

AVT Increases Mate Calling Probability in the Adult Male Gray Tree Frog,
Hyla versicolor

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Abstract

Mate calls are important reproductive behaviors among anuran amphibians and can be altered by specific hormones. In studies on newts, AVT has raised corticosterone levels and, in doing so, has reduced reproductive behaviors. However, at least two other anuran species (Rana catesbeiana and Hyla cinerea) have been stimulated to call when injected with AVT. In this experiment, we found that stress caused by handling and injections stopped mate calls in the adult male gray tree frog, Hyla versicolor. The AVT-injected frogs continued calling 60-90 min after treatment. We will prove that AVT independently increased corticosterone levels while prompting sexual behaviors in the Hyla versicolor. AVT's stimulation on mate calling temporarily overrode corticosterone as an inhibiting factor.

Introduction

One of the most important courtship behaviors for anurans is the mate call. We will test the adult male Hyla versicolor because its mate call is species specific and is influenced by many circumstances. Stress can cause negative changes in calling behavior. Marler, Chu, and Wilczynski (1995) found that handling and injecting the cricket frog Acris crepitans decreased the probability of calling. In turn, we expect our saline calling rate to significantly decrease compared to our saline baseline and our corresponding AVT groups. The saline frogs may cease calling altogether.

In several anuran amphibians, arginine vasotocin (AVT) has been shown to affect the levels of calling (Boyd 1994) and is one of the most powerful neuropeptides involved in reproductive behavior. Strong evidence, from experiments involving newts, indicates that AVT is involved in activating sexual behaviors--nanogram injections of synthetic AVT stimulate behavior, injections of AVT antagonists inhibit behavior, and the brain concentrations of AVT are higher in those that are sexually active than in those that are not (Moore 1987). In 1979 Moore and Zoeller discovered that AVT prompted male Taricha granulosa that were passive during the mating season to begin amplexic clasping. In 1986, Moore discovered that levels of AVT in the optic tectum peak during mating season then abate the rest of the year. Bullfrogs (Rana catesbeiana) with high AVT levels show increase in mate calling. However, these treatments worked only during the spring which correlates to the timing of their mating season (Boyd 1992). We expect the AVT group to continue calling as prior to injection.

The gray tree frog Hyla versicolor is a native to the eastern United States. It can measure up to 60mm in length, can vary in color from gray to green depending on the environment, and has orange-colored areas on its lower hind legs. The males calling in the field will indicate the duration of the mate-calling period under normal conditions on the night of the experiment.

Materials and Methods

At the University of Notre Dame Environmental Research Center (UNDERC), in Vilas County, Wisconsin and Gogebic County, Michigan, the gray tree frog, Hyla versicolor, was studied. The experiment concentrated on the effect of AVT on the males' mate callings.

On June 10, 1996, collecting was done at Hank field, a wet, muddy, and relatively open area surrounded by trees. Between 9:30-10:30pm, 14 frogs were captured. The temperature was 63°F.

The 14 frogs were brought to a screened-in porch and separated randomly into control and experimental groups. Each frog was isolated into individual styrofoam (.3x.3x.5)m coolers. After a 30-min acclimation period was allotted, baseline callings and frogs were injected. The control group was given 0.1mL Ringers' solution [0.14g KCl, 6.5g NaCl, 0.12g CaCl, and 0.2g NaHCO in 1L of distilled water] while the AVT group was treated with 100ug of AVT in 0.1mL Ringers' solution.

Tape recordings of calls/min were made at 30-, 60-, and 90-min intervals following the injections. Notebook entries of calls/min were taken to coincide with the tape recordings. Field callings were recorded after the 90-min interval. The rates of calls/min between the control and experimental groups will be compared.

Results

Two tests were used to analyze the data to distinguish calling changes due to the injections and changes due to AVT. According to the Mann-Whitney U test ($P < 0.05$), pre-injection behaviors showed no significant differences. Baseline calls/min were equivalent and within standard error. Comparisons between and within treatment groups were made using Fisher's exact test, $P < 0.5$. Both groups consisted of the same number of callers versus the total before injection (Table 1).

After injection no saline frogs called. Thirty minutes after injection, the saline population significantly differed from its baseline standard and from the comparable AVT group. The AVT-injected males continued to call as they had before injection or, in two cases, began calling once injected. By sixty minutes after injection, the entire AVT group called. AVT significantly affected the calling rate sixty to ninety minutes after injection. However, after ninety minutes only three AVT frogs called sporadically. Each averaged 1-3 calls/min with at least a two-minute latency period. At approximately this time, the field-callings frogs had stopped calling.

Discussion

Although AVT is found in all vertebrates, its functional purpose may be modified according to the species. All injections increased stress, which raised corticosterone levels. Saline-injected gray tree frogs significantly differed from their mean baseline and from their corresponding AVT groups. Albeit corticosterone has been proven to decrease behavior, we found that AVT-injected frogs continued to call as they had before injection. The calling males chorused among each other despite fatigue. We found that AVT acted independently on corticosterone and calling behavior in the adult male Hyla versicolor.

