and has been used previously in frogs (Behrends et al. 2010) and other vertebrates (Fusani et al. 2007). Immediately following injection, two males that received the same treatment and were approximately the same size were placed in a transparent mesh arena ($16.5 \times 12.5 \times 12.5$ cm) with one randomly chosen adult female. Four females were used in this experiment, with each female represented twice as a social stimulus in each treatment group. One female was used three times in the T plus FLUT treatment group. In total, we included eight mesh arenas in the T plus Veh treatment group and nine arenas in the T plus FLUT treatment group. Each arena was placed in a larger enclosure ($\sim60 \times 35 \times 35$ cm) containing an artificial waterfall and lined with Styrofoam and 40-mm thick acoustic foam padding to reduce sound reverberation. Overall, the setup mimicked the natural breeding environment (Preininger et al. 2012) and forced males in close proximity to stimulate agonistic interactions and

the full range of behaviors in their signaling repertoire (Grafe et al. 2012; Preininger et al. 2013).

After a 2-h acclimation period, we played back a recording of an individual male *S. parvus* vocalization (dominant frequency = 5.5 kHz, call duration = 8.5 s, 34 notes), which was repeated once every 5 min throughout the behavioral testing period. The playback was recorded in the field and therefore also included background stream noise from the frog's natural environment in Borneo. The peak sound pressure level of the playback was 67 decibels, measured at 70 cm from the speaker (Sony SRS-M 30, Japan), which corresponds to the location of the male frogs in the arena. We chose this playback amplitude in order to simulate another male *S. parvus* calling from <1 m away, which has been shown to elicit foot-flagging from male receivers (Grafe et al. 2012). Most interactions between wild *S. parvus* males do occur at close range, often within 50 cm (D. Preininger, personal observation). Following the 2-h acclimation period, social interactions that occurred in the arena were videotaped continuously for 7 h using a Panasonic Digital Camcorder (SDR-SW21, Japan).

Behavioral observations

We first examined all videos to identify periods of social interaction, which we call "bouts." We defined a bout as a period of continuous signaling activity including at least one foot flag or call. The end of a bout was defined as >1 min of no activity. For males in both treatment groups, almost all bouts occurred during the 3 h of peak signaling activity (4–6 h post-injection); therefore, we restricted our analyses to this time period. An observer blind to treatment group watched the videos and counted the display behaviors (Table 1) produced by both males in the arena during previously identified bouts. Behaviors included prominent visual displays, such as a foot flag, foot flash, upright posturing, and vocal sac inflation without audible vocalizations. In these videos, it was not possible to distinguish between individual males, thus, all behavioral measures were recorded on a per arena basis. We identified vocalizations produced by males in an arena by finding the call's acoustic signature on spectrograms of the video's audio track and then confirming observable movement of an individual's vocal sac at the corresponding time on the video. The observer also recorded the total duration of each bout and the number of times one male aggressively initiated contact with another male (e.g., jumped on the other individual's back, or kicked the other male) during the bout.

To confirm that FLUT did not impact the general health or overall activity levels of the frogs, we assessed levels of physical activity within each treatment group as previously described (Mangiamele et al. 2016). Briefly, we randomly chose 1 h of video during peak hours of signaling activity (4–6 h post-injection). We then sampled the video every 30 s and scored activity in 10-s intervals. If any of the males in the arena showed movement during the 10 s, that interval was scored with a 1; no movement was scored with a 0. Movement was defined as locomotion or a significant change in body position. We then totaled the number of minutes of video for which we recorded a score of 1 and used this metric as our measure of overall activity in each arena.

