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This project made excellent progress toward most of the goals outlined in the original proposal, as well as several new goals that arose since the inception of this project. Many of the technical aspects of the proposal, which were at first separate methods for analyzing distinct issues are now increasingly working together to answer questions that cannot be answered by a single technique alone. The biochemical investigation of infrared signal absorption and transduction is proceeding well. Our biochemical analysis of thei nfrared receptor terminals has revealed distinct biochemical differences between these cells and retinal photoreceptors. The results indicate that the IR receptors are not simply modified photoreceptors, one of the major hypotheses we originally set out to test. The data also point toward identified chemical components of the signal transduction pathway, and indicate that calcium, acting through calcium-binding proteins, is one of those components.

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Objectives:

The goals of this project are:

(1) elucidate the physiological and ultrastructural properties of infrared-sensitive neurons in the facial pits of pit vipers and boid snakes

(2) determine how these features change over the course of the development of infrared sensitivity

(3) identify and study the roles of candidate molecular and/or structural components involved in infrared detection.

(4) study the fine structure of infrared-sensitive terminals with respect to: (a) dynamic changes in the mitochondria of individual receptors, and (b) the static optical properties of surface structures of the organs

(5) determine the spectral sensitivity of the pit organ.

Status of Effort:

This project made excellent progress toward most of the goals outlined in the original proposal, as well as several new goals that arose since the inception of this project. Many of the technical aspects of the proposal, which were at first separate methods for analyzing distinct issues are now increasingly working together to answer questions that cannot be answered by a single technique alone. The biochemical investigation of infrared signal absorption and transduction is proceeding well. Our biochemical analysis of the infrared receptor terminals has revealed distinct biochemical differences between these cells and retinal photoreceptors. The results indicate that the IR receptors are not simply modified photoreceptors, one of the major hypotheses we originally set out to test. The data also point toward identified chemical components of the signal transduction pathway, and indicate that calcium, acting through calcium-binding proteins, is one of those components. We are now actively involved in using modern imaging techniques to directly test the functional roles of calcium, calcium-binding proteins, and mitochondria in IR signal transduction. Our electrophysiological system and stimulus source are now operational, and we are using this also as a tool in conjunction with pharmacological manipulations to investigate the functional biochemistry of IR receptors. In addition, both of these techniques, imaging and electrophysiology, are being used to further investigate the spectral sensitivity of the IR receptors.

Our structural analyses have been very productive. The ultrastructural analysis of IR receptor mitochondria has revealed that, contrary to a published report, IR receptor terminal mitochondria do not radically change morphology in response to physiological stimulation. These results not only help us understand function of the IR receptors, but also help us better design experiments using reasonable stimuli. Surface structural analysis has yielded unexpectedly promising new results. First, we now know that the surface structure of the pit organ and the other imaging organ (the eye) may function as a spectral filter or antireflective coating. These findings may help in the design of new artificial antireflective or spectral filter coatings, and also help us understand the spectral properties of the IR-sensitive pit organ as a whole. Furthermore, surface analyses have yielded important information about the frictional properties of the complicated surface structures found outside the pit organ. Again, these results may aid in the development of novel surface coatings with defined frictional properties.

This project trained 3 students, each on different aspects of the problem. These students each performied independent and original research which will ultimately lead to publications. The project has also yielded 6 published papers and chapters, 1 publication in press, 2 submitted for publication, 3 in preparation, and 5 presentations (with published abstracts) at major international scientific conferences.

Accomplishments/New Findings (University of Virginia):

Biochemistry

In a comparison of pit organ and retina, I localized opsins (the protein portion of the retinal photoreceptor photopigments) to photoreceptors of the retina. I used 5 different anti-opsin antisera and found that different classes of retinal photoreceptors react with different antisera (as expected). However, none of these anti-opsin antisera labeled the IR-sensitive terminals in the pit. This data shows that the IR receptor entity is very unlikely to be an opsin-like molecule. This answers a major question regarding the relationship between these two electromagnetic receptors, showing that they are biochemically very distinct, and that if IR sensitivity is photonic, the receptive molecule is probably unlike known retinal photopigments.

