

The Effects of Arginine Vasotocin on the Calling Behavior of Male Cricket Frogs in Changing Social Contexts

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We investigated the effects of the neurohypophysial peptide, arginine vasotocin (AVT), on the calling behavior of male *Acris crepitans* during and immediately following a simulated acoustic agonistic encounter. AVT did not block the aggressive response to agonistic calls, as the changes in temporal call characteristics in response to the encounter were similar to those of saline-treated males. However, AVT caused males to begin calling sooner during the agonistic encounter and to call significantly more than saline males during and after the agonistic encounter. In addition, AVT-treated males maintained a higher dominant frequency compared to saline animals during and following the agonistic encounter. Changes in temporal characteristics in the period following the agonistic encounter indicated that control males were more likely to exhibit a rebound effect which resulted in larger changes in calling parameters compared to AVT-treated animals. The results indicate that AVT causes changes in calling behavior in male *A. crepitans* during and following an agonistic encounter that are consistent with animals highly motivated to maintain vigorous active calling throughout changing social conditions. © 1998 Academic Press

The behavioral significance of the neuropeptide arginine vasotocin (AVT) and its mammalian homologue, arginine vasopressin (AVP), has been investigated in many vertebrate taxa. AVT and AVP have been implicated in the control of many behaviors including aggression (reviewed in Ferris and Delville, 1994), pair bonding (Winslow *et al.*, 1993; Insel *et al.*, 1994; reviewed in Carter *et al.*, 1995), learning and

memory (reviewed in Bohus *et al.*, 1993), and selective attention (Bunsey *et al.*, 1990; Bunsey and Strupp, 1990; Strupp *et al.*, 1990). In particular, AVT and AVP have been implicated in regulating male reproductive behavior and aggressive behavior in several taxa including rodents (Everts *et al.*, 1997; Delville *et al.*, 1996; Ferris *et al.*, 1984), primates (Winslow and Insel, 1991), birds (Voorhuis *et al.*, 1991; Viglietti-Panzica *et al.*, 1992; Ritters and Panksepp, 1997), and fish (Wilhelmi *et al.*, 1955; Pickford and Strecker, 1977). AVT increases amplexic clasping in *Taricha granulosa*, the rough skinned newt (reviewed in Moore, 1987, 1992), and evokes calling in four species of frogs, *Hyla cinerea*, *Rana catesbeiana*, *Acris crepitans*, and *Bufo cognatus* (Penna *et al.*, 1985; Boyd, 1994; Marler *et al.*, 1995; Propper and Dixon, 1997). AVT also influences release calling in male and female leopard frogs (Diakow, 1978; McClelland and Wilczynski, 1986). In our previous study, we reported that AVT increases the probability of calling (compared to saline-treated males) in male cricket frogs (Marler *et al.*, 1995). In addition, AVT treatment made their calling characteristics more similar to calls of less aggressive males.

The communication system of male cricket frogs, *A. crepitans*, has been thoroughly investigated (Nevo and Capranica, 1985; Perill and Shepard, 1989; Wagner, 1989a,b,c, 1991, 1992; Ryan and Wilczynski, 1991; Ryan *et al.*, 1992). As with most anuran amphibians, calling behavior in males serves both inter- and intra-sexual communication. Male *A. crepitans* call from established calling sites during the breeding season in order to attract mates or to repel intruding males. *A. crepitans* calls consist of short pulsatile calls grouped together to form call groups (Fig. 1). The spectral and temporal characteristics of the calls are altered in a

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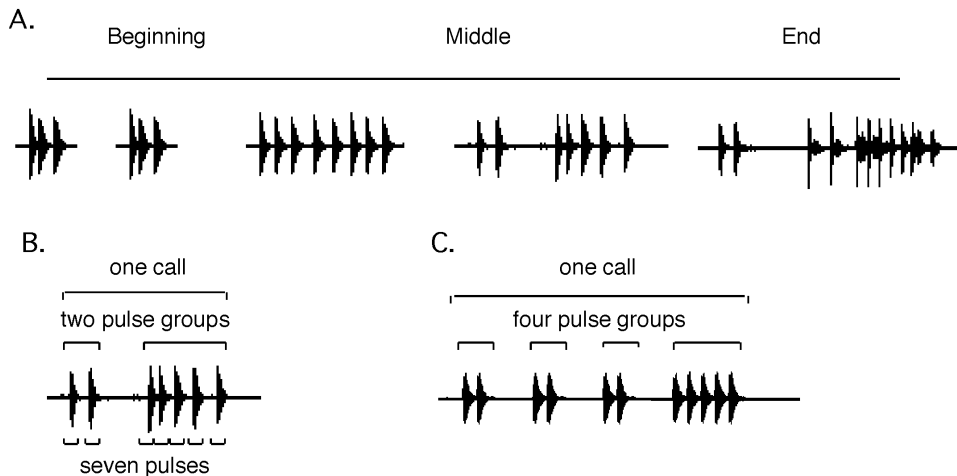


FIG. 1. A waveform of a representative call group. Call characteristics of individual calls differ quantitatively in beginning, middle, and end of the call group (A). An example of an individual call divided into pulse groups and pulses (B). The waveform of the digitized stimulus call used in the playback experiments (C).

graded manner depending upon social context (Wagner, 1989a,b,c). Aggressive changes include an increased call duration, increased number of pulses and pulse groups per call, and decreased pulse repetition rate within the call. Calls located in the middle and end of the call group tend to undergo more dramatic change than calls located in the beginning of the call group. Other changes in temporal characteristics include a decrease in the rate that both calls and call groups are produced and an increase in the duration of call groups. The dominant frequency of the call is also significantly lowered during agonistic interactions.

In our previous report (Marler *et al.*, 1995), we demonstrated that AVT administration increased calling in male *A. crepitans* and altered both spectral and temporal characteristics of the calls. Calls located in the middle and end portions of the call group underwent the most change compared to animals treated with a saline injection. AVT-treated animals exhibited reduced duration of calls located in the middle and end of the call group. Calls from the middle of the call group also exhibited a decreased number of pulses and an increased pulse rate. AVT-treated animals also significantly increased the dominant frequency of their calls. All of these changes are characteristic of less aggressive males (Wagner, 1989a,b).

Animals in the previous study were randomly selected from, and tested within, their breeding chorus and allowed to produce calls naturally. Although this procedure allowed a clear demonstration of AVT's effects under natural conditions, it did not allow us to

investigate if AVT's effects were specific to a particular behavioral context or to test several possibilities as to why AVT was changing the calls in the manner it did.

In this current study, we assessed whether the effect of AVT on male *A. crepitans* calling behavior was context-specific by examining its effect on calling males during and after a simulated agonistic challenge. This procedure allowed us to assess three possible reasons for the effects of AVT seen in previous work.

1. AVT may increase selective attention to surrounding social stimulation. Bunsey *et al.* (1990) showed that AVT appears to increase selective attention to relevant stimuli in rats. In this scenario, AVT may be causing males to become more reactive to changing social stimuli, in which case AVT-treated males in natural advertisement choruses would produce more calls, and call less aggressively, as we found in the previous study, but would call more aggressively than controls in an agonistic encounter.

2. AVT may inhibit aggressive responding. AVT treatment of male cricket frogs in a natural chorus resulted in males calling more frequently, but less aggressively (Marler *et al.*, 1990). These males may have appeared less aggressive than controls because control animals were responding to subtle cues from neighbors in the chorus that hormone-treated animals were not. Under this scenario, AVT-treated males in an simulated agonistic encounter would not change their calls, while control animals would respond in a normal aggressive manner.

