

## SHORT COMMUNICATION

Exogenous octopamine increases antipredator aggression in scorpions (*Centruroides vittatus*)

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**Abstract.** In many invertebrates, the monoamine octopamine (OA) acts as a hormone and neurotransmitter, regulating “fight-or-flight” behavior and aggression. Though well-studied in some taxa (e.g., insects and crustaceans), the role of OA in arachnids is little understood, and information on any hormones within scorpions is lacking. Given its effects in most arthropods, we expected elevated levels of octopamine to increase aggressive antipredator behaviors in scorpions. We experimentally raised octopamine levels in striped bark scorpions (*Centruroides vittatus* (Say, 1821)) and measured changes in their behavioral responses to simulated predator attack. Elevated octopamine levels increased aggressive behaviors in *C. vittatus* scorpions, whereas an octopamine antagonist (phentolamine) had no effect on behavior. Overall, this study begins to extend our understanding of behavioral endocrinology to scorpions, finding general similarities with spiders. Future work promises to better elucidate the role of monoamines and other hormones in regulating scorpion behavior and their interactions with predators.

**Keywords:** Behavioral endocrinology, defensive behavior, hormones, monoamine, neurotransmitter

While behavioral endocrinology has been best studied in vertebrates, several hormones are known to influence the behavior of arthropods (Beenackers et al. 1985; Chung et al. 1999; Lee & Park 2004). Notably, monoamine hormones influence a diverse array of behaviors (Libersat & Pflueger 2004). Octopamine (OA) is a monoamine derived from a tyrosine precursor, and OA acts as a hormone and neurotransmitter through G-coupled receptors (Roeder 2005). The effects of OA have been investigated thoroughly in insects and it has been suggested to mediate general arousal in response to stress, including “fight-or-flight” behaviors (Verlinden et al. 2010). For this reason, OA has been considered the protostome analogue to epinephrine (another monoamine) in vertebrates (Verlinden et al. 2010; Bauknecht & Jékely 2017).

In addition to influencing intraspecific behavioral interactions (e.g., aggression towards conspecifics; Kravitz & Huber 2003), OA also influences responses to predators. For example, crickets (*Gryllus texensis* Cade & Otte, 2000, Gryllidae) were found to spend more time immobile and under shelters when treated with injections of OA (Adamo et al. 2013) while the orb-weaving spider (*Larinioides cornutus* (Clerck, 1757), Araneidae) was found to significantly reduce the duration of immobility behavior (thanatosis) after OA treatment (Jones et al. 2011).

To the best of our knowledge, no study has evaluated the potential role of OA – or any other hormone – in scorpions (Order Scorpiones). We would expect OA to play a role in stress and arousal in scorpions, based on similar activities of OA in spiders (Order Araneae), which diverged from scorpions ca. 397 mya (Jeyaprakash & Hoy 2009), and in insects and crustaceans (the “Pancrustaceans”), which together share a last common ancestor with all arachnids ca. 725 mya (Pisani et al. 2004). We tested the effects of exogenous OA, phentolamine (PH, a competitive antagonist of octopamine receptors; Stevenson et al. 2005), and a control treatment (vehicle only) on the antipredator aggression of the striped bark scorpion (*Centruroides vittatus* (Say, 1821) Buthidae). We hypothesized that OA administration would result in an increase in aggression towards the threat, concordant with research in spiders (Jones et al. 2011; Hebets et al. 2015). We also expected that PH administration would result in a decrease in aggression by blocking the effect of endogenous OA.

*Centruroides vittatus* (the striped bark scorpion) is a common, medium-sized (approximately 300–800 mg) scorpion that occupies a

variety of habitats in the south-central United States and northeastern Mexico (Shelley & Sissom 1995). Unlike many other scorpions, *C. vittatus* are considered “errant” – highly mobile and actively foraging (McReynolds 2008), and sprinting can be an effective antipredator behavior along with stinging, especially if escape is difficult (Carlson et al. 2014). Scorpions in this study were collected in the Organ Mountains in New Mexico, USA in July and August 2014. After initial housing in groups of 50 with monthly feedings of crickets, scorpions were transferred into individual containers (16.5 × 11.5 × 7.5 cm) with a sand substrate and were kept undisturbed for one week in a temperature-controlled room (24° C) dimly lit with natural sunlight.

