

The role of thermal contrast in infrared-based defensive targeting by the copperhead, *Agkistrodon contortrix*

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ARTICLE INFO

Article history:

Received 26 July 2009

Initial acceptance 18 September 2009

Final acceptance 7 January 2010

Available online 21 February 2010

MS. number: A09-00501R

Keywords:

Agkistrodon contortrix

copperhead

defensive behaviour

imaging sensory system

infrared vision

pit organ

pit viper

The facial pit organs of the copperhead are the end organs of a complex infrared-imaging system that allows accurate and precise strikes on potential prey. Anecdotal and recent experimental observations show that pit vipers can use their infrared-imaging systems to discriminate between cool and warm features in the environment. We tested the hypothesis that the infrared-imaging system is a thermal contrast detector and determined whether behavioural responses of copperheads differ with contrast type. We found that blindfolded copperheads responded behaviourally towards both warm objects moving against cool backgrounds and cool objects moving against warm backgrounds. Responses towards the former were more robust than those towards the latter. Furthermore, oscillating warm targets generated rhythmic following behaviour in-phase with target motion, while oscillating cool targets generated rhythmic behavioural movement in antiphase to target motion. The results show that the infrared-imaging system of pit vipers operates on the basis of thermal contrast, but the differences in behavioural responses with respect to contrast type indicate that pit vipers preferentially target the warm aspect of thermal differentials. Our results also demonstrate that the infrared-imaging system allows defensive targeting of potential endothermic predators.

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Studies of sensory systems are fundamental to understanding behaviour because environmental perception informs decisions that allocate time and energy to competing activities, resulting in differing rates of reproduction and survival (Dunham et al. 1989; Goris et al. 2007). Sensory systems that spatially map environmental cues, including vision, audition in owls, and infrared reception in snakes, are especially important because they allow precise targeting of predatory (and potentially defensive) behaviours (Knudsen 1982; Grace et al. 2001). Infrared-imaging snakes of the family Crotalinae (the pit vipers) and the families Boidae (the boas) and Pythonidae (the pythons) possess heat-sensitive pit organs that allow accurate and precise targeting of endothermic (i.e. warm) prey (Lynn 1931; Noble & Schmidt 1937; Bullock & Cowles 1952). Breidenbach (1990) argued that some pitless vipers (including ambush predators of the genus *Bitis*) also use infrared-imaging systems, but through comparative behavioural experiments, Safer & Grace (2004) concluded that infrared-imaging capability is probably confined to pit vipers, pythons and boas.

In addition to their utility for predatory targeting, snake infrared-imaging systems may serve to localize cool thermal retreats (Krochmal & Bakken 2003; Krochmal et al. 2004) and to detect potential predators (Greene 1992; Roelke & Childress 2007; Rundus et al. 2007). Thermal information perceived by the pit organs allows detection of objects by their irradiance contrast with a thermal background (see Ebert & Westhoff 2006; Bakken & Krochmal 2007), and thermal information ultimately merges spatiotopically with visual information in the optic tectum (Hartline et al. 1978; Newman & Hartline 1981). As a result, Goris et al. (2007) suggested that snakes equipped with pit organs use thermal imaging information for all aspects of life.

Because pit organs were considered historically only an adaptation for capturing warm endothermic prey, nearly all investigations have focused on neurological and behavioural responses to warm stimuli. However, pit vipers are capable of perceiving cool thermal retreats (Krochmal & Bakken 2003; Krochmal et al. 2004), but neurological and behavioural responses to other cool stimuli are poorly understood. Cold stimuli suppress electrophysiological discharge from terminal nerve masses in the pit organ, and removal of a cold stimulus causes an instantaneous burst of neural activity similar to warm stimulation (Goris & Nomoto 1967). One anecdotal report indicates that pit vipers may respond behaviourally in an antiphase manner to the motion of cold stimuli (de Cock Buning et al. 1981). That is, directional behavioural responses (head turns,

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tongue flicks, etc.) reportedly occurred in the direction opposite the movement of a cool object. Together, these observations suggest that pit organs may allow pit vipers to discriminate both cool objects from warm backgrounds and warm objects from cool backgrounds.

