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13. ABSTRACT (Maximum 200 words) Aim and scope of the project was the investigation of infrared (IR) sensory systems in insects. It was intended to learn more about the principles and mechanisms of uncooled biological IR receptors. Investigations were performed on: (i) two species of "pyrophilous" jewel beetles (Buprestidae), which show the behaviour to fly to forest fires. (ii) Certain bloodsucking bugs (so called Chagas bugs, Reduviidae), which approach their warm blooded hosts at night. The IR sensilla in the metathoracic IR-pit organs of <i>Melanophila</i> -beetles were investigated physiologically and with respect to their ultrastructure and material properties. Results corroborated the so called "photomechanic model" of IR reception which was suggested in the literature. In the Australian "fire-beetle" <i>Merimna atrata</i> abdominal IR receptors were discovered which are different from the <i>Melanophila</i> -receptors and function according to a bolometer system. Thermal cues play an important role in host finding behaviour of bloodsucking bugs. However, there was only weak evidence that bugs can perceive IR radiation. In behavioural experiments it was found that Chagas bugs use IR radiation to approach a thermal source and that possible IR receptors most probably are located under the abdominal cuticle. In a biomimetical approach results were utilized to build a prototype of a technical photomechanic IR detector.				
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Final Report

Introduction:

Aim and scope of the project was the investigation of infrared (IR) sensory systems in insects. It was intended to learn more about the principles and mechanisms of uncooled biological IR reception. Investigations were performed on:

- “pyrophilous” jewel beetles (Buprestidae), which show the behaviour to fly to forest fires. It was known that buprestid beetles of the genus *Melanophila* have unique metathoracic IR pit organs. Nothing was known about possible IR receptors in the “fire-beetle” *Merimna atrata* which approaches forest fires in Australia.
- certain bloodsucking bugs (Reduviidae), which approach their warm blooded hosts at night. Thermal cues play an important role in host finding behaviour. However, there was only weak evidence that bugs can perceive IR radiation.

In a biomimetical approach results were utilized to build a prototype of a technical photo-mechanic IR detector.

People involved in this research:

Mr. Stefan Trenner : PhD student. Mr. Trenner has investigated the bloodsucking bugs. His Phd thesis will be finished within the next couple of months.

MURI at the University of Texas at Austin. The work was integrated into a MURI on infrared reception in animals (F49620-98-1-0480). The MURI is coordinated by Prof. Dr. A J. Welch (Biomedical Engineering, ENS 639, Austin, TX 78712)

During the term of the grant 10 papers were published in peer-reviewed journals (Appendix I). In 6 cases the PI Helmut Schmitz was the first or the last author.

I. The thoracic infared organs of pyrophilous beetles of the genus *Melanophila*

Physiology of the photomechanic IR sensilla [see papers 4, 5, 7 in Appendix I]
Responses of single photomechanic IR sensilla housed in the metathoracic pit organs of *Melanophila acuminata* were recorded in various electrophysiological experiments.

Material and Methods:

For the experiments male and female *Melanophila acuminata* (Coleoptera, Buprestidae) were used. For electrophysiological recordings the beetles were fastened upside down to an experimental platform. To make the pit organs accessible, all legs were removed. Preparations were tested at room temperature (20 – 24 ° C). Neuronal activity was recorded with electrolytically sharpened tungsten electrodes (tip diameter < 1 µm). A silver wire, inserted into the abdomen of the beetle, served as a reference electrode. The tip of the recording electrode was carefully inserted into the bottom of the pit organ with a micromanipulator. Penetration of the cuticle was also monitored with aid of an binocular microscope (magnification 80x). Neural activity was AC amplified (DAM 80, WPI; action potentials 300 - 3000 Hz), displayed on an oscilloscope (Hameg digital scope HM 205-3) and stored on a digital tape recorder (Biologic DTR 1200). For off-line analysis spikes were AD converted.

Signals were fed to a data acquisition system (GW Instruments, MacAdios II; Power Macintosh, Sampling rate 10 kHz) and further processed with the software SuperScope and Igor.

To stimulate the IR sensilla broadband as well as monochromatic radiation emitted by an IR laser was used.

Results:

Table I summarizes important results obtained in the experiments with broadband and monochromatic IR stimulation. Because an action potential (AP) is a well defined physiological event, the generation and number of action potentials were used as monitor for the responses of the strictly phasic photomechanic IR sensilla.

