

RESEARCH ARTICLE

Directional sensitivity in the thermal response of the facial pit in western diamondback rattlesnakes (*Crotalus atrox*)

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SUMMARY

Recent work published in the accompanying paper used a combination of 3D morphological reconstruction to define optical spread functions and heat transfer physics to study how external heat energy would reach the sensory membrane within the facial pit of pitvipers. The results from all of the species examined indicated asymmetric directional sensitivity, e.g. the pit would preferentially respond to stimuli located below and behind the snake. The present study was intended as a test of these findings through a quantitative neurophysiological analysis of directional sensitivity in the facial pit of the western diamondback rattlesnake, *Crotalus atrox*. An infrared emitter was positioned through a coordinate system (with varying angular orientations and distances) and the response it evoked measured through neurophysiological recordings of a trigeminal nerve branch composed of the afferents from the sensory membrane of the facial pit. Significant differences were found in the strength of the membrane's neural response to a constant stimulus presented at different orientations (relative to the facial pit opening) and over different distances. The peak sensitivity (at 12 deg above and 20 deg in front of the facial pit opening) was in good agreement with the predicted directional sensitivities based on optical spread functions and 3D topography. These findings support the hypothesis that the topography, and functional performance, of the facial pit has undergone an adaptive radiation within the pit vipers, and that differences in the behavioral ecology of the pit vipers (i.e. terrestrial versus arboreal) are reflected within the facial pits.

Key words: viper, sensory, behavioral ecology, thermal imaging.

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INTRODUCTION

The pit vipers of the subfamily Crotalinae are characterized by the presence of a facial (or loreal) pit located on the lateral surface of the head between the external nares and the eye. The basic architecture of the facial pit – an anterior chamber that opens to the surface of the snake's head, a posterior chamber and a membrane of sensory receptors suspended between them – is well known (e.g. Noble and Schmidt, 1937; Barrett et al., 1970). Neural receptors located on a membrane suspended within this pit respond to changes in relative thermal energy in the environment (e.g. Noble and Schmidt, 1937; Barrett et al., 1970; Newman and Hartline, 1982). The facial pit is frequently described as analogous to a chamber-type eye or pinhole thermal camera (e.g. Bakken and Krochmal, 2007). There is evidence of multispectral image formation; neural signals from the facial pit are integrated with those from the visual system (Hartline et al., 1978). Workers have explored aspects of this sensory system ranging from the molecular activation (Gracheva et al., 2010), the role of the vascular system (Goris et al., 2000) and the mechanics of neural integration within the trigeminal system (Stanford and Hartline, 1984). Despite these advances, little is known about the adaptive role of this system in the behavioral ecology of the snake.

While other functions have been proposed, there is empirical support only for the pit organ functioning during predator–prey interactions (e.g. Kardong and Mackessy, 1991; Van Dyke and Grace, 2010) and when locating thermal refugia (Krochmal and

Bakken, 2003). One of the hindrances to a deeper appreciation of the functional role of the pit organ is uncertainty regarding the thermal and directional sensitivity of the system, i.e. the quality of its spatial representation or 'image' of the environment.

The thermal sensitivity of the infrared (IR) pit has been explored using various approaches. The sensitivity of 0.003°C found experimentally (Bullock and Diecke, 1956) using water flowing over the membrane is frequently cited, but their apparatus could not resolve temperature differences of less than 0.0025°C. The authors suggested that the pit membrane's sensitivity might equal or exceed 0.001°C, based on their analysis of an earlier behavioral study (Noble and Schmidt, 1937). Similar sensitivity is suggested by more recent behavioral studies (e.g. Ebert and Westhoff, 2006). Further, a first-principles study based on optical and heat transfer physics and highly simplified facial pit anatomy (Bakken and Krochmal, 2007) indicated that sensitivity of 0.001°C and image sharpening, as found previously (Stanford and Hartline, 1980; Stanford and Hartline, 1984) may be necessary to explain the results of behavioral experiments. However, threshold sensitivities remain uncertain and the imaging properties of actual facial pit systems are unknown. More importantly, these studies presume a uniform thermal sensitivity across the facial pit; the presence of significant directional asymmetry in thermal sensitivity is particularly relevant to the design of behavioral studies.

Specifically, the use of generalized anatomy in early imaging studies (Bakken and Krochmal, 2007; de Cock Buning, 1984; Otto,

1972) obscured the potential significance of interspecific variation in the exact position and orientation of the external opening of the facial pit and in the topography of the adjacent scalation (Gumprecht et al., 2004). The 3D geometry of the facial pit determines its directional sensitivity and imaging properties. Consequently, interspecific variation might be adaptive to specific habitats or predation strategies.

