MATERIALS AND METHODS
Scorpions, *Heterometrus fulvipes* variety I (Geethabali and Rao, 1973) supplied by the procurer were maintained in glass vivaria, containing a three inch layer of moist soil at the bottom. Scorpions were fed with cockroaches once in four days. Only those scorpions which were active and apparently normal in their behaviour as was assessed by their ready and alert responses such as the defensive strike (Palka and Babu, 1967; Ramakrishna and Rao, 1970) were utilized for the experiments.

**SOURCE OF LIGHT**

A tungsten filament bulb (12 V, 5.2 Watts) from a microscope lamp was the source. Power was supplied through a transformer from the stabilized mains supply of 220 V. The bulb was fixed at one end of a light guide, which had two condenser lenses. At the distance of 12 inches, the source emitted a circular spot of light with a diameter of 0.5 mm.
**STIMULUS PARAMETERS**

**Intensity:**

Neutral density filters (NDF) were used to obtain different intensities. Each layer of the filter cuts the light by 50%. These filters were fixed in a metal frame through sliding. The metal frame was held in front of the light guide through a retort stand. Intensity measurements were made through a lux-meter (Technolab Co.). In each experiment, intensities were varied in an ascending as well as descending order.

**Wavelength:**

Interference filters (IF) were used to obtain different wavelengths. These filters were obtained from Klett Mfg. Co. (Filter No. 54-540 nm, No. 69-690 nm) and Universal Biochemicals (BG 37-440 nm, BG 28-470 nm, BG 18-490 nm, VG 6-520 nm, VG 5-550 nm, RG 630-640 nm). These were fixed in a similar metal frame as was used for the NDF. Filters were used in a random order.

**Duration:**

Duration of stimulus was controlled through a sectored disc, attached to a DC motor. Power was supplied from a storage
battery through a linear potentiometer. Different durations were obtained by varying the speed of the motor through a voltage change i.e., from 1 to 3 V at the source. The motor and the disc assembly was fixed in front of the metal holder carrying the intensity and wavelength filters.

**Flicker:**

Flicker rate was varied by using discs with sectors of different sizes. A wide range of flicker rates (1-100 per sec.) could be obtained by a proper combination of sector-size and the power to the motor. Flicker-fusion frequency was measured at different intensities. Each experiment was done once with an increasing flicker rate starting from 1 per sec. and then repeated with a decreasing rate, starting from a high rate (about 100 per sec.).

**EFFECT OF CO₂**

CO₂ was obtained using Kipp's apparatus. A cup of plasticene was built around the scorpion and a small tube inserted through the base of the cup. The light-stimulus was allowed to incident over the eye through the open top of the cup. At any time during an experiment CO₂ could be passed or stopped by means of a pinch-cock.
ELECTRODES

Glass pipette electrode with the inside tip diameter pulled to 70-80 µ through a vertical electrode puller and filled with scorpion Ringer (Naidu, 1967) was used as the recording electrode (RE). Platinum wire, inserted into the glass pipette was connected to the grid $G_1$ of a preamplifier (Grass P9B). A minuten steel pin etched electrolytically and insulated except at the tip with the same diameter as of the active electrode served as an indifferent electrode (IE) and was connected to the $G_2$ grid of the preamplifier.

RECORDING DEVICES

Potentials were displayed on a dual beam oscilloscope (Tektronix 502A) and were photographed with a Grass C4 camera. Photocell-response of the stimulus-light was fed into one of the beams, directly, whereas the ERG was fed into the other beam from the preamplifier. Simultaneously, stimulus and the response were recorded on a magnetic tape using a stereo-recorder (Ampex F 4470, Sunnyvale, California). This served as a useful memory-storage device that enabled the play-back of any experiment into the oscilloscope for verification.
PREPARATION

As a routine the scorpion was chilled to about 10°C for about 15-25 minutes to make it quiescent for handling. Scorpion made quiescent in such a manner was restrained with the ventral side on a metal base by using plasticine. With a fine micro-scalpel, the surface of the eyes were scraped gently to remove the wax-coat in order to allow the electrode to make proper contact. While the active electrode was placed over the illuminated eye, the indifferent electrode was placed over the adjacent unilluminated eye. The latter was covered with black paper.

All these operations took 20 to 30 minutes during which the chilling effects on the scorpion are known to wear off, as was judged from the behavioral responses.

The preparation as well as the stimulus assembly were enclosed in a metal cage which was grounded to a common point as the other instruments (Figs. 1 and 2).

ERG was displayed on the oscilloscope every 5 minutes. When the potential reached a stable level of amplitude, which it did usually after 40-60 minutes, the experiment was begun. All the experiments were carried out in a dark room, under a dim red light and at the temperature 26°C ± 1°C.
Fig. 1

Schematic diagram showing the preparation, stimulus-assembly and the recording devices used.
RE - Recording electrode. IE - Indifferent electrode. Unilluminated eye in scorpion was shown as a closed spot.
Photograph of the set-up used.
Fig. 2
PROFILE OF ERG AT DIFFERENT DEPTHS

The active electrode used in these studies had a tip diameter of about 2-3 μ. Before placing the active electrode, the eye was gently pierced with a steel pin tapered electrolytically to a depth of about 50 μ, with the help of a micromanipulator. This helped the placement of the glass-pipette electrode and piercing of the tissue through different depths without breaking the tip.

The depth of penetration was determined in two ways. In the first method the verniers on the micromanipulator was read to estimate the depth from which a particular recording was obtained. In the second method the recording electrode was inserted to a depth at which a particular response was obtained. The electrode was then sprayed in situ with the fast drying opaque India ink and withdrawn. The length of the unpainted tip was measured with an ocular micrometer on a compound microscope. These methods were used by Kampa et al. (1963).

HISTOLOGY

Scorpions were dark adapted for about 3 hours. Cephalic part of the prosoma with the median eyes intact was removed under a dim red light and preserved in Bouin's fixative. Paraffin blocks were made of the median eyes and transverse as well as
longitudinal sections of 6-8 μ in thickness were cut and stained with eosin-hematoxylin (Agee, 1971a, 1972). Measurements made of the total depth of the eye through serial transverse sections were compared with those taken of the whole eye.

**DARK ADAPTATION**

Dark adaptation was studied after adapting the preparation to a light source of 100 Watts slide-projector lamp at least for one hour. Recordings of ERG were made soon after switching off the adapting lamp and at each minute thereafter till the response shows a stable magnitude at least for a duration of 5 minutes successively.

**LIGHT ADAPTATION**

The time course of light-adaptation was studied in preparations kept in total darkness at least for 3 hours. The source of ambient light was the same, as was used for dark-adaptation studies. Recordings were made soon after switching on the ambient source and each minute thereafter till the response shows a stable magnitude at least for a duration of 5 minutes at a stretch.
Each aspect of the study has been worked out using a minimum of 4 and a maximum of 9 scorpions. Totally, the results were based on 72 scorpions. No single scorpion was used in general for more than 3-4 hours and for more than one aspect of the study, in order to keep the variability to a minimum. As the figures and tables indicate, the results were expressed as the mean ± standard deviation of the specified number of experiments.