One photoreceptor antiserum, CERN-911, did react with IR-sensitive terminals. This antiserum is sometimes referred to as an "anti-opsin", but is more appropriately characterized as an "anti-phototransduction" antiserum. It was produced against a crude preparation of photoreceptor outer segment material, and recognizes multiple components of the phototransduction cascade. This antiserum was highly effective in labeling infrared receptor neuron terminals in the pit organ, and also labeled retinal photoreceptors. Given the broad specificity of CERN-911, and the lack of IR receptor labeling with opsin-specific antisera, the CERN-911 immunoreactivity likely represents one or more non-opsin components. It is unclear as yet whether any of the immunolabeled proteins are common to both IR receptors and photoreceptors.

While the pit IR terminals may contain no opsin, I have discovered other proteins in these terminals. One of these, phosducin, regulates transduction in photoreceptors, and analogous proteins have been discovered in other neuronal cells. This phosducin-like immunoreactivity likely represents a conserved protein functional in multiple classes of receptor signal transduction systems. Therefore, the discovery of this regulatory protein in the IR terminals identifies another potential component of the IR transduction apparatus. Further analysis of this protein and its functional significance should provide insight into the molecular mechanism underlying IR reception, as well as into the evolution of sensory receptors in general and radiant electromagnetic energy receptors in particular.

Assuming that calcium is probably involved in IR transduction at some level (it is involved in all other sensory systems), I applied a similar approach using antisera against calcium-binding proteins. I found several calcium-binding proteins in the retina (among them calretinin and calbindin), and others in IR receptors (calmodulin and S-100). Those found in photoreceptors are not found in IR receptors, and those in IR receptors do not exist in photoreceptors. This data, together with that showing the distribution of opsin immunoreactivity, shows that IR receptors are biochemically distinct from photoreceptors. From a functional point of view, the calcium-binding proteins found in IR receptors of the pit organ are calcium-regulated proteins. That is, binding of calcium to these proteins causes them to change their functional activity. These proteins therefore are likely components of the IR transduction cascade. They may function by transducing the IR signal into an electrochemical signal through which neurons communicate, or they may be involved in regulating the sensitivity of the IR receptors or their recovery after stimulation. The calcium-binding proteins found in the retinal photoreceptors are not calcium-regulated proteins, but rather calcium-sequestering proteins (that is, they actively regulate calcium concentration in the cellular cytoplasm).

We have also applied the antisera that labeled IR terminals to the trigeminal ganglia, the location of the soma of the IR receptors. The trigeminal ganglia contain other cell types as well, and there has never been an attempt to distinguish IR receptor cell bodies from those subserving other senses such as pressure and pain. One of our major new goals (that has arisen since the inception of this project) is to isolate and culture IR-sensitive neurons. Such a preparation will be invaluable for functional and biochemical analyses of the IR system. Our immunocytochemistry on the trigeminal ganglion represents (along with other new experiments; see below) the first attempt to characterize IR-receptive vs. -non-receptive cells in the trigeminal ganglia, and this is a prerequisite to producing a defined cell culture system for investigation of IR receptor function.

We have found that all of the calcium-binding proteins that label IR receptor terminals also label soma in the trigeminal ganglia. In addition, antisera that were not functional on the terminals label subpopulations of cells in the trigeminal ganglia. The different antisera label morphologically distinct classes of trigeminal neurons. We are now attempting to determine whether all of the cells labeled with S-100 and calmodulin are in fact IR receptors, or whether these antisera recognize multiple cell types. If they are exclusive to IR receptors, then calcium-binding protein content will provide a convenient and effective means of distinguishing IR receptors from other cell types in the ganglion. It is possible that IR receptor soma contain calcium-binding proteins not found in their terminals. This result would suggest that those CaBPs found in the terminals play a specific role in IR signal transduction, while those in the soma function for other purposes.

In order to more fully characterize and ultimately isolate the proteins identified as prospective components of the IR signal transduction pathway, we have begun separating proteins from pit organ, non-pit organ epidermal, retinal, and brain homogenates by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). We originally began our analysis of proteins by SDS-PAGE while looking at the phosphoprotein content of the pit organ in collaboration with USAF Research Labs scientists (primarily Drs. Morley Stone and Tom Cooper) at WL/MLPJ, Wright-Patterson AFB. We have now begun a specific analysis of ospin-like proteins, calcium-binding proteins, and the proteins labeled by CERN-911 and phosducin antisera. We have prepared homogenates from these tissues, separated them by SDS-PAGE, and are now performing Western blot analysis to determine relative molecular weights. These data will help us characterize these proteins and will help determine their relatedness to known signal transduction components, and their functional significance in the IR receptor cell. We have also set up for 2-dimensional SDS-PAGE in order to determine isoelectric points, and to help purify prospective proteinaceous transduction components.