Parameter examined	<u>Action potentials [AP's]</u>
	Generation and number
Threshold (Sensitivity)	5 mW/cm ² (broadband) 14,7 mW/cm ² (at 3,4 μm)
Latencies	5 – 9 ms at 15 mW/cm ² 2 – 3 ms at 100 mW/cm ² < 2 ms at 270 mW/cm ²
Saturation	At about. 250 mW/cm ² (8 - 9 AP's evoked)
Dynamic range	14,7 – 250 mW/cm ² = 12 dB
Dependence of receptor response on wavelength	Between 2,8 – 3,5 μm no dependence on wavelength detectable at 5 μm number of AP's reduced by 20 %; latencies increased by 55 %
Repetitive Stimulation (chopperwheel)	AP's up to about 100 Hz at each ON-event within the dynamic range (broadband)

TABLE I

A theoretical calculation based on the results yielded the result that a *Melanophila* beetle should be able to detect a fire of 320 x 320 m extension from a distance of about 12 km (7.5 miles).

Neuroanatomical studies [see paper 1 in Appendix I]

A first approach to investigate the neuronal network which processes the input from the IR sensilla within the central nervous system was done by tracing of the primary afferences of the IR sensilla in the central nervous system (CNS) of the beetle.

Methods

For staining of the sensory axons and their terminals in the CNS, beetles were first immobilized ventral side up and their middle legs fastened by using warm resin so as to obtain access to the IR pit organs at the mesothoracic coxae. Some of the globular IR sensilla in the pit organ were scraped off by using a broken glass capillary. Immediately afterwards,

the pit was filled with a tiny droplet of fluorescent tracer (Lucifer yellow or the dextran tracer, Fluoro Ruby; Molecular Probes) dissolved in water. Alternatively, a tiny crystal of the dye was placed on the damaged sensilla and covered with a water droplet. The entire organ was then sealed with silicone (medium viscosity; Bayer) to prevent it from drying out.

The animals were then left at ambient room conditions, for different times of 16 – 60 h. Afterwards, the animals were killed, and their thoraces and heads were processed according to standard routines, viz., paraformaldehyde fixation, ethanol dehydration, plastic embedding (Fluka Durcupan), horizontal or sagittal sectioning at 15-25 μm , and epifluorescence micrography. Graphical reconstructions were made either from color slides (Kodak Elitechrom 400) or from monochrome videographs by means of a digital storage camera (Kappa CF 8/1).

Results

The axons of the IR sensilla primarily terminate in the central neuropil of the fused second thoracic ganglia where they establish putative contacts with ascending interneurons. Only a few collaterals appear to be involved in local (uniganglionic) circuits. About half of the neurons send their axons further anterior to the prothoracic ganglion. A subset of these ascend to the subesophageal ganglion, and about 10% project to the brain. The arborization patterns of the IR afferents suggests that IR information is processed and integrated upstream from the thoracic ganglia. The small diameter of the IR afferents allows the beetle to be equipped with many parallel channels, thus probably increasing the spatial resolution, without investing much metabolic energy in the construction and maintenance of the neuronal evaluation circuitry. Rather than building a fast dedicated pathway or labeled line system, IR information seems to be channelled into the “normal” ascending-descending pathways. Descending neurones are well equipped for the integration of different stimulus modalities and for the control of flight manoeuvres.

Function of the photomechanic sensillum [see papers 4, 5, 7 - 9 in Appendix II]

When summarizing all results about morphology, physiology and material composition of the photomechanic sensilla the following model about the function of the *Melanophila* sensillum could be established:

IR radiation is absorbed by the cuticular apparatus of the IR sensilla at the bottom of the pit organs. Absorption capacity highly depends on the material composition of the receiver. When frequencies of vibration of a chemical bonding between two atoms and the incoming radiation are identical, absorption will be maximal. It is known that the mid-IR region between 1 μm and 10 μm is the spectral region where most organic molecules show vibrational absorption bands. The cuticular spherules of the *Melanophila* sensilla like insect cuticle in general consist of a protein matrix in which long-chained chitin (N-acetylglucosamines) molecules are embedded, all having many C-H, N-H and O-H groups. Molecules with these atomic groups swing with a frequency of about 100 THz and therefore have stretch resonances at wavelengths in the range of 3 μm which corresponds to the emission maximum of a forest fire. Molecules which have stretch resonances in the IR region convert the vibrational energy within fractions of a millisecond into translational energy, i.e. heat, by non-radiative de-excitation processes. Any heating must cause a change in spherule volume which finally must induce a deformation of the dendritic tip of the mechanosensory cell innervating each sensillum. We also speculate that the spherule is designed to exert a maximal impact on the dendritic tip. This principle of converting IR radiation into a micromechanical event which is measured by a mechanoreceptor was called photomechanic. This principle was also used for the first time to build a technical IR detector (see below).

II. The abdominal infrared organs of pyrophilous beetles of the genus *Merimna* [see papers 6 and 10 in Appendix I]

During the term of the grant a hitherto unknown IR receptor was discovered in the Australian buprestid "fire-beetle" *Merimna atrata*. It was found that *Merimna* has two pairs of abdominal IR organs which are totally different from the thoracic IR pit organs of *Melanophila* beetles (see Overview in Appendix II). This was an unexpected finding, because both buprestid beetles obviously use their IR receptors for the same purposes.