In a recent study, described in the accompanying paper (Bakken et al., 2012), we examined the effect of interspecific variation in facial pit geometry on imaging. Using topographic information derived from X-ray computed tomography (CT) sections defining the facial pit's membrane, external opening and adjacent scalation, coupled with heat transfer physics, we were able to generate the optical spread functions (i.e. plots of the area on the pit membrane illuminated by thermal radiation from a point source) needed to compute the distribution of temperatures over the facial pit membrane (Bakken et al., 2012). Spatial maps of these spread functions indicate the directional sensitivity of the pit organ (Fig. 1) [see also fig. 3 in Bakken et al. (Bakken et al., 2012)], and suggest that this directional sensitivity may vary among species. While rooted in sectional anatomy, the directional sensitivity profiles that we generated (Bakken et al., 2012) have not been tested experimentally to see whether they predict sensory response. The purpose of the present contribution was to use neuroelectrophysiology to quantitatively test the spatial map of directional sensitivity for the pit organ of *Crotalus atrox* (Fig. 1).

MATERIALS AND METHODS

Live animals

This study examined six adult (snout–vent length, SVL, range 45–70 cm) long-term captive western diamondback rattlesnakes (*C. atrox* Baird and Girard 1853). The head geometry of these animals was proportionately similar, so the angular results presented would not be affected by the range in specimen size. The snakes were maintained in a specially constructed snake room, on a 12h:12h light:dark photoperiod, 22–30°C temperature range and a diet of pre-killed rodents, with water *ad libitum*. Care and maintenance of these animals followed all established guidelines for reptiles and venomous snakes, and their use in these experiments was approved by the LANUVNRW (50.203.2-BN 7, 5/03 issued 31.05.2006).

Stimulus

The experiments were performed on a vibration isolation table (TMC, Peabody, MA, USA), upon which was anchored a rattlesnake head holder modified from a previous design (Blum et al., 1978). The head holder allowed for precise positioning of the snake's head without obstruction of either the right-hand side of the head (which was used for all trials) or the tracheal tube needed to maintain anesthesia (see below). Using this head holder – more precisely the position of the center of the rattlesnake's facial pit when in the apparatus – a modified polar coordinate system (consisting of vertical and horizontal angular coordinates) was mapped out on the surface of the vibration isolation table, and the inner walls of the surrounding Faraday cage. The coordinate system was designed in accordance with the topographic data on the facial pit of *C. atrox* (Bakken et al., 2012). The coordinate system extended horizontally from 0 to 80 deg in front of the pit (using increments of 10 deg) and vertically from –18 deg below the pit to 30 deg above the pit (using increments of 6 deg); the center of the facial pit was used as a reference point for both planes (Fig. 2). The combination of the vertical and horizontal ranges creates a coordinate system of 117 points.

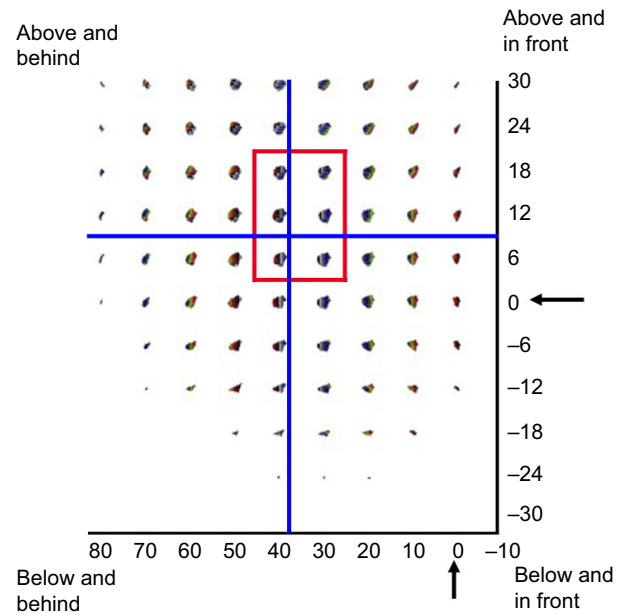


Fig. 1. Theoretical spatial map of directional sensitivity for the pit organ of *Crotalus atrox* (modified from Bakken et al., 2012). Units on the x-axis indicate the azimuth in the horizontal (frontal) plane with 0 deg straight ahead along the parasagittal plane passing through the middle of the facial pit. Units on the y-axis are angular elevation or depression relative to the frontal plane passing through the middle of the facial pit. The area of greatest sensitivity (indicated by the red square) falls roughly between 30 and 40 deg behind and 5–20 deg above the pit. The blue axes indicate the angular locations of the experimental transects used in this study.

Even before the incorporation of a range of distances and replications at each trial configuration, the number of points within this coordinate system was untenable given the influence of anesthesia on neurophysiological responses in reptiles (e.g. Moiseenkova et al., 2003; Carr et al., 2009). To avoid this potential complication, we documented directional sensitivity using a single horizontal axis (located at 10 deg vertically above the pit organ) and a single azimuthal axis (located at 40 deg in front of the pit organ). Each of these 22 coordinate points was divided into 5 cm lengths beginning at a distance of 5 cm from the surface of the pit and extended away to a maximum distance of 30 cm, for a total of 132 trial configurations.