At least two other anuran species have been stimulated to call when injected with AVT. Under laboratory conditions, Boyd (1994) intraperitoneally injected the bullfrog Rana catesbeiana of 500ug AVT (Sigma) in 0.1ml saline and found significant effects when exposed to playbacks of advertisement calls 0.5-2 hr after injection. Penna *et al.* (1992) subcutaneously injected the Hyla cinerea with 25ug AVT (Sigma) in 0.1ml Ringer's solution. Castrated implanted and castrated non implanted male frogs

experienced a threshold elevation after AVT injection, regardless of androgen treatment received.

Kloas and Hanke (1990) found AVT injections to increase corticosterone in the frog Xenopus laevis, a response similar to that found in mammals when given artificially increased levels of AVP (e.g., Gillies, Linton, and Lowry, 1982). Three nmol AVT (SERVA, FRG) per 100g body weight was dissolved in 100ul frog Ringer's solution and injected into the dorsal lymph sac. A significant increase of corticosterone and aldosterone concentrations in serum within 1 hr was caused by the AVT injection and gradually reached control levels after 12 hrs. There is evidence that corticosterone mediates the stress-induced inhibition of reproductive behaviors. Fifteen minutes after being injected with corticosterone, amplexic behaviors in T. granulosa disappeared completely (Moore 1983). This supports the mechanism that AVT increases corticosterone levels, which then decreases sexual behaviors. However, this response has been found in newts only.

Our data indicates that stress-induced injections may have raised the corticosterone level and physiologically inhibited the gray tree frog from calling while AVT lessened stress by an overriding factor. The calling AVT group potentially could have prompted saline males to call. The groups were within 3.3m of each other, and the AVT group continued to call as it had before injection. However, the stress caused by handling and injections was a significant inhibitor. While calling behavior of male Acris crepitans was influenced to some extent by caller density (higher call rate and number of calls per call group as density increased) and nearest neighbor distance (increased duration and number of pulses in their calls), a greater number of changes occurred when males were exposed to specific cues (a challenge by another calling male) (Marler, Chu, and Wilczynski 1995). Indeed, the calling Hyla versicolor had systems of rapid calling punctuated by periods of silence, and the chorus responded when a single frog called. Despite the "energetically costly" calls (Schwartz 1991), AVT-injected gray tree frogs served as each other's stimuli to chorus. We reason that AVT prompted calling behavior while simultaneously increasing corticosterone levels.

Moreover, Marler, Chu, and Wilczynski (1995) found evidence that AVT blocked or compensated some of the effects of handling in adult male Acris crepitans. Intraperitoneal injections of 20ug AVT (Sigma) in 50ul saline were given. AVT increased the cricket frogs' probability of calling after injection under natural conditions. We suggest that AVT temporarily eased the gray tree frog from stress. The Hyla versicolor mate call continued for 60-90 min after AVT injection as before treatment. After 90 min the AVT group ceased calling. At that time, the experimental and saline groups were behaviorally indistinguishable, denoting stress a factor. However, stress from handling and injections kept the saline group from calling. Only AVT-injected males called as they had before treatment, yet they did so for a limited time. For these reasons, we believe that AVT temporarily induced a behavioral response in the gray tree frog.

In conclusion, we have shown that stress and AVT both increase corticosterone levels and that behavioral responses tend to decrease. In spite of being handled and injected, the male gray tree frog had a greater probability of calling when treated with AVT. Thus, we suggest that AVT affects corticosterone and behavior independently.

Whether AVT temporarily blocked or compensated for the stress of the injections or actually stimulated calling is not certain. Furthermore, it is uncertain if the treated males would have continued to call had the field animals called longer. Future studies are necessary in these areas.

Table 1. AVT and Saline Groups' Mate Calling Behavior Before and After Injections. ^{a, b}=Fisher's Exact test. Groups which do not share the same superscript letter are significantly different (p<0.5). ^{c, d}=Mann-Whitney U test pair-wise comparison. Groups which do not share the same superscript letter are significantly different (p<0.05).

	<u>AVT</u>	<u>SALINE</u>
<u>BASELINE</u> CALLING FROGS/TOTAL CALLS/MIN	5/7 ^a 9.571428±/3.2 ^c	5/7 ^a 8.428572±/2.7 ^c
<u>30-MIN</u> CALLING FROGS/TOTAL CALLS/MIN	6/7 ^a 4.424286±/1.3 ^c	0/7 ^b 0±/0 ^d
<u>60-MIN</u> CALLING FROGS/TOTAL CALLS/MIN	7/7 ^a 5.557143±/1.3 ^c	0/7 ^b 0±/0 ^d
<u>90-MIN</u> CALLING FROGS/TOTAL CALLS/MIN	7/7 ^a 5.287143±/1.5 ^c	0/7 ^b 0±/0 ^d
<u>ALL TIMES</u> CALLING FROGS/TOTAL CALLS/MIN	7/7 ^a 5.11±/1.2 ^c	0/7 ^b 0±/0 ^d

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