# Arginine Vasotocin (AVT) Influences Aggressive Call Behavior in the Gray Treefrog, *Hyla versicolor*

Independent Research Project

Amy K. Evans and Sunny K. Boyd
Department of Biological Sciences
University of Notre Dame, Notre Dame, Indiana 46556

August 10, 1999

## Abstract

Arginine vasotocin (AVT), a neurohypophysial peptide hormone which controls aspects of reproductive physiology in all vertebrates, has been recently identified as a regulator of behavior in many species. In some amphibians, AVT plays an unknown role in the regulation of social and reproductive vocal behavior. In this study, the effects of experimentally administered AVT on the stimulated and non-stimulated aggressive calling behavior of the male gray treefrog, *Hyla versicolor*, and the male green frog, *Rana clamitans*, were to be determined through a series of playback experiments in the field. No real or significant results were obtained, however, due to an unusually cold mating season and several technical difficulties. Some suggestions are offered for eliminating some of these problems and easing the mechanics of future studies.


## Introduction

Arginine vasotocin (AVT), a neurohypophysial peptide hormone in the same family as arginine vasopressin and oxytocin, has been localized throughout the brain, spinal cord, and cerebrospinal fluid of all vertebrates (Moore 1987). It performs the "traditional" hormonal functions of physiological reproductive control, regulating processes such as lactation, uterine contractions, and hydromineral balancing, but in recent research attention has turned increasingly toward the evidence for the effects of AVT on vertebrate behavior. In killifish, AVT has been shown to influence spawning behavior, while it affects oviposition-associated behaviors in several species of geckos. It has been

suggested that AVT plays a role in imprinting and memory processes in ducks and in the sexual behavior of pigeons. In mice and rats, AVT affects memory and learning, copulatory performance, and maternal behavior (reviewed in Moore 1987). Arginine vasotocin has been repeatedly identified as one of the predominant regulators of social and reproductive vocal behavior in amphibians (Boyd 1994; Hilscher-Conklin 1998; Tito 1998). The hormone's concentration in the brain may be affected by levels of gonadal steroid hormones in some animals, further implying its role in controlling sexual behaviors (Boyd 1994). When experimentally administered to vertebrates, AVT can elicit certain behaviors in the absence of normal releasing stimuli. Arginine vasotocin may also function as a neurotransmitter, as it can also increase or decrease an animal's sensitivity to various stimuli (Moore 1987).

Arginine vasotocin clearly influences vocal behavior in frogs, but it has not been determined whether the variability it produces results from differences in frog sex, species, or call type. Due to their heavy reliance on vocalization for communication with conspecifics and their behavioral sensitivity to neuropeptide regulation, anuran amphibians provide excellent model systems in which to study further the effects of AVT on the calling behavior of frogs. The call repertoire of the male gray treefrog, the anuran *Hyla versicolor*, has been extensively studied and characterized (Fellers 1979; Schwartz 1987; Tito 1998) as three basic call types: the advertisement, release, and aggressive calls. Male treefrogs use the advertisement call for purposes of species recognition and attraction of females. Both male and female treefrogs give the release call when inappropriately clasped by a conspecific. Male gray treefrogs are highly territorial and defend their territories by means of the aggressive, or encounter, call in order to maintain spacing and

to establish dominance among themselves (Fellers 1979). The effects of AVT on the advertisement call and, to a much lesser degree, on the less important release call of *H. versicolor* have been recently studied (Tito 1998); however, the influence AVT exerts on the aggressive call has not yet been thoroughly examined. In frogs with distinctly different multiple call types, it is suspected that each call type may be differentially affected by AVT (Tito 1998). The stimulus conditions most likely to elicit an aggressive call response in male gray treefrogs will therefore be determined and utilized in order to examine the effects of AVT on the characteristics of stimulated and non-stimulated aggressive calling behavior in this species.

