DIFFERENT STIMULI INFLUENCE CALL TYPES IN THE GRAY TREEFROG, *Hyla versicolor*

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Introduction:

Hyla versicolor, otherwise known as the gray tree frog, is an amphibian species which is prevalent in the northern regions of the upper peninsula of Michigan and Wisconsin where UNDERC is located. I chose the species not only because of their plenitude at UNDERC but also because these frogs are good subjects because their vocal behavior is easily quantified and manipulated (Wells and Taigen, 1986). The vocalizations of frogs are utilized for communication. There are three different types of calls which can be observed from the Hyla versicolor. One is an advertisement call which the males use to attract females during the breeding season. The advertisement call has been extensively studied in the gray treefrog (e.g., Gerhardt, 1978; Wells and Taigen, 1986; Schwartz, 1987; Gerhardt, et.al,1996; Welch, et.al,1998). Another type of call is the aggressive call which is much less studied than the advertisement. Finally, Hyla versicolor also uses a release call which is generally given when inappropriate clasping has taken place. It is likely that there are differences in the neural and hormonal control of these different call types (Tito, *unpublished* 1998). The experiment is divided into three components. The first was created to observe preferences in how male Hyla versicolor react to two different calls played to them in a controlled lab setting. The second component deals with how male Hyla versicolor react to calls played back to them in their natural habitat. The third component handles the effects of vasotocin (AVT) on the calling behavior of the frogs.

Phonotaxis experiments have been run extensively with respect to a female responding to calls of males; Phonotactic responses can be readily elicited in the laboratory by the playback of appropriate acoustic stimuli, including synthetic sounds (Gerhardt, 1987). Although much research has been done in the area of female selection of male companions, much less has been published as to males responses to other male calls (aggressive, advertisement, or release). Thus, we tried to determine how male Hyla versicolor reacted behaviorally through a preference system

to various calls of males of their own species which were prerecorded.

The playback experiments which we ran were designed to test the effects of different call types on the call rate, duration, and pulse rate of male Hyla versicolor in their natural habitat. For instance in the male Hyla ebraccata the response to the calls of neighboring males or to the approach of females was often a change of rate, duration, or complexity of their own call (Wells and Schwartz, 1984). Also in the Hyla ebraccata it was observed that playbacks typically evoked advertisement calls from the males while aggressive calls only comprised a small portion of the responses (Allan and Simmons, 1994). Different types of calls were played to the frogs in the field and then their own call back was recorded, thus determining the type of calls evoked by the playbacks as well as some of the features of that call so as to determine whether the animal changed any feature of the call.

Research on a wide variety of vertebrates, from fish to mammals, reveals that corticosteroid hormones and vasotocin-like neuropeptides can potently modulate reproductive behaviors (Rose, Kinnaird, Moore, 1995). This being the case, the experiment with AVT was designed to test the effects of AVT on the calling behavior of male Hyla versicolor with respect to different properties of their calls. It has been found that AVT increases mate call frequency and decreases calling latency in male bullfrogs (Boyd 1997). Although it has been found that AVT has a profound effect in eliciting advertisement calls from male amphibians, it has not yet been shown what effects this drug has with respect to other call types in the Hyla versicolor.

Methods:

This project was separated into three experiments which were testing different hypotheses. The three parts to this project include a phonotaxis study, a play back experiment in the field, and a calling experiment with the use of AVT.

Collection and Treatment of Animals:

For all three experiments we caught Hyla versicolor from Bog Pot bog at UNDERC in Land O'Lakes, WI. Fifty five male and two female Hyla versicolor were collected between the hours of 2100 and 2300 on the days between May 19, 1998 and June 15, 1998. Each animal was housed in an individual clear plastic cage (1'X6" X4") with a metal screen top and fed moths or meal worms every 3 days.

