BLOOD IS NOT A CUE FOR POSTSTRIKE TRAILING IN RATTLESNAKES

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1. INTRODUCTION

Rattlesnakes in the wild usually strike, envenomate, and release rodents immediately (Klauber, 1956) avoiding potential injury from retaliation, but this may allow the struck rodent to scamper some distance from the site of initial envenomation while the cocktail of venom components immobilizes and eventually kills the rodent. Because the envenomated rodent often breaks visual contact, relocation of the dispatched prey may rely upon chemosensory cues emitted by the struck prey. On theoretical and on experimental grounds, these chemosensory cues may be carried in the blood. Theoretically, the strike includes deep penetration of the fangs (Kardong and Bels, 1998), which may carry away distinctive chemical cues used next to relocate the envenomated prey. Experimental trials with blood suggest that it prompts elevated interest in colubrid snakes (Chiszar et al., 1992a) and prompts high-rates of tongue flicking in rattlesnakes (Chiszar et al., 1993b).

Consequently, the purpose of our experiments was to see if blood *alone* carried chemical cues used during poststrike trailing by the rattlesnake to relocate its envenomated prey.

2. MATERIALS AND METHODS

Twenty-three individually housed northern Pacific rattlesnakes, *Crotalus viridis oreganus* (adult, long-term captives) collected locally in Whitman Co., WA, under State permits, were used in each of four experiments. Snakes were maintained on white laboratory mice (Balb/c or Swiss Webster), fed twice a month, and provided water *ad*

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libitum. All mice were fed Harlan Teclad 8640 Rodent Diet mouse chow, and kept on the same bedding of hard wood shavings. Safety procedures for snakes generally followed those of Gans and Taub (1964).

Trials were conducted in an arena (1.25 m each side) described elsewhere in detail (Alving and Kardong, 1996; Lavín-Murcio et al., 1993; Robinson and Kardong, 1991). Temperature in the test arena room was held between 25-30 C°. A Y-shaped outline made of black tape was placed on the floor of the arena and covered with a new piece of white butcher paper before each trial. Hatch-marks were placed perpendicular to the main Y-outline at 10 cm increments. The Y-outline, a 40 cm base and 40 cm each arm, could be seen through the white paper and was used to guide the placement of the paired scent trails. Before each trial, a snake was removed from its home cage and placed in a holding box stationed at the beginning of the Y-maze for a period of time no shorter than 12 hours, including overnight.

Treatment 1 – (Struck). Each snake was presented a choice of two trails, one of a struck mouse, the other of a water trail. At the onset of all trials, the room lights were dimmed and a removable chute was placed in front of the holding box. A pre-weighed mouse, secured with fishing line by its tail, was introduced down the chute to the snake. After the strike, this mouse was removed and used to make the prey odor trail. Pairs of non-overlapping scent trails were made for each trial immediately after the strike. First, de-mineralized water was used to make the control trail applied to the maze with a cotton-tipped applicator along the base and out one arm. Next, while being held in forceps by the nape of the neck, the struck mouse, belly-side in contact with the paper, was moved along the main branch, taking care not to overlap the water trail, and out the other arm of the Y-maze.

Treatment 2 – (Unstruck). The two trail choices consisted of an unstruck mouse versus water, using methods as in Treatment 1, except the mouse trail was made with an unstruck mouse. After making the trail with the unstruck mouse, the snake was only then allowed to strike this same mouse. (The strike is necessary to release poststrike trailing behavior (Chiszar et al., 1992b; Smith et al., 2000).)

Treatment 3 – (Unstruck plus blood). The two trail choices consisted of unstruck mouse odor out both arms, but blood taken from the struck mouse (see next for technique) was stroked over one of the unstruck choice trails. To do this, the unstruck mouse was slid along the base of the Y-maze and out one arm; then this same mouse was slid along the base of the Y-maze and out the other arm. This mouse was next presented to the snake and struck. After death, blood was drawn from it by cardiac puncture, placed on a cotton-tipped swab, and stroked over one of the two previously laid unstruck odor trails.

Treatment 4 – (Blood). The same group of snakes was presented with two trail choices, the first made with distilled water and the second made with blood drawn from the struck mouse. Blood was taken from the dead mouse just struck by the snake (about 2 min before) by cardiac puncture and then deposited onto a cotton-tipped applicator for making the trail. To prevent transfer of integumentary cues, the needle used in cardiac puncture was removed before loading collected blood from the syringe onto the cotton-tipped applicator.

