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Vasotocin Maintains Multiple Call Types in the Gray Treefrog, *Hyla versicolor*

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The neuropeptide arginine vasotocin (AVT) influences vocalizations in some anuran amphibians but it is unknown whether AVT alters all vocal behaviors of a species similarly. We first characterized the vocal repertoire of male gray treefrogs (*Hyla versicolor*). Three different call types were distinguished by unique sets of temporal and spectral features. Second, we examined the effects of AVT on each call type by injecting frogs with either AVT (100 μg; intraperitoneal) or saline and recording subsequent behavior. In the field, AVT maintained advertisement calling, whereas calling ceased in saline-injected animals. Advertisement call rate in AVT-injected males fell significantly and dominant frequency of the call was significantly higher. In the laboratory, AVT induced advertisement calling in males that were not initially vocalizing and dominant frequency was also significantly higher in these males. AVT maintained aggressive calling similarly but the characteristics of aggressive calls were not altered by AVT. There were no significant differences in release call behavior between AVT- and saline-injected groups; however, release call duration decreased significantly in AVT-injected animals, compared with preinjection values for the same animals. The effects of AVT on vocal behavior in this species are therefore not the same for each call type. AVT may act at more general motivational levels in the central nervous system and other neural or endocrine factors may control choice of call type and direct motor output.

Key Words: **reproductive behavior; aggression; vasopressin; frog; vocalization; neuropeptide.**

Neuropeptides in the vasopressin and vasotocin family have been consistently implicated in the control of reproductive and social behaviors in vertebrates. Vasopressin in mammals, for example, modulates paternal behaviors, pair-bonding behaviors, and aggressive or territorial behaviors (e.g., Winslow, Hastings, Carster, Harbaugh, and Insel, 1993; Wang, Ferris, and De Vries, 1994; Bamshad, Karom, Pallier, and Albers, 1997; Ferris, Melloni, Koppel, Perry, Fuller, and Delville, 1997). Vasotocin (AVT), the homologue of vasopressin in nonmammalian vertebrates, alters similar types of behavior including copulatory behaviors of birds and newts (Moore, 1992; Castagna, Absil, Foidart, and Balthazart, 1998), aggressive behaviors in birds (Goodson, 1998a, b; Goodson and Adkins-Regan, 1999), and several sexual behaviors of frogs (Boyd, 1997). In nonmammals, the influence of AVT on vocal behaviors has been recently more closely examined. AVT modulates singing in three bird species (Maney, Goode, and Wingfield, 1997; Castagna, *et al.*, 1998; Goodson, 1998a) and calling in five frog species (see below). This common theme suggests that AVT may have a relatively nonspecific and context-independent effect on motor areas controlling vocal output. This may be the case in female white-crowned sparrows, for example, in which singing and solicitation behaviors are induced by AVT even in the absence of males (Maney *et al.*, 1997). On the other hand, AVT induces the display of aggressive song specifically in male field sparrows (and not other song types), inhibits crowing in Japanese quail, and does not affect singing in limited studies with zebra finches and violet-eared waxbills (Castagna *et al.*, 1998; Goodson, 1998a, b; Goodson, and Adkins-Regan, 1999). AVTs influence on singing in birds is thus species-specific and call type-specific. In frogs, whether there is variability across species or call types in the effects of AVT is unclear.

Anuran amphibians are advantageous models for the study of the neuroendocrine control of social behavior because they rely heavily on vocalization for communication with conspecifics. Male frogs and toads usually have a small set of stereotyped calls that are used for species recognition, mate attraction, in-

