

Corticosterone Inhibition of Release Calls in Male *Rana clamitans*

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Introduction

Corticosterone steroid and neuropeptides have increasingly been looked into as inhibitory controls for male reproductive behavior. Seasonal neural interactions of gonadal steroids and neuropeptides have been identified to facilitate male courtship behavior (Moore, Lowry, and Rose, 1994). Research on various vertebrates has revealed that corticosteroid hormones play an important role in modulating reproductive behaviors (Rose, Kinnaird, and Moore, 1995). Amphibian research on the interaction of the drug with behaviors has been concentrated primarily on the rough-skinned newt, *Taricha granulosa*. The reproductive behavior of male newts begins with courtship and the capture of a female in a dorsal amplexic clasp. The amplexus will last from a few hours to several days. Completion of amplexus occurs when the male deposits a spermatophore for the female to pick up in her cloaca (Moore and Miller, 1984). Stressful events or a harsh environment has been shown to disrupt reproductive behaviors (Moore and Miller, 1984). Moore and Miller (1984) noted that newts exposed to stressors, displayed a decrease in courtship activity as well as showed increased plasma corticosterone concentrations. Thus, the assumption that elevated levels of corticosterone, induced by stress, were the main inhibition to courtship behaviors (Moore and Miller, 1984). Male clasping of females is promptly blocked by exposure to corticosterone (Rose, Moore, and Orchinik, 1993). When male newts are injected peritoneally with corticosterone (1, 5, 10, 15, 20, 25 μ g), sexual behavior decreases in proportion to the administered dose of corticosterone (Moore and Miller, 1984). The control of male *Taricha* reproductive behaviors was found to be part of an inhibitory pathway (Moore and Orchinik, 1992). Later, this pathway was expanded to focus on the action of corticosterone as it binds to a

neuronal membrane receptor, inducing rapid neurophysiological effects which inhibit the male reproductive behaviors.(Moore, Lowry, and Rose, 1994).

In the green frog, *Rana clamitans*, mating behavior is similar to the rough-skinned newt in that courtship is begun with clasping and amplexus occurs over a period of hours to days. The behavior of a green frog is characterized by two types of calls, an advertisement call and a release call. The release call is typically given in response to the male clasping of another male. The release call functions within the realm of courtship behavior. This study will allow a determination to be made as to whether or not the release call is under the control of corticosterone.

MATERIALS & METHODS

All animals and organic materials used came from various property lakes at the University of Notre Dame Environmental Research Center (UNDERC) in Gogebic County, Michigan. The project took place during June and July of 1998.

Several strategies were undertaken to elicit release calls from *Rana clamitans* over the ten-week period. The first strategy attempted was to capture male green frogs from Roach Lake and maintain them in captivity. The frogs would then be massaged to imitate clasping. This activity on the male frogs would result in the elicitation of release calls. The first collection brought in 7 green frogs. These were medium in size with a snout-vent length (SVL) ranging from 4.0 cm to 6.6 cm. The frogs were housed in plastic cages with a metal screen cover, containing water and sediment from Roach Lake. The frogs discontinued giving mating calls while in captivity except for a one hour period during the second day in confinement when the cages were taken outside (temperature: 76°F). The second collection of frogs at Roach Lake brought in 11 green frogs. The frogs would give release calls while in the cages as individuals jumped on top of males. The frogs were removed from the cage one at a time and massaged to induce calling. Only one frog of eighteen gave calls after being handled in a manner not conducive to experimentation. No calls were given as individuals were manually placed on top of males. The focus of the research remained on keeping the frogs alive by feeding them leaf worms and massaging them frequently to see if calls could be obtained. The frogs were maintained in captivity by feeding them primarily leaf worms purchased at Whitey's Bait and Tackle on Palmer Lake Road. Each frog consumed approximately 1-2

leaf worms every three days. The green frogs would also eat wax worms however in larger quantities than the leaf worms due to their smaller size.

The plan was to become skilled at getting the calls. Once this happened, corticosterone could be administered to the male frogs. The test group was starved for five days prior to the experiment in order that they consume the drug. The experiment involved injecting leaf worms with 0.25 micrograms of corticosterone dissolved in 0.1 ml of vegetable oil. The male frogs would be massaged for release calls for a 2 minute period. This would be their baseline calling rate. The injected leaf worms would be immediately fed to the frogs and after 30 minute period, the males would again be massaged to elicit release calls. Half of the frogs would receive leaf worms injected with the corticosterone/oil solution, while the other half would receive leaf worms injected with 0.1 ml of oil. The reciprocal experiment would occur with the same animals following a 24 hour period. During the first 7 weeks of the research period, the frogs would not give release calls and slowly died off due to living in captivity.

