STRIKE-INDUCED CHEMOSENSORY SEARCHING (SICS) AND TRAIL-FOLLOWING BEHAVIOR IN COPPERHEADS (*AGKISTRODON CONTORTRIX*)

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ABSTRACT: We performed three experiments that documented: (1) that the occurrence of strike-induced chemosensory searching (SICS) in the Copperhead (*Agkistrodon contortrix*), (2) this species will follow trails deposited by rodents, provided that cover objects are present in the vicinity of the trail, and (3) that trail-following occurred after snakes struck rodent prey, but not after presentations of prey that did not permit delivery of strikes. Copperheads performed more like rodent-specializing rattlesnake species than like congeneric Cottonmouths (*A. piscivorus*). Implications of this fact for the conceptualization of viperid evolution are discussed.

INTRODUCTION

Studies of predatory behavior in vipers have revealed two strategies. In one, the snake strikes and holds the struggling prey in its jaws (Chiszar et al., 1989; Chiszar and Radcliffe, 1989); in another, the snake strikes and immediately releases the envenomated prey, allowing it to wander from the site of attack, and follows the trail deposited by the prey (Klauber, 1956; Kardong, 1986). In the latter, the strike is followed by a pronounced elevation rate of tongue-flicking coupled with searching movements of the snake's head, collectively called strike-induced chemosensory searching (SICS; Dullemeijer, 1961; Chiszar et al., 1977, 1991). When the snake contacts the trail left by the departing mouse, tongue-flicks are restricted to the trail area, and the snake moves methodically along the trail until the prey carcass is discovered (Burghardt, 1970; Golan et al., 1982; Diller, 1990; Halpern, 1992). These behaviors have been studied in rattlesnakes that feed on rodents, but the same pattern was seen with lizard prey when a lizard managed to extricate itself from the jaws of a snake.

For example, Prairie Rattlesnakes (*Crotalus viridis*) frequently prey upon lizards early in life, consume neonatal rodents opportunistically, and later shift to adult rodents. Lizards are generally held whereas adult rodents are released, but an occasional lizard manages to escape after envenomation (Chiszar et al., 1993). Further, SICS and trail-following were seen in these cases, much as they occurred with adult rodent prey. The predatory strike triggers subsequent searching behaviors that generally do not occur unless a successful (envenomating) strike has been delivered; thus, a strong sequential dependency exists between striking prey and following the prey's post-strike trail (Cooper, 1989, 1993, 1994; Cooper et al., 1989, 1994).

Cottonmouths (*Agkistrodon piscivorus*), in contrast, exhibited an intermediate pattern of behavior when feeding upon adult rodents (O'Connell et al., 1981). Striking led to increased tongue-flicking and searching movements (Chiszar et al., 1985), but they found and followed rodent trails even if they had not struck (Chiszar et al., 1986). Thus, SICS was not a prerequisite for trail following behavior as it was in rattlesnakes (see Kardong, 1982). Copperheads (*Agkistrodon contortrix*) are closely related to *A. piscivorus* and share dietary habits (Gloyd and Conant, 1990), and might be expected to behave like their congeners in trail-following experiments. The purpose of this study is to assess this prediction.

Experiment I

Methods.—Six adult *A. contortrix* were maintained in individual cages (L32 x W61.5 x H31 cm) and observed in two conditions. In condition NS ("no strike") an adult mouse (*Mus musculus*; freshly euthanized by cervical dislocation) was suspended into the cage for 3 sec, but held out of striking range (about 15 cm from the snake's snout). The mouse was removed and tongue-flicks were counted for the next 30 min. In condition S ("strike"), the snake was permitted to strike the euthanized mouse after the 3 sec presentation; otherwise, this condition was exactly like the previous one. Similar to rattlesnakes (Kardong, 1986), *A. contortrix* always struck and immediately released rodent prey. Three snakes received condition NS first and condition S one week later; the remaining snakes received the reverse order. Snakes were deprived of food for one week prior to trials. They had been accustomed to striking and eating euthanized mice; hence, the procedures used in this experiment were similar to those for a normal feeding session. Tongue-flicks were recorded for a 10 min baseline (pre-test) period prior to rodent presentations.

