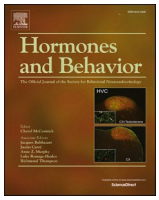
Hormones and Behavior 174 (2025) 105797

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh



A dopamine agonist affects the social decision-making of calling male túngara frogs

Logan S. James [a,b,c,d,*], Sarah C. Woolley [c,d,1], Michael J. Ryan [a,b,1]

[a] Smithsonian Tropical Research Institute, Balboa, Ancón, Panama
[b] Department of Integrative Biology, University of Texas at Austin, 2515 Speedway, Austin, TX 78712, USA
[c] Department of Biology, McGill University, 1205 Av. Docteur Penfield, Montreal, QC H3A 1B1, Canada
[d] Centre for Research on Brain, Language, and Music, 3640 de la Montagne, Montreal, QC H3G 2A8, Canada

ARTICLE INFO

Keywords:
Animal behavior
Neuromodulation
Vocal communication
Courtship
Lekking
Bioacoustics

ABSTRACT

When vocalizing, many animals engage in decision-making processes that integrate information regarding the current social context. The midbrain dopaminergic system may provide a conserved mechanism underlying this process. For instance, in songbirds, modulation of dopamine release appears to contribute to social context-dependent changes to song. However, relatively little is known about the degree to which dopamine may contribute to similar vocal production and decision-making processes in other taxa, particularly the highly vocal anurans (frogs and toads). Here, we treated wild-caught male túngara frogs (*Engystomops pustulosus*) with a general dopamine agonist (apomorphine) and assessed its effects on motor performance and motivation as well as vocal decision-making in response to auditory stimuli that varied in social relevance. We found that the dopamine agonist generally increased vocal speed, with decreases in response latencies and call durations. Additionally, we found that dopamine increased call complexity, but only in response to the most socially relevant auditory stimulus (conspecific call). Finally, dopamine treatment and auditory stimulus interacted to affect decision-making regarding call timing and overlap with the stimulus. Compared to controls, frogs with apomorphine were more likely to overlap the playback stimulus in a manner predicted to be more attractive to females. These results highlight a role of dopaminergic circuits in modulating vocal outputs based on social inputs within a species of basal tetrapod.

1. Introduction

During communication, animals integrate sensory and social information to produce signals tuned to their audience and context. Attending to external information can modulate when animals choose to communicate as well as the structure of their communication signals (Bernal and Page, 2023; Greenfield, 2002; Logue and Krupp, 2016; Snijders and Naguib, 2017). For example, Ueno's brown frog, a diurnal species of frog, ceases calling in the presence of birds (Kim et al., 2020), a common strategy among prey to avoid detection from eavesdroppers (Bernal and Page, 2023). Conversely, nuthatches produce alarm calls in response to predators, and the acoustic features of the alarm calls vary depending on both the threat level of the predator as well as the source (self or public) of the predator information (Carlson et al., 2020). During courtship, *Drosophila* use visual cues about motion and female position to guide transitions between pulse and sine song, and the choice of song is significant for influencing female responses (Clemens et al., 2018; Coen et al., 2014). In zebra and Bengalese finches, males adjust acoustic features of their songs depending on their social context, producing longer, faster, and more stereotyped songs during courtship interactions compared to when males sing while alone (Kao and Brainard, 2006; Sakata et al., 2008; Sossinka and Böhner, 1980; Woolley and Doupe, 2008). However, while many species modify their signals depending on context, there are few instances in which the mechanisms by which signals are modified have been studied.

Dopamine is well-positioned to drive context-dependent changes to vocal behavior across species through its actions across multiple receptor subtypes (Berke, 2018). Throughout vertebrates, dopamine is produced in conserved neuronal populations and dopamine neurons share similar projections to sensory, motor, and cognitive regions across

* Corresponding author at: Smithsonian Tropical Research Institute, Balboa, Ancón, Panama.
E-mail addresses: loganjames@utexas.edu (L.S. James), sarah.woolley@mcgill.ca (S.C. Woolley), mryan@utexas.edu (M.J. Ryan).
[1] Co-senior authors.

https://doi.org/10.1016/j.yhbeh.2025.105797
Received 14 January 2025; Received in revised form 10 July 2025; Accepted 17 July 2025

taxa (O'Connell and Hofmann, 2011, 2012; Yamamoto and Vernier, 2011). Dopamine affects the vigor and performance of motor behaviors, for example, depletion of dopamine synthesizing neurons leads to an overall reduction of movement speed (e.g., Parkinson's disease; Bologna et al., 2020; Panigrahi et al., 2015). Additionally, dopamine shapes learning and plasticity, leading to learned place preferences in frogs (Presley et al., 2010), as well as vocal plasticity in birds (Hisey et al., 2018; Kubikova and Košťál, 2010; Macedo-Lima et al., 2021; Woolley, 2019; Xiao et al., 2018). Social and environmental context can drive activity of dopaminergic neurons, thereby producing context-dependent changes to motivation, motor performance, and plasticity. For example, the shift in song performance that occurs from singing alone to directed courtship song in zebra and Bengalese finches (such as increases in stereotypy and speed) may depend on dopamine release into sensorimotor and basal ganglia regions (Ben-Tov et al., 2023; Hara et al., 2007; Matheson and Sakata, 2015; Singh Alvarado et al., 2021; Woolley et al., 2014).

While dopaminergic modulation of context-dependent vocalizations has been especially well-studied in birds, few studies have addressed a similar role in other species. Frogs (anurans) offer a particularly useful group for these investigations given that they produce relatively simple and stereotyped vocalizations that are modulated by social context, and, as basal tetrapods, they offer key phylogenetic insight into the evolution of dopaminergic systems during the vertebrate transition to land. Administration of a general dopamine agonist in frogs induces behavioral effects such as modulation of sleep-wakefulness cycles (Aristakesyan, 2011), feeding behaviors (Glagow and Ewert, 1999, Glagow and Ewert, 1996), and general movement (Chu and Wilczynski, 2007), but little is known about effects on communication behaviors. A dopamine D2 agonist has been shown to reduce calling in green tree frogs (Creighton et al., 2013), however, whether dopamine modulates social decision-making aspects of communication in frogs is unknown.

Túngara frog males produce calls that attract females, and this well-studied sexual communication system is ideal to investigate the role of dopamine in frog social decision-making during acoustic communication (Ryan, 1985). For a calling male túngara frog, there are two primary decisions he must make that are influenced by the social context: *when* to call and *what* call to produce. Frogs aggregate into choruses (leks) where they vocally interact. Males typically avoid overlapping their calls with each other, but also time their calls to occur during specific windows relative to other males which can increase their attractiveness to females (Larter and Ryan, 2024a). They also dynamically adjust the quality of their calls in response to other calling males, most notably by appending 'chucks' to the ends of their calls. The simple call of this species consists of a 'whine', and males will typically append 1–3 chucks to the end of the whine to produce a 'complex' call which induces a five-fold increase in attractiveness to females (Ryan, 1985; Ryan et al., 2019).

Here, we used a general dopamine agonist (apomorphine) to pharmacologically activate dopamine sensitive neurons in male túngara frogs during evoked vocal responses to playback stimuli. Because we used a broad-spectrum agonist and did not target a specific brain area, we anticipated broad and diverse effects including modulation of motivational, vocal motor, and cognitive aspects of calling. We specifically hypothesized that treatment with a dopamine agonist would (1) increase overall calling (vigor), (2) increase vocalization speed (performance), and (3) affect the social decision-making processes during competitive vocal interaction with the playback stimuli, enhancing the attractiveness of calls (cognitive effects).

2. Material and methods

2.1. Animals

We collected wild adult male túngara frogs (*Engystomopos* [formerly *Physalaemus*] *pustulosus*; $n = 132$) from sites in and around Gamboa, Panama and conducted our experiments in the Gamboa Acoustics Laboratory within the facilities of the Smithsonian Tropical Research Institute (STRI). All frogs were caught in amplexus (mating position with the male clasped onto the back of the female) and remained in amplexus until the male was removed just prior to the experiment.

All frogs were returned to their mate and released at their site of capture within 24 h of collection. All procedures were approved by the University of Texas at Austin (IACUC: AUP-2022-00012), STRI (ACUC: SI-21012), and the Ministry of the Environment of Panamá (MiAmbiente: SE/A-39-2020).

