Identifying the Physiological Effect of Introduced Stress to Frogs and Quantifying Behavioral Response

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Introduction

Physiological stress response in amphibians is regulated by the glucocorticoid hormone corticosterone, secreted by the hypothalamo-pituitary interrenal axis. Secretion of corticosterone causes changes in physiology and behavior in order to survive with the stressor. These changes include hiding in defense, increased locomotor activity, increased thermoregulatory behavior, and tonic immobility or “playing dead.” In this study, we investigated whether an environment of poverty or the threat of capture induced a corticosterone response in the leopard frogs using both behavioral analysis and corticosterone enzyme-immunoassay (EIA) to measure conjugated urinary corticosterone metabolites.

Methods

Captive Housing

Leopard frogs were randomly assigned into two tanks labeled as “enriched” or “impoverished.” The enriched environment included upgrades to standard laboratory housing such as moss, bamboo, water, an abundance of rocks, branch roots, dirt, lighting and hiding areas. The impoverished environment is the standard care for laboratory frogs.

Experimental Design

Frogs were named and photographed to organize data and experimental control. They were housed in the same room to control for temperature and auditory differences and handled by one experimenter consistently and only interrupted for feeding, cleaning, or experimental purposes. The responses of frogs to two experimental treatments were examined for 8 weeks after an adjustment to an environment adjustment period of one week. Half of the frogs from each tank underwent the capture stimulus prior to tonic immobility testing and all frogs underwent urine collection process for EIA analysis of corticosterone metabolites secretion.

Behavior

Capture stimulus was conducted through protocol of first the frog identification and selection from tank. They were then gently placed down on starting position on a damp open laboratory floor. Experimenter attempted to, and when captured held frog for 3 seconds and placed in start position. This was done 10 times with the Tonic Immobility (TI) test conducted immediately after. After capture stimulus or after identification and selection from the tank for non-capture stimulus frogs the frog was held in experimenter hands for 10 seconds before being placed in starting position on damp, marked, open laboratory floor. Timer was begun immediately, and frog was given a maximum time of 120 seconds or maximum distance traveled of 2.5 feet. Once one of these maximums is reached testing was concluded, frog was replaced into housing, distance traveled and time elapsed was recorded.

Corticosterone enzyme-immunoassay

This was used to quantify corticosterone urine concentrations and was used following procedure from manufacturer.

Results

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Discussion

The original hypothesis was that those with increased stressful stimulation would display a longer tonic immobility, or period of playing dead. Results show the opposite, with those in the stressful impoverished tank having increased locomotor activity.

Corticosterone enzyme-immunoassay could not be utilized for data analysis as Alvernia did not have a plate reader to analyze results.

Conclusions

Frogs in the intended more stressful environment show increased locomotor activity. Using only behavioral analysis, this locomotor increase included both a lower amount of time to travel, and a larger overall distance traveled, on average. It is unknown if this was in response to urine corticosterone concentrations, as this data could not be quantified using the corticosterone enzyme-immunoassay due to absence of a plate reader in the laboratory.

References