3. AVT may specifically motivate advertisement calling. In this scenario, the ability to produce an aggressive response would not be blocked. However, the tendency to respond aggressively would be countered by the motivation to maintain vigorous advertisement calling. The result would be less of a change in calling characteristics that normally decrease during an aggressive encounter, such as call rate, call group rate, pulse rate, and dominant frequency (for which a decrease corresponds with lowered call amplitude; Bee and Perrill, 1996; Lopez *et al.*, 1988; R. Dudley, personal communication). Calling characteristics that typically increase during an aggressive encounter, such as call duration and number of pulses, would not be affected. In addition, AVT-treated animals would recover faster from an agonistic encounter to resume vigorous advertisement calling.

To examine these possibilities, we documented the response of calling male cricket frogs under a simulated agonistic encounter, and immediately after the cessation of that simulated encounter. We examined the effects of AVT treatment on the vocal responses of these males during both social conditions. Our statistical analysis asked two questions: (1) How do both AVT-treated and control animals change their calling behavior during both social conditions? and (2) How do AVT-treated and control animals differ in the way they change their calling behavior?

MATERIALS AND METHODS

Experimental Design

All animals tested were located in a permanent creek in McKinney Falls State Park in Austin, Texas, from May 5 through May 31, 1994. Individual males were located using headlamps and hand-held flashlights and randomly selected from an active chorus. Calls from each subject were tape recorded using a Marantz PMD420 field recorder, and a Sennheiser ME80 directional microphone with a K3-U power module and a MZW 415 windscreen.

The animals' baseline calling behavior was recorded for 5 min. Animals were then caught by hand and given an injection of either 50 μ l of 400 μ g/ml AVT, ip (Sigma Chemical Co., St. Louis, MO) ($N = 13$), or an equivalent volume of saline ($N = 14$). This volume resulted in the equivalent dosage of 10 μ g/g body weight (each subject used weighed approximately 2 g). Our previous study indicated that this dose elicited changes in calling behavior within the normal

range of variation. This dose also is within the range of doses used in other studies examining AVT's effects on amphibian reproductive behavior (Penna *et al.*, 1985; Boyd, 1994; Marler *et al.*, 1995; Propper and Dixon, 1997). One saline-treated male did not call under the playback condition, and two animals under both AVT and saline conditions did not call under the post-playback condition. These subjects were not included in the analyses of their respective conditions. Following the injection each male was marked on the back with a small amount of white liquid correction fluid and returned to his original calling site. The animals were left undisturbed for 30 min, after which the experimenter returned and located the subject.

The recording microphone and small broadcasting speaker (Model No. SME SC-A9, Saul Mineroff Electronics, Elmont NY) were placed 15 cm in front of the subject. A call stimulus was broadcast for 5 min at 90–100 dB sound pressure level (SPL), while calls from the subject male were recorded. SPL was measured at the location of the subject male. The call stimulus consisted of a digitized call 112 ms in duration, consisting of 11 pulses divided into 4 pulse groups, with a pulse repetition rate of 0.1 pulses per second. This call was arranged into a call group consisting of 15 calls, broadcast at a rate of 120 calls/minute. Each call group lasted 2.9 s. Previous studies by Wagner (1989b) indicated that this stimulus simulated the calls of an aggressive male. Dominant frequency was set at the population average of 3.70 kHz. This stimulus was broadcast at a rate of 8 call groups/minute during the 5-min playback interval while the subject male's calls were recorded.

After 5 min, the call stimulus was discontinued. Calls from the subject male were then recorded for an additional 5 min. Animals were recaptured and snout-vent length was measured with calipers. Air and water temperature in the immediate area of each subject was recorded and sound pressure level of the call stimulus was checked using a hand-held sound level meter (Realistic, Radio Shack).

All experimental procedures were performed in accord with NIH standards of animal use and approved by the University of Texas IACUC.

Call Analysis

All call variables measured were based on original descriptions by Wagner (1989a). Call parameters were divided into three qualitatively distinct groups for analysis: Calling Rate Characteristics, Call Group Characteristics, and Call Characteristics. Calling rate

characteristics consist of call rate (number of calls/minute) and call group rate (number of call groups/minute). Call group characteristics consist of call group duration (s), mean number of calls per call group, and call rate within call group (number of calls/call group/call group duration). Call characteristics consist of call duration (s), number of pulses, number of pulse groups, and pulse rate (pulses/s). Call characteristics were determined for calls from the beginning, middle, and end of a call group. In addition, the dominant spectral frequency (the frequency with the most energy) was determined for all calls used in call characteristic measurements. The latency to begin calling during the playback interval was also measured.

All calls and call groups were counted using a Uniscan sonograph (Multigon Industries, Mt. Vernon, NY). All temporal and spectral analyses were performed using either a DATA 6100 universal waveform analyzer (Data Precision, Peabody, MA), or using SIGNAL (Engineering Design, Belmont, MA) for IBM. Calls analyzed using SIGNAL were initially digitized using SoundEdit (Macromedia, San Francisco, CA) on a Macintosh PowerPC. Call analysis obtained using the waveform analyzer was based on six call groups sampled from each 5-min recording interval. Call analysis performed using SIGNAL was based on 10 call groups per recording interval.

For each group of variables, two general analyses were made for both social conditions (playback and post-playback). To measure changes within each treatment group, statistical comparisons were made comparing baseline to playback and post playback calling behavior using multiple analysis of variance (MANOVA). Follow-up ANOVAs were performed to examine changes of individual variables. To compare the effects of hormone treatment on changes in calling behavior, statistical comparisons of the two hormone conditions were performed for both social conditions using data that were calculated as a relative change from baseline (baseline values subtracted from playback values). The effects of hormone treatment on all calling variables were determined for the playback and post-playback conditions using MANOVA for the three groups of calling characteristics. Follow-up ANOVAs were performed on each individual variable. The strength of using a multivariate test in this experimental design is that it allows for the statistical detection of overall changes in related groups of variables that may not be evident when using simpler direct comparisons.

All statistical tests were performed using SPSS*

(SPSS Inc., Chicago, IL) for Macintosh. All values reported in the text and graphs are means \pm SEM.

RESULTS

Playback Condition

Call latency. Animals receiving AVT showed significantly shorter latencies to begin calling compared to saline-treated animals ($F(1, 24) = 20.21, P < 0.001$). AVT-treated animals began calling 19.58 ± 6.63 s following the onset of the playback stimulus. In contrast, call latency for saline-treated males was 148.14 ± 6.63 s.

Calling rate variables: Change with respect to social condition. MANOVA for each hormone condition indicated both AVT- and saline-treated groups altered calling rate variables relative to baseline (AVT, $F(2, 11) = 20.09, P < 0.001$; saline, $F(2, 13) = 60.95, P < 0.001$). Follow-up ANOVAs indicated that AVT and saline-treated animals significantly decreased both call rate (AVT, $F(1, 12) = 38.15, P < 0.001$; saline, $F(1, 14) = 60.41, P < 0.001$) and call group rate (AVT, $F(1, 12) = 10.58, P = 0.007$; saline, $F(1, 14) = 104.4, P < 0.001$) during the interaction time period (Figs. 2A and 2B).

Change with respect to hormone treatment. Overall MANOVA for hormone treatment was not significant for calling rate characteristics under the playback condition (Figs. 3A and 3B).

Call group characteristics: Change with respect to social condition. MANOVAs under each hormone condition indicated that for saline-injected animals, Call Group Characteristics overall differed significantly from baseline calling during the interaction time period ($F(3, 11) = 5.44, P = 0.015$). MANOVA for AVT-treated animals was not significant. Follow-up ANOVAs for individual variables indicated that call group duration significantly increased in saline- ($F(1, 13) = 16.89, P = 0.001$) and AVT-treated males ($F(1, 12) = 6.97, P = 0.022$), compared to baseline (Figs. 4A–4C).

Change with respect to hormone treatment. Overall MANOVA for hormone treatment for Call Group Characteristics was not significant under the playback condition.