We tested the hypothesis that the infrared-imaging system is fundamentally a thermal contrast detector and determined experimentally how pit vipers respond behaviourally to different forms of thermal contrast. Specifically, we tested the hypothesis that pit vipers can detect moving objects that present thermal contrast with respect to the background, regardless of contrast type (i.e. warm object against a relatively cool background, or cool object against a warm background). A no-contrast treatment, in which targets were the same temperature as background, was included as a control. Behaviour directionality (tongue flicks, head turns and strikes performed in-phase with target motion, in antiphase with respect to target motion, or indeterminate, meaning neither in-phase nor out-of-phase with target motion) was quantified to test the hypothesis that directional behaviour varies predictably with respect to contrast type. Finally, we quantified the incidence of strikes and tail vibration to test the hypothesis that thermal information alone is sufficient to elicit defensive behaviour in pit vipers.

METHODS

Study Animals

Five adult copperheads were purchased from Glades Herp Farms, Inc. (Ft Meyers, FL, U.S.A.) and housed individually in 1750 cm² ventilated plastic enclosures on a 12:12 h dark:light cycle. We offered adult laboratory mice as food twice per month but always at a minimum of 1 week before and after we conducted behavioural tests. Water was available ad libitum, and environments were enriched with hide boxes. Each copperhead's eyes were occluded with small squares of black electrician's tape to remove the potential effects of visual stimuli on behavioural responses. Before tape was applied, we slowed copperheads by cooling them to approximately 10 °C in a refrigerator, a temperature commonly experienced during hibernation (Ernst & Ernst 2003). The copperheads were then immobilized at the neck with foam-padded Pilstrom tongs (Pilstrom, Fort Smith, AR, U.S.A.), and tape squares were gently applied to the spectacle and smoothed around the edge to eliminate vision. Copperheads were allowed at least 1 week to acclimate to occluded vision before behavioural trials were conducted. The snakes shed the tape squares along with spectacles once they entered ecdysis. All copperheads continued to feed normally while vision was occluded.

All animal procedures reported here were approved by the Institutional Animal Care and Use Committee (IACUC) of the Florida Institute of Technology (protocol number 041201). All experiments were conducted under a venomous reptiles permit issued by the State of Florida (license number VRL 716). At the end of the study, animals were returned to the animal care facility.

Experimental Arena

We conducted behavioural tests in a temperature-controlled cylindrical arena, constructed by bolting half of a 30-gallon (113.5-litre) drum inside half of a 55-gallon (208.1-litre) drum (Fig. 1). Bolting the drums together created an outer jacket surrounding the test arena, with the inner 30-gallon drum providing the actual test arena (area = 1752 cm²). We sealed the bolt hole connecting the drums with silicone, and painted the surfaces of both drums with Rust-Oleum primer and flat black enamel (Rust-Oleum Corporation, Vernon Hills, IL, U.S.A.). We filled the hollow jacket with water

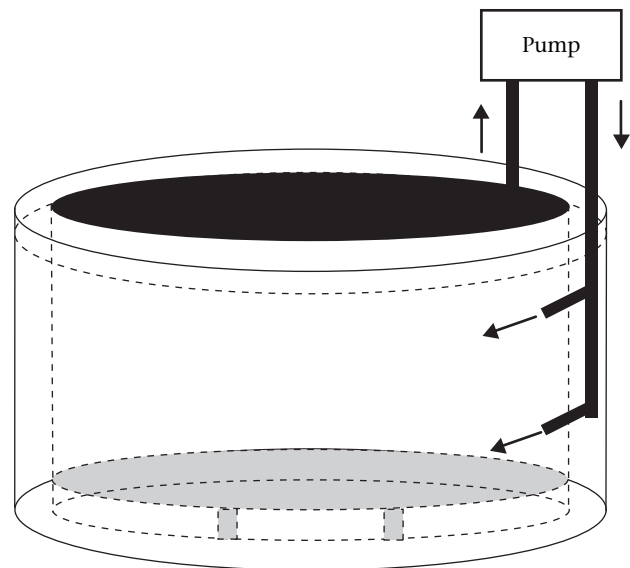


Figure 1. Schematic of the test arena. The thermostatically temperature-controlled water jacket provided stable background temperature for behavioural tests.

and connected a pump (Model R106; Water Ace Pump Co., Ashland, OH, U.S.A.) to two PVC tubes that pumped water continuously around the jacket. This ensured uniform water jacket temperature during all behavioural trials. Within the water jacket, we placed a 100 W submersible aquarium heater opposite the pump and connected it to a temperature controller (Thermo Fisher Scientific, Waltham, MA, U.S.A.) to warm the water jacket to 24 °C. This elevated the jacket temperature to slightly higher than ambient (20–22 °C) and removed effects of room temperature fluctuations. We painted the circular arena floor flat black with a 4 cm² square silver grid pattern and raised the floor above the bottom of the water jacket to prevent direct heat conduction to the snakes during behavioural trials. Styrofoam pads under the entire arena minimized external (i.e. building) vibration.