Acoustic recordings and call analysis

To better characterize any AR-dependent changes in male vocalizations, in July–August 2018, we replicated the experiment described above, except that we placed only one male and one female in an arena at a time so that we could record vocalizations in response to playback and be able to identify the individual male that produced each call. The body size (SVL) and weight of all adult males was similar to that in our first experiment (mean SVL = 20.19 mm, range = 18.7–21.85 mm; mean body weight = 0.86 g, range = 0.7–1.0 g). Four different females were used in this experiment. Each female was used only once, but all females were represented as social stimuli in both treatment groups. Males ($n = 4$ per group) heard a looped playback throughout the 3-h period in which we recorded vocalizations, which consisted of five individual *S. parvus* males' vocalizations recorded in the field (mean dominant frequency = 5.66 $\pm$ 401.22 kHz, mean call duration = 7.31 $\pm$ 2.36 s, mean number of notes per call = 26.6 $\pm$ 7.2) with a 2-min intercall interval. Background stream noise was also present in the playback. The peak sound pressure level of the playback was 67.7 decibels measured at a distance of 70 cm from the speaker, which is approximately the distance from the speaker to the male frog. We recorded the focal male's calls from a distance of ~12 cm directly above the animal using an omnidirectional mini condenser microphone (Pro 70, Audio-Technica, Stow, OH) and a digital audio interface (U-Phoria UMC404HD, Behringer, Germany) that was controlled via a laptop computer. We counted the number of calls per male on the recordings during the period of peak signaling identified previously (4–6 h post-injection) and measured dominant frequency, call duration, and number of

Table 1. Component signals of the multimodal display of *S. parvus* frogs

Signal	Description
Foot flag	Full extension and rotation of one hind limb. White foot webbing visible.
Foot flash	White webbing of one or both hind feet visible without full hind limb extension
Call	Audible sound recorded and vocal sac inflation visible
Vocal sac inflation	Repeated inflation and deflation of white throat sac without audible vocalization
Upright posture	Back arch and head pointed upward. White skin on ventral side visible.

notes in each vocalization using Raven Pro (Cornell Lab of Ornithology, Ithaca, NY).

Statistical analysis

We analyzed differences between treatment groups in the total number of each behavior observed, total number of times contact was initiated by a male, and activity score during peak signaling using Mann–Whitney U-tests because, as is often the case for count data, the data did not conform to a normal distribution (Shapiro–Wilk test, all $P < 0.05$). We analyzed differences in bout duration between treatment groups using *t*-tests. In our second experiment, each frog produced a different number of calls ($n = 1$–9 calls per male), so we calculated the mean dominant frequency, mean call duration, and mean number of notes per call for each male and used these values in our statistical analyses. We analyzed differences in call characteristics between treatment groups using *t*-tests. All statistical analyses were conducted using Prism version 8.4.3.

Data accessibility

The data underlying this article are available in the article or are available upon request to the corresponding author.

Results

The multimodal signaling behavior we observed in males treated with T alone was consistent with previous reports of *S. parvus* behavior in the wild, in that visual signaling behaviors were more commonly observed than vocalizations (Grafe et al. 2012). When we grouped all visual signaling behaviors together, we found a large decrease in visual signaling with FLUT treatment (Mann–Whitney $U = 9$, $P = 0.007$; Fig. 1A), but no change in acoustic signaling (Mann–Whitney $U = 33.5$, $P = 0.96$; Fig. 1B). The main effect of FLUT on visual signaling is likely driven by the significant decrease in foot-flagging behavior (Mann–Whitney $U = 11.5$, $P = 0.03$; Fig. 1C) and upright posturing (Mann–Whitney $U = 14$, $P = 0.03$; Fig. 1E). In particular, frogs treated with T + FLUT performed on average 81% fewer foot flags

compared to T-treated controls. FLUT did not appear to significantly decrease the number of foot flashes (Mann–Whitney $U = 18$, $P = 0.08$; Fig. 1D) or vocal sac inflations (Mann–Whitney $U = 25$, $P = 0.14$; Fig. 1F) in males' multimodal displays, though we observed fewer of these behaviors than other visual signals.