We conducted behavioral assays before and after hormone treatments ( $n = 32$ , including 14 females and 18 males). We developed behavioral assays to quantify the extent to which antipredator responses were characterized by flight behavior versus defensive aggression, based on previous scorpion behavior experiments (see van der Meijden et al. 2013; Carlson et al. 2014). We first lifted the scorpion with forceps from its housing container by the first metasomal segment. We then recorded whether or not the scorpion attempted to sting the forceps within a 3 second time interval (Table 1), and then placed the scorpion in a 15 × 25 cm arena with a paper substrate and translucent plastic walls. We recorded the response of the scorpion within the first 3 seconds after placement in the arena, ranging from fleeing to remaining stationary and with varying degrees of defensive (raised) positions of chelae/metasoma (Table 1). Next, a 3 ml plastic pipette was used to direct a puff of air toward the anterior prosoma from a distance of approximately 5 cm by rapidly compressing the bulb of the pipette with the experimenter’s fingers in a consistent manner. We recorded the immediate response, ranging from fleeing to “startling” (repositioning or backing away), to remaining stationary, with varying degrees of defensive positions of the chelae and metasoma (Table 1). We then grasped the scorpion’s prosoma and held it in place with forceps for 3 seconds. We recorded responses ranging from no attack followed by fleeing, to attacking (with either or both of the chelae [pinching] or metasoma [stinging]) and fleeing, to attacking and remaining in position (Table 1). Finally, the scorpion was again grasped by the first segment of the metasoma and lifted for 3 seconds with forceps, and we noted whether it stung the forceps or not (Table 1). During the behavioral assay, the experimenter was

Table 1.—Behavioral scoring system used to rate defensive aggression of scorpions. Stimuli are shown here in the order they were presented to the scorpions. Scores are assigned to each potential response (with only one response possible per stimulus), listed and ranked in order of increasing aggression towards the simulated threat. The sum of the five scores constituted individual scorpion aggression scores. See text for definitions of terms.

Stimulus	Possible responses	Aggression score
Lifting scorpion by metasoma	No sting	0
	Sting	1
Placing scorpion in arena	Flee (metasoma down)	-1
	Flee (metasoma raised), or rest	0
	Stationary (either metasoma or chelae raised)	1
	Stationary (both metasoma and chelae raised)	2
Puff of air	Flee (metasoma down)	-2
	Flee (metasoma raised), or startle (metasoma down)	-1
	Rest	0
	Startle (either metasoma or chelae raised)	1
	Startle (both metasoma and chelae raised)	2
	Stationary (either metasoma or chelae raised)	3
	Stationary (both metasoma and chelae raised)	4
	Restraint with forceps, and release	No aggression, then flee
No aggression, then stationary	0	
Pinch or sting, then flee	1	
Pinch and sting, then flee	2	
Pinch or sting, then stationary	3	
Pinch and sting, then stationary	4	
Lifting scorpion by metasoma	No sting	0
	Sting	1

aware of which treatments were administered to each scorpion. Therefore, there is a small possibility of biased data collection due to the lack of blinding, but any such bias is unlikely to have had much influence on our findings, given the occurrence of some null results that were counter to expectations. Additionally, variability in the administration of the assay (such as the amount of force used when grasping the scorpions with metal forceps) could introduce additional error into our measurements, which would limit the ability to detect weak effects or weak associations between variables. Future work could employ mechanical equipment calibrated to administer steps of the assay in a consistent manner.

We then calculated an overall score by summing the values assigned to each displayed behavior over the 5 steps of the assay, using the system shown in Table 1, with higher values indicating greater aggression. In general, we considered behaviors to be more or less aggressive using the following rules: fleeing was less aggressive than remaining stationary or startling, startling was less aggressive than remaining stationary, no defense was less aggressive than defense (stinging or pinching), postures with the metasoma or chelae lowered were less aggressive than postures with them raised, and postures or defensive behaviors employing one of the metasoma or chelae were less aggressive than employing both. We centered the scale for each stage of the assay on 0 (0 = no response, except during initial placement in the arena, when resting or fleeing with the metasoma raised were both considered 0, as most scorpions exhibited some movement when introduced to a new environment and it was unclear

which response should be considered more aggressive). We performed this behavioral assay both prior to (“pre-treatment”) and after (“post-treatment”) with monoamines.

Immediately after the pre-treatment behavioral assay and before treatment application, we temporarily limited the scorpions’ mobility by placing them in a -20 C freezer for approximately 60 seconds and then weighed them to the nearest milligram. We randomly assigned the treatment conditions: octopamine (OA), phenolamine (PH), and the vehicle – dimethylformamide (DMF) – alone as a control. Both OA and PH were dissolved in DMF vehicle to yield a 4  $\mu\text{mol/ml}$  (= 0.004 mol/L) solutions. DMF readily penetrates arthropod cuticles and is effective at introducing monoamines into the hemolymph (Jones et al. 2011). We tested the DMF vehicle alone as a control because it has been shown to affect spider behavior (DiRienzo et al. 2015), and therefore its presence must be controlled for in all treatment groups. We used a Hamilton syringe to place drops of one of the treatments on the dorsal surface of the mesosoma at a concentration of 1  $\mu\text{l}$  (4 nmol of OA or PH) per 100 mg body mass (dosing was based on pilot trials and other studies; Adamo 2010). After treatments, we returned the scorpions to their housing containers for 120 minutes to recover and allow circulation of the monoamines. We then performed a post-treatment behavioral assay.