Stimulation was supplied using a constant IR source (SA727-5M InfraRed emitter, Cal Sensors, Santa Rosa, CA, USA). This is a near-IR source, not a blackbody emitter, with peak emittance at 2.5 μm and little output above 5.0 μm . This emitter provided a stable stimulus that could be switched rapidly, but its spectral distribution was not ideal for absolute sensitivity analyses (which were beyond the scope of this study). The consistency of the IR emitter was confirmed, and the unit calibrated (using a Lab Master Ultima Powermeter from Coherent, Dieburg, Germany) in the laboratory of Dr Manfred Mürtz. The IR emitter was anchored on a rigid platform that also supported an LED laser (TIM 203, BLV Electronic, Aylesbury, Bucks, UK) that was used to ensure that the IR emitter was centered on the facial pit regardless of its position within the coordinate system. At the edge of the platform was an electronic shutter (VS25, Vincent Associates, Rochester, NY, USA). When the shutter was activated by a pulse generated by Spike2 (CED, Cambridge, UK) and routed through the AD/DA converter (Micro 1401 mkII, CED) it would open for 1 s then close for 2 s, and repeat this pattern 10 times. The 10 repetitions of the constant

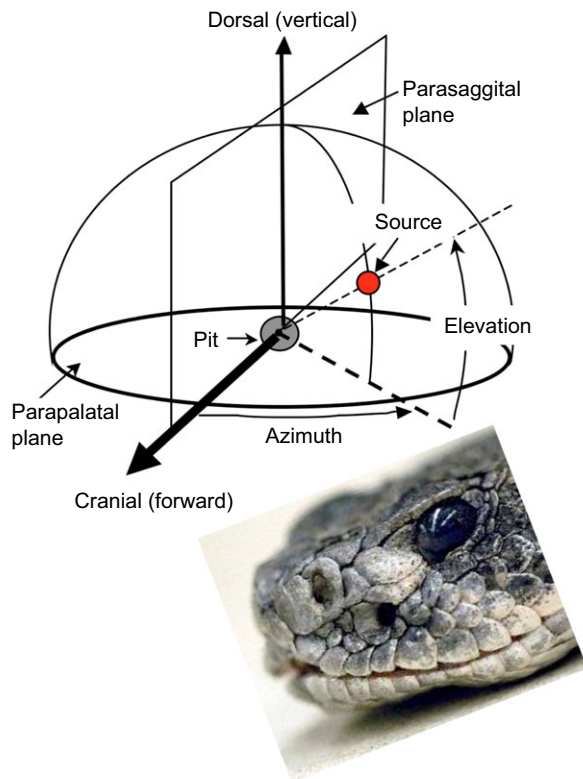


Fig. 2. Coordinate system used in this study. The intersection of the frontal and parasagittal planes passing through the middle of the right facial pit was used as the reference point around which a series of horizontal and vertical angles were defined. The infrared (IR) emitter was positioned at intersects between specific horizontal and vertical angles, then adjusted to a specific distance from the surface of the pit membrane.

IR source, presented at each of the 132 coordinate points, formed the stimuli for this experiment.

Predicted sensitivity of the facial pit

Three branches of the trigeminal nerve originate in the sensory membrane of the facial pit. Only one branch (the superficial branch of the maxillary nerve) is surgically amenable to experimentation; as the receptor associated with this branch has been shown to cover the majority of the sensory membrane in other pit vipers (Lynn, 1931; Bullock and Fox, 1957), this analysis was restricted to this one nerve branch. The spread functions determined for the sensory membrane (Bakken et al., 2012) treated the membrane as a whole; to accurately assess the fit between the biophysical predictions and the experimental results, a subset of the spread functions (representing the distribution of receptors associated with the superficial branch) had to be created. The first step in this process was to document the distribution of the superficial branch on the sensory membrane. Two adult specimens were anesthetized and killed, then exsanguinated and perfused with 10% neutral buffered formalin (NBF) in snake Ringer solution; after 2 days of fixation the sensory membranes were dissected out of the maxilla, with the superficial branch intact. The nerve was trimmed close to the edge of the membrane and a microstrip of NeuroVue Red (Molecular Targeting Technologies, West Chester, PA, USA) applied to the distal end of the nerve. The sensory membrane, with the microstrip in place, was incubated at 37°C for 36 h after which time the NeuroVue dye was visible in the fine nerve branches on the sensory

membrane. Two other specimens were anesthetized with Isoflurane and the superficial and deep branches of the maxillary nerve exposed. Alexa Fluor dextran 488 (green; excitation 495 nm, emission 519 nm) and Alexa Fluor dextran 546 (red; excitation 556 nm, emission 573 nm) were applied to the nerves; after a 5 day recovery period the snakes were killed, and the sensory membrane was excised from the maxilla and examined under a DM RBE fluorescence microscope (Leitz, Wetzlar, Germany).

