

# Infrared detection in the rattlesnake *Crotalus atrox* – from behavioural studies to midbrain recordings

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**Abstract.** We investigated the infrared detection range of *Crotalus atrox* for a mouse like stimulus for the first time in a behavioural approach. The results propose a detection range of merely 1 m which greatly exceeds all detection distances previously reported for Crotalinae. In addition we report from physiological experiments based on multi unit recordings and recordings of evoked potentials within the midbrain of rattlesnakes while applying infrared stimuli. The two different ways of measuring response profiles revealed evidence for an enhancing mechanism in the midbrain Tectum opticum. This enhancing mechanism is proposed as a possible reason for differences reported in detection ranges for mouse-like infrared stimuli.

## Introduction

Pitvipers (Crotalinae) like rattlesnakes have a name giving pit organ between their nostril and eye on each side of the face (Bullock, 1957; Lynn, 1931; Noble and Schmidt, 1937). These organs enable the snakes to detect minute thermal differences within their environment as they are highly sensitive to infrared (IR) radiation (Bullock and Diecke, 1956; de Cock Buning, 1983a, b; Molenaar, 1992). In physiological experiments different detection thresholds have been determined in crotaline snakes (Bullock and Diecke, 1956; de Cock Buning, 1983b). Based on these values the calculated detection range for crotaline snakes for a mouse-bourne stimulus varies from less than 5 cm (Jones et al., 2001) to 66 cm (de Cock Buning, 1983b). Thus, the actual detection distance remains more or less unclear. In an attempt to fill this gap we investigated the IR detection threshold and sensitivity of the rattlesnake *Crotalus atrox* for a mouse-like stimulus in a behavioural approach.

To understand the striking differences of determined threshold levels we made physiological recordings within the midbrain of rattlesnakes. The Tectum opticum of the midbrain is the most important brain area for the integration of visual and IR sensory cues as well as for representing a topographic map of the visual and IR field of view (Hartline et al., 1978; Newman und Hartline, 1981; 1982). Besides several qualitative analysis (de Cock Buning, 1983b; Hartline, 1971; Kass et al., 1978; Newman and Hartline, 1981; Terashima and Goris, 1976; Wells et al., 1971) the neural tuning characteristics of IR sensitive tectal units are not understood.

We investigated the tuning characteristics of IR sensitive tectal units of pitvipers by means of multi-unit recordings and recordings of evoked potentials. By comparing tuning characteristics of evoked potentials as well as afferent fiber units we found evidence for enhancing mechanisms for IR stimulus processing on the tectal level.

## Material and methods

Sixteen captive born *Crotalus atrox* (snout-vent lengths of 40 to 70 cm) were used for the studies. The animals were housed individually in terrariums with a hide box as a shelter at an ambient temperature of  $28 \pm 1$  °C and at a 12-hour light/12-hour dark cycle. The snakes were provided with water *ad libitum* and maintained on a diet of one mouse every 10 to 12 days.

Twelve individuals were blindfolded with a strip of black duct tape. Animals were tested in a circular arena (200 cm in diameter) shielding the snake from unwanted IR stimuli. The experimental setup was as described elsewhere (Ebert and Westhoff, 2006). The snakes had a body temperature of  $24 \pm 1$  °C (room temperature was  $23 \pm 1$  °C), measured with an IR-thermometer (minitemp TM, Raytech, USA).

The IR stimulus was presented to the snakes and set into oscillation (0.5 Hz, amplitude 30 cm) randomly at distances from 10-150 cm ( $\pm 0.5$  cm) whenever the snakes faced the stimulus. A number of behavioural responses (head jerks, tongue-flicking, freezing in a movement and rattling) were evaluated as "positive response" and pooled together. As these behaviours may also occur spontaneously the spontaneous behaviour was quantified in sham experiments. Controls for hidden cues with blocked pits and/or switched off heat element were carried out as well.

Sixteen individuals were kept under anaesthesia as described elsewhere (e.g., Moiseenkova et al., 2003) and the preparation of the midbrain Tectum opticum was carried out as has been described by Goris and Terashima (1973). A red diode laser (TIM 203/5 mW, ELV Elektronik, Germany) with a wavelength of 640-650 nm focused with a lens into position within the snakes' pits was used as heat stimulus (stimulus duration 1 s). A visual barrier of black plasticine was applied between the loreal pit and the eye. A razor blade attached to a micromanipulator was driven into the laser beam to reduce the power from 5 mW to 3  $\mu$ W (measured with Fieldmaster FM, Coherent). Recordings were done in

the Tectum opticum of twelve animals as described elsewhere (Westhoff and Morsch, submitted). Evoked potentials (EP) were recorded in four animals in the middle of the midbrain contralateral to the site of stimulation (for methodology see Westhoff and Morsch, submitted).

