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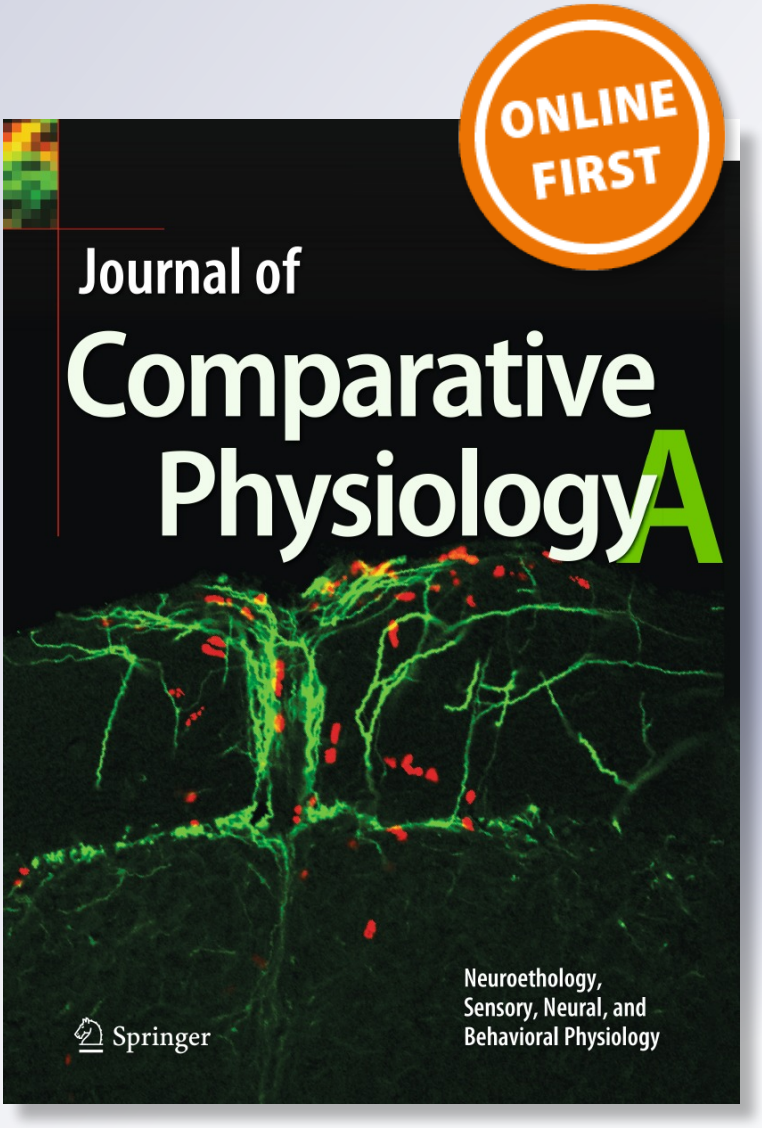

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ORIGINAL PAPER

Vasotocin induces sexually dimorphic effects on acoustically-guided behavior in a tropical frog

Alexander T. Baugh[1] · **Michael J. Ryan**[2,3]

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Abstract The neuropeptide arginine vasotocin (AVT) promotes sexual advertisement and influences vocalization structure in male anuran amphibians. In the present study, we used wild túngara frogs (*Physalaemus pustulosus*) to investigate the effects of AVT on phonotaxis in males and females—thereby controlling for potential task differences between the sexes. Using a combined within- and between-subjects design, we showed that acoustic choice behavior in female frogs is not influenced by injection *per se* (vehicle) or by AVT. Latency to choice in females, however, tends to decrease after AVT injection, supporting the hypothesis that AVT promotes female sexual arousal. In contrast, male choice behavior and latencies are negatively impacted by injection (vehicle) but rescued to pre-injection levels if administered with AVT. The sexes differed in area restricted searching (ARS) following choice—a measure of locomotor perseverance—with females but not males exhibiting ARS. AVT did not influence ARS behavior but ARS frequency was positively associated with the attractiveness of the acoustic stimulus. Finally, we showed that a female's latency behavior is correlated with her partner's behavior. Collectively we show that AVT promotes phonotaxis in both sexes in a dimorphic manner—a result that is consistent with sex differences in the neural vasotocin system.

