THE SURFACE ARCHITECTURE OF SNAKE INFRARED RECEPTOR ORGANS

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ABSTRACT

The surface of the epithelium in snake infrared receptor organs is covered with a characteristic array of tiny pores that is different from any other surface structure in squamate reptiles. The measurements and density of the pores differ slightly according to family and species, but the array is characteristic and immediately recognizable. In boids without pits, the array covers the entire surface of each scale that contains infrared receptors. In boids with pits, the array covers the fundus of each receptor pit organ. In crotaline pit organs the array is present on both the outer and inner surfaces of the receptor-containing membrane, and on the epithelium of the wall of the inner chamber. This inner chamber wall is sculpted into a tight array of large and small domed structures, on the surface of which the pore array appears. We speculate that the array of domes in the crotaline pit organ functions as a light trap to prevent infrared rays that penetrate into the inner chamber from being reflected back onto the receptors in the pit membrane. On the other hand, the array of pores, present in all species, appears to reflect away and diffuse visible radiation that might have enough energy to heat-stimulate the receptors and interfere with the target stimulus, i.e., infrared radiation.

Two groups of snakes possess infrared receptors: those of the family Boidae (the boids), and those of the subfamily Crotalinae (the crotalines) of the family Viperidae. The boids have the receptors in the labial scales, some without specialized structures, and others in the fundus of specialized labial pits. The crotalines, on the other hand, have the receptors in a thin membrane suspended between inner and outer chambers of a pair of pits in the loreal region. The three different types are illustrated in Fig. 1.

While doing a series of comparative anatomical studies on the structure of the infrared receptor organs, we were struck by certain surface features that seemed to be possessed in common by all species having these organs, and that have never before been reported in the literature. Since these surface features were common to all species with infrared reception, and were found only in regions associated with the infrared receptors, we surmised that they played a major role in the receptor function. As a first step in clarifying this role, we did a scanning and transmission electron microscope study of all surface areas associated with these receptors.

MATERIALS AND METHODS

As representative of the evolutionarily advanced crotaline snakes, we used 3 Agkistrodon blomhoffii, a common pit viper of Japan, Korea, and the eastern regions of China. For the boids with pits, we used 4 Python regius, the ball python, and 1 Python molurus, the Burmese python. For a representative of the boids that do not have labial pits, we used 1 Boa constrictor, the common boa con-
microscopy were embedded in a mixture of Epon and Araldite or in Luft’s Epon mixture. One μm semithin sections stained with toluidine blue were used for light microscopy and for selecting typical areas for ultrathin sections. Ultrathin sections of the chosen areas were then stained with uranyl acetate and lead citrate. Specimens for scanning electron microscopy were critical-point dried and sputter-coated with gold-palladium or platinum-palladium alloy.

The control animal was anesthetized with halothane and one upper labial scale and one parietal scale were dissected away. The snake was then allowed to recover. As further controls, we cut away a parietal and some dorsal scales from B. constrictor, and the scales outside one of the pits of P. molorus. The dissected tissues were fixed and prepared for scanning electron microscopy as described above.

In crotaline snakes it has long been known that the infrared receptors are entirely contained in the membrane suspended between the two chambers of the loreal pit (see 5, for review). The receptors in the membrane contain large numbers of mitochondria, and can be visualized by staining for the succinate dehydrogenase (SDH) present in the mitochondria (2). To locate the receptors in the boid snakes, we also used SDH staining. The method and results have been published elsewhere (1).

RESULTS

Boids without Pits

In Boa constrictor, our SDH staining confirmed the work of von Dürring (9), who described the presence of infrared receptors in this species in supralabial scales 8, 9, and 10, and in the 3 subocular scales (6) above these. Anticipating the presence of receptors in most, if not all, of the labial scales, we stained right supralabials 3 and 13, and found receptors localized in the rostral upper corner and caudal lower corner of each scale (Fig. 2A). We did not examine the subocular scales because the labials alone provided more than enough material for the work planned.

The entire surface of scales containing infrared receptors presented a pitted appearance, due to the presence of an array of microscopic pores (Fig. 2, B–D). The pores were roundish or elliptical, 0.3–0.5 μm in diameter at the mouth and 0.15–0.25 μm in depth, occupying only the outermost portion of the cornified layer. They were more or less crater-like in cross section (Fig. 2B), and spaced at inter-
Fig. 2  Surface structure of the pit organs of boids without pits (here, B. constrictor). A: Partial section of an infrared receptive scale. SDH staining of the receptors shows them grouped in the epidermis in only a part of the scale (between arrowheads). The rest of the epidermis is unstained. The black staining in the dermis is due to the presence of melanin. Bar = 200 \mu m. B: A transmission electron microscope (TEM) image shows the microscopic pores of the surface in cross section (arrows). Note the crater-like shape. Bar = 1 \mu m. C: A low magnification scanning electron microscope (SEM) image of an infrared receptive scale. The ridge marked with an arrow is the raised border between oberhauchten cells. Bar = 1 \mu m. D: A high magnification SEM image of the same scale. Bar = 1 \mu m
vals of 0.15–0.3 μm, with an average density of 3.34/μm² (Fig. 2, C and D). Scales not containing infrared receptors had a quite different surface architecture (Fig. 3, C and D). The pores were not confined to the areas of each scale containing receptors, but extended over the entire surface of the scale.

