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# Combined Effects of Experimentally Elevated CORT and Predation Threat on Exploratory and Foraging Behavior of *Desmognathus ochrophaeus*

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**ABSTRACT.**—Amphibian responses to stress are generally mediated through glucocorticoids produced by the hypothalamic–pituitary–interrenal axis. Chronic elevation of glucocorticoids can result in delayed wound healing and growth, but less is known about its influence on behavioral responses to predators. We examined the effect of acute and chronic CORT elevation on exploratory and foraging behaviors of Allegheny Mountain Dusky Salamanders (*Desmognathus ochrophaeus*) exposed to kairomones from a predatory snake. We established ‘No CORT,’ ‘Acute CORT,’ and ‘Chronic CORT’ conditions during an 18-d preparation phase by varying salamander exposure to an exogenous source of CORT (0.51 mg/mL) delivered via cutaneous patch. During the trial phase, we conducted assays to evaluate exploratory and foraging behavior of salamanders from the different CORT groups when exposed to snake kairomones or control. Exploratory behavior was examined within a circular arena with a series of surmountable concentric barriers, and foraging behavior was observed within Petri dishes containing *Drosophila* prey. Salamanders experiencing both Chronic CORT and snake kairomones exhibited the least exploratory behavior, escaped the array with the lowest frequency, climbed the fewest barriers, and delayed their movement. However, foraging behavior (latency to strike at prey, number of strikes, number of prey captured) was not affected by exposure to CORT or snake kairomone. Our findings suggest that artificially elevated CORT can modify some behavioral responses to predator kairomones, but only when individuals experience multiple CORT applications in the period preceding the trials.

Responses to stress are mediated chiefly through the production of glucocorticoids by the hypothalamic–pituitary–adrenal (HPA) axis in mammals and birds, and the hypothalamic–pituitary–interrenal axis (HPI) in amphibians and reptiles (Sapolsky et al., 2000; Reeder and Kramer, 2005; Sopinka et al., 2015). Corticosterone (CORT) is a key glucocorticoid produced by the HPI and exerts effects through changes to metabolic fuels, cardiac output, blood perfusion, and behavior (Sapolsky et al., 2000; Wingfield and Sapolsky 2003; Wingfield, 2005; Sheriff et al., 2011). While short-term glucocorticoid production allows an organism to respond to transient stressors and maintain homeostasis, prolonged elevation of CORT can lead to declines in reproduction (Wingfield and Sapolsky, 2003; Carr, 2011; MacLeod et al., 2018), growth (Boonstra et al., 1998), wound healing (Padgett et al., 1998; Romana-Souza et al., 2014) and regeneration (Lewis and Sullivan, 2020), and survival (Dahl et al., 2012).

Given its role in maintaining homeostasis and mitigating stressors, it is not surprising that CORT is produced by amphibians and reptiles in response to a variety of environmental factors. In some species, changes to the abiotic environment—such as elevated ambient temperature (Narayan and Hero, 2014; Navarro et al., 2018), exposure to chemical contaminants (Hopkins et al., 1997; Burraco and Gomez-Mestre, 2016), and ambient CORT in urban habitats (Gabor et al., 2018)—influence stress responses. In others, biotic stressors in the form of predators and competitors may stimulate CORT production and elicit behavioral or metabolic changes (Narayan et al., 2013; Jessop et al., 2015). Although the presentation predator stimuli can elevate CORT in some herptiles (Thaker et al., 2009b; Bennett et al., 2016), predator detection does not

guarantee an increase plasma CORT or corresponding behavioral changes for all species (e.g., Fonner and Woodley, 2015).

