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Central Processing of Plant Volatiles in *Agrotis ipsilon* Males is Age-independent in Contrast to Sex Pheromone Processing

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Abstract

Male moths rely on female sex pheromones to find their mating partner and on plant volatiles for the detection of food sources. In the noctuid moth, *Agrotis ipsilon*, plasticity of central sex pheromone processing has been shown previously in the antennal lobe. The sensitivity of antennal lobe interneurons increases with age and juvenile hormone level. Here we investigated whether age affects not only central sex pheromone processing, but also central processing of plant volatiles in *A. ipsilon* males. Intracellular recordings of antennal lobe interneurons were made in males of different ages after stimulation of the antennae with seven different plant volatiles. The sensitivity and specificity of the antennal lobe interneurons for any of the plant volatiles tested did not change with age. From these results we conclude that the sensitivity of the antennal lobe interneurons involved in central plant volatile processing is age-independent and that the action of juvenile hormone is specific for central sex pheromone processing in *A. ipsilon* males.

Introduction

Moths use mainly olfactory cues to orient in their environment. Males rely on the female species-specific sex pheromones to find their mating partners, whereas females are attracted by volatiles emitted from host plants in their search for oviposition sites. Both sexes locate food sources by flower volatiles.

Males and females of the noctuid moth *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) were observed to be highly attracted to flower volatiles from blooming plants in field experiments (Wynne *et al.*, 1991; Zhu *et al.*, 1993). Blooming linden (*Tilia americana*) was the most attractive of the plants tested for *A. ipsilon* and other moths (Zhu *et al.*, 1993). Heptanal, identified as a component of linden flower extracts, was found to be attractive to *A. ipsilon* in the field and elicited clear electroantennograms (Li, 1988; Zhu *et al.*, 1993). Insect host odour detection has been of interest for a long time [for reviews see (Boeckh and Ernst, 1983; Visser, 1986; Bernays and Chapman, 1994)], but only a few studies have dealt with the central processing of plant volatiles (Anton and Hansson, 1994, 1995; Roche King *et al.*, 2000).

The behavioural response of *A. ipsilon* males to the sex pheromone was shown to be age- and juvenile hormone (JH)-dependent (Gadenne *et al.*, 1993). Newly emerged males do not respond to the sex pheromone; however, their responsiveness increases with age and increasing JH level (Gadenne *et al.*, 1993; Duportets *et al.*, 1998). Peripheral electrophysiological studies displayed a fully functional antennal sensory system in newly emerged, non-mature males (Gadenne *et al.*, 1993). However, intracellular recordings performed in the male-specific macroglomerular complex of the antennal lobe (AL) revealed that the sensitivity of AL interneurons for the sex pheromone was age- and JH-dependent, thus suggesting a neuronal plasticity of central sex pheromone processing (Anton and Gadenne, 1999; Gadenne and Anton, 2000). As males feed regularly throughout their life (C. Gadenne and M.C. Dufour, personal observation), the behavioural attractivity and central processing of plant volatiles were expected to be age independent and therefore, in contrast to sex pheromone processing, not likely to be regulated by JH.

We tested the effect of age on central processing of plant volatiles by performing intracellular recordings of AL interneurons arborizing in the ordinary glomeruli while stimulating the antennae with various amounts of common plant volatiles. Our results show that the sensitivity of AL interneurons for the tested plant volatiles does indeed not change with age in *A. ipsilon* males.

Materials and methods

Insects

Male *A. ipsilon* moths originating from a laboratory colony in Bordeaux, France, were reared as described previously (Gadenne *et al.*, 1993). Adult moths were fed with 20% sucrose solution throughout their adult life.