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termale spacing, or territory defense; Bogert, 1960). In some species, a single invariant call type likely serves all these purposes (e.g., *Bufo canorus*; Karlstrom, 1962). In other species, one basic call type is varied depending on whether the context is one of male–male interaction or male–female interaction (e.g., *Acris crepitans;* Wagner, 1989a, b, c; Wagner, 1992). Finally, in some species, males give distinctly different advertisement calls (for species recognition and female attraction) and aggressive calls (for intermale spacing or territory defense) (Bogert, 1960; Wells, 1977). In the gray treefrog, *Hyla versicolor*, the advertisement call has been extensively studied (Gerhardt, 1978; Wells and Taigen, 1986; Schwartz, 1987; Gerhardt, Dyson, and Tanner, 1996; Welch, Semlitsch, and Gerhardt, 1998). Two other call types, an aggressive call and a release call (given by both males and females when inappropriately clasped), have also been briefly described in these treefrogs (Pierce and Ralin, 1972; Fellers, 1979; Schwartz, 1987). The male gray treefrog thus has a call repertoire of 3. It is likely that there are differences in the neural and hormonal control of these different call types. We characterized the spectral and temporal features of all three call types because a complete quantitative description of aggressive and release calls has not been reported and because there may be significant geographical variability in call characteristics (e.g., Snyder and Jameson, 1965; Asquith, Altig, and Zimba, 1988; Ryan and Wilczynski, 1991).

Vocal behavior in several anuran species is modulated by AVT (Table 4). Advertisement calling is stimulated by AVT in bullfrogs (*Rana catesbeiana,* Boyd, 1994), green treefrogs (*H. cinerea,* Penna, Capranica, and Somers, 1992), cricket frogs (*A. crepitans,* Marler, Chu, and Wilczynski, 1995; Chu, Marler, and Wilczynski, 1999), and the Great Plains toad (*B. cognatus,* Propper and Dixon, 1997). The effect of AVT on aggressive calls has not been reported. In cricket frogs, the advertisement call can also function as an aggressive call and this combination call is altered by AVT to produce a call typical of a less aggressive animal (Marler *et al.*, 1995). This suggests that AVT may inhibit an aggressive call in frogs with distinctly different call types but this has not been tested. The release call of female leopard frogs (*R. pipiens*) and bullfrogs is inhibited by AVT (Diakow, 1978; Boyd, 1992). AVT stimulates release calling in male bullfrogs (Boyd, 1992) but inhibits it in male leopard frogs (Raimondi and Diakow, 1981). AVT thus clearly alters vocal behavior in frogs but it is impossible to determine whether the variability observed is due to sex differences, species differences, or call type differences. We therefore examined the effects of AVT on the entire call repertoire of male gray treefrogs. This allowed us to determine whether AVT altered a particular subset of call types or call characteristics in this species.

MATERIALS AND METHODS

Characterization of Male Gray Treefrog Call Types

All animals used were collected in Gogebic County, Michigan, during May and June of 1996 and 1998. Care and use of animals conformed to NIH guidelines. We recorded calls (between 10:30 PM and 12:30 AM) from sexually mature male frogs that were captured while calling in the field. Advertisement and aggressive calls were recorded within 4 h of capture from single animals that were calling spontaneously and isolated in Styrofoam tanks ($30 \times 30 \times 50$ cm). Release calls were elicited with tactile stimulation (procedure from Pierce and Ralin, 1972) from individual males (kept in the laboratory for 10 days; see below for conditions). Different animals were used for each call type. Recordings (at least 5 min) were made with a Dan Gibson EPM parabolic microphone and Marantz tape recorder. Calls were digitized using Interactive Laboratory System software (ILS; Signal Technology). A bandpass filter (Butterworth) removed frequencies below 500 Hz and above 5000 Hz. At least five calls from each individual were analyzed and averaged to yield single mean values for each characteristic from each animal.

Effects of AVT on Advertisement, Aggressive, and Release Calling

The effects of AVT on the advertisement call were examined in both the field and the laboratory. Effects of AVT on aggressive calls were tested only in the field and effects on release calls were tested only in the laboratory. For field experiments, calling male treefrogs were captured at dusk on 2 nonconsecutive nights (17 and 19°C; mean snout–vent length 4.2 cm ± 0.05 SEM). Animals were isolated in individual Styrofoam tanks at the collecting site and the experiments run immediately each night on the edge of the bog. On the first night, males gave only advertisement calls while on the second night the males captured also gave aggressive calls spontaneously. Five-minute baseline (immediately before injection) recordings of the calls from each frog were made using the equipment described. Half the males were then injected intraperitoneally with 100 μg AVT (Sigma Chemical Co.; dose chosen from preliminary experiments) diluted in 0.1 ml amphibian Ringer's saline or an equivalent volume of saline alone. We alternated AVT and saline tanks to expose all animals equally to vocal neighbors (tanks about 0.5 m apart). Five-minute segments of advertisement calls and aggressive calls given by calling males were recorded at 90 min following injection. Response to AVT was maximal at this time point, rather than earlier times, in preliminary experiments. At least five calls from each vocal male were analyzed with ILS as described above.