Chemical stimuli from predators deposited in the environment (i.e., kairomones) can act as stressors that elicit behavioral, morphological, and physiological responses in amphibians. For example, plethodontid salamanders exposed to kairomones from predators decrease both courtship and foraging (Watson et al., 2004; Fonner and Woodley, 2015; Gildemeister et al., 2017) while increasing shelter use (Sullivan et al., 2002, 2005). Both McCollum and Leimberger (1997) and Hossie et al. (2010) show that tadpoles (Gray Treefrogs, *Hyla chrysoscelis*, and Leopard Frogs, *Rana pipiens*, respectively) exposed to chemical stimuli from dragonfly nymphs (*Aeshna* spp.) exhibited morphological changes not seen in control groups, and Allegheny Mountain Dusky Salamanders (*Desmognathus ochrophaeus*) exposed to kairomones from snakes regenerated less of their autotomized tail volume than those exposed to water (Payette and Sullivan, 2019). Although elevated threat can influence amphibians in a variety of ways, relatively little is known about the combined effects of elevated CORT on prey responses to predator stimuli.

Under natural conditions, organisms simultaneously contend with environmental stressors including predation threat (Lima and Dill, 1990; Lima, 1998; Clinchy et al., 2013). In some cases, herptile stress responses to obnoxious agents and defensive responses to elevated threats can appear antagonistic. For instance, some species exhibit increased activity and foraging (Belluire and Clobert, 2004), or extended periods of activity resulting in greater food intake (Cote et al., 2006) when plasma CORT is elevated. Conversely, when threatened, many prey decrease activity (Thoen et al., 1986; Ortega et al., 2018), reduce foraging (Roberts and Liebgold, 2008; Johnson and Sullivan, 2014), or increase refuge use (Sullivan et al., 2002; Amo et al., 2004). However, it is probable that concurrent elevation of stress and threat result in relatively subtle modifications of defensive behavior rather than drastic changes. For example, in one of relatively few studies to experimentally elevate both CORT and threat of predation, Thaker et al. (2009a) found that the type of antipredator behavior performed by lizard prey was not altered

in stressed individuals, but the responsiveness of prey to predators was increased. Obviously, behavioral responses to elevated CORT are variable and cannot be applied universally to all species or contexts (see Ricciardella et al., 2010; Bliley and Woodley, 2012), so research establishing links between experimentally elevated CORT and threat-mediated behaviors could provide insight into the effect of stress on activity and behaviors such as foraging in a range of species.

In this study we examined the effect of CORT manipulation on the exploratory and foraging behaviors of *D. ochrophaeus* while exposed to kairomones from predatory Common Garter Snakes (*Thamnophis sirtalis*). Working with *D. ochrophaeus* provides an excellent opportunity to concurrently manipulate stress and threat because previous work with this species indicates that exogenous sources of CORT applied via cutaneous patch alters plasma CORT levels (Wack et al., 2010), and individuals of this species alter their locomotor and foraging behavior in response to kairomones from predators (Johnson and Sullivan, 2014; Gildemeister et al., 2017). CORT does not appear to be immediately released by amphibians in response to predators, and increased activity would be maladaptive in most predator-prey contexts; therefore, we hypothesized that elevated CORT would enhance the responses of individual salamanders to predator kairomones, resulting in the further suppression of exploratory behavior. Predictions related to the effect of stress on plethodontid salamander foraging behavior are more difficult considering their low metabolic requirements (Pough, 1980), decreased foraging when threatened (Whitham and Mathis, 2000; Gildemeister et al., 2017), and reduced food conversion efficiency under duress (Novarro et al., 2018). We ultimately hypothesized that elevated stress also would exaggerate the inhibitory effects on foraging behavior by salamanders exposed to predator stimuli.

## MATERIALS AND METHODS

*Collection and Maintenance of Salamanders.*—To evaluate the combined effects of experimentally elevated CORT and predator kairomones on behavior, we collected 180 individual *D. ochrophaeus* by hand in a wooded area of Houghton College (Houghton, New York, USA) from 9 to 17 September 2019. We excluded individuals that showed obvious signs of recent tail autotomy (i.e., obvious tail discoloration or distal bluntness) because autotomy can influence foraging and activity (Gildemeister et al., 2017; Lewis and Sullivan, 2020). Salamanders were placed individually in 15-cm-diameter Petri dishes cleaned with Tergazyme detergent (Cat. No. Z273287, Sigma-Aldrich), provided acid-free paper toweling moistened with spring water, and stored in a climate-controlled chamber on a 13.5 h L: 10.5 h D cycle (lights on at 0700 h EST) at 15.5°C (day) and 12.8°C (night). All test subjects were returned to the field site on 17 October 2019.