The second strategy was to take remaining male frogs and place them in outdoor pens. Two pens were constructed of wooden frames 8' x 4' x 3 ½', covered with wire mesh, leaving the top open. This would allow insects to enter the pen and nourish the frogs. The pens were placed on the shore of Roach Lake. This second strategy was much like the first except that it would be conducted outdoors, allowing the frogs to acclimate to their environment, acting as a stimulus for calling. Nine male frogs were placed in the outdoor pens. After one week of no calling, either mating or release, the frogs were either dead or had escaped out an overlooked hole in the mesh.

The last attempt to get frogs to call involved collecting more males and placing them directly in the outdoor pens. Then, the experiment would continue as planned, having bypassed the indoor captive period. Five males were caught from various property lakes and left in the cages for three evenings. During this collection which took place in mid-July, few green frogs were of a suitable size. The majority of the populations were small, 2-3 cm SVL, especially at Hummingbird Lake. No calling of any kind occurred during this period. The frogs were released and the pens were placed in the storage shed.

An AVT experiment was performed using green frogs. Males from the group used during the first strategy were used in the injection experiment. The frogs had been kept in captivity for 6 days prior to the injection experiment. The mean SVL was 5.53 cm. The frogs were housed separately in styrofoam coolers from approximately 30 minutes prior to injection and through the duration of the night. Four frogs were injected with 100 micrograms of AVT in 0.1 ml of saline. Four different frogs were injected with 0.1 ml of saline. The injections began at approximately 10:00pm. The frogs' calling was observed for a period of 90 minutes after injection. No calls were given by any of the frogs.

Discussion

No results were obtained on how corticosterone or AVT interact in the control of reproductive behaviors of green frogs. In order to do the experiment, release calls must be elicited on a reliable basis and the frogs must be maintained suitably in captivity. Both of these tasks are extremely difficult to achieve with green frogs.

The AVT experiment obtained a result of no calling from the green frogs. Two possible explanations exist. The first is that green frogs stop giving mate calls after a disturbance even when remaining in their natural environment as in the case of the outdoor pens. Secondly, perhaps AVT doesn't play a role in the control of male mate calling in green frogs. Since only a 100 milligram dosage was given, a high dosage could be administered. This may however become too expensive for purposes at UNDERC.

During the first strategy, release calls could not be elicited by either massage or frog-to-frog interactions. One frog exhibited calling only when in the tight confines of a fist which is not an experimentally valid procedure. While in the cages however, away from human contact, several frogs gave calls frequently during the first few days of captivity. Two approaches remain to trying this experiment again. The first is to gather a large number of males together and mark individuals. Place them in a cage together and perform the experiment. Gather baseline calling by manipulating the frogs to jump on each other yet keep human contact to a minimum. Feed the frogs in isolation to insure that each frog receives the appropriate variable. Observe and record calling patterns at the 30, 60, 90 minute intervals following consumption. The second and better approach to eliciting release calls is through stressful exercise. Chase the frogs along a standard distance to heighten activity and metabolism. Then use massage to elicit the release calls.

Following this calling baseline acquisition, feed the frogs the injected worms and perform the activity and calling sequence at the time intervals following consumption. This should produce more reliable eliciting of release calls. The procedure has worked with *Hyla versicolor* tree frogs who have been maintained in captivity for a short period of time. One key to the project is to work quickly after capturing the frogs and do the experiment within the first 48 hours. If the frogs have been in captivity after 5 days, the chances of eliciting behaviors are greatly reduced.

The outdoor project resulted in failure for two main reasons. First, the frogs released to the pens from captivity were no longer suitable for the project after the extended stay in the laboratory. Secondly, the mesh of the cages was not conducive to the feeding patterns of the frogs despite the open top design. The frogs that were placed in the pens immediately after capture showed signs of malnutrition by the time they were released. Had these circumstances not played a role in the experiment, the project would still have been difficult and nearly impossible. Tracking the individual frogs in the pens posed a problem. In one case, the outdoor pen project would require the use of inhibiting the mating call rather than the release call. This is due to the fact that the frogs were not eliciting release calls. Had the release call still been desired, the use of outdoor pens becomes worthless since the acclimated environment is infringed upon by human intervention and laboratory conditions are much more conducive to experimentation. Therefore the mating call is required in the outdoor pen situation. Assuming that the frogs had called when contained in the pen, a strong chorus in that population was not heard until the hours of 10:00 or 11:00 pm. Thus, the frogs would be extremely difficult

to track in the darkness and un-intrusive red-hue lights would not be of much assistance.

For these reasons, the outdoor pen method is not advised.

Taking into consideration, all the attempts with and the experiences of the green frogs, the final and most important recommendation is to ditch the green frogs altogether. The project would be much easier to conduct with *Hyla versicolor* frogs when care in captivity considerations are analyzed. These tree frogs are receptive to consuming crickets of a decent size, making them a good candidate for this project. These frogs have been shown to give release calls to human stimulation much more readily than the green frogs and are considerably easier to maintain in captivity.