Phonotaxis:

For the phonotaxis experiment a grid was created on the floor of the wet lab room. The grid, made out of black lab tape was 12X14 squares. Each square was 6"X6". Two identical Radio Shak tape recorders (Marantz) were used. The speakers were placed on the floor at the corners of the grid propped up back two identical black trash cans. The speakers were set up vertically against the trash cans. The four fluorescent overhead lights in the room were always on during the experiment. The room door was closed during the experiment and garbage bags were placed underneath the door to muffle any outside sounds. The temperature on the days of phonotaxis ranged between 60-70 degrees F. Only male Hyla versicolor were tested. Each frog was taken out of the cage and the snout vent length was taken. Two tapes were put in the recorders and the tapes were started simultaneously. The tapes were 90 minute loop tapes which had recordings taken from field research in 1996 on the male Hyla versicolor. There were 3 different loop tapes. One tape had just the advertisement call. One tape had just the aggressive call. One tape had the

advertisement and aggressive calls alternating and hence was referred to as the mix tape. The volume at which the tapes were played was standardized each time to level 10 and the tone was standardized at 5. The animal was placed in the exact middle of the grid while the observers sat off to the side of the grid and observed the animal's behavior. This process was repeated for 5 different animals. The experiment was run until the frog left the grid or 10 minutes had expired. The two tapes were swapped to avoid any bias according to speaker. The speakers were then switched to two opposite corners to eliminate variance in the room and again 5 animals were tested. This process was repeated for 90 trials.

FieldPlayback Experiments:

In the play back experiment equipment, was taken out into Bog Pot in order to play and record calls from the calling male Hyla versicolor. The experimenters measured the distance from the calling male with a meter stick. The microphone and the tape recorder were approximately one meter away from the calling animal. While one experimenter played a call to the animal, the other experimenter used the microphone to record the animal's response to the call. The same loop tapes described above (recordings in 1996) were used for this experiment as well. After 5 minutes of one type of call, the tape was changed to another other call and the process was repeated. Towards the end of the experiment it was realized that the mixed tape was useless in this particular experiment and thus only the aggressive and the advertisement were used. Again the volume and tone were set at 10 and 5 respectively. The calls were later analyzed in the lab with the Interactive Laboratory System software (ILS; Signal Technology).

AVT injections:

The experiment which was testing the effects of AVT on the calling behavior of male Hyla versicolor took place in the wet lab at 2200 for several nights. The Hyla versicolor used for this experiment, were caught in Bog Pot bog they were kept in captivity from 3-14 days before this experiment was run. The first night of the experiment half of the frogs were injected with AVT and the other half with saline. The second night of the experiment (24 hours after) the frogs which were previously injected with AVT were injected with saline and vice versa. The snout vent length of the animal was taken with a ruler. The mean snout vent length in this study was 4.7cm +- .1SEM. The frogs were placed into individual Styrofoam coolers (30X30X50 cm) 12 hours prior to the injections to acclimate themselves to their new environment. The coolers were arranged so that every other cooler had a frog in it which was going to be injected with AVT. The other frogs were injected with saline. The experimenters injected the animals with either 100ug AVT (Sigma Chemical Co) or.1ml amphibian ringer's saline. The experimenters then waited for the drug to kick in. After about 30 minutes the AVT injected frogs would begin to call and the experimenters recorded calls with a Marantz microphone and tape recorder for at least 5 minutes, as well as recording by hand calls/minute. The calls were analyzed by ILS (as described before).

Results:

The phonotaxis experiment gave some insight into the reaction of male Hyla versicolor to hearing other same species and sex call types. The same calls which were played simultaneously caused the animal to either move off the grid all together or to stay on the grid showing no preference. This was true across the board with the (adv-adv, agg-agg, mix-mix, and static static) trials, except for the second trial of the agg-agg tape where 3/5 frogs chose speaker 1. When faced with speakers playing opposite calls the Hyla versicolor showed some preferences by clinging on to the speakers playing specific calls. Of the 30 times that frogs were tested with one speaker playing the aggressive and one playing the advertisement call, 7 of the animals chose the speaker playing the advertisement call while only 3 chose the speaker playing the aggressive call. The rest of the animals either stayed on the grid without choosing a speaker or they moved off the grid. The sequence of playing the mixed loop with the advertisement loop resulted in 2 fogs choosing the mix tape and one choosing the advertisement, while the 17 other either stayed on the grid or jumped off the grid. When the aggressive loop and the mixed loop were played simultaneously 2 frogs jumped on to the aggressive speaker and one clung to the mixed speaker, while the 17 others again either stayed on or jumped off the grid. When static was played with the advertisement loop 4/10 animals jumped onto the speaker with the advertisement call and the other 6 either stayed on the grid or jumped off. When the aggressive call was played along with the static one animal chose the speaker with the aggressive call and one chose the speaker with the static. The remaining 8 either stayed on or jumped off the grid.