2.2. Drug

Following removal from amplexus, males were administered either a 0 (saline control), 10, or 20 mg/kg dose of apomorphine (Sigma) dissolved in 0.9 % sterile saline. The high dose was based on a previous study of anurans (Chu and Wilczynski, 2007). However, because that study reported some impairment to locomotor behavior at this dose, we also chose to include a half dose in this study. The drug solution was prepared in advance and immediately frozen in small vials to be defrosted just prior to the injections. All injections were administered subcutaneously into the back of the thigh on one of the frog's hind limbs. Because all drug doses derived from the same frozen stock solution, the total injection volume was double for the high dose relative to the low dose. We administered saline at a volume to match the low dose.

2.3. Recording

We recorded males within custom built wooden sound attenuating chambers fitted with acoustic foam (30.5 × 46 × 30.5 cm). Small broad-range microphones were fitted in the ceiling, and speakers were placed on the far side of the box opposite the door. Speakers were calibrated to ~76 dB SPL (re. 20 μPa) at the center of the box each night, and all stimuli were peak normalized. Frogs were recorded using Audacity and digitized at 44.1 kHz. During recording, each frog was in a plastic cage that was modified by replacing most of the wall with fine mesh to ensure acoustic transparency to the speaker and microphone.

2.4. Experimental protocol

We were interested in testing how male túngara frogs would respond to call playbacks and how these responses could be modulated by a dopamine agonist. The timeline for the experiment was: (1) Inject (drug or saline), (2) Wait 15 min with the frog in a dark, dry cooler, with this wait time being determined by our preliminary experiment (see Supplementary Information), (3) Place the frog into the recording chamber in 1 cm of water, (4) 10 min silence and, (5) stimulus playback (7 stimuli, 10 min each; Fig. 1A). We note that only the first 6 playbacks were included in our analysis (see below).

There were three playback stimuli: Conspecific calls (C), contoured Noise calls (N), and Heterospecific calls (H). For each stimulus, we concatenated a single token of the call with approximately 1.5 s of silence between each call. The conspecific call was randomly selected from recordings from a previous study and contains a whine plus one chuck (James et al., 2021). The noise call was white noise with the amplitude envelope of a typical whine, which we generated to provide a stimulus with familiar amplitude structure and the ability to mask a frog's own vocalizations, but with less social salience than a conspecific call (Rand et al., 1992). For the heterospecific call we used a randomly selected exemplar from the Macaulay Library. The species we chose, *Physalaemus riograndensis,* is from the sister genus to *Engystomops*, but does not geographically overlap with the túngara frog. We previously tested phonotaxis responses in females for 10 heterospecific calls and found this call from *P. riograndensis* to be one of the least recognized (20 % acceptance rate) (James and Ryan, 2025).

The stimuli were arranged into sets that enabled us to control the stimulus order, the between stimulus transitions, and the number of

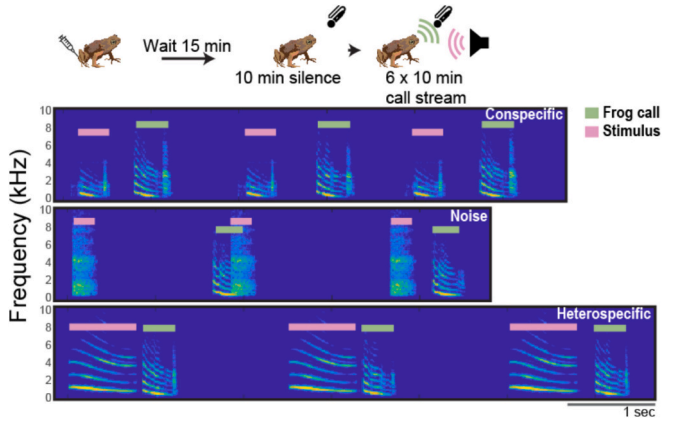


Fig. 1. Experimental protocol. Shown are the protocol and example spectrograms of a saline-treated frog calling during the conspecific (top), noise (middle) and heterospecific (bottom) playbacks. The playback stimuli are indicated with pink bars, and the live frog's calls are indicated with green bars. The conspecific playback stimulus was a whine followed by a single chuck. Frog illustrations by Damond Kyllo.

times each stimulus was heard. To this end, we created six different sets where each set contained all six possible transitions between the three stimuli, and each stimulus occurred at least twice within the set (Supplementary Table 1), with the first stimulus occurring three times.

Frogs in the low and saline doses were tested in November 2023, while the high dose males were tested in June 2024. This frog breeds during the rainy season (May – December). All frogs had their belly photographed to subsequently identify all recaptures. In total, there were 12 recaptures, and the frog ID was included as a random effect in models to account for this (see below). We tested 24 frogs per dose (n = 72 total).

2.5. Call labeling and measurement

Calls were manually labeled using custom built scripts in MATLAB. Sounds were segmented semi-automatically using an amplitude-based threshold. All calls that overlapped the stimulus were manually separated to identify the onsets of both the call playback and the live frog's call. We categorized the calls that overlapped the stimulus as either 'leading' (beginning before the onset of the stimulus call) or 'lagging' (beginning during the stimulus call), and we note that no call was long enough to overlap more than one stimulus call. The túngara frog call onset is marked by a rapid rise in amplitude that is identifiable even in the presence of background noise, but then slowly tapers down to silence, making the identification of offsets difficult when competing against the playback stimuli. Therefore, we only analyzed the duration of calls that did not overlap the playback stimulus. Calls were labeled based on the number of chucks (0 to 3). In total, we labeled 47,564 calls. After labeling, we extracted the time stamps of all call and stimulus onsets and offsets.

2.6. Analysis

We conducted preliminary analyses to initially assess the amount of calling within the experiment to decide which data to include for primary analysis. In particular, the study design ensured that each frog experienced all possible transitions among the stimuli, but heard one stimulus 3 times compared to 2 for the other stimuli. To understand whether the transitions between stimuli and the number of times a stimulus was heard predicted the amount of calling, we ran a full factorial model with stimulus count (ordered factor: 1–3) and previous stimulus type as well as our primary variables of interest (current stimulus and drug dose) as predictors and the total number of calls produced (with zeroes excluded) as the dependent variable. Frog ID was included as a random effect. We found no significant interactions between any of the four predictors ($p > 0.14$ for all; see Supplementary Fig. 2 for full results). We observed significant main effects of stimulus ($F_{2,166.8} = 3.4$, $p = 0.0055$, $\eta_p^2 = 0.09$), dose ($F_{2,108.3} = 6.5$, $p = 0.0022$, $\eta_p^2 = 0.11$), stimulus count ($F_{2,165.0} = 13.8$, $p < 0.0001$, $\eta_p^2 = 0.14$) and a non-significant effect of previous stimulus ($F_{2,164.1} = 2.6$, $p = 0.0734$, $\eta_p^2 = 0.05$). Importantly, the lack of interaction between stimulus and previous stimulus indicates that the transition between stimulus types did not have a large impact on calling behavior. In contrast, the large effect of stimulus count was the result of decreasing numbers of calls across repeat presentation, with fewest calls produced on the 3rd rendition of a stimulus playback (Supplementary Fig. 2). Thus, to avoid the confound that each frog had a 3rd rendition of only a single stimulus playback, we pooled the data from the first two repetitions of each stimulus and removed calls during the 3rd repetition from all further analyses. We note that the weak (but non-significant) effect of previous stimulus type was because frogs called more during the conspecific playback (see Results), thus, called less if the previous stimulus was conspecific (since no stimulus was repeated). Finally, when assessing data distributions, we found a highly skewed distribution of response latency data during the heterospecific stimulus; thus, we limited this analysis to values <0.25 s (which included ~88 % of the data; see Results).

2.7. Statistics

We were generally interested in how dose and/or stimulus affected behavior. Consequently, most of our mixed-effects models contained dose, stimulus, and the interaction between dose and stimulus as the fixed effects. Frog ID was included as a random effect both to account for the same frog being tested across all stimuli, as well as some frogs being recaptured and tested across multiple nights. We included all calls produced by frogs in models when possible: overlaps (binary), response latency (s), chucks per complex call (count), and duration (s). For the remaining variables, we calculated summary stats (e.g., means, percentages) within each frog before running mixed-effects models.

We used package 'lme4' (Bates et al., 2007) to conduct the models and assess the significance of fixed effects using Type III ANOVAs with the Satterthwaite's method. In cases of a significant interaction between dose and stimulus, we re-ran separate models with only dose or only stimulus as the fixed-effect with the data limited to a single dose or stimulus. This was because we were not interested in pairwise comparisons that span across multiple doses and stimuli simultaneously (preplanned contrasts). For our binary response variable (overlap), we conducted generalized linear mixed-effects models with a binomial error family and assessed the significance using Type III Wald chi-square tests. We report partial eta squared (η_p^2) values as a measure of effect size using the effect size package (Ben-Shachar et al., 2020) for all linear mixed-effect analyses with significant main effects. We then conducted post-hoc Tukey's test contrasts and calculated the magnitude of Cohen's d as a measure of effect size for significant pairwise contrasts using the emmeans package (Lenth, 2022). All models were conducted using R v4.2.1.