Results.—Table 1 shows that baseline (pre-test) rates of tongue-flicking were low and that the two

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Table 1. Mean rates of tongue-flicking per min (± SE) in six adult Copperheads (*Agkistrodon contortrix*) in experiment I. NS = No strike; S = strike; * = $P < 0.05$. See text for explanation of experimental procedures.

Condition	Pre-test (baseline)	Post-test
NS	5.3 (5.1)	1.0 (0.7)
S	0.0 (0.0)	17.9 (5.4)
t-test (*df* = 5)	1.04 (ns)	3.44*

conditions did not differ significantly during the baseline period. Hence, snakes were quiescent prior to rodent presentations. After these presentations the rate of tongue-flicking was significantly higher in condition S than in condition NS, revealing that SICS occurred in *A. contortrix*. Outcomes of student's *t*-tests for paired comparisons are shown in Table 1.

Discussion.—Although the magnitude of SICS observed in the *A. contortrix* was small compared with effects seen in rattlesnakes, the difference between the NS and S conditions was significant and comparable to the difference seen in *A. piscivorus* (Chiszar et al., 1985). The next step was to observe *A. contortrix* in a trail-following situation after NS and S presentations of rodent prey.

We performed an experiment under conditions comparable to those used with *C. viridis*, but discovered that *A. contortrix* would not move from their starting positions. The snakes would strike in condition S and a high rate of tongue-flicking would follow, but they would not venture out of their resting places to search for the trail that was only a few cm away. In similar experiments with Lower California Rattlesnakes (*Crotalus enyo*), we noticed that they moved toward or along trails only when cover was available (Duvall and Chiszar, 1990). When no cover objects were present in the trailing compartment, individuals of *C. enyo* stayed in their starting locations, much like *A. contortrix* appeared to do. In order to assess whether or not cover was a critical precondition for trail-following behavior in *A. contortrix*, experiment II was performed.

Experiment II

Methods.—The subjects, cages, and maintenance conditions were the same as those in experiment I. The trailing apparatus was a glass terrarium (L90 x W45 x H43 cm) divided by a black PlexiGlas® partition into two compartments, one L15 x W45 x H43 cm, the other L75 x W45 x H43 cm. The smaller compartment contained a paper floor cover and a vessel filled with water, and a snake was allowed to live there for one week prior to commencement of trials. The larger compartment contained a paper floor cover on which a meandering trail was deposited by dragging a euthanized mouse between a pair of parallel lines 4 cm apart. The trail began at the black PlexiGlas® partition and ended at a rock on the opposite side of the large compartment. Two identical rocks were positioned at opposite ends of the large compartment, each near a corner. The trail ended at one of these rocks (randomly selected prior to each trial by the flip of a coin). The euthanized mouse used to make the trail was placed behind the rock, with the trail leading to the rock, around it, and ending at the carcass.

Each subject was observed in two conditions, both involving a strike delivered at a freshly euthanized mouse that was suspended into the snakes' living compartment, and then used to make a trail. In condition NC, the trailing compartment was empty except for the paper floor covering, the trail, and the two rocks in corners opposite the black PlexiGlas® partition. In condition C, the trailing compartment contained five additional rocks, two sticks, and a handful of dried leaves. These items were arranged randomly in the compartment, but not directly on the trail, and were more-or-less distributed equally throughout the trailing compartment. Hence, a snake could move along the trail and remain within 10–15 cm of cover. Further, it was possible for a snake to have its head on the trail while the posterior half of its body contacted cover.

After a snake struck a mouse and a trail was deposited, the black PlexiGlas® partition was removed, giving the snake access to the trailing compartment. During the next 30 min or until the snake found the mouse, whichever occurred first, we recorded the time spent on the trail (i.e., the number of sec that the snake's head was between the trail boundary lines). When a snake found the carcass within 30 min, we recorded the number of sec between the start of the trial and the moment the snake made contact with the carcass. The snake was permitted to ingest the carcass before returning to the living compartment or its home cage. When a snake did not locate the carcass, a score of 1800 sec was assigned as its latency to contact the prey; and, the prey was moved toward the snake at the end of the trial. In order to keep all snakes on the same feeding schedule, the snake was allowed to ingest the carcass to verify that it was hungry and willing to eat. All snakes ate.

