HORMONAL REGULATION OF CALLING PERFORMANCE IN THE GRAY TREE FROG

Hyla versicolor

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Abstract

Mating calls are very important reproductive behaviors among Anuran amphibians. They help to insure the success of mating by attracting a mate and broadcasting the location of the male to the female and to other males. Temperature, humidity, and precipitation have all been found to influence mating calls. Mating calls can also be altered by specific hormones. The neurohypophyseal hormone arginine vasotocin (AVT) is one such hormone that is suggested to affect mating behaviors such as mating calls. AVT is an amphibian hormone related to the mammalian hormones Oxytocin and Vasopressin.

In this experiment, AVT was injected into tree frogs of the species Hyla versicolor. The calling rates of the AVT injected frogs and the control frogs were then recorded and compared. It was found that AVT did significantly increase the calling rates in frogs sixty and ninety minutes after injection.

Introduction

In most vertebrates, courtship behaviors are very important rituals that lead to the creation of the next generation and thus, continue the evolutionary line. These reproductive behaviors can differ not only among the various species of vertebrates, but also between the two sexes of these species.

In Anurans, the most important of these courtship behaviors is generally the advertisement call. Anuran calls have been classified into three general categories: mating calls, aggressive calls, and territorial calls. Mating calls help determine the reproductive success of the species by allowing females to find the breeding site. This unites the males and the females. Mating calls are also used to demonstrate the locations of other males. This permits the frogs to space themselves accordingly (Schwartz, 1987).

Mating calls are produced in the laryngeal apparatus. This consists of the larynx, which is composed of two vocal cords. Sound is produced when air, forced out of the lungs, causes the vocal cords to vibrate. This vibration results in a reverberation of the air column discerned as sound.

Calls tend to be species specific. The length, frequency, pitch, and rate of calling are all factors that have been found to differ from species to species. Individuals can recognize the specific calls by means of distinctive characteristics such as pulse and repetition rate.

Mating calls are influenced by many circumstances. Wells and Schwartz (1982) found that the call rates tend to increase in closed environments as opposed to open ones. Climatic conditions such as humidity and temperature have been discovered to modify not only the air medium through which the calls travel, but also the calls themselves. Gerhardt (1978) determined that females kept at a specific temperature were more responsive to the calls of males maintained at the same temperature. Thus, there is a correspondence between the mating call system in males and the recognition system in females in that both are regulated by temperature.

Hormones are another factor that influences mating calls. Schmidt (1966) noted that the injection of Rana pituitaries induced calling behaviors in Hyla versicolor and Hyla cinerea. This treatment brought about a reaction to recorded calls. These injections have also been found to increase call frequency in Rana pipiens.

Moore (1987) studied the different behavioral effects of three specific hormones: arginine vasotocin (AVT), arginine vasopressin (AVP), and oxytocin (OXY). Of these, only AVT is found in all vertebrates. He reports that the actions provoked by AVP and OXY in mammals are mimicked by AVT in all other vertebrates. It is suggested that these hormones regulate some sexual behaviors. In 1979 Moore and Zoeller discovered that AVT prompted males of T. granulosa that were passive during the mating season to begin ampletic clasping. Diakow (1978) determined that injections of AVT in females of Rana pipiens brought about reproductive behavior. AVT was also found to stimulate the oviducts to contract in females of Salamandra salamandra causing pregnant females to give premature birth (Heller et al, 1970). In 1986, Moore discovered that levels of AVT in the optic tectum peak during mating season then abate the rest of the year.

In male frogs, AVT influences the transmission of the calls. In females, the response to the calls is brought on by AVT. The impact of AVT on auditory and vocalization regions was explored in an experiment conducted on bullfrogs (Rana catesbeiana) (Boyd, 1994). It was found that an injection of AVT caused the male frogs to increase the frequency of their calls and , in females, decreased the amount of time spent locating the source of the calls, thus increasing the chances that mating would occur. These results led to the conclusion that AVT may have a direct effect on the auditory processing area of the brain in bullfrogs and consequently bring about these two sex-specific reproductive behaviors. AVT has also been shown to directly influence the rate and frequency of mating calls in the gray tree frog, Hyla versicolor (Leo, unpublished data).

