The Journal of Experimental Biology 215, 1542-1551

doi:10.1242/jeb.068064

RESEARCH ARTICLE

Olfactory coding in five moth species from two families

Sonja Bisch-Knaden[1,*], Mikael A. Carlsson[2], Yuki Sugimoto[1], Marco Schubert[1,†], Christine Mißbach[1], Silke Sachse[1,‡] and Bill S. Hansson[1,‡]

[1]Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, D-07745 Jena, Germany and [2]Department of Zoology, Stockholm University, SE-106 91 Stockholm, Sweden

*Author for correspondence (sbisch-knaden@ice.mpg.de)

†Present address: Department of Biology, Chemistry and Pharmacy, Institute of Biology/Neurobiology, Free University Berlin, D-14195 Berlin, Germany

‡These authors contributed equally to this work

Accepted 16 January 2012

SUMMARY

The aim of the present study was to determine what impact phylogeny and life history might have on the coding of odours in the brain. Using three species of hawk moths (Sphingidae) and two species of owlet moths (Noctuidae), we visualized neural activity patterns in the antennal lobe, the first olfactory neuropil in insects, evoked by a set of ecologically relevant plant volatiles. Our results suggest that even between the two phylogenetically distant moth families, basic olfactory coding features are similar. But we also found different coding strategies in the moths' antennal lobe; namely, more specific patterns for chemically similar odorants in the two noctuid species than in the three sphingid species tested. This difference demonstrates the impact of the phylogenetic distance between species from different families despite some parallel life history traits found in both families. Furthermore, pronounced differences in larval and adult diet among the sphingids did not translate into differences in the olfactory code; instead, the three species had almost identical coding patterns.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/215/9/1542/DC1

Key words: calcium imaging, olfactory glomeruli, odour coding.

INTRODUCTION

Among animals from remote phyla, the organization of olfactory systems is strikingly similar with regard to reception, transduction and initial processing centres (Eisthen, 2002; Hildebrand and Shepherd, 1997). In the case of insects, odorant molecules are detected by odorant receptors present in the dendritic part of olfactory sensory neurons (OSNs) on the antennae. The olfactory information is then transferred *via* the axons of OSNs to the antennal lobe (AL), the first olfactory processing centre of the insect brain. The AL consists of a specific number of glomeruli, structural and functional units in which synaptic contacts between OSNs and output neurons take place, modulated by mostly inhibitory local interneurons. All OSNs expressing the same type of odorant receptor usually converge on the same glomerulus in the AL, as has been shown for the fruit fly *Drosophila melanogaster* (Gao et al., 2000; Vosshall et al., 2000). If the odorant receptors were strictly specific, the number of receptor types would limit the number of odorants that the animal is able to detect. However, most odorant receptors respond to a variety of generally chemically related molecules, i.e. the receptive range of different receptors partly overlaps (Hallem and Carlson, 2006). Therefore, each odorant activates a unique combination of receptors and thereby glomeruli (Malnic et al., 1999). This combinatorial coding strategy allows insects to discriminate among an almost unlimited number of volatiles.

In this study we asked whether odour-evoked activity patterns in the AL depend on the particular lifestyle of a given species or whether coding strategies follow phylogenetic relationships. Recent comparative studies revealed OSNs with mainly conserved receptive ranges or conserved representation patterns of odorants in the first olfactory neuropil across species, with only little impact of species-specific life histories. In these studies, however, only species belonging to the same family [Nymphalidae (Carlsson et al., 2011; Ômura and Honda, 2009)], subfamily [Heliothinae (Rostelien et al., 2005; Stranden et al., 2003); Murinae (Johnson et al., 2009; Soucy et al., 2009)], or genus [*Drosophila* (de Bruyne et al., 2010; Stensmyr et al., 2003)] were investigated. Remarkable similarities in olfactory coding were also found across the ant *Camponotus fellah*, the bee *Apis mellifera* and the rat *Rattus norvegicus*, i.e. between species belonging to different families (ant *versus* bee), or even to different phyla (ant *versus* rat) (Dupuy et al., 2010). However, only a small set of straight-chain aliphatic compounds was tested. Between *D. melanogaster* (Drosophilidae) and *Anopheles gambiae* (Culicidae), in contrast, divergent coding strategies were found using a large range of chemically diverse odorants (Carey et al., 2010). In this case, however, the impact of different feeding and oviposition sites could not be untangled from the phylogenetic distance between flies and mosquitoes.

To address our research question, the choice of suitable species was therefore crucial. We decided to use moths because these insects have a conserved and small number of olfactory glomeruli, thereby facilitating cross-species comparisons [moth ALs usually contain 60–70 glomeruli (see e.g. Couton et al., 2009; Grosse-Wilde et al., 2011; Kazawa et al., 2009)]. As most moths are active at dusk and at night, they rely mainly on olfactory cues, although vision does play a role for some species (Balkenius et al., 2006). Moreover, as females have to accomplish more complex olfactory tasks than males

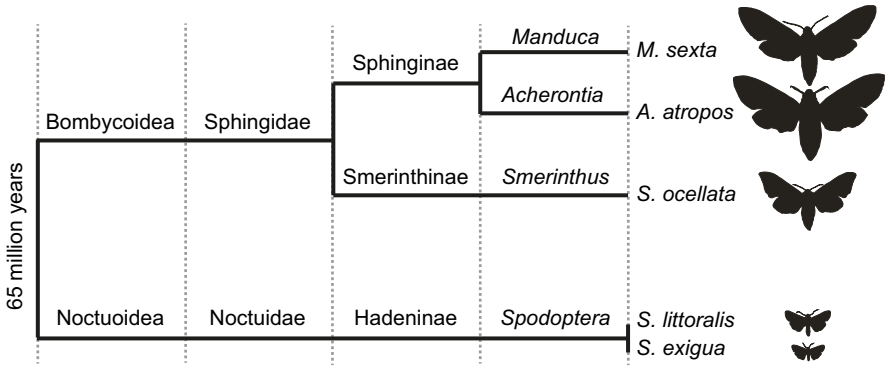

Fig. 1. Phylogenetic relationships between the moth species studied. The schematic dendrogram is based on previous studies (Kawahara et al., 2009; Kristensen and Skalski, 1999). Silhouettes of the species are drawn proportionally; for actual wingspans, see Table 1.

– finding foraging sites for themselves as well as suitable host plants for their herbivorous larvae – we tested only female moths. We chose five species belonging to the phylogenetically distant families Sphingidae (the tobacco hornworm *Manduca sexta*, the death's-head hawk moth *Acherontia atropos* and the eyed hawk moth *Smerinthus ocellata*) and Noctuidae (the Egyptian cotton leafworm *Spodoptera littoralis* and the beet armyworm *Spodoptera exigua*; Fig. 1). Sphingidae and Noctuidae have been separated for 65 million years (Kristensen and Skalski, 1999) and some of their features differ according to foraging and oviposition, whereas other life history parameters are similar between members of the two families (Table 1).