**Boids with Pits**

Both species examined had arrays of pores similar to those described above for *B. constrictor*, but different in size, shape, and density. The pores were cylindrical in cross section (Fig. 3A), 0.1–0.25 μm in diameter and 0.1–0.25 μm in depth, spaced at intervals of 0.15–0.45 μm, with an average density of 5.45/μm² (Fig. 3B). The pores were present only at the bottom of the receptor pits, in the area where SDH staining revealed the presence of receptors.

**Crotaline Snakes**

As mentioned in Materials and Methods, the crotaline infrared organs consist of an inner and outer chamber separated by a thin (15 μm in *Trimeresurus flavoviridis*, see 8) membrane which contains the receptor terminal nerve masses (*ibidem*). We found an array of pores on both the outer and the inner surfaces of this membrane (Fig. 4). We also found specialized structures on the surface of the wall of the inner chamber (Fig. 5).

The outer surface of the pit membrane was divided into mostly pentagonal, sometimes hexagonal, oberhauchten cells with well-defined, raised borders (Fig. 4B). The cells measured 15–30 μm at the widest point between opposing sides. All cells were covered with an array of pores similar to those of the boids with pits described above (Fig. 4A), pores were crater-like in cross section (Fig. 4A), 0.25–0.5 μm in diameter at the mouth and 0.2–0.25 μm in depth, and spaced at intervals of 0.15–0.4 μm, with an average density of 3.66/μm².

The inner surface of the pit membrane was also divided into pentagonal or sometimes hexagonal oberhauchten cells. In this case the cells were larger than the outer ones, measuring 20–40 μm at the widest point between opposing sides. This inner surface was also covered with an array of pores that were crater-like in cross section. They were 0.06–0.25 μm in diameter at the mouth and spaced at intervals of 0.25–0.5 μm, with a density of 1.97/μm². In contrast to the outer surface, the pentagonal or hexagonal cells had a slightly raised center where the density of the pores was reduced to about 1.50/μm² (Fig. 4C). These central pores were also smaller than the others, being 0.06–0.1 μm in diameter. We were unable to measure the depth of the pores on the inner surface of the membrane due to lack of suitable transmission electron microscope specimens.

The surface of the inner chamber presented the most specialized, complex picture. The entire surface was covered with domed structures of fairly regular size (Fig. 5). There were 2 classes of these domes: large (15–20 μm in diameter at the base and 8–11 μm from base to apex), and small (2–6 μm in diameter at the base and 1–3 μm from base to apex) (Fig. 5A). The large domes were spaced so closely that they were nearly touching, at an average density of 3,160/mm². The small domes packed the spaces occurring between the round bases of the large domes. There were 3–4 small domes in each space, depending on their size. At the base they appeared to be in direct contact with each other and with the large domes, and their density per unit area was 13,400/mm². Thus the wall of the inner chamber was completely covered with domes, with no open spaces whatever (Fig. 5, B–D).

A striking feature of the domes was that their surfaces, both those of the large domes and those of the small domes, were covered with an array of pores that were similar in shape, size, and density to those of the outer surface: crater-like, 0.25–0.5 μm in diameter at the mouth, and spaced at intervals of 0.25–0.5 μm, with a density of 2.41/μm² (Fig. 5C).

The dome-covered epithelium was not confined only to the wall of the inner chamber, but continued on through the pre-ocular pore (2) into the portion of the orbit immediately caudal to the pit (Fig. 6A). However, as one proceeded away from
Fig. 4  Surface of the pit membrane in crotaline snakes (here and in Figs. 5 and 6, A. blomhoffii). A: A TEM image of the outer keratinized layer of the pit membrane. Arrows point to the pores. Bar = 1 μm. B: A SEM image of the outer surface of the pit membrane. Arrows here and in C point to the raised borders of the oberhauchten cells. Bar = 5 μm. C: A SEM image of the inner surface of the pit membrane. The image is quite similar to that of B, with the exception of the raised centers of the oberhauchten cells (arrowheads). Bar = 5 μm
the pit itself, the specialization of the epithelium became less and less apparent: the small domes eventually disappeared entirely, the large domes became more or less distorted, and the pores covering their surface became smaller, shallower, and less regular in form and distribution (Fig. 6B).