Overall, FLUT appears to have a specific effect on the use of visual signaling behaviors during social interactions, as we did not find evidence of a general decrease in activity in males treated with FLUT (median number of scan samples with observed movement: TVeh = 30.5, TFLUT = 42; Mann–Whitney $U = 25.5$, $P = 0.07$). FLUT treatment also did not have an effect on the number of times males aggressively initiated contact with another male during social interactions (Mann–Whitney $U = 32$, $P = 0.73$) or on the total duration of signaling bouts ($t = 0.16$, df = 15, $P = 0.87$).

We also found no evidence that treatment with FLUT changed the dominant frequency of males' calls ($t = 0.23$, df = 6, $P = 0.82$; Fig. 2B). Dominant frequency is a species-specific call characteristic that is usually closely related to male body size in anurans (Gerhardt and Huber 2002), although *Staurois* frogs call with a much higher frequency than expected for their body size (Boeckle et al. 2009). FLUT similarly had no effect on the overall call duration ($t = 0.20$, df = 6, $P = 0.85$; Fig. 2C) or number of notes in males' calls ($t = 0.97$, df = 6, 0.37; Fig. 2D). In *S. parvus*, call duration and number of notes are positively related to one another and are known to increase with increasing loudness of background stream noise (Grafe and Tony 2017).

Discussion

We examined how AR influences the multimodal displays of male Bornean rock frogs (*S. parvus*). We found that blocking AR using FLUT inhibits visual signaling behavior overall, with the largest effect on foot-flagging and upright posturing. We show that FLUT does not affect visual signal output by suppressing all body movements or by altering the overall duration of signaling bouts. Rather, FLUT
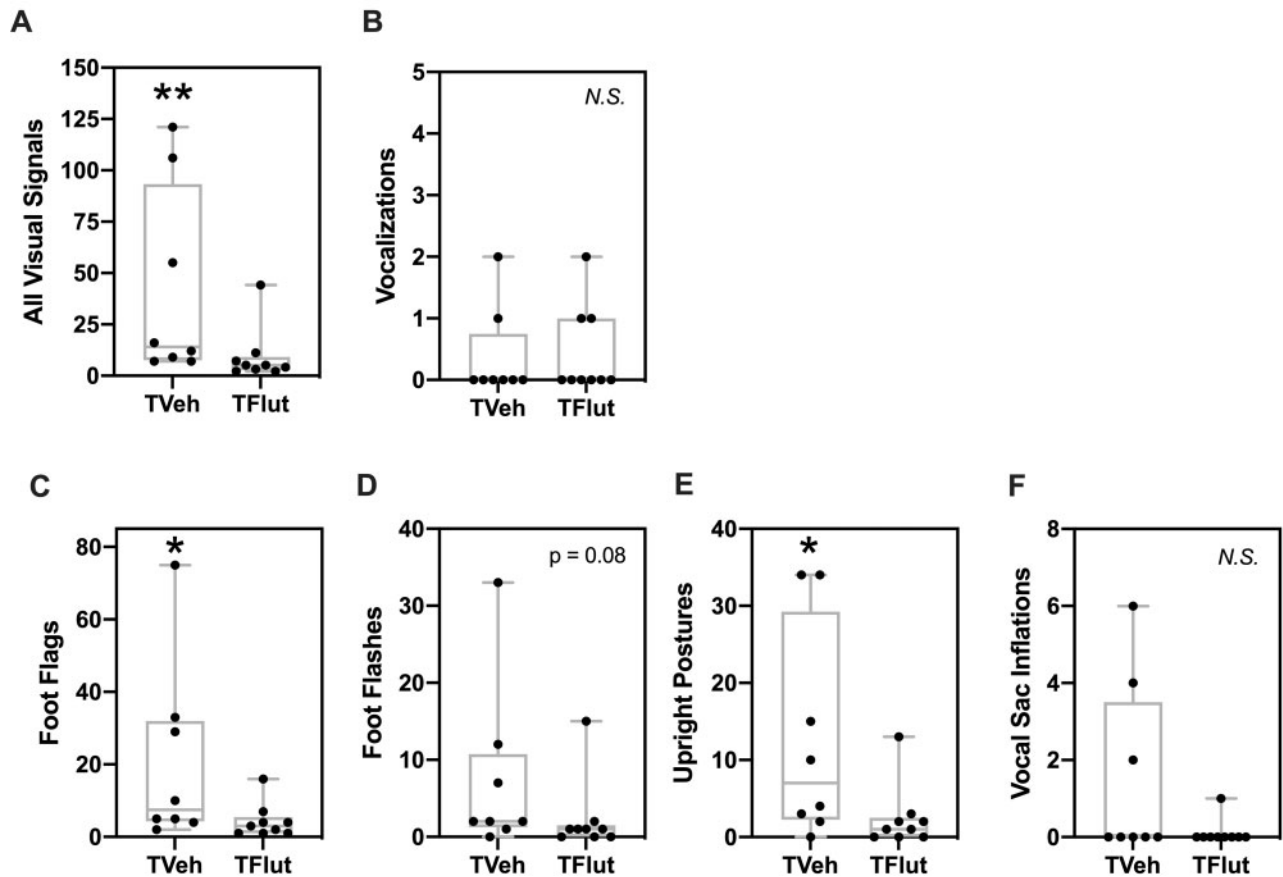
Fig. 1 Effects of FLUT on the number of multimodal display behaviors in *S. parvus*. Compared to Veh controls, FLUT decreases overall visual signaling (**A**), but vocalizations appear unaffected (**B**). Differences between treatment groups in individual visual signaling behaviors, including foot flags (**C**), foot flashes (**D**), upright posturing (**E**), and vocal sac inflations without audible vocalizations (**F**) are also shown. All males in both treatment groups were also treated with T to stimulate signaling behavior. Data points represent total number of behaviors observed for both males in each arena (T Veh, $n = 8$ arenas; T FLUT, $n = 9$ arenas). Asterisks indicate significant differences between treatment groups (Mann–Whitney U, *$P < 0.05$, **$P < 0.01$). N.S. refers to non-significant treatment effect.