Keywords Arginine vasotocin · Phonotaxis · *Physalaemus pustulosus* · Sex differences · Túngara

Introduction

The neuropeptide arginine vasotocin (AVT) and its mammalian homologue arginine vasopressin (AVP) are known to modulate social behavior in vertebrates—in addition to their role in osmoregulation (Caldwell and Young 2006)—including mammals (Young et al. 1998), teleosts (Oldfield and Hofmann 2011), birds (Kabelik et al. 2009) and anuran amphibians (Boyd 1997). The role of AVT in regulating social behavior in anurans has focused on male signaling, where it has been shown to promote vocal behavior in grey treefrogs, *Hyla versicolor* (Semsar et al. 1998; Tito et al. 1999; Klomberg and Marler 2000), green treefrogs, *Hyla cinerea* (Penna et al. 1992), the Puerto Rican coquí frog, *Eleutherodactylus coqui* (Ten Eyck and Haq 2012), American bullfrogs, *Rana catesbeiana* (Boyd 1994), great plains toads, *Bufo cognatus* (Propper and Dixon 1997), cricket frogs, *Acris crepitans* (Marler et al. 1995; Chu et al. 1998), and túngara frogs, *Physalaemus pustulosus* (Kime et al. 2007, 2010). Further, higher AVT immunoreactivity (AVT-ir) in the nucleus accumbens—a brain area associated with motivation—is positively correlated with vocal behavior in male cricket frogs (Marler et al. 1999). Further, in bullfrogs, the two sexes are known to exhibit a dimorphism in neural AVT systems, which is likely maintained by sex differences in gonadal steroid hormones (Boyd

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✉ Alexander T. Baugh
abaugh1@swarthmore.edu

[1] Department of Biology, Swarthmore College, 500 College Avenue, Swarthmore, PA 19081, USA

[2] Department of Integrative Biology, The University of Texas at Austin, 1 University Station, C0930, Austin, TX 78712, USA

[3] Smithsonian Tropical Research Institute, Balboa Ancon, Panama

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1997). In particular, nuclei in the auditory midbrain (e.g. torus semicircularis), which are known to play key roles in anuran auditory processing and phonotaxis, exhibit greater AVT receptor expression in adult males compared to adult females (Boyd 1997).

To date, only a few studies have examined the influence of AVT on female anuran sexual behavior. In leopard frogs (*Rana pipiens*), AVT influences the probability of release call production through the accumulation of body fluids that cause a mechanical distention of the abdomen (Diakow 1978), even in the absence of ovarian tissue (Diakow et al. 1978). Likewise, the only study that has explored the influence of AVT on female phontaxis demonstrated that the neuropeptide appears to decrease phonotaxis latencies to male calls (i.e. increases female sexual arousal; Boyd 1994). Although this previous study simultaneously examined the influence of AVT on male sexual behavior, the tasks for males (evoked vocal response) and females (phonotaxis) were different, thus constraining inferences about sex differences in the effects of AVT (Bernal et al. 2009). However, in a few anuran species (*P. pustulosus, R. sylvatica and Spea bombifrons*), females *and* males are known to exhibit positive phonotaxis to conspecific vocalizations (Ryan 1985; Bee 2007; Baugh and Ryan 2010b; Bernal et al. 2009; Pfennig et al. 2013). Whereas, female phonotaxis in anurans is widely considered an expression of sexual receptivity and mate choice, male phonotaxis is assumed to function as a means to locate and join male breeding aggregation (Baugh and Ryan 2010a), or a more general mechanism for conspecific cueing—using the presence of conspecifics as an indicator of habitat quality (Keister 1979; Stamps 1988, 1991). Therefore, male vocal responsiveness and phonotaxis are behaviors that we predict are linked due to their shared life history function. Indeed, preliminary evidence in individual male túngara frogs suggests that vocal and phonotactic responsiveness are positively correlated (see Results). This link could be achieved if the physiological systems underpinning each behavior are shared. Furthermore, because phonotaxis is shared between the sexes, there is also an opportunity to compare the influence of AVT in males and females while controlling for task differences.

In the present study, we manipulate AVT levels simultaneously in males and females from mated pairs collected in the field and examine the effects of this manipulation on the expression of phonotaxis, including choice frequencies and two measures of arousal: latency to choice and area-restricted searching following choice (Baugh and Ryan 2010a). In doing so, we test the hypothesis that AVT promotes sexual behavior in both sexes, but does so in a dimorphic manner with stronger effects observed in males due to the reported bias in AVT expression in the anuran brain (Boyd and Moore 1992; Boyd 1997; Marler et al. 1999; reviewed in; Wilczynski et al. 2005). More specifically, we predict the following: (1) AVT will differentially elevate choice frequency, with a larger effect observed in males; (2) latency to choice—which is presumably linked with sexual arousal—will decline (i.e. arousal will increase) following AVT administration in females (Boyd 1994); and (3) area-restricted searching—which is presumably linked with sexual arousal or urgency (Baugh and Ryan 2010a)—will be present in females but not males and will be enhanced by AVT.

