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ORIGINAL PAPER

Octopamine and serotonin have opposite effects on antipredator behavior in the orb-weaving spider, *Larinioides cornutus*

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Abstract In this study, we experimentally elevated levels of octopamine and serotonin in an orb-weaving spider, and observed the effects on the antipredator behavior thanatosis (death feigning), activity level, and running speed. We found that octopamine significantly shortened the duration of thanatosis, and its effect wore off over 24 h. We also found that serotonin significantly lengthened thanatosis, but in this case, the effect persisted for over 24 h. Neither octopamine nor serotonin affected the general activity or running speed of the spiders. To our knowledge, this is the first study to directly explore the role of biogenic amines on a specific antipredator behavior in spiders. Given that spiders must be both aggressive toward prey, yet wary of predators, we believe that this system will be an outstanding model to explore connections between behavioral ecology and neurochemistry.

Keywords Aggression · Biogenic amines · Thanatosis · Neurohormones · Neuromodulation

Introduction

There is a growing body of evidence that a wide variety of behaviors in arthropods is modulated by circulating levels of biogenic amines. In fact, neurotransmitters such as serotonin (5-HT) and octopamine (OA) have such broad behavioral effects they are commonly referred to as neurohormones (Kravitz and Huber 2003). Acting both centrally and peripherally, these neurohormones are known to affect a wide range of behaviors including the likelihood of foraging (Schulz et al. 2003), division of labor (Schulz and Robinson 2001), and waggle-dance communication (Barron et al. 2007b) in honeybees; posture and locomotion in crayfish (Tierney et al. 2004); and courtship behavior in male *Drosophila* (Certel et al. 2007). One area of particular interest is the role of neurohormones on aggression-related behaviors in arthropods. In general, higher (or experimentally increased) serotonin levels are associated with elevated aggression and dominance in crustaceans (Antonsen and Paul 1997; Huber et al. 1997; Huber and Delago 1998; but see Peeke et al. 2000), and insects (Dyankonova et al. 1999; Chen et al. 2002; Dierick and Greenspan 2007). Octopamine is considered to be an analog of norepinephrine in arthropods (Roeder 1999), and studies have demonstrated mixed effects on aggression. In crustaceans, OA is usually associated with lower aggression or more submissive behavior (Livingston et al. 1980; Antonsen and Paul 1997), but in insects, OA typically increases aggression (Stevenson et al. 2000 Stevenson et al. 2005; Dierick and Greenspan 2006; Hoyer et al. 2008). In spiders, agonistic encounters reduced the levels of both 5-HT and OA in the brains of male tarantulas (Punzo and Punzo 2001), but to our knowledge no studies have directly examined the role of these neurohormones on specific behaviors in spiders.

Despite the paucity of such studies, spiders are potentially an excellent model system to examine the role, and ecological context, of biogenic amines on behavior. Ecologically, spiders are both predators and prey, and therefore must balance the classic trade-off of wariness and foraging (reviewed in: Lima and Dill 1990). Orb-weaving spiders while foraging in their webs are exposed to predation (usually by wasps and birds), but may also be parasitized by wasps and flies (Foelix 1996). A common antipredator

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behavior in response to airborne attack is 'bailing-out', in which the spider drops from the web and hangs motionless from a dragline with its legs tightly huddled (Rayor 1996). Uetz et al. (2002) found that a colonial orb-weaving spider would 'bail-out' in response to constant vibrations at 95 Hz (from a battery-operated source touched to a support silk line), and that the likelihood of exhibiting the response increased in those spiders whose neighbors had already been coaxed to bail-out. Recently, Pruitt et al. (2008, 2010) found that this huddle response (thanatosis, or death feint) is part of an overall behavioral syndrome (sensu Sih et al. 2004) related to aggression. In their studies of social cob-web building spiders, they found that spiders which are more aggressive towards prey and conspecifics, would not hold the huddle posture as long as less aggressive spiders.