Imaging Analysis: spectral sensitivity and functional biochemistry

We are involved in two types of imaging analysis, one of the pit organ as a whole with the goal of understanding spectral absorption, and the other of IR receptor terminals in microscopic preparation with the goal of understanding the functional biochemistry of IR signal transduction.

The first investigation, of IR absorption/reflectance, is essentially complete. We imaged pit organs and other surface areas of Python using two infrared-sensitive video cameras. One of these cameras was sensitive in the 3-5μm range, and the other in the 8-12μm range. We found that pit organs were significantly more absorptive and/or of lower emissivity than surrounding tissues with both cameras. Absorption appeared significantly higher and/or emissivity appeared lower in the 8-12μm range than in the 3-5μm range. These results support the hypothesis that pit organs are IR absorptive in two major atmospheric transmission windows. Further, the suggest that 8-12μm is an especially important region of absorption, and this waveband encompasses the peak emission of prey (37°C mammals and birds) normally targeted by these snakes. Data from this investigation is currently in press (Biosensors and Bioelectronics).

We are also investigating the functional biochemistry of infrared imaging by *Python*, using fluorescent markers of cell function. We are currently imaging thick slices of pit organ containing receptor terminals using confocal laser scanning microscopy and digital deconvolution analysis. These techniques allow us to produce high resolution time-resolved images of IR receptors loaded with fluorescent probes. We are currently investigating two aspects of the IR receptors: calcium transients and mitochondrial potential in response to IR stimulation. This work is ongoing, and we are currently building a base of experimental results to begin probing protein function pharmacologically, and are also incorporating our narrow bandwidth stimulus source into our experimental setup in order to produce spectral response curves for calcium and mitochondrial activation. This work is being done in the state-of-the-art Advanced Cellular Imaging Facility (ACIF) at the University of Virginia. I have been asked by the director of this facility to present our infrared work at an upcoming ACIF Imaging Workshop.

Neuroanatomy of IR Imaging:

We are in the middle of an intensive effort to map input from the pit organ to the trigeminal ganglion, and from there to the brainstem (lateral descending trigeminal nucleus). This pathway is the initial route of IR information input to the central nervous system, and the first potential level of IR information processing. This has never before been done. The goals of this investigation are: (1) to determine whether IR receptor soma are morphologically distinct from other trigeminal ganglion neurons, (2) to determine whether IR receptor soma are biochemically distinct from other trigeminal ganglion neurons, and (3) to determine whether there is spatial mapping in the trigeminal ganglion and lateral descending trigeminal nucleus. Ultimately, these data will form the foundations for understanding neural processing within and beyond the trigeminal ganglion (for example, are there interneurons in the trigeminal ganglion that process IR information before it reaches the brain?).

We have investigated several methods of analyzing the neuroanatomy of this system, and are currently performing neuronal tract tracing in two different ways. These experiments are being coupled with immunocytochemistry to determine conclusively the biochemical characteristics of identified IR receptor cells within the trigeminal ganglion. We are using two different tract tracers initially: one, a retrograde tracer to label from the pit organ to the trigeminal ganglion, and the other, a bi-directional tracer to label the entire IR sensory neuron. Our best results are coming from the bi-directional fluorescent tracer DiI. We have either applied DiI, a lipophylic fluorescent tracer, within the pit organ of anesthetized animals, and allowed them to recover for tracer transport, or to the cut ends of trigeminal nerves and optic nerve in fixed isolated brains with trigeminal ganglia intact. We have produced excellent tract tracing results and are now analyzing this material by confocal laser scanning microscopy. We are producing computer-based 3-dimensional maps of (1) pit organ input to the trigeminal ganglion, (2) trigeminal input to the brainstem, and (3) optic nerve input to the brain. These maps will tell us exactly where visible light and infrared information is sent in the initial stages of processing, and in conjunction with immunocytochemistry, electron microscopy, and electrophysiology, to determine the extent and nature of information processing in the trigeminal ganglion and brainstem. Ultimately, these analyses will address the fundamentally important issues of IR signal amplification and spatial mapping of the infrared environment.

