Arginine Vasotocin Injection Increases Probability of Calling in Cricket Frogs, but Causes Call Changes Characteristic of Less Aggressive Males

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Male cricket frogs, Acris crepitans communicate to males and females using advertisement calls, which are arranged into call groups. Calls at the middle and end, but not the beginning of the call group, are modified in response to male–male aggressive interactions. We found in this field study of male cricket frogs in natural breeding choruses that the peptide hormone arginine vasotocin (AVT) not only increased the probability that males called after injections, but also caused modifications in middle and end calls to produce calls characteristic of less aggressive males. Moreover, AVT-injected males showed significantly greater increases in call dominant frequency than saline-injected males, again, a characteristic of less aggressive males. Cricket frog calls are used to both repel males and attract females, thus call changes may relate to male–male and/or male–female interactions. Saline-injected males also demonstrated significant changes in several call traits, including changes that occurred in the beginning and middle calls of the call groups, but not the end calls. AVT appeared to block some call changes produced through handling. These data suggest that AVT can influence acoustic communication in frogs in several ways, including effects on call characteristics and dominant frequency, as well as potentially blocking some handling effects.

The major focus for studies examining hormonal control of reproductive behavior has classically been the sex steroids. More recently, however, an interesting role for peptide hormones has been emerging. For example, studies have examined the effects of arginine vasotocin (AVT) and its homologue vasopressin (AVP) on male social behaviors. These studies indicate that there is variation in the effects of AVP on aggression. In golden hamsters Mesocricetus auratus (Ferris, Albers, Wesolowski, Goldman, and Leeman, 1984; Albers and Ferris, 1985, 1986; Ferris, Pollack, Albers, and Leeman, 1985; Ferris, Meenan, and Albers, 1986; Ferris, Meenan, Axelsson, and Albers, 1986;
Albers, Pollack, Simmons, and Ferris, 1986; Albers, Liou, and Ferris, 1988; Ferris and Potegal, 1988; Ferris, Axelson, Martin, and Roberge, 1989) and tryon maze dull S3 rats (Koolhaas, van den Brink, Roozendaal, and Boorsma, 1990), AVP injections stimulate behaviors associated with aggression and dominance while, in contrast, in squirrel monkeys (Saimiri sciureus) and mice (Mus musculus domesticus), AVP injections cause a decrease in aggression (Roche and Leshner, 1979; Winslow and Insel, 1991). The reasons for these differences are unclear.

AVP and AVT have also been demonstrated to influence male mating behavior, but this has been studied in detail only in rough-skinned newts (Taricha granulosa) (Moore and Zoeller, 1979; Zoeller and Moore, 1982; Moore and Miller, 1983; Zoeller and Moore, 1986a,b; Zoeller and Moore, 1988; Tripp and Moore, 1988; Deviche, Propper, and Moore, 1990; Boyd and Moore, 1991; Moore, Wood, and Boyd, 1992). These newts have been an excellent model system for studying AVT control of mating behavior and its interactions with other neurochemicals. AVT in this model system modifies the expression of clasping behavior and appears to increase mating behavior by acting directly on the brain (Moore and Miller, 1983). Mating behavior in the frog Hyla cinerea and Rana catesbeiana, may also be stimulated by AVT since it increases calling behavior in males, as well as female Hyla cinerea (Penna, Capranica, and Somers, 1992; Boyd, 1994). Release calls in females of the frog Rana pipiens are inhibited by AVT (Diakow, 1978), although the physiological regulation of these functionally different call types may well differ. Further study is required to clarify the role that AVT and its homologues have in the control of aggressive and mating behavior [for AVT effects on other behaviors see Moore (1987)]. One common theme that has emerged, however, is that there are widespread sexual dimorphisms in the AVT and AVP neurochemical systems (e.g., De Vries, Buijs, and Swaab, 1981; Stoll and Voorn, 1985; van Leeuwen, Caffe, and De Vries, 1985; Voorhuis, De Kloet, and De Wied, 1991; Boyd, Tyler, and De Vries, 1992; Boyd and Moore, 1992; Viglietti-Panzica, Anselmetti, Balthazart, Aste and Panzica, 1992). The differences in AVT/AVP distribution in the brains of males and females suggest that these peptide hormones may broadly influence sexually dimorphic behaviors in several taxa including anurans.