3. Results

We measured the evoked vocal responses (EVRs) of frogs during playback of three call types: conspecific calls, noise calls (white noise with the amplitude envelope of the conspecific whine), and heterospecific calls. For each call type, calls were played back with ~1.5 s between calls for 10 min. We measured the responses of frogs across two 10-min sessions of each stimulus type (presented in pseudorandom order, see Methods). Frogs were treated with peripheral injections of a general dopamine agonist (apomorphine; at a high or low dose) or a saline control solution prior to the start of playback and recording.

3.1. Dopamine has minimal effects on the total number of calls

We predicted that frogs given the drug would call more to all stimuli, and we observed a significant main effect of dose on the total number of calls produced ($F_{2,89.8} = 4.7$, $p = 0.0112$, $\eta^2_p = 0.10$). However, contrary to our expectation, we found that frogs with the low dose of apomorphine called significantly less than frogs given saline ($p = 0.0082$, d = 0.64). There was also a significant effect of stimulus on the total number of calls produced ($F_{2,98.1} = 24.5$, $p < 0.0001$, $\eta^2_p = 0.33$; Fig. 2) but no significant interaction between dose and stimulus ($F_{4,97.9} = 0.9$, $p = 0.4794$). Post-hoc contrasts indicated that frogs called more to the conspecific stimulus than either the noise or heterospecific stimuli (Tukey's tests: $p < 0.0001$, d > 0.72 for both).

3.2. Dopamine increases the speed of vocalizations

Dopamine agonists often increase the speed of motor behaviors. To investigate whether dopamine modulates the speed of vocal-motor behavior, we measured call durations and response latencies.

For call duration, we found significant main effects of both stimulus ($F_{2,71.6} = 18.1$, $p < 0.0001$, $\eta^2_p = 0.34$) and dose ($F_{2,88.5} = 28.2$, $p < 0.0001$, $\eta^2_p = 0.39$; Fig. 3A,B), but no significant interaction between stimulus and dose ($F_{4,71.6} = 0.1$, $p = 0.983$). Call durations were significantly shorter following both doses of apomorphine treatment (mean ± S.D. across frogs = 0.36 ± 0.03 s for the low dose and 0.32 ± 0.05 s for the high dose) compared to saline treatment (0.41 ± 0.04 s; $p < 0.0001$, d > 1.1 for both). Call durations also varied across stimuli: for all treatments, males produced the longest calls in response to the conspecific playback (0.37 ± 0.06 s) compared to both the noise and heterospecific playbacks (0.35 ± 0.6 s noise and 0.35 ± 0.05 s het; $p \leq 0.0001$, d > 0.47 for both).

We found that the latency to call back to a stimulus was affected by the type of stimulus, the dopamine treatment, and their interaction (Stim: $F_{2,39,154} = 6894.4$ $p < 0.0001$, $\eta^2_p = 0.26$; Dose: $F_{2,103} = 70.3$, $p < 0.0001$, $\eta^2_p = 0.58$; Stim*Dose: $F_{4,39,154} = 171.6$, $p < 0.0001$, $\eta^2_p = 0.02$; Fig. 3C,D). We took the onset of each call produced by a frog and calculated the time since the end of the most recent stimulus playback (excluding calls that overlap the stimulus). Regardless of the drug treatment, frogs produced extremely rapid responses to the heterospecific playback (mean ± S.D. = 0.17 ± 0.10 s), slower responses to noise (0.32 ± 0.12 s), and even slower responses to the conspecific playback (0.61 ± 0.19 s; $p < 0.001$, d > 0.43 for all post-hoc Tukey's tests). At the same time, regardless of the stimulus, we found that frogs with a low dose of apomorphine responded more quickly than frogs with saline ($p < 0.001$, d > 0.54 for all post-hoc contrasts). High-dose males responded more quickly than saline males to the noise stimulus ($p = 0.0004$, d = 0.50) while low-dose males responded more quickly than high-dose males to the conspecific stimulus ($p = 0.0291$, d = 0.51).

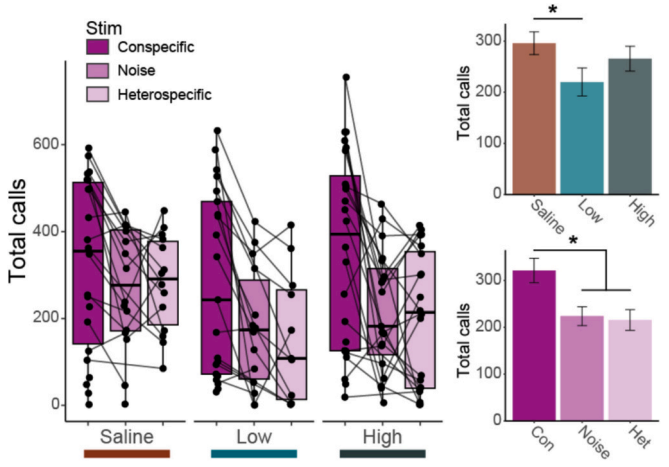


Fig. 2. Overall calling. Total calls (y-axis) produced by each frog summed across the two playbacks of each stimulus type and separated by dose (x-axis). Boxplots (left) depict the median, and Q1/Q3 ± 1.5*IQR and each dot and connecting lines depict the values from one frog on one night. Bars ± SE (right) group the same data as in the main panel to illustrate the significant main effects of dose (top) and stimulus (bottom; asterisks indicate $p < 0.05$).

3.3. Dopamine increases call attractiveness in a context-dependent manner

In addition to affecting the vocal motor features analyzed thus far, it is possible that dopamine may modulate calling in response to different external stimuli through actions on cognitive or motivational processes regarding social decision-making. Male túngara frogs typically compete antagonistically with other males in the chorus to attract females. Phonotaxis assays in females have highlighted that females prefer calls with greater complexity and with specific timing relative to other calls in the chorus, and males adjust both of these features while in a chorus (Larter and Ryan, 2024a; Ryan, 1985; Ryan et al., 2019).

Males increase their call complexity by adding additional elements to the end of the call (Fig. 4A). A túngara frog call begins with a "whine" that contains downward sweeping frequencies. Males often append one or more "chucks" to the end of a whine to produce a complex call. Such complex calls are five-fold more attractive to females than simple calls (Ryan et al., 2019). Here, we measured the percent of complex calls for each frog within each stimulus (with at least 10 calls produced) and found that the percent of complex calls a frog produced was predicted by the stimulus ($F_{2,107.7} = 22.3$, $p < 0.0001$, $\eta^2_p = 0.29$) but not the drug treatment or the interaction between dose and stimulus. In particular, frogs produced a greater number of complex calls during the conspecific stimulus compared to either the heterospecific or noise stimuli ($p < 0.0001$, d > 0.68 for both). There were no significant effects of body mass on the percent of calls with chucks, nor any interaction with body mass and stim or dose ($p > 0.15$ for all). We next measured the number of chucks in each complex call, and found that dose, stim, and their interaction significantly predicted the number of chucks (Dose: $F_{2,106} = 11.3$, $p < 0.0001$, $\eta^2_p = 0.18$; Stim: $F_{2,34,199} = 1524.9$, $p < 0.0001$, $\eta^2_p = 0.08$; Stim*Dose: $F_{4,34,199} = 48.8$, $p < 0.0001$, $\eta^2_p = 0.006$; Fig. 4B). Post-hoc contrasts on separate models revealed dose-dependent effects were only present during the conspecific stimulus, where we observed a dose-dependent increase in the number of chucks ($p < 0.005$, d > 0.28 for all comparisons) with males given a higher dose of apomorphine producing the most chucks. Indeed, while rare, the only 3 males to produce 3 chucks were males with the high dose of apomorphine. In addition, as was the case for the percent of complex calls, we found significant effects of stimulus in all doses, with frogs producing more chucks during the conspecific stimulus than either the noise or heterospecific playback ($p < 0.001$, d > 0.38 for all). Finally, frogs given either saline or the high dose produced more chucks in the noise stimulus relative to the heterospecific stimulus ($p < 0.02$, d > 0.05 for both).