Immediately (<2 min.) after laying the trails in all treatments, the door to the holding box was removed and all ensuing behaviors were recorded via a Panasonic camera capable of filming under low light. The experimenter stepped out of view and recorded

tongue-flicks from a monitor. Additional data were collected later from replay of the videotape.

The snake was considered to be following a trail if its head stayed within the 10-cm guidelines placed on either side of the black tape Y-maze trail. If the snake went outside these guidelines for over 30 seconds or if it did not leave the holding box within the 20 minute trial period, the snake was scored as out of bounds (OB) or not trailing (NT), respectively. Three main variables were scored: CHOICE, whether or not the snake followed an arm of the Y-maze to completion; TRAIL DISTANCE, the distance traveled along the Y-maze expressed as a percentage of the number of hatch marks crossed during the trailing episode out of the total of nine, evenly spaced hatch marks; EMERGERTF, rate of tongue-flicking per minute immediately upon emergence from the holding box. Results were analyzed using Statmost (Datamost Corp.) statistical software package. Appropriate nonparametric statistical tests were used (McNemar, Wilcoxon signed-rank tests, Krustal-Wallis). These protocols met guidelines for animal care and were approved by the local IACUC.

3. RESULTS

For Treatment 1 (struck mouse versus water), all snakes (23/23) trailed and exhibited characteristic poststrike chemosensory trailing, showing sustained high rates of tongue-flicking (RTF) and movement consistent with strike-induced chemosensory searching (SICS) (Chiszar et al., 1977; Chiszar et al., 1983; Chiszar et al., 1992b); for a review, (Gillingham and Clark, 1981; Golan et al., 1982; Haverly and Kardong, 1996). Having trailed 100% of the time in all trials, TRAIL DISTANCE averaged 100% (9/9 hatch marks) (Figure 1). Trailing of the prey odor trail was close and continuous. The average EMERGRTF was 77.9 tf/min. Total time of trailing averaged 127.6 seconds for 23 trials.

For Treatment 2 (Unstruck versus water), snakes trailed the mouse odor 73.9% (17/23), none of the snakes trailed the water trail; 21.7% (5/23) snakes were scored as out-of-bounds (OB), and one (4.4%) snake failed to leave the holding box and was scored as not trailing (NT). For all trials including OB and NT, TRAIL DISTANCE averaged 88.4% (8.8 hatch-marks). EMERGERTF was 80.8 tf/min. For those trials that the snake completed trailing (17) the average time of trailing was 194.1 seconds.

For Treatment 3 (Unstruck versus unstruck plus blood), snakes trailed 65.2% (15/23), although 8 of these were out the unstruck mouse odor trail (no overlaying blood); 7 were of the unstruck mouse odor trail (overlaid with blood). For all trials, TRAIL DISTANCE averaged 74.4 (6.6 hatch-marks). EMERGERTF was 83.3 tf/min. For those trials that the snake completed trailing (15) the average time of trailing was 106.1 seconds.

For Treatment 4 (blood versus water), none out of the 23 (0%) snakes successfully trailed along the main branch and out one arm to completion. In six of the trials (26.1%), the snakes moved very little out of the holding box and were scored as not trailing (NT). In the other 17 trials (73.9%), the snakes left the holding box and imprecisely trailed along the main branch, but did not reach the intersection before veering completely off and abandoning trailing, scored as out-of-bounds (OB). For all trials of blood cues,

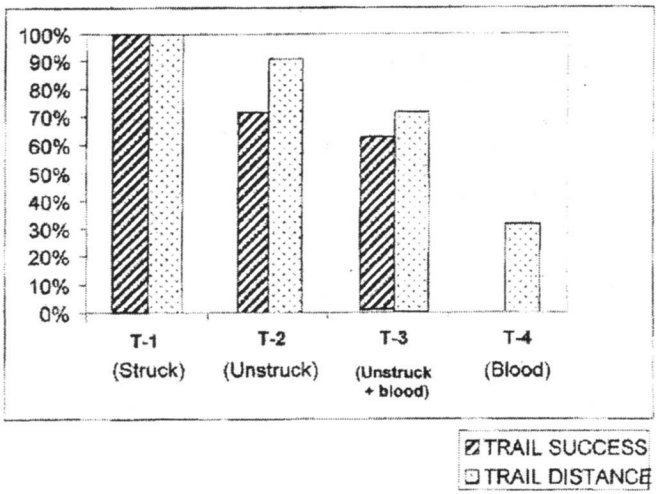

Figure 1. Trailing. Trail Success (CHOICE): whether or not the snake followed an arm of the Y-maze to completion, expressed as a percentage of average overall success for all trials. TRAIL DISTANCE: the distance traveled along the Y-maze expressed as percentage of the number of hatch marks crossed during the trailing episode out of the total hatch marks possible (9). Treatment 1 (T-1), Treatment 2 (T-2), Treatment 3 (T-3), Treatment 4 (T-4). Both trailing success and distance were significantly different in Treatment 1 compared to the other three treatments.

