

RATTLESNAKES CAN USE AIRBORNE CUES DURING POST-STRIKE PREY RELOCATION

M. Rockwell Parker and Kenneth V. Kardong*

1. INTRODUCTION

Rattlesnakes are a unique vertebrate system for studying chemosensory behaviors because unlike other venomous snakes such as elapids (Kardong, 1982), rattlesnakes break physical contact with the prey following an envenomating strike (Klauber, 1956) to reduce injury from retaliation by the prey (Chiszar et al., 1977; Estep et al., 1981; Golan, 1982; Cundall and Beaupre, 2001). Once released, the prey may then travel significant distances before succumbing to the effects of the venom (Estep et al., 1981; Kuhn et al., 1991). This presents an immediate problem to the snake: how to relocate the prey. Previous studies have shown that rattlesnakes are extremely efficient at relocating envenomated prey using substrate trails (see Kardong and Smith, 2002 for review). Rattlesnakes are capable of discriminating between unstruck and struck prey substrate trails (Furry et al., 1991), and they can successfully relocate struck prey when deprived of visual cues (Chiszar et al., 1977). However, cues such as urine (Chiszar et al., 1990) and blood (see Smith and Kardong, this volume) are of little importance to the snake during post-strike relocation.

Following the strike, rattlesnakes exhibit stereotyped innate behaviors termed strike-induced chemosensory searching (SICS) (Chiszar et al., 1977), including an increased tongue flick rate. This increase is indicative of subsequent increased stimulation of the vomeronasal organ (VNO) via cues received and transferred by the tongue (Burghardt and Pruitt, 1975). Previous work on rattlesnakes has suggested that the envenomating strike is the mechanism by which the snake acquires a partial chemosearching image (Melcer and Chiszar, 1989; Chiszar et al., 1991) and releases SICS behavior (Chiszar et al., 1977).

Relocation of the envenomated prey is crucial to the survival of the snake. Therefore, multiple cues could be used by the rattlesnake during relocation to insure

* M. Rockwell Parker and Kenneth V. Kardong, School of Biological Sciences, Washington State University, P.O. Box 644236, Pullman, WA, 99163.

recovery. Furthermore, substrate cues could be of primary utility to rattlesnakes, but it is doubtful that such cues are used exclusively in the process of prey relocation. To date, all previous studies investigating rattlesnake post-strike trailing behavior have only presented substrate-deposited chemical trails to the snakes. Although work has been conducted on rattlesnake olfaction (Cowles and Phelan, 1958), there was no distinction made between pre-strike and post-strike reactions of snakes to the odors presented. Therefore, our purpose was to examine the abilities of rattlesnakes (*Crotalus viridis oreganus*) to use airborne cues to relocate envenomated prey.

2. MATERIALS AND METHODS

Twenty-three northern Pacific rattlesnakes (*Crotalus viridis oreganus*) were used as the test animals. All individuals were housed in separate 10 gallon aquaria and kept on a 12h/12h l:d cycle at 30°C. Water was provided *ad libitum*, and the aquaria were lined with newspaper. Prey items used during all experiments were Swiss-Webster mice obtained the day of the trial from a large breeding colony.

The arena used for all trials has previously been described in detail (Lavín-Murcio et al., 1993). A Plexiglas Y-maze was created through which a moving airstream could be directed, and the maze fit directly over the Y-outline on the bottom of the arena (Figure 1). Small computer cooling fans (6 cm X 6 cm) were inserted into each arm of the Plexiglas Y-maze to provide and maintain air flow. Honeycomb flow blocks (1.5 cm-thick) were placed in front of the fans to reduce significant turbid air flow, and the holding box was fitted with a ¼" hardware cloth top to allow for unidirectional flow. The arena floor was covered with fresh butcher paper prior to each trial and then removed following the trial.

During a trial, the mouse's tail was tied with string, lowered down a vertical Plexiglas chute, struck by the snake, and then removed. Both fans were turned on immediately before removing the sliding door to the holding box, and both fans were on during all three treatments. All trials were recorded under low-light (Smith et al., 2000) using black and white security cameras and a VCR.