Behavioral Analyses: Roles of IR System and Vision in Targeting:

We have undertaken an extensive behavioral analysis of infrared imaging in Python, with the goals of (1) determining whether *Python* is capable of accurately targeting prey in the absence of visual information, (2) determining whether *Python* is capable of accurate targeting in the absence of infrared information, (3) determining the relative importance of vision and infrared imaging, and (4) assessing the effects of thermal

environment on targeting ability. These experiments are rapidly yielding valuable information on all of these issues.

We have discovered that *Python* is capable of accurate and precise prey targeting in the complete absence of visible light input to the retina. This result was obtained by quantifying targeting ability in snakes either congenitally missing both eyes, or by occluding both eyes of normally-sighted snakes. We also found that vision is relatively unimportant for prey targeting in these snakes, a result that was completely unexpected. We hypothesized that snakes normally use both senses in targeting, but that either alone may suffice for prey capture. We tested snakes either congenitally lacking one eye or snakes with one eye experimentally occluded, and expected that snakes would preferentially target on the sighted side. However, monocular snakes were just as accurate and precise in targeting prey as were normally-sighted controls, and did not preferentially target on the sighted side (strike angle was not significantly different between left eye occluded, right eye occluded, and normally-sighted snakes).

Finally, we tested whether snakes would more accurately target prey in a visually high contrast environment vs. a low contrast one (we hypothesized that they would preferentially target, or more accurately target in a visually high contrast environment). We tested the ability of snakes to target C3H mice (albino strain) and C57Bl6/J mice (black pigmented) against a visibly black background. The thermal environment was held constant, and the emitted radiation measured the same from the two mouse strains. We found that there was no significant difference (in any of the 6 variables quantified) between the ability to target albino mice on a black background and black mice on a black background. Together, these results indicate that *Python* is capable of accurate targeting in the complete absence of visible light, and that infrared sensitivity is used preferentially over visible light imaging by the lateral eyes.

There is an important practical application of these data. The fact that

Ultrastructure of the IR-sensitive Terminals:

Our basic ultrastructural analysis of the IR-sensitive terminals is complete. We have performed a detailed characterization of their ultrastructure prior to stimulation and after stimulation. We find that the terminals are densely packed with mitochondria, and with electron-lucent vesicles. We do not see any significant changes in the morphology of mitochondria with stimulation. The numbers of vesicles may or may not change; we are in the process of quantifying the numbers of vesicles before and after stimulation, and also the distribution of the vesicles within the terminals. We find no evidence of vesicle fusion with either the plasma membrane or with the membranes of mitochondria. Therefore, we hypothesize that these vesicles may not serve and endo- or exocytotic function, but rather may function as sources/sinks for calcium during neuronal excitation.

We will use the findings from this set of analyses to design experiments to investigate the functional roles of proteins identified as potential IR signal transduction components. For example, we will perform immunocytochemistry at the electron microscopic level to determine the subcellular distribution of proteins localize to the IR receptor terminal.

Surface Structural Analyses:

Our transmission electron microscopic (TEM) studies also revealed the presence of numerous invaginations in its surface the epidermis overlying infrared-sensitive nerve terminals in the *Python* pit organ. These invaginations were of random widths and depths. Invaginations were observed only over the portion of the pit containing IR-sensitive terminals, and only in the outermost layer of keratinized epithelium.

The spectacle overlying the eye exhibited a surface structure similar to that of the pit organ. The surface of the spectacle was composed of an array of plates, each covered by micropits. Micropits on the spectacle appear less regular in arrangement than those in the pit organ.

Outside the pit organ, the outer surface of the epidermis appeared to be composed of parallel rows of parallel ridges that may be attached at their anterior ends, and free and angled upward at their posterior ends. All of these structures (from pit organ, spectacle, and non-pit/spectacle epidermis) were confirmed by scanning electron microscopy (SEM) and by scanning probe atomic force microscopy (AFM).

