Snake Infrared Receptors: Thermal or Photochemical Mechanism?

Abstract. *It appears that the two most sensitive infrared receptors known in the biological world are found in two widely different families of snakes, the pit vipers and the boas. After an infrared stimulus from a carbon dioxide laser, which has a monochromatic output at 10.6 micrometers, we find evoked potentials in boas with chronically implanted electrodes. Our data suggest that the receptors operate on a thermal principle.*

In 1931 Lynn found (*1*) that pit vipers (Crotalidae) possess an intricate sense organ capable of detecting very subtle environmental stimuli. Lynn postulated that this pit organ was primarily a sensitive mechanical receptor which operated on fluctuations in air vibrations. In 1937 Noble and Schmidt (*2*) concluded on the basis of behavioral evidence that the pit organs of snakes receive information on both temperature and vibration from the environment. From the work of Bullock and Cowles (*3*) and Bullock and Diecke (*4*) came electrophysiological evidence that the pits were sensitive primarily to infrared radiation rather than to air temperature. Attempts to verify the vibrational characteristics of the facial pits failed.

Examinations by Bullock and Barrett (*5*) of the labial pits of Boidae revealed not only that these pits were sensitive to infrared stimuli, but also that they were quite insensitive to vibrational stimuli. As a matter of fact, representatives of the Crotalidae and Boidae families were the only snakes studied that were found to possess infrared-sensing organs. Comparative evaluations of the pits of the Crotalidae and the Boidae (*5*) indicated that the Crotalidae possess the more sensitive pits for infrared radiation. Values of the threshold stimulus for the Crotalidae were estimated to be 3.15×10^{-4} calorie cm^{-2} sec^{-1}, whereas such values for Boidae were estimated to be 1.3×10^{-3} calorie cm^{-2} sec^{-1}.

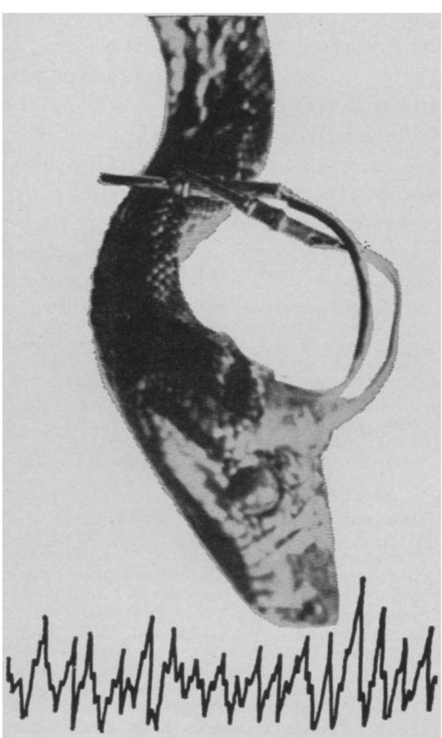

Fig. 1. A tree boa (*Corallus enydris cooki*) with implanted electrodes. At the bottom is a representation of the boa's electroencephalographic record.

A fascinating and still not fully answered question is whether the infrared pit receptors act as thermal or photochemical sense organs. Bullock and Diecke (*4*) stated that "the question cannot yet be answered unequivocally whether the receptors of the pit organs are normally stimulated, like the eye, by certain wavelengths of all those absorbed, or, like temperature receptors, by all wavelengths in proportion with which they heat the tissue." Histological studies of the nerve endings in the membrane from the facial pit of *Crotalus viridis* show that the axons fan out and taper until each "suddenly expands into a very broad palmate structure from which 3 to 7, usually 5 or 6, processes spring to branch repeatedly and end as exceedingly fine, free endings" (*6*). Barrett *et al.* (*7*) commented that "the unique geometrical pattern may allow the nerve fibers to fire after very small changes in temperature," thus suggesting that a thermal mechanism is at work. In 1967 Goris and Nomoto (*8*) studied Crotalidae and found that the absorption characteristics of the pit membrane are quite similar to those of a wide range of other epithelial tissues.