Methods

Adult *Merimna atrata* were collected in Western Australia. Beetles were kept alive in plastic containers for some time and finally fixed for light microscopical or for scanning and transmission electron microscopical (SEM, TEM) examinations.

For SEM-examinations beetles were either fixed in 70% ethanol or in 4% paraformaldehyde. Fixed beetles were cleaned in a sonicator for 2 minutes and air dried. The first four abdominal sternites were excised and mounted with carbon glue (Leit-C) on holders, sputtered with gold and examined with a Hitachi SEM 2610-N.

For light- and TEM examinations pieces of cuticle bearing the IR organs excised from the second and third abdominal sternites were immersed in iced 0.05 M cacodylate buffer with 3 % glutaraldehyde (pH 7.2, osmolarity 380-400 mOsmol l⁻¹) and fixed for 2h at 4°C. Sections were taken from five organs originating from three different beetles (one male and two females). After 2 h postfixation with 1.5% OsO₄ in the same buffer and rinsing in buffer solution, the specimens were dehydrated in ascending series of ethanol and embedded in Epon 812. Semithin and ultrathin sections were made with a Reichert Ultracut Microtome using glass- or diamond knives. The semithin sections were stained with a 0.05 % toluidin-blue/borax solution and examined with a Leitz DM RBE light microscope. The ultrathin sections were stained with uranyl acetate and lead citrate and examined using a Zeiss EM 109 transmission electron microscope.

Electrophysiological recordings from the multipolar neuron were made extracellularly with an electrolytically sharpened tungsten electrode. The electrode was inserted into the cuticle of the absorbing area of the receptor with a micromanipulator until an electrical contact to the hemolymph near the neuron was made. The airsac obviously shields the site of the neuron from electrical noise generated by neighboring units (especially moto activity); therefore, recordings with a good signal to noise ratio were routinely obtained.

Results

Two pairs of infrared (IR) organs are situated ventrolaterally on the second and third abdominal sternite of *Merimna atrata*. In ventral view each IR organ has a round IR absorbing area under which a sensory complex is attached to the hypodermis. The main component of the complex is a single large multipolar neuron and its mass of highly branched dendrites. All parts of this neuron are enveloped in glial cells. The proximal primary dendrites, which arise from the soma, finally branch into several hundred tightly packed terminal dendrites, which contain many mitochondria. We term this unusual morphology of the dendritic region a terminal dendritic mass (TDM). A TDM could be described for the first time in insects. Additionally, two chordotonal organs were found in each sensory complex. Their somata are integrated in the complex and the dendrites extend to the periphery of the absorbing area.

The electrophysiological recordings showed that the neurones were spontaneously active at room temperature (20 ° C) firing with frequencies of 7 – 10 Hz. The frequency of this spontaneous activity was unaffected by sound (voice or hand clapping), moderate air movements or gentle touch of the surrounding cuticle by a bristle. In contrast to this, frequency was strongly affected by changes in temperature. We heated the absorbing area by broadband IR radiation, warm air, and also visible radiation emitted from a light bulb and a red-light helium-neon laser (30 mW, $\lambda = 660$ nm). Independent of how the rise in temperature was achieved, any temperature increase of the receptor cuticle increased spike frequency in a phasic – tonic way. In order to measure the temperature of the cuticle of the IR organ the second sternite was excised from a freshly killed beetle. A small thermocouple (Type K: Chromega/Alomega, wire diameter: 13 μ m) was attached with a minimal amount of insect glue on the inner surface of the absorbing area of one IR receptor. Care was taken to place the sphere of the thermocouple (diameter about 30 μ m) in the center of the absorbing area where the neurone is situated in a living beetle. Neutral density filters and/or two polarizing foils were used to adjust different stimulus intensities. At each intensity, we measured the maximum excess temperature of the cuticle caused by the laser irradiation. A camera shutter allowed a defined exposure of the absorbing area. Because the metallic sphere of the thermocouple increased the thermal mass of the cuticle and because the digital thermometer (HH202, Newport Omega) needed two seconds for signal integration, the real-time increase of the temperature immediately after shutter opening could not be measured. When the shutter opened and the temperature of the cuticle rapidly increased, a sharp phasic increase in spike frequency occurred after a latency of about 20 ms. After the initial high peak frequencies were exceeded, the frequencies went back to lower values. In general, a higher steady state temperature was coded by a higher frequency. Especially when high stimulus intensities were applied, a gradual decrease in firing (adaptation) during the whole exposure time was observed. As the receptor may serve for the detection of high surface temperatures from short distances, we also tested high intensities which heated the cuticle to nearly 50 °C. Even when repeatedly stimulated with such high intensities, no deterioration of receptor performance took place. Responses could be recorded down to a temperature increase of 0.7 ° C. Cessation of the stimulus inhibited the generation of action potentials depending on the magnitude of the temperature drop. Cooling from high excess temperatures suppressed the generation of spikes for more than a second.