Males in this species typically avoid producing overlapping calls in small choruses (Fig. 5A), and females are sensitive to the overlap of calls between different males (Larter and Ryan, 2024a). To assess the precision of call timing during the playback stimuli, we measured the number of calls that overlapped the stimulus playback. Overall, frogs overlapped the stimulus far less frequently than expected by chance (mean ± S.D. across frogs = 6.0 ± 4.3 % overlaps; chance ≈ 40 % overlap) and the odds of calls overlapping the stimulus was affected by both drug treatment and stimulus (Dose: $\chi^2_2 = 258.8$, $p < 0.0001$; Stim: $\chi^2_2 = 282.4$, $p < 0.0001$) as well as an interaction between stimulus and dose (GLMM: $\chi^2_4 = 378.5$, $p < 0.0001$; Fig. 5B). For every stimulus, saline males differed significantly from both drug groups ($p < 0.03$ for all comparisons), however, interestingly, the pattern differed across stimuli. Saline-treated males overlapped the conspecific and noise playbacks more than either apomorphine dose, while they overlapped the heterospecific

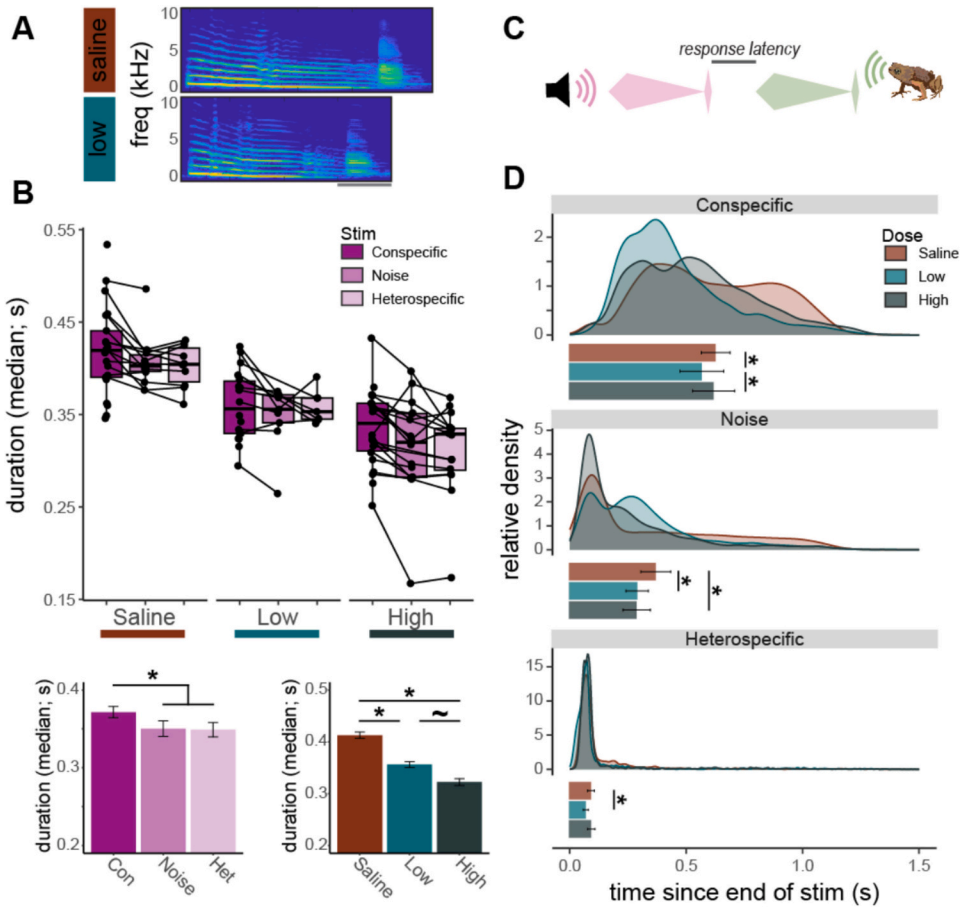


Fig. 3. Drug impacts vocal speed. A) Example spectrograms of whine chucks from a single male that was recaptured across multiple nights and randomly received different treatments each night. B) The median duration of complex calls containing a single chuck. Lines connect the values from a single male within a night (top) and bars ± SEM (bottom) depict significant main effects. C) Diagram depicts the response latency, as measured from the end of the preceding stimulus call to the onset of a frog's call. D) Density plots depict the overall distribution of all response latencies for each stimulus (in rows) and dose (in colors). Bars depict the mean ± 95 % confidence interval across mean values for each frog. See text for differences within each dose. For all plots, asterisks depict significant differences ($p \leq 0.05$) from linear mixed effects models and tildes depict non-significant trends ($p < 0.10$). Boxplots depict the median, and Q1/Q3 ± 1.5*IQR.

playback less than either dose. The low-dose males overlapped the conspecific playback less than the high dose males ($p = 0.0035$). Relatedly, there were effects within each dose. Saline males overlapped the noise playback the most and the heterospecific playback the least ($p < 0.003$ for all), low-dose males overlapped the conspecific playback less than the noise and heterospecific playbacks ($p < 0.001$ for both), and high-dose males overlapped the conspecific and noise playbacks less than the heterospecific playback ($p < 0.0001$ for both).

Interestingly, the time course of overlap rates differed by stimulus and dose when analyzing the overlap rates within each minute of the stimulus. A full factorial model with minute into the stim, stim, and dose predicting percent overlap revealed significant interactions between stim and dose ($F_{4,1271.9} = 3.9$, $p = 0.0040$, $\eta^2_p = 0.01$; Fig. 5C), stim and minute ($F_{2,1262.0} = 20.7$, $p < 0.0001$, $\eta^2_p = 0.03$) as well as significant main effects of stim and dose (Stim: $F_{2,1273.2} = 37.1$, $p < 0.0001$, $\eta^2_p = 0.06$; Dose: $F_{2,207.6} = 22.4$, $p < 0.0001$, $\eta^2_p = 0.18$). Interestingly, saline males begin and end the conspecific and noise playbacks with higher levels of overlap than either drug dose. However, during the heterospecific playback, males with the high dose of drug start with low levels of overlap but ramp up across the course of the playback.

Frogs can overlap the stimulus in two ways: (1) *leading*, by calling before the overlapped playback call, or (2) *lagging*, by initiating a call during a playback call (Fig. 5D). Overall, most overlapping calls (84 %) were produced in a *leading* manner relative to the stimulus. We found a significant interaction between stim and dose on the percent of overlaps that were considered leading vs lagging (binomial GLMM: $\chi^2_4 = 54.8, p <$ 0.0001), as well as significant main effects of dose ($\chi^2_2 = 43.9$, $p <$ 0.0001) and stim ($\chi^2_2 = 112.3$, $p < 0.0001$; Fig. 3E). Post-hoc contrasts analyzing for effects of stimulus independently indicated that during both the conspecific and noise stimuli, saline males produced fewer lagging overlaps than both low- and high-dosed males ($p < 0.02$ for all). During the heterospecific stimulus, high-dose males produced fewer lagging overlaps than both low-dose and saline males ($p = 0.002$ and 0.039, respectively). Overall, these results align to the overall percent overlap results indicating that increased overlaps tend to occur due to an increase in leading overlaps. We also investigated the time course of the percent of leading vs lagging calls, finding a significant interaction between dose and minute into the stimulus ($F_{2,654.7} = 4.4$, $p = 0.0131$, $\eta^2_p = 0.01$). Analyzing each dose independently, we found that frogs with a low dose decreased the percent of overlaps that were leading (thereby increasing their percent of lagging calls: $F_{1,138.5} = 8.6$, $p = 0.0039$, $\eta^2_p = 0.06$), but no significant effect of minute for the saline and high dose frogs ($p > 0.55$ for both).