The gray tree frog is native to the eastern United States. It measures up to 60 mm in length and is characterized by orange colored areas on the lower hind legs. It can vary in color from gray to green depending upon the environment. The gray tree frog also exhibits a very unique mating call. The call of Hyla versicolor is defined as a trill and is pulsed. Schwartz (1987) found that female members of the species responded to calls with distinctive characteristics even if the calls were overlapped with calls from members of other species. Wallace (unpublished data) studied the effects of AVT on the calling performance of the gray tree frogs. This experiment continues that research by studying the effects of AVT on calling in males of the species. Hyla versicolor is an appropriate choice of frog for this experiment because of the unique trilling call it exhibits. This experiment is important because it studies one aspect of the mating behavior of a species of amphibian. In the past few decades, the number of amphibians in populations has declined dramatically. Scientists as of now are not sure why amphibian populations are decreasing so rapidly. One hypothesis suggests that increased ultra-violet radiation exposure due to the reduction of the ozone layer. Another explanation is increased pollution in areas inhabited largely by amphibians. By studying an aspect of the mating behavior of amphibians, perhaps a way will be discovered to reverse the situation or increase the existing population.

Materials and Methods

The experiment was conducted on the UNDERC property in Gogebic County, Michigan, and Vilas County, Wisconsin. During the last weeks in May and the first week in June, the property was explored to find an appropriate site to conduct the experiment. The area surrounding Bog Pot in the central part of the property was found to be suitable due to the wet and muddy terrain and the abundance of Hyla versicolor calls heard there. The area was explored during the day and at night. Frogs were captured the night of Saturday June 3, 1995. They were put into buckets and later transferred to an aquarium in the laboratory. They were observed such that all people involved in the project became familiar with the calls, the appearance of the frogs themselves, and the general terrain of the area.

On June 13, 1995 the experiment was carried out. At 9:30 PM, a group of volunteers was sent to Bog Pot. By 11:00 PM, 15 frogs had been captured. The frogs were placed in styrofoam containers with lids. Baseline calls from the frogs were recorded as were calls from the general chorus. AT 11:35 PM, eight frogs were injected with 100 uM in 0.1 ml of AVT. The remaining seven were injected with 100 uM in 0.1 ml of saline. Thirty minutes after injection, the calls were recorded using a parabolic microphone and a tape recorder. The calls were then recorded at thirty minute intervals for one and one-half hours following injection. The frogs were taken from the site of Bog Pot back to the main camp where they were left on the porch of the laboratory overnight. It was later determined that the microphone had stopped working at approximately 1:15 AM. The frogs were observed again at 5:30 AM (six hours after injection) and 12:00PM on June 14, 1995. The experiment was tried again on Sunday, July 9, 1995 but the mating season for Hyla versicolor had ended. The experiment was tried on Tuesday July 11, 1995 with Green frogs (Rana clamitans), but the frogs did not call. The data was then analyzed using Fisher's Exact Test (Systat for DOS, ver. 6) to answer the question of how the calls of gray tree frogs injected with AVT compared with the calls of those injected with saline.

Results

Baseline calls were collected from two frogs AVT#1 and AVT#6. All AVT frogs called at least once during the night, but only one saline frog called at all (Fig. 1). One half hour after the injection, AVT frogs called with an average of 1.75 calls per minute, while no saline frogs called at all as seen in Fig. 2. At 12:35AM, one hour after injection, AVT frogs called 3.583 calls per minute while one saline frog called with an average of 1 call per minute. All of the AVT frogs called ninety minutes after injection at an average of 2.874 calls per minute, while none of the saline frogs called at all during this time period. It was observed that the AVT-injected frogs continued to call six and twelve hours after injection. Calls of AVT and saline injected frogs were compared with each other using Fisher's exact test (Fig. 3). A value of $p<.05$ was considered significant.

Discussion

Using Fisher's Exact test, it was determined that AVT significantly affected the calling rate of the grey tree frog only at periods of sixty and ninety minutes after injection as compared to the calling rates of frogs thirty minutes after injection and the calling rates of saline injected frogs. This data follows the hypothesis that AVT does affect the calling rate of the gray tree frog Hyla versicolor . However the hypothesis must be modified to include the calling rates of frogs sixty and ninety minutes after injection only.