Another important issue was the choice of odorous stimuli. Plants emit distinct blends of volatile compounds from both leaves (Visser, 1986) and flowers (Knudsen and Tollsten, 1993). We used 14 common plant-released volatiles from five chemical classes to study coding patterns across moth species. Furthermore, we quantified the physico-chemical properties of our olfactory stimuli by using more than 1000 mathematical descriptors (see Materials and methods), and were thus able to relate the physico-chemical similarity of odorants to their neural response similarity in moths.

Possible outcomes of our comparative study could be formulated based on either the phylogeny of the tested species or differences in their life histories (larval host plant range, adult diet, activity phase). Phylogenetic relationships imply that different coding strategies exist in each of the two distantly related families. Within the three sphingid species, *S. ocellata* might have slightly different features because it belongs to a different subfamily than *M. sexta* and *A. atropos*, whereas coding patterns in the two noctuid species were expected to be very similar as they belong to the same genus (Fig. 1). Regarding the range of larval host plants, the biggest discrepancy might again be expected between the two moth families, as *S. littoralis* and *S. exigua* larvae are wide generalists feeding on 49 and 37 plant families, respectively, whereas *A. atropos* (27 families), *M. sexta* (10 families) and *S. ocellata* (seven families) larvae are more restrictive in their food choice (Table 1). However, host plants widely overlap between species (e.g. all 10 host-plant families of *M. sexta* are also exploited by *S. littoralis* and/or *S. exigua*); only *S. ocellata* and *M. sexta* have mutually exclusive host ranges (supplementary material Fig. S1), suggesting that these two sphingid species might have differing coding patterns. With regard to the diet of the imagos, both *Spodoptera* spp. and *M. sexta* share the same trophic niche, i.e. they feed on floral nectar, whereas *A. atropos* is a food parasite on honey from beehives. *Smerinthus ocellata*, however, does not feed after emergence and therefore is exceptional among the five moth species. If we instead compare the daily activity phase of our moths, only *M. sexta* is regularly foraging before sunset and after sunrise, whereas the other species

Table 1. Life history and morphological parameters of the species studied

	Manduca sexta	*Acherontia atropos*	*Smerinthus ocellata*	*Spodoptera littoralis*	*Spodoptera exigua*
Main larval host-plant families	Solanaceae	Solanaceae, Bignoniaceae, Verbenaceae	Salicaceae, Rosaceae	Polyphagous (Leguminosae, Compositae, Malvaceae, Solanaceae, etc.)	Polyphagous (Leguminosae, Graminae, Compositae, Malvaceae, Amaranthaceae, Solanaceae, etc.)
Number of known host-plant families (genera)[a]	10 (27)[b]	27 (54)[b,c,d]	7 (11)[b,d]	49 (100)[b,e]	37(104)[b,e]
Diet of the imagos	Floral nectar	Honey	Non-feeding	Floral nectar	Floral nectar
Wingspan (cm)	9–12	10–13	7–9	3–4	2.5–3
Width of the AL (μm)[f]	477±38	554±34	363±46	229±29	191±28
No. of glomeruli	69–71[g]	65–68[h]	64–65[h]	60–63[i]	69[h]

AL, antennal lobe.
[a]For a detailed list of host plants and their overlap between moth species, see supplementary material Fig. S1.
[b]Robinson et al., 2010.
[c]Attie et al., 2010.
[d]Pittaway, 1993.
[e]Brown and Dewhurst, 1975.
[f]Largest width of the AL as seen in the imaging experiments (mean ± s.d., *N*=6–12).
[g]Grosse-Wilde et al., 2011.
[h]See supplementary material Fig. S2.
[i]Couton et al., 2009.

are strictly nocturnal (Leppla et al., 1979; Pittaway, 1993; Theobald et al., 2010). Therefore, *M. sexta* might have particular coding strategies as it depends on visual cues in addition to olfactory cues when searching for foraging and oviposition sites. Furthermore, the olfactory code might be basically similar across all five moth species because the coding of odorants is supposed to be widely determined by the physico-chemical properties of odorous molecules (Haddad et al., 2008), and because of the structural similarity of the moths' ALs.

To our knowledge, this is the first detailed comparative study of olfactory coding strategies within a group of phylogenetically closely and more distantly related species. Using functional brain imaging, we recorded the response of AL glomeruli to ecologically relevant odorants in five moth species. We showed that basic features of the olfactory code were similar across species, and that the most prominent difference observed might reflect the phylogenetic distance between sphingid and noctuid moths. Among the three sphingids, the crepuscular hawk moth *M. sexta* had a particular coarse coding strategy, which might parallel the higher weight this moth gives to visual *versus* olfactory cues. Divergent larval host plant ranges or dietary demands of the imagos, however, did not seem to translate into different olfactory coding strategies.

MATERIALS AND METHODS

Animals

Manduca sexta (Linnaeus 1763), *Spodoptera littoralis* Boisduval 1833 and *Spodoptera exigua* (Hübner 1808) were reared in the laboratory on species-specific artificial diet based on corn, wheat and beans, respectively. *Acherontia atropos* (Linnaeus 1758) and *Smerinthus ocellata* (Linnaeus 1758) larvae were purchased (Worldwide Butterflies, www.wwb.co.uk) and fed the leaves of privet and willow, respectively. It was previously shown for honeybees that different diets (winter bees *versus* summer bees, or bees from different hives with different food resources) did not change the coding of odorants (Galizia et al., 1998); a bias induced by feeding artificial or natural diets therefore seems unlikely. Female pupae of each species were kept separated in plastic boxes or paper bags and moths were tested 2 to 7 days after hatching.

Odorants

The olfactory stimuli were chosen because of their ecological significance to moths (i.e. common floral volatiles and ubiquitous odorants present in the vegetative headspace), and also because they were chemically diverse. We used 14 monomolecular odorants (Sigma-Aldrich, St Louis, MO, USA) from five chemical classes: methyl salicylate (CAS number: 119-36-8), phenyl acetaldehyde (122-78-1), hexanol (111-27-3), octanol (111-87-5), nonanol (143-08-8), hexanal (66-25-1), octanal (124-13-0), nonanal (124-19-6), 2-hexanone (591-78-6), 2-octanone (111-13-7), 2-nonanone (821-55-6), geraniol (106-24-1), (±)-linalool (78-70-6) and β-caryophyllene (87-44-5). Most of these odorants have been found to evoke responses in the antennae of female *M. sexta* and *S. littoralis* (Anderson et al., 1995; Fraser et al., 2003; Shields and Hildebrand, 2000) and to be behaviourally active in these two species (Daly et al., 2007; Fan and Hansson, 2001; Riffell et al., 2009). The main larval host-plant families of our sphingid species and most of the known hosts of the two *Spodoptera* species emit at least one of the tested odorants (The Pherobase, www.pherobase.com). Because the most common volatiles are the same in typical sphingid and noctuid hosts [(±)-linalool, β-caryophyllene, methyl salicylate, hexanol, nonanal and geraniol)], the volatiles we tested represent a subset of a wide range of ecologically significant olfactory cues. The odorants were diluted in mineral oil (Sigma-Aldrich) to a concentration of $1{:}10^3$ or $1{:}10^4$ (v/v). These concentrations were chosen to evoke specific glomerular responses, i.e. threshold responses (indicated by sparse activity patterns; see Fig. 2). Pilot experiments showed that stimulus concentrations of $1{:}10^5$ or lower did not consistently evoke responses, and higher concentrations ($\geq 1{:}10^2$) elicited activity in the majority of glomeruli for some odorants and were thus not meaningful for our purpose.