*Preparation of Predator Kairomones.*—To collect the predator kairomone used in behavioral trials, three different *T. sirtalis* were collected from the site where the salamanders were collected. The collection of kairomones followed the general methodology of Sullivan et al. (2005) whereby snakes were held individually within 18.9-L aquaria littered with crumpled paper towels for cover, given access to water ad libitum, and fed 1–3 intact *D. ochrophaeus*. Thirty minutes after feeding, each *T. sirtalis* was transferred individually to a clean 3.8-L beaker and covered with cheesecloth for 48 h. At the end of this period, snakes were returned to home aquaria while beakers were rinsed with 200 mL

of spring water. Rinses were filtered through glass wool and then stored at –20°C until use. Previous studies indicate that freezing does not affect the efficacy of the kairomone because frozen samples have elicited antipredator responses by plethodontids in both laboratory (Madison et al., 2002, 2005; Gildemeister et al., 2017) and field trials (Sullivan et al., 2002, 2005). In this case, samples were thawed and allowed to reach room temperature prior to use in behavioral trials.

*Creation and Application of Cutaneous CORT Patches.*—We used rectangular patches (1.5 × 3.0 mm) prepared from low protein binding filter paper (Cat. No. 1820-070, Whatman) and applied to skin to deliver CORT to salamanders (e.g., Wack et al., 2010; Lewis and Sullivan, 2020). These patches were applied to salamanders along the dorsal midline anterior to the pectoral girdle using blunt forceps and then saturated with 1.5-μL of either a blank (sesame oil) or CORT solution. The CORT solution was prepared by adding 25.5 mg of crystallin CORT (Cat. No. C2505, Sigma-Aldrich) to 1.5 mL of 100% ethanol in a microcentrifuge tube (Cat. No. 05-408-129, Fisher Scientific) and subsequently vortexed for 1 min to ensure dissolution. Next, the ethanol solution was combined with 50 mL of a carrier oil (sesame oil) and stirred overnight. The period of stirring provided time for the ethanol to evaporate, leaving a stock solution with 0.51 mg CORT/mL carrier oil to serve as the CORT treatment. This dosage of CORT applied for 30 min via dermal patch elevated plasma CORT levels in *D. ochrophaeus* to approximately 200 ng/mL (Wack et al., 2010), although levels were trending downward within 3 h of patch removal. Lastly, CORT doses were not adjusted for the size of the individual because Wack et al. (2010) suggest no significant relationship between body weight and plasma CORT concentrations in *D. ochrophaeus* receiving exogenous CORT via cutaneous patches.

*Preparation and Trial Phases.*—The preparation phase was an 18-d period immediately prior to the commencement of behavioral trials when we established treatment groups and began the manipulation of CORT levels of designated individuals. We randomly assigned salamanders to one of three treatment groups that varied according to the number of CORT applications to be received over the course of the study: ‘No CORT,’ ‘Acute CORT,’ and ‘Chronic CORT’ ( $n = 60/\text{treatment}$ ). Stimuli that occur over relatively short periods or in a single occurrence typically are labelled ‘acute,’ whereas those that extend over a relatively long time (or are repeated) are considered ‘chronic’ (Burchfield, 1979; Sapolsky et al., 2000; Romero, 2004). However, as McCormick et al. (2015) note, the point at which an acute (or repeated) stressor becomes chronic is not well-defined (see also Busch et al., 2008a, b). Despite the nebulous boundaries of these terms, we defined ‘chronic’ and ‘acute’ based on the number of otherwise identical CORT exposures received by each test subject during preparatory and trial phases. During the preparation phase, salamanders were transferred to polystyrene (6-cm diameter × 1.0-cm height) dishes, and those assigned to No CORT and Acute CORT received patches containing 1.5 μL of sesame oil (blank), while those in Chronic CORT received patches with 1.5 μL of the CORT solution. We applied patches to test subjects on 20, 26 September, and 2 October 2019, then removed them with clean blunt forceps after 1 h.