Table 2. Mean values on five measures of trailing behavior (± SE) in six adult Copperheads (*Agkistrodon contortrix*) in experiment II. + = 0.10 > P > 0.05; * = P < 0.05; ** = P < 0.001. See text for experimental procedures.

Condition	% snakes locating mouse	Mean time on trail (sec)	Mean time to find mouse (sec)	Mean % time on trail	Mean % time on trail from first contact
No Cover	17 (15.2)	19.2 (14.2)	1560 (196.0)	2.7 (2.3)	5.0 (3.2)
Cover	100 (0.0)	98.3 (26.0)	372 (54.6)	26.4 (6.9)	38.0 (8.4)
t-test (*df* = 5)	*P* = 0.03 (binomial test)	2.24	4.88**	2.80*	2.95*

Each subject received both conditions with one week separating the trials. Thus, the snakes were under one week of food deprivation at the start of a trial. Three snakes received condition NC followed by condition C, and the remaining snakes received the reverse order.

Results.—Table 2 presents five measures of trail-following behavior for each of the two conditions, along with associated inferential statistics. Subjects performed better when cover was available than when it was absent. In condition NC, only one snake located the mouse carcass within 30 min, and this occurred not by following the trail but by moving along the walls of the trailing compartment. Little time was spent on the trail in condition NC. In condition C, all snakes found the mouse carcass in less than seven min., and they all moved along the trail.

Discussion.—Accordingly, we conclude that the presence of cover is required for *A. contortrix* to exhibit trail-following behavior. Further, we interpret this finding as a reflection of the presence in captivity of behavioral predispositions characteristic of predatory tactics in nature. The alternative interpretation would be that captive maintenance induced this psychological dependence on cover, but we reject this view because the snakes were acclimated and generally undisturbed by human presence or other stimuli in captivity. Observing cover-dependent behavior in *A. contortrix* appears most consistent with our first interpretation. Research along these lines would be useful in identifying traits that are influenced by captivity, and might constitute problems for captive snakes released into natural habitats.

The main implication of experiment II is that cover must be provided in the trailing compartment of our apparatus. Another experiment (experiment III) was performed to assess the extent to which SICS is a critical determinant of trail-following in *A. contortrix*. All tests wereare conducted with cover items amply provided in the trailing compartment.

Experiment III

Methods.—To establish the presence or absence of a connection between SICS and trail-following behavior, an experiment containing four conditions is necessary (Golan et al., 1982). We must measure snake response to trails after S and NS presentations of rodent prey, as well as when no trail is present in the apparatus. In order to verify trail following, we must show stronger responses to the trail area (i.e., the space between the parallel lines that form borders) when a chemical trail (T) is present than when one is absent (NT), and to assess the effect of striking prey, the differences in snake response to NT vs T must be compared after NS and S presentations of rodent prey. If S presentations lead to trail following while NS presentations do not, as is the case for rattlesnakes, then the NT vs T difference will be large after S presentations and small or non-significant after NS presentations. Conversely, if equal intensities of trail following occur after NS and S presentations, as is the case for *A. piscivorus*, then the NT vs T difference will be equal after these presentations (Chiszar et al., 1986). In short, we must test the significance of the interaction between NS vs S and NT vs T.

The six subjects, apparatus, and cover objects used in experiments I and II were used here. Snakes received four trials: NS-NT, NS-T, S-NT, and S-T. The NS and S treatments were like those described in experiment I, except that the snake was in the living compartment of the trailing apparatus rather than in its home cage. Deposition of the trail was done as in experiment II, except that a non-envenomated mouse was used to make trails in order to avoid confounding NS vs S with quality of trail (i.e., envenomated vs nonenvenomated). In the case of NT, a non-envenomated mouse was moved through the air about six cm above the trail area and placed behind one of the terminal rocks.