Intracellular recording and stimulation

Preparation and intracellular recordings were performed on 1- and 5-day-old, unmated *A. ipsilon* males according to standard methods (Christensen and Hildebrand, 1987; Anton and Gadenne, 1999). Plant-specific AL interneurons were randomly penetrated within the array of ordinary glomeruli. A glass microelectrode filled with 2 M KCl or 4% Lucifer Yellow (*Sigma*) backfilled with 2 M LiCl was used as recording electrode. Seven different plant odours were tested while the solvent, hexane and clean air served as controls. The following secondary plant compounds, behaviourally active in various insect species (Werner, 1972; Guerin and Visser, 1980; Anderson *et al.*, 1993; Hartlieb and Rembold, 1996), were tested: (*E*)-2-hexenal, 1-hexanol, heptanal, geraniol, eugenol, *trans*-caryophyllene and linalool. The sensitivity to the sex pheromone blend was tested in some 5-day-old moths and the same sensitivity distribution was found as in previous studies (thresholds between 1 pg and 100 ng) (Anton and Gadenne, 1999; Gadenne and Anton, 2000). Pasteur pipettes with pieces of filter paper were loaded with the specific plant odours dissolved in hexane between 1 ng and 1 mg or with hexane only. The antennae of the moths were stimulated with a 0.5 s air pulse containing clean air, the solvent or the different test compounds. The stimuli were presented in a random order separated by inter-stimulus intervals of at least 10 s.

Data analysis

Responses were analysed manually as described previously (Anton and Gadenne, 1999). Non-responding neurons, inhibitory neurons ($n = 12$ neurons) and neurons responding to the sex pheromone blend ($n = 18$ neurons) were not included in the data analysis. The net difference of spike counts was calculated according to Gadenne and Anton (Gadenne and Anton, 2000). Differences in the number of responding neurons to each odour and in response specificity between 1- and 5-day-old males were analysed pairwise for each threshold/response specificity and each odour. The χ^2-test was used on the number of responding neurons at the significance level of $P \leq 0.05$, applying the Bonferroni correction to limit the overall experiment-wise error rate. Differences in dose-dependent responses were analysed with ANOVA followed by the Fischer exact probability test ($P \leq 0.05$).

Results

General response characteristics of AL neurons

In this study, recordings from 206 AL neurons in 127 *A. ipsilon* males are included. A further 209 neurons did not respond to any of the stimuli. The spike frequency in the active AL interneurons increased in a dose-dependent manner with increasing stimulus amounts presented, as shown for responses to heptanal (Figure 1). The net difference at stimulus threshold ranged between 5 and 10 Hz above spontaneous activity and increased between 5 and 20 Hz from the threshold to the next higher amounts. Intracellular staining of physiologically characterized neurons revealed AL projection neurons ($n = 14$ neurons) with uniglomerular arborizations in ordinary glomeruli (data not shown).

The effect of age on the response thresholds and dose–response characteristics of AL neurons

One hundred and nine neurons in 46 1-day-old males and 97 AL interneurons in 81 5-day-old males responded to one or more of the tested stimuli. For all seven stimuli, thresholds were similar in 1- and 5-day-old animals. For heptanal, (*E*)-2-hexenal, eugenol and geraniol, no significant difference between thresholds of the two age groups was found (Figure 2). For the other three compounds the number of responses was too low ($n = 10$ neurons, $n = 20$ neurons, $n = 10$ neurons, for 1-hexanol, linalool and caryophyllene, respectively) to perform statistical tests. Dose–response relationships did not differ between 1- and 5-day-old *A. ipsilon* males, as shown for heptanal (Figure 1).

The effect of age on compound specificity of AL neurons

A clear majority of the tested AL neurons responded to heptanal and (*E*)-2-hexenal (Figure 3a). No significant difference in the representation of any of the tested stimuli was found between the two age groups. Response specificity

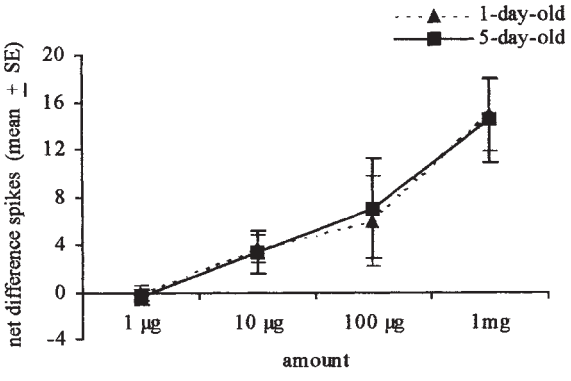

Figure 1 Dose–response curves of AL neurons in response to heptanal in 1- and 5-day-old *A. ipsilon* males ($n = 5$ neurons with 10 µg threshold). The dose–response curves do not differ significantly between the two age groups.