treatment appears to produce the observed effects on multimodal signaling behavior by inhibiting androgenic activity via AR on the multiple neuromotor systems that regulate foot flagging and other visual signaling behaviors. Thus, activation of AR could possibly serve as a mechanism for coordinating these movements into a larger multimodal display.

In contrast, we find that FLUT did not have a dramatic effect on vocal performance, and blocking ARs did not affect call dominant frequency or the fine-scale temporal patterning of individual calls. However, we caution that male frogs called much less than they foot-flagged under these experimental conditions, and several interacting male pairs did not call at all. We suspect that environmental context is an important factor that modulates vocal signaling in this species. For example, if males use vocalizations more frequently as long-distance signals (Grafe et al. 2012; Preininger et al. 2013), being forced to interact at close range may have suppressed male calling in

our experimental setup. It, therefore, remains possible that AR does in fact influence vocal behavior in *S. parvus* in other contexts, particularly those in which males would be expected to produce more calls than foot flags. Nonetheless, our data are consistent with our prior work, which showed that exogenous administration of T dramatically increases the number of foot flags produced by male *S. parvus*, but does not change the number of vocalizations (Mangiamele et al. 2016). Studies in many species of frogs indicate that other hormonal systems, including the neuropeptide arginine vasotocin (Boyd 1994; Marler et al. 1995; Chu et al. 1998) and pituitary gonadotropins (Yang et al. 2007; Miranda et al. 2015), contribute to calling behavior and may interact with androgens in complex ways. Thus, our results leave open the possibility that different endocrine mechanisms, or different threshold levels of hormone sensitivity, may regulate visual and vocal signaling behaviors in foot-flagging frogs.
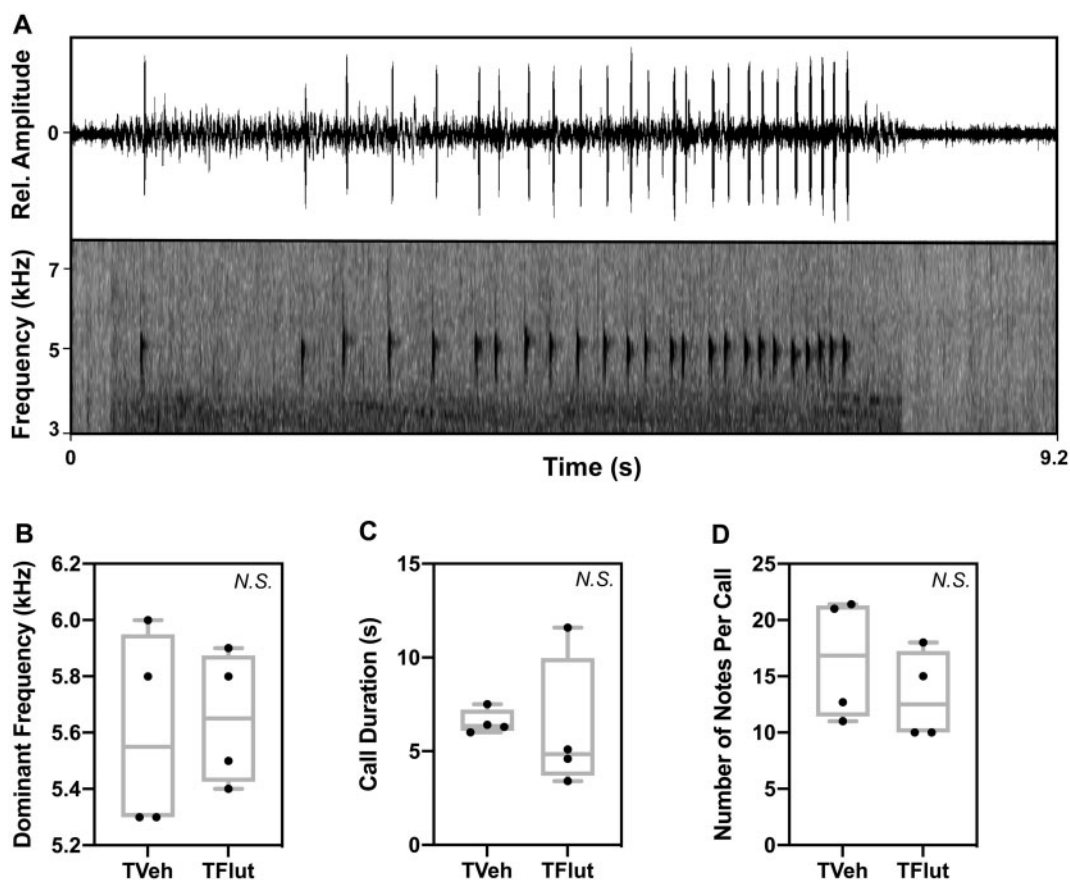