## Materials and Methods

*Experiment 1: Determination of Optimal Conditions for Eliciting Aggressive Call Response in* Hyla versicolor

All animals used were collected in Gogebic County, Michigan, during May, June, and July of 1999. Care and use of animals conformed to NIH guidelines. Between 9:30 pm and 11:30 pm, sexually mature male gray treefrogs (*Hyla versicolor*) were located in the field. The experiments were meant to be run immediately where each frog was found. In order to determine the stimulus conditions that would optimally elicit an aggressive call response from the frogs, a pre-recorded audiotape was prepared with both advertisement and aggressive calls from male *H. versicolor* at high call rates. Using a Dan Gibson EPM parabolic microphone and Radio Shack tape recorder, the initial stimulus tape was played to each frog so that he heard a total of six stimulus types, presented in random order (five minutes each): the advertisement call recording at low, medium, and high volumes (to

mimic varying proximity of another calling male frog) and the aggressive call at low, medium, and high volumes. The stimulus tape was played to an isolated frog (n=10, ideally) while his response was simultaneously tape-recorded using the previously described equipment. The initial stimulus tape contained too much background noise, however, so one male frog was recorded in the field and his advertisement calls used to produce a new stimulus tape of three identical five-minute call segments. This tape was then played to an isolated frog, and his response simultaneously recorded, in the format of one 5-minute call segment followed by a 3-minute interval of silence, followed by another call segment, then 3 minutes of silence, then the third call segment (call segments presented randomly at low, medium, and high volumes for each frog so that each frog was exposed to each call volume). Results were to be analyzed by listening to recordings and counting the number and rate of aggressive calls given by each frog after each stimulus presentation.

*Experiment 2: Effect of AVT on Stimulated Aggressive Calling in* Hyla versicolor

To determine the effects of AVT on the stimulated aggressive calling behavior of male gray treefrogs, frogs were to be captured in the field between 10:30 pm and 12:30 am and isolated in individual Styrofoam tanks (30 x 30 x 50 cm) at the collecting site. Five-minute baseline recordings of the calls given by each frog were to be made using the equipment previously described. The frogs would then have been injected intraperitoneally either with 0.1 mL amphibian ringer's saline alone (n=10, ideally) or with 100 ug AVT (Sigma Chemical Co.) diluted in 0.1 mL saline (n=10). There would also have been a control group of non-injected frogs (n=10). AVT, saline, and control tanks

would have been alternated between each frog in order to expose all animals equally to environmental conditions, including vocal neighbors. At 90 minutes following injection, the optimal stimulus type recording (determined by call type and volume and derived from the previous baseline experiment) would have been broadcast for 10 minutes to each isolated frog while his response was simultaneously recorded. Recordings would have been analyzed by counting the number and rate of each call type. Effects of AVT were to have been determined by comparing post-AVT-injection recording results with those results obtained from saline-injected and non-injected frogs.

*Experiment 3: Determination of Optimal Conditions for Eliciting Aggressive Call Response in* Rana clamitans

An experiment identical to Experiment 1 was meant to be carried out in the same locations, at the same times, and under the same conditions with sexually mature male green frogs (*Rana clamitans*), a territorial ranid species with distinct advertisement and aggressive calling behavior. One male was initially recorded in the field for purposes of creating a stimulus tape identical to that used in the playback experiment with the Hylas. The tape was to be played to other isolated male frogs (n=10, ideally) in the same manner as in Experiment 1 while they were simultaneously recorded using the equipment previously described. Results were to be analyzed by listening to recordings and counting the number and rate of aggressive calls given by each frog after each stimulus presentation.

*Experiment 4: Effect of AVT on Non-Stimulated Aggressive Calling in* Hyla versicolor

To determine the effects of AVT on the non-stimulated aggressive calling behavior of male gray treefrogs, frogs were to be captured in the field between 10:30 pm and 12:30 am on a single night and isolated in individual styrofoam tanks (30x30x50 cm) at the collecting site. Ten-minute field baseline recordings of the calls given by each frog were to be made using the equipment previously described. All frogs would then be moved, in their tanks, to the wet lab where ten-minute lab baseline recordings would be made of each frog. On the next night, half of the frogs (n=10, ideally) would have been injected intraperitoneally with 0.1 mL amphibian ringer's saline, while the other half (n=10) would have received injections of 100 ug AVT diluted in 0.1 mL saline. Five-minute recordings would be made of each frog's calls at 30, 60, and 90 minutes following injection. This injection and recording procedure would be repeated exactly on each subsequent night, with individual frogs receiving the same treatment each night, until all frogs ceased calling. Results were to be analyzed by comparing post-injection recording results between AVT-injected and saline-injected frogs.