Controls

All controls, i.e. scales from snakes without infrared reception, and boid and crotaline scales from areas not related to infrared reception, showed features common to squamate reptiles, as reviewed aptly by Landmann (4) (Fig. 3, C and D). But none of the controls showed the characteristic array of rounded pores that we have described above.

DISCUSSION

Significance of the Pores

The scale surface in squamate reptiles generally presents a sculpted, pitted appearance (see 4, for review). However, the array of rounded pores that we have described in this paper can be seen only in snakes that possess infrared receptors; and in these snakes only in structures immediately associated with the receptors themselves, but nowhere else: For example, in the crotaline A. blomhoffii, not even the scales at the immediate edge of the pit organs have the array of rounded pores, but instead show the sculpted appearance common to other body scales, with scattered, small, irregular pores (Fig. 3, C and D). In the boid without pits, B. constrictor, the pore array is seen in places where there are no infrared receptors, but it is still confined within the borders of the scales that contain infrared receptors. Other scales show the common sculpted appearance.

The pore arrays are seen even in the tissues behind the receptors in crotaline snakes, i.e., in the domed epidermis of the inner chamber of the pit organs. They even appear on the domed surface of this epidermis as it continues on through the precingular pore to the orbit of the eye, although outside the confines of the pit they become shallower and less regular in appearance (Fig. 6B).

This fact, i.e., that representative species of the family and subfamily possessing infrared reception, although of widely separated evolutionary stock (cf. 1), all possess a similar array of pores covering or backing up the infrared receptors, can mean only one thing. That is, the pores serve in some way to facilitate infrared reception. We speculate that the pores serve as a form of filter, reflecting away the shorter wavelengths of light in the visual spectrum, while allowing the longer infrared wavelengths free passage through the keratin to the receptors below. This could be necessary because some of the longer visual wavelengths could have enough energy to raise the temperature of the receptors and cause a nerve potential that would diminish the sharpness of the infrared ‘image’. As a matter of fact, Terashima and Goris (7) were able to do very precise electrophysiological measurements of receptor performance using a visible laser (He-Ne, 632.8 nm wavelength) that had sufficient energy. This speculation is strengthened by simple observation of the pit organs with the naked eye. Compared to surrounding skin structures the pit membranes of crotalines and the fundus of boid pits are extremely reflective of visual light, to the extent that when photographing at close range with a flash or spotlight, one must pay attention to the angle at which the light strikes the pit organ in order to avoid unseemly glare.

To obtain this effect of rejecting visible light to enhance the receptivity of infrared wavelengths, the precise measurements of the array of pores do not seem to be critical. As a matter of fact, there is a certain amount of difference among species in the diameter, depth, distribution, and density per unit area of the pores, as illustrated in Table 1. Shape and all measurements are roughly equal in the labial scales of B. constrictor, and in the outer surface of the pit membrane and the domed wall of the inner chamber of the pit organ in A. blomhoffii. In P. regius and P. molurus, on the other hand, the pores are cylindrical instead of crater-like, smaller and shallower, and distributed at greater density. The reason for this difference is not readily apparent. However, the similarity is such that the array is immediately recognizable at suitable magnification, so that it is possible to predict the presence or absence of infrared receptors by observation of the skin surface with, e.g., a scanning electron microscope.

In boids without pits the pores were distributed over the entire surface of scales containing receptors, including sections of the scale where there were no receptors. This is not surprising if we consider the scale in its entirety as the infrared receptor organ. Thus the specialization of the epidermis continues over the entire surface of the organ, in order to cut down unwanted reflections.

Significance of the Domes

The domed structure of the inner chamber epitheli-
Fig. 5 Surface structures on the wall of the inner chamber of the crotaline pit organ. A: A light micrograph of a semithin cross section through the pit organ stained with toluidine blue. Arrows point to the large domes, arrowheads to the small domes. PM, pit membrane; IC, inner chamber. Bar = 50 μm. B: A SEM image of the surface of the inner wall. The large and small domes can be clearly distinguished. Bar = 10 μm. C: B at higher magnification, showing the characteristic pores on the surface of both the large and small domes. Bar = 5 μm. D: A SEM image of the underside of the keratinized surface of the domes of B. The measurements of dome diameters were made on images such as this. Bar = 10 μm

um of crotaline snakes undoubtedly also functions to enhance the sharpness of infrared 'imaging', but in a slightly different way from the pore array. In crotalines, evolution has increased the sensitivity of infrared reception by an order or two of magnitude by drastically reducing the heat capacity of the receptor-bearing structures. That is, by segregating the receptors from the rest of the body and suspending them in an extremely thin membrane, it has become possible for a given nervous response to be elicited by a far smaller amount of radiant energy than is necessary to obtain the same response in boid pits where the receptors are in contact with the rest of the head tissues. This increase in sensitivity, however, carries with it a heavy cost. The pit membrane is now so thin that it will not absorb all the infrared radiation that impinges on it. A large amount penetrates through the membrane (3), with the danger that it will be
Table 1  Dimensions of the Surface Structures of Snake Infrared Receptor Organs