The neural labels permitted a demarcation of that portion of the sensory membrane that gives rise to the superficial branch of the maxillary nerve. This demarcation was then superimposed onto the spread function distribution determined in the accompanying paper (Bakken et al., 2012). One final correction was made; though both studies defined horizontal as passing through the middle of the facial pit (the parapalatal plane of Fig. 2), the anesthetized rattlesnakes used in this study (see below) were supported by a platform that caused a slight change in orientation of the head compared with the preserved specimens examined in the companion paper (Bakken et al., 2012). We corrected for this by tilting the horizontal line of the spread function map by 14 deg. Using the same procedures as before (Bakken et al., 2012), it was then possible to predict the directional asymmetry of only the portion of the sensory membrane associated with the superficial branch of the maxillary nerve.

Recordings

Each specimen was placed in an induction chamber and exposed to Isoflurane until unresponsive. The snake was removed from the induction chamber, intubated and connected to a regulated gas source (carbogen 95% O₂; 5% CO₂) and maintained on Isoflurane (this typically required a 2% mix of Isoflurane). Anesthesia was maintained throughout the experiments. When the animal was stabilized on the ventilator, and unresponsive to tactile stimulation, an incision was made in the scalation below the eye. Through this surgical window the superficial branch of the maxillary nerve (a component of the trigeminal nerve), which originates in the sensory membrane of the facial pit, could be surgically isolated, cleared of connective tissue, then severed proximally to leave an approximately 1 cm long cleaned stump of this nerve.

The exposed nerve was pulled into a custom-built suction electrode. Silver wire (0.38 mm diameter, WPI, Sarasota, FL, USA) was used both to couple the suction electrode to the amplifier and as a reference electrode, which was positioned in the exposed tissue of the surgical site. Once the suction electrode and reference electrodes were in place the surgical site was covered in a mix of petrolatum and mineral oil to prevent desiccation. The neural responses were bandpass filtered (low 300 Hz, high 1 kHz) and amplified 1000× using a DAM 80 amplifier (WPI). Amplified signals were then routed through the AD/DA converter and recorded using Spike 2.

All experimental preparations were tested by placing the stimulus source in the same coordinate point (30 deg vertically above the horizon, 40 deg in front of the pit, and 10 cm distance) and verifying the signal-to-noise ratio. Following this initial stimulation, die rolls were used first to randomize the order of the angular coordinates and then to randomize the order of the distances over which the trials were conducted at each angular coordinate. If no response was observed (traces were visualized using a PM 3335 oscilloscope (Philips, Amsterdam, The Netherlands) at a given distance we would proceed to the next greater (+5 cm) distance for the next trial; if no response was observed at the greater distance, no greater distances were used for that particular angular coordinate. Upon completion of the last coordinate stimulation trial, the stimulator was returned

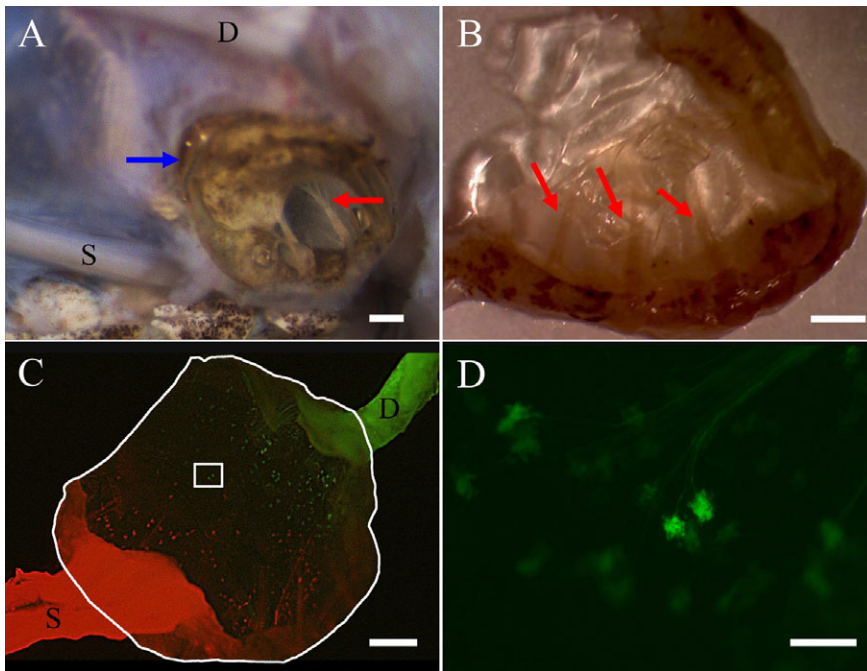


Fig. 3. Sensory receptors in the facial pit of *C. atrox*. (A) Deep dissection in which the margin of the sensory membrane (blue arrow) is viewed from the posterior; this exposes one of the sensory rami (red arrow) as well as the superficial (S) and deep (D) branches of the maxillary branch of the trigeminal nerve. (B) The membrane has been removed from the maxilla and the superficial branch retrograde labeled with NeuroView Red to highlight the series of sensory rami on the ventral portion of the membrane (arrows). (C) Fluorescent dextran labeling in the superficial (S) and deep (D) branches of the maxillary branch of the trigeminal nerve clearly reveal the division in the receptor fields within the pit membrane (outlined in white). (D) Increased magnification of the area within the white box in C demonstrates the labeling of the individual receptor organs. Scale bars: A–C, 500 μm ; D, 100 μm .

to the initial point, and a final stimulus response recorded. This final trial was compared with the initial trial for that specimen using the data analysis procedure detailed below. None of the specimens showed a significant difference between the initial and final response; accordingly, we have no evidence for neural fatigue and/or any physiological condition that could have compromised the integrity of our data set.