TRAIL DISTANCE averaged 30.9% (3.5 hatch-marks). EMERGERTF was 73.0 tf/min for those snakes where it was possible to record this rate (n= 20 trials). Since no snake completed trailing, there were no total times of trailing.

CHOICE was significantly different between Treatment 1 (Struck) and Treatment 2 (Unstruck) (100% vs. 73.9, X^2 = 4.17, P= 0.04) and between Treatment 1 (Struck) and Treatment 3 (Unstruck plus blood) (100% vs. 65.2%, X^2 = 6.12, P= 0.01). TRAILING DISTANCE was also significantly different between Treatment 1 (Struck) and both Treatment 2 (Unstruck) (Z= 2.2, P= 0.028) and Treatment 3 (Unstruck plus blood) (Z=2.5, P=0.01). Both CHOICE and TRAILING DISTANCE were significantly higher in all treatments compared to Treatment 4 (blood trail) (Z=3.97, P>0.001). However, there was no significant difference in CHOICE (X^2 =0.125, P=0.72) or in TRAILING DISTANCE (Z=1.24, P=0.21) between Treatment 2 (Unstruck) and Treatment 3 (Unstruck plus blood). The average rates of tongue flicking (RTF) upon first emergence from the holding box were significantly elevated above prestrike values (78.7 EMERGERTF up from 0.36 RTF prestrike) indicating that in all treatments the strike had released normal SICS behavior. Across the four treatments, the elevated EMERGERTF were not significantly different (H=5.10, P=0.16).

Overall, rattlesnakes did not follow the blood trail. In some trials with blood, snakes began to trail, exhibiting a predatory interest and SICS, but did not completely follow a blood (or water) trail to its end. TRAIL DISTANCE was used to express this level of trailing. Snakes completed significantly more trailing distance when the odor of a struck

mouse was used than in any other treatment. This was especially true compared to the blood trail, wherein snakes, on average, ventured only about a third of the way along the trail. Note that adding blood to an unstruck mouse odor trail (Treatment 3) did not improve rattlesnake performance compared to the unstruck mouse odor trail only (Treatment 2). In fact, rattlesnakes in Treatment 3, when choosing an odor trail, made no significant discrimination between the two choices, (unstruck trail, n=8; unstruck plus blood, n=7). For total time of trailing, snakes trailed faster in Treatment 1 (Struck) trials than in Treatment 2 (unstruck) (127.6 sec. versus 194.1 sec., z-score = 2.44, p-value = 0.015, Wilcoxon sign-rank).

4. CONCLUSIONS

First, although the rattlesnakes in our study exhibited SICS, as defined by increased RTF and movement (Chiszar et al., 1977), they did not follow a poststrike odor trail made using only blood. This was not likely a consequence of low blood concentration along the trail. The blood trails presented to snakes were often still very wet when the snake first exited the holding box, whereby the snake body actually smeared the blood trail over which it had moved.

As in previous studies (Chiszar et al., 1983; Chiszar et al., 1992b; Smith and Kardong, 2000), we found a statistically higher EMERGERTF after the strike. However, in all four treatments, the elevated EMERGERTF's were statistically equivalent. Past studies showed increased RTF and other behaviors related to SICS when presented general biological materials (Chiszar et al., 1999a; Chiszar et al., 1992b; Chiszar et al., 1983; Chiszar et al., 1981; Chiszar et al., 1991; Chiszar et al., 1982). Other studies have also shown that snakes exhibit moderate rates of tongue-flicking, prestrike, when presented with biological odors of interest (Smith et al., 2000). Rattlesnakes relying on ambush for prey capture must first locate an area of high prey density to set up – sit-and-wait (Duvall et al., 1990; Duvall et al., 1985). Certainly in the wild, rattlesnakes use general chemosensory information prestrike to locate habitats occupied by rodents and there wait in ambush (Duvall et al., 1985) so that SICS in response to blood is not surprising. But it is important to note that SICS found in our study were elicited by the strike, and that blood added (or subtracted) nothing significant to (or from) the poststrike, RTF. As significant amounts of blood are very seldom lost by an envenomated mouse (Kardong, 1986), a blood trail in the wild would be an infrequent and quite unreliable consequence of envenomation. Therefore, it is not surprising that northern Pacific rattlesnakes, *Crotalus viridis oreganus*, do not use blood, alone, as a poststrike cue for successful chemosensory trailing.