Methods

The system

Túngara frogs are small anurans distributed throughout Mesoamerica (Weigt et al. 2005). Males advertise vocally to attract females during the breeding season (May–December) using a species-typical call, known as the 'whine' or simple call (Ryan 1985). Males can ornament the whine with one to seven suffixes know as 'chucks', thereby producing a complex call or whine-chuck. In nature, females use calls to localize an individual male amongst a chorus and then select a mate by making physical contact, after which the male mounts and clasps the female in a posture known as amplexus. Males also perform selective phonotaxis, presumably to locate and join an existing chorus (Baugh and Ryan 2010a, b; Bernal et al. 2009). In the laboratory and field, the whine-chuck calls are strongly preferred to whine-only calls in both females and males (Ryan 1985; Ryan et al. 2003; Baugh and Ryan 2010a, b). In addition, túngara frogs show strong preferences for conspecific compared to heterospecific calls, and this can be tested with synthetic calls that vary along a graded continuum between conspecific and heterospecific (Baugh et al. 2008; Ryan et al. 2003; Baugh and Ryan 2010a).

Animals

Protocols for collecting and testing frogs were approved by the Institutional Animal Care and Use Committees of the University of Texas at Austin (06041701) and Autoridad Nacional del Ambiente approved scientific permits in the Republic of Panamá. We conducted all experiments during the breeding season in July 2007 at facilities for the Smithsonian Tropical Research Institute in Gamboa, Panamá (9°07.0′ N, 79°41.9′ W). Protocols for collecting and testing females followed those we have described previously (Ryan et al. 2003). Frogs were returned to their site of collection within 12 h. To prevent resampling, frogs were marked with a unique toe-clip combination following the

Guidelines for the Use of Live Amphibians and Reptiles in Field Research (Beaupre et al. 2004).

Apparatus

Experiments were conducted inside a rectangular, sound-attenuating chamber (2.7×1.8×1.78 m^3, $L \times W \times H$; ETS Lindgren, Cedar Park, TX) equipped with acoustic foam to reduce reverberation and located inside a temperature-controlled (~27 °C) laboratory. Acoustic stimuli were output through the soundcard of a desktop (Dell Dimension 4600) using SIGNAL 4.0 (Engineering Design, Berkeley, CA), amplified with a Crown XLS 402 amplifier (Crown Audio USA, Elkhart, IN), and broadcast through one or two A/D/S L210 speakers (Directed Electronics, Vista, CA) located 2.6 m apart at opposite ends of the sound chamber at equal distances from the center of the chamber. Stimulus SPLs were calibrated in dB SPL (re 20 µPa) using a GenRad, 1982 sound level meter (SLM; IET Labs Inc., Roslyn Heights, NY), with its microphone pointed toward a speaker from a central release point at the center of the chamber, equidistant from the two playback speakers. During all treatments both speakers, including "silent" speakers, were amplified to control for any low frequency sound present in the system. SPLs of "silent" speakers were inaudible and below the threshold for detection using a GenRad SPL meter (General Radio Corporation, West Concord, MA; Model No. 1982). The frogs' behavioral responses were monitored using a wide-angle infrared camera (Fuhrman Diversified Inc., Seabrook, TX) mounted from the center of the sound chamber ceiling and connected to a TV monitor located outside the chamber.

Acoustic stimuli

We used three synthetic stimuli throughout this study (Fig. 1), which were matched for peak amplitude before playback: a simple whine (*W*), the *W* stimulus with one chuck appended to the end (W_c) and an artificial 'hybrid' whine (*PE25*) which is characterized by seven acoustic parameters that are intermediate between the mean túngara call an its sister taxon *P. enesefae* (25% *P. enesefae*, 75% *P. pustulosus*). The chuck is twice the peak amplitude of the whine (Fig. 1). The whines in these signals consist of only the fundamental frequency; it has been shown previously that the upper harmonics of the whine do not influence mate choice in the laboratory (Ryan and Rand 1990; Rand et al. 1992), and that these synthetic conspecific signals are as attractive as natural signals (Ryan, unpublished data). We synthesized these stimuli based on the mean values for the parameters of the calls in the population by shaping sine waves using custom software (J. Schwartz, Pace University at Pleasantville, NY, U.S.A.; sampling rate 20 kHz and 8 bit depth). We calculated mean values for the population based on the calls from 50 males recorded in July 1996 with a Marantz PMD 420 recorder and a Sennheiser ME 80 microphone with K3U power module on magnetic cassette tape. Additional information on the call parameters used

