

Modification of the Calling Behavior of *Hyla Versicolor* by AVT

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Abstract

Arginine vasotocin (AVT), a natural amphibian neurohormone, activates many reproductive behaviors. Studies have shown that AVT affects the mating calls of several different species of frogs. As a consequence of the changes that result from AVT treatment, the reproductive success of the particular frog could be altered due to the differences in their calling behavior.

This experiment examined the effects of AVT on the calling behavior of the gray tree frog *Hyla versicolor*. From the research conducted, the mating calls of *Hyla versicolor* were influenced by many factors, such as, temperature, environmental conditions, stress levels, and the experimentally controlled increased levels of AVT. AVT was shown to induce calling in males that were otherwise not actively calling in the experiment. Results conflicting with previous experiments were obtained regarding the differences in calling rates. Compared to field calls from the same temperature, the AVT induced calling rates were significantly slower. In the study done in the previous year, results indicated that AVT calling rates were higher than those not treated with AVT. The inconsistency of the results from this experiment could have been due to the small sample size, incomplete data, and the large number of uncontrollable variables in the field.

Introduction

Mating calls of frogs, also known as advertisement calls, are vocalizations associated with reproductive behavior. The purpose of these mating calls is to draw the attention of the females so they can find the males during the breeding season. Males increase their chances of mating success by producing a call that females show a greater preference toward. The degree to which a frog can produce a call that will stand out is limited by the costs of vocalization which include the cost of the actual energy used in the call production and the increased risk of predation (Pough 1989). Individuals that produce calls that balance these factors out will have the ability to mate with the most females. Those individuals with the greatest reproductive success have a high fitness level and their genes will be propagated to the next generations.

The vocalizations of frogs are produced by the vocal cords which are a pair of elastic bands that extend across the larynx longitudinally. Air is pushed out of the lungs which causes the edges of the vocal cords to vibrate. These vibrations produce the sound, and variations in the call are controlled by changing the tension of the vocal cords with the laryngeal muscles (Holmes 1954).

The characteristics of these calls are species specific. Female frogs are programmed to respond to conspecific calls. There is speculation that during the breeding season, the hormones that are involved with ovulation also sensitize certain cells in the auditory pathway. These cells are sensitized to the characteristics of the calls of males of the same species (Pough 1989 and Ryan 1993). In this way, the female is able to pick out a conspecific male from various other calls of frogs that are present in the environment. The calls also appear to attract other conspecific males to join in the chorus (Capranica 1965).

The characteristics of the calls are affected by a variety of factors. Temperature, time of the year, amount of light and the weather can change the frequency and the rate of the calls (Gerhardt 1978 and Stetson). The rate and frequency of a call can also be changed by changes in the levels of hormones and neuropeptides in the frog's body. Arginine vasotocin is one of these substances (Leo, unpublished data).

Arginine vasotocin (AVT) is a small neuropeptide that activates certain reproductive behaviors in some amphibians (Stetson). AVT has been shown to increase amplexic clasping, egg laying behavior, calling rate, mate call phonotaxis, and female receptivity. The mechanism for how AVT works is unknown (Boyd 1992).

For this experiment, *Hyla versicolor*, also known as the common gray tree frog, will be used as the experimental subject. *H. versicolor* is found in the eastern half of the United States, extending from Ontario to the Gulf of Mexico. The frogs are 3.2 to 6.0 cm long with rough skin that is greenish or grayish in color. There are characteristic bright yellow orange markings under the thighs. *H. versicolor*'s natural habitat is up in trees and shrubs that are near water. Their vocalization is characterized as a resonating trill and is heard in spring and early summer (Bebler and King 1979). They descend at night into the water in the vegetation to call and breed (Bogart 1960).

The mating call of *H. versicolor* is made up of two frequencies -- a secondary frequency of 1000 Hz and a dominant frequency of 1300 Hz. In a previous experiment

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conducted by Harvey Leo, the secondary frequency of the calls of the AVT injected males had a drop in pitch and the call rate increased. The dominant frequency remained constant (Leo, unpublished data). Since these mating calls are species specific, changes in the characteristics of the calls would probably result in a change in reproductive success.

The main purpose in the experiment was to determine the direct effects of AVT on the calling behavior of *Hyla versicolor*. The calls of saline injected males, AVT injected males, and natural field males were examined. Characteristics such as the calls per minute and whether or not the subjects called or not were used as the basis for comparison.