Physico-chemical properties of the odorants tested

To quantify the physico-chemical properties of our odorants, we fed the three-dimensional structure of each odorant molecule (generated in MDL SDFiles format, PubChem, http://pubchem.ncbi.nlm.nih.gov/) into software for molecular descriptor calculations (Dragon 5.5, Talete, http://www.talete.mi.it/). This software is able to compute 3224 values per molecule, but as airborne chemicals have a relatively low molecular weight and thus cover only a small portion of chemical space; 1919 of these descriptors had constant values for each of the test odorants and were therefore not meaningful. The remaining 1305 values per molecule were normalized (*z*-score), and pairwise correlations between the odorants were calculated based on these descriptors to determine their physico-chemical similarity.

Preparation and staining

Moths were gently pushed into a 15 ml plastic tube (sphingids) or in a 1000 µl pipette tip (noctuids) with the tip cut open. The protruding head at the narrow end was fixed with dental wax. Labial palps and probosci were also fixed or removed to reduce movement artefacts. A window was cut in the head capsule between the compound eyes, and the tissue covering the brain was removed to uncover the ALs. A fluorescent calcium indicator (Calcium Green-2 AM, Invitrogen, Carlsbad, CA, USA) was dissolved in physiological saline solution (Christensen and Hildebrand, 1987) with 6% Pluronic F-127 (Invitrogen) to a concentration of 30 µmol. The exposed brain was incubated with 20 µl of this solution at 4°C. The dye was accumulated by OSNs that contribute largely to the neural circuits of a glomerulus but are shown to actively expel dye molecules (Manzini and Schild, 2003), and also by glial cells that tightly surround each glomerulus and retain the fluorescent dye. Glial cells respond with an influx of Ca^{2+} upon odorant stimulation of OSNs projecting to the respective glomerulus (Heil et al., 2007), thus leading to an increase of fluorescence at the site and reflecting the activity of OSNs. After incubation for 90 min, the brain was rinsed several times with physiological saline to remove excessive dye.

Optical imaging

The imaging setup was controlled by the software Tillvision 4.0 (Till Photonics, Munich, Germany) and consisted of a CCD camera (Sensicam Qe, PCO, Kelheim, Germany) mounted to an upright microscope (Olympus BX51WI, Hamburg, Germany) with a water immersion objective (Olympus, 10×/0.30 for sphingids and 20×/0.95 for noctuids). Calcium Green™-2 was excited at 475 nm (500 nm SP; xenon arc lamp, Polychrome V, Till Photonics) and fluorescence was detected at 490/515 nm (DCLP/LP). Fourfold symmetrical binning resulted in an image size of 344×260 pixels with 1 pixel corresponding to 2.5×2.5 µm (sphingids) or 1.25×1.25 µm (noctuids).

Six microlitres of the diluted odorants were applied to a circular piece of filter paper (12 mm diameter, Whatman, Dassel, Germany). Filter papers were inserted into glass pipettes and were