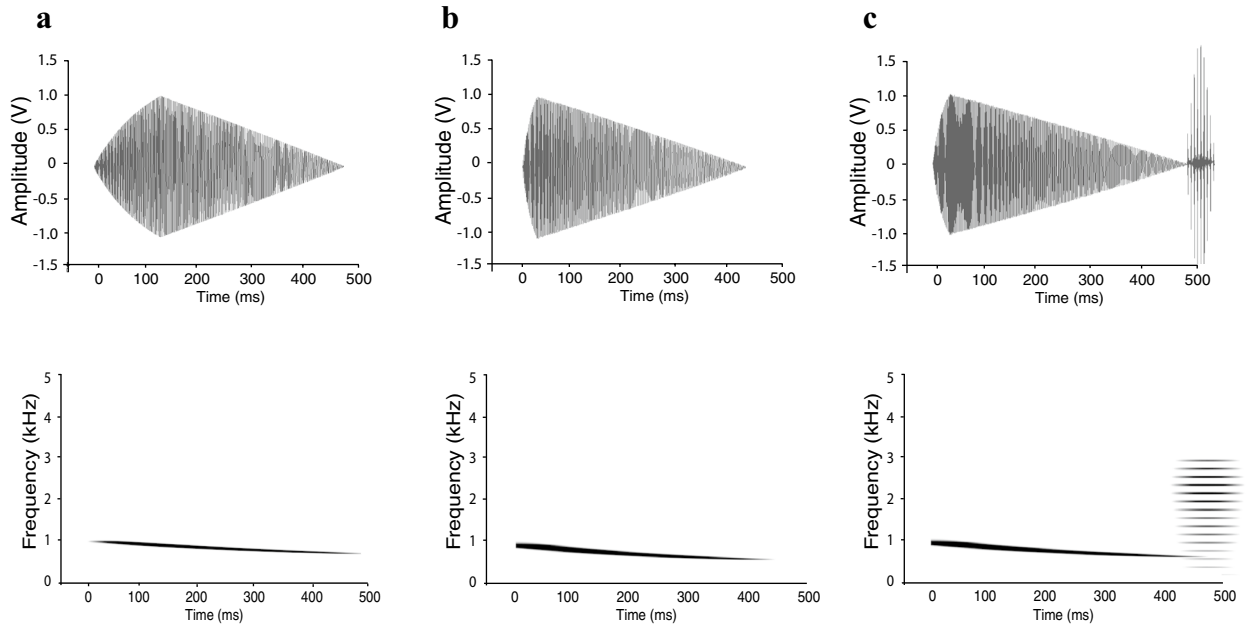

Fig. 1 Oscillograms (*top*) and spectrograms (*bottom*) of the three synthetic stimuli used in this study: **a** hybrid intermediate call (*PE25*); **b** conspecific whine (*W*); **c** conspecific whine-chuck (W_c)

and the synthesis procedure can be found in Ryan et al. (2003).

Hormone treatment

Immediately following the pre-injection testing, each frog was given an intraperitoneal injection (30 gauge needle attached to 1-cc syringe) with either AVT (Sigma-Aldrich; 25 µg AVT diluted in 25 µL of 0.9% saline) or a vehicle control (25 µL 0.9% saline). This dose and delivery method was previously validated in a dose–response study conducted in male túngara frogs (Kime et al. 2007) and shown to be effective in the similarly sized male cricket frogs (Marler et al. 1995; Chu et al. 1998). After injection, frogs were held singly in small plastic bags in darkness for 40 min prior to the post-injection testing. Both sexes were handled identically before and after injection, thus reducing the potential influence of stress on sex comparisons. Solutions were stored at 20 °C.

Experimental protocol

We used a combined within- and between-subjects experimental design with individual nested within mated pair to control for the possible influence of date and time of day on sex differences. The first mated pair (male and female) tested each night was randomly assigned to either the control (vehicle) or experimental treatment (AVT). All subsequent mated pairs per night alternated between these two treatments (1–3 mated pairs tested per night). The male and female within a mated pair were tested sequentially with a random assignment for the first sex to be tested. Each frog was tested pre- and post-injection in three acoustic test conditions: (1) *Responsiveness:* W_c versus silent; (2) *Permissiveness: PE25* versus silent; (3) *Discrimination: W* versus *PE25*. In previous experiments, we have shown that silent speakers are treated identically to a positive control sound such as white noise (Baugh and Ryan 2010a; Ryan, unpublished data). The order of these tests was randomized between pairs. The male and female from each pair were separated at the beginning of testing and not reconvened until the end of the experiment.

Each trial began by placing a single frog under an acoustically transparent cone at the center of the chamber (origin). We then broadcast the two alternative stimuli antiphonally (180° out of phase) for 2 min at a rate of one stimulus per 2 s—simulating two actively calling males. Following this 2-min period, the cone was lifted remotely and the frog could move freely within the sound chamber. We scored a "choice" if the frog entered within 15 min a response zone that extended 10 cm from the speaker in all directions without simply following the chamber wall. After a choice was made, stimulus presentation was continued for 3 min. A "no choice" was recorded if the frog (a) remained motionless at the origin for 5 min after the cone was raised; (b) remained motionless for 2 min after exiting the origin; or (c) failed to make a choice within 15 min (Ryan and Rand 1993). We periodically alternated the side of the chamber broadcasting each alternative stimulus to eliminate any potential side bias. Across frogs, we systematically alternated which stimulus in a pair of alternatives was broadcast first to control for a potential leading caller preference (Bosch and Márquez 2002).