The *bauplan* of the dendritic region of the multipolar thermoreceptor is reminiscent of the thermosensitive trigeminal nerve fibres innervating the absorbing structures in the IR receptors in boid and crotalid snakes. The terminal nerve masses (TNM's) of the trigeminal nerve fibres are characterized by large number of mitochondria which was also found in the TDM in *Merimna*. Because this multipolar neuron also functions as a thermoreceptor, another example of a functional analogy between insect and vertebrate sensory systems could be demonstrated. In *Merimna* as well as in IR sensitive snakes the IR receptors work as microbolometers.

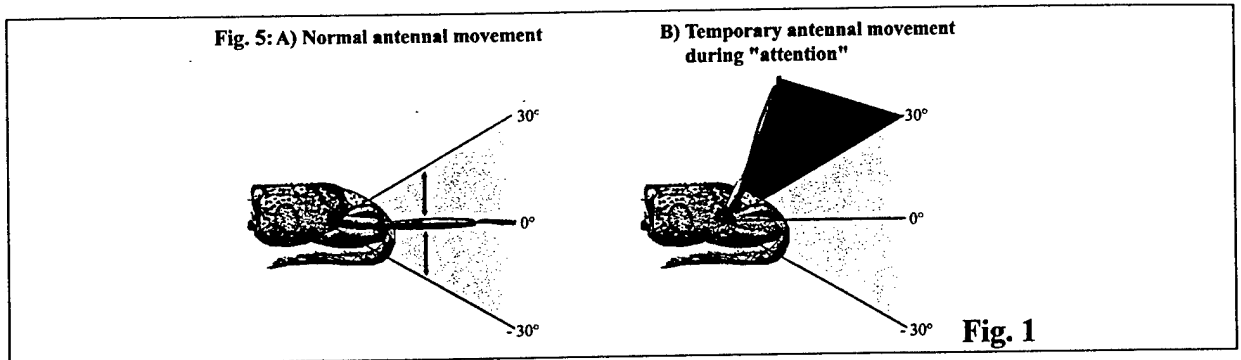
III. Infrared sensitivity of bloodsucking bugs

[see paper 3 in Appendix I]

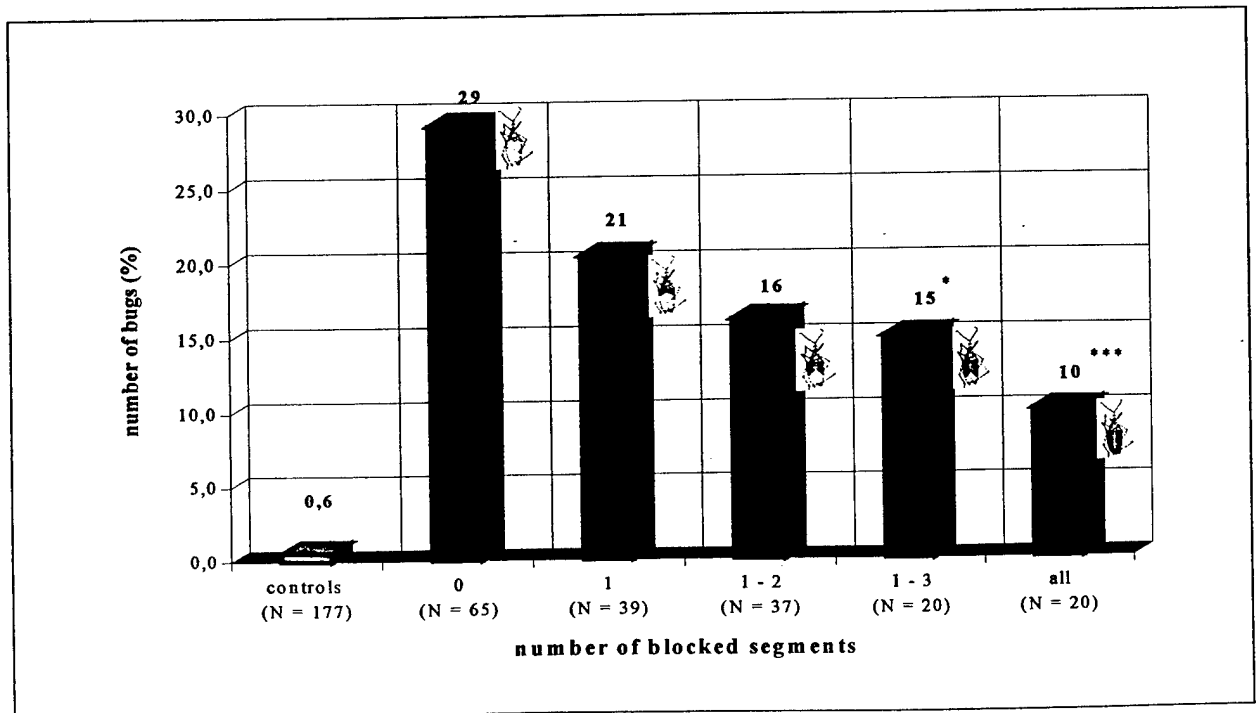
Bloodsucking Chagas bugs like *Rhodnius prolixus* and *Triatoma infestans* respond to infrared radiation, but nothing was known about IR receptors. To find the IR receptor we (A) designed a new setup for behavioural experiments and (B) investigated the innervation pattern of the abdomen of adult *Triatoma infestans* to find possible IR sensitive structures.