<table>
<thead>
<tr>
<th></th>
<th>Boids without pits</th>
<th>Boids with pits</th>
<th>Crotaline snakes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter</td>
<td>0.3 –0.5</td>
<td>0.1 –0.15</td>
<td>0.25–0.5/0.06–0.25</td>
</tr>
<tr>
<td>Depth</td>
<td>0.15–0.25</td>
<td>0.05–0.1</td>
<td>0.2 –0.25/no data</td>
</tr>
<tr>
<td>Interval</td>
<td>0.15–0.3</td>
<td>0.15–0.45</td>
<td>0.15–0.4/0.25–0.5</td>
</tr>
<tr>
<td>Density</td>
<td>3.34/μm²</td>
<td>5.45/μm²</td>
<td>3.66/1.97/μm²</td>
</tr>
<tr>
<td>Shape</td>
<td>crater-like</td>
<td>cylindrical</td>
<td>crater-like/crater-like</td>
</tr>
<tr>
<td><strong>Domes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>Diameter</td>
<td></td>
<td>15.0–20.0</td>
</tr>
<tr>
<td></td>
<td>Height</td>
<td></td>
<td>8.0–11.0</td>
</tr>
<tr>
<td></td>
<td>Density</td>
<td></td>
<td>3,160/μm²</td>
</tr>
<tr>
<td>Small</td>
<td>Diameter</td>
<td></td>
<td>2.0–6.0</td>
</tr>
<tr>
<td></td>
<td>Height</td>
<td></td>
<td>1.0–3.0</td>
</tr>
<tr>
<td></td>
<td>Density</td>
<td></td>
<td>13,400/μm²</td>
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All values in μm except where specified. Outer/inner = pit membrane outer surface/pit membrane inner surface

Fig. 7  Schematic summary of the function of the surface structures as typified in a crotaline snake infrared receptor organ. Infrared rays (IR) pass through the surface layer of the pit membrane and stimulate the receptors (R). Some of the IR rays pass entirely through the membrane, but are absorbed by the light trap formed by the large (ld) and small (sd) domes (enlarged view at bottom). Visual light (V) is largely reflected by the pores (p) at the surface of the pit membrane, but some passes through. This light is in turn diffused by the surface pores of the inner chamber domes. Any visual light that is reflected back to the pit membrane is further diffused by the pores on the inner surface of the membrane. The final result is an excellent signal-to-noise ratio for the infrared stimulus.

reflected back from the head tissues to re-stimulate the receptors, causing a large amount of amorphous, background ‘noise’.

To obviate this difficulty, the crotalines have evolved the domed wall of the inner chamber to serve as a trap for visual and infrared radiation, analogous to the ‘light trap’ (10) antireflective coating used on lens hoods and other such optical instruments. In other words, the combination of large and small domes forms a sort of maze in regard to impinging infrared rays. Once they enter the maze they bounce back and forth among the domes interminably but are unable to exit. This prevents an infrared stimulus which has excited a receptor from bouncing straight back from the opposite direction to cancel out the signal it has just generated. The tiny pores on the surface of the domes probably serve the same function as they do on the outer surface of the pit membrane. That is, they effectively disperse rays in the visual spectrum that have passed through the membrane, improving the signal-to-noise ratio. The same function can be attributed to the pores on the inner surface of the pit membrane. They most likely serve to further disperse any random reflections from the wall of the inner chamber, improving the signal-to-noise ratio even further.

As recorded in Results, the domed structure could be seen also inside the preocular pore and in the epithelium of the orbit. Since there is no clear demarcation between the epithelium of the inner
chamber and that of the preocular pore and the orbit, this does not seem strange: the epithelium of the pore and the orbit is simply a continuation of that of the pit. However, since neither the inside of the pore nor the orbit epithelium are exposed to infrared radiation, the domes of these two areas probably have no direct connection with the functioning of the pit. In fact, the irregularity of the domes in these extralimital areas, and the lack of small domes between them, strengthens the hypothesis that the regular array of large and small domes inside the inner chamber does indeed function as a light trap to heighten signal-to-noise ratio.

Conclusion

The physical configuration of the surfaces of snake infrared receptor organs serves to make the organs eminently suitable for their purpose. I.e., they are highly selective in regard to the portion of the electromagnetic spectrum that they have been evolved to ‘see’. Among the various forms of these organs, the pit organs of the crotaline snakes, with their combination of pores and domes, can be said to be the most effective infrared sensors known.

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REFERENCES