Additionally, we measured two behavioral variables as indicators of arousal: (1) latency to choice: the duration of time elapsed between raising the cone and entering the choice zone (trials not resulting in a choice were omitted); (2) area restricted searching (ARS): if a choice was made we estimated the amount of movement inside the choice zone during the 3 min period of continued stimulus presentation following choice by counting the number of times the frog crossed an invisible line projecting from the center of the speaker's face out to the 10 cm choice boundary line (Hyde and Jerussi 1983; Hills et al. 2004). All of these measurements were recorded for behavior at either pole of the chamber, irrespective of whether stimuli were broadcast from both speakers.

We tested 12 individuals/sex/treatment on each of the three acoustic test conditions (pre- and post-injection) for a total of 288 tests on 48 frogs. In this species, there is no evidence of carry-over effects for repeated testing (Kime et al. 1998), and memory of stimuli decays to null expectations in less than 120 s (Akre and Ryan 2010). Frogs were never tested more than once in the same test condition.

Statistics

To analyze the dichotomous choice results, we first performed a series of binomial exact tests (two-tailed) to determine whether preferences were exhibited in each test condition and group (choices for silence were combined with "no choice" results to permit a dichotomous test). To determine the effects of sex, injection and treatment on choice behavior we used a generalized linear mixed model (SPSS version 21). Individual was specified as a random factor nested within mated pairs to permit sex comparisons that controlled for date and time of day of testing. Pre-/post-injection was specified as a within-subjects factor and crossed with acoustic condition. Treatment (AVT, saline) and sex were specified as fixed factors. A logit link function was used with a binomial distribution and Kenward-Roger degrees of freedom correction.

Latency data were transformed ($\log_{10}$) to improve the normality of model residuals and were analyzed with the model specifications listed above for a continuous dependent variable. Specifically, a general linear mixed model

using REML estimation and planned comparisons for pre-/post-injection for both sexes and treatments. Multiple comparisons were corrected using Tukey–Kramer adjustments.

ARS data were analyzed using a generalized linear mixed model (SAS, GLIMMIX Procedure) with a log link function, a negative binomial response distribution and Kenward-Roger degrees of freedom correction. Multiple comparisons were corrected using Tukey–Kramer adjustments.

Results

Preliminary findings

To identify any potential for side bias in the acoustic chamber, we performed trials ($N=235$) in which both speakers broadcast the identical standard call (W vs W and W_c versus W_c). We found no evidence of a side bias (left:right choices: 122:113; $p=0.60$). In a separate experiment (July 2006), we showed that W is preferred to *PE25* (18 and 2 choices, respectively) in females (zero "no choices"; $p=0.0004$, binomial exact test, two-tailed). Females and males in the present study also significantly preferred W to *PE25* (Table 1). Finally, in a separate study (2006) using captive male túngara frogs ($N=5$), we found a positive correlation between an individual male's vocal responsiveness to playback of the stimuli used in this present study and his phonotaxis towards those stimuli (Online Resource 1), suggesting that the physiological systems that subserve these two behaviors may overlap.

Choices

Binomial exact tests showed significant preferences in 7 of the 24 test groups (Table 1). Choice frequencies and preference patterns were consistent across the three acoustic test conditions. The main finding was that vehicle and AVT injections did not influence female choice frequency, whereas male choice frequencies decreased substantially (−80%) after injection with vehicle and were rescued to pre-injection levels when administered AVT (Table 1). The generalized linear mixed model confirmed this result: there were main effects of sex ($F_{1,41.2}=36.3$, $p<0.0001$), acoustic condition ($F_{1,278}=10.2$, $p<0.0001$), and pre-/post-injection ($F_{1,278}=4.93$, $p=0.02$) but no main effects of treatment ($F_{1,40.3}=0.35$, $p=0.56$). There were significant interactions for sex*pre-/post-injection ($F_{1,278}=4.41$, $p=0.03$), treatment × pre-/post-injection ($F_{1,278}=4.00$, $p=0.04$) and most importantly, the three way interaction of sex*treatment*pre-/post-injection ($F_{2,278}=4.78$, $p=0.009$). Nesting individual within mated pairs resulted in an improved model fit and Gaussian model residuals.

Latencies

There was a significant main effect of sex ($F_{1,46.9}=35.2$, $p<0.0001$); all other main effects were not significant (all $p>0.09$). There were significant interactions of

Table 1 Choice behavior in male and female tungara frogs under three acoustic conditions