We have now completed a detailed investigation of the surface structural dimensions on all of these structures. This work was performed in collaboration with USAF Research Labs scientists (primarily Dr. Angela Campbell) at WL/MLPJ, Wright-Patterson AFB. We have found that there are significant differences between the dimensions of micropit structures and their associated plates in pit organ, spectacle, and interscale skin. We hypothesize that these differences play a role in spectral filtering of incedent electromagnetic radiation, and/or that they serve as an antireflective coating. This hypothesis is currently under investigation. The results of this investigation are the subject of two manuscripts, one being submitted to the Journal of Structural Biology, and the other to the Journal of Herpetology.

We are also investigation the frictional coefficients of different aspects of these unique surface structures, with the goal of understanding why such elaborate structures as the parallel rows of parallel ridges exist in *Python* epidermis. This line of investigation developed as a tangent to the infrared project, but is revealing useful information that merits mention here. We find that there is significant frictional anisotropy on a nanoscale level on the epidermal ridges. This frictional anisotropy may be a structural phenomenon, or may be a product of chemical distinct regions of the microfibril ridge. We are now using functionally modified AFM probe tips to analyze chemical composition at a nanoscale level. These results may help in the design of new structural surface coatings with unique frictional properties. This work has led to 2 publications, and had provided training for one graduate student.

Accomplishments/New Findings (Yokohama City University):

The receptors

Snake infrared receptors are discrete masses of free nerve endings called terminal nerve masses (TNMs). We used a combination of light microscopy and transmission and scanning electron microscopy to determine the location and morphology of the TNMs.

Location

In the Boidae the TNMs are located in the epidermis of the labial scales, either just at the surface without any specialized skin structures, as in Boa constrictor, or at the bottom of pits in the labial scales. We determined by transmission electron microscopy that in these snakes the TNMs are renewed with each shedding cycle, the old ones being replaced by new ones which form in the new epidermis.

In the Crotalinae, the TNMs are located in the dermis, not the epidermis, of a thin (10-15 micrometers) membrane suspended in a deep pit in the loreal region between the eye and the nostril. We determined by scanning and transmission electron microscopy that they are not affected by the shedding cycle. This indicates that Crotaline pits are much more efficacious than the pits of the Boidae, because the TNMs can remain active throughout the shedding cycle. The pit vipers can use the pit organs to see infrared images even when the snake is otherwise blinded during the opaque phase of the shedding cycle, because infrared rays can penetrate the very thin epithelium about to be shed. In the Boidae performance of the TNMs is probably significantly degraded during the last days of the shedding cycle due to blockage of infrared rays by the old, dying receptors.

Morphology

Transmission electron microscopy showed that the TNMs are fundamentally similar in both the Boidae and Crotalinae, being masses of free nerve endings densely packed with

mitochondria. We were able to distinguish clearly 3 states of the mitochondria: charged, discharged, and discharging. These 3 states were present simultaneously in all preparations. Since the pit receptors, in contrast to other sensory organs, are constantly producing nervous discharges, this observation of the simultaneous occurrence of the 3 states of the mitochondria confirms the hypothesis that the mitochondria are actively involved in producing the nervous discharges.

We also used scanning electron microscopy to obtain 3-dimensional views of the receptor morphology. We were able to do this by using potassium hydroxide to remove collagen and expose the nervous structures within the pit membrane. Previous descriptions were only plausible reconstructions from TEM micrographs. With our work we were able to view in 3 dimensions all structures previously reported from the pit membranes: a monolayer of terminal nerve masses formed from free nerve endings supported within by Schwann cell cytoplasm, unmyelinated and myelinated nerve fibers, a capillary bed, and vacuole cells

Surface morphology

We used light microscopy and scanning electron microscopy to view the surfaces of pit organ structures. In both boids and crotalines the outward-facing epithelium 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 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.

The microvasculature of the receptor-containing membrane

The infrared sensory membrane of crotaline pits and the fundus of boid pits have an extremely rich capillary vasculature, which has been noted passim in the literature, but never illustrated or studied in detail. Using pit vipers, we rendered the pit vasculature visible in various ways, namely, by microinjection of India in, by a combination of ink and succinate dehydrogenase staining, and by making resin casts for scanning electron microscope study. We also used transmission electron microscopy for identifying the types (arterioles, venules, capillaries) of blood vessels. Then we compared the pit vasculature with that of the retina and the dermis.

