Effects of Exogenous Corticosterone on Locomotor Activity in the Red-Eared Slider Turtle, *Trachemys scripta elegans*

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ABSTRACT We investigated the effects of exogenous corticosterone on the locomotor activity of captive red-eared slider turtles, *Trachemys scripta elegans*. An increase in plasma corticosterone often increases locomotor activity in mammals and birds, but there are no reported findings for turtles. In this study turtles implanted with corticosterone-filled Silastic® implants showed a significant increase in caged locomotor activity when compared to control animals with empty implants. Corticosterone-treated turtles also showed a significant increase in plasma corticosterone concentration when pre-trial plasma samples were compared to post-trial plasma samples, while control turtles exhibited no such increase, validating the effectiveness of our implants to deliver corticosterone. Although corticosterone remained high at the end of the activity trials, the increase in activity was ephemeral in nature, peaking within 48 hr after the implant was in place. This suggests that the effects of corticosterone on behavior may be context-dependent (i.e., whether the turtles can find food) and concentration-dependent, and that there are underlying physiological mechanisms, perhaps mediated at the receptor level in the brain, involved in locomotor activity behavior in slider turtles. Environmental perturbations that cause a reduction in available food resources may cause the organism to increase its level of locomotor activity to increase food encounter rate but later reduce activity to conserve energy reserves. These data are important when considering behavioral and physiological mechanisms involved in a turtle’s response to changing conditions in habitat quality. J. Exp. Zool. 284:637–644, 1999. © 1999 Wiley-Liss, Inc.

Like many other vertebrates, reptiles show an increased plasma concentration of corticosterone in response to a dynamic array of stressors, both acute and chronic (Guillette et al., ’95; Gregory et al., ’96; Cash et al., ’97). The effects of acute and chronic corticosterone release have been linked to a number of important physiological and behavioral functions that help individuals meet their energy demand. Corticosterone affects energy balance by promoting gluconeogenesis, resulting in glucose substrates from non-carbohydrate resources such as skeletal muscle protein (Harvey et al., ’84; LeNinan et al., ’88). Corticosterone can also have anabolic effects by promoting lipogenesis if the energy demand is being met by adequate food availability (Berdanier, ’89; Boswell et al., ’94; Holberton et al., ’96). Foraging activity, such as food searching or feeding, has been shown to increase in response to exogenous corticosterone in several species of mammals and birds (Nagra et al., ’63; Wingfield and Silverin, ’86; Berdanier, ’89; Asheimer et al., ’92; Challet et al., ’95).

The availability of resources in some systems may be inherently stochastic, and activity patterns of many animals often reflect this (Taylor and Taylor, ’78). During declining habitat conditions (like droughts), and presumably declining food availability, animals may be faced with few options to respond to the situation. Some animals must attempt to maintain their energetic condition, which may mean increasing their level of activity in order to increase food encounter rate (e.g., optimal foraging theory, Stephens and Krebs, ’86; Wingfield, ’94) or decrease or cease activity until conditions improve (Ruby et al., ’94; Dunlap, ’95).

Turtle activity and movement have been classified in terms of their purpose and extent (Morreale et al., ’84; Gibbons, ’86; Gibbons et al., ’90). Local (intrapopulation) activities associated with homeostasis, reproduction, and predator avoidance can continue as long as habitat requirements meet the existing energetic demands of those activities...
Our objective was to determine the effects of exogenous corticosterone on locomotor activity in the freshwater turtle. Many freshwater turtles inhabit ephemeral habitats and thus make an excellent model for studying the effects of changing environmental conditions on their physiology and behavior. Our objective was to determine the effects of exogenous corticosterone on locomotor activity in the freshwater turtle, Trachemys scripta elegans. Specifically, we wished to see if an increase in plasma concentration of corticosterone would result in a significant increase in locomotor activity in captive slider turtles.

METHODS

Turtles (n = 17 male; n = 13 female) were captured from ponds using baited hoop nets in Lafayette Co., Mississippi and were acclimated for 1–3 weeks in the laboratory prior to initiation of each activity trial. Turtles were held individually in 76-liter glass aquaria and fed fresh catfish and collard greens once every 5 days. Two activity trials took place from June 18 to July 14, 1996, and one trial from May 18 to May 30, 1997 (n = 30; 10 turtles per trial). Ambient temperature for trials 1 and 2 ranged from 24 to 25°C and Trial 3 temperature ranged from 29 to 30°C. Photoperiod was held equivalent to natural photoperiod at the time of each respective trial.