Because the blackbody radiation from a mammal or bird (the natural prey of most of these snakes) occurs at a wavelength of about 10 μm and drops off precipitously at wavelengths of less than 5 μm (*9*), we thought it was imperative to verify that snakes can indeed respond to wavelengths corresponding to the maximum thermal radiation of their prey.

Conventional heat lamps are, in general, inadequate because of their low power density in the far-infrared region. With lasers, however, one can now deliver almost any power density at any desired wavelength. For this study we used a carbon dioxide laser which has a maximum power output of 30 watts at 10.6 μm. The beam is attenuated by means of a diverging lens (Irtran 2, Eastman Kodak) that spreads the beam's intensity over an area of many square feet. By means of a thermopile and power meter (Scientech) we found that the power density in the vertical plane (where the snake's head was located) was between 1.9×10^{-3} and 3.4×10^{-3} calorie cm^{-2} sec^{-1}. This range was a result of the power variation occurring in the laser beam itself. The laser beam was mechanically pulsed with a camera shutter speed of 33, 18, or 8 msec. The snakes used in this study were red-tailed boa constrictors (*Boa constrictor*). A response to a discrete stimulus was determined by measuring the evoked potential from the brain. Standard methods of electrode implantation were used (*10*); Fig. 1 is a photograph of a tree boa (*Corallus enydris cooki*) with implanted elec-

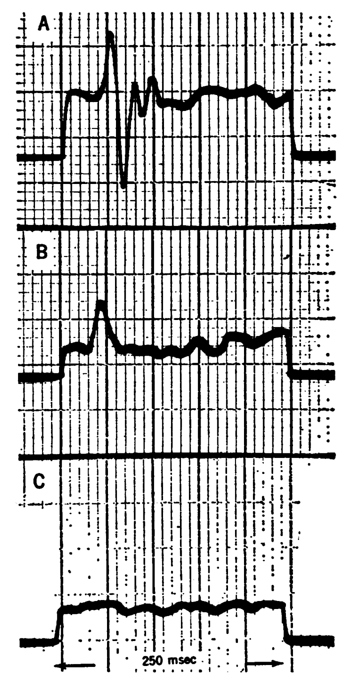

Fig. 2. (A) The averaged evoked potential to a series of light flashes. (B) The averaged evoked potential to an infrared stimulus. (C) The control situation with no stimuli reaching the snake. The signal-to-noise ratio was averaged over a period of 250 msec in all cases. The initial deflection represents the initiation of the stimulus.

trodes with a typical electroencephalographic record shown below. For implantation the snakes were anesthetized with ~ 30 mg per kilogram of sodium pentobarbital administered intraperitoneally. With a dental hand drill small holes were made in the cranium. Small stainless steel jewelers' screws with electrical leads were self-tapped through the skull to the surface of the telencephalon. In all experiments reported here each snake was allowed sufficient time to recover fully from the electrode implantation before any responses were measured. The evoked potentials (EP) were measured with a signal averager (Princeton Applied Research waveform eductor). The eductor was set to average the signal-to-noise ratio 250 msec after the initiation of the stimulus. Signal amplification was recorded in the conventional manner. Evoked potentials to visible stimuli were obtained by the use of a photoflash with an electronic trigger to the eductor. Figure 2A is a typical visual EP with a latency of 30 msec. This latency is a little longer than the 21- to 24-msec latency reported for garter snakes (*10*). Figure 2B is a typical EP record from an 8-msec infrared laser pulse. The total energy given was between 1.5×10^{-5} and 2.7×10^{-5} calorie per square centimeter. The EP latency averaged from three different snakes was between 25 and 30 msec. The snakes were tested on consecutive days with essentially no variance in their record. Electroencephalographic records were obtained routinely during every experiment.