The trial period began 6 d after the final application of patches during the preparation phase and consisted of assays to evaluate both exploratory and foraging behavior. We separated each CORT treatment into two groups of 30 salamanders each. For instance, the No CORT group ( $n = 60$ ) was separated into two subgroups of  $n = 30$  so that during the trials to evaluate

exploratory behavior, one subgroup was exposed to the snake kairomone (while the other received the water control), but during the trials to observe foraging behavior, that same subgroup was exposed to water. This process ensured that each individual was tested once each with the snake kairomone and control. Immediately prior to each trial (described below), salamanders in No CORT received cutaneous patches with 1.5  $\mu$ L of sesame oil, but those in both Acute and Chronic CORT received patches with 1.5  $\mu$ L of CORT solution. As a result, salamanders in the No CORT received no CORT, Acute CORT received two doses (immediately prior to each trial), and Chronic CORT received five doses (beginning 18 d prior and continuing through trials). One individual died from unknown causes over the course of the study so that the final sample sizes were No CORT ( $n = 59$ ), Acute CORT ( $n = 60$ ), and Chronic CORT ( $n = 60$ ).

*Effects of CORT and Kairomones on Exploratory Behavior.*—We evaluated the exploratory behavior of salamanders from one of the three CORT treatment groups under the threat of predation in assays conducted on 8–9 October 2019. To begin, salamanders were moved from the climate-controlled chamber to the laboratory for the application of cutaneous patches. The application and removal of patches was timed so that patches remained in contact with the salamander for 1 h prior to transfer to the test dish. Once patches were removed, test subjects were transferred to individual polystyrene dishes (6-cm diameter  $\times$  1.0-cm height) lined with filter paper (Cat. No. WHA1001150, Sigma-Aldrich) and saturated with 1 mL of either spring water (control) or predator kairomone solution, and moved to the test arena.

The circular arena used to evaluate exploratory behavior consisted of a series of surmountable concentric barriers. The design was based on the methodology of (Lynn et al., 2019) and used by Lewis and Sullivan (2020) to examine the combined effects of autotomy and elevated CORT on *D. ochrophaeus* exploratory behavior. This array consisted of five 1.5-cm-high rings of polyvinyl chloride (PVC; 9101 Duct Pipe, Harrington Industrial Plastics LLC) ranging from 10.2 to 50.8 cm in diameter and arranged in a concentric manner on a substrate of acid-free paper towels moistened with spring water. The arrangement provided a central space for the placement of the 6-cm-diameter dish containing the test subject, with an approximately 5.1-cm gap between each PVC ring.

The behavioral trials were conducted over two consecutive nights (8–9 October 2019) beginning at 2000 h in a laboratory with red LED bulbs installed overhead to provide enough light to observe the movement of salamanders. Individuals from different treatment groups were stratified so that an equal number of individuals in each treatment were tested each night. Test subjects within the 6-cm-diameter Petri dishes were placed in the center of the arena and provided 3 min to adjust to the disturbance and ensure their detection of the control or kairomone treatment. After this adjustment period, Petri lids of dishes were removed and salamanders were free to scale barriers and move through the arena for 5 min. During each trial, we recorded the (a) latency to climb the first barrier (defined as the amount of time required for an individual's pectoral girdle to pass over the vertical wall of the test dish), and (b) total number of barriers climbed. After each trial, we removed paper towel substrates and thoroughly wiped the rings of PVC and tabletop with 95% ethanol to minimize contamination by CORT, salamander odors, or potential pathogens (Lynn et al., 2019). Individuals were returned to

home dishes, measured for snout–vent length (SVL), and placed in the climate control chamber until their release at the point of capture at the conclusion of testing.

*Combined Effects of CORT and Kairomones on Foraging Behavior.*—We evaluated the foraging behavior of salamanders from each of the three CORT treatment groups under the threat of predation in assays conducted on 14–15 October 2019. Salamanders were moved from the climate-controlled chamber to the laboratory for the application of cutaneous patches, and timed so that patches remained in contact with the salamander for 1 h prior to transfer to a test dish. To observe salamander foraging, we used 15-cm-diameter Petri dishes lined with filter paper and saturated with 3 mL of either control or kairomone. In this case, the control or kairomone treatment selected was the opposite that the individual received during the experiment to examine exploratory behavior. Each salamander was transferred to the center of the test dish, provided a 3-min adjustment period, then supplied 10 wingless *Drosophila* sp. prey. During each trial, we recorded the (a) latency to strike, (b) number of strikes, and (c) flies successfully captured. We defined strikes as rapid movements of the salamander in the direction of the prey with mouth open and/or tongue extended, and successful captures as instances when flies were ingested (Jaeger and Rubin, 1982; Maerz et al., 2001).