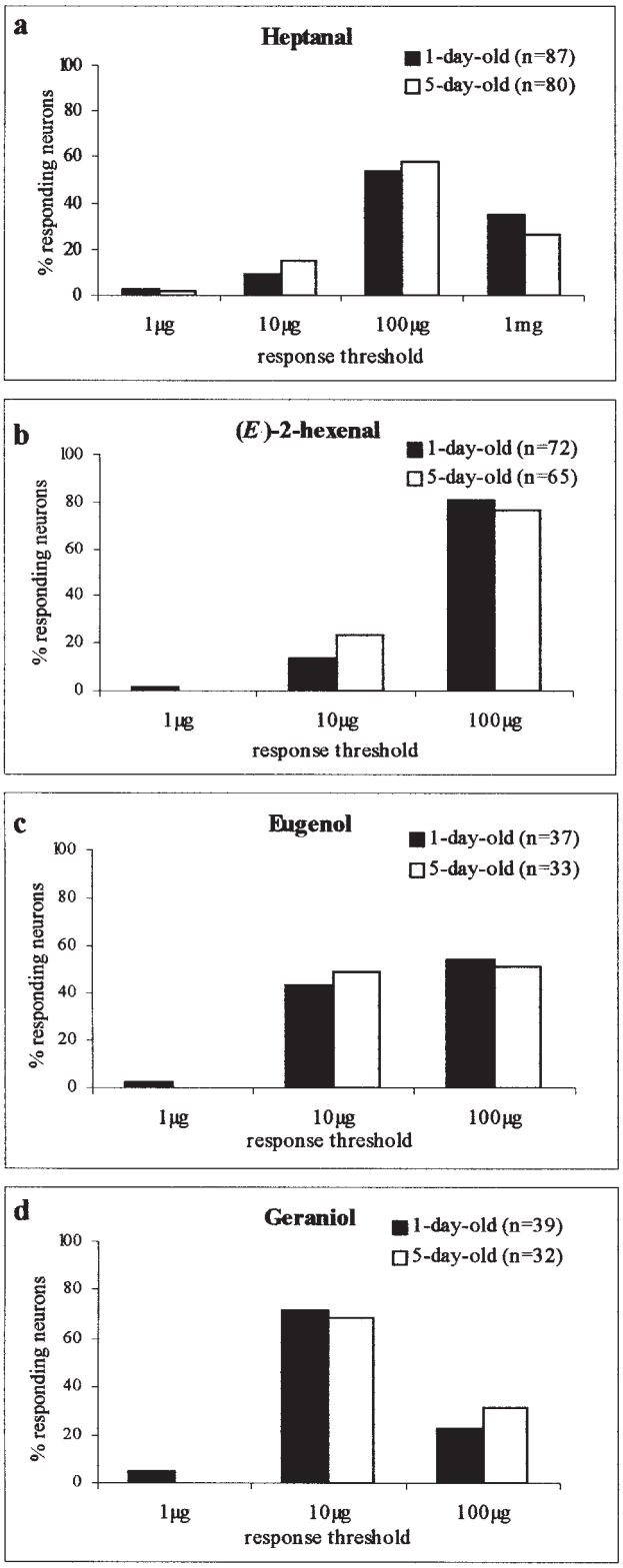

Figure 2 Response thresholds for plant volatiles of AL neurons in 1- and 5-day-old *A. ipsilon* males. No significant differences (χ^2-test, on the number of responding neurons, n.s., $P < 0.05$) between the thresholds of the two age groups could be determined in pairwise comparison for heptanal **(a)**, (*E*)-2-hexenal **(b)**, eugenol **(c)** and geraniol **(d)**. *n* = number of neurons responding to the respective compound.