Turtle locomotor activity was measured in specially designed chambers described in detail in Cash (unpublished manuscript). Briefly, 75-liter plastic tubs were fitted with platforms that pivoted upon a fixed central ball (4 cm diameter) secured to the center of the chamber floor. Each platform was suspended using four strands of 20-gauge wire from levers on four microswitches at equidistant points around the chambers. These were then wired in electrical circuit to a mechanical counter. Turtles were placed in their respective activity chamber and the water level was adjusted based on the size of the turtle (to the top of the turtle's carapace). When the turtle moved, regardless of its direction, the platform would be displaced thus depressing one or more switch levers. The number of counts by each turtle over a 24-hr period was recorded daily at 09:00 CDT.

To make two groups of similar body size, pairs of turtles were matched based on total carapace length and then randomly assigned to either group by coin toss. Turtles were allowed to adjust to the chambers for 24 hr prior to the initiation of each activity trial. After the completion of 5 full 24 hr intervals (referred to as the “pre-implant” activity period), turtles were implanted with either empty (“control”) or corticosterone-filled (“experimental”; Sigma Chemical Co., St. Louis, MO) Silastic® implants (10 mm length; 1.5 mm bore, 0.5 mm wall). Each implant was placed subcutaneously anterior to the hind limb. An L-shaped incision was made and the connective tissue separated from the skin creating a cavity large enough for the implant to be inserted. The incision was then sealed with Nexaband®, a surgical adhesive, and allowed to dry thoroughly (up to 20 min) before placing the turtle back in its respective activity chamber. After all implant procedures had been completed, turtles were fed and allowed a 24 hr recovery period before the recording of activity resumed. Twenty-four-hour activity counts were then recorded daily for an additional 5 days (referred to as the “post-implant” activity period) as before. Turtles were weighed at the beginning of the 5-day pre-implant period, on the day of implant, and at the end of the 5-day post-implant period to the nearest 0.01 g.

To validate corticosterone delivery by our implants, blood samples were collected at the beginning of the pre-implant period and 10 days later, at the end of the post-implant period, via venupuncture of the forelimb using 23 or 26 gauge...
needles, depending on turtle size. A 100–150-µL blood sample was collected in heparinized microcapillary tubes and placed immediately on ice. Blood samples were then centrifuged and the plasma removed with a 50-µL Hamilton® syringe. The samples were kept frozen (–5°C) until assayed for corticosterone concentration by radioimmunoassay.

Radioimmunoassay

The radioimmunoassay for plasma corticosterone concentration was based on that described by Wingfield et al. (’92). Specifically, each plasma sample (15–50 µL) was diluted with distilled water up to a total volume of 200 µL and treated with 2,000 cpm of radiolabelled corticosterone (New England Nuclear, Boston, MA). Four ml of freshly distilled dichloromethane (Fisher Scientific, Pittsburgh, PA) were added to each sample to extract corticosterone for at least 2 hr. The steroid-containing organic phase was then aspirated and the solvent evaporated under nitrogen in a 40°C water bath. The extracts were reconstituted in 550 µL of phosphate-buffered saline. Replicates of each sample were set up using 200 µL aliquots of the extract. One hundred microliters of the reconstituted extract were added to 4 ml of scintillation fluid (Packard Ultima Gold) in 9 ml scintillation vials to determine the extraction efficiency (% recovery) of each sample.

Each aliquot received 100 µL of tritiated corticosterone (approx. 10,000 cpm) to compete with the endogenous corticosterone for binding with 100 µL of corticosterone antibody (B21-42, Corticosterone-21-succinate-bovine serum albumin, Endocrine Sciences, Calabasas, CA) for 24 hr. Separation of unbound from bound radiolabelled hormone was done with the addition of 500 µL of dextran-coated charcoal for 12 min at 4°C. The samples were then centrifuged at 2,000 rpm for 10 min at 4°C. The resulting supernatant containing the bound fraction was decanted into scintillation vials, 4 ml of scintillation fluid were added, and each vial was counted on a Beckmann LS6500 system for up to 10 min or until 2% accuracy was reached. Three tubes (in replicate) were set up containing the buffer and labelled steroid; buffer, labelled steroid, and charcoal; and buffer, labelled steroid, and antiserum, in order to measure total cpm, non-specific binding and maximum binding, respectively. A series of nine pairs of replicates containing decreasing concentrations of unlabelled corticosterone (Sigma) and 100 µL of antibody and 100 µL of radiolabelled corticosterone was set up to create a standard curve from which all sample values were determined. Both a water blank and a standard containing 2,000 pg of unlabelled corticosterone were placed at the beginning and at the end of each assay to check for contamination and reliability. In addition, 50 µL of a plasma pool was included in each assay to determine inter-assay variation. The sensitivity of each assay was 7.8 pg, the average recovery efficiency was 83%, the inter-assay coefficient of variation (3 assays) was 9.6%, while the intra-assay coefficient of variation based on sample replicates was 3.2%.