Call characteristics: Change with respect to social condition. MANOVAs and follow-up ANOVAs under each hormone condition indicated significant interaction effects between time period and call position. How cricket frog call characteristics varied depended strongly upon the location of the call within the call

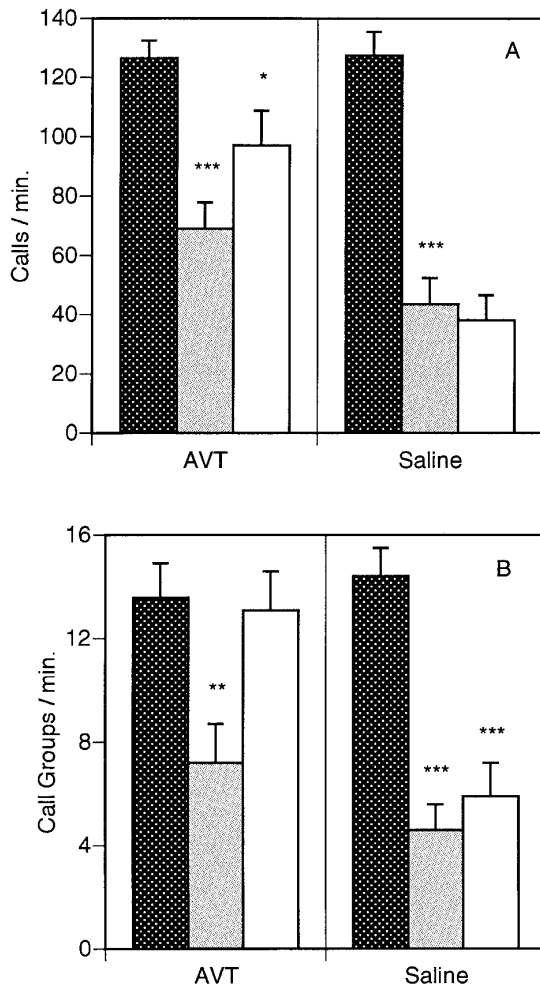


FIG. 2. Calling rate variables in each time interval for AVT and saline-treated animals: baseline (dark stipple), playback (light stipple), and post-playback (white). Calling rate variables include (A) calling rate and (B) call group rate. All statistical differences were comparisons to baseline levels of calling. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

group. Overall MANOVA for call characteristics for saline-treated animals indicated a significant time by call position interaction ($F(8, 6) = 12.59$, $P = 0.003$). The same interaction effect for AVT-treated animals was not significant. Follow-up ANOVAs indicated that call duration increased in animals responding to the playback recording (Fig. 5A). AVT-treated animals increased call duration in calls from the beginning ($F(1, 12) = 8.02$, $P = 0.015$) and middle ($F(1, 12) = 7.53$, $P = 0.018$) of the call group. Saline-treated animals increased call duration significantly in the middle ($F(1, 13) = 41.89$, $P < 0.001$) and end ($F(1, 13) = 10.61$, $P = 0.006$) of the call group.

The number of pulses in a call also increased during the playback encounter (Fig. 5B). AVT- and saline-treated animals increased the number of pulses in calls from the middle (AVT, $F(1, 12) = 12.93$, $P = 0.004$; saline, $F(1, 13) = 40.56$, $P < 0.001$) and end of the call group (AVT, $F(1, 12) = 14.34$, $P = 0.003$; saline, ($F(1, 13) = 11.12$, $P = 0.005$). The number of pulse groups in each call also increased during the playback encounter (Fig. 5C). AVT- and saline-treated animals increased the number of pulse groups per call in the beginning (AVT, $F(1, 12) = 12.12$, $P = 0.005$; saline, $F(1, 13) = 8.98$, $P = 0.01$) and the middle (AVT, $F(1, 12) = 6.29$, $P = 0.028$; saline, $F(1, 13) = 15.67$, $P = 0.002$) of the call group.

Pulse rate decreased during the playback interval (Fig. 5D). Overall changes in pulse rate were significant for AVT- and saline-treated animals for the time period by call position interaction (AVT, $F(2, 11) = 6.78$, $P = 0.012$; saline, $F(2, 12) = 9.13$, $P = 0.004$), indicating that the degree to which pulse rate decreased depended upon where the call was located within the call group. Follow-up tests for AVT-treated animals indicated that no specific call position varied significantly. Saline-treated animals significantly decreased the pulse rate in calls from the middle of the call group ($F(1, 13) = 11.5$, $P = 0.005$).

Change with respect to hormone treatment. Overall MANOVA for hormone treatment was not significant under the playback condition. Follow-up ANOVA indicated that AVT-treated animals produced pulse rates that were significantly higher than saline animals in the end of the call group ($F(1, 25) = 5.41$, $P = 0.03$) (figure not shown).

Dominant frequency: Change with respect to social condition. The overall MANOVA for comparing the dominant frequency under the playback condition to the baseline condition indicated a significant decrease in dominant frequency in saline-injected control animals ($F(1, 13) = 15.78$, $P = 0.002$). In comparison, AVT-treated animals exhibited little change from baseline calling (baseline, 3.67 ± 0.05 kHz; playback, 3.68 ± 0.04 kHz). Follow-up tests for saline-treated animals indicated that dominant frequency was significantly lowered for calls located in the beginning ($F(1, 13) = 13.92$, $P = 0.003$), middle ($F(1, 13) = 13.57$, $P = 0.003$), and end ($F(1, 13) = 14.18$, $P = 0.002$) of the call group (Fig. 7).

Change with respect to hormone treatment. Overall MANOVA for hormone treatment was significant ($F(1, 24) = 10.14$, $P = 0.004$). Follow-up ANOVAs indicated that saline-treated animals lower the dominant frequency of their calls significantly compared to