Materials and Methods

Preliminary steps to the experiment included general behavioral observations of the frogs on different occasions. Special notes were taken as to what time calling began, where the frogs were calling from, and the weather conditions. Several recordings of natural chorus field calls were made for later reference.

The experiment was run on June 13, 1994 with clear weather conditions at a temperature of 20 C at 8:00 pm. Field calls were strong and numerous. Twenty male frogs were captured in an hour and a half. The frogs were caught by shining a flashlight on a calling frog and quickly grabbing them with hands.

Styrofoam rectangular containers with net tops were spread out along the side of the site. One frog was randomly placed in each container. Ten frogs were injected with 100 µg of AVT in the peritoneal cavity. The other ten frogs were injected with .1 ml of Ringer's Amphibian solution.

Tape recordings of the frogs' calling behavior were made immediately after injection. Two minute recordings of each individual subject were made 30 minutes and 60 minutes after the injection of AVT or saline. Temperature, humidity, and time were both recorded throughout the experiment. Observations of the behavior of the individual frogs were also noted. The rate of calling was counted for each individual frog for one minute from the tape recordings of the experiment.

A male frog that was in close proximity to the location of the experiment site was also intentionally recorded. The frog (referred to as "random frog") was not manipulated in any way, and his calls were used as another control reference.

Results

The frogs injected with saline did not call for the entire time of the experiment. Only one AVT frog called immediately after injection. After 30 minutes, six out of the ten AVT frogs called. The calling of the random frog was also recorded at this time. See Table 1 for calling rates. For the recordings at 60 minutes, only half of the experimental subjects were actually recorded on tape. Half of the data was lost due to faulty equipment. The results that were obtained for 60 minutes are in Table 2. With the data that was recorded for 60 minutes, five out of six AVT frogs called. Even though the calling was not able to be recorded after 60 minutes for the experimental subjects, the calling rate dropped dramatically after 90 minutes had elapsed. The field chorus calls that were recorded on a

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Table 1. Results of AVT frogs 30 minutes after injection.
17° C and 70% humidity

| AVT Frog | Call/Not Call | # calls per min |
|----------|---------------|-----------------|
| 1 | + | 1 |
| 3 | + | 14 |
| 5 | + | 12 |
| 7 | + | 7 |
| 9 | - | - |
| 11 | + | 6 |
| 13 | - | - |
| 15 | + | 1 |
| 17 | - | - |
| 19 | - | - |
| random | + | 8 |

+ frog called 30 minutes after injection
- frog did not call 30 minutes after injection

Table 2. Results of AVT frogs 60 minutes after injection.
17° C and 75% humidity

| AVT Frog | Call/Not Call | # calls per min |
|----------|---------------|-----------------|
| 1 | + | 4 |
| 3 | + | 8 |
| 5 | + | 9 |
| 7 | + | 12 |
| 9 | - | - |
| 11 | + | 4 |

* the rest of the data was able to be recorded

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different night but at the same temperature of 17° C had an average of 15.0 calls per minute. This rate was only an estimate since it was hard to distinguish individual frog's calls in the chorus.

The comparison of the average calling rates for the different experimental groups can be seen in Table 3. The averages of the AVT frogs were less than those of the field calls. The rates of the random frog and the AVT frogs overlapped.

Based on the data collected after 30 minutes, AVT induced *Hyla versicolor* males to call in comparison to the saline treated frogs. See Table 4 for statistical analysis. The AVT calls sounded different from the natural field calls. They sounded more metallic and probably at a different frequency. Computer analysis would have to be done to determine if there is a true difference in the frequencies of the AVT calls and the field calls.

The frogs that were injected with AVT did not stop calling when flashlights were shown in their eyes. They did not seem to be disturbed by the experimenters presence. The frogs out in the field responded much differently. When someone would come in close proximity to one of the calling frogs, the frog would stop calling momentarily and wait until it thought the threat had disappeared. Shining a flashlight in their eyes also immediately stopped them from calling and they would freeze for a few seconds.

Both the AVT treated males and the field males called in chorus. When one frog called, the others would follow. The random frog outside the experiment would call following the calls of the other AVT experimental frogs.

Discussion

Observations during the experiment showed that the calling behavior of *Hyla versicolor* was very sensitive to temperature and stress. Some preliminary trials ended in failure due to the temperature dropping too low too quickly during the evening. The temperature had to be at least 50° F for the frogs to be calling out in the field. Once the temperature dropped below this temperature, their calling would slow down substantially and even cease during the course of the evening. In Wells and Taigen's research, *Hyla versicolor* males called at a rate of 20 - 22 calls per minute at a temperature of 23° C (Wells and Taigen 1985). Lower temperatures slow down the rate of calling as seen by the results from the field calls that had a rate of about 15 calls per minute at 17° C.