Statistical analysis

Changes in activity counts, hormone concentration, and turtle mass were analyzed using repeated sampling measures analysis of variance (ANOVA) (Sokal and Rohlf, ’81). Due to the heteroscedasticity of the data, activity counts were square root transformed (Sokal and Rohlf, ’81). The change in locomotor activity within each treatment group was analyzed using a single factor ANOVA. All statistical analyses were performed using Statview version 4.5 and SuperANOVA version 1.11 (Abacus Concepts, Berkeley, CA).

RESULTS

Locomotor activity

Temperature did not influence the response to the implant within the first 48 hr; therefore, the data from the three trials were pooled (treatment × time × temperature interaction, F_{1,26} = 0.001, P = 0.975). The sexes did not differ in their level of locomotor activity across the 5-day pre-treatment period of the study (F_{1,28} = 3.252, P = 0.08) and were pooled for all analyses. Turtle locomotor activity in both the control and experimental groups significantly declined over the entire trial period (F_{1,9} = 6.151, P < 0.0001, Fig. 1). However, the two groups showed a significant difference in their short-term (within 48 hr) response to the implant treatment (treatment × time interaction, F_{1,26} = 4.84, P = 0.037, Fig. 1). Turtles implanted with corticosterone showed a significant increase in activity levels within the first 24–48 hr following implantation (F_{1,14} = 14.78, P = 0.002), while control turtles showed no such increase (F_{1,14} = 0.219, P = 0.65, Fig. 1). When comparing the activity levels of the period immediately before (Day -1, Fig. 1) and the period immediately following (Day 1, Fig. 1) implantation, the experimental turtles experienced a 211 ± 49% SE increase in locomotor activity. This increase was ephemeral, declining to
pre-treatment levels within 48 hr after implantation (Fig. 1). Control turtles did show an increase in activity following the implant event (7.9 ± 3.8\% SE), but this increase was not significant.

**Effect of implant on plasma hormone concentration**

There was a significant difference in the magnitude of change in plasma corticosterone concentration between the control and experimental groups for pre-treatment and post-treatment samples (F$_{1,26} = 7.121$, $P = 0.013$, Fig. 2). At the beginning of the trials, plasma corticosterone concentrations were relatively low for both the control and experimental groups of turtles and were not significantly different from each other (F$_{1,27} = 0.304$, $P = 0.59$, Fig. 2). However, when sampled at the end of the activity trial, the experimental turtles that received exogenous corticosterone had significantly elevated plasma corticosterone as compared to the control group (F$_{1,27} = 12.76$, $P = 0.001$, Fig. 2).

Turtle mass declined significantly over the activity trial for both groups combined (F$_{2,56} = 17.51$, $P < 0.0001$, Table 1). This change in mass was unaffected by treatment (treatment × time interaction, F$_{1,28} = 0.37$, $P = 0.55$). Both the control and experimental groups lost an average of 4.4 ± 0.9\% SE and 3.9 ± 1.2\% SE body mass, respectively, over the 10-day period.

**DISCUSSION**

Exogenous corticosterone resulted in a significant increase in locomotor activity in our experimental turtles. These results support our hypothesis that an increase in plasma corticosterone concentration can facilitate, either directly or indirectly, an increase in locomotor activity in freshwater turtles. These findings are comparable to those of other studies in which increases in locomotor activity were observed as a result of the administration of exogenous corticosterone (mammals, Challet et al., '95; birds, Astheimer et al., '92; Breuner et al., '98) and constitutes the
CORTICOSTERONE AND ACTIVITY IN TRACHEMYS SCRIPTA

