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Innate and Changed Responses to Plant Odours in Moths and Weevils

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Introduction

The challenge for the olfactory system in animals is to detect the large diversity of molecules released by food or host plants and to discriminate these odours from irrelevant ones. Molecular biological studies of vertebrates and insects have shown that the olfactory information is not handled by a few, but by a large and species-specific number of receptor proteins (Vosshall, 2003; Mombarts, 2004). Furthermore, the olfactory receptor neurons (ORNs) are divided into subpopulations, each expressing only one type of receptor protein and converging in one or two specific glomeruli in the primary olfactory centres, i.e. the antennal lobe in insects and olfactory bulb in vertebrates. Questions of interest in our studies of insects are how receptor and central interneurons encode plant odour information leading to behavioural responses as well as the neuronal mechanisms underlying olfactory learning. By comparing related species of moths (subfamily Heliothinae) with distantly related moths and weevils, our intention is to identify similarities and differences of the olfactory coding mechanisms across species. Recognition of odours requires learning and memory of the odour, as shown in the honeybee (Menzel, 2001). Heliothine moths also can learn odours, which seems to be important in the selection of host plants for nectar feeding and egg-laying (Cunningham *et al.*, 1998; Hartlieb *et al.*, 1999; H.T. Skiri *et al.*, unpublished data).

Identification of biologically relevant plant odorants

The first question to be asked is which compounds of the complex mixtures of volatiles released by host and non-host plants are detected by the ORNs. Naturally produced volatiles are trapped by adsorbents during aeration of intact plants or cut plant materials ('head-space' technique) (Røstelien *et al.*, 2000a). The compounds are then eluted by solvents and used as test samples. Gas chromatography with two parallel columns linked to electrophysiological recordings from single neurons is employed to test the stimulation of the ORNs by the separated compounds. The use of one chiral column for separating optical isomers has enabled tests of pure enantiomers on single ORNs (Stranden *et al.*, 2002, 2003a). The effective compounds are then identified by GC-MS and NMR, followed by retesting authentic materials on the ORNs. This provides information not only on which odorants are detected, but also about the molecular receptive ranges of the ORNs.

The molecular receptive range and enantioselectivity of the olfactory receptor neurons in closely and distantly related species

The results have shown that the ORNs in these insect species primarily respond by excitation to the odorants and fall into distinct types according to their molecular receptive ranges (Røstelien *et al.*, 2000a,b; Stranden *et al.*, 2002, 2003a,b). They show strong responses to one or two primary odorants and weak responses to a few chemically related compounds (secondary odorants), demonstrating a narrow tuning. These results correlate well with the principle that one type of receptor protein is expressed in each ORN. The molecular receptive ranges show no or minimal overlap for neurons responding to the same chemical group. For instance, the monoterpene alcohol, (+)-linalool, being the primary odorant for one ORN type, is a secondary odorant for the ORNs primarily responding to another monoterpene alcohol, geraniol. In three related species of heliothine moths the same functional types of ORNs have been found. One example is the germacrene D type, for which (–)-germacrene D has a 10-fold stronger effect than (+)-germacrene D, which differs in the direction of the isopropyl group in relation to the 10-carbon ring (Stranden *et al.*, 2003a,b). The much weaker effect of three other sesquiterpene molecules seems to be due to their less flexible ring systems. These properties are similar for all germacrene D types of ORNs in three heliothine species. In addition, the strawberry weevil (*Anthonomus rubi*) has one ORN type tuned to (–)-germacrene D, responding with lower sensitivity to (+)-germacrene D (Bichao *et al.*, unpublished data). However, in this species the secondary odorants are different molecules to those in the heliothine moths, suggesting that the germacrene D ORNs in the two insect groups have evolved independently in the adaptation to their host plants. Species differences are also shown in respect to the presence of different ORN types tuned to the opposite enantiomers of linalool (Røstelien *et al.*, 2000a,b). Altogether, the results obtained in these moth and weevil species show a narrow tuning and a minimal overlap of the molecular receptive ranges of the ORNs. These data on insects are in contrast to the results of broadly tuned ORNs reported in vertebrates, and may reflect the low homology found between the receptor proteins in insects and vertebrates (Breer, 2003).