Good visualization of the vasculature was obtained with both ink and resin injection. Arterioles, venules, and capillaries could be distinguished with all methods used. The monolayer vasculature is denser in the pit membrane than in the retina or skin. Each loop of the network encloses a small number of infrared receptors so that all receptors are in contact with a capillary on at least one side. The forward-looking areas of the pit, the "fovea",so to speak, of the infrared retina, have a denser network than side-looking areas. Since infrared rays cause nerve impulses by raising the temperature of individual receptors, the capillary network functions not only as a supplier of energy but also as a cooling mechanism to reduce afterimages. Thus the denser network in the "foveal" areas causes these areas to be more sensitive and have better image resolution than the rest of the membrane.

Bloodflow measurements

We next attempted to determine the dynamics and mechanics of bloodflow in the crotaline pit membrane. The pit organ serves as an infrared retina, processing infrared information by the degree to which the temperature of the terminal nerve masses is raised. As stated above, the capillary network both supplies energy to the terminal nerve masses and serves as a heat exchange mechanism. This mechanism maintains the receptors at a stable temperature level to increase or decrease their sensitivity and to reduce to a minimum the afterimage effect of a moving stimulus. We used a Doppler laser bloodflow meter to measure the local changes in bloodflow in response to a general heat stimulus (a small soldering iron) and a point stimulus (visible and infrared lasers). Three main patterns were recorded with an extremely short lag time (5-15 msec): a rising curve, a falling curve, and a biphasic curve, depending on the relative positioning of the stimulus and recording probe, and on the relative strength and nature of the stimulus. Using histochemical and immunohistochemical staining, we were able to determine that the pit membrane contains autonomic and sensory C-fiber innervation in addition to the main sensory A-delta fibers. But surgical and chemical blocking of the C-fibers had no influence on the bloodflow changes. Therefore, on the basis of the rapid response time and the similarity of the bloodflow curves to electrophysiological recordings from the receptors, we surmised that all bloodflow changes were due to a vasomotor reaction, modulated by the terminal nerve masses directly, resulting in a change in local heat capacity that cools the stimulated receptors back to a basal temperature.

The role of capillary pericytes in local bloodflow control

Our Doppler bloodflow curves and direct observation with a high-power dissecting microscope showed that bloodflow changes were very discreet and very local. Therefore we focussed on the capillary pericytes as the possible mediators of local blood control. We studied their ultrastructure with scanning and transmission electron microscopy, and used immunohistochemical staining to search for two contractile proteins, smooth muscle alpha-actin and desmin, in their cytoplasm.

Morphologically, pit capillary pericytes have two major cytoplasmic processes: thickened primary processes that radiate to embrace the endothelial tube, and flattened secondary processes that are distributed diffusely on the endothelium. We observed coexpression of smooth muscle alpha-actin and desmin in the pericytes of entire capillary segments, and the smooth muscle alpha-actin was characterized by prominent filament bundles directed mainly at right angles to the capillary long axis. In contrast, in capillary pericytes of the snake scales, which are not involved in infrared imaging, smooth muscle alpha-actin in the cytoplasm is very diffuse. Again, in a series of electron microscopic sections, we often observed the primary processes depressing the endothelial wall. We concluded, on the basis of all the work summarized above, that pit capillary pericytes, in response to some signal from the terminal nerve masses, regulate capillary bloodflow through their contractile and relaxing activity. The next task in this line of investigation will be to determine how the terminal nerve masses send signals to the capillary pericytes.

Summary

The results described in this report are completely new findings on a novel and essentially unexplored sensory system. The infrared imaging capabilities of boid and crotalid snakes have many significant advantages over other natural IR sensors, and over current artificial infrared detection devices. The work described herein was approached from six standpoints: (1) identification of structural features of the IR sensing organ that contribute to its optical properties, (2) investigation of the IR transmission and absorption properties of the organ, (3) biochemistry of the transduction process, (4) behavioral correlates of infrared imaging, (5) neuroanatomy of infrared imaging, and (6) role of