Data analysis

The superficial branch of the trigeminal nerve is composed of 3000–6000 axons (Bullock and Fox, 1957) so the responses we

recorded were summations of numerous action potentials. This precluded the use of a spike-sorting or threshold detection approach; instead, we used the calculation of power spectral densities utilized in an earlier study of the facial pit (Moiseenkova et al., 2003). The background levels (defined as the value for 1.0 s duration prior to each stimulus) and the neural response to the stimuli were quantified for each coordinate point. Therefore, 10 stimuli and background responses from each point were fast Fourier transformed (at 16,384 points) and the power spectrum density (hereafter PSD) of each quantified between 300 and 1000 Hz (additional analyses were performed with the 350–650 Hz

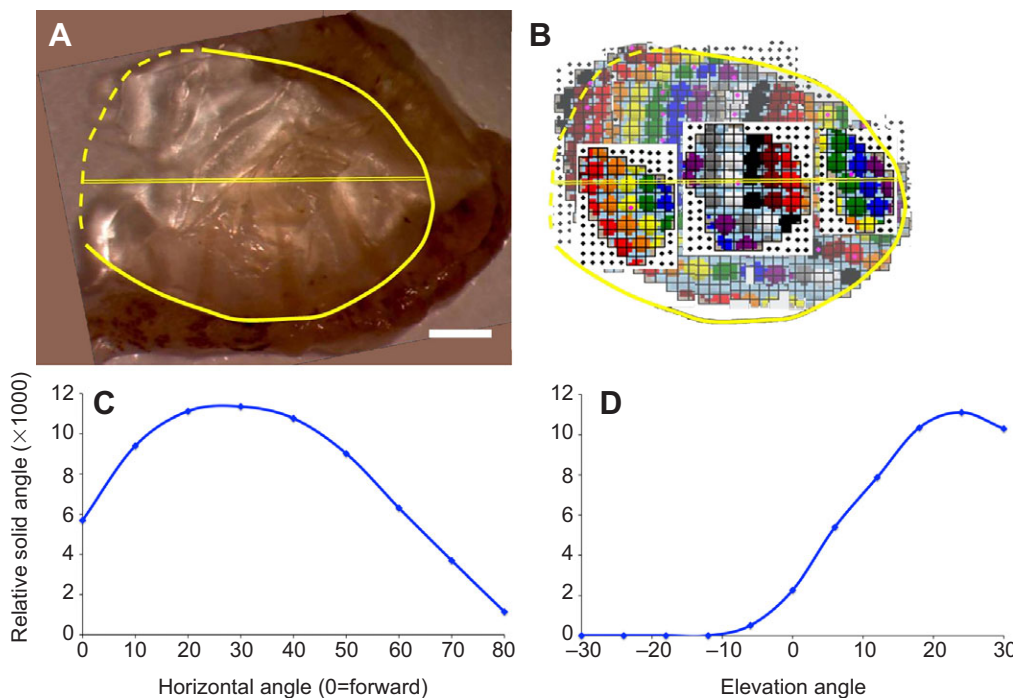


Fig. 4. Basis for predicted thermal sensitivities. (A) Excised sensory membrane with the ramifications of the superficial branch of the trigeminal nerve labeled with NeuroVue Red (scale bar, 500 μm); the ovoid yellow line indicates the margins of the sensory membrane (dashed on the posterior edge where the supporting epithelium was removed) and the parallel yellow lines demarcate the superior extent of the sensory field of the superficial branch. (B) Spread functions determined in the accompanying paper (Bakken et al., 2012) placed in the same size and orientation as in A; the colored markers identify points on the facial pit membrane. The distribution of the spread functions under the parallel yellow lines permitted a prediction of the directional sensitivity in both the vertical (C) and horizontal (D) planes.

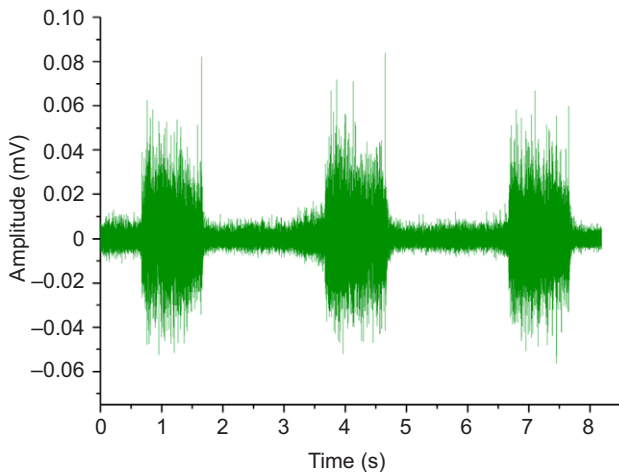


Fig. 5. Raw data tracing showing the clear neural response to the IR emitter. Three of the 10 stimuli are shown; note the relative consistency of the response, and the clear distinction between the response and background activity.

interval used previously (Moiseenkova et al., 2003); these yielded no significant differences from the initial analysis). For each coordinate point the 10 PSD values for the stimuli and the background were then compared using the Wilcoxon signed-rank test; trials in which $p < 0.05$ were taken as exhibiting no neural response to the IR stimuli presented. The coordinate points from those trials in which a significant difference was found between the background and stimulus responses were used to construct a

second coordinate map, this one representing the spatial sensory field of the facial pit of *C. atrox*.