In this field study we investigate the effects of AVT on changes in acoustic communication involved in aggression and mate attraction in the cricket frog Acris crepitans. The acoustic communication of these frogs has been studied in detail (Nevo and Capranica, 1985; Perill and Shepherd, 1989; Wagner, 1989a,b,c; Wagner, 1992; Ryan and Wilczynski, 1991; Ryan, Perrill, and Wilczynski, 1992). Male cricket frogs call to attract females, but in response to an encounter with a male conspecific will modify components of these advertisement calls, resulting in graded aggressive signals (there is no separate aggressive call) (Wagner, 1989a). The basic unit of male vocalization is the
advertisement call, which is composed of a variable number of pulses and pulse groups [see Wagner (1989a) for schematic representation of calls and call groups]. Calls, in turn, are produced in distinct call groups. The temporal and spectral characteristics of the calling behavior can vary within a call group and in response to social conditions. Calls vary over a call group in a continuum such that calls at the end of a call group are longer in duration, contain more pulses and pulse groups, and decrease in pulse rate. In aggressive calls, defined as ‘‘calls produced during wrestling contests over call sites (Wagner, 1989b),’’ the middle and end calls of the call group are longer, with more pulses and pulse groups, and the call groups are longer and produced at a lower repetition rate (Wagner 1989a) (see summary in Table 1). Because females may prefer less aggressive calls, Wagner (1989a) has proposed that the calls at the beginning of the call group are used to attract females, because these calls typically do not change in aggressive encounters. Thus, the calling behavior of cricket frogs is stereotyped, quantifiable, and varies in a predictable way with social context and aggressive state. The detailed studies by Wagner provide a solid background that allow us to compare calling behavior between males given AVT injections under natural conditions and males in aggressive encounters studied by Wagner. Analyses of these data allow us to examine in detail how AVT can influence vocal communication under natural conditions.

MATERIALS AND METHODS

Hormone Manipulations

We conducted hormone manipulations in the cricket frog Acris crepitans from April through August 1992 and April through July 1993 at a semi-permanent pond, in Travis County, Texas, used in the studies by Wagner (1989a,b,c, 1992). We observed frogs using handheld flashlights and 6-V headlamps between 2100 and 2400 h. The observer located a calling male and tape recorded his calls for a 5-min period using an MZW 415 windscreen and a Sennheiser ME 80 microphone with a K3-U power module attached to a Marantz PMD420 field recorder. The observer placed the microphone approximately 30 cm from the test animal. After the recording period males were caught by hand and given intraperitoneal injections, (using a 30-gauge needle and a 1-cc syringe) of either 20 μg AVT (Sigma Chemical Co.) diluted in 50 μl saline or an equivalent volume of saline alone (50 μl). Solutions were stored at 20°C and fresh solutions were made approximately every 2 weeks. The AVT injection levels are similar to those used by Penna et al. (1992) and preliminary studies indicated that this level of AVT resulted in behavioral changes. There were no behavioral indications that this level of AVT causes water balance problems because the frogs did not typically move toward or away from the pond after the AVT injection.
We marked males by placing a small drop of Papermate Office Products Correction Fluid on the back and then returned the frog to its calling site less than 5 min after being caught. The observer moved out of sight of the pond for a 30-min interval to increase the likelihood that the frog would resume calling. After this interval, the observer located the male and recorded the calls for a second 5-min period. The observer recaptured the males after the second recording and measured SVL to the nearest 0.1 mm and air temperature at the calling site (all frogs were on land surrounding the pond).

Call Analysis

We assessed probability of calling by noting the number of males that were observed to be calling during the post-injection recording period. For the calling males from which we obtained recordings, we analyzed call characteristics as described by Wagner (1989a). The following call group characteristics were measured: call rate (number of calls/min), call group rate (number of call groups/min), calls per call group, call group duration (s), and call rate within a call group (number of calls/call group/call duration). For call characteristics we measured three calls from each call group chosen from the beginning, middle, and end of the call group (again, calls vary qualitatively over the course of each call group). For each call we measured call duration (ms), number of pulses, number of pulse groups, pulse rate (number of pulses/call duration = pulses/ms), and dominant frequency (kHz). The spectral frequency with the most energy was defined as the dominant frequency and was determined by Fourier analysis.

We counted all calls and call groups within each 5-min recording using a Uniscan sonograph (Multigon Industries, Inc., Mt. Vernon, NY). From each 5-min recording we randomly chose six call groups for further analysis of spectral and temporal characteristics. We did not choose call groups containing one or two calls. We analyzed all other spectral and temporal characteristics using a DATA 6000 Universal Waveform Analyzer (Data Precision) at a sampling rate of 10 kHz with a frequency resolution of 13–26 Hz (depending on call duration). Call duration was measured using the Uniscan. Each variable was adjusted to 20°C using the regressions of each variable on temperature empirically determined by Wagner (1989a). The adjusted data were analyzed using the same statistical tests as those used by Wagner (1989b), except that the computer program Statistica was used. See results for specific statistical tests used. Data presented in figures are mean ± SEM.