The ease in which the huddle response can be elicited and quantified in the lab makes it an excellent behavioral assay to explore the effects of neurohormones on anti-predator behavior. In this study, we begin to explore the role of biogenic amines on aggression in spiders. *Larinioides cornutus* is an orb-weaver in the family Araneidae, and is common to Europe and North America (Levi and Levi 1990). It is a nocturnal spider, constructing its web at dusk and actively foraging throughout the night while remaining hidden in a retreat during the day (Bellmann 1997). We manipulated levels of 5-HT and OA in individual spiders and observed the effects over time on the anti-predator huddle response, and also on two assays of more generalized behavior.

Materials and methods

Collection and housing

Adult *L. cornutus* were collected in September and October 2009 from buildings and shrubbery in east Tennessee. The spiders were removed from their webs and individually housed in 250 ml plastic deli containers. The spiders could not build orbs in these containers, but they would lay silk and attack/consume prey within them. Within two days of collection, the spiders were fed a cricket and given distilled water with a spray bottle. Following that, the spiders were given water once a week, fed crickets every other week, and kept at ambient light and temperature. All spiders were fed and watered the evening prior to testing.

Treatment with biogenic amines

Barron et al. (2007a) demonstrated that topical application of biogenic amines is as effective as oral delivery or microinjection at modifying honeybee behavior. For this study, we adjusted the topical method to deliver serotonin and octopamine to the spiders. Serotonin hydrochloride (5-HT) and octopamine hydrochloride (OA) were dissolved in the carrier solvent dimethylformamide (dMF) at 2 mg/ml. Solutions were made fresh on each day of application, and 1 µl of the solution was applied to the dorsal opisthosoma with a pipetor. One control group was given 1 µl of dMF only, and a second control group was simply touched with a dry pipette. Fifteen spiders were included in each experimental group and in both control groups. The spiders were individually weighed, and evenly distributed by mass across the treatment groups. Behavioral pre-trials were conducted between 0630 and 0800, and the spiders were dosed between 0800 and 0830.

Quantifying the huddle response

These spiders exhibit an anti-predator huddle response in which they draw their legs in tight to their body and remain motionless (Fig. 1). If left undisturbed, the spiders will break out of the huddle after a period of time. The method of Pruitt et al. (2008) was modified to quantify

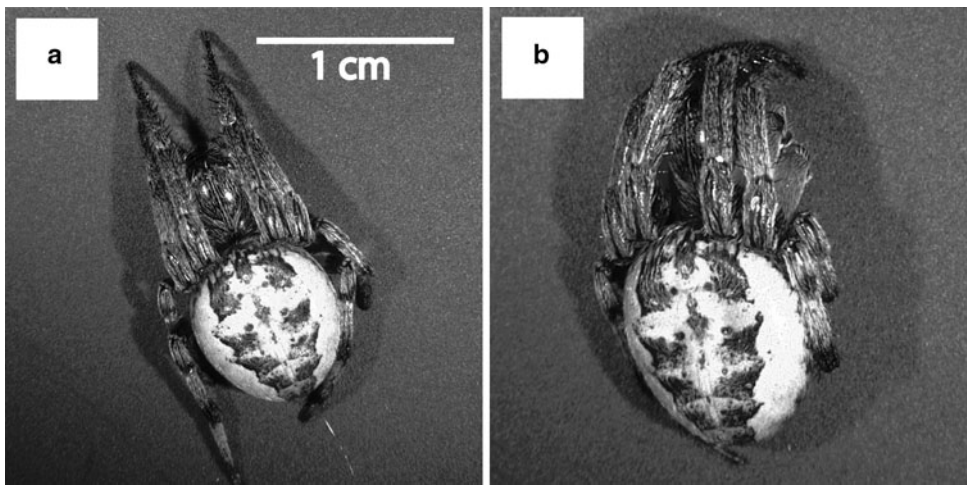

Fig. 1 Typical postures of *L. cornutus*. Spider in a resting posture (**a**) sits with their tarsi extended, while huddled spiders (**b**) have their metatarsi flexed