As the experiment went on during the night, it was observed that the calls of the AVT injected frogs sounded metallic and seemed to increase in length as compared to calls made earlier and calls made by the chorus. The general calling pattern of the frog chorus was also observed during the night. The frogs of the chorus usually followed a system of rapid calling punctuated by periods of silence. Once a single frog called, the chorus would quickly respond. Schwartz (1991) referred to this calling strategy as unison bout singing. He suggested that the periods of silence represent intervals the frogs use to save energy. However, Schwartz rejected the theory that increased muscle lactate levels were the reason for the pauses in calling. Unison bout singing illustrates that males not only call to attract females, but also to respond to the calls of other males. In 1984, Schwartz demonstrated how males respond to synthetic calls or any other sound as long as it is made at the appropriate frequency. The AVT injected frogs followed the calling pattern of the chorus frogs to a certain degree. The calls of members of the chorus were generally the stimulus that triggered the AVT frogs to call, but the AVT frogs usually called more and for a longer period. This would imply that AVT compels the frog to continue calling despite resulting fatigue. The observation of the metallic sound and longer call duration of AVT calls supports this idea in that the calls may be altered due to the stress of continued calling despite tiredness. The calls of the gray tree frog have been described as "energetically costly calls" (Haustater et al, 1990). When the frog chorus ceased calling at approximately one o'clock on the morning of June 14, the AVT frogs continued to call.

The experiment illustrated the effects of other factors on the calls of Hyla versicolor. The frogs began calling at approximately nine o'clock on the evening of the experiment. This was about the time of sunset. During all my observations prior to and following the experiment, I never heard the call of a grey tree frog during the day (except in captivity). However, the frogs injected with AVT continued calling throughout the night of the experiment and into the afternoon of the next day. Thus, AVT must have overcome the factor that inhibits frogs from calling during daylight hours. Another consideration that also influenced the calls of the gray tree frog was stress. The saline frogs most likely refrained from calling during the duration of the experiment due to the stress of being caught and injected. The stress of being captured would also serve as an explanation of why there were so few baseline calls. Although there was no precipitation and it was warm on the night of the experiment, the frogs abstained from calling on many nights when it rained or temperatures were extremely low.

Conclusions

Advertisement calls have a major impact on Anurans. For many species they are the only way a mate is attracted. Thus, mating calls have an overall influence on the survival of Anurans in that they directly affect the reproduction rate of the species.

Mating calls also broadcast the location of the calling males. In this way, frogs can space themselves accordingly. Many factors influence mating calls. Among these are light, stress and temperature. In this experiment the affect of stress upon gray tree frogs was seen. Frogs that had been captured called very infrequently and frogs that were injected did not call at all unless prompted by another stimulus. The impact of AVT on calling was tested in this experiment. AVT was found to increase the rate of mating calls in Hyla versicolor sixty and ninety minutes after injection. Questions of why AVT did not act immediately to increase calling significantly would have to be answered with further research.

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Fig. 1 Calls per minute in AVT and saline injected frogs

Calls/min.	Baseline	12:05 AM	12:35 AM	1:00 AM
AVT #1	0.00	0.00	1.00	3.00
AVT #2	0.00	0.00	0.00	5.00
AVT #3	1.00	0.00	1.00	7.33
AVT #4	0.00	1.00	4.50	0.67
AVT #5	0.00	1.00	6.00	2.33
AVT #6	2.00	3.00	5.00	1.00
AVT #7	0.00	0.00	0.00	1.67
AVT #8	0.00	2.00	4.00	2.00
Saline #5	0.00	0.00	1.00	0.00

Fig. 2 Average call rates in AVT and saline injected frogs.

Treatment	n	% calling	mean calls/min.	st. dev.	SEM
Baseline	15	25	1.500	0.707	0.500
AVT 12:05 AM	8	50	1.750	0.957	0.479
AVT 12:35 AM	8	75	3.583	2.108	0.860
AVT 1:00AM	8	100	2.874	2.245	0. 794

Fig. 3 Statistical analysis of calling using Fisher's Exact test.

BASELINE	Call	No Call
AVT	2	6
SAL	0	7

p=0.467

12:05 AM	Call	No Call
AVT	4	4
SAL	0	7

p=0.077

12:35 AM	Call	No Call
AVT	6	2
SAL	1	6

p=0.041

1:00 AM	Call	No Call
AVT	8	0
SAL	0	7

p=0.0002

	Call	No Call
AVT 12:05	4	4
AVT 12:35	6	2

P=0.608

	Call	No Call
AVT 12:05	4	4
AVT 1:00	8	0

p=0.077

	Call	No Call
AVT 12:35	6	2
AVT 1:00	8	0

p=0.47

	Call	No Call
SAL 12:05	0	7
SAL 12:35	1	6

p=1.00

	Call	No CAll
SAL 12:05	0	7
SAL 1:00	0	7

p=?

	Call	No Call
SAL 12:35	1	6
SAL 1:00	0	7

p=1.0