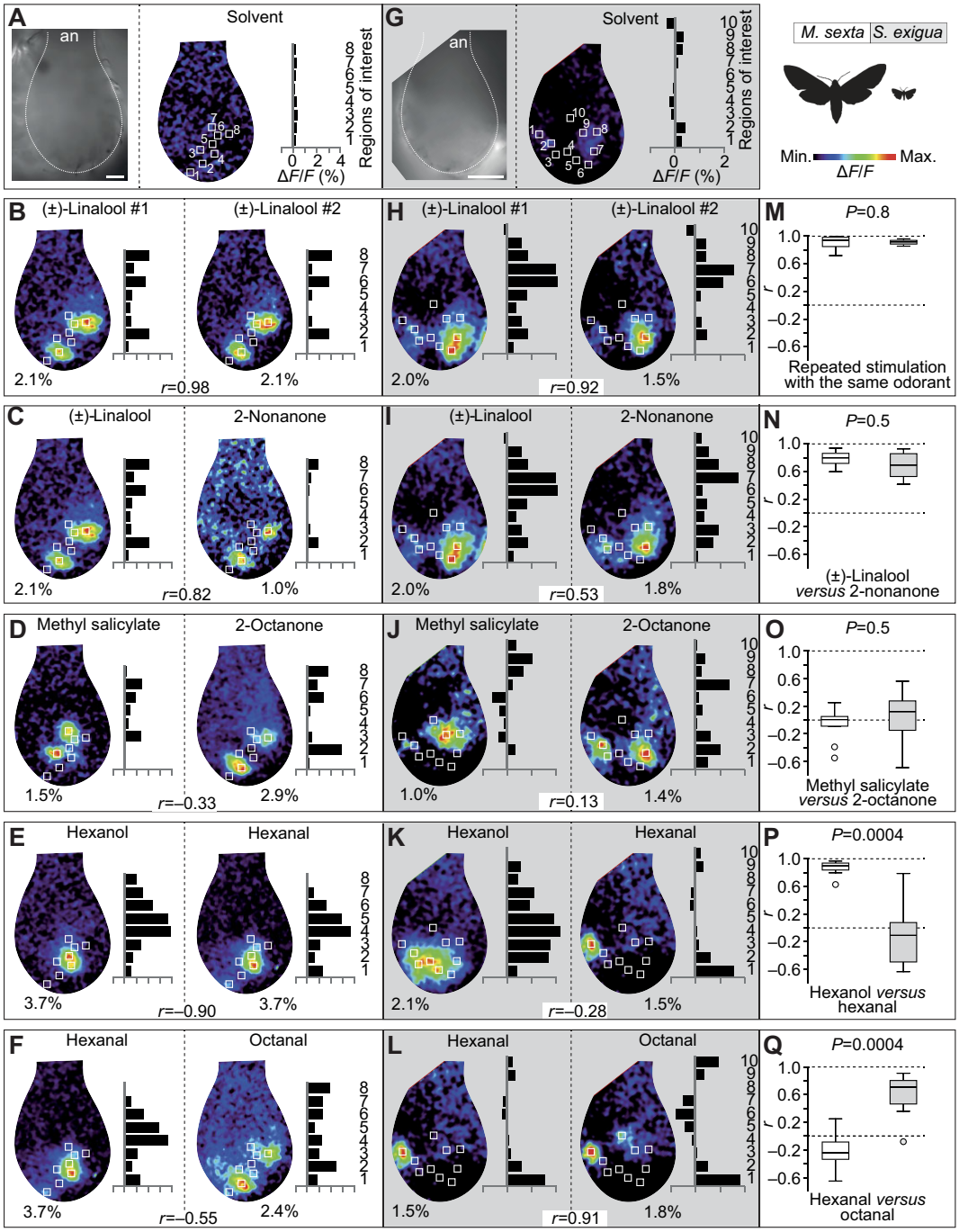

Fig. 2. Calcium imaging of odour-evoked neural activity in the moth antennal lobe (AL). Recordings from the left AL of *Manduca sexta* (A–F) and *Spodoptera exigua* (G–L), and a summary of the results for these two example species (M–Q). (A,G) Left: view of the AL under fluorescent illumination. an, Entrance of the antennal nerve; white line, contour of the AL. Scale bar, 100 μm. Right: stimulation with the solvent mineral oil did not increase calcium activity in the AL; white squares with numbers, regions of interest, i.e. regions of increased activity evoked by any of the 14 tested odorants in this particular animal; bar graph, response to the solvent expressed as the relative increase of fluorescence over background fluorescence ($\Delta F/F$) at the regions of interest. (B,H) Repeated stimulations with (±)-linalool [(±)-linalool #1 and (±)-linalool #2] in the same animal evoked congruent activity patterns. *r*, Correlation coefficient between the two glomerular activity patterns. False colour-coded images were generated by subtracting the frame before stimulus onset from the frame with the maximum response; calcium activity was normalized for each individual image and colour-coded (see colour bar; the maximum $\Delta F/F$ is given close to the bottom of each AL image. (M) Responses to repeated stimulations with the same odorant in *M. sexta* (six odorants) and *S. exigua* (four odorants), respectively. (C,I) Stimulations with (±)-linalool (left) and 2-nonanone (right) resulted in similar activity patterns. (D,J) Methyl salicylate (left) and 2-octanone (right) activated non-overlapping AL regions. (E,K) Hexanol (left) and hexanal (right) evoked similar activity patterns in *M. sexta* but not in *S. exigua*. (F,L) Hexanal (left) and octanal (right) activated similar regions in *S. exigua* but dissimilar regions in *M. sexta*. (N–Q) Summary of the correlation coefficients between odorant pairs shown in C–F and I–L from all tested *M. sexta* (N=10–12) and *S. exigua* (N=6–8) individuals, respectively. Boxplots show the median correlation coefficients of a pair of odorants (horizontal line in the box), the 25th and 75th percentiles (lower and upper margins of the box) together with the minimum and maximum values (whiskers), and outliers (circles) in *M. sexta* (white boxes) and *S. exigua* (grey boxes); the Mann–Whitney *U*-test was used to test for differences between species.

renewed every day. The immobilized moth was placed upright under the microscope. A glass tube was directed to one antenna (5 mm diameter; ending 10 to 15 mm from the tip of the antenna), delivering a constant stream of clean, moistened air (1 l min^{-1}). Two glass pipettes were inserted through small holes in the tube. One pipette (inserted 5.5 cm from the end of the tube) was empty and added clean air to the continuous airstream (0.1 l min^{-1}). This airstream could automatically be switched (Stimulus Controller CS-55, Syntech, Kirchzarten, Germany) to the second pipette (inserted 3.5 cm from end of tube), which contained an odorant-laden filter paper.

One odorant stimulation experiment lasted 10 s and was recorded with a sampling rate of 4 Hz using the following protocol: 2 s clean airstream, 2 s odorant airstream and 6 s clean airstream. The odorants were presented first at the low (1:10^4) and then at the high (1:10^3) concentration, with an interval of at least 1 min between stimulations. The animal's response to the lower stimulus concentration was usually analyzed; the response to the higher concentration was chosen if the lower one did not evoke a response. Additionally, two to three stimulations with the solvent were carried out, alternating with the odorant stimulations. The sequence of stimulations was changed from animal to animal. In one to four moths per species, some of the odorant stimulations were repeated after the set of odorants was completed to test for the reproducibility of the evoked activity patterns.

Analysis of optical imaging data

The imaging data were processed with custom-written software (IDL, ITT Visual Information Solutions, Gilching, Germany) to enhance the signal-to-noise ratio. First, background activity was defined as the average fluorescence (F) before stimulus onset, and was subtracted from the fluorescence of each frame. This background-corrected value (ΔF) was divided by the background fluorescence to obtain the relative changes of fluorescence over background fluorescence for each frame ($\Delta F/F$). Second, to compensate for bleaching, we subtracted from each frame an exponential decay curve that was estimated from the bleaching course of the frames before and after the stimulus and the response. Third, a spatial median filter with a width of 7 pixels was applied to remove outliers. Finally, possible shifts of the brain between the experiments were adjusted by aligning the middle frame of each stimulation experiment to the middle frame of a reference stimulation experiment with the help of the outline of the AL and the remains of tracheae.

Increased neural activity upon odorant stimulation led to spatially restricted spots of increased fluorescence in the AL. In the centre of each activity spot, the average $\Delta F/F$ was recorded in an area smaller than a small glomerulus (sphingids: 38×38 μm; noctuids: 19×19 μm; white squares, Fig. 2). Time traces of $\Delta F/F$ were averaged over three successive frames for each activity spot. In these smoothed time traces, the maximum $\Delta F/F$ after stimulus onset was determined. The average of the maximum value and the value before and after the maximum was calculated and was termed the response of the animal to the odorant stimulation at the given activity spot. In each animal, the responses were normalized to the maximal response and were considered for evaluation if they reached >30% of the maximal value in at least one activity spot.

Histology

The ALs of three out of the five moth species studied were investigated for the first time (*A. atropos*, *S. ocellata* and *S. exigua*); therefore, one to three ALs per species were reconstructed and the glomeruli were counted (supplementary material Fig. S2).