We recorded the same measures of trail-following behavior as in experiment II. Each snake lived in the

Table 3. Mean values on five measures of trailing behavior (± SEM) on six adult Copperheads (*Agkistrodon contortrix*) in experiment III. + = $0.10 > P > 0.05$; * $P < 0.05$. ^{a} = binomial test for comparison of each of the first three conditions with the last condition. See text for experimental procedures.

Condition	% snakes locating mouse	Mean time on trail (sec)	Mean time to locate mouse (sec)	% mean time on trail	% mean time on trail from first contact
NS - NT	17 (15.2)	0.0 (0.9)	1040 (160)	0.0 (0.0)	0.0 (0.0)
NS - T	17 (15.2)	9.5 (9.5)	1130 (70)	1.2 (1.2)	2.0 (2.0)
S - NT	17 (15.2)	11.8 (7.5)	1170 (30)	1.1 (0.7)	2.0 (1.4)
S - T	83 (15.2)	43.2 (14.9)	630 (179)	11.4 (4.8)	16.8 (6.8)
F_{NSvsS}; *df* = 1, 15	$P = 0.06^a$	5.27*	4.98*	5.60*	6.00*
F_{NTvsT}; *df* = 1, 15		4.25^+	3.87^+	5.89*	6.02*
$F_{\text{inter.}}$; *df* = 1, 15		1.21	7.58*	4.81*	4.98*

apparatus for four weeks, receiving one trial per week. If a snake began ecdysis, the animal was allowed to remain undisturbed in the living compartment until the old skin was shed before receiving the next trial. The snake was fed after every trial, either by finding and eating the mouse hidden in the trailing compartment, or by offering the mouse to the snake at the end of the trial. This procedure allowed us to conclude that snakes were hungry and ready to eat on every trial, and that they remained at the same level of food deprivation (1 wk) prior to each trial.

Results.—Five of six snakes found the hidden mouse in the S-T condition, whereas only one of six did so in each of the other conditions (see Table 3), indicating that Copperheads used chemical trails only after striking prey. This inference was also strongly justified by most of the other measures shown in Table 3.

Discussion.—The time to find the mouse was significantly less in condition S-T than in the three other conditions, which did not differ among themselves. This pattern of results gave rise to significant interaction between NS vs S and NT vs T. Inferential statistics from 2 x 2 ANOVAs treating NS vs S and NT vs T as repeated measures are presented in Table 3. In these ANOVAs, three error terms involving subjects (Subjects x NS vs S, Subjects x NT vs T, and Subjects x NS vs S x NT vs T), each bearing 5 degrees of freedom, did not differ from each other and were pooled into a single error term with 15 degrees of freedom (Hicks, 1964). The interaction between NS vs S and NT vs T was significant for three of the four ANOVAs reported in Table 3. Accordingly, we conclude that trail-following occurred after successful predatory strikes were delivered to rodent prey but not after NS presentations of prey.

GENERAL DISCUSSION

When *A. contortrix* preyed on rodents in these experiments, they behaved much like rattlesnakes in that (1) rodents were struck and released, (2) a high level of chemosensory searching occurred following S presentations of prey but not after NS presentations, and (3) trail-following behavior was well-developed and contingent upon the presence of a chemical trail, and upon the prior delivery of a strike and activation of SICS. Copperheads, therefore, resembled rodent-feeding rattlesnakes more closely than their congener *A. piscivorus* that followed trails whether or not strikes were delivered (Chiszar et al., 1985, 1986). We suggest that this difference between *A. contortrix* and *A. piscivorus* is related to the natural diets of these species. Although their diets overlap, *A. piscivorus* preys on frogs and fish more consistently than *A. contortrix*, which consumes more mammals (Gloyd and Conant, 1990). The strike-release-trail strategy appears to characterize vipers that have no anatomical protection to guard them from injury while holding a rodent after an envenomating strike. Releasing the envenomated rodent and following its trail is assumed to be a strategy for avoiding costly damage to predator eyes, pits, nostrils or other tissues. This strategy in *A. contortrix* infers that this species depends upon rodents to such a degree to select for rattlesnake-like predatory behavior. Shine and Covacevich (1983) made a similar argument in explaining the occurrence of strike-release-trail behaviors in several Australian elapid species of the genus *Oxyuranus*.