first such report for reptiles. The slight increase
in locomotor activity of our control turtles might
be explained by the temporary endogenous rise
in corticosterone due to handling and surgery. We
chose not to obtain blood samples immediately fol-
lowing implant to reduce the effect of handling.
The post-trial corticosterone concentration of our
experimental turtles was approximately the mean
corticosterone concentration observed after 60 min
of handling stress in free-living T. scripta (Cash
et al., '97). This suggests that the implants were
delivering a realistic dose during our treatment
time, but the plasma corticosterone concentration
immediately following the implant was unknown.
Time-course delivery of corticosterone-filled im-
plants as used in this study was monitored in a
group (n = 5) of turtles. This trial revealed that
the implants delivered doses within the physiologi-
cal range observed in this species (0 hr, before
implantation, 0.24 ± 0.21 SE ng/ml; 24 hr, 21.9 ±
2.81 SE ng/ml; 48 hr, 15.61 ± 3.96 SE ng/ml; and
72 hr, 13.49 ± 3.32 SE ng/ml).
The behavioral effects of elevated corticoster-
one in wild-caught birds include hyperphagia and
an increase in captive locomotor activity, the lat-
ter interpreted as an increase in food searching
behavior to meet an increase in energy demand
(Wingfield and Silverin, '86; Berdanier, '89; Gray
et al., '90; Astheimer et al., '92; Challet et al., '95).
Our results suggest that turtles respond in a simi-
lar manner. Because of the diversity in the type
and extent of perturbation an individual may en-
counter, one would expect diverse responses that
allow animals to adjust physiologically and behav-

**TABLE 1.** Mass (g) for control (n = 15) and experimental (n = 15) turtle groups taken at the beginning, at the time of implant,
and at the end of the activity trials

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean pre trial mass (g)</th>
<th>Mean mass at implant (g)</th>
<th>Mean post trial mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>782.5 ± 74.1 SE</td>
<td>759.0 ± 70.9 SE</td>
<td>749.4 ± 69.6 SE</td>
</tr>
<tr>
<td>Experimental</td>
<td>708.5 ± 89.6 SE</td>
<td>691.8 ± 91.3 SE</td>
<td>681.8 ± 88.3 SE</td>
</tr>
</tbody>
</table>

Turtle mass did change significantly over the course of the trials ($F_{2.56} = 17.51, P < 0.0001$), however, treatment groups did not significantly differ from each other ($F_{1.26} = 0.37, P = 0.55$).
iorally to potential costs and benefits of the higher concentrations of corticosterone that may be a result of changes in food availability. A change in locomotor activity may be expressed in different ways. If there is a decline in habitat quality a turtle might experience an increase in corticosterone initially, accompanied by an increase in locomotor activity to increase food encounter rate locally. If habitat decline is widespread or long-term, a turtle might then decrease its activity if food cannot be found locally in order to conserve energy reserves until conditions improve. This "riding out" strategy may ultimately be abandoned if poor conditions persist and the turtle must then emigrate from its home range. Ectothermic organisms can reduce metabolic activity on a diel or even seasonal basis, allowing them to survive through extreme environmental conditions (e.g., drought) that may be accompanied by unpredictable periods of food availability (Bradshaw, '86; references in Pianka, '94).

When viewed in an ecological context, these physiological and behavioral responses could be responsible for helping an organism respond to inherently stochastic environments. In a study involving the western fence lizard (Sceloporus occidentalis), animals deprived of food and water, resulting in comparatively poorer energetic condition than control animals, had higher plasma corticosterone concentrations and were more active than their control counterparts (Dunlap, '95). Similarly, fasted white-crowned sparrows (Zonotrichia leucophrys) showed increased locomotor activity when implanted with exogenous corticosterone, while fed birds showed no such increase, suggesting that this response could be dependent on the energetic condition of the animal (Astheimer et al., '92; Wingfield et al., '97). Our results suggest that an increase in corticosterone, either short- or long-term, may play an important role in mediating these behaviors in freshwater turtles.

Although increased plasma corticosterone resulted in increased locomotor activity in this study, the response was ephemeral or short term (<48 hr). This could be interpreted as the initial food searching response, and as turtles encountered no food after receiving implants in this study, they may have shut down activity to conserve energy reserves (i.e., a switch from a food searching/energy expending strategy to a "riding out"/energy conservation strategy). We do not know if these turtles would have initiated another bout of activity in response to a critical depletion of energy reserves, as we determined the length of the experiment a priori. Although turtles did lose a significant amount of mass during the entire period, it is not known whether this loss was significant in a biological sense. The lack of significant differences in mass loss between the two groups indicates that exogenous corticosterone at the concentrations observed here had no effect on energy utilization during the 5-day post implant period. This is in contrast to Gray et al. ('90) who found that dark-eyed juncos (Junco hyemalis) implanted with corticosterone lost significant amounts of fat and muscle mass, although these results may not be wholly comparable due to the metabolic differences between birds and reptiles. Experiments are ongoing to test for a potential threshold for condition-dependent changes in locomotor activity and corticosterone levels.