this response. Individual spiders were removed from their containers and placed in a glass bowl, and were given 30 s to acclimate to the dish. To trigger the huddle response, we delivered a puff of air using a squeeze bulb from 10 cm away. The fine sensory hairs (trichobothria) on the spider's legs respond to airflow, initiating the stereotypical huddle response (Foelix 1996). The huddle response was pre-tested 30–60 min prior to chemical manipulation, then again at 1, 3, 6, and 24 h after application of the chemical. We cleaned each dish with ethanol between trials.

Test for habituation in the huddle response

To test for habituation to the stimulus, 10 spiders were induced to huddle 10 consecutive times. In this case, as soon as a spider broke its huddle it was immediately induced to huddle again and the duration of each huddle was recorded. The procedure followed the squeeze bulb method described above, except that the bowls were not cleaned between trials for individual spiders.

Quantifying general activity level

To test whether the chemical manipulation grossly affected behavior (i.e. lethargy or hyperactivity), we examined the effects of the treatments on general activity levels. When placed in a novel environment, *L. cornutus* will begin wandering around the enclosure intermittently. This behavior was quantified and termed 'exploratory behavior' by Pruitt et al. (2008). To quantify the behavior, we placed spiders in a 25 × 10 cm glass bowl, which usually resulted in an initial huddling. After the spiders first initiated exploratory behavior, we recorded the amount of time they spent walking during a 3-min period. Activity trials were run immediately after each huddle trial, and the bowls were cleaned with ethanol between trials.

Quantifying running speed

Larinioides are web building spiders, and are more adept at walking on silk than on a foreign substrate (Foelix 1996). Nevertheless, these spiders are quite capable of walking on a horizontal surface, particularly if there is some texture to the substrate. We used running speed on a flat surface to test if chemical treatment was having any gross effects on coordination of motion/locomotion in the spiders. The test arena consisted of concentric 3 and 15 cm radius circles on white drawing paper. The spiders were initially held inside the smaller circle with a clean plastic container. When released and prodded, they would usually run in a straight line across the paper. The amount of time taken for the spider to cross from the inner circle (3 cm) to the outer circle (15 cm) was recorded. If the spider stopped between the lines, or did not run in a straight line, it was moved back to the center and re-run. At each observation time, three trials were recorded, and the quickest time was used in analysis. Running trials were conducted after each activity trial, and silk draglines were removed between trials with a dry paper wipe. A few spiders were crushed during this experiment and removed from further trials.

Results

Huddle response

Considering the duration of the huddle response, there was no overall evidence of habituation to the stimulus over ten consecutive trials (Fig. 2a, one-way ANOVA: $F_{9,99} = 0.51$,