Importantly, previous research has measured female responses to overlapping complex calls (whines followed by a single chuck), revealing that lagging calls are preferred if they begin during the second half of the leading call, likely because the portion of the whine with higher amplitude masks the chuck of the leading call (Fig. 5F) (Larter and Ryan, 2024a). Therefore, we could categorize all whine chucks that overlapped with the conspecific stimulus according to whether we predict the call to be preferred ("good") or dispreferred ("bad") by a female compared to the stimulus call. We found a significant interaction

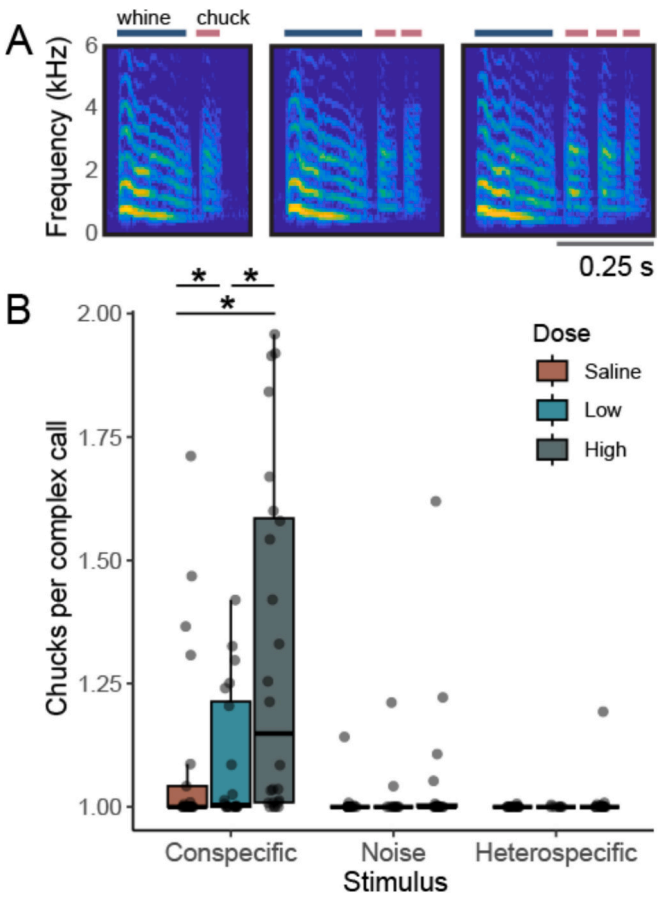


Fig. 4. Drug affects call complexity. A) Example spectrograms of a whine followed by one, two, or three chucks produced by the same male given the high dose of apomorphine. B) The average number of chucks in each frog's complex calls across doses and stimuli. Boxplots depict the median, and Q1/Q3 ± 1.5*IQR and asterisks indicate significant differences ($p < 0.05$) between doses within each stimulus. See text for differences within each dose.

between overlap type and dose on the percent of each overlap type ($F_{6,196} = 12.3$, $p < 0.0001$, $\eta_p^2 = 0.27$; Fig. 5G). Analyzing each overlap type independently, we found effects of dose for both types of leading calls, where saline treated males produced more "bad" leading calls than those with either dose of apomorphine, and fewer "good" leading calls than high dose males ($p < 0.05$, d > 0.79 for all). Finally, we asked whether males that produced more chucks also produced more "good" leading calls. However, we did not find a significant correlation between the increased chucks per complex call and the increased "good" leading calls within the high dose frogs ($F_{1,11.5} = 0.0$, $p = 0.9354$, $\eta_p^2 < 0.0001$).

4. Discussion

In vocal communication, signalers need to integrate sensory and social information to modify their signals for a specific partner, audience, or social context (Bernal and Page, 2023; Greenfield, 2002; Logue and Krupp, 2016; Snijders and Naguib, 2017). Depending on the context, animals can adjust multiple aspects of their signals, including features of vocal production as well as the timing and content of the signal. We hypothesize that dopamine may be a key neuromodulator in driving these changes to vocal behavior across species. Dopamine is produced in conserved neuronal populations that share similar projections to sensory, motor, and cognitive regions across taxa (O'Connell and Hofmann, 2011, 2012; Yamamoto and Vernier, 2011). While dopamine has been shown to modulate aspects of vocalizations in rodents, birds, and fish across multiple receptor subtypes (Allen et al., 2023; Kubikova and Košťál, 2010; Woolley, 2019; Wright et al., 2013), few studies have addressed a role for dopamine in calling in frogs. Here, we found that systemic injection of apomorphine, a general dopamine agonist, modulates multiple aspects of calling behavior in túngara frogs. In particular, apomorphine treatment led to changes to vocal motor behavior, including increases in measures of the speed of call production. In addition, apomorphine treated frogs showed systematic changes to the timing and quality of evoked vocal responses to stimulus playback.

Interestingly, some of the changes to calling induced by apomorphine treatment modulated the competitive behaviors of frogs in a manner likely to increase call attractiveness to females (Figs. 4, 5). Specifically, all frogs were more likely to produce complex calls by adding one or more chucks to their whine when interacting with a conspecific playback. However, the amount of increase in the average number of chucks appended was dose-dependent, with frogs given the high-dose of apomorphine producing the most chucks. Furthermore, we found that, compared to males injected with saline, males with apomorphine injections overlapped the conspecific stimulus in such a way that more of their overlaps would be expected to be preferred by females when pitted against the overlapped stimulus call (Larter and Ryan, 2024a). The fact that a dopamine agonist induces calls of potentially higher quality is consistent with a previous study in songbirds, where amphetamine administration led males to produce songs that contained some aspects of the more attractive female-directed songs (Matheson and Sakata, 2015).

Contrary to our predictions, we found no support that apomorphine treatment led to increased calling. Rather, frogs generally called to all stimuli and, if anything, drug treatment reduced the number of calls that frogs produced (Fig. 1, Supplementary Information). However, we did find effects on vocal motor speed, with increases in multiple measures of the speed of call production, including the call duration and latency to call, in males given apomorphine treatment (Fig. 3). Specifically, males given a low dose of apomorphine responded more quickly to playbacks than frogs given saline. We also found a dose-dependent decrease in call durations, which we expect is due to more rapid inflation and deflation of the vocal sac. Finally, we found that frogs had highly stereotyped intrinsic call rates which were unaffected by apomorphine (Supplementary Information).

There were also differences in how frogs respond to the three auditory stimuli that were independent of the drug treatment. We saw large variation in the latency between the end of each stimulus and the beginning of the frogs' responses, with extremely rapid responses to the heterospecific call and slow responses to the conspecific call. Túngara frogs are known to be sensitive to drops in amplitude when deciding when to call (Larter and Ryan, 2024a, 2024b, 2024c). The heterospecific call is longer in duration and has a later amplitude descent compared to the conspecific call and these differences may be responsible for the rapid response latencies to the heterospecific call; i.e., by the time the heterospecific stimulus starts to decline in amplitude and end, males are ready to produce their own call. To test this hypothesis, future work testing the frogs' responses to a range of heterospecific stimuli, and stimuli with modulations of specific acoustic features will be of particular interest (James and Ryan, 2025).

These results differ from a previous study measuring the effects of dopamine agonists on the calling behavior of green tree frogs (Creighton et al., 2013). They found that a D2 receptor-specific agonist generally inhibited calling, while apomorphine or a D1-receptor specific agonist had little effect. This could be due to species differences in dopamine receptor expression or dopaminergic regulation of calling behavior, or could be due to methodological differences in the two studies. Creighton et al. injected males in the field, and noted if frogs resumed calling, and how many calls they produced over time. In contrast, for our study, we subjected frogs to a battery of playback stimuli aimed specifically to evoke calling, and then quantified differences in fine-scale measures of subsequent calling behavior. Also of interest is the fact that, in our