RESULTS

To test the role of AVT in calling behavior, we injected adult males with either AVT (recordings from 15 males) or, as a control, saline solution alone (recordings from 14 males). We analyzed data in two ways to distinguish between changes induced by injection and/or handling (‘‘handling effects’’).
versus changes induced by AVT. Comparisons within treatment groups refer to differences seen before and after injection of either AVT or saline. Comparisons between treatment groups refer to differences seen when changes (post-injection minus pre-injection) in the AVT and saline groups were compared to each other. For both kinds of comparisons, call group and call characteristics (see Introduction and Materials and Methods) were analyzed. There were no significant differences (Mann–Whitney U tests, $P < 0.05$) in pre-injection levels of behavior for any call or call-group characteristics, or in dominant frequency (see Figs. 1, 3–7), although there were two nonsignificant trends ($0.05 < P < 0.10$) among two of the call traits: mean number of calls per call group ($P = 0.08$) and call duration of the end calls of the call group ($P = 0.06$). We identified two categories of AVT-induced changes. In some cases AVT appears to be blocking or compensating for the effects of handling on calling. In other cases it appears that AVT causes changes in traits that are not influenced by handling or the AVT-induced changes that occur are significantly greater than that caused by handling (although note that AVT and stress interactions cannot be ruled out here because it is possible that synergistic interactions between handling and AVT caused the changes seen in this group).

### Probability of Calling

We assessed the effect of AVT on calling probability by catching males and injecting them with either AVT or saline. Males immediately ceased calling upon capture, and, in the case of the saline-injected males, only 50% (11/23) resumed calling after being released and undisturbed for 30 min. In contrast, 78% of the AVT-injected males (21/27) resumed calling, significantly more than the saline-injected males ($X^2 = 4.84, P < 0.05$).

#### Call Group Characteristics

**Call rate.** Call rate significantly decreased in both saline-injected (Fig. 1; Wilcoxon matched-pairs signed-ranks test, $Z = 2.07, P = 0.05$) and AVT-injected males ($Z = 2.58, P = 0.01$). There was a nonsignificant trend for a greater decrease in the AVT-injected males compared to the saline-injected males (Fig. 2; Mann–Whitney U test, $U = 62, P = 0.06$). Thus handling caused a decrease in call rate, but it is not clear whether AVT has an effect differing from that of the saline injections.

**Call group rate.** There were no significant changes in call group rate in the saline-injected males (Fig. 1; $Z = 1.44, P = 0.15$), AVT-injected males ($Z = 0.52, P = 0.61$), or when the differences between the groups were compared (Fig. 2; $U = 80, P = 0.28$).

**Mean number of calls per call group.** There was a significant decrease in the mean number of calls per call group in both the saline-injected (Fig. 1; $Z = 1.9, P = 0.05$) and AVT-injected males ($Z = 2.07, P = 0.04$). However,
there was no significant difference between groups (Fig. 2; $U = 75, P = 0.19$) suggesting that the changes in both groups are a response to handling.

Call group duration. There were no significant changes in call group duration in the saline-injected males (Fig. 1; $Z = 0.80, P = 0.42$). However, in AVT-injected males there was a significant decrease in call duration ($Z = 2.07, P = 0.04$). The final difference between groups was not sufficient to reveal differences between the two groups (Fig. 2; $U = 81, P = 0.30$). Thus AVT decreases call group duration, but there are no apparent effects of handling alone.
Call rate per call group. There were no significant changes in call group rate in the saline-injected males (Fig. 1; $Z = 0.80, P = 0.42$), AVT-injected males ($Z = 1.03, P = 0.30$), or when the differences between the groups were compared (Fig. 2; $U = 79, P = 0.26$).

