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Calcium responses to pheromones and plant odours in the antennal lobe of the male and female moth Heliothis virescens

Accepted: 3 October 2000 / Published online: 7 November 2000 © Springer-Verlag 2000

Abstract In male moths, the primary olfactory integration centre, the antennal lobe, consists of two systems. The macroglomerular complex processes pheromone information, while the ordinary glomeruli process plant odour information. Females lack a macroglomerular complex. We measured the spatial representation of odours using in-vivo optical recording. We found that: (1) pheromone substances elicited activity exclusively in the MGC. No response was found in female antennal lobes. (2) Plant odours elicited combinatorial activity patterns in the ordinary glomeruli in both males and females. No response was found in the MGC of male moths. (3) A clean air puff often led to activity, in both males and females, suggesting that mechano-sensory information is also processed in the antennal lobe. (4) With an interstimulus interval of 5 or 10 s, strongly activated glomeruli were able to follow the temporal structure of the stimulus, while others lost their phase-locking. Some glomeruli showed “off” responses. These properties were odour dependent. This confirms and extends previous studies, showing the functional significance of the two subsystems for processing olfactory information. Pheromones are coded in a combinatorial manner within the macroglomerular complex, with each glomerulus corresponding to one information channel. Plant odours are coded in an across-glomeruli code in the ordinary glomeruli.

Key words Optical imaging · Calcium green · Spatial coding · Olfactory representation · Repetitive stimulation


Introduction

The olfactory system in many vertebrate and insect species can be subdivided into a “main” and an “accessory” part, involved in detection of food odours and pheromones, respectively. In male moths, the primary olfactory integration centre, the antennal lobe of the deutocerebrum, relays both kinds of information into two separate systems of glomerular structures (reviewed by Homberg et al. 1989; Masson and Mustaparta 1990; Hildebrand and Shepherd 1997). The macroglomerular complex (MGC) is involved in processing pheromone information, as demonstrated by electrophysiological recordings combined with stainings of antennal lobe neurones (Boeckh and Boeckh 1979; Christensen and Hildebrand 1987; Christensen et al. 1989, 1991, 1995a; Hansson et al. 1991). The antennal lobe projection neurones responding to olfactory stimulation with pheromones have dendritic arborizations exclusively or mainly in the MGC. The input to the MGC by receptor neurones responding to pheromones is demonstrated by staining of sexually dimorphic sensilla (Koontz and Schneider 1987; Christensen et al. 1995b) and also by stainings combined with recordings from the cut tip of olfactory sensilla, showing projections mainly in the MGC (Berg et al. 1998; Hansson et al. 1992, 1995). A functional subdivision of the MGC has been demonstrated by electrophysiological recordings from receptor and antennal lobe neurones combined with stainings in several species of moths and other insects (Christensen et al. 1991; Hansson et al. 1991, 1995; Berg et al. 1998; Vickers et al. 1998).

Well separated from the MGC are the numerous ordinary glomeruli that seem to be involved in the processing of general odours. The antennal lobes of
female moths lack an MGC. It consists entirely of “ordinary” glomeruli, and a “modified glomerular complex”, which is possibly involved in host-odour recognition (King et al. 2000). In the (female) honeybee, optical recordings from the antennal lobe during stimulation with plant odours have demonstrated a spatial pattern of activity characteristic for each odour. These patterns are conserved within the species, and can be mapped to identified glomeruli (Galizia et al. 1999a; Sachse et al. 1999).

In the tobacco budworm moth, Heliothis virescens, receptor neurones responding specifically to insect- or plant-produced compounds have been identified. In males, four sex-specific receptor neurone types have been classified, each projecting to one of the four MGC compartments (Berg et al. 1995, 1998). Neurones tuned to the major pheromone component, Z-11-hexadecenal (Z11–16:AL), project to the largest compartment, cumulus, whereas the neurones responding selectively to the second pheromone component, Z9-tetradecenal (Z9–14:AL) project to the dorso-medial compartment. The other two neurone types are each tuned to one of the interspecific signals, Z11-hexadecenol (Z11–16:OH), and Z11-hexadecenylacetate (Z11–16:AC), produced by sympatric females, and project to one of the smaller ventral compartments, respectively. In this species there seems to be a correspondence between input and output of the MGC, i.e. that antennal lobe projection neurones responding to one of the four compounds have at least one dendritic arborisation in the compartment receiving that particular information (Berg et al. 1998; Vickers et al. 1998). In the female H. virescens several types of plant odour receptor neurones have been identified by the use of gas chromatography linked to recordings from single neurones (Røstelen et al. 2000a, b). For each neurone type, selective responses were demonstrated to one or two compounds, monoterpenes or sesquiterpenes, of the volatiles naturally produced by plants. One frequently occurring receptor neurone type responded selectively to the sesquiterpene germacrene D. Another neurone type responded selectively to the monoterpenes trans-β-ocimen and β-myrcene and a third type selectively to linalool, all present in host and non-host plants. These results raise the question of how these odours are represented in the antennal lobe. Neuron staining, combined with the electrophysiological recordings, has not yet been performed. In the present study optical recordings from the antennal lobes of females and males have been used to study the spatial pattern of activity elicited by antennal stimulation with odours. We tested the four insect produced compounds, three of the four identified plant compounds as well as naturally produced plant volatile mixtures and an isolated fraction of a plant extract.