*Statistical Analyses.*—We used separate  $2 \times 6$  contingency tables to analyze the frequencies of salamanders that climbed the first barrier, and climbed all barriers (i.e., 'escaped' the circular array) during the test of exploratory behavior. Furthermore, we used an additional  $2 \times 6$  contingency table to analyze the frequencies of salamanders that made an attempt to forage (i.e., number of strikes  $\geq 1$ ).

In order to analyze the combined effects of CORT and predator kairomones on exploration and foraging, we performed generalized linear model factorial analysis of variance (ANOVAs) using Statistica (Statsoft, Inc. 2001, version 6.0) because our data failed to meet the assumptions of normality and homoscedasticity. In Experiment 1, we examined the latency to climb first barrier and number of barriers climbed with CORT and predator treatment as main effects. In Experiment 2, we evaluated latency to strike at prey, total number of strikes, and number of prey captured using the same independent variables. Although SVL can influence the behavioral responses of *D. ochrophaeus* to predator stimuli (e.g., Gildemeister et al., 2017), we did not include size as a covariate in our analyses because there were no significant correlations between the SVL of test subjects and the latency to climb the first ring ( $r = 0.023$ ;  $P = 0.757$ ), nor the total number of rings climbed ( $r = -0.007$ ;  $P = 0.925$ ). Furthermore, there were no significant correlations between SVL of test subjects and the latency to strike at prey ( $r = 0.011$ ;  $P = 0.876$ ), number of strikes ( $r = 0.014$ ;  $P = 0.855$ ), or number of successful prey captures ( $r = -0.009$ ;  $P = 0.908$ ). When main effects were significant, we conducted post hoc pairwise multiple comparisons while using the Bonferroni adjustment.

## RESULTS

The frequency of salamanders that escaped the array was lower when exposed to both Chronic CORT and snake kairomone compared with the other treatment groups (Table 1). There were no significant effects of CORT or kairomone on the frequency of individuals that failed to climb the first barrier or forage (Table 1).

TABLE 1. Frequencies of *Desmognathus ochrophaeus* that climbed at least one ring barrier of the circular array ('Climbed?'), climbed all of the barriers of the array ('Escaped?'), and made at least one attempt to forage ('Foraged?') in one of three CORT treatment groups (No CORT, Acute CORT, or Chronic CORT) and exposed to one of two chemical stimuli (water or kairomone from snake predator). Separate contingency tables were used to calculate the  $\chi^2$  test statistics for each category of behavior.

| Treatment               | Climbed? |    |          |          | Escaped? |    |          |          | Foraged? |    |          |          |
|-------------------------|----------|----|----------|----------|----------|----|----------|----------|----------|----|----------|----------|
|                         | Yes      | No | $\chi^2$ | <i>P</i> | Yes      | No | $\chi^2$ | <i>P</i> | Yes      | No | $\chi^2$ | <i>P</i> |
| No CORT + water         | 26       | 3  | 9.59     | 0.088    | 16       | 13 | 11.40    | 0.044    | 18       | 11 | 6.40     | 0.269    |
| No CORT + predator      | 29       | 1  |          |          | 20       | 10 |          |          | 14       | 16 |          |          |
| Acute CORT + water      | 29       | 1  |          |          | 16       | 14 |          |          | 17       | 13 |          |          |
| Acute CORT + predator   | 28       | 2  |          |          | 14       | 16 |          |          | 20       | 10 |          |          |
| Chronic CORT + water    | 26       | 4  |          |          | 12       | 18 |          |          | 23       | 7  |          |          |
| Chronic CORT + predator | 23       | 7  |          |          | 8        | 22 |          |          | 19       | 11 |          |          |