For the laboratory experiment on advertisement calls, calling males were captured and brought into a laboratory about 2 km from the bog. These males ceased spontaneous calling within hours and such calling never reappeared on subsequent nights in untreated animals. In the laboratory, males were housed individually in clear plastic tanks (30 × 16 × 10 cm) with water and vegetation from their home bog. Frogs were fed small crickets ad libitum daily and exposed to natural temperature and light conditions. Males that had been in the laboratory between 3 and 12 days were used for this study (mean snout–vent length 4.7 cm ± 0.1 SEM) and the study was run on 2 different nights (between 10 PM and 1 AM; temperature 18°C; both treatment groups on both nights). Treatment and data collection were otherwise identical to the field experiment.

The release call experiment was run on males that had been in captivity for 2 weeks (mean snout–vent length 4.5 cm ± 0.1 SEM). Data were collected on a single evening (24°C). Procedures were identical to previous studies except that release calls were induced at baseline (before injection) and at 30-, 60-, and 90-min time points after injection with tactile stimulation (Pierce and Ralin, 1972). Only data from the baseline and 90-min time points are presented (results at 30 and 60 min were similar to those at 90 min).

Data Analysis

Temporal parameters of calls were determined within bouts of calls for advertisement and release call types. A "bout" was defined as a group of calls. Bouts were separated by periods of silence that were significantly greater than the silence between calls within a bout. Aggressive calls were given infrequently, at widely varying intervals, and not within bouts. Call rate and intercall interval were thus not calculated for aggressive calls. In the call type characterization study (Table 1) each characteristic was compared across call types with a one-way ANOVA (when parameter was measured for all three call types) or *t*-test (when parameter was measured in only two call types). Fisher's least significant difference test was used for pairwise comparisons after the ANOVA. In all AVT experiments (Tables 2 and 3), ratios of number of males calling out of total males in the group were compared with Fisher's exact test. Wilcoxon signed-ranks test was used to compare baseline and 90-min values within treatment groups and Mann–Whitney *U* test was used to compare AVT and saline treatment groups at the same time points.

RESULTS

Characterization of Male H. versicolor Call Types

Male gray treefrogs gave three significantly different types of calls (Fig. 1, Table 1).). The advertisement call was the most complex. This call type had a significantly greater call duration and pulse number, than the other two call types. The call rate for advertisement calling was significantly less than for release calling. Intercall interval was significantly longer for advertisement calls than for release calls.

Aggressive calls were given singly in our isolated animals (Fig. 1, Table 1). Call rate and intercall interval measurements could not thus be compared with the other call types. The duration of the aggressive calls was intermediate between advertisement and release calls and differed significantly from both. Pulse number was relatively low and not significantly different from pulse number in release calls. Pulse number in aggressive calls was about 25% of the pulse number in advertisement calls and differed significantly.

The release call was the simplest call type (Fig. 1, Table 1). Each individual call was very short and call duration for release calls was significantly less than duration for advertisement or aggressive calls. Calls were typically produced in bouts with three to five calls per bout. The intercall interval within bouts of release calls was significantly shorter than the interval between advertisement calls. Call rate was thus significantly greater for release calls. The number of pulses per call for release calls was significantly less than the pulse number for advertisement calls, but did not differ from pulse number for aggressive calls.

