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INFLUENCE OF PREY ODOR CONCENTRATION ON THE POSTSTRIKE TRAILING BEHAVIOR OF THE NORTHERN PACIFIC RATTLESNAKE

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ABSTRACT: Rattlesnake predatory behavior is defined by a strike and then release of rodent prey. Poststrike, the released envenomated prey dashes off and dies. Therefore, the snake must relocate the prey relying largely upon chemosensory cues emitted by the struck prey. Assessing these poststrike chemosensory cues is important to the snake as these cues may contain significant information about the effectiveness of the preceding strike and hence the likelihood that trailing the odor left by the envenomated prey will allow successful recovery of the prey. One possible cue in this scent trail is concentration of the distinctive odor generated in the prey during envenomation. To test this, we presented snakes with different poststrike choices of low and high prey-odor concentrations. We found that although rattlesnakes could trail each concentration level, they preferred the high odor concentrations. These results imply that rattlesnakes respond to concentration levels, not just to a threshold level of prey odor. This adjustment to different odor concentrations is not accomplished by varying rate of tongue flicks (RTFs). Based on this study and previous work, we propose a behavioral mechanism by which rattlesnakes assess the chemosensory quality of a poststrike prey odor trail.

Key words: Chemoreception; *Crotalus viridis oreganus*; Predatory behavior; Poststrike trailing; Rattlesnakes; SICS

RATTLESNAKES strike and usually immediately release envenomated rodent prey (Kardong, 1986; Klauber, 1956). The strike itself is necessary to trigger the subsequent poststrike trailing behaviors (Chiszar and Scudder, 1980; Chiszar et al., 1977; Chiszar et al., 1983*a*,*b*; Chiszar et al., 1986; Chiszar et al., 1990; Chiszar et al., 1991; Chiszar et al., 1992; Furry et al., 1991; Haverly and Kardong, 1996; Melcer and Chiszar, 1989; Robinson and Kardong, 1991; Scudder et al., 1992), which are accompanied by elevated tongue flick rates and the preference for the odor trail of the struck mouse (Burghardt, 1980; Chiszar and Scudder, 1980; Duvall et al., 1980; Haverly and Kardong, 1996). In particular, a rattlesnake is able to discriminate the odor trail of the mouse it struck from all other competing environmental odors including the prestrike odor trail of the same mouse (Kardong and Smith, 2002; Smith and Kardong, 2000). Successful envenomation not only enhances the perceptibility of the trail of the envenomated rodent (Chiszar et al., 1983*a*; Lavín-Murcio and Kardong, 1995) but also induces the distinctive odor released by the mouse that the rattlesnake uses to track and relocate the particular mouse it envenomated (Kardong and Smith, 2002; Smith and Kardong, 2005).

A few studies indicate that the chemosensory system of rattlesnakes processes the information from envenomated prey in a qualitative way to adaptively change predatory behaviors. For example, it is suggested that rattlesnakes can use the "quality" of the odor trails to assess their chances of trailing success (Lavín-Murcio and Kardong, 1995). Airborne odors bring a different response to envenomated prey than substrate deposited odors leading to differences in trailing behavior (Parker and Kardong, 2005). Varying doses of injected venom bring varying responses in the poststrike behavior of rattlesnakes. Further, the interest of the snakes in this poststrike odor is directly correlated to its concentration (Chiszar et al., 1999). However, it is not clear whether trailing rattlesnakes respond to any level above threshold perceptibility equally or if they prefer trails with higher odor concentrations.

Therefore, the purpose of this study was to examine the effects of odor concentration left

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by a struck mouse on the trailing behavior of the snake. To do this, we offered the snake the choice of two paired poststrike prey odors in a Y-maze, one low concentration and one high concentration.

Methods

We collected 21 northern Pacific rattlesnakes, *Crotalus viridis oreganus* (SVL: 33.5–82.5 cm) in Whitman County, Washington under state permits, housed individually in glass aquaria, and held in captivity for at least one year prior to the onset of feeding trials, a length of time that produces no significant change in predatory behavior (Alving and Kardong, 1994). Maintained on white laboratory mice (Swiss Webster), snakes were fed twice a month with water provided ad libitum. Safety procedures for snakes generally followed those of Gans and Taub (1964).