With regards to exploratory behavior, there was a significant CORT  $\times$  kairomone interaction ( $Wald = 6.27, P = 0.044$ ; Fig. 1A) in the latency to climb the first barrier (Table 2). Post hoc comparisons indicate that salamanders in the Chronic CORT group showed a significantly higher latency to climb when exposed to kairomones versus those in No CORT ( $P = 0.005$ ). Latency to climb by individuals in Acute CORT did not differ from the Chronic ( $P = 0.163$ ) or No CORT treatments ( $P = 0.210$ ) when exposed to kairomones. The mean number of barriers climbed also exhibited a significant CORT  $\times$  kairomone interaction effect ( $Wald = 6.33, P = 0.042$ ; Fig. 1B; Table 2). In this instance, post hoc comparisons show that salamanders in Chronic CORT climbed significantly fewer rings when exposed to kairomones than those in Acute CORT ( $P = 0.004$ ) and No CORT ( $P < 0.001$ ) treatments. The number of rings climbed by individuals in the Acute CORT group did not differ from those in the No CORT treatment ( $P = 0.221$ ) when exposed to kairomones.

The analysis of foraging behaviors indicates no significant main effects of CORT application or kairomone on the latency to strike at prey (Fig. 1C), total number of strikes (Fig. 1D), or number of *Drosophila* prey captured (Fig. 1E; Table 2).

## DISCUSSION

To determine how salamander exploratory behavior and foraging were influenced by periods of stress and elevated predation risk, we manipulated CORT levels via cutaneous patch and exposed individuals to kairomones from a predatory snake. We hypothesized that chronically elevated CORT would enhance the defensive responses of individual salamanders to predator kairomones, resulting in the further suppression of exploratory behavior and foraging. Our results support this hypothesis because salamanders experiencing Chronic CORT escaped the circular array with a lower frequency (Table 1), exhibited a greater latency to climb (Fig. 1A), and climbed fewer barriers during trials (Fig. 1B) when exposed to the snake kairomone. Conversely, no aspect of foraging measured in this study was affected by the experimental manipulation of CORT and predator threat (Fig. 1C–E).

In our study, *D. ochrophaeus* did not exhibit increased locomotor activity that accompanies CORT production in some species. Instead, Chronic CORT appeared to intensify the suppression of activity that is characteristic of predator avoidance in this species (Fig. 1A–B; Brodie et al., 1974). An observable behavioral response to the combination of CORT and predator stimulus elucidates features of the stress response in an antipredator context because both Ricciardella et al. (2010) and Wack et al. (2012) show that elevated plasma CORT alone has no

discernible influence on activity levels in *D. ochrophaeus*. The tendency of CORT to enhance a defensive response is consistent with the findings of Thaker et al. (2009a), and the suppression of exploration supports the hypothesis that CORT is not a component of the immediate antipredator response in amphibians (Davis and Gabor, 2015; Woodley, 2017). The suppression of an immediate CORT response could facilitate the inactivity that is characteristic of amphibians under threat (Fraker et al., 2009; Hossie et al., 2010; Florencio et al., 2020). How repeated applications of exogenous CORT augment a typical response to predators is unknown, but could provide valuable insight given the many stressors encountered by amphibians within their environment.

We found that individual *D. ochrophaeus* did not display the changes in foraging that often accompany an increase in CORT (Fig 1C–E). This response is likely adaptive for ectotherms with low energy budgets (Pough, 1980; Woodley, 2017), which respond to threats through avoidance and decreases in overall activity. Glucocorticoids such as CORT contribute to the regulation of metabolism, which may help individual amphibians and reptiles recover from prior stressors or prepare for future stressors through the renewal of energy reserves (Dallman and Bhatnagar, 2000; Sapolsky et al., 2000; Crespi and Denver, 2004). As a result, elevated CORT could alter patterns of foraging resulting in greater food intake (Cote et al., 2006) or time spent foraging (Crespi and Denver, 2004). The presence of a predator and ensuing behavioral change may be prioritized over the increased foraging often linked to elevated CORT, in part because of the low metabolic requirement in this species. The differential prioritization of these two chemical stimuli leads to behavior that selects short-term survival over an increase in energy reserves for long-term survival.