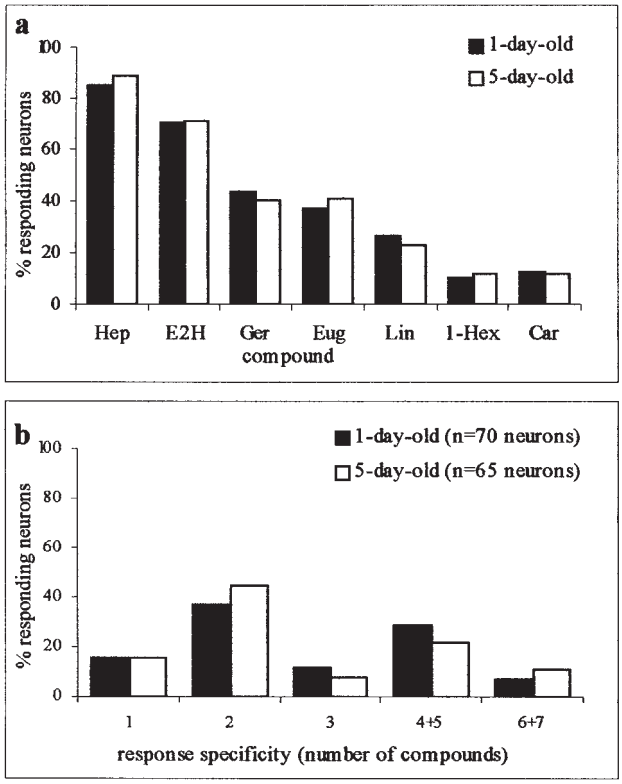

Figure 3 Compound specificity of AL neurons in 1- and 5-day-old *A. ipsilon* males. **(a)** Representation of responses to the compounds tested in AL neurons in 1- and 5-day-old *A. ipsilon* males (*n* = 75–102 neurons/compound). Hep, heptanal; E2H, (*E*)-2-hexenal; Eug, eugenol; Ger, geraniol; Lin, linalool; 1-Hex, 1-hexanol; Car, *trans*-caryophyllene. **(b)** Response specificity of AL neurons. No significant difference was found (χ^2-test, on the number of responding neurons, n.s., $P < 0.05$) between the two age groups in (a) and (b).

of AL neurons varied. A large proportion of the AL neurons, however, responded to more than one compound, among which many responded specifically to two compounds (Figure 3b). No significant difference in neuron specificity was found between the two different age groups.

Discussion

The results of the present study show that the sensitivity and compound specificity of plant volatile processing AL interneurons are age-independent in *A. ipsilon* males for the tested plant compounds. This is in contrast to the age- and JH-dependent central sex pheromone processing in this species (Anton and Gadenne, 1999; Gadenne and Anton, 2000), and the aggregation pheromone processing in locusts (Ignell *et al.*, 2001). JH-linked neuronal plasticity of odour processing in *A. ipsilon* males seems therefore likely to be restricted to the sex-specific macroglomerular complex of the AL as the site for sex pheromone processing. This plasticity is important for male moths undergoing a maturation process of their reproductive system, while such plasticity may not be needed regarding plant volatile processing, as

males seem to use plant volatiles to detect food sources throughout their adult life.

Heptanal, a behaviourally relevant plant volatile for *A. ipsilon* (Li, 1988), elicited responses in a large proportion of AL interneurons innervating ordinary glomeruli in males of different ages, which underlines the important role of this compound. The most common threshold for the plant volatiles tested was between 10 and 100 μg of the compounds in the source. These values are similar to plant volatile thresholds of AL interneurons in *Manduca sexta* females (Roche King *et al*., 2000) and in *Spodoptera littoralis* males and females (Anton and Hansson, 1994, 1995).

In this study we show that there is no change of central plant volatile processing with age, thus suggesting that the JH-linked plasticity of central olfactory processing is sex-pheromone-specific in *A. ipsilon* males. Further investigations will be needed to show whether mating makes plant volatiles emitted from oviposition sites more attractive to *A. ipsilon* females and whether the ordinary glomeruli could be a site of neuronal plasticity for central plant volatile processing in this context too.

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