A. Behavioural experiments

During normal walking, the bugs move their antennae in an angle of $\pm 30^\circ$ around their heads. Confronted with a sudden IR stimulus their walking is stopped abruptly. Simultaneously they lift their antennae $> 30^\circ$. We call this behaviour a "combined behavioural pattern" (CBP; Fig. 1 A, B).



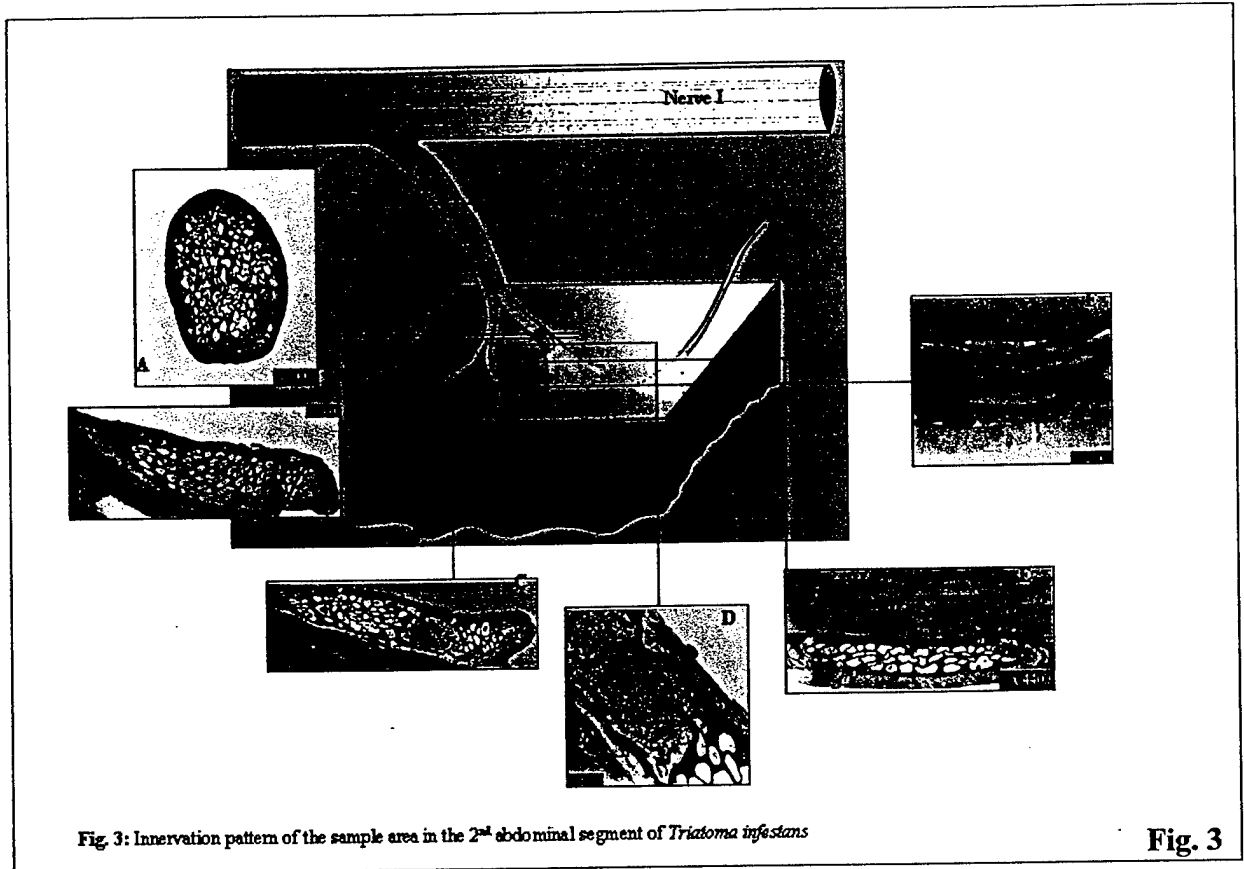
When an IR stimulus was applied for 1 second to unforced animals, 29% of 65 tested animals showed a CBP (Fig. 2). In the controls only one bug showed this behaviour ($p < 0.001$). In blocking experiments we covered single abdominal segments of the bugs with water soluble metallic varnish. The number of CBP's decreased (29 to 10%) when single abdominal segments were blocked. Compared with unforced animals, the decrease in the behavioural pattern was significant when at least three segments were blocked (Fig. 2).