Given that the application of CORT patches was a significant factor in the observed behavioral differences among test subjects, the distinction between chronic and acute stress in our study should be carefully considered. Our Chronic CORT would likely not be considered 'chronic' in the traditional sense in the field of stress physiology, but resembled the 'high-dose acute' category of McCormick et al. (2015), who varied treatments according to intensity (dosage of CORT) and duration. Certainly our approach to applying weekly patches with a relatively high dose of CORT is consistent with this approach, but we cannot state with certainty how the repeated weekly application of patches and predator kairomones influenced salamander physiology and subsequent behavior. The patches in our study likely increased plasma CORT levels in *D. ochrophaeus* based on the work of Wack et al. (2010), who show that this concentration of CORT applied via patches

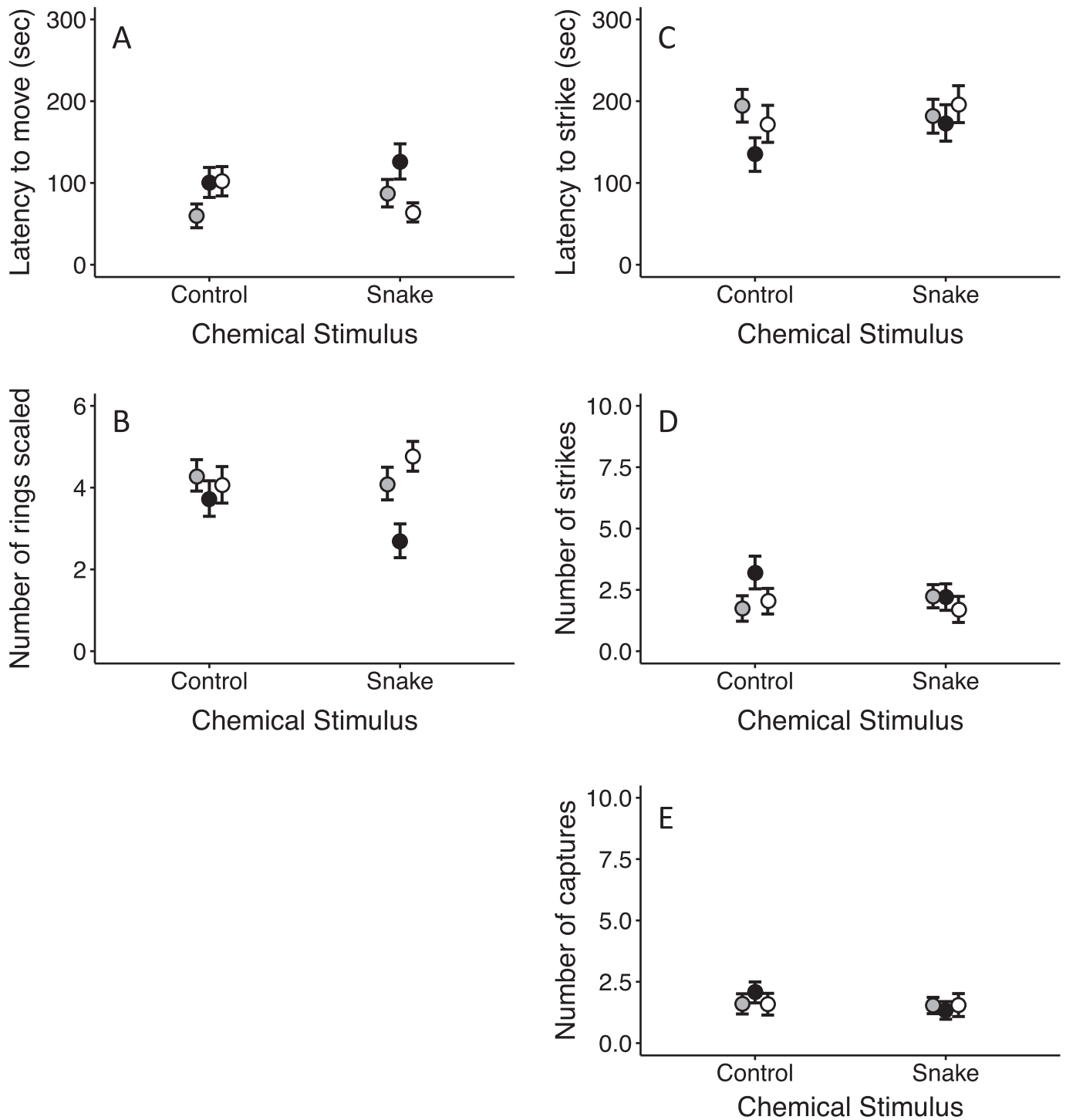


FIG. 1. Behavioral responses of Allegheny Mountain Dusky Salamanders (*Desmognathus ochrophaeus*) in one of three CORT treatments exposed to a control or kairomone from Common Garter Snakes (*Thamnophis sirtalis*). Points represent mean  $\pm$  standard error (A) latency to climb the first barrier of a circular array, (B) number of barriers climbed, (C) latency to strike at prey, (D) number of strikes at prey, and (E) prey successfully captured. CORT regimens: No CORT (white circle), Acute CORT (gray circle), and Chronic CORT (black circle).

elevated plasma CORT to approximately 200 ng/mL. This level is three to four times higher than the level of stress experienced during collection or handling in the laboratory, but not sufficient on its own to alter exploratory activity in *D. ochrophaeus* (Lewis and Sullivan, 2020). When patches containing CORT at an equivalent concentration were applied to *D. ocoee* for 30-min periods over 9 consecutive days, plasma CORT was elevated 1 h after patch removal, but indistinguishable from control animals

by 8 h. Additionally, the repeated application of transdermal CORT for 9 d did not alter baseline concentrations of plasma CORT, although it is unknown whether each subsequent stressor resulted in higher plasma corticosterone production (e.g., Cordero et al., 2003). Whether exposure to kairomones from snakes promotes plasma CORT production in salamanders is unknown, although Fonner and Woodley (2015) suggest that kairomones from a different predator (Northern Spring Sala-