It is possible that SICS and its association with trail-following behavior evolved independently within *Agkistrodon*, *Crotalus*, and *Sistrurus*, as apparently happened within Viperidae and Elapidae (Schuett et al., 1984; Chiszar et al., 1990; Shine and Covacevich, 1983; for convergence of predatory traits in Elapidae and Viperidae, see Shine, 1980). It is also possible these behavioral traits were well-developed in the *Agkistrodon*-like ancestors of modern *Agkistrodon* and *Crotalus*. Although trail-following has not been studied in Old World vipers, they are well known to exhibit SICS (Burghardt, 1970; Chiszar et al., 1982, 1999). Presuming that these snakes follow rodent trails only after striking prey, then the conclusions will be tempting (1) that the strike-release-SICS-trail strategy was an ancestral condition developed before emergence of New World vipers from their Old World antecedents, and (2) that the pattern observed in *A. piscivorus* represents a derived condition, probably evolving as a result of decreased reliance on rodent prey.

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LITERATURE CITED

BURGHARDT, G. M. 1970. Chemical perception in reptiles. Pp. 241–308 *In* J. W. Johnson, Jr., D. G. Moulton, and A. Turk (Eds.), Communication by Chemical Signals. Appleton Century Crofts, New York.

CHISZAR, D., C. ANDRÉN, G. NILSON, B. O'CONNELL, J. S. MESTAS, JR., H. M. SMITH, AND C. W. RADCLIFFE. 1982. Strike-induced chemosensory searching in old world vipers and new world pit vipers. Anim. Learn. Behav. 10:121–125.

———, D. BOYER, R. LEE, J. B. MURPHY, AND C. W. RADCLIFFE. 1990. Caudal luring in the southern death adder, *Acanthophis antarcticus*. J. Herpetol. 24:253–260.

———, J. BROWN, A. CHISZAR, AND H. M. SMITH. 1999. Strike-induced chemosensory searching in the Caucasus viper (*Vipera kaznakovi*) and the ocellate mountain viper (*Vipera wagneri*). Bull. Maryland Herpetol. Soc. 35:85–91.

———, J. B. MURPHY, C. W. RADCLIFFE, AND H. M. SMITH. 1989. Bushmaster (*Lachesis muta*) predatory behavior at Dallas Zoo and San Diego Zoo. Bull. Psychon. Soc. 27:459–461.

———, AND C. W. RADCLIFFE. 1989. The predatory strike of the jumping viper (*Porthidium nummifer*). Copeia 1989:1037–1039.

———, ———, R. BOYD, A. RADCLIFFE, H. YUN, H. M. SMITH, T. BOYER, B. ATKINS, AND F. FEILER. 1986. Trailing behavior in cottonmouths, (*Agkistrodon piscivorus*). J. Herpetol. 20:269–272.

———, ———, R. OVERSTREET, AND T. POOLE. 1985. Duration of strike-induced chemosensory searching in the cottonmouth (*Agkistrodon piscivorus*), and a test of the hypothesis that striking prey creates a specific search image. Can. J. Zool. 63:1057–1061.

———, ———, AND K. M. SCUDDER 1977. Analysis of the behavioral sequence emitted by rattlesnakes during feeding episodes. I. Striking and chemosensory searching. Behav. Biol. 21:418–425.

———, H. M. SMITH, AND R. DE FUSCO. 1993. *Crotalus viridis viridis* (prairie rattlesnake) Diet. Herpetol. Rev. 24:106.

———, ———, J. L. GLENN, AND R. C. STRAIGHT. 1991. Strike-induced chemosensory searching in venomoid pit vipers at Hogle Zoo. Zoo Biol. 10:111–117.