B. Morphological studies

To find potential IR receptors, we examined the abdominal innervation of 12 adult *Triatoma infestans*. With the stereo microscope a sample area was found in the middle of the second segment. This area was dissected and fixed for further investigations. The fine structure was evaluated on combined serial semithin and ultrathin sections using the light and electron microscope, respectively.

The observed nerve (nerve I in Fig.3) branches for several times. One branchlet innervates more proximal parts of the abdomen, another one innervates the pleurite near the stigma whereas the most thin part of it can constantly be found in the middle of the segment (Fig. 3 A and B). Some hundred microns after the furcation, a soma of a of a multipolar neuron (about 15 μ m in diameter) was found inside the branchlet. Often the somata can be seen immediately beneath the cuticle (Fig. 3 C and D). Axons and dendrites build a bundle of 40 – 50 profiles which runs within the epidermis under the cuticle (Fig. 3 E). After 50-100 μ m the dendrites immediately terminate (Fig. 3 F). More than 20 bundles can be found in the second abdominal segment of adult *Triatoma infestans*.



It is well known that multipolar neurons act as thermoreceptors in insects (Schmitz et al. 2000, 2001). Our hypothesis is that there is a meshwork of multipolar neurons all over the abdomen of *Triatoma infestans* which is sensitive to IR radiation.

IV. Building of a technical photomechanic IR detector [see Appendix III]

The *Melanophila*-IR sensillum consists of an solid IR absorbing structure (mainly a little cuticular sphere) and a mechanoreceptor which measures the micomechanical deformation (expansion) of the sphere due to absorption (see Chapter I: Function of the photomechanic sensillum). In a biomimetic approach, a technical IR detector was established which works according to this unique biological transduction mechanism. Construction of the sensor mainly served for two aims:

- to demonstrate that it is possible to detect IR radiation with a detector which works according to the photomechanic *Melanophila* sensillum.
- to demonstrate that a photomechanic IR detector can be simple and cheap, and can be operated at room temperature.

Material and Methods

Two prototypes of detectors were build: a simple basic type (see Appendix III, A) and an improved version (see Appendix III, B). For the detector element, Small Teflon®-plates were used as IR absorbers. Teflon® has many C-F and C-C bondings which show vibrational resonances at 9 and at 11 μm respectively. This most probably results in a broad peak of absorption in the mid IR around 10 μm . Therefore the sensor can be used to detect IR radiation emitted from warm-blooded man or animal (surface temperatures at about 37 ° C) against a cooler background. Expansion of the plates was measured with a little Piezo-transducer which was driven against one side of the Teflon plate by a micrometer- screw. The Piezoelement is a sensitive electro-mechanical transducer made from bi-morph ceramics which changes mechanical enery into electrical energy. The Piezoelement was permanently held under a minimal pretension so that any additional expansion of the absorbing plates could be detected. The voltage output of the element was fed into an ampifier and visualized on an oszilloskope.

Results

Because Teflon absorbs IR radiation best at about 10 μm , a human hand was taken as IR source for demonstratation purposes. Depending on signal amplification, it was possible to detect a hand from a distance up to 2 m. In the example shown in Appendix III C, a hand was detected with a good signal to noise ration from a distance of 30 cm. This corresponde to a radiation intensity at the detector of about 100 $\mu\text{W} / \text{cm}^2$.

With a relatively small expenditure it was possible to build a technical IR detector. It is expected that it will be possible to build highly sensitive photomechanic IR sensors when thermal masses are considerably reduced (miniaturization) and highly sensitive mechanosensory devices are used.

V: Investigating smoke sensitivity in pyrophilous beetles [see paper 2 in Appendix I]

In a cooperation with Dr. Stefan Schütz (formerly University at Giessen; now University at Göttingen), sensitivy to smoke was tested for the first time in *Melanophila acuminata*. Like in IR sensitive snakes, which use infrared, visual and olfactory cues for the detecion of warm blooded animals at night, it was postulated that the beetles use not only IR radiation but also olfactory cues for the detection of a forest fire.

Materials and Methods:

Isolated antennae of *Melanophila*-beetles were connected to a gas chromatograph equipped with parallel flame ionization and electroantennographic detectors. Volatiles generated by smouldering splint wood from *Pinus sylvestris* were collected on a charcoal trap, chemodesorbed by an organic solvent, and injected into the apparatus.