2.1. Treatments

Treatment 1 (substrate-only) was used as a baseline to test whether the snakes could use mouse-deposited substrate odors to relocate prey post-strike. To create substrate trails following the strike, the struck mouse was slid in one continuous motion, ventral surface down, along the base of the maze and out one of the two arms. The mouse was then removed from the maze so as not to present visual cues to the snake. Treatment 2 (airborne-only) presented snakes with airborne odors from struck mice to test whether snakes could relocate prey using only airborne information. To provide airborne cues, the struck mouse was placed in a wire-mesh basket, and that basket was placed on the intake side of one of the fans. The arm containing the odor was alternated from one trial to the next. Treatment 3 (substrate vs. airborne) presented snakes with both substrate and airborne cues from struck mice to determine if there was a preference for one cue over the other. To present both substrate and airborne cues in concert, the substrate trail was deposited along the base and out one arm as in Treatment 1, and then the same struck mouse was placed in a wire-mesh basket on the intake side of the fan of the opposite arm.

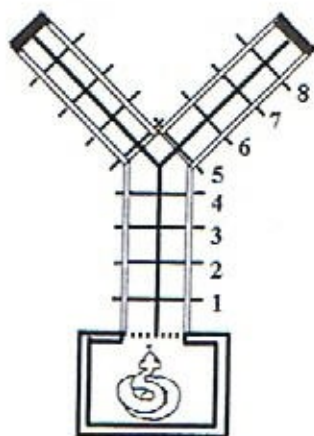


Figure 1. Generalized arena setup with Plexiglas Y-maze, holding box, and hatch-marks. The dashed line represents the location of the sliding door, and the black bars at the ends of the arms of the Y-maze represent the fans and honeycomb blocks.

We recorded several variables throughout a trailing episode: choice, tongue flicks, emergences, turnarounds, and temporal measures. Snakes were considered to have made a choice when the tip of the snake's rostrum passed the eighth hatch-mark (Figure 1), and choice was scored as either arm A, arm B, or no choice. Tongue flicks were observed as protrusions of the tongue, counted for every 10 cm section of the maze, and expressed as rate of tongue flick per minute. Tongue flicks were recorded per section of the maze until the snake's head passed the next sequential hatch-mark. An emergence was scored when the snake's head came out of the holding box during post-strike trailing. While trailing in the maze, snakes would often change their direction of travel, which provided another quantifiable character for analysis: a turnaround. A turnaround was defined as a deviation within the maze during a trailing episode where the snake moved past 90° in the left lateral or right lateral directions. Lastly, temporal measures were taken: time from last emergence to finish (total trailing time) and time spent per 10 cm section of the maze. Trials were considered finished when the snake's head passed the eighth hatch-mark in either arm (choice) or when 25 min. had elapsed (no trail).

All statistical analyses were done using SAS. Cochran's Q was used to analyze differences in choice between the three treatments. Friedman's signed-rank test was used to analyze differences between treatments in all other quantifiable measures.

3. RESULTS

In Treatment 1 (substrate-only), 22 snakes chose the "substrate" arm ($z = 4.38$, $p < 0.0001$) and one chose the "blank air" arm. In Treatment 2 (airborne-only), 19 snakes chose the "airborne" arm ($z = 3.13$, $p = 0.0017$) and four chose the "blank air" arm. In Treatment 3 (substrate vs. airborne), all 23 snakes chose the substrate arm ($z = 4.80$, $p <$

0.0001). The number of emergences was greater for Treatment 2 than in either Treatments 1 ($p = 0.0017$) or 3 ($p = 0.0001$). Also, the number of turnarounds was greater in Treatment 2 than in either Treatments 1 ($p < 0.0001$) or 3 ($p = 0.0003$) (Figure 2).

Rattlesnakes that trailed did so more quickly (mean = 61.61 sec) in the substrate-only treatment than in both the airborne-only (107.29 sec, $p < 0.0001$) and substrate-airborne treatments (88.0 sec, $p = 0.0016$). Tongue flick rates were lower at sections 1 (first 10 cm) and 2 (second 10 cm) in the airborne-only treatment than in both the substrate-only (section 1, $p = 0.038$; section 2, $p = 0.0263$) and substrate-airborne treatments ($p = 0.0108$; $p = 0.0079$).