Call Characteristics

Call duration. There was a significant increase in call duration in the beginning and middle calls of the call group in the saline-injected males (Fig. 3; Wilcoxon matched-pairs signed-ranks test, beginning: $Z = 2.07, P = 0.05$, middle: $Z = 2.40, P = 0.02$), but not in the AVT-injected males (beginning: $Z = 0.00, P = 1.00$, middle: $Z = 1.03, P = 0.30$). In the end call of the call group the pattern was reversed or diminished, but there was only a nonsignificant decrease in call duration occurring in the AVT-injected males ($Z = 1.76, P = 0.08$) and not the saline-injected males ($Z = 1.34, P = 0.18$). This weak support for an effect of AVT on decreased call duration is strengthened when the two groups are compared. There was a significantly greater decrease in the middle and end calls of the call group in the AVT-injected males as compared to saline-injected males (Fig. 3; middle: $U = 47.5, P = 0.01$, end: $U = 58, P = 0.04$). As mentioned earlier, for the end calls there was a nonsignificant trend (Fig. 3; $U = 62, P = 0.06$) for pre-injection levels to be greater in the AVT-injected males, and this could potentially have contributed
to the differences found between the AVT- and saline-injected males. Thus handling caused an increase in call duration in the beginning and middle calls of the call group, while AVT appeared to both block or compensate for the effects of handling in the beginning and middle calls and also appeared to decrease call duration in the middle and end calls (the significant difference between groups appears to be influenced by opposite changes in the saline and AVT groups that do not cause a sufficient change within each group but do cause sufficient changes between groups).

**Number of pulses.** There were no significant changes in the number of pulses produced by the saline injections in the beginning (Fig. 4; \( Z = 1.11, P = 0.27 \)) , middle (\( Z = 0.27, P = 0.79 \)) , or end calls (\( Z = 0.29, P = 0.77 \) ). In AVT-injected males there were no significant changes in either the beginning (\( Z = -0.00, P = 1.00 \)) or end calls (\( Z = 0.27, P = 0.79 \) ), but there was a significant drop in number of pulses in the middle calls (\( Z = 2.07, P = 0.04 \) ). Support for the effect of AVT on call duration is also supported by comparisons between groups. There was no significant difference in the beginning calls of the call group (Fig. 4; \( U = 95, P = 0.66 \) ), but there was a significantly greater decrease in number of pulses produced in the AVT-injected males as compared to the saline-injected males in the middle.
calls ($U = 58.5, P = 0.04$) although not in the end calls ($U = 74, P = 0.18$). Thus AVT caused a decrease in the number of pulses per call in the middle calls, but handling alone has no effect.

**Number of pulse groups.** In the saline-injected males there was no change in number of pulse groups in the saline-injected in the beginning (Fig. 5; $Z = -0.71, P = 0.48$), middle ($Z = -0.35, P = 0.72$), or end calls ($Z = 1.06, P = 0.29$). In the AVT-injected males there were no changes in the beginning ($Z = -0.50, P = 0.62$), middle ($Z = 0.95, P = 0.34$), or end calls ($Z = 1.81, P = 0.07$). There were also no significant differences between the two groups in the beginning (Fig. 5; $U = 104.5, P = 0.98$), middle ($U = 84, P = 0.36$), and end ($U = 76.5, P = 0.21$) calls of the call group. Overall, there does not appear to be an effect of handling or AVT on the number of pulse groups.

**Pulse rate.** Saline injections caused a nonsignificant trend for a decrease in pulse rate in the beginning (Fig. 6; $Z = 1.87, P = 0.06$) and middle calls ($Z = 1.87, P = 0.06$), but no change in the end calls ($Z = 0.27, P = 0.79$). AVT injections did not cause any changes in the beginning (Fig. 6; $Z = -0.00, P = 1.00$), middle ($Z = 1.34, P = 0.18$), or end calls ($Z = 1.55, P = 0.12$). There was no significant difference between the two groups in the beginning (Fig. 6; $U = 73.5, P = 0.17$) or end calls ($U = 69.5, P = 0.12$).
but there was a significantly greater drop in the saline-injected males as compared to the AVT-injected males in the middle (Fig. 6; \( U = 49.5, P = 0.02 \)) calls. Thus it appears in this case that AVT blocked or compensated for the effect of handling, and provides weak support for AVT causing a further increase in pulse rate.

**Dominant frequency.** Saline injections had no effect on dominant frequency in the beginning (Fig. 7; \( Z = 0.27, P = 0.79 \)), middle (\( Z = -0.27, P = 0.79 \)), or end calls (\( Z = 0.29, P = 0.77 \)). In contrast, AVT injections caused a significant increase in dominant frequency in the beginning (\( Z = 2.41, P = 0.02 \)) and middle (\( Z = 2.07, P = 0.04 \)) and a nonsignificant trend for an increase in the end calls (\( Z = 1.87, P = 0.06 \)), although this difference between groups was not revealed by the between group comparison (Fig. 7; beginning: \( U = 72.0, P = 0.15 \), middle: \( U = 66, P = 0.09 \), end: \( U = 73.0, P = 0.16 \)). Thus AVT, but not handling alone, caused an increase in dominant frequency.