A square test arena (1.25 m side × 0.5 m high) described elsewhere in detail (Alving and Kardong, 1996; Lavín-Murcio et al., 1993; Robinson and Kardong, 1991) provided the context for experimental trials. Before each trial a new piece of white butcher paper was placed over a Y-shaped outline made of black tape affixed to the floor of the arena. The Y-outline, a 50-cm base and 50 cm each arm, visible through the white paper, guided the placement of the scent trails. We began each trial by placing one snake in a holding box located at the base of the Y-outline for an acclimation period of not less than 6 hours. After acclimation, we placed a removable chute in a slot in front of the holding box, and introduced a preweighed mouse through the chute to the rattlesnake in the holding box. Once struck by the snake, we retrieved the mouse via fishing line tied to the base of the rodent's tail, replaced the door to the holding box, and removed the chute. We placed pairs of nonoverlapping scent trails, from the holding box along the base of the Y then out one arm, for each trial. To make paired choices of nonoverlapping sent trails, we grasped the nape of the mouse's neck with long forceps and, belly down, slid it smoothly in one continuous motion slowly along the base and out an arm of the Y-maze, taking 10+/–3 s to complete the laying of a scent trail. For Baseline trials, we made the two odor trails with the same mouse, before (unstruck) and after (struck) being envenomated. We compared results of the baseline trials to those of the experimental trials.

In the Experimental trials, we tested for the effects of odor concentration using two paired trails, one low and one high concentration. To do this, we used a single pass of the mouse along the trail and out arm A; then we used the same mouse and passed it in 5 overlapping trails along the base and out the second arm, B. We assumed that, just as repeated passes of a paint brush add layers of paint, the 5 repeated passes of a struck mouse would deposit additional layers of its scent to the odor trail. This marking technique gave two choice trails, one (A) of low odor concentration produced by a single pass of the struck mouse, and one (B) of high odor concentration produced by five overlapping passes of the same mouse.

In theory, the odor concentration could be affected by the order of swipes of the struck mouse in making these paired trails. For example, the first pass of the struck mouse along the paper could remove most of the significant odor leaving little on the mouse to produce the alternative scent trail. To control for this possibility, we actually ran two variations of the experimental trials by varying which trail received the first pass of the mouse. In Experiment 1 (Exp 1), we first passed the struck mouse out arm B, then one pass out arm A, and then returned to arm B placing the remaining four swipes. For Experiment 2 (Exp 2), we made the first pass of the struck mouse out arm A and then used the same mouse to make five consecutive passes our arm B. This gave us three treatments—baseline, Exp 1, Exp 2—run in random order.

Based upon earlier trailing experiments (Alving and Kardong, 1996; Lavín-Murcio and Kardong, 1995), we ended a trial when a snake completed trailing (traveling to the end of either arm) or after 20 minutes, whichever came first. A snake was considered to be following a scent trail if its head remained, except for short departures (less than 30 s), within 10 cm of either side of the scent trail. We defined the total time of trailing as the time from when the head of the snake first emerged from the holding box until it crossed the end of one trail. Also, we scored the rate of tongue-flicking (RTF, tongue-flicks/min) for each trial as the

total number of tongue flicks observed divided by the total time of trailing. For statistical analysis, we assigned a score of 0 to a snake not choosing a trail, choosing A as 1, and B as 2. Conditions of data normality were not met, so we used binomial and nonparametric tests for statistical significance, as appropriate, with $\alpha = 0.05$ (Zar, 1999).