Results

The resulting components indicated that several components of these volatiles were biologically active. Most of the volatile perceived by the antennae are phenolic compounds, derivatives of 2-methoxyphenol (guaiacol) elicited the greatest response. Methoxylated phenols are released by the incomplete combustion of lignin and have also been identified as atmospheric markers for wood smoke. Because it is particularly sensitive to guaiacol derivatives, *Melanophila* beetles can detect remote fires and might even be able to use the pattern of volatiles to identify the species of tree. The beetle's antennae can detect these guaiacol derivatives at concentrations as low as a few parts per billion (ppb).

APPENDIX I

Papers published in peer-reviewed journals under the grant

- 1.) W. Gronenberg and **H. Schmitz** (1999) Afferent projections of infrared sensilla in the beetle *Melanophila acuminata* (Coleoptera: Buprestidae). *Cell Tissue Res.* **297**: 311-318
- 2.) S. Schütz, B. Weissbecker, H.E. Hummel, K.-H. Apel, **H. Schmitz** and H. Bleckmann (1999) Insect antennae as a smoke detector. *Nature* **398**: 298-299
- 3.) **H. Schmitz**, S. Trenner, M. H. Hofmann and H. Bleckmann (2000) The ability of *Rhodnius prolixus* (Hemiptera: Reduviidae) to approach a thermal source solely by its infrared radiation.. *J. Insect Physiol* **46**: 745-751
- 4.) **H. Schmitz**, M. Mürtz, and H. Bleckmann (2000) Responses of the infrared sensilla of *Melanophila acuminata* (Coleoptera: Buprestidae) to monochromatic IR-stimulation. *J. Comp. Physiol. A* **186**: 543-549
- 5.) **H. Schmitz** and S. Schütz (2000) Waldbrandortung durch *Melanophila acuminata*: Die spezialisierten Sinnesorgane des „Feuerkäfers“. *BIUZ 5/2000*: 266-273 [in German]
- 6.) **H. Schmitz**, A. Schmitz, and H. Bleckmann (2000) A new type of infrared organ in the Australian „fire-beetle“ *Merimna atrata* (Coleoptera, Buprestidae). *Naturwissenschaften* **87**: 542-545.
- 7.) D. X. Hammer, **H. Schmitz**, A. Schmitz, H. G. Rylander and A. J. Welch (2001) Sensitivity threshold and response characteristics of infrared perception in the beetle *Melanophila acuminata* (Coleoptera: Buprestidae). *Comp. Biochem. Physiol. A* **128**: 805-819
- 8.) J. Hazel, N. Fuchigami, V. Gorbunov, **H. Schmitz**, M. Stone, V. Tsukruk (2001) Ultra-microstructure and microthermomechanics of biological IR detectors: material properties from biomimetic prospective. *Biomacromolecules* **2**: 304-312
- 9.) L. A. Sowards, **H. Schmitz**, D.W. Tomlin, R.R. Naik, M.O. Stone (2001) Characterization of beetle *Melanophila acuminata* (Coleoptera: Buprestidae) infrared pit organs by high-performance liquid chromatography/mass spectrometry, scanning electron microscope, and Fourier transform-infrared spectroscopy. *Ann. Entomol. Soc. Am.* **94**: 686-694
- 10.) **H. Schmitz**, A. Schmitz, and H. Bleckmann (2001) Morphology of a thermosensitive multipolar neuron in the infrared organ of *Merimna atrata* (Coleoptera, Buprestidae). *Arthropod Struct. & Develop.* **30**: 99-111

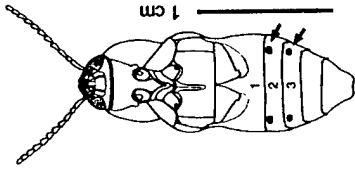
Appendix II

Overview: Infrared receptors in pyrophilous buprestid beetles

Beetle:

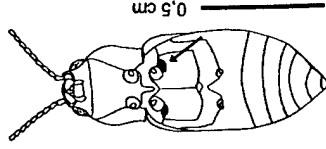
ventral view, legs
not shown

IR-organs in red




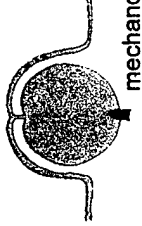
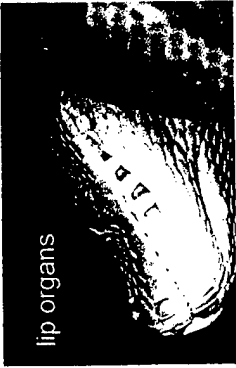
Merimna atrata

(Jewel beetle, Buprestidae)