microvasculature in regulating infrared sensor function. The surface structure of the pit organ contributes to the transmissive, absorptive and reflective properties of the pit organ. IR spectrometric and imaging studies support this conclusion, and indicate that both atmospheric IR transmission windows (3-5μm and 8-12μm) are probably particularly important to IR sensitivity. The results of the IR spectrometric and imaging studies, together with biochemical analyses suggest that 10μm radiation may be particularly important. We hypothesize that protein phosphorylation and calcium regulation are important components of infrared reception, and that calcium exerts its effects through calcium-regulated calcium-binding proteins. This, together with our protein phosphorylation analysis, provides an inroad to dissecting the entire transduction process. Ultimately, this will lead to understanding of both the infrared receptive molecule, and the signal amplification process, both fundamentally important components in the development of biomimetic infrared sensors. Our neuroanatomical studies will provide the basis for understanding how IR information is processed, ultimately leading to a highly sensitive infrared mapping system. Finally, our behavioral analyses show that infrared imaging by these snakes may be the most important spatial mapping sense, surpassing vision in importance for prey targeting. Behavioral experiments are also helping to elucidate the thermal contrast sensitivity of this system. The results of these investigations continue to be very productive, and provide the foundation for ongoing collaborative studies between myself and researchers at Wright Laboratories, with Systems Research Laboratories, and now with biomedical engineers at the University of Texas. This last collaboration is specifically aimed at the engineering novel infrared sensors and imagers.

Relevance to AF mission; Potential technological applications:

Artificial infrared image-forming devices have tremendous practical applications in the military, in medicine, and in industry. While artificial infrared (IR)-sensing technology has improved dramatically in recent years, there are many reasons to seek even greater improvements in this technology. Highly sensitive image-forming IR detectors are bulky, complicated (often necessitating frequent repair or modification), and costly to maintain. In order to function with reasonable sensitivity they often must be super-cooled, adding to the their bulk, cost, and potential failure. Boid and crotalid snakes, on the other hand, "see" spatial images of their thermal environments using infrared detectors that are microscopic in dimensions, self-repairing, and that function at normal ambient temperatures. Perhaps most importantly, snake infrared detectors are at least an order of magnitude more sensitive than the best artificial ones. In addition, snake infrared detectors are necessarily different from all artificial IR detectors in both mechanism and construction. An analysis of the extremely sensitive snake image-forming IR detector should provide insight for the development of new technologies that will result in greater sensitivity, operation at higher temperatures, and reduced in size and weight.

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Publications:

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Interactions/Transitions:

a. Participation at meetings, conferences, seminars, etc.:

(1) Society for Neuroscience
(2) Japanese Society of Neuroscience
(3) Defense Advanced Research Projects Agency workshop on *Tissue Based Biosensors*, George Washington University, Reston, Virginia (presentation, participation at meeting)
(4) Department of Biology, Old Dominion University, Norfolk, VA (seminar)
(5) University of Washington, Dept. of Zoology, Seattle, Washington (seminar)
(6) University of South Florida School of Medicine, Tampa, Florida (seminar)
(7) Materials Lab WUD (Work Unit Directive), Wright-Patterson AFB, talk: *Mechanisms of Infrared Detection by Snakes*,
(8) Wright Laboratories Education Outreach (summer high school teacher apprentice program) seminar
(9) Research Seminar, Wright-Patterson AFB (base-wide and off-base

invitation)
(10) University of Florida, Florida Museum of Natural History, Gainesville, Florida (presentation, participation at meeting)
(11) Presentation: NSF Science and Technology Center for Biological Timing, University of Virginia, Charlottesville, Virginia
(12) Association for Research in Vision and Ophthalmology (annual international conference), Ft. Lauderdale, Florida (participation at meeting)
(13) 5th World Congress on Biosensors and Bioelectronics, Berlin, Germany
(14) Japanese Association of Anatomists
(15) XVth Federative International Congress of Anatomy
(16) Herpetological Society of Japan
(17) 6th Asia-Pacific Congress of Electron Microscopy, Hong Kong
(18) Workshop on infrared reception in nature and its practical application, Yokohama, 1996
(19) Third World Congress of Herpetology, Prague

b. Consultative and advisory functions to other laboratories and agencies:
(1) Strategy and planning session with MLPJ staff at University of Virginia (October, 1997)
(2) Ongoing collaboration with scientists from WL/MLPJ, Wright-Patterson AFB
(2) Development of large-scale interactive collaboration: FY1998 AFRL MURI project based at University of Taxas, Austin

c. Transitions:

New discoveries, inventions, or patent disclosures:

Honors/Awards:

Michael S. Grace
signature, Principal Investigator

6/13/00
***Revised** Date*