Autofluorescence (*A. atropos*)

For *A. atropos*, brain sections were prepared using autofluorescence. Animals were dissected in ice-cold phosphate-buffered saline (PBS; 0.1 mol, pH 7.4) and their brains were fixed in a mixture of formaldehyde (26% v/v), acetic acid (7%) and ethanol (67%; Roth, Karlsruhe, Germany) for 4 days at 4°C. The brains were washed several times in PBS and fixed in 4% paraformaldehyde for 4 days at 4°C (Kuebler et al., 2010). After repeated washing, the brains were fixed in 4% glutaraldehyde (Electron Microscopy Sciences, Munich, Germany) for 4 days at 4°C. After several changes of PBS, the brain was dehydrated in an ascending ethanol series (50, 70, 80, 90, 96 and three times at 100%, for 10 min each) and cleared in methyl salicylate (Sigma-Aldrich). The brains were scanned in metal objective slices in methyl salicylate and stored in methyl salicylate.

Immunohistochemistry (*S. ocellata* and *S. exigua*)

For *S. ocellata* and *S. exigua*, brain sections were prepared using immunohistochemistry. The moths' brains were dissected in ice-cold PBS and fixed in 4% paraformaldehyde (Roth) in PBS overnight at 4°C. After fixation the brains were washed several times with PBS for at least 4 h followed by pre-incubation in PBS containing 0.3% Triton X-100 (PBST; Sigma-Aldrich) and 1% bovine serum albumin (Sigma-Aldrich) for 24 h at 4°C. Afterwards the brains were incubated in primary antiserum (3C11, anti SYNORF1 concentrate, 372 μg ml^{-1}, 1:10^3 in PBST; Developmental Studies Hybridoma Bank, University of Iowa) for 3–4 days at 4°C. The monoclonal mouse anti-*Drosophila* synapsin SYNORF1 antibody was raised against a *D. melanogaster* GST-synapsin fusion protein and recognizes at least four synapsin isoforms (ca. 70, 74, 80 and 143 kDa) in western blots of *Drosophila* head homogenates (Klagges et al., 1996). The antibody was also used in moths to selectively label neuropil areas (Couton et al., 2009; El Jundi et al., 2009). After incubation in primary antiserum, brains were washed several times with PBS for 4 h at room temperature and then incubated in secondary antiserum containing conjugated Alexa Fluor 488 (1:500, Invitrogen) for 3–4 days at 4°C. The brains were then washed several times with PBS for 4 h and mounted in embedding material (MOWIOL, Calbiochem, Darmstadt, Germany) using several layers of spacer slides (Grace Bio-Labs, Bend, OR, USA) to prevent compression of brains.

Image acquisition and three-dimensional reconstruction

Pictures of brain sections were taken with a laser-scanning microscope (Zeiss LSM 510 Meta, Jena, Germany). ALs were scanned at a 1024×1024 pixel resolution in the x–y direction and 1 μm in the z direction using an argon laser (488 nm). According to the size of the AL, we used a 10× water immersion objective (*A. atropos*, Zeiss C-APOCHROMA, 10×/0.45), a 20× air objective (*S. ocellata*, Zeiss PLAN-APOCHROMAT, 20×/0.8) or a 40× water immersion objective (*S. exigua*, Zeiss C-APOCHROMA, 40×/1.2 W Korr UV-VIS-IR). Glomeruli were reconstructed and counted in Amira 4.1.1 (Visage Imaging, Berlin, Germany) using the same dimensions (voxel size) as the corresponding image stacks; for water immersion objectives the z direction was corrected with the factor 1.2. The outlines of the glomeruli were traced in the LabelField module and surfaces were generated by the SurfaceGen module in Amira 4.1.1.

Statistical analysis

Statistical tests were performed with PAST (Palaeontological Statistics, http://folk.uio.no/ohammer/past/), Instat (GraphPad