## Results

No real or significant results were obtained from this study. An unusually cool summer severely limited the number of frogs calling in the field on any given night, and frogs seemed not to be out at all on most nights of the season. In the beginning of Experiment 1, the original stimulus tape proved to consist of too much background noise to be useful in the field, and adequate equipment for making the new stimulus tape loud enough for playback use could not be found. As a result of these setbacks, no treefrogs

were recorded consistently enough to yield valuable data . Experiment 2 was never initiated due to the failure of Experiment 1 to determine the optimal conditions for eliciting an aggressive call response in the male gray treefrog. However, frogs were not present in great enough numbers to have allowed execution of the entire experiment on any given night .

Due to these difficulties in conducting the planned experiments, focus was turned to the male green frog, *Rana clamitans* in an attempt to conduct a study identical to Experiment 1 with the Hylas. *R. clamitans* was a suitable subject because, like *H.versicolor,* it is territorial and has distinct advertisement and aggressive calling behavior. However, the weather seemed to be too cold for these animals as well as very few were out calling in the field. Also, it proved extremely difficult to learn to get close enough to these frogs to get good recordings without scaring them away. One male frog was finally recorded and a stimulus tape made, but then frogs apparently ceased to be out and no subsequent recordings were made.

Fortunately, another Hyla population was found in a new location (Gravel Pit) and Experiment 4 was devised. The very next night, however, absolutely zero frogs could be heard anywhere on the property, a trend which continued until the end of the last research period. The experiment was never initiated and no data obtained.

## Discussion

No conclusions were drawn from these experiments due to lack of any real or significant results. In order to maximize quality data and results in future field studies of similar nature, several guidelines are suggested. Make sure that all materials and

equipment to be used in the field are functioning properly and as expected, possibly testing them in model field conditions before relying on them during the actual experiments. Before starting any experiments, scout out the property to find the largest, loudest, most promising frog populations so that if the animals cease calling in one location, it may be possible to begin again at a new location with a minimal time loss. It may be a good idea to become familiar with more than one species and to plan more than one possible experiment, determining upon arrival at the property which approaches will work best under given conditions.

The effects of arginine vasotocin on the stimulated and non-stimulated aggressive calling behavior of the male gray treefrog, *Hyla versicolor*, remain unknown. Future studies may address these questions, possibly repeated experiments attempted and described in this paper, or may focus on the effects exerted by AVT on the calling behavior of the male green frog, *Rana clamitans*.

## References Cited

Boyd, S. K. (1994). Gonadal steroid modulation of vasotocin concentrations in the bullfrog brain. *Neuroendocrinology* 60: 150-156.

Fellers, G. M. (1979). Aggression, territoriality, and mating behavior in North American treefrogs. *Anim. Behav.* 27: 107-119.

Hilscher-Conklin, C., Conlon, J. M., and Boyd, S. K. (1998). Identification and localization of neurohypophysial peptides in the brain of a caecilian amphibian, *Typhlonectes natans* (Amphibia: Gymnophiona). *J. Comp. Neurol.* 394: 139-151.

Moore, F. C. (1987). Behavioral actions of neurohypophysial peptides. *Psychobiology of Reproductive Behavior: An Evolutionary Perspective*. D. Crews, ed. New Jersey: Prentice Hall. 61-87.

Schwartz, J. J. (1987). The function of call alternation in anuran amphibians: a test of three hypotheses. *Evol.* 41: 461-471.

Tito, M. B., Hoover, M. A., Mingo, A. M., and Boyd, S. K. (1998). Vasotocin maintains multiple call types in the gray treefrog, *Hyla versicolor*. (in press).