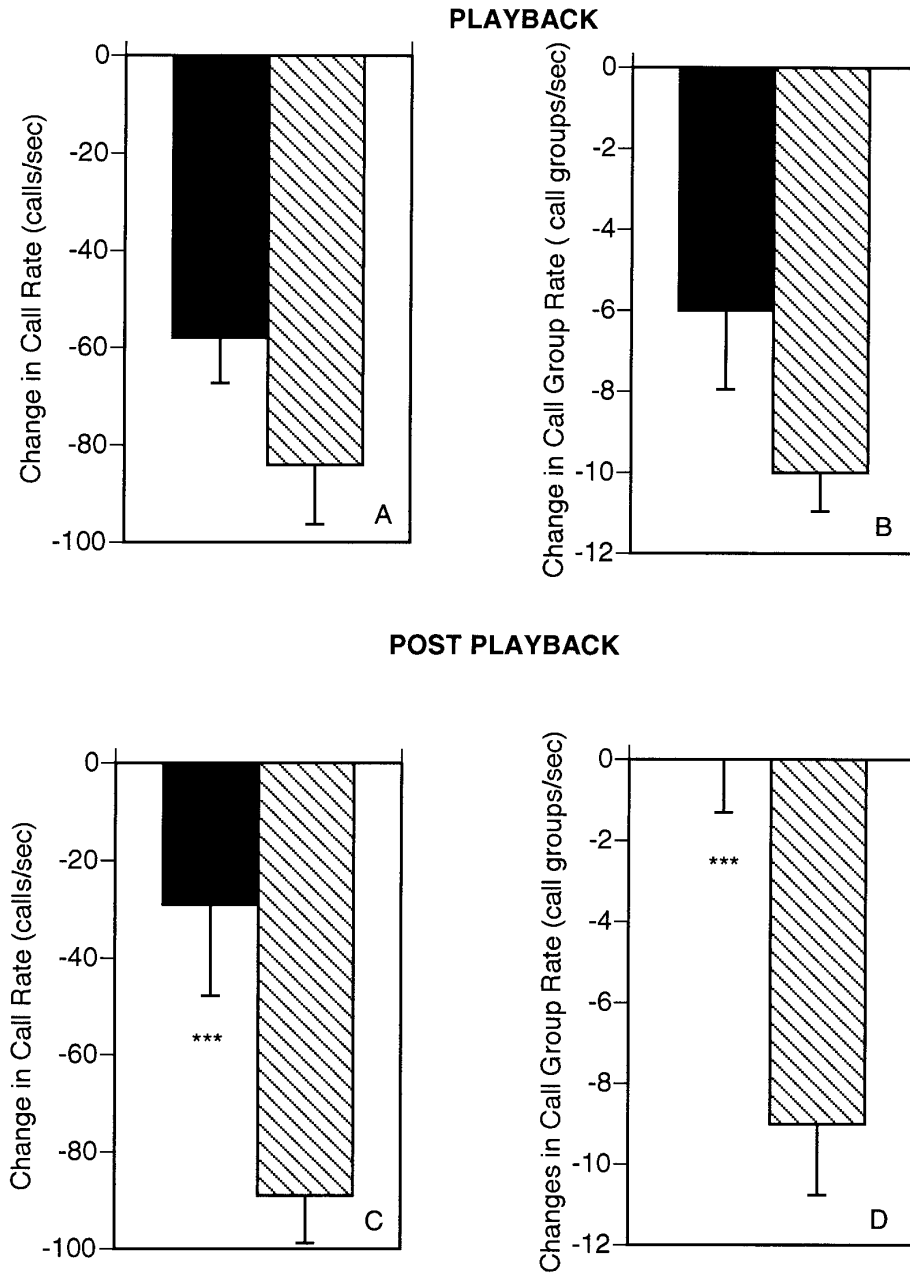


FIG. 3. Changes in calling rate variables during playback (A and B) and post-playback (C and D) intervals for AVT (solid bar) and saline (hatched bar)-treated animals. Characteristics include call rate (A and C) and call group rate (B and D). *** $P < 0.001$.

AVT-treated animals. This difference was significant for calls from the beginning ($F(1, 24) = 8.04, P = 0.009$), middle ($F(1, 24) = 9.34, P = 0.005$), and end ($F(1, 24) = 9.01, P = 0.006$) of the call group (Fig. 8A).

Post-playback Condition

Changes in cricket frog calling during the interval immediately following an agonistic encounter have not

previously been examined. These changes indicate what lingering effects previous social encounters have on calling and whether hormonal state influences such effects.

Calling rate characteristics: Change with respect to social condition. Calling rate characteristics were significantly depressed for saline-injected animals during the period following the playback encounter ($F(2, 13) = 40.00, P < 0.001$). In saline-treated males,

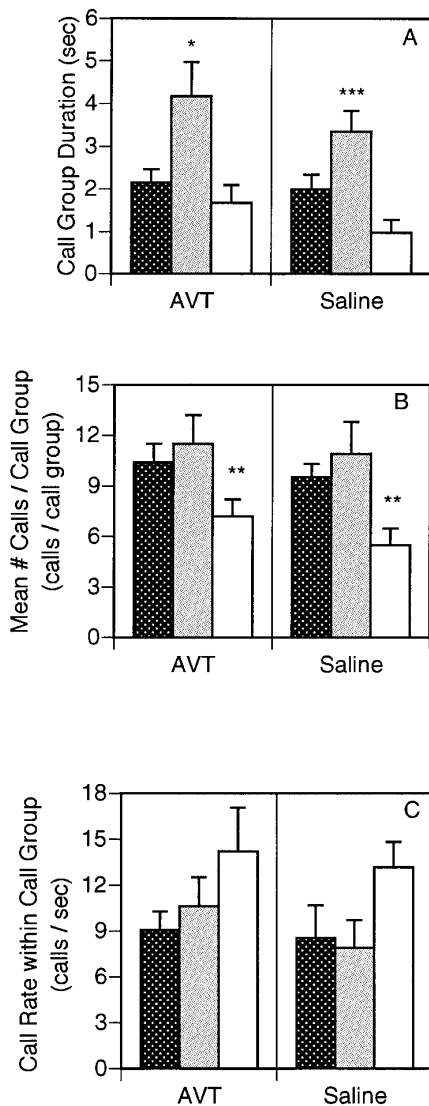


FIG. 4. Call group variables for each time interval: baseline (dark stipple), playback (light stipple), and post-playback (white). Call group variables include (A) call group duration, (B) mean number of calls per call groups, and (C) call rate within call group. All statistical differences are comparisons to baseline levels of calling. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

both call rate ($F(1, 14) = 83.47$, $P < 0.001$) and call group rate remained depressed ($F(1, 14) = 23.41$, $P < 0.001$) relative to baseline calling. In comparison, the overall calling rate characteristics of AVT-treated animals were not different from baseline. Follow-up analyses on specific call variables indicate that AVT-treated males maintained lower than baseline calling rates ($F(1, 12) = 5.73$, $P = 0.03$), but the call group rate was not statistically different from baseline values

(baseline, 13.6 ± 1.13 call groups/min; post-playback, 13.1 ± 1.5 call groups/min) (Figs. 2A and 2B).

Change with respect to hormone treatment. Overall MANOVA for hormone treatment was significant during the time interval following playback ($F(2, 25) = 9.59$, $P = 0.01$). Calling rate ($F(1, 26) = 14.75$, $P = 0.001$) and call group rate ($F(1, 26) = 12.84$, $P = 0.001$) were significantly higher in AVT-treated animals compared to saline-treated animals (Figs. 3C and 3D).

Call group characteristics: Change with respect to social condition. MANOVA indicated a significant residual effect of the agonistic encounter under the post-playback condition in both AVT- ($F(3, 9) = 5.78$, $P = 0.02$) and saline-treated ($F(3, 10) = 3.75$, $P = 0.049$) animals for call group characteristics. Follow-up ANOVA's indicated that the mean number of calls per call group was lower compared to baseline in both AVT- ($F(1, 12) = 9.93$, $P = 0.008$), and saline-treated animals ($F(1, 14) = 11.76$, $P = 0.004$) (Figs. 4A–4C).

Change with respect to hormone treatment. Overall MANOVA for hormone treatment was not significant during the interval following the playback.

Call variables: Change with respect to social condition. MANOVAs indicated only AVT-treated animals produced call characteristics that were statistically different during the post-playback interval compared to baseline values (AVT, $F(4, 8) = 3.91$, $P = 0.048$) (Figs. 5A–5D). Follow-up tests indicate that the change in call duration was significant in both groups depending on call position (AVT, $F(2, 10) = 9.30$, $P = 0.005$; saline, $F(2, 8) = 16.31$, $P = 0.002$), although the effect was not significant for any particular call position (Fig. 5A).

The number of pulses in each call also differed according to call position (AVT, ($F(2, 10) = 6.36$, $P = 0.016$; saline, $F(2, 8) = 8.35$, $P = 0.011$), but not for any particular call position (Fig. 5B). AVT animals significantly differed in the number of pulse groups produced ($F(1, 11) = 8.29$, $P = 0.015$) (Fig. 5C). Saline animals did not exhibit a significant overall effect for this variable, but the interaction between social condition and call position was significant ($F(2, 8) = 9.80$, $P = 0.007$) (Fig. 5C).

Pulse rate for both AVT- and saline-treated animals indicated a significant social condition by call position interaction (AVT, $F(2, 10) = 7.64$, $P = 0.04$; saline, ($F(2, 8) = 8.44$, $P = 0.011$), although there were no statistical differences for any particular call position (Fig. 5D).