Sex	Drug	Injection time point	Responsiveness W_C | Sil | NC	Permissiveness PE25 | Sil | NC	Discrimination W | PE25 | NC	Total choices	% Relative change (i.e. injection effect)	% Relative net change (i.e. AVT effect per se)
Female	Vehicle	Pre	**11** | 0 | 1 (***p* < 0.01**)	8 | 0 | 4 ($p=0.39$)	**10** | 0 | 2 (***p* < 0.01**)	29 (80.5%)	-3.4%	
		Post	**11** | 0 | 1 (***p* < 0.01**)	9 | 0 | 3 ($p=0.15$)	**8** | 0 | 4 (***p* < 0.01**)	28 (77.8%)		
	AVT	Pre	9 | 0 | 3 ($p=0.15$)	6 | 0 | 6 ($p=1.0$)	2 | 5 | 5 ($p=0.45$)	22 (61.1%)		
		Post	**11** | 0 | 1 (***p* < 0.01**)	7 | 0 | 5 ($p=0.77$)	5 | 0 | 7 ($p=0.06$)	23 (63.8%)	+4.5%	+7.90%
Male	Vehicle	Pre	9 | 0 | 3 ($p=0.15$)	6 | 0 | 6 ($p=1.0$)	4 | 1 | 7 ($p=0.38$)	20 (55.5%)		
		Post	3 | 0 | 9 ($p=0.15$)	1 | 0 | **11** (***p* < 0.01**)	0 | 0 | 12 (n/a)	4 (11.1%)	-80.0%	
	AVT	Pre	7 | 0 | 5 ($p=0.77$)	1 | 1 | **10** (***p* < 0.01**)	3 | 2 | 7 ($p=1.0$)	13 (36.1%)		
		Post	6 | 0 | 6 ($p=1.0$)	3 | 0 | 9 ($p=0.15$)	5 | 0 | 7 ($p=0.06$)	14 (38.8%)	+7.7%	+87.7%

Total choices: number of choices for acoustic stimuli (choices for silent speakers omitted) out of the 36 possible choices (%). % Relative change: i.e. the effect of injection: (Post − Pre)/(Pre). % Relative net change: i.e. the effect of AVT per se: % Relative change (AVT) minus % relative change (vehicle). Bolded choice counts and p values indicate statistically signficant preferences (binomial exact test, two-tailed; silent and NC counts were combined for the Responsiveness and Permissiveness conditions; for the Discrimination condition, NC were not included)

W_c synthetic whine-chuck; *Sil* silent; *NC* no choice; *PE25* synthetic intermediate stimulus; *W* synthetic whine

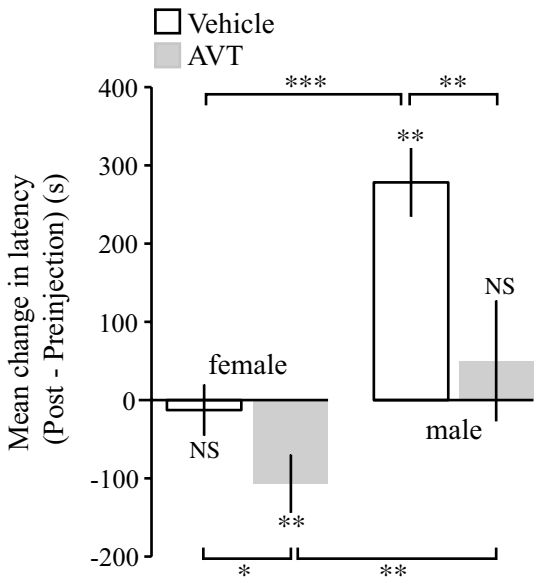

Fig. 2 Sex differences in the effect of AVT and vehicle injections on latencies to choice. Latencies to choice were averaged across all three acoustic conditions and then change values (post-injection minus pre-injection) were calculated and plotted (±SE). *Values near zero* indicate that injection had no effect. *Values below zero* (decrease in latency) indicate that injections increased arousal and *values above zero* (increase in latency) indicate that injection suppressed arousal. *p* values *above and below each column* indicate whether injection significantly influenced latencies. *p* values *above and below the cross bars* indicate pairwise comparsions from the two and three-way interactions. All *p* values estimated from post-hoc contrasts in the general linear mixed model. $*p<0.05$, $**p<0.01$, $***p<0.001$

sex × pre-/post-injection ($F_{1,137}=14.4$, $p=0.0002$) and treatment × pre-/post-injection ($F_{1,125}=4.4$, $p=0.03$). Planned comparisons showed that vehicle injection had no effect of female latencies but AVT injections resulted in decreased latencies (Fig. 2 and Online Resource 2). Conversely, in males, vehicle injections significantly increased latencies whereas AVT injections rescued normal (pre-injection) latencies (Fig. 2 and Online Resource 2). Nesting individual within mated pairs resulted in an improved model fit and Gaussian model residuals.

Area restricted searching

ARS behavior was virtually absent in males but common in females (main effect of sex: $F_{1,142}=49.0$, $p<1\times10^{-8}$). There was a significant interaction between sex × acoustic condition ($F_{1,142}=3.0$, $p=0.03$), which was driven by a higher frequency of ARS behavior in females after selecting W_c compared to W ($p<0.0001$) or PE25 ($p<0.0001$), but no difference between W and PE25 ($p=0.98$; Fig. 3). There were no effects of injection per se (pre- vs post-injection) or treatment (vehicle versus AVT) on female ARS behavior (all $p>0.27$).