**DISCUSSION**

Two important findings emerge from this study demonstrating AVT-induced changes in acoustic communication. First, AVT caused male cricket
frogs to increase their probability of calling after injection under natural conditions, although whether or not AVT is blocking or interacting with stress effects in this case is unclear. Other anuran species, the treefrog *Hyla cinerea* (Penna et al., 1992) and the bullfrog *Rana catesbeiana* (Boyd, 1994), housed under laboratory conditions can also be stimulated to call when injected with AVT and exposed to playbacks of advertisement calls. Here we also demonstrate, using a more detailed analysis, that the calls produced after these AVT injections are characteristic of less aggressive males. There was a decrease in call group duration and the calls were characterized by decreased call duration and number of pulses, as well as an increase in pulse rate and dominant frequency (for summary see Table 1). Thus AVT can cause changes characteristic of less aggressive calls when males are in a chorus (but not interacting directly with another male). It is also interesting to note that AVT selectively influenced the parts of the call group (middle and end) that typically change in male–male encounters. In addition, AVT did not result in a change in the number of pulse groups, which also do not influence a male’s response to an opponent’s call, even though they do change during an encounter (Wagner, 1991). Wagner (1989a) found considerable individual variation in dominant frequency in male cricket frogs and an ability to actively alter
their dominant frequency (Wagner, 1989b, 1992). Thus here we present evidence that one of the physiological factors potentially influencing this behavioral variation is AVT.

It is also possible that these calls were more attractive to females, although this possibility remains speculative. Females prefer calls with fewer pulses and more pulse groups (Wagner, 1991). While AVT did not influence number of pulse groups, it did cause a significant decrease in number of pulses. The effect of AVT on dominant frequency is more difficult to evaluate. Females prefer calls with a lower dominant frequency (Ryan et al., 1992). However, Wagner (1991) has found that an increase in dominant frequency occurs with an increase in call amplitude in this species, which would increase the call active space (Brenowitz, 1982, 1986) and could therefore potentially increase the probability of mating by attracting females from a larger area. The increased probability of calling after an injection is also consistent with an increased emphasis on attracting females. Further studies need to be performed to confirm that AVT-influenced calls are more attractive to females.

The changes in call traits observed with AVT administration, that is, calls characteristic of less aggressive males, are consistent with the effects of AVP (note that these mammalian studies used AVP, not AVT) on aggression in
TABLE 1
Summary of Effects of (a) Handling and Injecting Saline (Saline Injections), (b) AVT (Effects Separate from Blocking or Compensating for Effects of Saline Injections), (c) AVT Blocking or Compensating for Handling Effects, (d) Natural Encounters (Wagner, 1989a), and (e) Effects of Playbacks of Male Calls (Wagner, 1989b). AVT Effects Separate from Blocking or Compensating for Effects of Handling Are Defined as Those Causing Either a Difference between the Two Groups and/or Changes within the AVT Group That Did Not Occur in the Saline-Injected Group. NC Means That No Changes in the Trait Were Caused by This Factor.