Change with respect to hormone treatment. Overall MANOVA for hormone treatment was significant ($F(4, 17) = 3.40$, $P = 0.03$). Follow-up ANOVAs indicated that individual tests for specific call characteris-

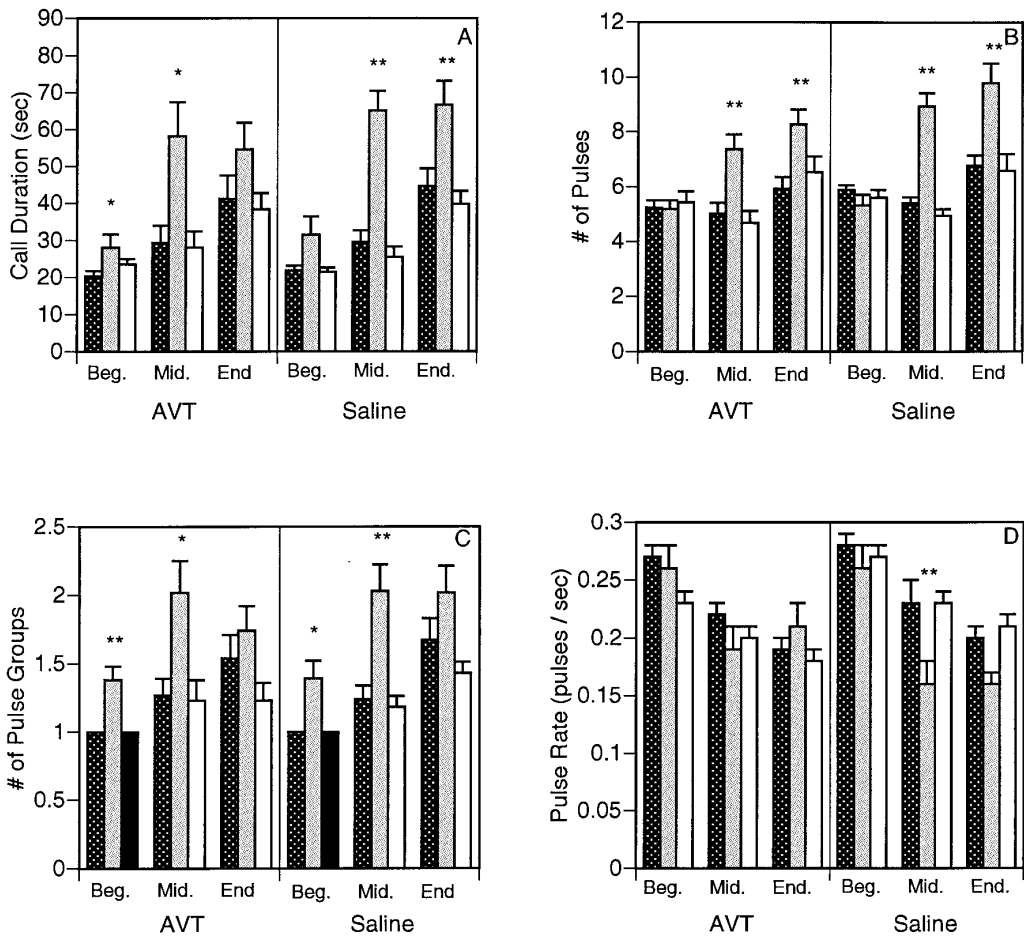


FIG. 5. Call variables in each time interval for AVT and saline-treated animals: baseline (dark stipple), playback (light stipple), and post-playback (white). Call variables include (A) call duration, (B) number of pulses, (C) number of pulse groups, and (D) pulse rate. Calls from the beginning (Beg), middle (Mid), and end (End) of the call group were analyzed. Statistical differences are comparisons to baseline levels of calling. ** $P < 0.01$, * $P < 0.05$. Post-play interaction effects not noted on graph: call duration \times call position, $P < 0.01$ (both AVT and saline); No. pulses \times call position, $P < 0.05$ (both AVT and saline); No. pulse groups, $P < 0.05$ (AVT); No. pulse groups \times call position, $P < 0.05$ (saline); pulse rate \times call position, $P < 0.05$ (AVT and saline).

tics did not differ significantly with treatment (Fig. 6). Although the individual tests did not result in statistically significant differences between drug treatments, it is important to bear in mind that the strength of the MANOVA test lies in its ability to detect overall changes in the data set as a whole. Therefore a significant overall MANOVA value indicates that the overall pattern of call variables differed between drug treatments. No subjects altered the number of pulse groups in the beginning call (number of pulse groups = 1); therefore this parameter was not included in the multivariate analysis.

Dominant frequency: Change with respect to social condition. Differences in the dominant frequency of calls produced in the postcounter were not signifi-

cant for either AVT- or saline-treated animals. Follow-up ANOVAs for AVT animals indicated a non-significant trend toward increases in dominant frequency for calls from the middle ($F(1, 10) = 4.33$, $P = 0.06$).

Change with respect to hormone treatment. Overall MANOVA for hormone treatment was not significant. However, follow-up ANOVAs indicated a significant hormone effect on the dominant frequency of calls located in the middle of the call group ($F(1, 21) = 4.70$, $P = 0.04$) (Fig. 8B).

Relative change between the playback and post playback conditions. Relative changes in all call variables under playback and post-playback conditions are summarized in Table 1. Wilcoxon matched-pairs

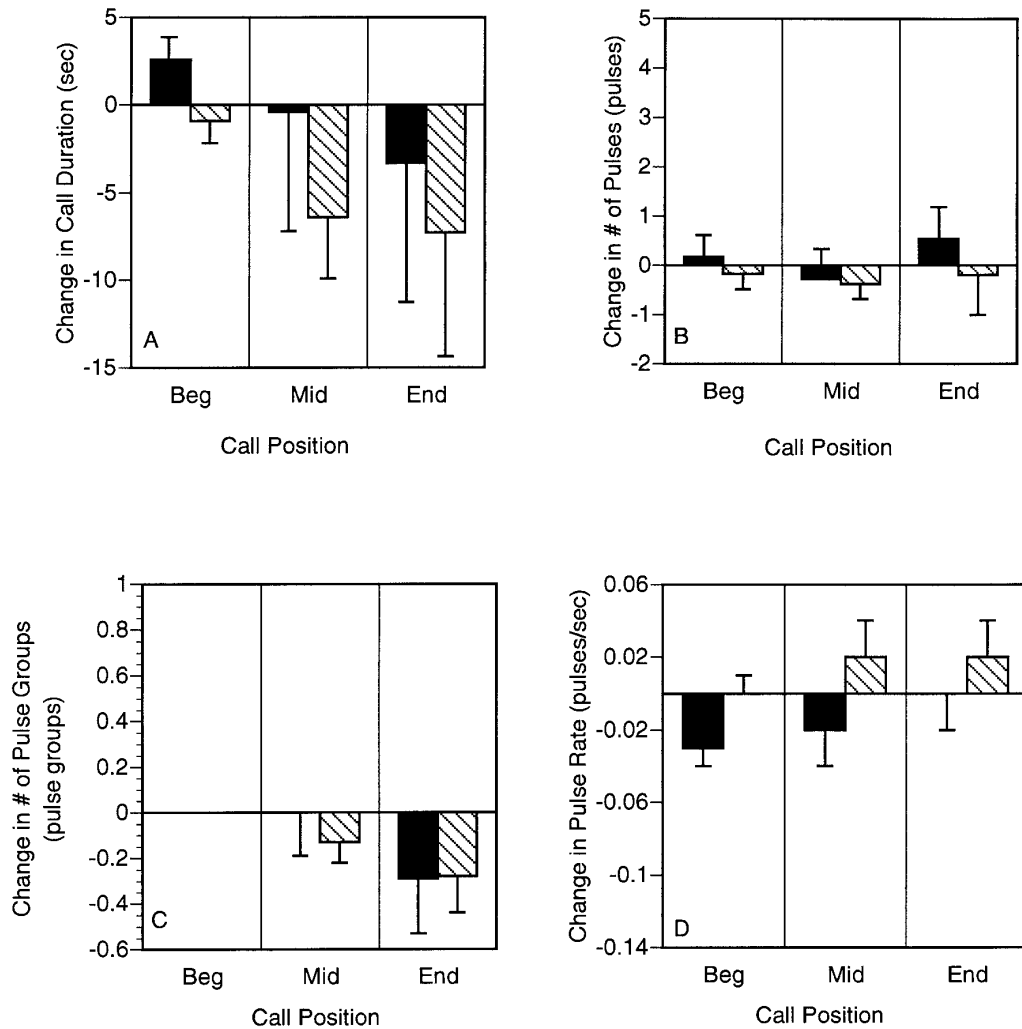


FIG. 6. Changes in call variables during the post-playback interval for AVT (solid bar) and saline (hatch bar)-treated animals. Characteristics include changes in (A) call duration, (B) number of pulses, (C) number of pulse groups, and (D) pulse rate, relative to preinjection baseline calling. Calls from the beginning (Beg), middle (Mid), and end (End) of the call group were analyzed.