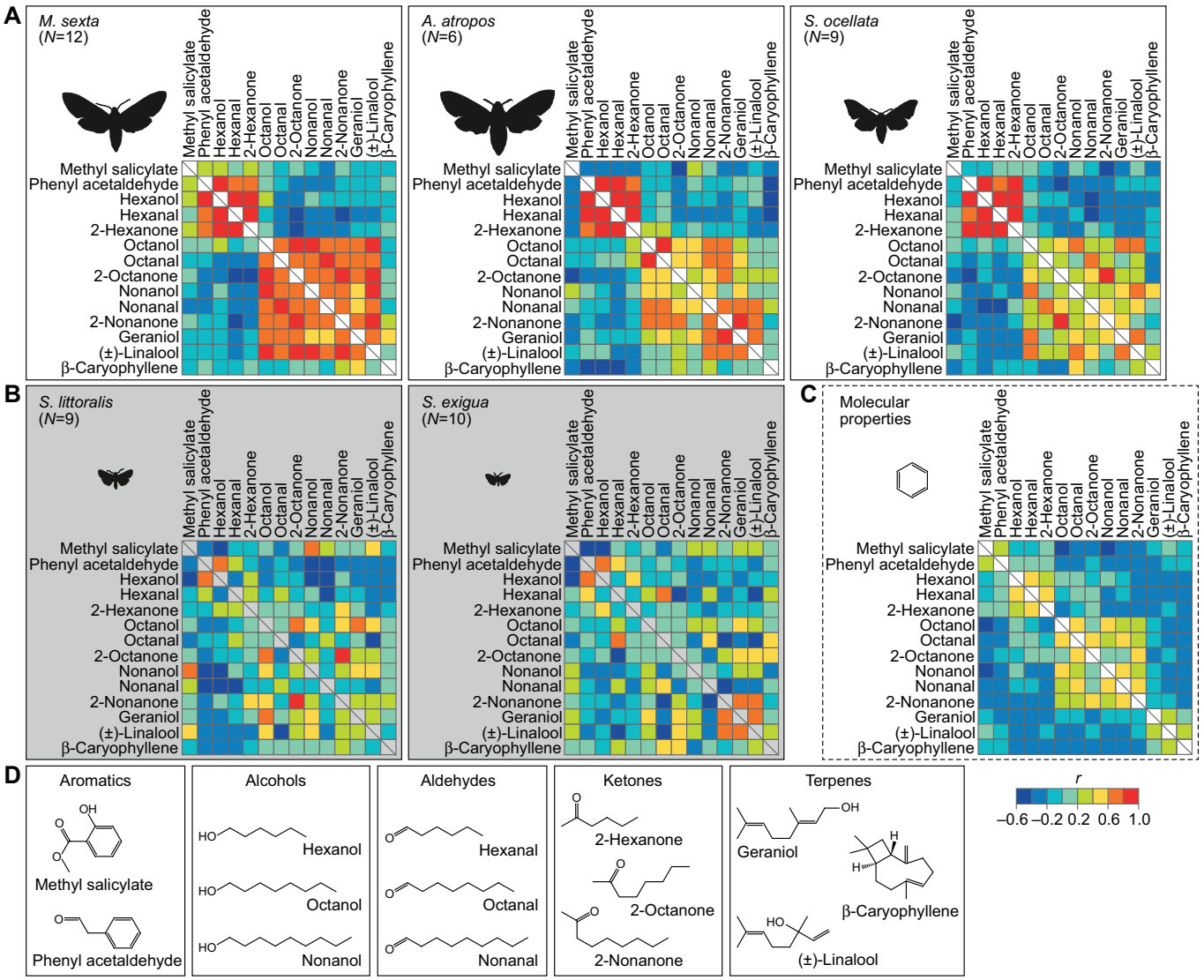

Fig. 3. Neural response similarities in moths and molecular similarities of the tested odorants. Sphingid moths (A) and noctuid moths (B) were exposed to 14 volatiles in succession. In each animal, the evoked glomerular activity patterns in the AL were compared for any pair of odorants, resulting in one correlation coefficient per odorant pair. The median correlation coefficients for each odorant pair in each species are displayed in a matrix; the colour-coded values range from blue (exclusive activity pattern) to red (congruent activity pattern). (C) Similarity between pairs of odorants from our experimental set based on the physico-chemical properties of the molecules. Both enantiomers of linalool had very similar molecular properties (r=0.75) and yielded identical similarity matrices, and were therefore not listed separately. For the calculation of molecular descriptors, see the Materials and methods. (D) Structural formulas of the tested odorants.

Software, La Jolla, CA, USA) and XLSTAT (Addinsoft, New York, NY, USA) with a significance level of α=0.05.

RESULTS

Comparing neural activity patterns among species

We recorded the neural representation of plant volatiles in the female moth's AL of five moth species from two phylogenetically remote families (Fig. 1) using functional brain imaging. Although varying considerably in size – the average width ranged from 191 µm in *S. exigua* (Noctuidae) to 554 µm in *A. atropos* (Sphingidae) – the structure of the ALs was comparable in the five moth species studied: it consisted of a similar number of glomeruli (60 to 71; Table 1) arranged in a single layer around a central fibre core. From a dorsofrontal view, the same view as that observed during the imaging experiments, the number of accessible glomeruli was 20 to 24 in each species, corresponding to 30 to 40% of the glomeruli of one AL (Carlsson et al., 2002; Grosse-Wilde et al., 2011) (supplementary material Fig. S2). Because this percentage is higher than that from imaging experiments performed with honeybees (24%), in which odorant identity could consistently be predicted from the activity patterns across the accessible subset of glomeruli (Galizia et al., 1999), conclusions can be drawn about odour coding strategies in different species.

Each of the 14 odorants induced a mosaic of activated glomeruli in a moths' AL. As glomerular contours were invisible under fluorescent illumination (Fig. 2A), we first determined all glomerulus-sized regions (see Materials and methods) that showed increased activity after stimulation with any of the tested odorants in a particular animal. The activity in each of these regions of interest was then calculated for each odorant separately to obtain the odorant-

specific glomerular activity pattern. The observed activity patterns were sparse (Fig. 2B–F,H–L), as we stimulated the moths with low odorant concentrations. To check the reproducibility of these patterns, we repeatedly stimulated individuals with the same odorants and correlated the resulting spatial response patterns. We found that stimulations with the same odorant evoked very similar responses (Fig. 2B,H), showing that the observed patterns were consistent within an individual during an experiment (median correlation coefficient of repeated stimulations: r=0.93, range: r=0.72 to 0.99, N=10 animals with a total of 36 repeated stimulations). However, if we correlated the activity patterns evoked by different odorants, we found not only values resembling those obtained by repeated stimulations with the same odorant, but also negative correlation coefficients, indicating the presence of almost exclusive sets of activated glomeruli (Fig. 2C–F,I–L). Different species had sometimes similar (Fig. 2C,D,I,J) and sometimes opposite responses for pairs of odorants (Fig. 2E,F,K,L). After correlating the activity patterns evoked by all possible combinations of odorants (91 pairwise comparisons) within each individual, we calculated the corresponding median values for each species (Fig. 2M–Q). To visualize our results, these median correlation coefficients were colour-coded and displayed in one similarity matrix per species (Fig. 3A,B).

Differences in glomerular activity patterns between moth species

A first visual inspection of the similarity matrices revealed a striking difference between species belonging to the two moth families: many olfactory representation patterns were rather similar in each of the three sphingid species (orange and red cells, Fig. 3A), whereas in each of the two noctuids, more distinct patterns were found (green and blue cells, Fig. 3B). One group of odorants with consistently similar representations was conspicuous across all the three sphingid species but less similar in the two noctuids: the aromatic phenyl acetaldehyde and the short-chain aliphatic compounds hexanol, hexanal and 2-hexanone, respectively (median correlation coefficient in sphingid species: r=0.82–0.86; in *S. littoralis* and *S. exigua*: r=0.23 and 0.25; Fig. 4A). Furthermore, stimulations with aliphatic compounds with eight and nine carbon atoms (octanol, octanal, 2-octanone, nonanol, nonanal and 2-nonanone), and the terpenes geraniol and (±)-linalool evoked remarkably similar activity patterns in the AL of *M. sexta* (r=0.75), and at least partly overlapping activity patterns in *A. atropos* (r=0.46) and *S. ocellata* (r=0.38). In each of the two noctuid species, however, the corresponding representation patterns were more dissimilar than in the sphingid species (*S. littoralis*: r=0.28, *S. exigua*: r=0.18; Fig. 4B).

Physico-chemical properties of the tested odorants

Do the more similar representations of odorants in the three sphingids, especially pronounced in *M. sexta*, imply an unusually coarse olfactory resolution? To answer this question, we examined the nature of the tested odorants in detail by using chemometric tools, i.e. a large set of mathematical values that comprehensively describe the physico-chemical properties of molecules. These properties include simple qualities such as chain length, functional group and molecule shape, which are commonly used to classify chemicals (Fig. 3D), but in addition numerous molecular descriptors consider characteristics such as hydrophobicity, connectivity, topological charge, etc. Based on these mathematical descriptors that we computed for each of our odorants, we calculated the physico-chemical similarity between each pair of odorants, colour-coded the resulting correlation coefficients, and displayed them in a matrix (Fig. 3C). When comparing this matrix with the neural response similarity matrices of each moth species (Fig. 3A,B), we found that molecular similarity reflected the ‘unspecific’ neural responses of sphingids. The ‘specific’ patterns of noctuids, however, were only weakly predicted by the molecular properties of the odorants (molecular properties *versus* neural response patterns in *M. sexta*: r=0.53, *A. atropos*: r=0.46, *S. ocellata*: r=0.53; P<0.0001 in all three species; molecular properties *versus S. littoralis*: r=0.22, P=0.03 and *S. exigua*: r=0.14, P=0.06; Mantel test, Spearman rank correlation).