<table>
<thead>
<tr>
<th>Call trait</th>
<th>Handling</th>
<th>AVT</th>
<th>Natural encounter</th>
<th>Playback</th>
</tr>
</thead>
<tbody>
<tr>
<td>Call rate</td>
<td>↓</td>
<td>NC*</td>
<td>NC</td>
<td>↓</td>
</tr>
<tr>
<td>Call-group rate</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>↓</td>
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<tr>
<td>Calls per call group</td>
<td>↓</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>Call-group duration</td>
<td>NC</td>
<td>↓</td>
<td>NC</td>
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<tr>
<td>Call rate within call group</td>
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<td>Call rate within call group</td>
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<tr>
<td>Call duration</td>
<td>↑&lt;sub&gt;B&lt;/sub&gt;, ↑&lt;sub&gt;M&lt;/sub&gt;</td>
<td>↓&lt;sub&gt;M&lt;/sub&gt;, ↑&lt;sub&gt;E&lt;/sub&gt;</td>
<td>↑&lt;sub&gt;B&lt;/sub&gt;, ↑&lt;sub&gt;M&lt;/sub&gt;</td>
<td>↑&lt;sub&gt;B&lt;/sub&gt;, ↑&lt;sub&gt;M&lt;/sub&gt;, ↑&lt;sub&gt;E&lt;/sub&gt;</td>
</tr>
<tr>
<td>Number of pulses</td>
<td>NC</td>
<td>↓&lt;sub&gt;M&lt;/sub&gt;</td>
<td>NC</td>
<td>↑&lt;sub&gt;M&lt;/sub&gt;, ↑&lt;sub&gt;E&lt;/sub&gt;</td>
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<td>Number of pulse groups</td>
<td>NC</td>
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<td>NC</td>
<td>↑&lt;sub&gt;B&lt;/sub&gt;, ↑&lt;sub&gt;M&lt;/sub&gt;, ↑&lt;sub&gt;E&lt;/sub&gt;</td>
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<tr>
<td>Pulse rate</td>
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<td>↑&lt;sub&gt;M&lt;/sub&gt;</td>
<td>↑&lt;sub&gt;B&lt;/sub&gt;, ↑&lt;sub&gt;M&lt;/sub&gt;</td>
<td>↓&lt;sub&gt;M&lt;/sub&gt;, ↑&lt;sub&gt;E&lt;/sub&gt;</td>
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<tr>
<td>Dominant frequency</td>
<td>NC</td>
<td>↑</td>
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* Note that P value was 0.06 for a difference between the two groups.
arginine vasotocin and calling

To begin testing whether AVT has a general depressant effect on aggression in cricket frogs or whether or not it is context dependent, we are currently manipulating the aggressive context under which males receive AVT.

AVT also appeared to either block or compensate for some of the effects caused by handling and injecting such as the increase in call duration and decrease in pulse rate that occur in the beginning and middle calls of the call group (see Table 1; note also that the changes caused by AVT in this category were also in the direction of decreased aggression). This response could either be a result of AVT blocking stress effects or blocking an increase in aggression after being temporarily removed from the calling site (for less than 5 min). AVT and AVP are not typically thought to block stress. AVT injections increase corticosterone in the frogs *Xenopus laevis* (Kloas and Hanke, 1990) and *Rana temporia* (Hanke and Maser, 1985), a response similar to that found in mammals when given artificially increased levels of AVP (e.g., Gillies, Linton, and Lowry, 1982). However, the effect of AVP on behavioral stress responses can have varied results depending on whether or not an animal is resting or being stressed (e.g., Buwalda, Nyakas, Koolhaas, and Bohus, 1993). That is, there is evidence that AVP may actually suppress stress-related changes. The other potential interpretation is that AVT was blocking a temporary increase in aggression after being returned to the calling site. Further studies will be necessary to examine these two possibilities.

Whether the site of action for the behavioral effects of AVT that we describe is in the brain or peripheral target tissues is not known for this species. Moore and Miller (1983), however, demonstrated that AVT acts on target tissues in the brain and that peripheral and intracranial AVT injections in rough-skinned newts cause the same qualitative changes in mating behavior. To our knowledge, similar studies have not been performed with frogs and we cannot rule out the possibility that intracranial injections could have a different effect on calling behavior.

It is worth noting that handling and injecting had effects on calling behavior, suggesting that stress can cause changes in the calling behavior. Since all males were calling before being injected, but only 50% were calling after the saline injection, the handling obviously decreased the probability of calling [the stress hormone corticosterone can also decrease the probability of calling in the tungara frog *Physalaemus pustulosus* (Marler, Ryan, and Wilczynski, 1991)]. In this case, however, the behavioral changes induced by handling are more complex than simply calling or not calling. In males that did call, handling appeared to influence some call (call duration, and pulse rate) and call group (call rate and number of calls per call group) characteristics. The changes that occurred varied with respect to the aggressive nature of the calls. The decreases in call rate, the increase in call duration, and decrease in pulse rate were characteristic of more aggressive males. In contrast, the decrease
in number of calls per call group is characteristic of less aggressive males. Because it is unknown which of the handling factors influenced these changes in calling behavior it is difficult to interpret. Two major possibilities are (1) that the changes are a response to stress or (2) that since all males stopped calling for at least a short period of time, the change in calling behavior is a response to reestablishing male–male spacing.

In summary, AVT not only increases the probability that a male will call, but it also appears to have separate effects on the aggressive nature of calls in call components typically receptive to social factors. Specifically, it causes changes in call characteristics that are similar to those of less aggressive males but may also be making the calls more attractive to females. Apart from the effects of AVT on aggression, AVT may also block or compensate for some of the effects of handling.

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