signed-ranks test for the mean values indicated that the changes observed in AVT-treated animals between social conditions was significantly different from saline-treated animals ($T = 56$, $P < 0.025$). Examination of the means indicates that the temporal characteristics of AVT-treated males were less varied in response to changing social conditions. These results demonstrate that saline-treated males were more likely to exhibit a rebound effect of changes in calling behavior following the agonistic encounter time interval.

DISCUSSION

Our data support the conclusion that AVT stimulates calling, but does not increase aggressive behavior

or stimulate the production of more aggressive calls. These results are consistent with results from other studies indicating that AVT increases the amount of calling (Penna *et al.*, 1985; Marler *et al.*, 1995; Propper and Dixon, 1997) as well as decreases latency to begin calling (Boyd, 1994) in frogs. Furthermore, the social context in which the comparisons between AVT- and saline-treated males were made affected the degree to which the hormone effects were apparent. Although some effects of AVT may be seen during the agonistic interaction period, more robust effects of the neuropeptide are seen in the time interval immediately following the playback encounter.

We found changes in calling parameters under the playback condition that were consistent with those

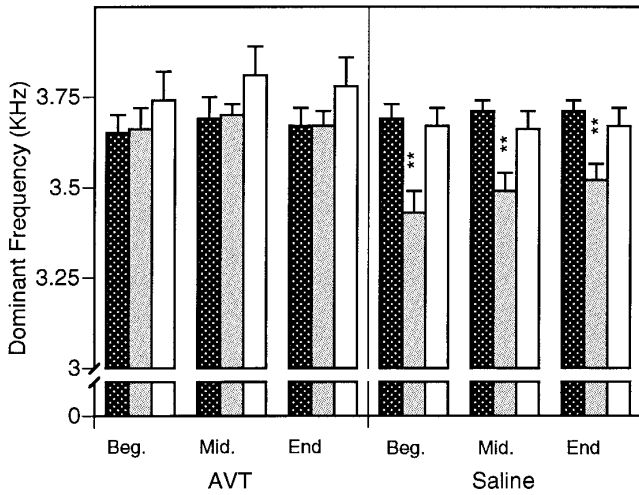


FIG. 7. Dominant frequencies for saline- and AVT-treated animals for each time interval: baseline (dark stipple), playback (light stipple), and post-playback (white). Calls from the beginning (Beg), middle (Mid), and end (End) of the call group were analyzed. Statistical differences are comparisons to baseline calling. $**P < 0.01$.

reported by Wagner (1989a,b). Among these call characteristics, AVT animals generally changed in a direction similar to saline males during the playback interval, although AVT males displayed less of a change. That is, both AVT- and saline-treated animals responded with largely similar aggressive calls once they began calling in response to the stimulus tape.

Although multivariate analyses comparing the degree of change during playback in AVT- vs saline-treated animals indicated no significant effect for hormone treatment for the temporal calling parameters during the playback encounter, there were some important differences due to AVT treatment. Under the playback condition, AVT-treated males began calling sooner in response to the playback stimulus than saline-treated males. This finding is consistent with AVT's effects on advertisement calling in the male bullfrog, *R. catesbeiana* (Boyd, 1994). Another important exception was a significant effect of AVT on dominant frequency. Male cricket frogs engaged in agonistic encounters typically lower the dominant frequency of calls in response to the social challenge (Wagner, 1989a,b,c, 1991, 1992). Results from our study showed that although calls from control animals decreased dominant frequency as expected, the spectral characteristics of calls from AVT-treated animals were virtually unchanged relative to baseline during the agonistic encounter even though the temporal characteristics of their calls did change in the manner expected of more aggressive animals. In our previous study, we

demonstrated that AVT significantly increased the dominant frequency of calls compared to baseline calls under normal chorusing conditions (Marler *et al.*, 1995). Our current study suggests that during the playback encounter, AVT's tendency to increase call dominant frequency was strong enough to completely reverse an animal's normal response to lower the dominant frequency when faced with a social challenge.

Although AVT had little effect on the temporal components of calling behavior during the playback time interval, there was a significant effect for hormone treatment during the time interval immediately following. Multivariate analyses indicated AVT signifi-

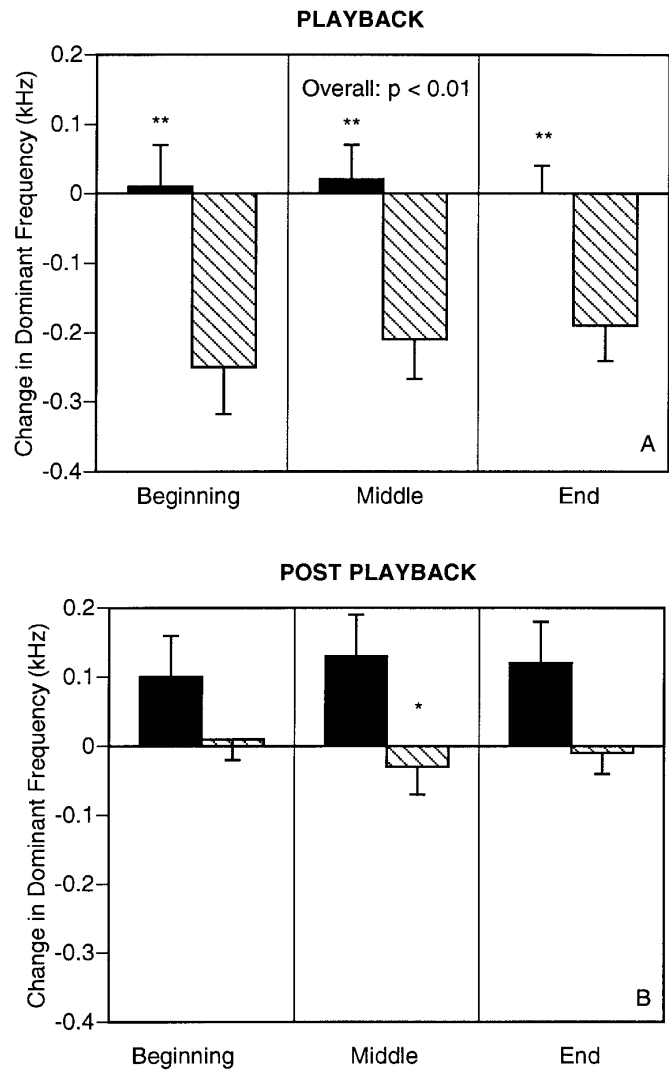


FIG. 8. Change in dominant frequency during (A) playback and (B) post playback interval for AVT (solid bar) and saline (hatch bar) treated animals. $**P < 0.01$ $*P < 0.05$.