TABLE 2. Results of the factorial ANOVA in the Generalized Linear Model for the effect of frequency of CORT application (No CORT, Acute CORT, or Chronic CORT) and exposure to chemical stimulus (water or kairomone from snake predator) on the latency to climb, number of rings climbed, latency to strike, number of strikes and prey captures by *Desmognathus ochrophaeus*.

|                 | df | Exploratory behavior |       |             |       | Foraging behavior |       |             |       |              |       |
|-----------------|----|----------------------|-------|-------------|-------|-------------------|-------|-------------|-------|--------------|-------|
|                 |    | Latency climb        |       | No. climbed |       | Latency strike    |       | No. strikes |       | No. captures |       |
|                 |    | Wald                 | P     | Wald        | P     | Wald              | P     | Wald        | P     | Wald         | P     |
| CORT            | 2  | 5.902                | 0.052 | 12.964      | 0.001 | 1.838             | 0.399 | 0.603       | 0.970 | 0.068        | 0.967 |
| Predator        | 1  | 0.153                | 0.696 | 0.866       | 0.352 | 0.896             | 0.344 | 1.203       | 0.273 | 0.643        | 0.423 |
| CORT × Predator | 2  | 6.266                | 0.044 | 6.332       | 0.042 | 1.131             | 0.568 | 0.124       | 0.940 | 0.854        | 0.652 |

mander, *Gyrinophilus porphyriticus*) do not contribute to a significant increase in CORT in *D. ochrophaeus* despite suppressing salamander activity (Johnson and Sullivan, 2014).

Our study suggests that repeated application of CORT to individual *D. ochrophaeus* heightens their response to predator kairomones (in terms of exploratory behavior), but does not affect foraging. These data support the hypotheses that CORT is not a component of the immediate antipredator response in amphibians, and a CORT-mediated increase in metabolism may be detrimental to a species adapted to low metabolic requirements. Our study also suggests that the effects of combined stressors on behavior may be relatively subtle and represent a balanced response to conflicting stimuli.

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