Experimental Procedure

A Sony CCD-TRV82 Hi8 video camera, mounted directly above the arena on a tripod, recorded behavioural activity during each trial. Targets were black latex balloons filled with 90 ml of water and incubated at 36 °C in a Fisher Scientific Isotemp Incubator (warm target, or what we refer to as 'positive contrast'), at 12 °C in a Kenmore household refrigerator (cool target or 'negative contrast'), and at 24 °C (background temperature) in the arena water jacket itself (no-contrast). We incubated all balloons for at least 1 day within clear Ziploc plastic bags to ensure thermal equilibrium and standardize chemical cues among treatments. Before each trial, targets were suspended via monofilament fishing line from a black PVC support device. Even though snakes were visually blinded with eye patches, black balloons were used and the support device was painted black to ensure very low visible contrast in the test.

We placed snakes in the centre of the test area under an opaque cover to allow acclimation. After 5 min, we began videorecording and measured the surface temperatures of both the arena's inner surface (background) and the test balloon (target) with a Raytek PM Plus infrared thermometer (Raytek Corp., Santa Cruz, CA, U.S.A.). Targets were suspended just above the arena floor in between the snake and the background and oscillated in a 20 cm horizontal arc perpendicular to the longitudinal axis of the snake's head. We

presented targets between 5 cm and 10 cm from the snake's rostrum, and approximately 10 cm in front of the arena wall (background). If the snake turned away from the target area to face in a new direction, the target was repositioned in front of the snake and oscillation continued, but snakes were not manipulated during the experiment. Any tongue flicks or head turns that occurred after the snake moved and prior to target repositioning were counted as indiscriminate. Target oscillation was necessary to determine response direction, and the rate of movement was approximately 1 cycle/s. Each trial lasted 1 min. Target surface temperatures were measured again at the end of each trial. We noted no difference in target temperature between the beginning and end of any trial, but the Raytek thermometer was not sensitive to temperature changes of less than 1 °C.

After testing, we removed the target and allowed the snake to rest in the arena for at least 5 min. Individual snakes were tested with all three target temperature classes in random order each time they were placed in the arena. We ordered treatments randomly by flipping two coins simultaneously and assigning warm target trials to two heads, no-contrast trials to one head and one tail, and cool target trials to two tails. If the moving target and the snake collided during a trial, the trial was stopped and the snake was retested at a later date. Snakes were given a single series of trials in one day and were returned to home enclosures for at least 24 h before being tested again. Every snake was tested 10 times with each target/background temperature class, giving 30 trials per snake and 150 total trials. Videotaped trials were later played back on a 20-inch (50.8 cm) Sony Trinitron television to quantify tongue flicks and head turns made during each trial.

Behaviour Analysis

We categorized tongue flicks and head turns as in-phase, anti-phase, or indiscriminate. In-phase responses were defined as tongue flicks and head turns that occurred in parallel with movement of the oscillating target. Antiphase responses were defined as tongue flicks, head turns and strikes that occurred in opposition to target motion; these behaviours occurred rhythmically with the same period as target motion, but were out-of-phase (phase opposition) with target motion. In each of these cases, rhythmic behaviour that occurred with the same period as target oscillation indicated discrimination of the target temperature with respect to background temperature. Therefore, behaviours that were not directed at the region of target motion or did not show the rhythmic period of target motion were defined as indiscriminate. Strikes that hit or missed targets were also quantified. Strikes only occurred in 23 trials (of 150 total = 15.3%) and were performed by only two snakes. Every trial involving these two snakes was classified by the strike type that occurred (i.e. hit target, miss target, or no strike). The incidence of tail vibration in a trial was recorded as a binomial response. If copperheads performed tail vibration during a trial, a 'vibration' was recorded; if tail vibration did not occur, a 'no vibration' was recorded.