In the playback experiments, the aggressive and advertisement loop tape was played to male Hyla versicolor in the field and their response to the tapes was recorded. In every case an advertisement call was given in response to the call played at the frog. Although this was true, the characteristics of the call given in response to

the loop tape varied. The temperature recorded during the playback experiments ranged from 58 to 76 degrees Fahrenheit. When the advertisement call was played to the frogs the return calls/minute ranged from 3-20. The mean was 12.2267 and the Standard Error of the Mean was +/- 1.0430. The mean for the calls in response to the aggressive loop tape was 13.2018 with SEM +/- 1.0469. The call duration in response to the aggressive call was almost half of what it was to the advertisement loop. The intercall interval was also significantly smaller. It was 1.88 seconds in response to the aggressive loop and 4.162 in response to the aggressive loop. Both the fundamental and dominant frequencies of the advertisement loop were larger than the aggressive. Finally, the calls per minute and calls per 5 minutes were drastically different when comparing the advertisement (13, 65) and the aggressive (23, 115).

In the AVT experiment, 7 animals who had previously not been calling at all were injected with AVT. All but one of these animals began to call within the first 30 minutes of the injection. The animals injected with saline also had been silent. Of the seven animals injected with saline (combined on both days) none of the animals began calling. Before the second injection 2/7 males were giving spontaneous aggressive calls along with their advertisement calls. These aggressive calls were maintained in 3 of seven AVT-injected animals, but the saline injected animals stopped calling immediately. After the animals had been injected with saline the first day and then AVT the second day, their calls were not purely advertisement ones. There was a mixture of calls in the frog's repertoire after being injected the first day with saline and the second day with AVT. The fist night of injections the AVT animals gave only advertisement calls. The second night of injections, the animals who were injected with AVT gave a different call type along with the advertisement call. This different call type was not any of the classic three types (advertisement, aggressive, or release). This new call type was not interspersed between the advertisement calls at any regular interval and was not quantified. Hence, only the advertisement call type

could be compared. The first night of injections, May 31 1998 three of the four animals injected with AVT who called had call rates that were faster and fundamental and dominant frequencies which were larger than the animals who were injected on June 1, 1998. The animals injected on June 1 had been injected with saline 24 hours previous to the AVT injection (see Table 1).

Discussion:

The phonotaxis experiment attempted to determine whether male Hyla versicolor show preferences when presented with different types of calls from their own species. Thus, the experiment was designed to test the reaction of these frogs to the advertisement, aggressive, and mixed loop tapes and to somehow quantify these results. Interestingly enough, none of the animals called back to the loop tapes. The hypothesis was that the frogs would have a significant aversion to the aggressive call, mainly because the nature of the call and when the aggressive call is used. Six of the total number of frogs tested chose the aggressive call, while twelve chose the advertisement call at some point in the experiment. The most striking result came when the aggressive and the advertisement call were played simultaneously because it was at this time that seven animals chose the advertisement and three chose the aggressive. Although these results may seem not to be significant enough to discuss, there was a generally attraction of the frogs to the advertisement call. It was not quantified in this experiment because to show preference the animal had to come within 10cm of the speaker, which only a few frogs actually completed. When the results of the experiment were analyzed further though, many of the frogs ended up facing the advertisement call and many more frogs ended up leaving the grid away from the aggressive call. This shows one of the weaknesses in this experiment. A better way to quantify preference may need to be researched because many of the frogs turned away from the aggressive call and were facing the advertisement call- even hopping near the advertisement call, but never actually jumped onto the speaker, and these frogs were then left out of many of the statistics. In addition the room which was used for the phonotaxis experiment had some variability in it, which we thought was compensated for by switching the position of the speakers, but this aspect may need to be looked into further. The loop tapes also had some variability within them, some were faster than others and quieter than others. Although the volume of the