## Results

### *Behavioural experiments*

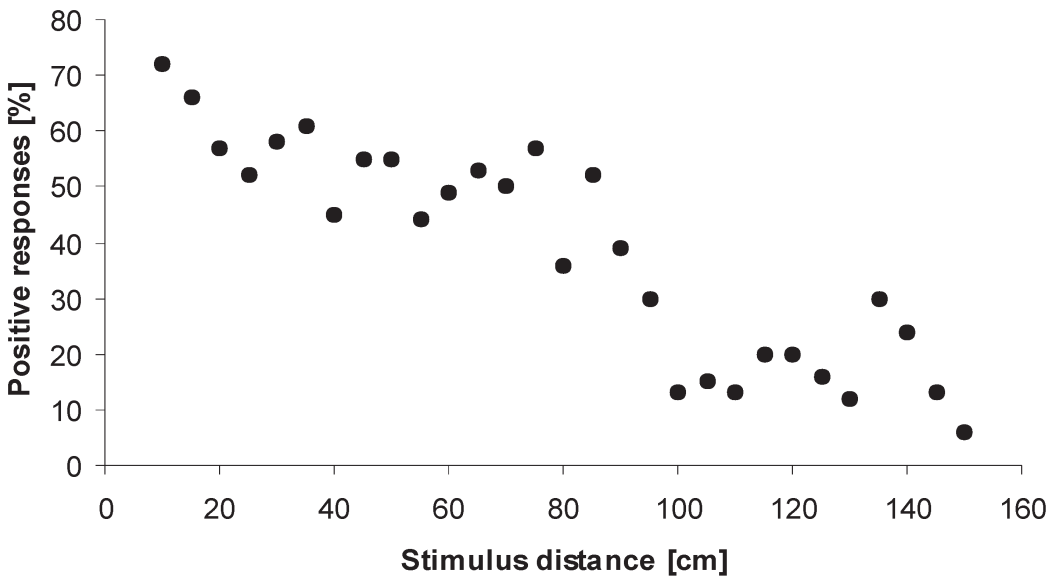
The snakes were exposed to 1705 IR stimuli, offered at distances between 10 cm and 150 cm in 130 sessions. Pooled in 5 cm distance intervals positive responses decreased from 72% (shortest source distance 10 cm) to 6% (farthest source distance 150 cm). At distances of less than 35 cm to the IR stimuli the snakes responded the stimulus in 52-72%. About 50% of the snakes responded at distances between 35 and 75 cm and the behavioural responses declined continuously at farther distances. However, there was a sudden drop in responses at about 100 cm and the responses finally dropped to 6%.

The data of the 465 control trials were pooled, 38 of which were scored as "positive response". The control trials were tested against the IR trials which revealed a significant positive response for distances up to 95 cm (Chi<sup>2</sup>-Test, Chi<sup>2</sup> = 20.367, df = 1, p < 0.05). There was no significant difference at the distance of 100 cm (Chi<sup>2</sup> = 0.839, df = 1, p = 0.36).

### *Physiological experiments*

None spontaneously active IR sensitive multi-units (N = 30) were recorded in twelve animals ( $1 \leq n \leq 4$ ) with interspike intervals of less than 2 ms. Individual spikes of the multi-unit responses had a duration of 0.15 – 1.40 ms (N = 5, n = 450,  $20 \leq n \leq 370$ ). Bimodal IR and visual multi unit responses or unimodal or exclusively visual multi units were not found. Testing the neural response for different stimulus intensities the maximal laser intensity was either 1600  $\mu$ W (N = 19) or 3200  $\mu$ W (N = 11, c.f. Fig. 2A). The spike rate (number of spikes) of the multi-units increased with increasing stimulus intensity. Saturation of spike rate was reached at intensities between 600  $\mu$ W and 1000  $\mu$ W. The slope of the increasing spike rate differed considerably between units and the maximal numbers of spikes varies from 14 to 100 (examples are given in Fig. 2A). Thus, the multi units reflect variable activity profiles covering a wide range.

Evoked potentials (EP) were recorded in three animals. IR stimuli of 1000  $\mu$ W were applied while recording in steps of 100  $\mu$ m from the tectal surface to a depth of 2000  $\mu$ m. Maximal amplitudes of EPs (peak to peak) were observed at a depth of 800  $\mu$ m to 1000  $\mu$ m. Recordings were obtained from the centre of the contralateral tectal



**Figure 1.** Percentages of positive responses as function of distances of IR stimuli. Note the sudden drop of positive responses at distances of 90–100 cm.

hemisphere whereas the laser stimulus was focused on the centre of the pits membrane. Depth profiles were obtained under same stimulus conditions at a number of slightly different sights in the Tectum opticum. Intensity profiles were recorded at the sight and depth of highest EP amplitudes for intensities of 3 - 2000  $\mu\text{W}$ . The amplitudes of evoked potentials were again measured peak to peak and plotted as function of stimulus intensity (Fig. 2B). Increasing stimulus intensities up to 350  $\mu\text{W}$  resulted in a steep increase of EP amplitudes. In contrast, stimulus intensities higher than 350  $\mu\text{W}$  resulted in almost no increase of EP amplitudes (c.f. Fig. 2B).