Data Analysis

All statistical tests were performed using SAS version 9.1 (PROC GLM; SAS Institute, Cary, NC, U.S.A.) or Microsoft Excel 2003 (Microsoft Corp., Redmond, WA, U.S.A.). We log-transformed all tongue flick and head turn data plus one to transform discrete count data into continuous data appropriate for continuous parametric statistics. All data were plus one to allow logarithmic transformation of trials in which no responses were recorded. We used repeated measures multivariate analyses of variance (rMANOVA) of log tongue flicks and log head turns to test for differences

in mean tongue flicks and head turns between treatments ($\alpha = 0.05$). Trial was included as the repeated effect. Because our sample size was small, the potential for an outlier biasing our results was strong. Therefore, we included individual copperhead in the rMANOVA models as a random effect to examine and account for differences between individual snake responses. Pillai's trace was used as the test statistic in all multivariate comparisons because it is the most robust to violations of assumptions and was most appropriate given our small sample size (Scheiner 2001). Post hoc orthogonal contrasts were used to examine pairwise factor effects on individual tongue flick and head turn responses, using a Bonferroni corrected $\alpha' = 0.05/3 = 0.017$. Differences between individual copperheads were significant in the rMANOVA (tongue flicks: $F_{4,134} = 6.75$, $P < 0.0001$; head turns: $F_{4,134} = 9.55$, $P < 0.0001$), so we used principal component analyses (PCA; PROC PRINCOMP) to visualize differences in head turns and tongue flicks between individuals. Prior to PCA, each individual's responses were averaged across all 10 trials, and separate PCA were run on tongue flick and head turn data.

Contingency tables and chi-square tests determined whether strike type (hit, miss, no strike) depended upon experimental treatment. Contingency tables and chi-square tests also determined whether the occurrence of tail vibration (vibration, no vibration) depended upon experimental treatment. Univariate Shapiro–Wilk normality tests and normal probability plots of residuals assured that the assumptions of normality and homogeneity of variance were not violated in all comparisons (Shapiro–Wilk test: $P > 0.15$ in all cases).

RESULTS

Tongue Flicks

Log-transformed counts of in-phase, antiphase and indiscriminate tongue flicks did not differ across trials (Pillai's trace = 0.221, $F_{27,402} = 1.18$, $P = 0.2444$), but they did differ significantly with treatment (Pillai's trace = 1.21, $F_{6,266} = 68.17$, $P < 0.0001$). Post hoc orthogonal contrasts showed significant differences between all treatment–response comparisons (Fig. 2). Warm targets elicited significantly more tongue flicks in-phase with target oscillation than did cool targets ($F_{1,134} = 341.14$, $P < 0.0001$) and no-contrast targets ($F_{1,134} = 146.21$, $P < 0.0001$). In-phase tongue flicks were also a more frequent response to no-contrast than to cool targets

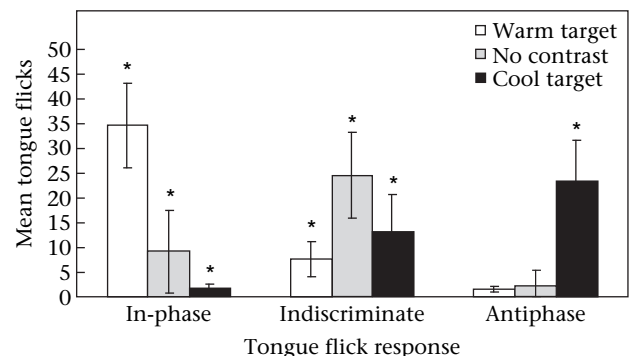


Figure 2. Mean \pm SE number of tongue flicks by copperheads towards targets in three thermal treatments: warm target against cooler background, cool target against warmer background and no-contrast target. In-phase tongue flicks were in parallel with oscillation of the moving target, antiphase tongue flicks were in opposition to target oscillation, and indiscriminate tongue flicks were not directed towards the target. Asterisks show significant within-response differences to the thermal treatments at Bonferroni corrected $\alpha = 0.017$.