Fig. 2 Example of a male *S. parvus* vocalization (**A**). Dominant frequency (**B**), call duration (**C**), and number of notes per call (**D**) were not affected by FLUT treatment. Males in both treatment groups were also treated with T. Data points represent mean values for each individual male ($n = 4$ per treatment). N.S. refers to non-significant treatment effect in independent-samples *t*-tests.

Androgens and motor control

Sex steroid hormones can shape signaling behaviors by acting at multiple levels in a neuromotor pathway. In anurans, most previous studies have focused on how androgens modulate vocal systems (Kelley 2002; Moore et al. 2005), primarily by influencing the morphology and physiology of laryngeal muscles (Sassoon and Kelley 1986; Tobias and Kelley 1987; Catz et al. 1992; Potter et al. 2005) and their associated neural control pathways (Zornik and Kelley 2011). These issues have been best studied in *Xenopus laevis*, in which T activates male vocal behavior (Wetzel and Kelley 1983), while treatment with FLUT tends to block calling and reverse T-induced laryngeal morphology when administered long-term (Watson et al. 1993; Behrends et al. 2010). This study raises the possibility that androgens may have similar and coordinated physiological effects on multiple neuromotor pathways involved in frog visual signaling behaviors, which require the precise control of limb movements and also involve specific posturing of the head, neck, and back to reveal a bright white ventral surface. Indeed, foot-flagging frogs express high levels of AR throughout the body compared with other frog species that produce only vocalizations (Mangiamele and Fuxjager 2018). Within *S. parvus* males, AR levels are highest in the spinal cord and hind leg muscle (Mangiamele et al. 2016), suggesting that these are especially critical sites at which androgens act to influence the motor control necessary for visual signaling behaviors. Thus, we hypothesize that AR in multiple neuromotor pathways supports the coordination of multiple visual display behaviors in foot-flagging frogs.