The stimuli we were presenting appeared to be well above the threshold values since a decrease in the shutter speed from 8 to 18 or 33 msec with no change in the laser output gave a record similar in every respect to the 8-msec stimulus. Before each stimulus the position of the snake's head was carefully noted, but here again, at the stimulating intensities used, no correlation with head position was noted, thus suggesting that we were operating in the range far above the threshold stimulus. Figure 2C is a click control; the recording situation is the same as that used in Fig. 2B except that the laser beam was blocked from the snake by means of a firebrick.

Our data strongly suggest that the receptor operates on thermal principles. This conclusion is based on the following observations: (i) there is a high sensitivity to stimuli in the far-infrared region; and (ii) the EP latency after an infrared stimulus is comparable to that of the visual EP. Since the eye is a multisynaptic structure in contrast to the single afferent nerve endings of the infrared receptor, we would expect a very much shorter latency if the infrared receptor operated on a photochemical basis. Bullock and Barrett (*5*) reported a threshold of 1.3×10^{-3} calorie cm^{-2} sec^{-1}, which is very close to our finding. The fact that the energy we used was entirely in the far-infrared region and that our responses were measured by means of evoked potentials from chronic preparations is strong evidence that the mode of operation is thermal.

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11. We thank Dr. R. Gallet for first making us aware of this interesting sensory system. We thank Dr. F. Barnes for use of the carbon dioxide laser and W. Bank for his excellent technical assistance. This work was supported in part by funds from the Research Corporation of Minneapolis, a National Science Foundation grant (GK-5682), and the University of Colorado Graduate School.

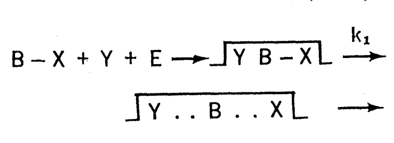
4 December 1970; revised 10 February 1971

Covalent Enzyme-Substrate Intermediates

Abstract. *A literature search reveals 60 cases in which there is strong evidence for covalent enzyme-substrate intermediates.*

The manner in which enzymes catalyze reactions is a fascinating problem, relevant both to an understanding of organic chemistry and to the biological role of these catalysts. In all cases, the enzyme must act by polarizing the electrons and the bonds undergoing reaction, but in some cases a covalent intermediate is formed during the course of the enzyme action. The question then arises as to the role of these covalent intermediates. [For general reviews of covalent intermediates, see (*1–7*).]

One possibility is that the covalent intermediate provides a catalytic function. This can be illustrated in Eqs. 1 and 2, where the substrate, BX,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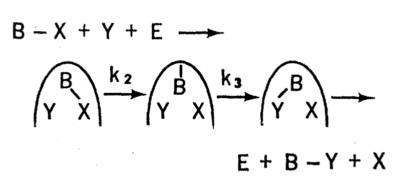
(2)

is converted to the product BY, either by direct attack of Y on BX (Eq. 1) or indirectly through an enzyme-substrate intermediate (Eq. 2). If the intermediate is to perform a catalytic role, the rate constants k_2 and k_3 must both be greater than k_1, that is, the group on the enzyme surface acts both as a better attacking group than Y and a better leaving group than X. These criteria have been satisfied in certain model systems. Thus the serine of many serine proteinases is both a better leaving group than the amide of peptides and a better nucleophile than water (*8, 9*). Similar properties of pyridoxal intermediates have been demonstrated (*10, 11*). Nevertheless, the data from model systems comparing nonenzymatic analogs for k_2, k_3, and k_1 does not indicate any very large catalytic advantage for the formation of a covalent intermediate.

There may be, of course, reasons other than catalytic ones for such intermediates. They might preserve the high energy character of a bond in a multistage reaction. They might serve the function of preserving the stereochemistry of an asymmetric atom or might provide a sterically more favored course for complex intermediates.

Although advantages can be listed, it is clear that covalent intermediates are not essential for enzyme action. A negative result such as the failure to isolate an intermediate is not conclusive, but the stereochemistry in a single dis-