## Discussion

### Behavioural responses

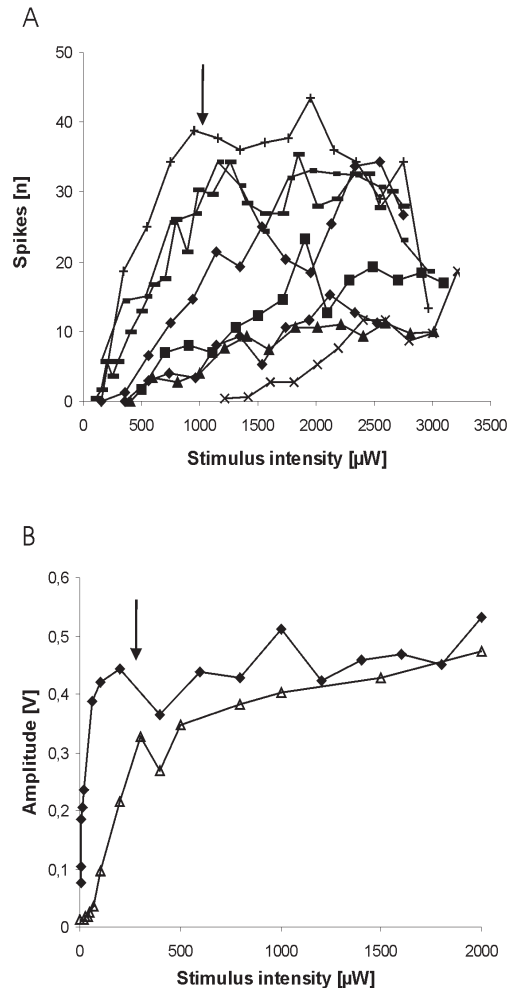
The type of behavioural response (head jerk, tongue flicking, rattling) was irrelevant for this study as the focus laid on a distinct change in behaviour during a 10 s stimulus presentation. The change in behaviour revealed whether the stimulus was perceived by the snake or not. However, conclusions can only be drawn from positive reactions. Thus, a snake that didn't respond might still have perceived the stimulus. In conclusion the actual detection threshold should be considered even more sensitive and the detection distance further than the provided values in this study. Our data support that *C. atrox* detected the IR stimulus at least up to a distance of 95 cm. This is a considerably greater distance than proposed by the calculations of de Cock Buning (1983) (for *Calloselasma*: 66.6 cm) or Jones et al. (2001) (for *Crotalus*: < 5 cm).

### Physiological experiments

The width of a spike can be used to distinguish a spike originating from a cell soma from fiber spikes (Zittlau et al., 1986). The spikes of the multi units recorded in the present study had durations of maximally 1.5 ms. In contrast, recordings of single cells within the optic tectum of rattlesnakes revealed a spike duration of 2 to 6 ms (unpublished data). We therefore conclude that the spikes recorded here are from afferent IR processing fibers entering the Tectum opticum rather than the tectal cells. The spike rates of multi units reached a steady state at a stimulus intensity of 600  $\mu\text{W}$  to 1000  $\mu\text{W}$ . In conclusion we consider these values as the saturation levels for IR stimulus intensities of the afferent signals entering the Tectum opticum.

Evoked potentials recorded here can be regarded as the output of IR-sensitive tectal cells. The amplitudes of

EPs increased rapidly with increasing stimulus intensity up to app. 300  $\mu\text{W}$ . This is a 2 to 3 times lower value in comparison to the saturation of spike rates recorded from multi units. Moreover, the increasing of EP amplitudes with increasing stimulus intensity is characterized by a strong slope. In contrast, the dynamic characteristics of spike rates over stimulus intensity are highly variable. Jones et al. (2001) calculated the detection range of a rattlesnake for a mouse like IR stimulus to be less than



**Figure 2.** A) Stimulus intensity depending response profile of 8 IR sensitive multi units recorded in the Tectum opticum. The spike rates are plotted as function of stimulus intensity. The arrow indicates the approximate stimulus intensity of spike rate saturation. Note the difference of response profiles. B) Stimulus intensity depending response profiles of evoked potential amplitudes. Arrow indicates approximate stimulus intensity at amplitude saturation of evoked potentials.

5 cm and they were aware that this did not meet the snakes' capabilities. As the calculation was based on an IR threshold determined at the trigeminal nerve, the authors suggested that there might be an enhancing mechanism in the central nervous system. The results of the present study favour the hypothesis that incoming signals of afferent IR sensitive fibers are enhanced by IR sensitive tectal neurons.

The highly variable dynamic range of spike rates in multi units again favours the interpretation that these multi units reflect afferent fibers and thus represent the tectal input. The variability of input units would enable the system to process different stimuli always with the maximum sensitivity and maximum dynamic enhancement. However, further studies are needed to understand the cellular mechanisms responsible for these enhancing processes.

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