Studies on Chemotactic Behaviour of Silkworms, *Bombyx mori* L. (Lepidoptera: Bombycidae) Using Electroantennogram

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ABSTRACT

Insects, the predominate life forms on earth, are quite fascinating owing to their vast complexity in behaviour and attributes. The olfaction system in insects is highly advanced with suitable olfactory sensors or receptors in the antennae, maxillary palps etc. that are triggered in response to intra-specific chemicals like pheromones or feeding stimulants.Therefore technique of electroantennogram (EAG) is primarily used for quantitative measurements of general odorants in model organisms like *Bombyx mori*. Odours/ chemical stimuli play a vital role in life cycle of insects influencing their behaviour and conduct in complex natural ecosystems.Olfaction, one of the key senses controlling insect behaviour, has been a successful pest management strategy. Thus, elaborate understanding of the EAG technique and set up under laboratory conditions is vital to figure out the response of insects to general chemical stimuli and the consequent behaviour. Therefore, this article aims to review the basic EAG instrumentation and parameters with B.mori as a model.

INTRODUCTION

Olfaction is the prime key in various behaviours of insects such as host habitat finding, host finding, foraging, oviposition, mate recognition, predation, etc. Therefore a broad understanding of the insect olfaction and communication process is exploited as a potential tool for pest management. Insects have well developed cephalic appendages called antennae to perceive various chemical stimuli. This is aided by maxillary palps and proboscis. The odorants from the surrounding environment binds to the water soluble proteins in the sensillar haemolymph called the odorant binding proteins (OBPs). As a result the dendrites of the olfactory neurons are triggered and induce suitable responses towards the chemical stimuli received. It is also hypothesised that sensory neuron membrane proteins (SNMPs) on the dendrites of olfactory sensory neurons (OSNs) are responsible for the perception of the pheromones by insects as fruitflies and moths. Therefore, editing of vital OBPs, SNMPs, and OSNs related genes using CRISPR/Cas9 or RNA interference tools results in effective disruption of insect communication process, reducing insect damage in agricultural and horticultural crops.

ELECTROANTENNOGRAM

The responses of the various receptor neurons to the specific chemical stimuli are recorded as slow potentials. These potentials from antennal preparations form the basis of electroantennogram investigations. Thus it is a popular bioassay technique in experimental entomology whichis based on the principle that there are minute voltage variations between the tip and base of antennae of insect when presented with pheromones (Schneider, 1957). This may be due to electrical depolarisation of various olfactory neurons in the antennae of insects. When the concentration of stimulus presented to the insect increases, the EAG signal peaks until it reaches saturation. The type of stimulus, species and sex of insect and surrounding environmental conditions are the factors that influence the peak, amplitude and patterns of the EAG signals.

PRACTICAL APPLICATIONS OF EAG SIGNALS

It helps in identification of physiologically active compounds, purification of extracts, synthesis and selection of active synthetic chemicals, concentrations measurement in the field and usage in gas chromatography as a detector. For a particular species, nature and strength of stimulus, conditions of the antennae, life time of preparation, number and strength of previous stimulations, quality of amplifier input, varying temperature and humidity of the surrounding and the physiological condition and stage of the insect is considered in EAG studies. Therefore the basic set up of the EAG setup includes preparation of the antennae and the use of manipulators to record electrodes, a electron processing signal and amplifier, signal display and recording system, stimulus application system etc (Fig. 1).A relatively stable baseline in the recording device is visualised if antennae makes a good contact with the receptor (Fig. 2).

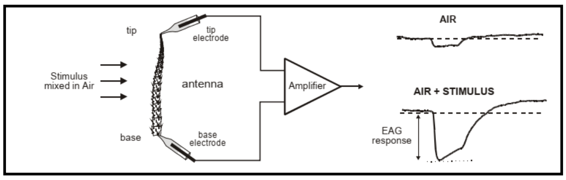


Fig. 1: Basic set up of EAG

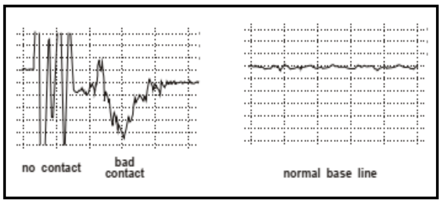
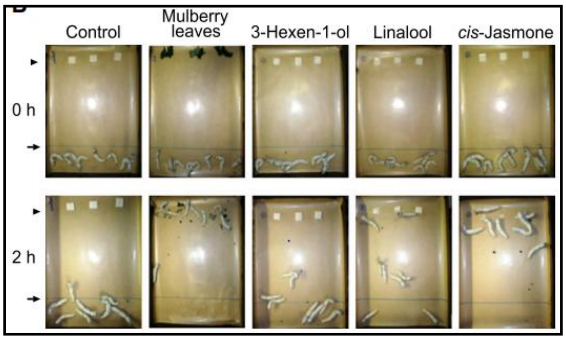