RESULTS

The neural tracers enabled clear identification of three large rami of the superficial branch, which are located adjacent to one another in the middle of the ventral half of the sensory membrane; posterior to these three large rami are two smaller rami that are also restricted to the ventral half of the membrane (Fig. 3). Using the distribution of the individual receptors (visible with the fluorescent labels, Fig. 3), the portion of the sensory membrane that constituted the receptive field of the superficial branch could be identified (Fig. 3).

The receptive field of the superficial branch was then superimposed onto the spread function determined in the accompanying study (Bakken et al., 2012) (Fig. 4). Using the distribution of receptors located within the superficial branch's receptive field, it was possible to predict the directional asymmetry of this portion of the facial pit membrane for the vertical and azimuthal transects used in this study (Fig. 4). Both transects are predicted to show directional asymmetry; peak vertical sensitivity is predicted around 20 deg elevation while peak azimuthal sensitivity is between 20 and 30 deg in front of the pit (Fig. 4).

The signal from the IR emitter, the positioning of the stimulus apparatus and the surgical preparation were all stable enough that there was little variation among the 10 stimulus–response couplings that comprised a single trial (Fig. 5). Constant voltage was applied to the IR emitter, which produced a constant energy level; accordingly, the strength of the stimulus applied to the receptors in the facial pit membrane was varied by distance and the orientation of the stimulus relative to the head/pit opening/pit membrane. When

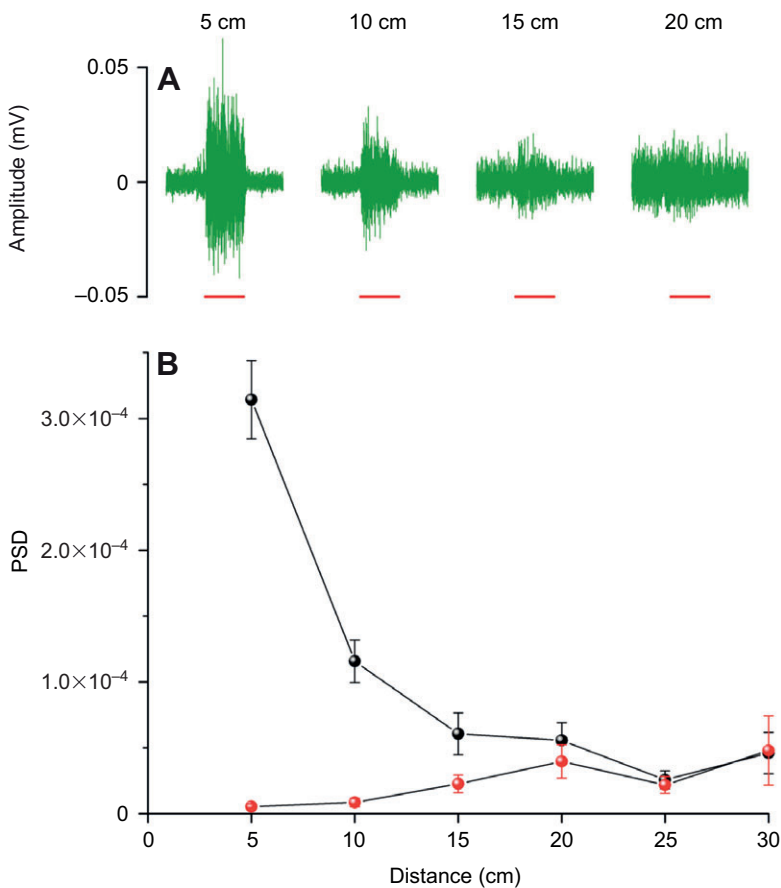


Fig. 6. Effect of varying stimulus distance on the neural response. (A) Cut data traces from the same angular intersect show the diminution of the neural response with increasing distance; red bars are 1 s durations. (B) Power spectral density (PSD) of the mean neural response (black) and background signals (red) against increasing distance. A Wilcoxon signed-rank test (using $p < 0.05$) revealed no significant difference between response and background at distances of 25 cm or greater.