It is also possible that the effect of FLUT on multimodal display output originates from the influence of AR on skeletal muscles. Few studies have examined how acute changes in androgenic signaling in mature frogs could influence muscle properties in such a way as to impact visual signal performance. It would be interesting to know whether AR-antagonist treatment affects not only the frequency with which males deploy visual displays, but also the speed, timing, and coordination of the movements.

Recent work in golden-collared manakins demonstrated that AR activation is necessary to maintain the exceptionally rapid contractile speed of the wing muscles that underlie their wing-snap courtship displays (Fuxjager et al. 2016, 2017); therefore, it is possible that androgen action also directly affects muscle performance in *S. parvus* to influence the precise control of movements related to visual signals, in particular the elaborate limb movements that make up the foot flag. In order to better understand the role of AR in modulating the performance of visual displays, future studies might experimentally alter T or DHT levels or block AR in *S. parvus* males and observe changes in the speed or shape of the foot flag.

Evolutionary implications

Here we demonstrate that blocking ARs primarily influences performance of visual signaling behaviors in male *S. parvus*; however, these and previous results do not provide clear evidence of androgenic effects on *S. parvus* vocalizations, despite the fact that vocalizations in many other anuran species are androgen-dependent (Wada and Gorbman 1977; Wetzel and Kelley 1983; Burmeister and Wilczynski 2001; Zornik and Kelley 2011). We argue that these results could indicate structural and functional modularity in the dynamics of this multimodal signaling system. Whereas "fixed" signals are linked by shared development or physiology—like the simultaneous visual and vocal signal produced by the inflating vocal sac of a frog—"free" signals that are produced by independent vocal motor and hind limb motor systems do not necessarily need to share mechanisms of endocrine regulation.

In fact, multimodal displays such as these in *S. parvus*, which are a combination of free and fixed signals, may evolve in a modular fashion, whereby different selection pressures may act on the neuro-motor pathways underlying each component signal (Hebets et al. 2016; Miles et al. 2017, 2018). For example, context-dependent selection may exert pressure to enhance foot-flagging performance in environments with a lot of noise or with variable noise levels, whereas pressure to enhance vocalizations may occur in environments with variable light regimes (Bro-Jørgensen 2010; Grafe and Tony 2017). If distinct hormonal pathways regulate these different communication signals, then they have the potential to act as individual, modular components on which divergent selection may act to shape the structure and function of the signal. Indeed, prior work on *S. parvus* and its close relatives suggests that vocal

and visual signals in *Staurois* are functionally distinct, in that they show non-redundant influence on receivers (Preininger et al. 2013). In fact, vocalizations may act as a simple alerting signal because calls most often precede the conspicuous visual signal of the foot flag (Grafe and Wanger 2007; Preininger et al. 2009, 2013; Grafe et al. 2012). Additionally, selection should favor the mechanistic uncoupling of display components that do not have the same adaptive value in a given environment (*sensu* Ketterson et al. 2009). Seasonal shifts in background stream noise levels cause male foot-flagging frogs to increase the frequency of visual display components and decrease the more easily obscured vocal components (Grafe and Tony 2017), therefore selection might favor multiple mechanistic controls in order to maintain this flexibility in the multimodal display. In short, additional studies are necessary to disentangle the complex interaction between sexually selected traits in multimodal displays and the androgenic modulation of those traits.

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Supplementary data

Supplementary data available at *ICB* online.

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