The dominant frequency did not differ significantly among the three call types (Table 1). The fundamental frequency of the aggressive call type

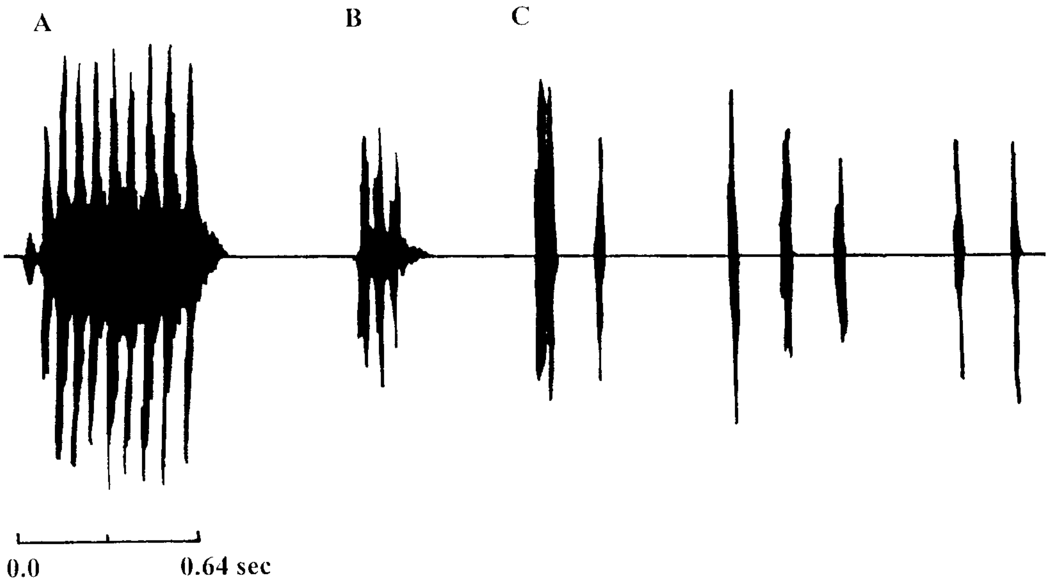

FIG. 1. Representative oscillograms of the three call types in male *H. versicolor*. (A) Advertisement call. (B) Aggressive call. (C) Release call.

was slightly (about 75 Hz) but significantly less than the fundamental frequency of the other two call types.

Effects of AVT on Advertisement, Aggressive, and Release Calling

Advertisement calling was observed significantly more often in AVT-injected male treefrogs than in saline-injected males. This was true in both laboratory and field experiments (Table 2). In the field study, all males were calling when captured and an equal number of males in each treatment group (five of seven) were calling from within individual tanks after capture but before injection. Before injection, there were no significant differences in any advertisement call parameter between frogs in each treatment group (Mann–Whitney *U* tests; $P > 0.05$). After injection, all saline-injected males ceased calling immediately. No calls were heard from any saline-injected male during

TABLE 1

Spectral and Temporal Characteristics (Mean ± SEM) of Advertisement, Aggressive, and Release Call Types from Male Gray Treefrogs, *H. versicolor*

Parameter	Advertisement call	Aggressive call	Release call	*P* value^a
Call rate (calls/min)	12.6 ± 1.8	—	45.0 ± 5.0	<0.0005
Pulse number (pulses/call)	12.7 ± 0.4^b	3.5 ± 0.2^c	2.66 ± 0.4^c	<0.0005
Call duration (s)	0.78 ± 0.05^b	0.41 ± 0.04^c	0.08 ± 0.003^d	<0.0005
Intercall interval (s)	2.53 ± 0.14	—	0.17 ± 0.006	<0.0005
Fundamental frequency (Hz)	1222.9 ± 17.4^b	1148.6 ± 20.9^c	1223.2 ± 13.9^b	<0.04
Dominant frequency (Hz)	2070.0 ± 17.0	2076.6 ± 30.5	2054.8 ± 12.4	<0.658
Calls analyzed	$n = 50$	$n = 16$	$n = 95$	
Males contributing calls	$n = 10$	$n = 5$	$n = 19$	
Snout–vent length (cm)	4.24 ± 0.07	4.30 ± 0.09	4.47 ± 0.1	<0.213
Temperature (°C)	17	20	24	

[a] Value for *P* is the result of a one-way ANOVA when data for all three call types exist and for a *t* test when only two call types were compared.