Second, envenomation does produce a chemical cue that is formed quickly after the strike and immediately secreted from the rodent into the environment to produce an odor trail followed poststrike by the rattlesnake (Chiszar et al., 1992b; Kardong, 2001). Poststrike, rattlesnakes can follow an odor trail produced by an unstruck mouse (Treatment 2). However, the poststrike trailing success of rattlesnakes significantly improves if provided with the odor trail of a *struck* mouse (Treatment 1 compared to Treatment 2). Whatever this strike-induced odor cue might be, it is not carried in the blood to surface sites on the rodent for release to the environment.

Third, blood odor added to the odor of an unstruck mouse does not increase the poststrike trailing success of rattlesnakes (Treatment 3 compared to Treatment 2).

Essentially, poststrike trailing rattlesnakes ignored the blood cue, as they exhibited no preference for it over an unstruck mouse odor trail (Treatment 3). Therefore blood odor and mouse odor do not interact in a synergistic way to produce a scent trail of increased perceptibility for the rattlesnake.

Fourth, the chemical cue reaching the environment and producing the chemosensory image followed by the rattlesnake may be binary, composed of two or more necessary chemical principles presented simultaneously or in temporal sequence. For example, the binary cues used for poststrike trailing might involve integumentary (individual mouse odor) cues together with some other component related to envenomation. Successful poststrike trailing is a multiple step process entailing the delivery of an accurate strike, initially locating the beginning of the odor trail, selecting the trail of the struck mouse over other odors, and following the correct trail in the correct direction to completion. Thus multiple cues--multiple classes of cues--may be used at different stages of the entire trailing episode. Viewed as a binary system, our results could be interpreted as follows:

The increased EMERGERTF we observed in treatment 4 might be a normal response under poststrike conditions, picking up a general biological odor such as mouse scent. However an additional cue was unavailable—the odor of the individual struck mouse. Individual mouse odor, of this binary system, would be obligatory and the first chemosensory cue. The second chemosensory cue, induced by the strike/envenomation, serves to enhance or produce the odor for specific (Chiszar et al., 1992b; Chiszar et al., 1999b) discriminatory trailing. In our trials, rattlesnakes successfully followed an odor trail of the unstruck mouse (although at lower rate of success then of struck mice), suggesting that the cue provided by envenomation is not always necessary. The strike is necessary to release poststrike trailing, but so initiated, rattlesnakes can distinguish mouse odor trails even between littermates (Chiszar et al., 1992b). Cues provided by envenomation might only play a role when discriminating two closely related trails such as from the same mouse, before and after being struck. However, if individual mouse odor is absent, then an important part of the chemosensory binary image is absent.

In addition to odors picked up during contact with the rodent, the *specific* chemosensory cue(s), used for trailing, might also include a chemical principle generated by the strike. Envenomation may stimulate the rodent to release some type of alarm pheromone, secreted into the air and/or deposited on the substrate over which the struck rodent runs. Along with the odor of the mouse, rattlesnakes sample this cue via tongue-flicking and deploy this cue as a kairomone (chemicals used advantageously by the receiver [predator] than the emitter [rodent]) (Weldon, 1980) to successfully discriminate the particular trail of the struck rodent. The binary chemosensory cues would therefore include one part identifying the particular mouse (picked up during the strike) and a second part indicating envenomation (kairomone). Since the blood contained neither of these binary cues, this would account for why blood trails were not followed successfully. This also explains discrepancies between our results with blood (no trailing interest) and the results of others (tongue-flick interest) (Chiszar et al., 1993b). Different cues are emphasized during different predatory phases (Kardong, 1992; Kardong, 2001). Although blood may be biologically significant for snakes during other phases in their predatory behavior (Chiszar et al., 1993a), it may not have any biological significance during the poststrike trailing phase because it lacks chemical cues with information about the specific prey struck and about the success of envenomation.

In summary, although snakes showed SICS, poststrike, consistent with results from previous studies, this increased predatory interest did not lead to successful poststrike

trailing of a blood trail. Therefore, whatever the chemical cue(s) used to trail poststrike, these are not carried in the blood to sites of surface release into the environment. Further, rattlesnakes do not use blood, alone, as a cue for poststrike trailing. We suggest that the chemosensory cue used in successful poststrike trailing is a binary chemosensory cue. This is consistent with the likely environmental chemosensory image produced by an envenomated rodent.

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