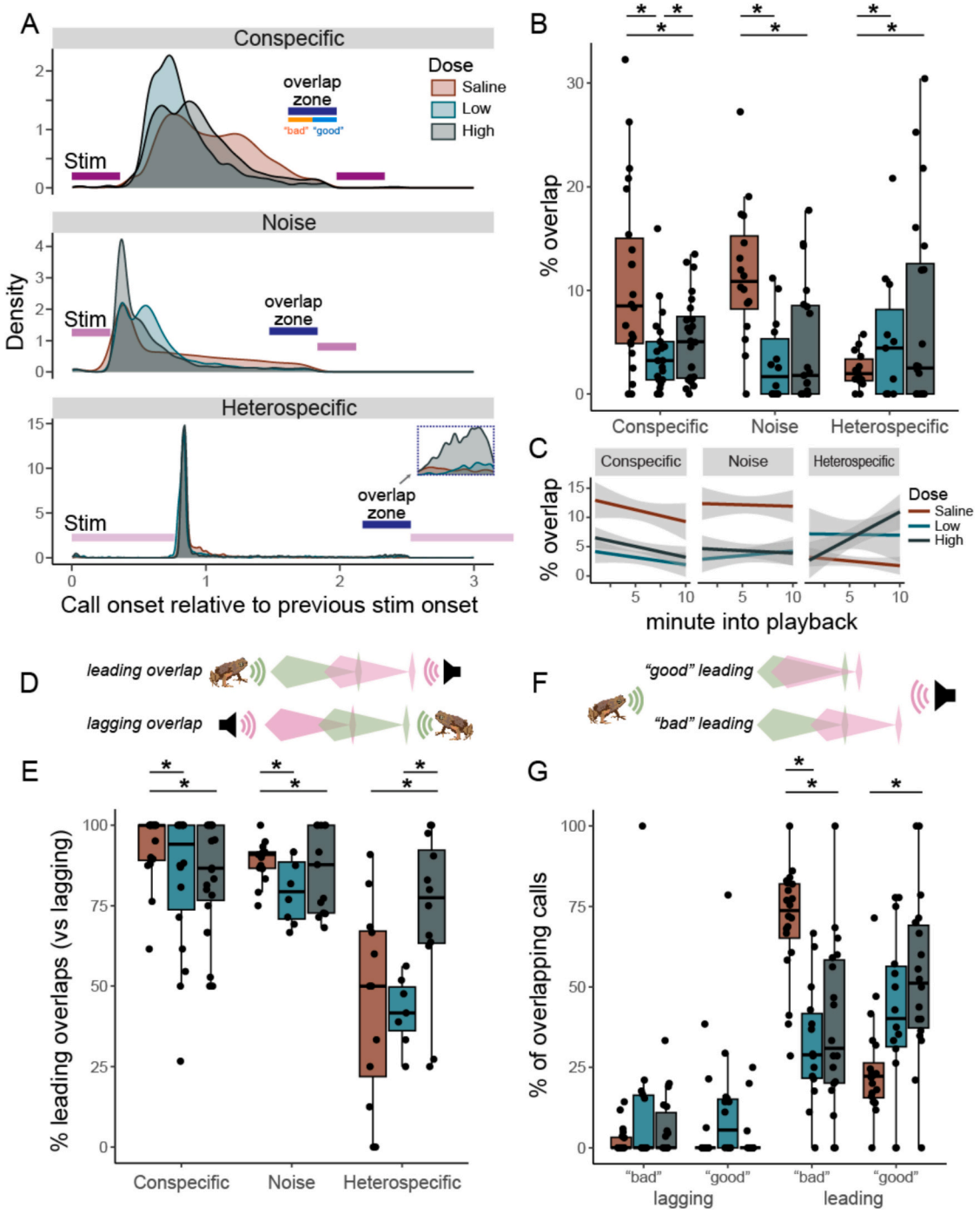


Fig. 5. Drug affects call overlap with the stimulus. A) Density plots depict the onset of calls produced by frogs relative to the previous stimulus onset, including those that overlap with the previous or subsequent stimulus. Pink bars indicate the duration and timing of the stimulus playback, and dark blue bars indicate the timing when a call onset is likely to overlap with the subsequent call. For the heterospecific stimulus, the inset depicts the relative density of calls across all three doses during this overlap zone. B) The percent of calls that overlapped the playback stimulus for each frog. C) Linear regressions depict the change in overlap percentage across the 10 min of playback stimuli. D) Examples of leading and lagging calls. E) The percent of all overlapping calls that led the stimulus (compared to those that were initiated during the stimulus playback). F) Examples of leading calls that are typically preferred by females ("good") compared to those that are dispreferred ("bad"). G) The percent of all whine chucks that overlapped the conspecific stimulus according to their predicted preference in a female choice assay. These data are limited to whines with a single chuck produced during the conspecific playback (which was also a whine with a single chuck). For all panels, boxplots depict the median, and Q1/Q3 ± 1.5*IQR and asterisks indicate significant differences ($p < 0.05$) between doses within each stimulus. See text for differences within each dose.

preliminary experiment (Supplementary information), there was a stronger reduction in calling with apomorphine treatment when frogs were stimulated to call by a large chorus playback, which may relate to the tendency of frogs with apomorphine to avoid overlapping the conspecific stimulus in the main experiment. Further studies are needed to understand how variation in social contexts affects the dopaminergic modulation of social decision-making behaviors across species.

Dopamine receptors are expressed throughout the frog brain, including in the auditory system (torus semicircularis), in the striatum and pallidum, in nuclei of the social behavior network, and throughout

the pallium (Burmeister, 2022; Endepols et al., 2000; O'Connell et al., 2011). Because our study utilized peripheral injections, we likely activated dopamine receptors across regions. It is possible that, like in birds and mammals, changes to measures of motor performance could be through actions on the basal ganglia (e.g. striatum) while changes to cognitive decision-making aspects of calling could be through actions on the pallium. However, compared to pallium in birds and mammals, frog pallium is less elaborated, has small, multimodal sensory areas, and has few descending connections (Laberge and Roth, 2007; Roth et al., 2007). This organization suggests that the pallium in frogs may be less involved in executive control and have lower resolution sensory representations. In contrast, the striatum has more substantial descending connections, which has led to the hypothesis that the striatum may be the "executive center" in amphibians (Burmeister, 2022). Future work, using markers of neural activity to uncover differential activity when males vary their calling behavior will be key in determining the neural substrates underlying the ability of these frogs to flexibly adjust their vocal signals.

While our use of apomorphine, a broad-spectrum dopamine agonist, allowed us to make direct comparison to similar studies of motivated behavior in other frog species (Chu and Wilczynski, 2007; Creighton et al., 2013; Glagow and Ewert, 1999), the variety of changes to calling we observed in this study may stem from differential activation of dopamine receptor subtypes. Anurans appear to have two broad classes of receptors similar to the mammalian D1 and D2 subfamilies although with some variation in the specific binding characteristics of the receptors (Chu et al., 2001), and apomorphine generally targets both receptor subfamilies with similar affinity (Millan et al., 2002). Both classes are g-protein coupled receptors but they have opposite intracellular effects with D1 receptors increasing adenylate cyclase (AC) and cAMP expression and D2 receptors inhibiting AC and decreasing cAMP (Kawahata et al., 2024). However, while receptors have clear, opposing actions on intracellular cascades, effects on behavior are more nuanced. For example, current models for the role of striatal D1+ ("direct" pathway) and D2+ ("indirect" pathway) spiny neurons hypothesize that these pathways may not act as a simple go/no-go for movement (Klaus et al., 2017; Tecuapetla et al., 2016). Similarly, both D1 and D2 type receptors in mammals modulate cortical and corticostriatal plasticity (Calabresi et al., 2007; Jay, 2003), and synergistic interactions between both receptor classes are important for long-term depression. Given variation between the receptor classes in the location of expression, with high expression of both classes in the striatum but lower expression of D2 receptors in the pallium (Kawahata et al., 2024; Kubikova et al., 2009), future work targeting specific receptor subtypes may not only offer clarity into the role of DA in modulating calling, but into the specific neural substrates responsible for the distinct effects on calling that we see here. Finally, apomorphine also acts on serotonin and adrenergic receptors, and we cannot rule out the possibility that some of the effects on calling that we see could be a consequence of activation of non-dopaminergic receptors. For example, in male coquí frogs, 5-HT receptor agonists reduce or eliminate territorial calling (Ten Eyck, 2008; Ten Eyck and Ten Eyck, 2020). Targeting of specific receptor subtypes will be key to disentangling the unique roles of these neuromodulators in calling behavior.

The species richness of anurans provides the opportunity to understand dopamine's role in diverse communication systems. Like túngara frogs, other species modulate aspects of their calling behavior based on the social context, which can include changes to repertoire use and sequencing (Bhat et al., 2022; Fang et al., 2014; Humfeld et al., 2009; Leverett et al., 2022). Unlike túngara frogs, some species produce calls in near-perfect synchrony with neighboring males (Clulow et al., 2017; Legett et al., 2021, Legett et al., 2019; Ryan, 1986) while others produce calls during rare explosive breeding aggregations (Rehberg-Besler et al., 2016). Furthermore, some species incorporate other motor behaviors like foot-flagging with their courtship signals (Love et al., 2023). This cross-species variation offers an ideal system for comparative studies of dopamine's role in how social decision-making shapes courtship communication signals. Finally, dopamine is known to be important in receivers, and future work on dopamine's role in modulating acoustic preferences in females will be of utmost interest.

CRediT authorship contribution statement

Logan S. James: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sarah C. Woolley:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Michael J. Ryan:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Funding

This work was supported by a Smithsonian Institute Postdoctoral Fellowship (L.S.J.); the National Science Foundation (IOS-1914646; M. J.R., R.A. Page, R.C. Taylor, and K.L. Hunter); and the National Science and Engineering Council of Canada (RGPIN2018-05267; S.C.W.).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sarah C. Woolley reports financial support was provided by Natural Sciences and Engineering Research Council of Canada. Michael J. Ryan reports financial support was provided by National Science Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Gregg Cohen, Rachel A. Page, Ryan C. Taylor, Kimberly L. Hunter, and the staff of the Smithsonian Tropical Research Institute for invaluable assistance with fieldwork, logistics, equipment, and support. We thank Luke Larter, Jon Sakata, and the Woolley, Ryan, and Sakata labs for feedback on the project. We thank Panama's Ministry of the Environment (MiAmbiente) and the Autoridad del Canal de Panamá (ACP) for permission to conduct this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2025.105797.

Data availability

Data are provided as supplementary material.