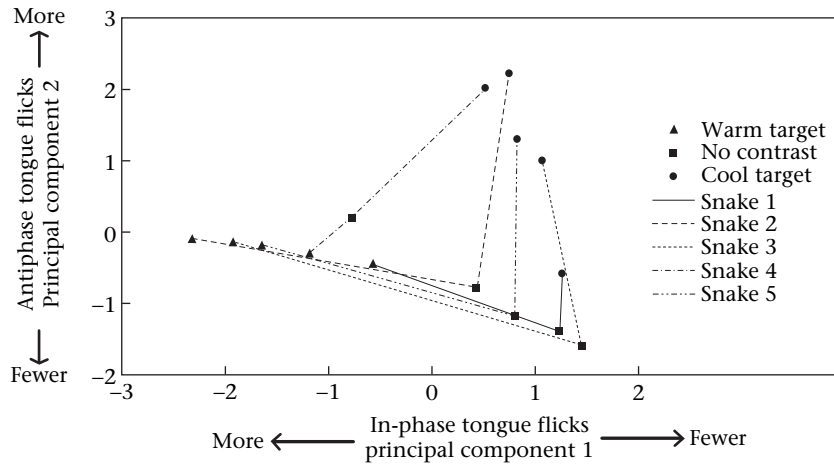


Figure 3. First and second principal component eigen scores calculated from in-phase, antiphase and indiscriminate tongue flicks showing that tongue flicks towards warm targets clustered at negative PC 1, those for cool target clustered at positive PC 1 and positive PC 2, and those for no-contrast targets clustered at positive PC 1 and negative PC 2.

($F_{1,134} = 40.68$, $P < 0.0001$). Cool targets elicited significantly more antiphase tongue flicks than did warm targets ($F_{1,134} = 201.62$, $P < 0.0001$) and indiscriminate tongue flicks ($F_{1,134} = 209.79$, $P < 0.0001$), but there was no difference between warm targets and no-contrast targets ($F_{1,134} = 0.08$, $P = 0.7762$). No-contrast targets elicited significantly more indiscriminate tongue flicks than did warm targets ($F_{1,134} = 40.39$, $P < 0.0001$) and cool targets ($F_{1,134} = 13.85$, $P = 0.0003$). Cool targets also elicited more indiscriminate tongue flicks than did warm targets ($F_{1,134} = 6.94$, $P = 0.0094$).

PCA revealed three principal component (PCs) axes from the tongue flick data. Because the first and second PC accounted for 99.5% of the variance in tongue flick data, the third PC was not considered. Eigenvectors showed that in-phase tongue flicks loaded almost entirely on negative PC 1 and only weakly on PC 2. Antiphase tongue flicks loaded most strongly on positive PC 2, but also loaded on positive PC 1. Indiscriminate tongue flicks loaded nearly equally on positive PC 1 and negative PC 2. Therefore, negative PC 1 eigen scores represented in-phase tongue flick responses, while positive PC 1 eigen scores represented indiscriminate and/or antiphase tongue flick responses (Fig. 3). Positive PC 2 eigen scores represented antiphase responses, while negative PC 2 Eigen scores represented indiscriminate and/or in-phase responses. Since PC 1 accounted for more than 50% of the variation, the difference between in-phase responses and antiphase and indiscriminate responses combined was the strongest relationship observed among tongue flick data. Plotting PC 1 and PC 2 eigen scores against each other showed that all five copperheads displayed similar responses to warm target treatments (Fig. 3). A single individual (Snake 4) responded to no-contrast treatments with more in-phase tongue flicks than did the other four individuals. Another individual (Snake 1) responded to cool target trials with fewer antiphase tongue flicks than did the other four individuals. Overall, PCA supported the rMANOVA analysis in showing that PC eigen scores for warm targets clustered with in-phase tongue flicks, those for cool targets clustered with antiphase tongue flicks, and those for no-contrast targets clustered with indiscriminate tongue flicks.

Head Turns

Head turn results were nearly identical to tongue flick results. Log-transformed counts of in-phase, antiphase and indiscriminate head turns did not differ across trials (Pillai's trace = 0.184,

$F_{27,402} = 0.97$, $P = 0.5073$), but did differ significantly with treatment (Pillai's trace = 1.31, $F_{6,266} = 57.67$, $P < 0.0001$). As was the case for tongue flicks, post hoc orthogonal contrasts for head turns showed significant differences between all treatment–response comparisons (Fig. 4). Warm targets elicited significantly more head turns in-phase with target oscillation than did cool targets ($F_{1,134} = 291.73$, $P < 0.0001$) and no-contrast targets ($F_{1,134} = 192.47$, $P < 0.0001$). No-contrast targets also produced more head turns in-phase than did cool targets ($F_{1,134} = 10.28$, $P = 0.0017$). Cool targets elicited significantly more antiphase head turns than did warm targets ($F_{1,134} = 96.01$, $P < 0.0001$) and no-contrast targets ($F_{1,134} = 101.29$, $P < 0.0001$), but antiphase head turns did not differ between warm targets and no-contrast targets ($F_{1,134} = 0.07$, $P = 0.7909$). No-contrast targets elicited significantly more indiscriminate head turns than did warm targets ($F_{1,134} = 22.07$, $P < 0.0001$) or cool targets ($F_{1,134} = 8.17$, $P = 0.0049$). Indiscriminate head turns did not differ between warm and cool targets ($F_{1,134} = 3.39$, $P < 0.0680$).