[b,c,d] For each parameter, these superscripts are used to describe when a *post hoc* pairwise least significant difference test showed significant differences ($P < 0.05$) between call types. Calls with different superscript letters differ significantly from one another while those that share a letter do not.

TABLE 2

Effects of AVT or Saline Injection on Advertisement Calling in Male *H. versicolor* under Laboratory and Field Conditions (Means ± SEM, unless Otherwise Indicated)

Parameter	Field study		Laboratory study	
	AVT	Saline	AVT	Saline
Number of males calling/total number of males				
Baseline	5/7	5/7	0/7	0/7
90 min	7/7	0/7[a]	6/7	0/7[a]
Call rate (calls/min)				
Baseline	13.4 ± 2.9	11.8 ± 2.3	0	0
90 min	5.0 ± 2.1[b]	0	12.0 ± 1.1	0
Pulse number (pulses/call)				
Baseline	13.2 ± 1.2	12.1 ± 0.7	0	0
90 min	11.5 ± 0.6	0	11.5 ± 1.5	0
Call duration (s)				
Baseline	0.80 ± 0.10	0.71 ± 0.05	0	0
90 min	1.01 ± 0.05	0	0.74 ± 0.15	0
Intercall interval (s)				
Baseline	2.72 ± 0.30	2.70 ± 0.18	0	0
90 min	3.24 ± 0.30	0	2.78 ± 0.30	0
Fundamental frequency (Hz)				
Baseline	1218 ± 27	1228 ± 10	0	0
90 min	1242 ± 17	0	1185 ± 28	0
Dominant frequency (Hz)				
Baseline	2044 ± 25	2095 ± 26	0	0
90 min	2133 ± 8[b]	0	2185 ± 45[c]	0

[a] Fisher's exact test comparison of number of males calling in AVT- and saline-injected groups at 90-min postinjection: field study $P = 0.001$; laboratory study $P = 0.005$.

[b] Wilcoxon signed-ranks test comparing baseline and 90-min values on all parameters for the five males in the AVT-treated group that called at both times. For call rate and dominant frequency, values were significantly different ($P = 0.043$). Pulse number, call duration, intercall interval, and fundamental frequency did not differ.

[c] Mann–Whitney U test comparing each parameter from AVT-injected animals in the laboratory with the baseline preinjection values from the field experiment (AVT and saline groups combined, $n = 10$) showed that only dominant frequency differed significantly ($P = 0.042$).

the 90-min observation period that followed injection. On the other hand, AVT-injected males all called, including the two animals that had not called during the baseline recording period. Thus, in the field experiment, AVT maintained advertisement calling in injected animals.

Two parameters of the advertisement call were significantly different following AVT treatment (Table 2). The call rate, 90 min after AVT injection, was less than half the call rate of the same animals before injection. Dominant frequency of the calls was significantly higher after injection, compared with the baseline dominant frequency. Pulse number, call duration, intercall interval, and fundamental frequency were not altered (Table 2).

In the laboratory, AVT stimulated the display of advertisement calling in animals that were initially silent (Table 2). No frogs were calling before injection and no calls were heard during the 90-min observation period from the saline-injected males. Six of the seven AVT-injected frogs, however, began to call within 30 min of injection and called in a robust fashion through the first 90 min postinjection. The temporal and spectral characteristics of calls from these frogs were compared with the preinjection baseline call characteristics from the field study. There were no significant differences except that the dominant frequency of the call in the AVT-injected animals in the laboratory experiment was significantly greater than in uninjected animals in the field study.