References

Allen, A., Heisler, E., Kittelberger, J.M., 2023. Dopamine injections to the midbrain periaqueductal gray inhibit vocal-motor production in a teleost fish. Physiol. Behav. 263, 114131. https://doi.org/10.1016/j.physbeh.2023.114131.

Aristakesyan, E.A., 2011. Effect of apomorphine on the wakefulness-sleep cycle of the common frog *Rana temporaria*. J. Evol. Biochem. Physiol. 47, 348–359. https://doi.org/10.1134/S0022093011040062.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2007. Package "lme4." R Package.

Ben-Shachar, M., Lüdecke, D., Makowski, D., 2020. Effectsize: estimation of effect size indices and standardized parameters. JOSS 5 (56), 2815. https://doi.org/10.21105/joss.02815.

Ben-Tov, M., Duarte, F., Mooney, R., 2023. A neural hub for holistic courtship displays. Curr. Biol. 33, 1640–1653.e5. https://doi.org/10.1016/j.cub.2023.02.072.

Berke, J.D., 2018. What does dopamine mean? Nat. Neurosci. 21, 787–793. https://doi.org/10.1038/s41593-018-0152-y.

Bernal, X.E., Page, R.A., 2023. Tactics of evasion: strategies used by signallers to deter eavesdropping enemies from exploiting communication systems. Biol. Rev. 98, 222–242. https://doi.org/10.1111/brv.12904.

Bhat, A.S., Sane, V.A., Seshadri, K.S., Krishnan, A., 2022. Behavioural context shapes vocal sequences in two anuran species with different repertoire sizes. Anim. Behav. 184, 111–129. https://doi.org/10.1016/j.anbehav.2021.12.004.
Bologna, M., Paparella, G., Fasano, A., Hallett, M., Berardelli, A., 2020. Evolving concepts on bradykinesia. Brain 143, 727–750. https://doi.org/10.1093/brain/awz344.
Burmeister, S.S., 2022. Brain-behavior relationships of cognition in vertebrates: lessons from amphibians. In: Advances in the Study of Behavior. Elsevier, pp. 109–127. https://doi.org/10.1016/bs.asb.2022.01.004.
Calabresi, P., Picconi, B., Tozzi, A., Di Filippo, M., 2007. Dopamine-mediated regulation of corticostriatal synaptic plasticity. TINS 30, 211–219. https://doi.org/10.1016/j.tins.2007.03.001.
Carlson, N.V., Greene, E., Templeton, C.N., 2020. Nuthatches vary their alarm calls based upon the source of the eavesdropped signals. Nat. Commun. 11, 526. https://doi.org/10.1038/s41467-020-14414-w.
Chu, J., Wilczynski, W., 2007. Apomorphine effects on frog locomotor behavior. Physiol. Behav. 91, 71–76. https://doi.org/10.1016/j.physbeh.2007.01.019.
Chu, J., Wilczynski, W., Wilcox, R.E., 2001. Pharmacological characterization of the D1- and D2-like dopamine receptors from the brain of the leopard frog, *Rana pipiens*. Brain Behav. Evol. 57, 328–342. https://doi.org/10.1159/000047251.
Clemens, J., Coen, P., Roemschied, F.A., Pereira, T.D., Mazumder, D., Aldarondo, D.E., Pacheco, D.A., Murthy, M., 2018. Discovery of a new song mode in *Drosophila* reveals hidden structure in the sensory and neural drivers of behavior. Curr. Biol. 28, 2400–2412.e6. https://doi.org/10.1016/j.cub.2018.06.011.
Clulow, S., Mahony, M., Elliott, L., Humfeld, S., Gerhardt, H.C., 2017. Near-synchronous calling in the hip-pocket frog *Assa darlingtoni*. Bioacoustics 26, 249–258. https://doi.org/10.1080/09524622.2016.1260054.
Coen, P., Clemens, J., Weinstein, A.J., Pacheco, D.A., Deng, Y., Murthy, M., 2014. Dynamic sensory cues shape song structure in *Drosophila*. Nature 507, 233–237. https://doi.org/10.1038/nature13131.
Creighton, A., Satterfield, D., Chu, J., 2013. Effects of dopamine agonists on calling behavior in the green tree frog, *Hyla cinerea*. Physiol. Behav. 116–117, 54–59. https://doi.org/10.1016/j.physbeh.2013.03.012.
Endepols, H., Walkowiak, W., Luksch, H., 2000. Chemoarchitecture of the anuran auditory midbrain. Brain Res. Rev. 33, 179–198. https://doi.org/10.1016/S0165-0173(00)00029-1.
Fang, G., Jiang, F., Yang, P., Cui, J., Brauth, S.E., Tang, Y., 2014. Male vocal competition is dynamic and strongly affected by social contexts in music frogs. Anim. Cogn. 17, 483–494. https://doi.org/10.1007/s10071-013-0680-5.
Glagow, M., Ewert, J.-P., 1996. Dopaminergic modulation of visual responses in toads. J. Comp. Physiol. A. 180, 11–18. https://doi.org/10.1007/s003590050022.
Glagow, M., Ewert, J.-P., 1999. Apomorphine alters prey-catching patterns in the common toad: behavioral experiments and ^{14}C-2-deoxyglucose brain mapping studies. Brain Behav. Evol. 54, 223–242. https://doi.org/10.1159/000006625.
Greenfield, M.D., 2002. Signalers and Receivers: Mechanisms and Evolution of Arthropod Communication. Oxford University Press.
Hara, E., Kubikova, L., Hessler, N.A., Jarvis, E.D., 2007. Role of the midbrain dopaminergic system in modulation of vocal brain activation by social context. Eur. J. Neurosci. 25, 3406–3416. https://doi.org/10.1111/j.1460-9568.2007.05600.x.
Hisey, E., Kearney, M.G., Mooney, R., 2018. A common neural circuit mechanism for internally guided and externally reinforced forms of motor learning. Nat. Neurosci. 21, 589–597. https://doi.org/10.1038/s41593-018-0092-6.
Humfeld, S.C., Marshall, V.T., Bee, M.A., 2009. Context-dependent plasticity of aggressive signalling in a dynamic social environment. Anim. Behav. 78, 915–924. https://doi.org/10.1016/j.anbehav.2009.06.028.
James, L.S., Halfwerk, W., Hunter, K.L., Page, R.A., Taylor, R.C., Wilson, P.S., Ryan, M.J., 2021. Covariation among multimodal components in the courtship display of the túngara frog. J. Exp. Biol. 224, 1–10. https://doi.org/10.1242/jeb.241661.
James, L.S., Ryan, M.J., 2025. Time and place affect the acoustic structure of frog advertisement calls. Curr. Zool. zoae039. https://doi.org/10.1093/cz/zoae039.
Jay, T.M., 2003. Dopamine: a potential substrate for synaptic plasticity and memory mechanisms. Prog. Neurobiol. 69, 375–390. https://doi.org/10.1016/S0301-0082(03)00085-6.
Kao, M.H., Brainard, M.S., 2006. Lesions of an avian basal ganglia circuit prevent context-dependent changes to song variability. J. Neurophysiol. 96, 1441–1455. https://doi.org/10.1152/jn.01138.2005.
Kawahata, I., Finkelstein, D.I., Fukunaga, K., 2024. Dopamine D1–D5 receptors in brain nuclei: implications for health and disease. Receptors 3, 155–181. https://doi.org/10.3390/receptors3020009.
Kim, K., Macias, D., Borzée, A., Jang, Y., 2020. Ueno's brown frog *Rana uenoi* indiscriminately ceases calling in the presence of daytime birds. Ethol. Ecol. Evol. 32, 251–263. https://doi.org/10.1080/03949370.2020.1717638.
Klaus, A., Martins, G.J., Paixao, V.B., Zhou, P., Paninski, L., Costa, R.M., 2017. The spatiotemporal organization of the striatum encodes action space. Neuron 95, 1171–1180.e7. https://doi.org/10.1016/j.neuron.2017.08.015.
Kubikova, L., Košťál, L., 2010. Dopaminergic system in birdsong learning and maintenance. J. Chem. Neuroanat. 39, 112–123. https://doi.org/10.1016/j.jchemneu.2009.10.004.
Kubikova, L., Wada, K., Jarvis, E.D., 2009. Dopamine receptors in a songbird brain. J. Comp. Neurol. 518, 741–769. https://doi.org/10.1002/cne.22255.
Laberge, F., Roth, G., 2007. Organization of the sensory input to the telencephalon in the fire-bellied toad, *Bombina orientalis*. J. Comp. Neurol. 502, 55–74. https://doi.org/10.1002/cne.21297.
Larter, L.C., Ryan, M.J., 2024a. Female preferences for more elaborate signals are an emergent outcome of male chorusing interactions in túngara frogs. Am. Nat., 727469 https://doi.org/10.1086/727469.
Larter, L.C., Ryan, M.J., 2024b. Sensory-motor tuning allows generic features of conspecific acoustic scenes to guide rapid, adaptive, call-timing responses in túngara frogs. Proc. R. Soc. B 291, 20240992. https://doi.org/10.1098/rspb.2024.0992.
Larter, L.C., Ryan, M.J., 2024c. Túngara frog call-timing decisions arise as internal rhythms interact with fluctuating chorus noise. Behav. Ecol. 35, arae034. https://doi.org/10.1093/beheco/arae034.
Legett, H.D., Page, R.A., Bernal, X.E., 2019. Synchronized mating signals in a communication network: the challenge of avoiding predators while attracting mates. Proc. R. Soc. B 286, 20191067. https://doi.org/10.1098/rspb.2019.1067.
Legett, H.D., Aihara, I., Bernal, X.E., 2021. The dual benefits of synchronized mating signals in a Japanese treefrog: attracting mates and manipulating predators. Philos. Trans. R. Soc. B 376, 20200340. https://doi.org/10.1098/rstb.2020.0340.
Lenth, R., 2022. Emmeans: Estimated Marginal Means, Aka Least-Squares Means.
Leverett, M.C., McLister, J.D., Desaivre, S.S., Conway, S., Boyd, S.K., 2022. Social modulation of spatial dynamics in treefrog choruses. Behav. Ecol. Sociobiol. 76, 54. https://doi.org/10.1007/s00265-022-03163-z.
Logue, D.M., Krupp, D.B., 2016. Duetting as a collective behavior. Front. Ecol. Evol. 4. https://doi.org/10.3389/fevo.2016.00007.
Love, N., Preininger, D., Fuxjager, M.J., 2023. Social regulation of androgenic hormones and gestural display behavior in a tropical frog. Horm. Behav. 155, 105425. https://doi.org/10.1016/j.yhbeh.2023.105425.
Macedo-Lima, M., Boyd, H.M., Remage-Healey, L., 2021. Dopamine D1 receptor activation drives plasticity in the songbird auditory pallium. J. Neurosci. 41, 6050–6069. https://doi.org/10.1523/JNEUROSCI.2823-20.2021.
Matheson, L.E., Sakata, J.T., 2015. Catecholaminergic contributions to vocal communication signals. Eur. J. Neurosci. 41, 1180–1194. https://doi.org/10.1111/ejn.12885.
Millan, M.J., Maiofiss, L., Cussac, D., Audinot, V., Boutin, J.-A., Newman-Tancredi, A., 2002. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. J. Pharmacol. Exp. Ther. 303, 791–804. https://doi.org/10.1124/jpet.102.039867.
O'Connell, L.A., Hofmann, H.A., 2011. The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. J. Comp. Neurol. 519, 3599–3639. https://doi.org/10.1002/cne.22735.
O'Connell, L.A., Hofmann, H.A., 2012. Evolution of a vertebrate social decision-making network. Science 336, 1154–1157. https://doi.org/10.1126/science.1218889.
O'Connell, L.A., Matthews, B.J., Ryan, M.J., Hofmann, H.A., 2011. Characterization of the dopamine system in the brain of the túngara frog, *Physalaemus pustulosus*. Brain Behav. Evol. 76, 211–225. https://doi.org/10.1159/000321715.
Panigrahi, B., Martin, K.A., Li, Y., Graves, A.R., Vollmer, A., Olson, L., Mensh, B.D., Karpova, A.Y., Dudman, J.T., 2015. Dopamine is required for the neural representation and control of movement vigor. Cell 162, 1418–1430. https://doi.org/10.1016/j.cell.2015.08.014.
Presley, G.M., Lonergan, W., Chu, J., 2010. Effects of amphetamine on conditioned place preference and locomotion in the male green tree frog, *Hyla cinerea*. Brain Behav. Evol. 75, 262–270. https://doi.org/10.1159/000314901.
Rand, A.S., Ryan, M.J., Wilczynski, W., 1992. Signal redundancy and receiver permissiveness in acoustic mate recognition by the túngara frog, *Physalaemus pustulosus*. Am. Zool. 32, 81–90. https://doi.org/10.1093/icb/32.1.81.
Rehberg-Besler, N., Doucet, S.M., Mennill, D.J., 2016. Vocal behavior of the explosively breeding neotropical yellow toad, *Incilius luetkenii*. J. Herpetol. 50, 502–508. https://doi.org/10.1670/15-152.
Roth, G., Laberge, F., Mühlenbrock-Lenter, S., Grunwald, W., 2007. Organization of the pallium in the fire-bellied toad *Bombina orientalis*. I: morphology and axonal projection pattern of neurons revealed by intracellular biocytin labeling. J. Comp. Neurol. 501, 443–464. https://doi.org/10.1002/cne.21255.
Ryan, M.J., 1985. The túngara Frog - a Study in Sexual Selection and Communication. University of Chicago Press.
Ryan, M.J., 1986. Synchronized calling in a treefrog *(Smilisca sila)*. Brain Behav. Evol. 29, 196–206. https://doi.org/10.1159/000118681.
Ryan, M.J., Akre, K.L., Baugh, A.T., Bernal, X.E., Lea, A.M., Leslie, C., Still, M.B., Wylie, D.C., Rand, A.S., 2019. Nineteen years of consistently positive and strong female mate preferences despite individual variation. Am. Nat. 194, 125–134. https://doi.org/10.1086/704103.
Sakata, J.T., Hampton, C.M., Brainard, M.S., 2008. Social modulation of sequence and syllable variability in adult birdsong. J. Neurophysiol. 99, 1700–1711. https://doi.org/10.1152/jn.01296.2007.
Singh Alvarado, J., Goffinet, J., Michael, V., Liberti, W., Hatfield, J., Gardner, T., Pearson, J., Mooney, R., 2021. Neural dynamics underlying birdsong practice and performance. Nature 599, 635–639. https://doi.org/10.1038/s41586-021-04004-1.
Snijders, L., Naguib, M., 2017. Communication in animal social networks. In: Advances in the Study of Behavior. Elsevier, pp. 297–359. https://doi.org/10.1016/bs.asb.2017.02.004.
Sossinka, R., Böhner, J., 1980. Song types in the zebra finch. Z. Tierpsychol. 53, 123–132. https://doi.org/10.1111/j.1439-0310.1980.tb01044.x.
Tecuapetla, F., Jin, X., Lima, S.Q., Costa, R.M., 2016. Complementary contributions of striatal projection pathways to action initiation and execution. Cell 166, 703–715. https://doi.org/10.1016/j.cell.2016.06.032.
Ten Eyck, G.R., 2008. Serotonin modulates vocalizations and territorial behavior in an amphibian. Behav. Brain Res. 193, 144–147. https://doi.org/10.1016/j.bbr.2008.05.001.
Ten Eyck, G.R., Ten Eyck, L.M., 2020. Serotonin and vasotocin function in territoriality. Pharmacol. Biochem. Behav. 199, 173068. https://doi.org/10.1016/j.pbb.2020.173068.