Behavior within mated pairs

A χ^2 contingency table showed that there was no association between the presence or absence of choice behavior (≥1 choice across all three acoustic conditions) in a female and her male mate, before ($p=0.38$) or after injection

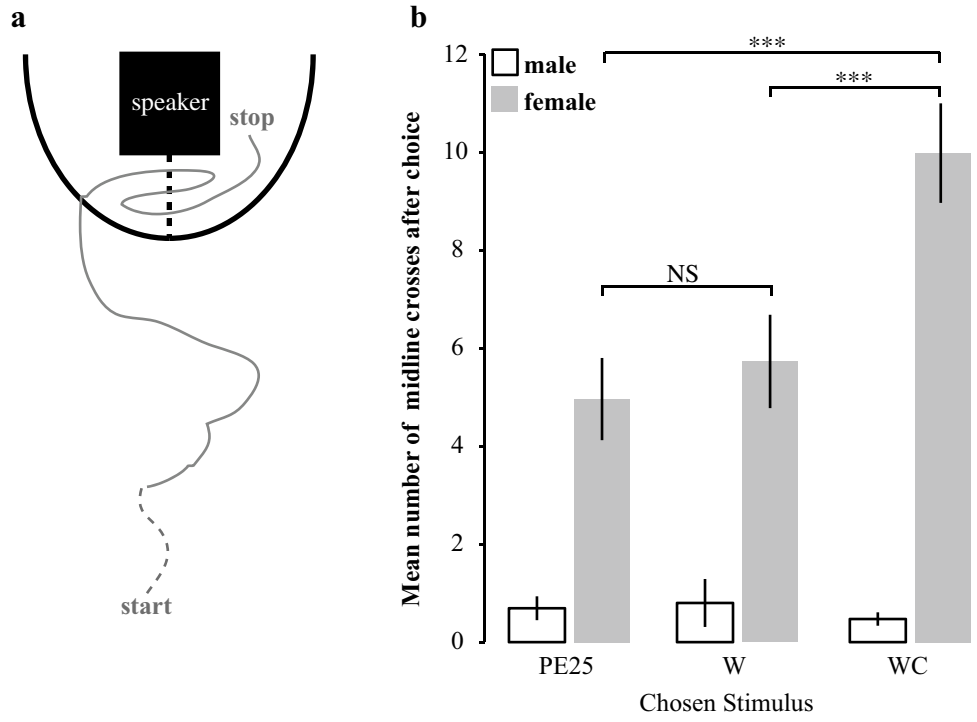

Fig. 3 **a** Illustration of the choice zone for one speaker and the invisible midline (*dashed black line*) projecting from the face of the speaker to the choice boundary (*solid line*) and a hypothetical frog that made three ARS midline crosses during its path (*grey line*). **b** The mean number (±SE) of midline crosses in front of the chosen speaker. For each female, the number of midline crosses was averaged across pre- and post-injection. Vehicle and AVT treatment groups are combined. Females exhibit significantly more area restricted searching than males, especially upon choosing a whine-chuck. $***p<0.0001$

($p = 1.0$). A female's latency to choice, however, was correlated with that of her male partner's. There was a significant positive correlation (Spearmans ρ, two-tailed) for the full dataset (vehicle and AVT groups combined and pre-/post-injections results averaged; $\rho = 0.507$, $p = 0.023$, $N = 20$ pairs; Online Resource 3) and this was driven by a strong positive correlation within the AVT group ($\rho = 0.721$, $p = 0.019$, $N = 10$ pairs); there was no correlation in the vehicle treatment group ($\rho = 0.139$, $p = 0.70$, $N = 10$ pairs). Further, this correlation was present in the pre-injection dataset in which no frog had experienced treatment ($\rho = 0.586$, $p = 0.011$, $N = 18$ pairs); but no correlation was observed in the combined treatments post-injection dataset ($\rho = 0.445$, $p = 0.17$, $N = 11$ pairs); there was a positive correlation in the post-injection AVT group ($R^2 = 0.25$), however the small sample size ($N = 4$ pairs) precludes p value estimation. In other words, there was a natural positive correlation between partners in sexual behavior and this correlation is retained after AVT injection but disrupted after saline injection (which inhibits male behavior). There were no correlations between male and female partners for the number of midline crosses (all $p > 0.50$), but males provided very little variance in this trait.