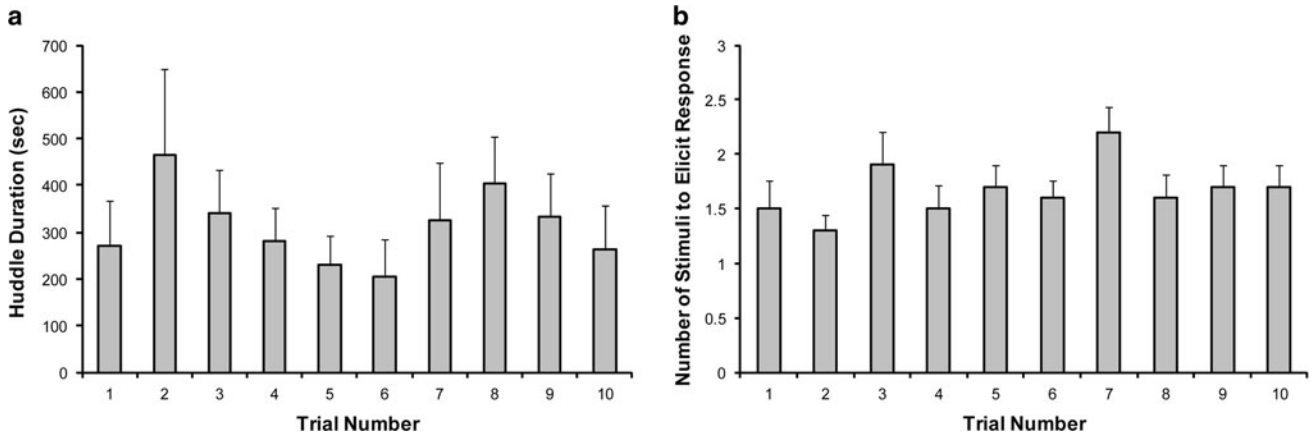

Fig. 2 Test for habituation in the huddle response to repeated stimuli. Spiders were induced to huddle by a standard puff of air ten consecutive times. Means with standard errors are reported. **a** Duration of huddle response over the ten trials, **b** the number of puffs used to trigger the response over the ten trials. *Open circles* represent individual in each trial, while the full squares are the mean for each trial

$P = 0.861$). However, one of the spiders did seem to habituate, having the two longest huddle durations among the spiders for the first and second trials, followed by a steady decrease in duration to having the three lowest durations of the last three trials. Overall, there were significant differences among individuals in huddle duration (one-way ANOVA: $F_{9,99} = 5.38$, $P < 0.001$). When considering the number of puffs required to elicit the huddle response, there was no significant effect of trial number (Fig. 2b, one-way ANOVA $F_{9,99} = 1.5$, $P = 0.223$); but there were differences among individuals in this measure (one-way $F_{9,99} = 6.4$, $P < 0.001$). There was no relationship between the number of stimuli required to elicit the response and the duration of the response (regression ANOVA $F_{1,99} = 0.25$, $P = 0.601$).

In the pre-trial of the neurohormonal manipulation experiment, spiders huddled for a mean of 75.9 s, and there was considerable variation among individuals (min = 1, max = 316, standard deviation = 98.4). The puff of air successfully initiated the response in all of the spiders, but some would immediately break out of the posture. A repeated-measure MANOVA of all the data found a significant effect of treatment ($F_{3,54} = 0.74$, $P < 0.0001$), and time ($F_{4,51} = 0.31$, $P = 0.007$), with a significant interaction between the two (Wilks' $\text{Lambda}_{12} = 0.52$, $P = 0.006$). Clearly, the huddle response was significantly affected by both OA and 5-HT (Fig. 3; Table 1). Dunnett's post hoc comparisons to the 'touch only' control showed that OA shortened the duration of huddling at both 1 and 3 h after dosing. Octopamine-treated spiders also huddled less time than all of the groups at 6 and 24 h, but these differences were not significant. The effect of OA seemed to wear off over time, with a significant positive relationship between huddle duration and time (regression ANOVA: $F_{1,52} = 6.08$, $P = 0.017$). Serotonin significantly lengthened the huddle duration at all of the times measured. The effect of 5-HT did not seem to wear off over time, and the increased huddle response was most pronounced 24 h after dosing leading to a nearly significant positive relationship with time (regression ANOVA: $F_{1,56} = 3.94$, $P = 0.052$). There were no significant differences between control spiders and those dosed only with dMF. In addition, there was no relationship between the duration of the huddle response in all of the pre-trials and the mass of the spider (regression ANOVA: $F_{1,56} = 0.05$, $P = 0.83$). The huddle response was not correlated with either activity level (Pearson = −0.213, $P = 0.10$), nor with running speed (Pearson = −0.169, $P = 0.20$).