As in the tongue flick data, PCA revealed three PC axes from the head turn data. PC1 and PC 2 accounted for 93.9% of the variance in head turn data, so the third PC was not considered. Eigenvectors showed that in-phase head turns loaded entirely on negative PC 1 and not on PC 2. Antiphase head turns loaded most strongly on

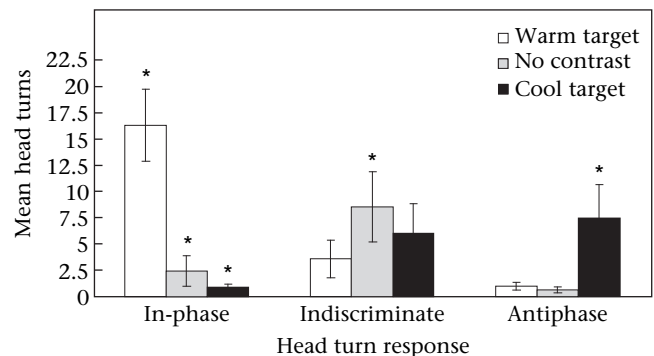


Figure 4. Mean \pm SE number of head turns by copperheads towards targets in three thermal treatments: warm target against cooler background, cool target against warmer background, and no-contrast target. In-phase head turns were in parallel with oscillation of the moving target, antiphase head turns were in opposition to target oscillation, and indiscriminate head turns were not directed towards the target. Asterisks show significant within-response differences to the thermal treatments at Bonferroni corrected $\alpha = 0.017$.

positive PC 2, but also loaded on positive PC 1. Indiscriminate head turns loaded most strongly on negative PC 2, but also on positive PC 1. Therefore, negative PC 1 eigen scores represented in-phase responses, while positive PC 1 eigen scores represented indiscriminate and/or greater antiphase responses. Positive PC 2 eigen scores represented antiphase responses, while negative PC 2 eigen scores represented indiscriminate responses. Since PC 1 accounted for more than 50% of the variation, the difference between in-phase responses and combined antiphase and indiscriminate responses was the strongest relationship observed among head turn data. All five copperheads displayed similar responses to warm target treatments (Fig. 5). A single individual (Snake 4) responded to no-contrast treatments with more in-phase head turns than did the other four individuals. Another individual (Snake 1) responded to cool target trials with fewer antiphase head turns than did the other four individuals. Overall, PCA supported the rMANOVA analysis and showed that PC eigen scores for warm targets clustered with in-phase head turns, those for cool targets clustered with antiphase head turns, and those for no-contrast targets clustered with indiscriminate head turns.

Strikes

Two copperheads performed strikes during the experiment. Thirteen strikes (54% of total strikes by all snakes in all treatment conditions) occurred during warm target treatments, and all hit the target. Seven strikes (29% of total strikes) occurred during cool target trials, and all missed the target. Only one snake struck during no-contrast trials. This snake made four strikes (17% of total), and all strikes hit the target. Chi-square contingency tables showed that hits occurred during warm target situations and misses occurred during cool target situations more frequently than would be expected by chance ($\chi^2_4 = 33.147, P < 0.001$). All strikes occurred simultaneously with tail vibration.

Tail Vibration

Copperheads displayed tail vibration during 36 warm target trials (72% of total), 18 (36%) no-contrast trials and 28 (56%) cool target trials. All five copperheads performed tail vibration in response to warm target and no-contrast treatments, and all but one individual (Snake 1) performed tail vibration in cool target situations. Copperheads displayed tail vibration more frequently during warm and cool target trials than would be expected by

chance ($\chi^2_2 = 13.128, P < 0.01$). Copperheads also displayed no tail vibration more frequently during no-contrast trials than would be expected by chance ($\chi^2_2 = 13.128, P < 0.01$).