AVT injection did not alter the likelihood that male treefrogs would give aggressive calls (Table 3). This call type was the rarest of the three call types in our isolated frogs. Before injection, two of the seven frogs in each treatment group were giving spontaneous aggressive calls, in conjunction with advertisement calls. The display of aggressive calls was maintained at about the same level in AVT-injected males (three of

TABLE 3

Effects of AVT or Saline Injection on Aggressive Calling or Release Calling in Male Gray Treefrogs (Mean ± SEM, unless Otherwise Noted)

	Aggressive calling		Release calling	
Parameter	AVT	Saline	AVT	Saline
Number of males calling/total number of males				
Baseline	2/7	2/7	9/9	9/9
90 min	3/7	0/7	9/9	9/9
Call rate (calls/min)				
Baseline	NA	NA	36.2 ± 6.4	41.3 ± 5.7
90 min	NA	NA	78.0 ± 15.4	60.8 ± 8.4
Pulse number (pulses/call)				
Baseline	4.5 ± 0.5	3.5 ± 0.0	1.9 ± 0.2	1.9 ± 0.2
90 min	3.3 ± 0.3	0	2.0 ± 0.1	2.0 ± 0.1
Call duration (s)				
Baseline	0.26 ± 0.02	0.47 ± 0.11	0.12 ± 0.01	0.10 ± 0.01
90 min	0.41 ± 0.03	0	0.07 ± 0.003[a]	0.09 ± 0.009
Intercall interval (s)				
Baseline	NA	NA	0.17 ± 0.02	0.13 ± 0.01
90 min	NA	NA	0.17 ± 0.01	0.14 ± 0.01
Fundamental frequency (Hz)				
Baseline	1066 ± 43	1098 ± 4	1056 ± 30	1106 ± 10
90 min	1243 ± 11	0	1076 ± 23	1111 ± 13
Dominant frequency (Hz)				
Baseline	1973 ± 27	2011 ± 4	2064 ± 21	2122 ± 31
90 min	2230 ± 103	0	2114 ± 23	2140 ± 20

[a] Comparison of baseline and 90-min values for AVT-injected animals showed a significant difference (Wilcoxon signed-ranks test; $P = 0.025$).

seven) but saline-injected males ceased calling immediately. The ratio of aggressive calls to advertisement calls was unaffected by AVT treatment. Before injection, frogs in both treatment groups were giving a mean of 8.5 advertisement calls for each aggressive call. At 90 min after AVT injection, those males were giving a mean of 6.4 advertisement calls for each aggressive call. There were no significant differences in any call parameter, between baseline and 90-min times, in AVT-injected males; however, the overall small number of animals giving aggressive calls likely decreased the power of the tests.

All males displayed release call behavior at both baseline and 90-min postinjection time points (Table 3). AVT or saline injection thus did not affect whether or not animals gave release calls. There were no significant differences in any call parameter between AVT-injected and saline-injected treatment groups (Mann–Whitney *U* test). Paired comparison (Wilcoxon signed ranks test) of baseline and 90-min values within each call parameter showed no significant treatment effects except that call duration in AVT-injected males was significantly lower 90 min after treatment.

DISCUSSION

Arginine vasotocin significantly altered certain aspects of the vocal behavior of male gray treefrogs. Although AVT has been previously shown to alter calling in a few species of frogs (see below and Table 4), this is the first time that the effects of the peptide on the complete vocal repertoire of a species have been reported. For the advertisement call type, AVT injection determined whether frogs gave any calls at all. Overall, in laboratory and field experiments, AVT appeared to maintain calling in the face of stressors (injection or captivity) that otherwise caused calling to cease. In some cases, AVT injection induced calling in silent frogs. Facilitation of advertisement calling by AVT has been shown in bullfrogs, Great Plains toads, cricket frogs, and green treefrogs (Penna *et al.*, 1992; Boyd, 1994; Marler *et al.*, 1995; Propper and Dixon,

TABLE 4

Summary of Studies on the Effects of AVT on Vocalizations in Anuran Amphibians

Species	Sex	Call type	Effect of AVT	References
Hyla versicolor	Male	Advertisement	Increase	This paper; Semsar *et al.* (1998)
		Aggressive	No effect	This paper
		Release	No effect	This paper
Hyla cinerea	Male	Advertisement	Increase	Penna *et al.* (1992)
Acris crepitans	Male	Advertisement	Increase	Marler *et al.* (1995); Chu *et al.* (1998)
Rana catesbeiana	Male	Advertisement	Increase	Boyd (1994)
	Male	Release	Increase	Boyd (1992)
	Female	Release	Decrease	Boyd (1992)
Rana pipiens	Male	Release	Decrease	Raimondi and Diakow (1981)
	Female	Release	Decrease	Diakow (1978)
Bufo cognatus	Male	Advertisement	Increase	Propper and Dixon (1997)