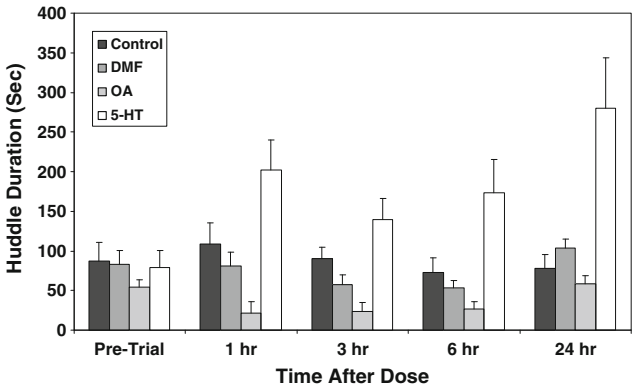

Fig. 3 Effects of OA and 5-HT on the duration of the huddle response at the pre-trial, and at four times after dosing. Control spiders were not given anything, DMF spiders were topically treated with 1 µl of the solvent, OA and 5-HT spiders were treated with 1 µl containing 2 µg of the biogenic amine

General activity

In the pre-trial, the spiders spent less than half of the three minute time trial actively moving, but there was considerable variation among individuals (mean = 76.8 s, min = 7.0, max = 180, standard deviation = 52.8). Unlike the huddle response however, general activity was not affected by treatment with biogenic amines (Fig. 4; Table 1). There was no relationship between activity and spider mass among all of the spiders in the pre-trial (regression ANOVA: $F_{1,56} = 0.03$, $P = 0.86$). Overall, the activity of the spiders appeared to be related to the time of the trial. When all of the treatment groups were analyzed in a

Table 1 Results of one-way ANOVA on individual behavioral trials by time after dosing

Time (h)	*F* statistic	*P* value	Dunnett's comparisons
Huddle response			
Pre-test	$F_{3,56} = 0.63$	0.60	
1	$F_{3,56} = 8.37$	<0.001	OA (lower), 5-HT (higher)
3	$F_{3,54} = 8.22$	<0.001	OA (lower), 5-HT (higher)
6	$F_{3,53} = 6.37$	0.001	5-HT (higher)
24	$F_{3,53} = 7.79$	<0.001	5-HT (higher)
General activity			
Pre-test	$F_{3,56} = 0.48$	0.70	
1	$F_{3,56} = 0.47$	0.70	
3	$F_{3,54} = 0.88$	0.46	
6	$F_{3,53} = 0.51$	0.69	
24	$F_{3,53} = 1.24$	0.30	
Running speed			
Pre-test	$F_{3,56} = 1.68$	0.18	
1	$F_{3,56} = 0.62$	0.61	
3	$F_{3,54} = 0.88$	0.46	
6	$F_{3,53} = 0.89$	0.45	
24	$F_{3,53} = 0.33$	0.80	

Included are Dunnett's post hoc comparisons which were statistically significant ($P < 0.05$) from the control spiders

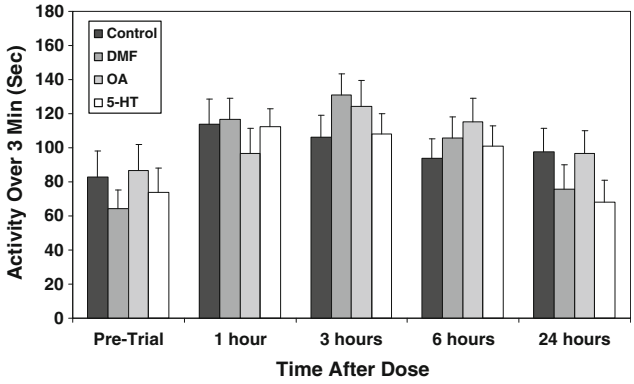

Fig. 4 Effects of OA and 5-HT on the general activity level of spiders at the pre-trial, and at four times after dosing. Control spiders were not given anything, DMF spiders were topically treated with 1 µl of the solvent, OA and 5-HT spiders were treated with 1 µl containing 2 µg of the biogenic amine

repeated-measures MANOVA, there was no effect of dosing ($F_{4,66} = 0.04$, $P = 0.61$), but there was a significant effect of test time on activity ($F_{4,63} = 0.39$, $P = 0.0003$), and a significant interaction between the two (Wilks' $\text{Lambda}_{16} = 0.60$, $P = 0.007$). Activity was not correlated with time to cover 12 cm (Pearson = 0.169, $P = 0.20$).