Results

Concentration of odor influenced the poststrike trailing of the snake, but order of placement of the cue did not. In baseline trials (unstruck versus struck), 19 out of the 21 snakes trailed. Of those that successfully trailed, 19 out of the 19 snakes followed the trail made from the struck mouse. In Exp1 (B trail received first pass), 20/21 snakes trailed. Of those that successfully trailed, 16 chose the B trail (higher concentration) and 4 chose the A trail (lower concentration), a significant difference in choice (binominal test, $k \leq 4$, 0.006, one-tailed; $P = 0.012$, two-tailed). In Exp 2 (A trail received first pass), 20/21 snakes trailed. Of those that successfully trailed, 17 chose the B trail and 3 the A trail, also a significant difference in choice (binominal test, $k \leq 3$, 0.001, one-tailed test; $P = 0.002$, two-tailed test). We found no significant difference between Exp 1 and Exp 2 (Wilcoxon, $Z = 0.314$, $P = 0.753$), suggesting that the odor is not wiped from the mouse on the first swipe. Furthermore, we documented no significant difference in trailing choice between the three treatments—baseline, Exp 1, Exp 2 (Friedman's test for homogeneity, $\chi^2_{0.05,2} = 0.595$, $P = 0.743$). In addition, the overall trailing behavior observed in the baseline and experimental trails did not differ as indicated by the trailing parameters of total time of trailing (emerge from the holding box to the end of one arm) ($x^{\text{Baseline}} = 114.1$, Exp 1 = 93.6, Exp 2 = 117.9 s; Friedman's test: $\chi^2_{0.05,2} = 1.50$; $P = 0.4724$) and by RTF (tongue flicks per minute) ($x^{\text{Baseline}} = 79.3$, Exp 1 = 80.5, Exp 2 = 80.1 RTF; Friedman's test: $\chi^2_{0.05,2} = 0.857$, $P = 0.6514$).

Discussion

Our results indicate that rattlesnakes preferentially follow the poststrike odor trails produced with multiple, overlapping swipes of the struck mouse compared to an odor trail made with a single swipe of the same mouse. Although we did not directly measure absolute odor concentrations in the two odor trails, single versus multiple swipes of the mouse seems a reasonable way to produce such choices in odor concentration for the rattlesnakes, low versus high. The "low" concentration trail in Exp's 1 and 2 represents the same level as used here in the baseline and the same as used in previous laboratory studies of poststrike trailing (e.g., Robinson and Kardong, 1991), and to which rattlesnakes have been shown to respond preferentially by following the trail of the struck compared to an unstruck mouse odor trail (Chiszar et al., 1992; Kardong and Smith, 2002).

We interpret these results as follows. First, snakes prefer a "high" to a "low" concentration poststrike rodent trail. Even though they could successfully trail a low concentration scent (baseline, previous literature), rattlesnakes prefer the higher concentration when given the choice. Therefore rattlesnakes respond not to just a threshold level of odor, but also to concentration levels above threshold perceptibility. Previous work documents a positive correlation between snake interest (tongue flicks directed at prey) and venom dose (Chiszar et al., 1999). Our results establish a similar positive correlation between poststrike trailing preference and odor trail concentration.

Second, although snakes change trailing preferences (from low to high concentrations), they do not alter the rates of tongue flicks (RTF) to adjust to differences in odor concentration. This result supports the view that poststrike rates of tongue flicking, released by the strike, are a stereotypic behavior (Chiszar et al., 1977; Chiszar et al., 1982) producing stimulation of the vomeronasal system (Halpern, 1988). Although different prey types may be accompanied with different RTFs (Haverly and Kardong, 1996), rattlesnakes in the context of our protocol here do not adjust to levels of trail odor by changing RTFs.

Third, our results imply that rattlesnakes possess the neurosensory ability to recognize concentrations of the prey odor produced by

envenomation and use it to assess risk/chance of prey recovery. Earlier work, using a different protocol, also argued that poor envenomation might produce an ambiguous or poor quality poststrike odor trail, prompting the rattlesnake to cease pursuit of an unpromising trail (Lavín-Murcio and Kardong, 1995). Our results herein suggest a chemosensory mechanism for making such a choice to trail or not. This could provide the neural basis of one behavioral mechanism by which the rattlesnake indirectly assesses through chemosensory cues the success of its own predatory strike. Specifically, the rattlesnake might evaluate the prey odor trail directly and hence the likelihood that the odor includes sufficient and specific cues to permit recovery of the envenomated prey. Proceeding to trail struck mice when there is little chance of recovery expends energy of the snake uneconomically and increases its exposure to its own community of predators. Such information, odor concentration, is one characteristic of the envenomation-induced odor trail available to the snake that determines whether poststrike trailing will be released or not. This would represent the proximate behavioral mechanism by which ecologically adaptive behaviors are initiated.

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