TABLE 1

A Summary of Mean Changes in Call Characteristics Relative to Baseline under Playback and Post-playback Conditions

Call variables	Drug condition	Relative changes from baseline		Absolute difference
		Playback	Post-playback	
Call duration (beginning) (s)	AVT	8.20	2.58	5.62
	Saline	7.52	-0.90	8.42
Call duration (middle) (s)	AVT	27.97	-0.37	28.34
	Saline	31.38	-6.40	37.78
Call duration (end) (s)	AVT	11.89	-3.30	15.19
	Saline	17.59	-7.24	24.83
No. of pulses (beginning)	AVT	-0.25	0.17	0.42
	Saline	-0.93	-0.18	0.75
No. of pulses (middle)	AVT	2.30	-0.29	2.59
	Saline	2.92	-0.38	3.30
No. of pulses (end)	AVT	2.25	0.54	1.71
	Saline	2.16	-0.2	2.36
No. of pulse groups (beginning)	AVT	0.38	0.00	0.38
	Saline	0.3	0.00	0.30
No. of pulse groups (middle)	AVT	0.73	0.00	0.73
	Saline	0.65	-0.13	0.78
No. of pulse groups (end)	AVT	0.18	-0.29	0.47
	Saline	0.22	-0.28	0.50
Pulse rate (beginning) (pulses/s)	AVT	-0.11	-0.03	0.08
	Saline	-0.04	0.00	0.04
Pulse rate (middle) (pulses/s)	AVT	-0.03	-0.02	0.01
	Saline	-0.09	0.02	0.11
Pulse rate (end) (pulses/s)	AVT	0.02	0.00	0.02
	Saline	-0.05	0.02	0.07

Note. Absolute differences between the two time intervals were obtained to assess the extent of the rebound effect following the playback condition.

cantly altered calling rate variables compared to saline-injected animals during the post-playback interval. In particular, call rate and call group rate were significantly higher in AVT-treated males. There was also a significant difference of dominant frequency during the post-playback time interval. Control animals returned the dominant frequency of their calls to baseline levels, whereas AVT-treated animals increased dominant frequency above baseline. These results indicate that AVT-treated animals called more vigorously than control animals once the agonistic encounter was completed. Although the individual call variables (call duration, number of pulses, number of pulse groups, and pulse rate) did not differ between AVT- and saline-treated animals, the multivariate analysis was able to detect changes in the call variables as a whole.

During the post-playback period, the stimulus tape was turned off, simulating a reestablishment of dominance for the resident male. Our analysis of calling behavior showed definite lingering effects of the previous acoustic encounter. These effects were present

for both experimental groups; however, these effects were seen to a greater extent in saline-treated animals. Saline animals showed significantly depressed call rate and call group rate throughout the post-playback time interval. However, AVT-treated males lowered only call rate and not call group rate relative to baseline. The calling behavior of both AVT and saline animals was also changed with respect to call group characteristics following the playback interval. These changes were due mostly to a decrease in the number of calls per call group, and did not reflect an effect of hormone treatment. Call variables were also changed during the post-playback interval. These changes not only reflected a return to a less aggressive state, but also to a state that the call variables indicated as even less aggressive than the baseline condition, especially in the saline condition. Examination of the changes in these temporal characteristics suggested there exists a rebound effect that resulted in males vocalizing at a lower level of aggression compared to calls produced prior to the "agonistic" encounter. Comparison of the changes in call variables indicated that both AVT- and

saline-treated animals demonstrated such a rebound effect, but that AVT animals in most cases exhibited calling behavior closer to baseline than did the saline-treated animals. Although the parametric values of these variables seemed to indicate that the AVT animals were more aggressive relative to saline control animals, a more accurate statement might be that the AVT simply motivated subjects to call more vigorously, and thus compensated to some degree for the post-aggression rebound (Table 1).

Several important observations from this study bear on the initial questions raised in the introduction. During playback, AVT facilitated subjects' responses to the tape stimulus by reducing the latency to begin calling. This facilitation was also indicated by the failure of AVT-treated animals to lower their dominant frequency, which would be an indication of lowered call amplitude (Bee and Perrill, 1996; Lopez *et al.*, 1988; Robert Dudley personal communication). However, multivariate analyses of the temporal structure of calls from AVT-treated males indicated that they were not distinguishable from calls produced by control animals, indicating an ability of both treatment groups to produce an aggressive response to the stimulus. Therefore, AVT clearly did not inhibit males from producing the appropriate aggressive response. Observations of noncalling behavior are also consistent with this. AVT- and saline-treated males were equally likely to engage in aggressive posturing, as 69% of males under both conditions approached the location of speaker under the playback condition.

AVT-treated animals did differ in several calling parameters following the playback interval compared to saline control animals; however, these changes were in the direction of returning to normal baseline levels of calling. In contrast, saline-treated animals appeared to react more strongly to the transition from the playback to post-playback period. Although maintaining depressed levels of calling, the temporal characteristics of the individual calls were more widely varied compared to AVT-treated males. Furthermore, during post-playback, AVT-treated animals maintained a slight increase in the dominant frequency of calls indicative of vigorous calling found during the playback. The observations that AVT-treated animals could respond normally to the aggressive playback and that AVT resulted in males being less variable to changing social environments suggest that this peptide is not causing males to become more reactive to social stimuli in general.

The results of this study are most consistent with the hypothesis that AVT has a specific effect in in-

creasing the motivation of male frogs to produce advertisement calls more vigorously. Call differences attributable to hormone treatment under both playback and post-playback conditions are largely due to AVT-treated animals showing behavior indicative of vigorous calling compared to saline-treated males: shorter latency to respond to calls, higher dominant frequency than saline-treated animals in both time intervals, higher call and call group rates during both time intervals, and a greater tendency to return to baseline condition during the post-playback period countering the depressive rebound effects of the simulated aggressive encounter. AVT-treated animals were capable of producing an aggressive response to the playback tape and were not significantly different from controls, except in terms of some features that were at odds with calling more vigorously, such as calling at a higher dominant frequency compared to control animals.

It is possible that the effects of AVT might be dose dependent in complex ways. However, at the present time the most likely explanation for the pattern of results we obtained over different social conditions in this and in our previous study (Marler *et al.*, 1995) is that AVT increases males' motivation to call and thereby increases the vigor of that behavior. Under some conditions within the cricket frog's particular graded advertisement call system, such an increased motivation may counteract the changes made to signal an increase in aggression, while in other respects it leaves aggressive signaling unimpeded. Regardless of this effect, our interpretation is that any effect on aggression is secondary to AVT's influence on the vigorous production of advertisement calls. This behavior is one of the primary components of male reproductive social behavior in frogs (Wells, 1977; 1988). The effect of higher calling rates on female preference has not been empirically demonstrated in *A. crepitans*. However female preference for high calling rates and increased signaling has been shown in other anuran taxa (Wells, 1988; Gerhardt, 1991; Bosch and Marquez, 1996). Therefore, our results examining the effects of AVT on social behavior in *A. crepitans* are consistent with those found in other amphibians (Penna *et al.*, 1985; Moore, 1987; Moore, 1992; Boyd, 1994) and other taxa (Wilhelmi *et al.*, 1995; Pickford and Strecker, 1977; Voorhuis *et al.*, 1991; Winslow *et al.*, 1993; Insel *et al.*, 1994) that suggest that AVT has specific effects in increasing the expression of social behavior related to male-female interactions and reproduction.