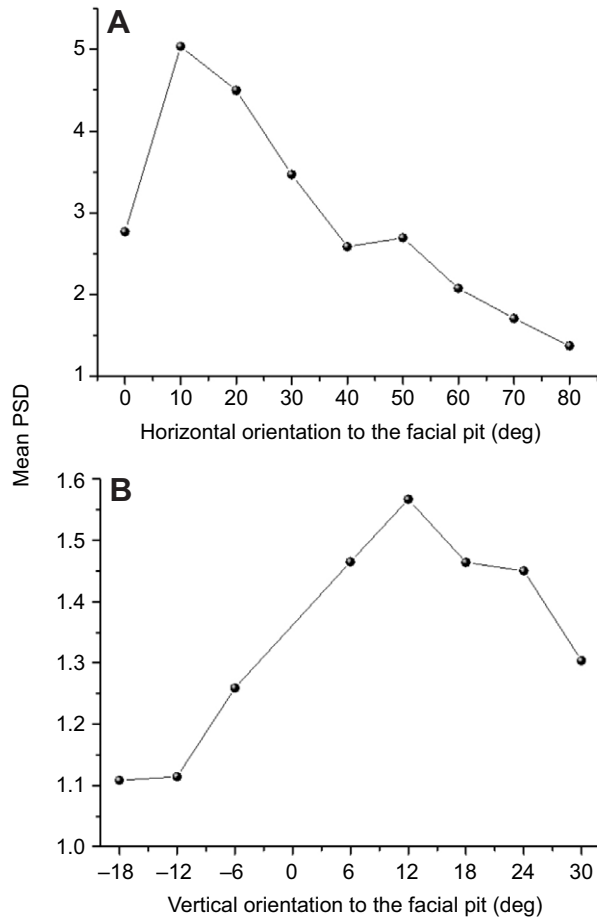


Fig. 7. Effect of varying the orientation of the IR emitter relative to the facial pit while keeping the distance between the emitter and the pit constant (at 10 cm). Values given are mean differential power spectral density, calculated by dividing the neural response by the background levels. Stimuli along the horizontal azimuths (A) were presented at a constant vertical angle of 10 deg above the horizon; stimuli along the vertical azimuths (B) were presented at a constant horizontal angle of 40 deg in front of the pit.

orientation was held constant along a single angular intersect, the decrease in response with increasing stimulus distance from the pit membrane (i.e. decreasing stimulus strength) was evident in the responses (Fig. 6).

If the distance between the IR emitter and the pit membrane is held constant, the influence of stimulus position can be seen by looking at the differential neural response obtained as the emitter moved along horizontal (Fig. 7A) or vertical (Fig. 7B) transects. When both emitter distance and orientation are taken into account, the experimental results can generate a 3D map of the directional sensitivity of the pit organ (Fig. 8). A key feature of this directional map is its asymmetry relative to the facial pit's opening on the lateral surface of the head. The sensitivity was greatest above (peaking at 12 deg vertical) and in front (peaking at 20 deg horizontal) of the pit's opening (Fig. 8). Similar directional asymmetry was found in all six specimens; the intraspecific variation in maximal sensitivity was between 6 and 30 deg vertical and 20 and 50 deg horizontal.

The maximum sensitivity we recorded during these experiments was when the stimulus was at a distance of 30 cm, which with the calibrations of the IR emitter would irradiate the membrane with approximately $220 \mu\text{W cm}^{-2}$.

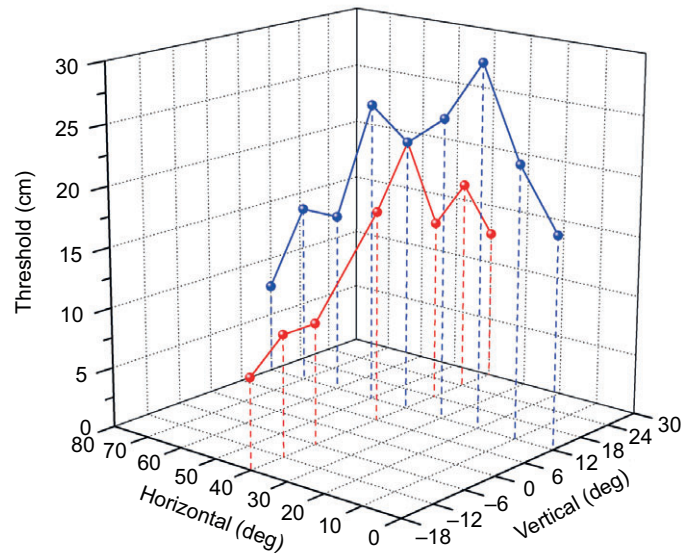


Fig. 8. Plot of directional sensitivity of the facial pit of *C. atrox*. Along both the horizontal (blue) and vertical (red) transects the facial pit exhibits clear directional sensitivity. Threshold (z-axis) represents the greatest distance (up to a maximum of 30 cm) between emitter and facial pit that generated a significant neural response of background activity.

DISCUSSION

Prior studies (e.g. Bullock and Diecke, 1956) exploring the response of the facial pit to thermal radiation have typically treated the membrane as an entity in isolation from the head of the snake. While this has led to advances in our understanding of the neurophysiology and cellular/molecular bases of thermal reception, it has provided limited insight into broader issues concerning the behavioral ecology of the snake.