Fig.2: Stable base line recording

EAG IN SILKWORMS

EAG was first used by Schneider to record chemo and mechano reception on the antennae of B. mori. Thus electroantennogram is used to study the chemotactic behaviour and response of B. morito various kinds of chemicals and volatiles emanated from the mulberry leaves. Gas chromatography and mass spectroscopy analysis has visualised that cis-jasmone is the most potent chemical attractant of B. moriat the working concentration of 0.3pg at a 20 cm distance. Also, out of the 66 olfactory receptors (ORs), *BmOr-56* possesses the greatest sensitivity to the attractant cis-jasmone (Tanaka et al., 2009) (Fig. 3). Similarly other studies reported the stimulation of silkworm antennae in response to chemicals as linalool and α-terpineol (Topazziniet al., 1990).



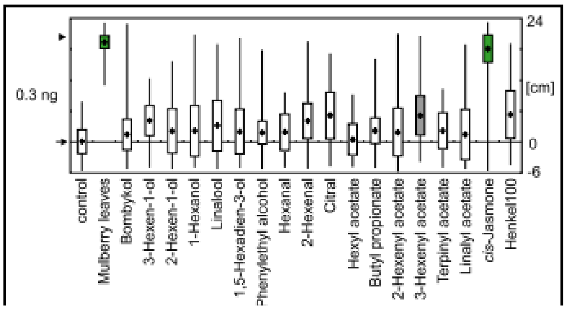


Fig. 3: Response of silkworms to cis-jasmone in mulberry leaves (Tanaka et al., 2009)

Antennae are also a vital insect organ that helps in perception of pheromone by the insects. The B. mori sex pheromone, Bombykol, is the oldest and first discovered insect pheromone. The EAG responses of the antennae to the major and minor pheromone components were studied and represented.

Since, olfaction is a vital process in insect life cycle, olfactory dysfunction caused due to exposure to volatile organophosphate dichlorovos was investigated. It is explained that an acute exposure of 12 hours significantly reduced EAG responses, resulting in non-identification of the pheromone source by the male moths. Transcriptomic profiles proposed that upregulations of *Arrestin1* and *nitric oxide synthase1* (NOS1) may inhibit cyclic nucleotide-gated channels and hinder calcium influx in the antennae. Similarly, in the central nervous systems (CNS), downregulations of *tyrosine hydroxylase* (TH) and *tyrosine decarboxylase* (TDC) may inhibit olfactory signal transduction by reducing the second messenger biosynthesis. Meanwhile, an abnormal increase of brain cell apoptosis was revealed by Annexin V-mCherry staining, often leading to persistent neurologic impairment (Chen et al., 2022) (Fig. 4).

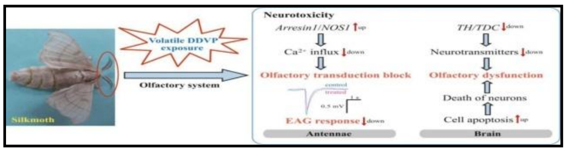


Fig. 4: Olfactory dysfunction under exposure to volatile DDVP (Chen et al., 2022)

CONCLUSION

Since olfaction is a complex process involved in insect foraging, host finding and predation, disruption of the same is an efficient tool for pest management. The class of *orco* genes are currently being targeted for knockout and disruption via CRISPR/Cas9 to hamper this olfaction process in many insects as fruit flies and a variety of fruit and shoot borers Also in beneficial organisms like B. mori genes expressing essential chemical stimulants like jasmone and linalool in host plants as mulberry can be manipulated for higher expression and induce insect feeding and growth providing a nutritional feed choice for the insect.

REFERENCES

Chen, J., Li, S. S., Fang, S. M., Zhang, Z. and Yu, Q. Y. (2022), Olfactory dysfunction and potential mechanisms caused by volatile organophosphate dichlorvos in the silkworm as a model animal. Journal of hazardous materials, 425: 127940.

Schneider, D.(1957), Electrophysiological investigation on the antennal receptors of the silk moth during chemical and mechanical stimulation. Experientia, 13(2): 89-91.

Tanaka, K., Uda, Y., Ono, Y., Nakagawa, T., Suwa, M., Yamaoka, R. and Touhara, K. (2009), Highly selective tuning of a silkworm olfactory receptor to a key mulberry leaf volatile. Current Biology, 19(11): 881-890.

Topazzini, A., Mazza, M. and Pelosi, P. (1990), Electroantennogram responses of five Lepidoptera species to 26 general odourants. Journal of insect physiology, 36(9): 619-624.