To test the effects of treatments on antipredator behavior in *C. vittatus*, we used an ANCOVA model with the post-treatment antipredator behavior score as the dependent variable, and pre-treatment score, monoamine treatment group, and sex as independent variables. We included pre-treatment score as a covariate because scorpions with already high pre-treatment behavioral scores are inherently more likely to decline than to increase in score from pre- to post-treatment assays, regardless of treatment (the well-known “regression to the mean” phenomenon; Galton 1886; Barnett et al. 2005). Scorpion sex was included due to previous findings of sexual dimorphism in aggression (Carlson et al. 2014). We initially included an interaction between sex and treatment, but it was not significant ( $F_{2,25} = 0.35$ ,  $P = 0.71$ ) and was dropped from the model.

Hormone treatment of *C. vittatus* scorpions significantly affected the change in anti-predator aggression scores from the pre-treatment to post-treatment period ( $F_{2,27} = 7.12$ ,  $P = 0.003$ ; Fig. 1). The OA-treated scorpions increased in aggression after treatment more than PH-treated scorpions (post-hoc Tukey test:  $P = 0.003$ ) or DMF vehicle-treated scorpions ( $P = 0.046$ ); the latter two treatments did not differ from each other ( $P = 0.53$ ). There was no effect of sex on changes in aggression scores ( $F_{1,27} = 0.95$ ,  $P = 0.34$ ). Pre-treatment aggression scores were not correlated with post-treatment aggression scores ( $F_{1,27} = 0.01$ ,  $P = 0.92$ ). Sexes did not differ in aggressive behavior scores overall either before (Welch’s  $t$ -test:  $t_{21,9} = 1.16$ ,  $P = 0.26$ ) or after monoamine treatment ( $t_{23,0} = 0.69$ ,  $P = 0.50$ ).

This work suggests that OA plays a role in modulating the state of arousal in scorpions, increasing aggressive defensive behavior, consistent with the “fight-or-flight” response (Verlinden et al. 2010). This adds to the mixed findings for the effects of OA administration on behavior in arachnids. In some spiders, OA reduced huddling or thanatosis behavior or increased aggression, consistent with an increase in boldness (Jones et al. 2011; DiRienzo et al. 2015; Hebets et al. 2015), but this response is not consistent (DiRienzo et al. 2015; DiRienzo & Aonuma 2017) and may depend on dosage.

Generally, the effects of OA on aggressive behavior in arachnids studied thus far are more consistent with insects (Adamo et al. 1995; Dierick 2008; Rillich et al. 2011) than crustaceans, in which increased OA is generally associated with submissive rather than aggressive behavior during conspecific encounters (Kravitz 1988; Roeder 2005). With the present data, it is difficult to determine the likely ancestral physiological role of OA. Additional sampling of other arachnids and arthropods could better elucidate the evolution of behavioral

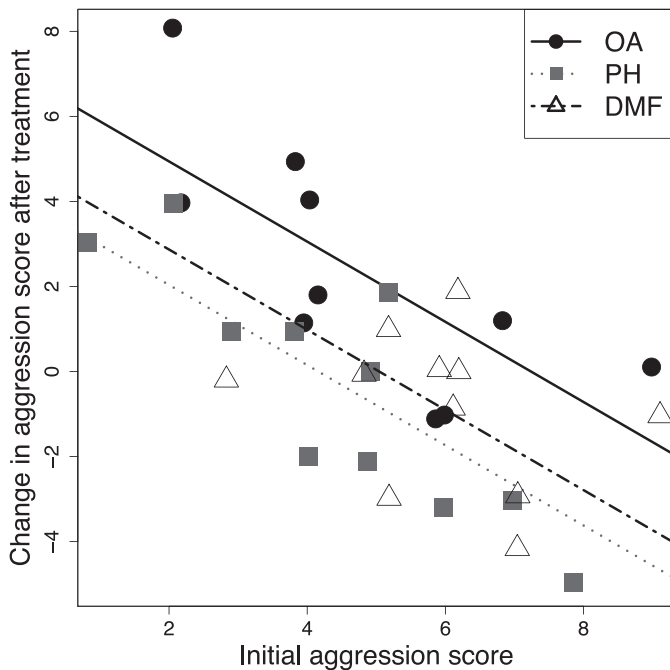


Figure 1.—Changes in *Centruroides vittatus* scorpion aggression scores from pre-monoamine treatment to post-monoamine treatment. The amount and direction of change in aggression from pre-treatment to post-treatment conditions is shown on the y-axis to correct for the tendency for increases in aggression in individuals that were initially more timid, and decreases in aggression in individuals that were initially more aggressive, regardless of monoamine treatments. Octopamine-treated scorpions had a more positive (or less negative change) from pre- to post-treatment conditions than other scorpions of similar initial aggressiveness, as indicated by the differences in the intercepts of the fitted lines. To reduce overlap of individual points, a small amount of random noise has been added to the plotted data. OA = octopamine treatment, PH = phentolamine (octopamine antagonist) treatment, and DMF = dimethylformamide vehicle only.

neuroendocrine systems in arthropods. Further work within scorpions to better characterize the roles of OA and other monoamines in individual, sex, and species differences in behavior promises to shed additional light on the neuroendocrine correlates of behavior in arthropods.

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