COOPER, W. E., JR. 1989. Strike-induced chemosensory searching occurs in lizards. J. Chem. Ecol. 15:1311–1320.

———. 1993. Duration of poststrike elevation in tongue-flicking rate in the Savannah monitor lizard, *Varanus exanthematicus*. Ethol. Ecol. Evol. 5:1–18.

———. 1994. Prey chemical discrimination, foraging mode, and phylogeny. Pp. 95–116 *In* L. J. Vitt and E. R. Pianka (Eds.), Lizard Ecology: Historical and Experimental Perspectives. Princeton University Press, Princeton, New Jersey.

———, C. S. DE PERNO, AND J. ARNETT. 1994. Prolonged post-bite elevation in tongue-flicking rate with rapid onset in the Gila monster *Heloderma suspectum*: relation to diet and foraging and implications for evolution of chemosensory searching. J. Chem. Ecol. 20:2867–2881.

———, S. G. McDowell, and J. Ruffer. 1989. Strike-induced chemosensory searching in the colubrid snakes *Elaphe g. guttata* and *Thamnophis sirtalis*. Ethology 81:19–28.

Diller, L. 1990. A field observation on the feeding behavior of *Crotalus viridis lutosus*. J. Herpetol. 24:95–97.

Dullemeijer, P. 1961. Some remarks on the feeding behavior of rattlesnakes. Kansas Med. Acad. Wetenschap., Series C 64:383–396.

Duvall, D., and D. Chiszar. 1990. Behavioural and chemical ecology of vernal migration and pre- and post-strike activity in prairie rattlesnakes: Field and laboratory experiments. Pp. 539–554 *In* D. W. MacDonald, D. Müller-Schwarze, and S. E. Natynczuk (Eds.), Chemical Signals in Vertebrates 5. Oxford University Press, Oxford.

Gloyd, H. K., and R. Conant. 1990. Snakes of the *Agkistrodon* Complex: A Monographic Review. Society for the Study of Amphibians and Reptiles, Contributions to Herpetology 6. Oxford, Ohio.

Golan, L., C. W. Radcliffe, T. Miller, B. O'Connell, and D. Chiszar. 1982. Trailing behavior in prairie rattlesnakes (*Crotalus viridis*). J. Herpetol. 16:287–293.

Halpern, M. 1992. Nasal chemical senses in reptiles: structure and function. Pp. 423–523 *In* C. Gans and D. Crews (Eds.), Biology of the Reptilia, Vol. 18. Academic Press, New York.

Hicks, C. R. 1964. Fundamental Concepts in the Design of Experiments. Holt, Rinehart & Winston, New York.

Kardong, K. V. 1982. Comparative study of changes in prey capture behavior of the cottonmouth (*Agkistrodon piscivorus*) and Egyptian cobra (*Naja haje*). Copeia 1982:337–343.

Kardong, K. V. 1986. Predatory strike behavior of the rattlesnake, *Crotalus viridis oreganus*. J. Comp. Psychol. 100:304–314.

Klauber, L. M. 1956. Rattlesnakes: Their Habits, life Histories, and Influence on Mankind, 2 Vols. University of California Press, Berkeley and Los Angeles.

O'Connell, B., D. Chiszar, and H. M. Smith. 1981. Poststrike behavior in cottonmouths (*Agkistrodon piscivorus*) feeding on fish and mice. Bull. Philadelphia Herpetol. Soc. 29:3–6.

Schuett, G. W., D. L. Clark, and F. Kraus. 1984. Feeding mimicry in the rattlesnake *Sistrurus catenatus*, with comments on the evolution of the rattle. Anim. Behav. 32:625–626.

Shine, R. 1980. Ecology of the Australian death adder *Acanthophis antarcticus* (Elapidae): evidence for convergence with the Viperidae. Herpetologica 36:281–289.

———, and J. Covacevich. 1983. Ecology of highly venomous snakes: the Australian genus *Oxyuranus* (Elapidae). J. Herpetol. 17:60–69.