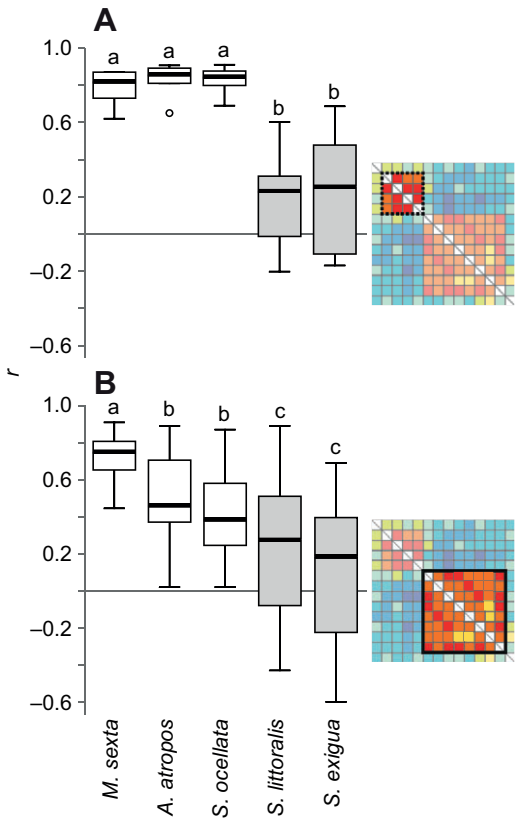

Fig. 4. Differences in odour-evoked activity patterns across species. Correlation coefficients within two groups of odorant pairs that evoked strikingly similar activity patterns in the antennal lobe of *M. sexta* were compared among all five species. (A) Summary of six pairwise comparisons between phenyl acetaldehyde, hexanol, hexanal and 2-hexanone (dotted outline in the colour-coded matrix to the right of the boxplots; cf. Fig. 3A); (B) Summary of 28 pairwise comparisons between octanol, octanal, 2-octanone, nonanol, nonanal, 2-nonanone, geraniol and (±)-linalool (solid outline in the matrix to the right). Different letters depict significant (repeated-measures ANOVA, P<0.05) differences between moth species (white boxes: sphingid species; grey boxes: noctuid species); for boxplot conventions, see legend to Fig. 2.

Basic similarities in olfactory coding across moth species

To further investigate the representation of odorants in the moths’ brains, we reduced the dimensions of our data by a principal component analysis (PCA). Thus each odorant was no longer defined by its pairwise similarity to all the other odorants (13 dimensions) but was described by the first two principal components (PC1 and PC2, two dimensions) that were plotted for each moth species and for the physico-chemical properties of the odorant molecules

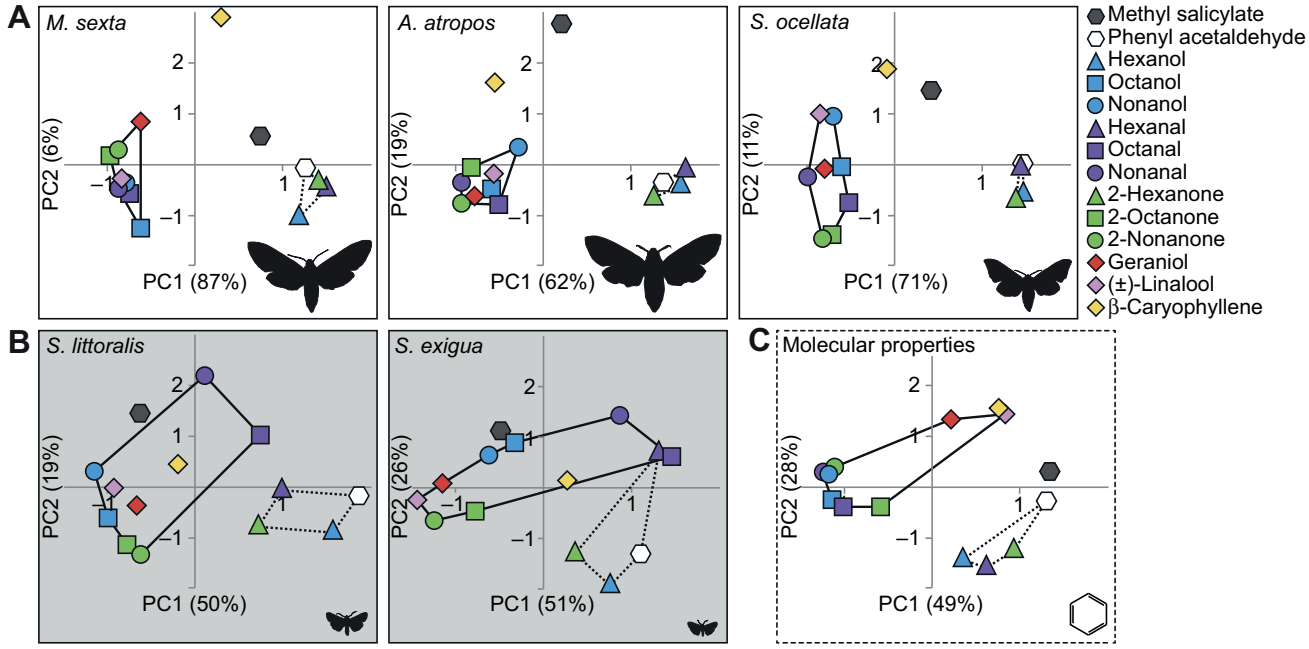

Fig. 5. Mapping of odorants in neural and physico-chemical space. Principal component analysis (PCA) of the tested odorants based on neural response similarities in sphingids (A) and noctuids (B), and on the physico-chemical properties of the odorant molecules (C). The first two principal components are shown; values in parentheses depict the proportion of the observed variance in the data that was explained by PC1 and PC2. Solid and dotted lines enclose two groups of odorants that had more similar representations in sphingids than in noctuids [cf. Fig. 4A,B; dotted line: phenyl acetaldehyde, hexanol, hexanal, 2-hexanone; solid line: octanol, octanal, 2-octanone, nonanol, nonanal, 2-nonaonone, geraniol, (±)-linalool]. The PCAs were based on the values shown in Fig. 3A–C.

(Fig. 5). In these scatterplots, the proximity of odorants implies similar neural representation (Fig. 5A,B), or similar molecular properties (Fig. 5C). Across sphingid species, two groups of odorants were tightly clustered (connected with solid and dotted lines, respectively), indicating their similar neural representation in each of the three species (compare orange and red cells in Fig. 3A; see also Fig. 4). Most of the tested odorants were similarly distributed when analyzed according to their physico-chemical properties. The only obvious exceptions were geraniol and (±)-linalool (red and pink diamond symbols), which have different physico-chemical properties than eight- and nine-carbon aliphatics (circles and squares), but which are mapped close to them in the neural space of each of the five moth species. In the two *Spodoptera* species, odorants were generally mapped farther apart from each other, pointing to more distinct odorant representations in the ALs of *S. littoralis* and *S. exigua*. The representations of octanal and nonanal (purple square and circle) were especially different between noctuids and sphingids. Nonetheless, the basic spatial arrangement of the 14 odorants along PC1, which explained 50 to 87% of the variance in the data, was remarkably similar across species (pairwise correlation of PC1 between sphingid species: r=0.83 to 0.89, P<0.0002; between noctuid species: r=0.78, P=0.001; between species from different families: r=0.53 to 0.65, P<0.05, Spearman rank correlation). This underlying correspondence indicates similar basic strategies of olfactory coding in each of the five moth species.