speakers was standardized, the tapes were not and therefore may have had some effect on the experiment. The room had a large window on one side of the room and a noisy experiment was being run in the room adjacent to the experiment room. Finally, the temperature range of when the phonotaxis was preformed was large and this coupled with the fact that the floor was cement and thus quite affected by temperature change may have had some bearing on the results of this experiment. Although the data we obtained does not easily lend itself to many conclusions or significant differences, phonotaxis itself has been studied for many years. There have been several studies done with female phonotaxis reacting to male call types. Preferences have been shown in these studies with respect to call rate, duration, and pitch (Gerhardt, 1987). Although the aggressive call has not been studied as intensely as the advertisement call, and the effect of the aggressive call in phonotaxis has not actually been tested on males before, there has been some text written on the call itself. The aggressive call has been documented briefly in some works (when males and females have been inappropriately clasped)(Pierce and Ralin, 1972; Fellers, 1979; Schwartz, 1987).

The playback experiments were also designed to test the reaction of male Hyla versicolor to other same species and sex calls. Specifically, we were interested in the frog's reactions to the aggressive call. Although no aggressive calls were elicited in the field there was significant difference in the characteristics of the response within the advertisement call to the played aggressive call. What the call duration in the playback experiment suggests to me is that there is a very different reaction of the animals to the aggressive call. The animals clearly recognized a difference in the two loop tapes, and the call characteristics then also varied. Perhaps when the male hears the aggressive call, he adapts to this by increasing the call rate either as a scared response or to attract females that might also hear the aggressive call of his companion. There were some obvious problems with this experiment because the

nature of working in a bog. Although one meter was standardized in each recording, the frogs were at somewhat different distances from the microphone whether they were in a shrub, or on the ground. The loop tapes that were used also had alternating calls, which was probably compensated for because they kept repeating themselves, but could be amended for more accurate results.

The final experiment tested the effects of AVT on Hyla versicolor. The animals responded after the initial injection by giving purely advertisement calls, while the frogs injected with saline gave no calls. Advertisement calling is stimulated by AVT in bullfrogs (Boyd, 1994), green treefrogs (Penna, Capranica and Somers, 1992), cricket frogs (Marler, Chu, and Wilczynski, 1995; Chu, Marler and Wilczynski, 1998), and the Great Plains toad (Propper and Dixon, 1997). The effect of AVT on aggressive calls, however has not been recorded. The second night when we injected the Hyla versicolor with AVT or saline according to what they had got the previous night, there were a mixture of call types along with the advertisement. The AVT ended up not giving a significant difference in call parameters because only a few of the animals gave aggressive calls. More often though the frogs would give different calls mixed in with their advertisement calls when given the AVT. The reason for the mixture of call types and the fact that a definite call type could not be determined on the calls given by the Hyla versicolor the second night was probably due to the fact that the animals were stressed. After being injected the first night with saline and then subjected to another injection 24 hours later while residing in a cooler most likely stressed out the animals to the point where they gave this different call. This call which was given was not given on a consistent basis and was very tense which leads me to believe that it was a result of the stress we put the animal through, rather than the AVT itself.

Table 1

Effects of AVT or saline injection on advertisement calling in male *H.versicolor* under laboratory conditions on two consecutive nights (means ± SEM).

	May 31 1998		June 1 1998	
Parameter	**AVT**	**Saline**	**AVT**	**Saline**
Number of males calling/total number of males				
baseline	0/4	0/3	0/3	0/4
90-min	3/4	0/3	3/3	0/4
Call Rate (calls/min)				
baseline	0	0	0	0
90-min	9.0±2.2	0	5.7±2.3	0
Pulse number (pulses/call)				
baseline	0	0	0	0
90-min	11.03±.42	0	9.43±.88	0
Call duration (sec)				
baseline	0	0	0	0
90-min	.997±.057	0	.859±.063	0
Intercall interval (sec)				
baseline	0	0	0	0
90-min	2.6±.24	0	3.27±.425	0
Fundamental frequency (Hz)				
baseline	0	0	0	0
90-min	1207.7±21.7	0	1088.3±13.3	0
Dominant frequency (Hz)				
baseline	0	0	0	0
90-min	2159.7±39.8	0	2005.0±132.6	0

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