Woolley, S.C., 2019. Dopaminergic regulation of vocal-motor plasticity and performance. Curr. Opin. Neurobiol. 54, 127–133. https://doi.org/10.1016/j.conb.2018.10.008.
Woolley, S.C., Doupe, A.J., 2008. Social context-induced song variation affects female behavior and gene expression. PLoS Biol. 6, 0525–0537. https://doi.org/10.1371/journal.pbio.0060062.
Woolley, S.C., Rajan, R., Joshua, M., Doupe, A.J., 2014. Emergence of context-dependent variability across a basal ganglia network. Neuron 82, 208–223. https://doi.org/10.1016/j.neuron.2014.01.039.
Wright, J.M., Dobosiewicz, M.R.S., Clarke, P.B.S., 2013. The role of dopaminergic transmission through D1-like and D2-like receptors in amphetamine-induced rat ultrasonic vocalizations. Psychopharmacology 225, 853–868. https://doi.org/10.1007/s00213-012-2871-1.
Xiao, L., Chattree, G., Oscos, F.G., Cao, M., Wanat, M.J., Roberts, T.F., 2018. A basal ganglia circuit sufficient to guide birdsong learning. Neuron 98. https://doi.org/10.1016/j.neuron.2018.02.020, 208-221.e5.
Yamamoto, K., Vernier, P., 2011. The evolution of dopamine systems in chordates. Front. Neuroanat. 5. https://doi.org/10.3389/fnana.2011.00021.