DISCUSSION

Pit Vipers Detect Both Warm and Cool Thermal Contrast

Our results support the hypothesis that pit vipers are capable of detecting thermal contrast, regardless of whether contrasts are formed by a warm target against a cooler background or a cool target against a warmer background. The results presented here agree with prior work demonstrating the ability of pit vipers to detect and target warm prey (reviewed in: de Cock Buning et al. 1981). In-phase behavioural responses and accurately targeted strikes occurred with highest frequencies during warm thermal contrast trials (warm target against a cooler background). More recent investigations documenting the abilities of pit vipers to detect relatively cold objects and cool refuges (Goris & Nomoto 1967; de Cock Buning et al. 1981; Krochmal et al. 2004) suggest that cool targets in motion against a warmer background should be detectable. In our study, antiphase tongue flicks and head turns and consistently inaccurate strikes all occurred with highest frequency during trials with cool targets, demonstrating that copperheads are capable of detecting the thermal contrast between a cool object and warm background. Reduced frequency of targeting behaviours and increased frequency of indiscriminate behaviour during no-contrast trials lend further support for the hypothesis that copperheads were targeting the thermal contrasts presented by warm and cool targets, rather than using other sensory cues to target the moving objects. Thus, our results support the conclusion that pit vipers use their infrared-imaging systems to detect objects by thermal irradiance contrast and can detect both warm objects against cooler backgrounds and cool objects against warmer backgrounds.

Pit Vipers Target the Warm Aspect of Thermal Contrasts

While copperheads detected thermal contrasts involving either relatively warm or cool targets, the types of responses to different contrasts clearly differed. Warm targets elicited in-phase tongue flicks and head turns, and all strikes hit targets. Cool targets elicited antiphase tongue flicks and head turns, and all strikes missed targets. Because emitted infrared radiation is proportional to

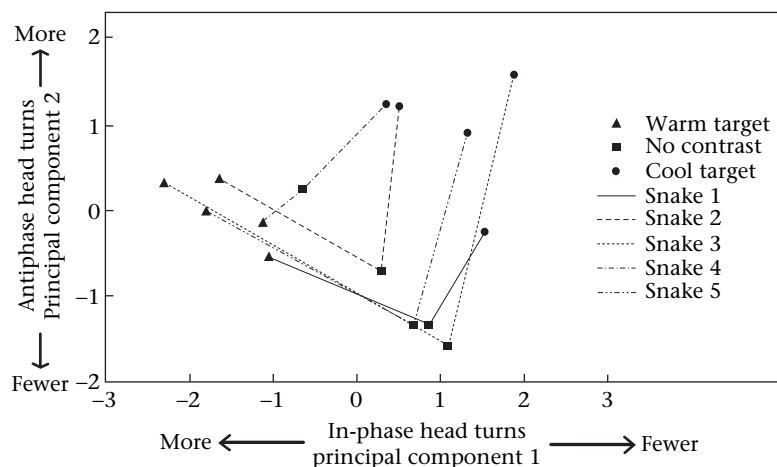


Figure 5. First and second principal component eigen scores calculated from in-phase, antiphase and indiscriminate head turns showing that head turns towards warm targets clustered at negative PC 1, those for cool targets clustered at positive PC 1 and positive PC 2, and those for no-contrast targets clustered at positive PC 1 and negative PC 2.

surface temperature, cool objects may temporarily block infrared signals emitted by warmer backgrounds, effectively casting an infrared 'shadow'. Neurological recordings from the trigeminal nerve show that terminal nerve mass (TNM) activity in pit organs is strongly depressed by such an infrared shadow (Goris & Nomoto 1967). Once the cool object moves out of pit organ range, removal of the infrared shadow causes a strong stimulation in the trigeminal nerve similar to stimulation by a warm object (Goris & Nomoto 1967). Antiphase behaviour and strike misses apparently occur in response to this secondary strong stimulation, which leads us to suggest the hypothesis that pit vipers target the warm aspects of thermal contrasts. In 'positive contrast' situations, pit vipers target the warmer object rather than the cooler background. In 'negative contrast' situations, pit vipers target the warmer background rather than the cooler target.

Infrared Information Alone Induces Defensive Behaviour

Greene (1992) suggested that infrared information may be used to detect endothermic predators as well as prey, and while pit viper responses to warm objects have been described as defensive (Roelke & Childress 2007), the role of infrared imaging in defensive targeting has never been tested directly. In our behavioural experiments, copperheads often displayed rapid tail vibration, a behavioural displacement activity (Tinbergen 1952) often shown by copperheads and a variety of other snake species in response to perceived threats. Tail vibrations in our blinded copperheads were induced by warm and cool thermal contrast situations more frequently than they were by no-contrast situations, and they often occurred as a precursor to strikes. We considered strikes to be defensive in our experiments because they were made against targets that lacked chemical cues associated with prey, and because they occurred in conjunction with tail vibration. Thus, the tail rattling and strike behaviours of blinded copperheads reported here support the hypothesis that infrared information alone is sufficient to guide defensive strikes against potential endothermic predators.

