INTRODUCTION

In recent times the physiologists have now focussed their attention upon insect chemoreceptors because these are especially well adapted for study of the basic mechanisms of chemoreception on a cellular level. Such physiological studies often have relevance and significance for understanding chemoreception in other invertebrates and in vertebrate animals as well. Accessibility for experimentation, high sensitivity and lack of trouble some protective barriers between receptor cell and chemical stimuli are the properties of insect chemosensory system that make them of special interest in general physiology.

Insect receptor cells, sensitive to chemicals, are important components of an insect's sensory system. Such cells are designated as chemoreceptors and the physiological processes which occur in these cells upon chemical stimulation are termed chemoreception. The significance of chemoreception has long been recognized by entomologists, for it is this sensory process more than the auditory or visual which triggers a wide variety of the most important behaviour patterns among insects. The change in the behaviour of the insect or the maintenance of some existing activity is the result of the transformation of the energy of a stimulus into a nervous impulse after transmission into one of the central ganglia (Dethier, 1963; Horridge, 1965; Carthy and Newell, 1968; Boeckh and Boeckh, 1979; Hildebrand et al., 1980; Matsumoto and Hildebrand, 1981).

Confirmation of the fact that a given organ is a receptor has, in many cases, been obtained by electrical recording of the nervous impulse which follow stimulation. However, some receptors have been classified as such only on the basis of structure and anatomical relations (Richards and Davies, 1977). The connection between a receptor and the effector organs which change or maintain insect's activity is ofton represented as a relatively simple reflex arc (Fig. 1) though it is probable that oven the simplest behavioral act is complex. The response of the effector organ is also governed by the central nervous system so that their functional activity depends not only on the quality of the stimulus, but also on the physiological state of the receptor and nervous system.

The olfactory sense (smell) in insects is stimulated by low concentration of the vapour phase of a variety of substances which are relatively volatile at ordinary temperature. The antenna which is the main site of olfactory receptors (Schneider and Steinbrecht,

1968) is located in front part of the body. attached to the head. The obvious advantage is to receive olfactory signals early enough to guide its behaviour.

Despite considerable variation in gross structural features, the olfactory sensilla of insects show many common structural features. A typical sensillum (trichoid) of Bombyx mori may serve as an example of the structural features of insect sensilla in general. The sensillum (Fig. 2) is 45 to 110 μ in length and are thin walled perforated by a large number of pores. These pores are 1000 - 3000 with a diameter of 100 - 150 A^o. A sensillum may have a variable number of bipolar neurons, from a single neuron upto 60 or more.

Olfactory stimulus act on the dendritic receptor which modulate the permeability of cation channels. Generally the influx of Na^+ leads to the development of a generator potential difference which is a depolarization of the potential at the dendritic membrane. It is electronically transmitted to the soma of the receptor neuron and is proportional to the input stimulus i.e. concentration of the chemical received through the sensillum pores. If the threshold for eliciting action potential is crossed at the spike initiating zones of the axonal hill, a number of action potentials are produced so long the depolarization is maintained. The frequency of the spike is proportional to the size of the generator potential. The amplitude of the spike differs for different receptor cells (Morita and Yamashita, 1959; Morita and Takeda, 1959; Wolbarsht and Hanson, 1965). At the synapse the quantum of neurotransmitter release is proportional to the frequency of the propagated spikes. The depolarization of the post synaptic membrane of a neuron of the CNS is proportional to the amount of neurotransmitter released by the synapse and thus, through this long chain the stimulus information about intensity is truthfully conveyed by the receptor neuron to the appropriate part of the CNS.

The threshold of different receptor vary in a wide range. It has been found that a very high sensitivity is displayed by certain receptor cells specific biological attractant such as bombykol (the female sex attractant of Bombyx mori). A single molecule of this compound is enough to elicit a nervous impulse in an antennal sense-cell of males (Steinbrecht, 1970).

Olfactory stimuli have been intensively studied through the search for attractant and repellant substances in economic entomology. The most remarkable feature of the olfactory responses is their considerable specificity and the major role played by these in regulating the life of the insect. Karlson and Butenandt (1959) introduced a term "Pheromone" for all such substances and they have extensively been studied in different insect species (Butler, 1967; Beroza, 1970; Wood et al., 1970; Jacobson, 1972; Shorey, 1973; and Birch, 1974). A pheromone is defined as "a substance secreted externally by an individual and received by other conspecific individuals, in which it elicits some behaviour pattern or developmental process". Originally, it was assumed that pheromones are species-specific, that is, each species produce its own specific sexual attractant. This is true in many cases but investigations have shown that very often the situation is much more complex. Closely related species may have the same pheromone and so they need additional means to secure their reproductive isolation, for example, differences in their diel rhythm, behavioural differences and/or different thresholds for the attractant (Kaae et al., 1973). In some cases, two closely related species were found to utilize the same two isomers of an acetate in different ratios (Minks et al., 1973; Klun et al., 1973). To what extent interspecific attraction occurs under normal field conditions is not known, but cross-attraction (Ganyard and Brady, 1972), and inhibition have been observed in field and laboratory experiments (Ganyard, 1970).

The number of pheromone molecules emanating from the gland surface in Bombyx has been estimated to 10^{11} molecules/sec, corresponding to a pheromone molecule density of $10^{8}/cm^{3}$ in a moderate airstream passing over the female gland (Kaissling and Priesner, 1970). The effective attraction distances vary from several kilometres to a few tens of meters (Schneider, 1975) depending on the pheromone output rate, the physical transfer conditions and eventually on the male insect's behaviour threshold, which in Bombyx has been found to be at 10^{3} bombykol molecules/cm^{3} of air (Kaissling and Priesner, 1970).