The results confirm and extend results from electrophysiological recordings of pheromone receptors and antennal lobe projection neurones, showing (1) the significance of the two subsystems, the MGC and the ordinary glomeruli, for processing information about insect and plant produced odours, (2) a functional subdivision of the MGC compartments in processing information about intra- and interspecific insect produced signals, and (3) specific patterns of activity in the ordinary glomeruli by stimulation with different odours.

### Materials and methods

#### Animal stocks

*Heliothis virescens* (Lepidoptera: Noctuidae) pupae, originating from a lab culture, were kindly provided by Novartis Crop Protection, Basel, Switzerland. Male and female pupae were separated into different containers and kept in an incubator on a phase-shifted light-dark 14 h:10 h photoperiod at 23 °C. Every day, moths that had emerged were placed in a separate container marked with the date. Animals were allowed to feed on honey-water. Most animals were studied 1–3 days after emergence.

#### Animal preparation and staining

The moths were prepared in a way similar to that for standard electrophysiological recordings. The animal was fixed into a Plexiglas stage and the antennae were fastened in the desired position with utility wax (Kerr). Scales were removed; a hole was cut into the head cuticle in order to expose the brain. Mouthparts, glands and trachea were gently removed. The brain was kept wet at all times with Ringer solution containing (mmol l⁻¹): 130 NaCl, 6 KCl, 4 MgCl₂, 5 CaCl₂, 160 sucrose, 25 glucose, 10 HEPES, pH 6.7; 500 mosmol l⁻¹; all chemicals from Sigma. The brain was then floated in dye solution (cysteamine-green-2 (AM), Molecular Probes; 50 µg dye was first dissolved in 50 µl Pluronic in DMSO and then diluted in 950 µl Ringers saline). After 1 h, the brain was rinsed in fresh Ringer solution, covered with a cover slip, and placed under the microscope. During the experiment, a constant Ringer flow was applied (1 ml min⁻¹, 22°C).

#### Odours used and stimulus application

The animals’ antennae were constantly puffed with a clean air stream, which was replaced by an odour-laden air stream for stimulation. Controlled amounts of odours were placed on filter paper in glass cartridges. Odours were dissolved in 1-hexane. Stimulus concentration was varied by using different dilutions, resulting in different effective amounts on the filter paper (0.2 µg, 2 µg, 20 µg in the case of the pheromones, and 0.01 µl, 0.1 µl and 1 µl for plant odours). These effective amounts are referred to as concentrations in this paper. Different amounts lead to different stimulus intensities. A clean glass cartridge was used as control.

The insect-produced substances used for olfactory stimulation were: two of the six identified pheromone components, cis-11-hexadecenol (Z11–16:AL) and cis-11-hexadecenyl acetate (Z11–16:AC), and two intraspecific disruptants, cis-11-hexadecenol (Z11–16:OH). These compounds, provided by Dr. JG Tumlinson, USDA, Gainesville, Florida, had a purity above 99% (checked by GLC). Plant odours tested were: (1) *Piper cubeba* oil (provided by Dr. G Schmaus, Dragoco, Holzminden, Germany), which is a sesquiterpene fraction containing mainly sesquiterpenes including germacrene D, but also small amounts of many other compounds (including linalool, cis-/-trans-β-ocimen, and β-myrcene); (2) headspace of sunflower and tobacco plants (flower and leaves); (3) synthetic materials of β-ocimen, trans-cis-β-myrcene (ratio 66:33); and (4) the pure substances linalool, (racemic mixture with purity 95–98%, supplied by Firmenich, Geneva), citral, hexanal and 1-hexanol (Sigma, purity 95%). Not all odours were tested in all animals. Generally, each odour was delivered two times in a row, with an