Running speed

Prior to chemical dosing, the spiders covered 12 cm by crawling in an average of 1.25 s (min = 0.64, max = 2.78, standard deviation = 0.46). We found no effect of treatment of biogenic amines on running speed (Fig. 5; Table 1), nor did there appear to be any affect of trial time. A repeated-measures MANOVA of all the data found no significant effect of dosing ($F_{4,66} = 0.08$, $P = 0.26$), or time ($F_{4,63} = 0.3$, $P = 0.30$), and there was no interaction between them (Wilks' $\text{Lambda}_{16} = 0.76$, $P = 0.31$). There

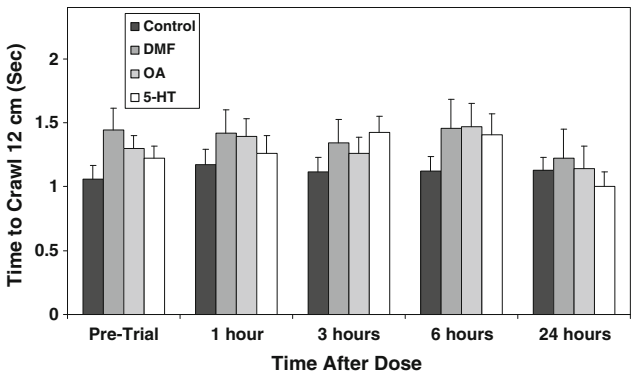

Fig. 5 Effects of OA and 5-HT on the maximum crawling speed of spiders at the pre-trial, and at four times after dosing. Control spiders were not given anything, DMF spiders were topically treated with 1 µl of the solvent, OA and 5-HT spiders were treated with 1 µl containing 2 µg of the biogenic amine

was an indication that larger spiders may be quicker, but this was not significant (regression ANOVA: $F = 2.82$, $P = 0.1$).

Discussion

This study found a clear effect of exogenously elevated levels of OA and 5-HT on the antipredator huddle response, with elevated OA decreasing the duration of the response, and elevated 5-HT prolonging it. A similar shortening of death feigning from elevation of OA is observed in the flour beetle *Tribolium castaneum* (Nishi et al. 2010), but in this case there was no observed effect of elevating 5-HT. In the same system dopamine and tyromine are also shown to shorten the duration of death feigning (Nishi et al. 2010), and lines artificially selected for shorter death feigning have lower naturally occurring levels of dopamine (Miyatake et al. 2008). Exploration of the role of these other biogenic amines in spider antipredator behavior is clearly warranted.

Spiders detect predators when air movement deflects fine innervated hairs called trichobothria (Foelix 1996), and it is likely that these trichobothria are involved in triggering the huddle response. Widmer et al. (2005) found that circulating OA increased the sensitivity of trichobothria to airbourne vibrations across a broad frequency spectrum. Treatment with OA increased the firing frequency of the associated neurons for several minutes. This suggests that elevating OA levels would increase the propensity of a spider to huddle in response to a stimulus, but does not explain why the huddle duration is shortened by OA. Decoupling of the triggering and duration of the huddle response would be consistent with our finding here that stimulus strength does not affect the duration of the behavior. It may be that OA may functions peripherally to increase sensitivity to airborne signals, while working centrally to decrease the duration of the antipredator response. This actually makes sense ecologically, as most orb-weaving spiders are only foraging at the hub of their web during part of the day. While foraging it would be to the spider's advantage to be particularly sensitive to airborne stimuli, but when they drop from the hub in a huddle, they would pay a 'cost' in lost foraging from prolonged periods out of their web. We are currently working to differentiate the effects of OA (and 5-HT) on specific components of antipredator and foraging behavior.