4. CONCLUSIONS

Rattlesnakes can use airborne cues to relocate envenomated prey. Although mechanically deposited substrate trails are preferred, airborne cues provide sufficient information to the snake following the strike. Previous research has shown that rattlesnakes are capable of a suite of complex behaviors before (Duvall et al., 1985), during (Kardong and Bels, 1998), and after (Chiszar et al., 1977) an envenomating strike (see Kardong and Smith, 2002 for review). Our research extrapolates further on the complexity of rattlesnake chemosensory behavior following the strike.

Rattlesnakes are a unique system for studying predatory behaviors, but it should also be noted that several other species of reptiles serve as potential chemical models in terms of airborne cue use. Garter snakes are capable of using odorized air currents to locate prey (Waters, 1993) and respond to such odors by using their nasal chemical senses (Halpern et al., 1997). Geckos are known to use airborne odors to find fruit (Cooper and Perez-Mellado, 2001), and iguanas are well studied in their abilities to use airborne cues during mate searching (Alberts and Werner, 1993).

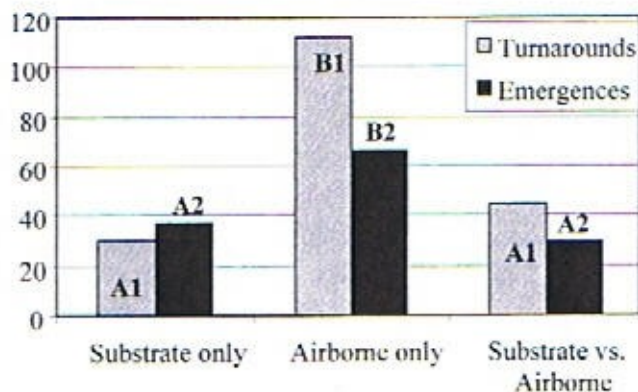


Figure 2. Numbers of turnarounds and emergences for all three treatments. Different letters with the same number (e.g. A1 and B1) represent statistically different groups.

Though rattlesnakes are capable of relocating prey using airborne cues, the process is very different than that occurring during substrate trailing. Differences observed between contexts may have several unique, plausible explanations. For example, tongue flick rates could have been lower in Treatment 2 (airborne-only) as a result of general ophidian neurological anatomy. Connections exist between the olfactory and vomeronasal systems at the hypoglossal nucleus which could result in residual signaling to the musculature of the tongue (Martinez-Marcos et al., 2002). Thus, tongue flicks seen during airborne cue presentation could be uninformative in that context. Conversely, there could be a difference in prey odor concentration from substrate trailing to airborne cue use, with airborne cues being more dilute. If such a concentration difference of the same cue were responsible for the differences in behavior seen, one would expect the snakes' behavior to also be notably different in Treatment 3 (airborne vs. substrate) where the snakes were presented with two informative cues. There was no such difference. Lastly, tongue flicks seen in the airborne-only treatment could be directed at cues in the moving air stream and/or at airborne cues that settled on the substrate in the maze. We did not attempt to distinguish between the tongue flick directions. Instead, the airborne-only treatment confirmed that cues carried in the air current could be used to successfully relocate prey following the strike.

The use of airborne cues post-strike is perplexing when we try to explain the origination of such a behavior. Selection should favor individuals capable of using all informative cues present to relocate prey. Therefore, could rattlesnakes be capable of airborne trailing because selection favored such a behavior secondarily, or were airborne cues of primary use at one time and substrate trailing arose in the more derived condition? Understanding how often and in what contexts such strategies have arisen is a difficult process. However, as these meetings highlight, two divergent research levels that characterize the study of chemosensory systems prove complementary: proximate and ultimate. As more species are studied and the proximate mechanisms responsible for prey relocation become better understood, comparative analyses will be possible and evolutionary events can be clarified.

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