Implications for Ecology and Evolution

Given the results reported by previous investigators (Goris & Nomoto 1967; de Cock Buning et al. 1981; Krochmal & Bakken 2003), it should come as no surprise that pit vipers are capable of detecting both warm ('positive') and cool ('negative') thermal contrasts. However, our results, together with those from previous investigations, allow specific predictions to be made about the utility of infrared imaging for the guidance of pit viper behaviours. For example, infrared information provides an extremely useful mechanism for targeting warm prey and predators, especially when visual cues are unavailable (Kardong & Mackessy 1991; Roelke & Childress 2007). Shine et al. (2002) even suggested that pit vipers may select foraging sites that minimize background temperature to maximize potential thermal contrast with prey. Such site selection could be particularly important to pit vipers preying on ectothermic prey, the surface temperatures of which may be very similar to ambient environmental temperature. While accurate predatory behaviour has always been considered an important product of snake infrared imaging, this link is still debated. Bakken & Krochmal (2007) argued that few predatory situations present a strong enough thermal contrast for the pit organs to detect prey at any but the closest of distances. Our results support the hypothesis that pit vipers can and do use their infrared-imaging systems to target moving prey and potential predators at behaviourally relevant distances.

Infrared information may support pit viper thermoregulation by allowing detection of cool thermal retreats (Krochmal & Bakken 2003; Krochmal et al. 2004) and warm basking sites. Krochmal & Bakken (2003) argued that thermoregulation was the primary adaptive force driving evolution of the pit organ. However, a diverse array of invertebrate and vertebrate ectotherms, including those basal to pit vipers, is capable of effective thermoregulation without any known means of infrared imaging. The utility of the infrared-imaging system for thermoregulation still remains largely untested; its utility for selecting warm basking sites in cool environment has not been experimentally documented, and the potential use of infrared information for thermoregulation in boas and pythons has not been investigated.

In our experiments, antiphase targeting behaviour largely prevented pit vipers from effectively targeting cool objects moving against warmer backgrounds. However, it may be very rare for prey and potential predators to appear relatively cool in contrast to the natural thermal background (Shine et al. 2002; Bakken & Krochmal 2007). First, when active, both endotherms and ectotherms are likely to show elevated body temperatures relative to the environment. Second, the infrared emittance of some backgrounds, especially the sky, is likely to be extremely low, which would allow nearly all prey or predators to present warm thermal contrast with respect to background. Low infrared emittance by the sky led Bakken & Krochmal (2007) to suggest that a predatory pit organ may have first evolved in snakes inhabiting arboreal habitats. The large pit organs observed in arboreal pit vipers, and elaborate arrays of pit organs in some arboreal boid snakes may support this hypothesis.

In at least one fascinating instance of predator-prey dynamics, the use of infrared imaging by pit vipers for defence may be used to advantage by potential prey. California ground squirrels, *Spermophilus beecheyi*, manipulate infrared emittance of their tails and thereby deter predation by Pacific rattlesnakes, *Crotalus oreganus*, which respond in a defensive manner to tail waving by the squirrels (Rundus et al. 2007). Our observations in the laboratory suggest that potential prey could avoid predation by manipulating the infrared-based predatory and defensive behaviours of snakes.

In conclusion, the infrared-imaging systems in pit vipers, boas and pythons are novel sensory systems that provide the ability to effectively detect and target thermal contrast. The results presented here show that copperheads can detect the thermal contrast presented by both warm objects against a cooler background (positive contrast) and cool objects against a warmer background (negative contrast). However, at least under the conditions of the experiments reported here, a cool moving target against a warmer background elicits behaviour that may be counterproductive (i.e. snakes are unable to effectively target a relatively cool moving object), but such 'negative' thermal contrast may be behaviourally irrelevant because of its rarity in nature. In addition, we provide experimental evidence that the infrared-imaging system can function not only as a tool for predation and thermoregulation, but also for defence from endothermic predators.

Acknowledgments

This work was supported in part by a grant from the US Air Force Office of Scientific Research to M.S.G. and a Sigma Xi Grant-in-Aid of Research to J.U.V. We thank Adam Safer, Gerri Talley and Scott Taylor for technical assistance, Steven J. Beupre for statistical advice, and Joseph Agugliaro, Lara Douglas, Marshall McCue, Jason Ortega, Matt Smith, Rod Wittenberg and three anonymous referees for helpful comments.

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