It is noteworthy that neither OA nor 5-HT seemed to affect the assays of general activity or running speed. This suggests that these neurohormones affect something specific about the huddle response rather than having a more general effect on the spider's physiology. In other words, the 5-HT spiders are not huddling longer simply because

they are lethargic (which would correlate with general activity) or have lost coordination (which would correlate with running speed). Conversely, it is not likely that the OA spiders are not huddling for a shorter time simply because of a general reduction in excitability. This is somewhat unexpected given that OA is considered the arthropod correlate of norepinephrine and more often associated with increased excitability (Roeder 1999).

The correlation of the huddle duration with aggressiveness toward prey and conspecifics seen in other spiders (Pruitt et al. 2008, 2010), suggests that higher aggression is associated with shorter huddling. From this, our data suggest that OA may be increasing aggression in the spider, which is similar to the insect model (Stevenson et al. 2000, 2005; Dierick and Greenspan 2006; Hoyer et al. 2008), but opposite what is observed in many crustaceans (Livingston et al. 1980; Antonsen and Paul 1997). The lengthened huddle response associated with experimentally elevated 5-HT suggests that the neurohormone makes the spiders more fearful, or less aggressive, which is opposite to what is the typical effect of 5-HT in both insects and crustaceans (Kravitz and Huber 2003).

This study found differences between OA and 5-HT in how long the respective effects lasted. Whereas the reduction in the huddle response from OA appeared to have worn off after 24 h, the prolonged huddling effect of 5-HT was still apparent. For the OA treated spiders, behavioral response over time is consistent with the extra biogenic amine being metabolized and leaving no lasting effects on sensitivity to naturally occurring levels of OA in the spider. The fact that the effect of 5-HT is even more pronounced at 24 h after dosing (with no prolonged effect observed in DMF-only treated spiders) suggests that either the extra biogenic amine is not being metabolized in that time, or that the treatment has effectively hyper-sensitized the spiders, causing them to respond more intensely to their natural levels of 5-HT. The latter case seems more likely as experimental manipulation of 5-HT in arthropods only elevates levels in the hemolymph for a short time (Pank-sepp et al. 2003), and most behavioral effects are similarly short-lived (Huber et al. 1997; Huber and Delago 1998; Livingston et al. 1980). Further work directly quantifying the natural and experimentally manipulated levels of biogenic amines, along with experiments involving sequential doses, will be needed to discern between these hypotheses. We do know from another study that *L. cornutus* shows circadian rhythmicity in the huddle response, and that the effects of a single dose of 5-HT prolongs the response for several days, but does not affect overall rhythmicity (unpublished data). It is therefore interesting that 5-HT is known to modulated circadian rhythmicity in photoreceptors in flies (Chen et al. 1999), crickets (Saifullah and Tomioka 2002), and locusts (Cuttle et al. 1995).

The results of this study clearly indicate that OA and 5-HT affect the huddle response of *L. cornutus*. Octopamine significantly shortened the duration of the response while 5-HT significantly lengthened it. Given the results presented here, we are confident that these differences were not due to habituation. Overall, we believe these results indicate great promise for further study of the roles of OA and 5-HT in spider behavior. An important caveat however, is that we do not know how our topical dosing of the spiders affected circulating levels of neurohormones relative to naturally occurring levels. As Zera (2007) points out, results of hormone manipulation must be interpreted cautiously, as manipulation of the chemical in question could cause a cascade of changes in other regulators which are actually responsible for the observed behavioral effects, or even cause aberrant effects when elevated outside of normal ranges. We are currently working to calibrate dosing levels with natural levels of neurohormones, and also to correlate behavioral variation with natural neurohormone levels in this species. Further manipulative experiments are warranted to identify the role of neurohormones on aggressive and antipredator behaviors. Given the tractability of spider behavior, this system should provide an excellent model to connect specific foraging and antipredator behaviors to their neurochemical underpinnings and ecological consequences.

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