1997). In these four species as well, AVT maintains or stimulates calling in the face of handling stressors. This may be due to an interaction between AVT and corticosterone as previously shown for control of amplectic clasping behavior in newts (Moore, Lowry, and Rose, 1994; Rose, Kinnaird, and Moore, 1995).

In gray treefrogs, we found that two specific parameters of the advertisement call were significantly different 90 min after AVT injection. Call rate fell to less than half the baseline values in AVT-injected males in the field experiment. This is likely due to the cumulative effect of slight increases in both the call duration and the intercall interval. These changes in the temporal characteristics of the call are typical of the changes that occur in dense choruses of gray treefrogs and longer calls at lower call rates are preferred by females (Wells and Taigen, 1986; Klump and Gerhardt, 1987; Gerhardt *et al.*, 1996; Welch *et al.*, 1998). A perceived increase in chorus density could not have been responsible for the change in call rate in our experiment because the total number of calling males had fallen (from 10 to 7) during the 90-min period. A significant decrease in call rate after AVT treatment has also been shown in the cricket frog (Marler *et al.*, 1995).

The second parameter altered was dominant frequency. The significant increase in dominant frequency that we found in gray treefrogs has also been observed in another hylid, *A. crepitans* (Marler *et al.*, 1995), but not in any other anuran. The biological significance of this small (about 100 Hz) change in frequency of the advertisement call of gray treefrogs is unclear. It is unlikely that this shift would affect the preferences of females and eventual mating success of the male (Gerhardt *et al.*, 1996). In *A. crepitans,* on the other hand, males can facultatively alter the dominant frequency of their calls during male–male aggressive encounters (Wagner, 1989a, 1992). AVT and saline treatment of cricket frogs results in changes in dominant frequencies between 100 and 250 Hz (Chu *et al.*, 1999). Shifts of this magnitude have been shown to alter aggression and competitive abilities in other studies in cricket frogs (Wagner, 1992).

Although the distinctive trilled advertisement call of gray treefrogs has been intensively studied (Gayou, 1984; Wells and Taigen, 1986; Gerhardt *et al.*, 1996), the other call types have not been quantitatively characterized and statistically compared with the advertisement call. The aggressive call of the gray treefrog is a short, frequency-modulated call (Pierce and Ralin, 1972; Fellers, 1979; Schwartz, 1987). In our study, the aggressive call type was given by isolated males. We termed this call type “aggressive” therefore after comparison of our oscillograms with those of Fellers (1979) and Schwartz (1987). They observed this call specifically during aggressive interactions between two males or as an “answer” to loud advertisement calls nearby. We found that the aggressive call differed significantly from the advertisement call in pulse number, call duration, and fundamental frequency. Differences in pulse number and call duration of this magnitude can be detected by other individuals (Gerhardt and Doherty, 1988; Allan and Simmons, 1994; Diekamp and Gerhardt, 1995; Gerhardt *et al.*, 1996) and potentially alter their behavior. The difference in fundamental frequency is probably not significant in the behavior of the frogs hearing these calls, especially since dominant frequency of the calls did not differ. Aggressive calls that we recorded were longer in duration than previously reported and were given singly (rather than in multiple call bouts; Pierce and Ralin, 1972; Fellers, 1979; Schwartz, 1987). This was likely a consequence of isolation of males for recording in our experiment. During encounters with other males or during playback experiments, male gray treefrogs will respond to increased threat with an increase in the number of aggressive calls (Fellers, 1979; Schwartz, 1987). Even in these situations, however, aggressive calls typically make up only 10–20% of the calls given by this species (Schwartz, 1987; Wells and Bard, 1987).