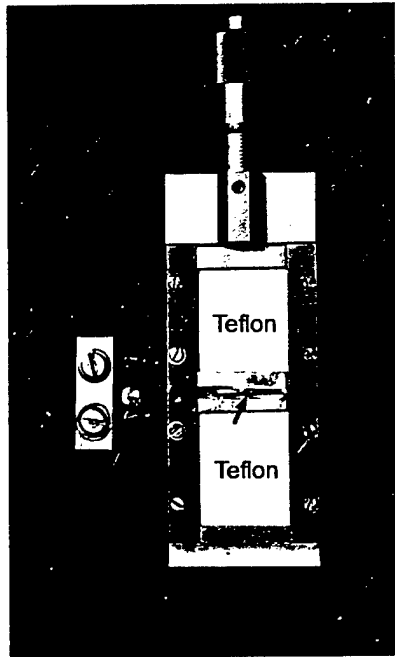


Melanophila acuminata

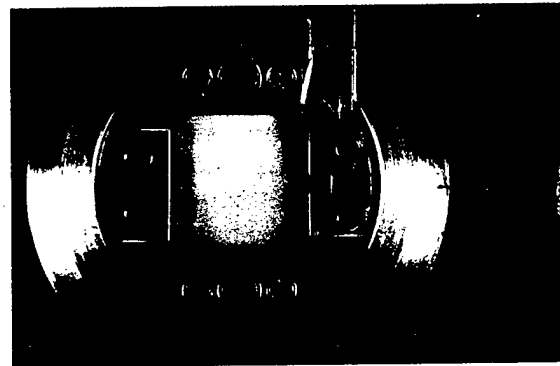
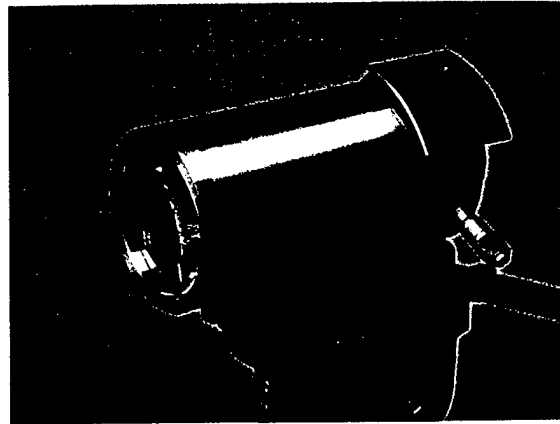
(Jewel beetle, Buprestidae)

Location	Second and third abdominal segment (abdomen)	Third thoracic segment (metathorax)
Mechanism	Bolometer 	Photomechanic receptor 
Comparable to IR receptors in:	Boid snakes (Boidae) 	no comparable receptors found in the animal kingdom up to now

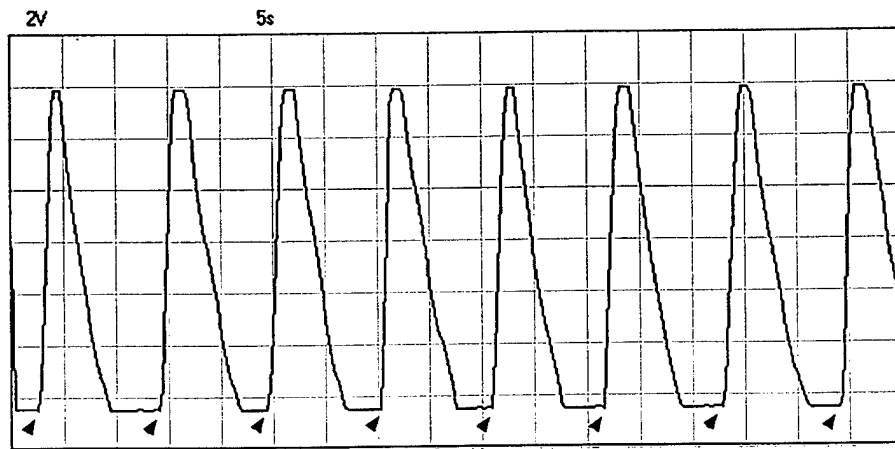
Appendix III: The Photomechanic IR Detectors



A. First prototype of a photomechanic IR detector. A small Piezoelement (red arrow) which is clamped between the two absorbing Teflon plates, measures expansion of the plates due to IR absorption. A micro-meter screw (above) allows an adjustable strain of the Piezoelement.



B. Second improved prototype of the detector. Above: Equipped with focussing lenses. Below: Inner view of the detector element.



C. Example for the detection of a human hand. Arrowheads mark the moment when a hand was held about 30 cm in front of the detector. When the maximum of the signal amplitude was reached, the hand was removed immediately. Amplification of the signal: 400 x. Time-scale: 5 s / unit.