Stress also stopped the frogs from calling. Frogs that had been in captivity for as short as one night would not call the following evening whether they were treated with AVT or not. The frogs had to be caught the same evening as the experiment. Even those frogs that were caught the same night as the experiment and were treated with saline would stop calling once they were caught. Stress has been shown to upset the normal levels of CRH, ACTH, corticosteroids, and opiate peptides. These are the chemical messengers that are involved with the stressed induced inhibition of sexual behaviors (Moore 1983 and Moore and Deviche). The effects of AVT seemed to overpower the influence of the stress on the frogs since they called despite the fact that they were experiencing the same stressful situation as the saline frogs.

The data obtained in this experiment suggests that AVT reduced the calling rate of the experimental frogs. This data is in contrast with the results of Harvey Leo's

Table 3. Comparison of average call rates at 17° C.

| | calls/min | SEM |
|-------------------------------|-----------|------|
| AVT 30 min after injection | 6.8 | ±2.2 |
| AVT 60 min after injection | 7.4 | ±1.2 |
| Random frog during experiment | 8.0 | NA |
| Field chorus calls at 17° C | 15 | ±0.8 |

Table 4. Statistical analysis of AVT and control frogs 30 minutes after the injection.

Observed values:

| | no call | call | totals |
|--------|---------|------|--------|
| AVT | 4 | 6 | 10 |
| Saline | 10 | 0 | 10 |
| Totals | 14 | 6 | 20 |

Expected values calculated from observed values:

| | no call | call | totals |
|--------|---------|------|--------|
| AVT | 7 | 3 | 10 |
| Saline | 7 | 3 | 10 |
| Totals | 14 | 6 | 20 |

$$X^2 = \sum \frac{(\text{obs}-\text{exp})^2}{\text{exp}} = 1.3 + 1.5 + 1.3 + 1.5 = 5.6$$

$$X_{1,\alpha=.05} = 3.84 \quad .01 < P < .025$$

Therefore, the null hypothesis that AVT has no effect on calling can be rejected.

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experiment in 1993. His results showed an increase in the AVT calling rates. Since the field calls and the experiment were recorded on different nights in this experiment, the differences in the average rates of calling could have been due to differences in environmental factors that were not measured. Different environmental variables and small sample size could also account for different results in the two experiments.

The random frog's calling rate was probably similar to the experimental frogs because he was following their lead. "Calls seem to attract other males whose calling adds, in turn, to the chorus." (Capranica 1965). Throughout the experiment, the *Hyla versicolor* males were observed to synchronize their calls in the same rhythm, both in the field and in the experiment.

Both the vocalizations of the experimental subjects and the natural field chorus frogs decreased as the night went on. Usually all calling in the field was completed by midnight or soon after. This decrease in calling could be due to the decrease in temperature during the night or to the high energy expenditure of calling. Other researchers have shown the dependence of sexual calling behavior on temperature and other environmental factors (Blair 1958 and Gerhardt 1978). Also, the vocalizations of *Hyla versicolor* are very energy expensive (Wells and Jaigen 1985) so the frogs may only have been able to call for a limited number of hours due to fatigue.

The quality of the results could have been improved greatly with a larger sample size, consistent environmental conditions, and a little luck with the technical equipment. Another improvement would include recording each individual for the entire time of the experiment which would require additional recording equipment. In addition, each frog that would be used as a possible experimental subject should be recorded calling in his natural environment before capture. This natural call could then be used for direct comparison of the effects of AVT on each individual.

Conclusions

The significance of this experiment is related to the factors and characteristics that make an animal successful in reproduction. Females choose their mates based on the mating call produced by the competing males. With the hormonal changes that occur from the injection of additional AVT, the modifications to the vocalizations could alter the male frog's attractiveness to the females.

From the results of this experiment, AVT induced *Hyla versicolor* males to call. AVT treated frogs also had a slower average calling rate than those of natural field calls. The preliminary conclusion is that AVT does change the calling behavior of *Hyla versicolor*. Other factors such as stress levels and temperature also greatly influenced vocalization behaviors. Further analysis of the data and additional experimentation is needed in order to draw more confident conclusions.

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