Gibbons et al. ('90) proposed that freshwater turtle species that have evolved in stochastic habitats exhibit two adaptive strategies for coping with changes in habitat quality: (1) remain in a quiescent state, or (2) emigrate in search of better habitat conditions. Evidence for different movement strategies in turtles is scarce but does exist. Turtles have been observed moving from habitats where food resources are in decline (after algacide application, Parker, '84) or when presumably better resources are available, as in the case of the seasonal movement of T. scripta in Panama in response to having access to flooded areas (Moll and Legler, '71). Comparatively more data exist concerning freshwater turtles and their response to drought conditions and pond drying. Trachemys scripta and Chrysemys picta moved from a drying pond in southern Illinois in response to drought-induced pond drying (Cagle, '44), whereas in an earlier study, Cahn ('37) observed that C. picta did not emigrate from a pond due to drying. Pseudemys floridana and T. scripta (again in response to drought-induced pond drying) emigrated from a pond while Deirochelys reticularia, Kinosternon subrubrum, and Sternotherus odoratus did not emigrate (Gibbons et al., '83). In a systematic test of pond drying, Gibbons et al. ('90) found that when the water level was dropped over a 7-day period (a relatively short time period when compared to drought-induced pond drying), Chelydra serpentina and K. subrubrum did not emigrate while T. scripta, P. floridana, and contrary to the earlier observation, D. reticularia emigrated from the pond, suggesting that condition-dependent responses to habitat change may exist for some turtle species but not for others.

A species-dependent response to habitat pertur-
bations on the scale of the complete drying of a pond (as discussed in Gibbons et al., '90), or a rapid decline in available food resources, may exist. It would be adaptive to have physiological mechanisms, particularly in those turtle species that have evolved in stochastic habitats, which allow for alternative response strategies when dealing with changes in the condition of the habitat. The mechanisms of corticosterone's effect on the physiology and behavior of vertebrates are poorly understood. In particular, the exact relationship between corticosterone, locomotor activity (either increase or decrease) and metabolic rate is unknown in reptiles. Our results suggest a link between corticosterone and locomotor activity and provide a foundation for further study. Research on mammals has revealed two different glucocorticoid receptors (type I and type II) with different levels of binding affinities for corticosterone. The different binding affinities for these two receptor types are believed to be responsible for the dynamic physiological and behavioral effects that can be observed in mammals (Ratka et al., '89). Knapp and Moore ('97) suggest that this receptor system could help explain the underlying physiological basis of behavioral differences within and among the different male morphotypes of the tree lizard, Urosaurus ornatus. Further study is required to determine whether turtles have the same receptor system. The results from the previously cited experiments suggest that this will be an important area of research in the understanding of the ecological physiology of freshwater turtles.

Turtles are among the oldest groups of living reptiles that have clearly demonstrated success in adapting to environmental change (Pritchard, '79). In addition, individual turtles are relatively long-lived (Congdon et al., '93). The unique metabolic activity of ectotherms makes the freshwater turtle an excellent model for investigating the relationships among the role of glucocorticoids, behavior and physiology, and changes in energetic demand. We will test the effects of energetic condition on the physiological and behavioral responses of the slider turtle in future studies. These characteristics, in conjunction with the ephemeral nature of freshwater habitats, make freshwater turtles an excellent model for studying behavioral and physiological mechanisms in response to environmental stochasticity. In such a model, one might expect a wide range of behavioral and physiological responses that reflect the various time scales of environmental change that an individual may encounter. However, the nature and timing of environmental change (from within-season perturbation or more predictable changes within the annual cycle, to long-term changes in climatic patterns) may impose different constraints on survival and reproductive success. By investigating the relationships between glucocorticoid levels, energetic condition, and movement patterns of the slider turtle, we will be able to determine how individuals respond physiologically and behaviorally to different levels of habitat change and/or disturbance.

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**LITERATURE CITED**