In the accompanying paper (Bakken et al., 2012), a different approach was taken, using the 3D topography of the sensory membrane relative to the opening of the facial pit and the topography of the adjacent scalation to generate optical spread functions. The results predicted, for the first time, that the sensory fields of the facial pits exhibited directional asymmetry in all of the species examined, including *C. atrox*. Including the null hypothesis of no directional asymmetry, and the nine general directional asymmetries (e.g. above and behind), the facial pit could exhibit one of 10 states with respect to directionality. In the accompanying study, it was hypothesized that in *C. atrox* the sensory field was asymmetric (Bakken et al., 2012), being centered above and in front of the facial pit (Fig. 4).

The present study was intended as a neurophysiological test of the predicted directional asymmetry (Bakken et al., 2012). We focused on the western diamondback rattlesnake (*C. atrox*) because of its ready availability, large (surgical-friendly) size, and the clear directional asymmetry predicted for the facial pit system of this species (Fig. 1).

Our results clearly demonstrate directional asymmetry in the sensitivity of the facial pit of *C. atrox*. As evident in Figs 7 and 8, changing the angular coordinates of the IR emitter relative to the facial pit produced significant changes in the neural response, despite constant output (and distance) from the emitter. Not only do our results support directional asymmetry but also all six of the *C. atrox* we examined had an IR sensory field that was centered above and in front of the facial pit. Our neurophysiological results thus provide

support for the hypotheses and underlying methodologies put forth in the companion paper (Bakken et al., 2012).

The pooled neural response data revealed peak sensitivities at 12 deg above (vertical) and 20 deg in front (horizontal) of the facial pit; looking at the intraspecific variation detected in our study, we saw high sensitivities extending from 6 to 30 deg above and from 20 to 50 deg in front of the facial pit (Fig. 8). The mean experimental results and biophysical predictions differed by less than 10 deg in either plane (compare Figs 4 and 8). Though we cannot provide a definitive explanation for this discrepancy, we hypothesize that it is due to heterogeneous distribution of receptor organs in the posterior portion of the sensory membrane. The spread functions were created assuming a homogeneous spatial distribution of receptors; previous workers have suggested that there is an area of increased receptor density in the posterior portion of the membrane (Amemiya et al., 1999). A localized cluster of increased receptor density could function like a thermal fovea; in any case it would increase local sensitivity that could skew the experimental results relative to the biophysical predictions.

In *Crotalus* the receptors in the facial pit's sensory membrane contribute to three branches of the trigeminal nerve (Fig. 3); our anatomical results suggest that *C. atrox* is similar to other pit vipers in both the position and minimal overlap of the three respective fields on the sensory membrane (Bullock and Fox, 1957; Amemiya et al., 1999). This study recorded from only one (the superficial maxillary) of these three branches. Several considerations led to this experimental design; the superficial maxillary covers the majority of the sensory membrane (Bullock and Fox, 1957), one of the branches (the ophthalmic) is virtually inaccessible surgically, and the time required to complete all of the different emitter positions and orientations did not allow us to repeat the process on the remaining branch (the deep maxillary) without compromising the experimental preparation. The superficial maxillary branch that we recorded from is formed by neurons in the ventral portion of the sensory membrane; these neurons are distributed across the anterior–posterior length of the membrane (though are more numerous posteriorly). The distribution of these sensory neurons could not produce the directional asymmetry we recorded during these experiments. Increasing vertical angles would preferentially stimulate the neurons of the superficial maxillary branch (as they are located on the inferior portion of the membrane), yet we found a decreasing response to vertical angles greater than 20 deg. At the same time, we have no experimental evidence regarding the directional sensitivity of the other two portions of the sensory membrane.

This study was not intended as an exploration of the maximum sensitivity of the facial pit system. The IR emitter was never positioned more than 30 cm away from the facial pit, even when significant responses were detected at 30 cm. At 30 cm, the IR emitter was exposing the pit membrane to approximately $220 \mu\text{W cm}^{-2}$, which is well above the minimum thresholds of $10.76 \mu\text{W cm}^{-2}$ (de Cock Buning, 1983) and $3.35 \mu\text{W cm}^{-2}$ (Ebert and Westhoff, 2006) reported previously. The higher threshold in the present study resulted, we believe, from the combination of three main factors: (1) sampling from the trigeminal nerve (rather than individual neurons), which produced an averaging effect; (2) the use of an IR emitter with a more restricted spectral distribution; and (3) performing the experiments on snakes anesthetized with Isoflurane, which has been shown to reduce the sensitivity of the IR system (Moiseenkova et al., 2003). For the goal of the present study it is important to note that the trigeminal preparations and the emitter

were 'constants' and that we controlled for the influence of anesthesia by randomizing the sequence of stimulus presentation and by assuring a continuity of neural response to a fixed stimulus presented at the onset and completion of each experiment. As such, the reduction in overall sensitivity of our experimental approach could not have biased our findings toward directional asymmetry, and certainly not toward the predicted form of directional asymmetry.

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