The antennal lobe: optical imaging and intracellular recordings from projection neurons

Models of the antennal lobes of three heliothine species have shown the presence of 62–64 glomeruli involved in plant odour information (Berg *et al.*, 2001; H.T. Skiri *et al.*, unpublished data). In one study, optical recordings of the glomerular activity elicited by some of the primary and secondary odorants have been studied (H.T. Skiri *et al.*, unpublished data). In these recordings, covering ~20 glomeruli of the anterior antennal lobe (AL), each primary odorant elicited activity in specific glomeruli. In addition, three glomeruli were found that responded to two or three primary odorants, which is in contrast to the principle of one ORN type converging in each glomerulus. The results from these optical recordings, indicating the input to the glomeruli, are compared with the responses of projection neurons (PNs) in the antennal lobe, representing the glomerular output. Responses of PNs to primary and secondary odorants are studied by the use of intracellular recordings combined with injection of fluorescent dyes for visualization of the morphology of the neurons by confocal laser scanning microscopy (CLSM). This is followed by reconstruction of the neurons and the innervated brain structures using the AMIRA software. Different types of PNs have been demonstrated. They are characterized by physiological responses to different odorants, dendrite arborizations in one or more glomeruli, axons in different antenno-cerebral tracts and projection patterns in the calyces of the mushroom bodies and lateral protocerebrum (Müller *et al.*, 2002). The responses of the PNs are compared with the molecular receptive ranges of the functionally characterized ORNs, and this indicates that there is excitatory input from one ORN type and inhibition mediated via local interneurons from another ORN type. Recordings from a multiglomerular PN with scarce innervation in each of many glomeruli indicated that specific blends of odorants might be required for spike firing. In further studies we hope to determine the overall representation of the odour quality of primary and secondary odorants in the glomeruli of the antennal lobe and find out whether the different antenno-cerebral tracts in these moth species convey different biologically relevant information.

Projections of gustatory receptor neurons in the brain and the possible neuronal connection with the olfactory pathway involved in associative learning

Associative learning is studied by the use of the proboscis extension response (PER) (Menzel, 2001). By pairing an odour stimulus (conditioned stimulus), followed by sucrose stimulation to the gustatory sensilla (unconditioned stimulus), the insects learn to associate the odour with the sucrose reward. In order to trace the neuronal connections between the pathways mediating the unconditioned and the conditioned information in heliothine moths, the projections of the gustatory receptor neurons have been traced. Two populations of gustatory receptor neurons are present, one located in the contact chemosensilla on the antennae (sensilla chaeticae) and another in sensilla styloconicae on the proboscis. By the use of fluorescent dyes for visualization in CLSM and reconstruction in AMIRA, the projections are found in two closely located fingerlike areas in the SOG (Jørgensen *et al.*, 2002; Kvello *et al.*, 2002). One neuron has been found that might form the connection between the taste and the olfactory pathway (Müller *et al.*, 2002). The morphology of this neuron resembles the VUMmx1 involved in associative learning in the honeybee (Hammer, 1993). Thus, it may mediate the connection between the US and CS in the heliothine moths.

In behavioural experiments we have started to study the importance of olfactory learning in moths and weevils. In moths, conditioning of the PER is used to determine the ability to learn the identified odorants, including concentration dependency and salience (H.T. Skiri *et al.*, unpublished data). By differential conditioning, the ability to discriminate between primary and secondary odorants are revealed, e.g. whether the moths may more easily distinguish different primary odorants than primary and secondary odorants activating the same ORNs, as indicated in the study by Skiri *et al.* The perception and discrimination of single odorants and mixtures are investigated in further experiments on PER conditioning. In another learning study of the pine weevil (*Hylobius abietis*), multiple-choice experiments are used to investigate how previous experience with food materials influences the preference for an odour. The weevils were found to change their preference for two attractive enantiomers, depending on the experience with different food materials (O. Roten *et al.*, personal communication). The enantiomeric content in the food plants correlated with the preferred enantiomer. Thus, olfactory learning in addition to the innate responses to odorants may play a significant role for host attraction in the pine weevil as well.

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