A large number of compounds have been identified and isolated in insects and other animals including mammals (Fig. 4). These olfactory stimulants have been classified by their biological functions. These are sex attractants, trail-making pheromones, Assembly and aggregation pheromones, Alarm pheromone, morphogenetic pheromone: Apart from these, there are some other biologically important odours produced by one species, but of benefit mainly to the other species that percieves them, called Kairomones (Brown et al., 1970). These are oviposition attractants, Food-plant attractants, attraction to animal hosts.

A major stimulus to research on pheromones has been the prospect that they might lead to increased selectivity in insect control, and also diminish the disruptive effects of insecticides on the environment. The sex attractants are most often considered for insect control programmes, although recruitment or aggregation preromones have also been used (Beroza, 1972). Three principal approaches to the use of pheromones in pest control are currently being explored. (i) The early detection and monitoring of infestation by a pest, employing traps baited with attractant, allows more judicious application of broad spectrum insecticides. (ii) Mass trapping technique employing traps baited with sex pheromone, which lures males to the trap rather than to the females. (iii) The male confusion technique by permeating the air with female sex pheromone, so that the male, surrounded with the attractant becomes confused and unable to locate the female.

The direct investigation of the olfactory receptors have been done by electrophysiological recording methods (Fig. 5). Both Electroantenogram (EAG) and single cell recording have been successfully used to elucidate the various aspects of olfactory perception and characterization of sex-pheromone in insects. The technique have been exhaustively discussed by Schneider (1957); Kaissling (1971); Rose et al., (1979); Palaniswamy et al., (1979) Bromley and Anderson (1982); Nishino et al., (1983); Vinard and Pichon (1984); Masson and Arnold (1984) and others. Inferences from the ultrastructure of the sensilla (Albert et al., 1974) and from the results of behavioural experiments can also be drawn with increasing confidence (Shorey, 1973).

The aim of the present work was multifold and to study the sex pheromone communication system and to find out the chemical nature of the pheromone in Rice moth, corcyra cephalonica, an important stored grain pest. Although, a number of studies are available (Jacobson, 1972; Kaissling, 1971) on the different aspects of pheromore biology and identification of pheromone in a large number of insects. However, so far no study has been reported in Rice moth on its sex-pheromone receptors, or its behavioral/electrophysiological responses in response to the sex pheromone stimuli. However, two closely related species of this insect, cadra cautella and plodia interpunctella, also stored grain pests, have been reported to emit a female sex-pheromone, which has been isolated and identified

It has been reported by behavioural experiments that rice moth males are being attracted towards the virgin calling females (Shamkumar et al., 1984) showing the possibility of possessing sex-pheromone communication system. Thus the first study was to see the general morphology and histology of the antenna of the male rice moth and to locate the presence of sex-pheromone receptors with the help of light and electron microscopy. This study was intended mainly to find out the presence of sensilla trichodea, which are reported to be sex-pheromone receptors (Schneider, 1957; Albert et al., 1974). This morphological identification of receptors, served as a prerequisite to electrophysiological experiments.

The second major study was undertaken to record the electrophysiological responses from the different hairs of male antenna. Responses were recorded with the following objectives in mind:

(i) To see which hair type is highly responsive to the extracts of the virgin females, so that recordings could be done specifically from these hairs for further studies.

(ii) To see the response pattern of the specific receptor after being stimulated by different synthetic pheromones (Both acetates and alcohols) which is known to be the sex-pheromones of the closely related Lepidopterans of Rice moth (Beroza, 1976). This screening of the different synthetic pheromones for receptors sensitivity was aimed at finding whether the rice moths also utilize the structually same pheromone for their communication as is done by their two closely related species, cadra cautella and plodea interpunctella (Ganyard and Brady, 1972).

(iii) To see that if there is any compound among these, which causes excitation or inhibition of the response pattern since there are evidences that excitatory odors induce dendritic deplorazlation of the sense-cell followed by the nervous impulses in the axon, while inhibitory odors hyperpolarize the cell and depress the impulses (Boeckh et al., 1965).

(iv) To see that what is the threshold value for the adaptation response of the receptors.

Third major study was regarding the behavioral response pattern and had the following objectives:

(i) To study the different behavioral patterns of the male and female insects and the attraction and inhibition of the male responses, in presence of the different synthetic pheromones and abdominal extracts used in the above studies. This was aimed to reach on some conclusion about the approximate nature of the compound to which the male is most responsive and can be said to be its specific sex pheromone.

(ii) To see the effect of the age of the female on sex-pheromone production, since the production of the sex pheromone is limited to certain age of the insect.

(iii) To see that what is the influence of the dark period and mating on the calling of virgin females.

(iv) Finally, to see the reduction in responsiveness of the males by the previous pheromonal exposure, because there are reports that the previous pheromonal exposure results in a reduction in responsiveness of male (Shorey and Gaston, 1964; Traynier, 1970).

The study on the effectiveness of different synthetic pheromones (ii above) was conducted with the aim of finding out the approximate chemical nature of the sex pheromone by using electrophysiological techniques. This has successfully been used by many workers for this purpose (Nishino et al., 1983; Roelof et al., 1971). The biochemical methods to isolate and characterize the sex-pheromones are very tedious involve GLC and NMR spectrophotometery and thousands of insects are required for in one experiment. These were the framework of the present study.