Arginine vasotocin maintained aggressive calling at preinjection baseline levels. Ratio of advertisement to aggressive calls was not altered by AVT injection. Aggressive calling disappeared, however, in saline-injected animals. Effects of AVT on likelihood of aggressive calling were thus similar to effects on likelihood of advertisement calling in that the number of frogs displaying a particular call type was maintained at preinjection levels after AVT (compared to saline injection) in both cases. This is the first time that the effects of AVT on a distinct aggressive call in frogs has been tested. The use of the advertisement call in aggressive interactions has been studied in gray treefrogs (Semsar, Klomberg, and Marler, 1998). AVT increased the ability of male intruders to take over calling sites from resident frogs. This ability apparently did not depend upon the aggressive call or actual physical aggression. In *A. crepitans*, the advertisement call is used in aggressive interactions with graded changes in call structure (Wagner, 1989b). The changes in *A. crepitans* advertisement call structure that are induced by AVT are changes that would indicate a less aggressive call (Marler *et al.*, 1995). Despite this, AVT does not block the aggressive response of male *A. crepitans* in staged agonistic encounters (Chu *et al.*, 1999). We found no indication that AVT increased or decreased aggressive vocalizations in our frogs. This suggests that AVT is not acting at a brain site involved in choice of call type. For example, AVT treatment did not cause frogs to switch from the advertisement call type to the aggressive call type. The effects of AVT on the aggressive call of *H. versicolor* should be more extensively studied, especially in the context of staged aggressive encounters between males.

Release calls were given readily by male gray treefrogs when tactile stimulation, to simulate inappropriate clasping, was given. This was a very short and rapid call, usually arranged into bouts of calls. This call type is very common in anurans (Bogert, 1960). Despite this, the release call of this species has only been briefly described (Pierce and Ralin, 1972). We found that the release call differed significantly from the advertisement call in every parameter we measured, except the dominant frequency. Neither AVT (at this 100 μg dose) nor saline injection altered the likelihood that male *H. versicolor* would give release calls. Release calling thus differed significantly from the other two call types in that saline injection alone did not cause this call type to disappear. The release calls given by AVT-injected males differed only slightly from calls of the same animals before injection. Specifically, there was a significant decrease in call duration 90 min after AVT injection. Call rate was also increased substantially in the same animals but this was not quite significant. These changes are opposite to AVT-induced changes in advertisement call characteristics. Effects of AVT are thus very specific to call type. AVT has been previously shown to alter release calling only in ranid amphibians (Table 4). In the bullfrog, AVT inhibits release calling in females but stimulates release calling in males (Boyd, 1992). This sexual difference, plus a seasonal difference (Boyd, 1992), suggests an interaction of AVT and gonadal steroids in the control of vocalizations in bullfrogs. AVT might thus affect release calling in female gray treefrogs although we saw no effect in males. In the leopard frog, AVT inhibits release calling in both sexes (Diakow, 1978; Raimondi and Diakow, 1981). Effects of AVT on release calling in male gray treefrogs thus differs from the effect of AVT on other call types in the same species and the effects of AVT on the same call type in other species.

Although the distribution of AVT and AVT receptors has not been described for gray treefrogs, such information is available for other anurans (Moore and Lowry, 1998). In the bullfrog, for example, AVT-producing cells are found in three areas that are part of the neural vocal pathway: the amygdala, preoptic area, and pretrigeminal nucleus (Boyd, Tyler, and DeVries, 1992; Boyd, 1997). AVT fibers and receptors are found in all parts of the vocal pathway, including more posterior pulse generator and motor output areas (Boyd, 1997; Emerson and Boyd, 1999). Some parts of the vocal pathway are shared in the generation of all three call types. Motor neurons in nucleus IX–X that control laryngeal muscles and those of spinal motor nuclei that control oblique muscles of the body wall are common to all vocalizations (Emerson and Boyd, 1999). Because the effects of AVT are different for each call type, it is unlikely that AVT is acting at these motor output levels. Effects of AVT are thus more likely to be at anterior motivational levels and choice of particular call types is controlled by other neural or hormonal factors.

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