Discussion

Our results confirm previous studies demonstrating that AVT facilitates sexual behaviors in anurans (reviewed in Wilczynski et al. 2005; Boyd 2013). We found support for the hypothesis that AVT promotes acoustically-guided sexual behavior in both male and female túngara frogs. Unlike most previous research, which has focused principally on the role of AVT in regulating male behavior (Wilczynski et al. 2005), we compared the sexes using the same task, thereby enabling inferences about sex differences *per se*. Male biases in AVT receptor expression in the midbrain and brainstem auditory nuclei of bullfrogs (Boyd 1997) and the forebrain of cricket frogs (Marler et al. 1999) suggest that males might be more sensitive (i.e. exhibit behavioral effects at lower dosages) to endogenous and exogenous AVT than females. Our results generally support this hypothesis. We found that AVT increases phonotactic readiness in both sexes, but in different ways. In females, the probability of exhibiting phonotaxis was not influenced by exogenous AVT, and likewise, the experience of being injected *per se* had no effect on female phonotaxis. On the other hand, the latency between stimulus onset and choice in females decreased following AVT administration, suggesting that motivational systems underlying this canonical female response to male calls is potentiated by AVT. In contrast, males experienced a large decline in choice behavior following injection with vehicle but pre-injection levels of phonotaxis were rescued by exogenous AVT. These results suggest that AVT facilitates phonotaxis in males and that this behavior in males is highly sensitive to the experience of being injected *per se*, presumably due to its effect as a potent stressor (males do not experience a decline in phonotaxis due to handling alone; Baugh and Ryan 2010a). This sensitivity in male túngara frogs appears to be specific to phonotaxis behavior, as evoked vocal responses appear to be normal following injection with saline (Kime et al. 2007). Further research is necessary to understand the basis for this sexual dimorphism in phonotactic sensitivity to stress. For example, it would be informative to describe in males the binding of glucocorticoids in the vocal motor circuits compared to the audiomotor centers of the anuran midbrain that underlie phonotaxis (Endepols and Walkowiak 1999, 2001; Endepols et al. 2003). The latter might be more sensitive to increases in circulating corticosterone, and these pathways could differentially express stress hormone receptors in AVT-immunoreactive (AVT-ir) neurons, thereby explaining the sensitivity in phonotactic but not vocal behavior following acute stress (Leary et al. 2004; Wilczynski et al. 2005). Finally, sex differences in these pathways could help explain the behavioral dimorphism observed here (Boyd 1997; Marler et al. 1999)—i.e. how AVT may function to rescue behavior that has been inhibited by acute elevations in stress hormones in males (Moore and Rose 2002; Coddington and Moore 2003). Based on sex differences in neural AVT expression and the influence of AVT on amphibian behavior, Wilczynski et al. (2005) proposed that AVT acts principally by potentiating sensory or sensorimotor systems, as opposed to motor control itself. Presumably the motor control of phonotaxis is similar between the sexes (but see ARS discussion below), while the sensory, sensorimotor and motivational circuits underlying phonotaxis are dimorphic. This hypothesis leads to the prediction that males and females will be affected differently by AVT. While our behavioral results are in agreement with this hypothesis, the neural basis remains to be tested.

Our results also confirm earlier findings in this species, including shorter latencies in females compared to males and the presence of ARS in females but not males (Baugh and Ryan 2010a). The latter result had previously been shown only in captive túngara frogs treated with human chorionic gonadotropin. That study also showed that adult males and females differed strictly in ARS and not path lengths to the choice zone, thus implying that the sex differences in ARS observed here are not due to general locomotor differences between the sexes. Although AVT did not influence ARS behavior—consistent with the idea that ARS is regulated perhaps by other neurotransmitters (Hills et al. 2004)—the wild-caught females in the present study showed a gradual increase in ARS in

response to more attractive stimuli. This relationship, as well as the absence of ARS in adult males and in juveniles of both sexes (Baugh and Ryan 2010a), indicates that ARS functions exclusively in a female mate choice context (e.g. mate searching).

To our knowledge this is the first study to (1) examine the influence of AVT on a shared behavioral task in male and female anurans, and (2) test for a correlation between a female and her male mate's behavior. The latter is important here because it demonstrates the value of statistically accounting for pair identity in our statistical models, but it is also an interesting—and understudied—topic itself. Here, we showed that choice latencies were positively correlated within mated pairs. Specifically, a female's reproductive motivation is linked with her male partner's sexual motivation; the inhibitory effects of injection with saline interfere with this underlying correlation and injection with AVT compensates for it. Although this correlation could arise due to active processes (e.g. assortative mating based on sexual arousal), we suspect that this result is due instead to more passive influences such as shared environmental experience within a pair (e.g. exact time of day for behavioral testing, duration of time in amplexus). This hypothesis requires further study.

Because selective phonotaxis is present and sexually monomorphic in juvenile anurans of both sexes (Baugh and Ryan 2010a; Pfennig et al. 2013), and the onset of this behavior occurs in very early stages of post-metamorphic growth, it is possible that the vasotocin system plays a principal role in facilitating this behavior prior to the growth and differentiation of the gonads. To test this, it will be necessary to manipulate AVT levels in gonadectomized juveniles. Future studies could test the idea that sexual dimorphisms in the vasotocin system during early development (Boyd 1991) interact with circulating gonadal steroid hormones such as estrogens and androgens—which are known to influence vasotocin receptor expression (Boyd 1997) and interact with circulating AVT in amphibians (Moore et al. 1992; Thompson and Moore 2003)—and together these interacting hormone pathways underlie the similarities and differences in acoustically-guided behavior between the sexes.

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