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REFERENCES

- Bee, M. A., and Perrill, S. A. (1996). Responses to nonspecific advertisement calls in the green frog (*Rana clamitans*) and their role in male-male communication. *Behavior* **133**, 283–301.
- Bohus, B., Borrell, J., Koolhas, J. M., Nyakas, C., Buwada, B., Compaan, J. C., and Roozendaal, B. (1993). The neurohypophysial peptides, learning, and memory processing. *Ann. N. Y. Acad. Sci.* **689**, 285–299.
- Bosch, J., and Marquez, R. (1996). Acoustic competition in male midwife toads *Alytes obstetricans* and *Alytes cisternasii*: Response to neighbor size and calling rate. Implications for female choice. *Ethology* **102**, 841–855.
- Boyd, S. K. (1994). Arginine vasotocin facilitation of advertisement calling and call phonotaxis. *Horm. Behav.* **28**, 232–240.
- Bunsey, M., Kramer, D., Kesler, M., and Strupp, B. (1990). A vasopressin metabolite increases attentional selectivity. *Behav. Neurosci.* **104**, 277–287.
- Bunsey, M., and Strupp, B. J. (1990). Vasopressin metabolite produces qualitatively different effects on memory retrieval depending on the accessibility of the memory. *Behav. Neurol. Biol.* **53**, 346–355.
- Carter, C. S., DeVries, A. C., and Getz, L. L. (1995). Physiological substrates of mammalian monogamy: The prairie vole. *Neurosci. Biobehav. Rev.* **19**, 303–314.
- Delville, Y., Mansour, K. M., and Ferris, C. F. (1996). Testosterone facilitates aggression by modulating vasopressin receptors in the hypothalamus. *Physiol. Behav.* **61**, 25–29.
- Everts, H. G., De Ruiter, A. J., and Koolhas, J. M. (1997). Differential lateral septal vasopressin in wild-type rats: correlation with aggression. *Horm. Behav.* **31**, 136–144.
- Diakow, C. (1978). Hormonal basis for breeding behavior in female frogs: Vasotocin inhibits the release call of *Rana pipiens*. *Science* **199**, 1456–1457.
- Ferris, C. F., Albers, H. E., Wesolowski, S. M., Goldman, B. D., and Leeman, S. E. (1984). Vasopressin injected into the hypothalamus triggers a stereotypic behavior in golden hamsters. *Science* **224**, 521–523.
- Ferris, C. F., and Delville, Y. (1994). Vasopressin and serotonin interactions in the control of agonistic behavior. *Ann. N.Y. Acad. Sci.* **19**, 593–601.
- Gerhardt, H. C. (1991). Female mate choice in treefrogs: static and dynamic acoustic cues. *Anim. Behav.* **42**, 615–636.
- Insel, T. R., Wang, Z. X., and Ferris, C. F. (1994). Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. *J. Neurosci.* **14**, 5381–92.
- Lopez, P. T., Narins, P. M., Lewis, E. R., and Moore, S. W. (1988). Acoustically induced call modification in the white-lipped frog, *Leptodactylus albilabris*. *Anim. Behav.* **36**, 1295–1308.
- Marler, C. A., Chu, J., and Wilczynski, W. (1995). Arginine vasotocin injection increases probability of calling in cricket frogs, but causes call changes characteristic of less aggressive males. *Horm. Behav.* **29**, 554–570.
- McClelland, B. E., and Wilczynski, W. (1986). The effects of testosterone and arginine vasotocin on release calling in male *Rana pipiens*. *Soc. Neurosci. Abstr.* **12**, 314.
- Moore, F. L. (1987). Behavioral actions of neurohypophysial peptides. In D. Crews (Eds.), *Psychobiology of Reproductive Behavior: An Evolutionary Perspective*, pp. 61–87. Prentice Hall, Englewood Cliffs, NJ.
- Moore, F. L. (1992). Evolutionary precedents for behavioral actions of oxytocin and vasopressin. *Ann. N.Y. Acad. Sci.* **652**, 156–165.
- Nevo, E., and Capranica, R. R. (1985). Evolutionary origin of ethological reproductive isolation. *Ecol. Biol.* **19**, 147–214.
- Penna, M., Capranica, R. R., and Somers, J. (1985). Hormone induced vocal behavior and midbrain auditory sensitivity in the green treefrog, *Hyla cinerea*. *J. Comp. Physiol. A.* **170**, 73–82.
- Perrill, M., and Shepard, W. J. (1989). Spatial distribution and male-male communication in the northern cricket frog, *Acris crepitans blanchardi*. *J. Herpetol.* **23**, 237–243.
- Pickford, G. E., and Strecker, E. L. (1977). The spawning reflex response of the killifish, *Fundulus heteroclitus*: Isotocin is relatively inactive in comparison with arginine vasotocin. *Gen. Comp. Endocrinol.* **32**, 132–137.
- Propper, C. R., and Dixon, T. B. (1997). Differential effects of arginine vasotocin and gonadotropin-releasing hormone on sexual behavior in an anuran amphibian. *Horm. Behav.* **32**, 99–104.
- Riters, L. V., and Panksepp, J. (1997). Effects of vasotocin on aggressive behavior in male Japanese quail. *Ann. N. Y. Acad. Sci.* **807**, 478–80.
- Ryan, M. J., Perill, S. A., and Wilczynski, W. (1992). Auditory tuning and call frequency predict population-based mating preferences in the cricket frog, *Acris crepitans*. *Am. Nat.* **139**, 1370–1383.
- Ryan, M. J., and Wilczynski, W. (1991). Evolution of intraspecific variation in the advertisement call of a cricket frog (*Acris crepitans*). *Biol. J. Linn. Soc.* **44**, 249–271.
- Strupp, B. J., Bunsey, M., Bertsche, B., Levitsky, D. A., and Kesler, M. (1990). Enhancement and impairment of memory retrieval by a vasopressin metabolite: an interaction with the accessibility of the memory. *Behav. Neurol.* **104**, 268–276.
- Viglietti-Panzica, C., Anselmetti, G. C., Balthazart, J., Aste, N., and Panzica, G. C. (1992). Vasotocinergic innervation of the septal region of the Japanese quail: Sexual differences and the influence of testosterone. *Cell Tissue Res.* **267**, 261–265.
- Voorhuis, T. A. M., De Kloet, E. R., and De Weid, D. (1991). Effect of a vasotocin analog on singing behavior in the canary. *Horm. Behav.* **25**, 549–559.
- Wagner, W. E. J. (1989a). Social correlates of variation in male calling behavior in Blanchard's Cricket Frog, *Acris crepitans blanchardi*. *Ethology* **82**, 27–47.
- Wagner, W. E. J. (1989b). Graded aggressive signal in Blanchard's cricket frog: Vocal responses in opponent proximity and size. *Anim. Behav.* **38**, 1025–1038.
- Wagner, W. E. J. (1989c). Fighting assessment, and frequency alteration in Blanchard's cricket frog. *Behav. Ecol. Sociobiol.* **25**, 429–436.
- Wagner, W. E. J. (1991). *Social Selection on Male Calling Behavior in Blanchard's Cricket Frog*. Univ. of Texas, Austin.
- Wagner, W. E. J. (1992). Deceptive or honest signaling of fighting ability? A test of alternative hypotheses for the function of

- changes in call dominant frequency by male cricket frogs. *Anim. Behav.* **44**, 449–462.
- Wells, K. D. (1977). The social behaviour of anuran amphibians. *Anim. Behav.* **25**, 666–693.
- Wells, K. D. (1988). The effect of social interaction on anuran vocal behavior. In B. Fritsch, M. J. Ryan, W. Wilczynski, T. E. Heatherrington, and W. Walkowiak (Eds.), *The Evolution of the Amphibian Auditory System*, pp. 433–454. Wiley, New York.
- Wilhelmi, A. E., Pickford, G. E., and Sawyer, W. H. (1955). Initiation of the spawning reflex response in *Fundulus* by the administration of fish and mammalian neurohypophysial preparations and synthetic oxytocin. *Endocrinology* **57**, 243–252.
- Winslow, J. T., Hastings, N., Carter, C. S., Harbaugh, C. R., and Insel, T. R. (1993). A role for central vasopressin in pair bonding in monogamous prairie voles. *Nature* **365**, 545–548.
- Winslow, J. T., and Insel, T. R. (1991). Social status in pairs of male squirrel monkey determines the behavioral response to central oxytocin administration. *J. Neurosci.* **11**, 2032–2038.