DISCUSSION

We studied the representation of plant-derived odorants in the brain of closely and distantly related moth species (Fig. 1). The method we used, calcium imaging, allowed us to monitor neural activity in the AL evoked by a set of 14 volatiles *in vivo* (Fig. 2). The basic mapping of these odorants in neural space was comparable across the five species (Fig. 5), indicating similar coding strategies between the two moth families. This is in accordance with the results of previous comparative studies in Lepidoptera (Stranden et al., 2003; Rostelien et al., 2005; Omura and Honda, 2009; Carlsson et al., 2011). These studies, however, were restricted to closely related species within one family or subfamiliy, and hence did not allow any conclusions about general olfactory coding strategies between families.

Besides this basic correspondence, we found that several odorants evoked very similar glomerular activity patterns in the ALs of each of the three sphingid moth species studied (*M. sexta*, *A. atropos* and *S. ocellata*), whereas in the two noctuid species *S. littoralis* and *S. exigua*, the patterns were more dissimilar and thus more odorant-specific (Figs 3, 4). However, the physico-chemical properties of the tested odorants correlated only weakly with the discrete responses of the two *Spodoptera* species but rather predicted the coarse coding patterns found in the sphingid moths. This result indicates that *Spodoptera* spp. appear to have a particularly pronounced ability to separate chemically similar odorants in their ALs. Accordingly, single-sensillum recordings revealed only narrowly tuned odorant receptor types in *S. littoralis* and other noctuid species (Anderson et al., 1995; Rostelien et al., 2005; Stranden et al., 2003), but both narrowly and broadly tuned receptor profiles in *M. sexta* (Shields and Hildebrand, 2000). A higher number of tested species representing each of the sphingid and noctuid subfamilies would of course be necessary to determine whether this difference is a consistent family-specific feature or only accidental, based on an unintentional bias in the selection of species.

If two odorants evoke very similar activity patterns in the AL, as observed particularly in the three sphingid species, are these odorants also perceived as being similar? In a behavioural discrimination task, *M. sexta* trained to octanol were not able to

distinguish this odorant from nonanol (Daly et al., 2001). Accordingly, the two odorants evoked almost identical representation patterns in the ALs of *M. sexta* (r=0.85; Fig. 3A). In contrast, *M. sexta* could easily discriminate hexanol from octanol (Daly et al., 2001), an odorant pair that elicited distinct activity patterns (r=0.26; Fig. 3A). Such a correspondence between neural response similarity and perceptual similarity was also found in honeybees (Guerrieri et al., 2005), rats (Youngentob et al., 2006) and fish (Valentincic et al., 2005). In light of these findings, we hypothesize that the three sphingid species studied here might have poorer olfactory discrimination abilities than *S. littoralis* and *S. exigua*, because the sphingids – especially *M. sexta* – had less distinct odour-evoked neural representation patterns. It would be interesting to test this hypothesis in a comparative behavioural experiment.

The two noctuid species use a similar and broad range of host plants (supplementary material Fig. S1), and both feed on floral nectar; therefore, we expected similar coding strategies to be found in these two sister species. Among sphingids, however, the overall high similarity in olfactory coding was surprising because phylogenetic relationships (*S. ocellata* is placed in a different subfamily than *M. sexta* and *A. atropos*) and, notably, life histories are diverse: the larval host plant range is much larger in *A. atropos* than in the other two species, larvae of *M. sexta* and *S. ocellata* exploit mutually exclusive host plants (*M. sexta* and *A. atropos* share three host plant families; *S. ocellata* and *A. atropos* have four host plant families in common), and the imagos of the three species all have particular dietary requirements. However, none of these differences was reflected in species-specific olfactory coding patterns in the moths' brain. An analogous result was reported in an electroantennogram study from several species of butterflies (Nymphalidae) that differ in their foraging preferences (floral nectar *versus* rotten fruits). Although the butterflies clearly preferred odorants that matched their feeding habits, the sensitivity to odorants from both food sources was similar across species (Ômura and Honda, 2009). Furthermore, a comparison of the molecular receptive range of OSNs among *Drosophila* species from diverse environments and with distinct feeding preferences revealed a high level of conservation (Stensmyr et al., 2003), showing that despite being adapted to different ecological niches, closely related animals might still share the same olfactory coding strategies.

The only species-specific feature that we found was an especially low olfactory resolution in the AL of *M. sexta* (Fig. 4), a crepuscular animal, which in turn has a higher spatial and temporal visual acuity than the strictly nocturnal *A. atropos* (Theobald et al., 2010). Furthermore, although *M. sexta* requires both visual and olfactory stimuli to forage from flowers, it clearly prefers the visual target to the odour source when given a choice between the two decoupled stimuli (Goyret et al., 2007; Raguso and Willis, 2005). This higher weighting of visual as compared with olfactory cues in *M. sexta* together with its rather unspecific olfactory coding patterns for plant volatiles lead to the hypothesis that the fineness of olfactory resolution might differ between crepuscular/diurnal and nocturnal species. To test this hypothesis, a further comparative study is needed that includes species with differing activity phases, preferably from each of the three sphingid subfamilies.

ACKNOWLEDGEMENTS

We thank Mathias Ditzen for programming the analysis tools; Frank David and Céline Heinl for initial help with imaging experiments; Angelika Berg, Sabine Kaltofen and Elisabeth Marling for rearing the moths; Just Vlak and Lutz Thilo Wasserthal for providing some of the *S. exigua* and *A. atropos* pupae; Leslie Vosshall and Ian T. Baldwin for comments on the manuscript; and Emily Wheeler for editorial assistance.

FUNDING

This research was supported by the Max Planck Society and by the German Federal Ministry of Education and Research (BMBF).

REFERENCES

Anderson, P., Hansson, B. S. and Lofqvist, J. (1995). Plant-odour-specific receptor neurones on the antennae of female and male *Spodoptera littoralis*. *Physiol. Entomol.* **20**, 189-198.
Attie, M., Kitching, I. J. and Veslot, J. (2010). Patterns of larval hostplant usage among hawkmoths (Lepidoptera, Sphingidae) from La Réunion, with a comparison of the Mascarenes with other regions of the world. *Terre Vie* **65**, 3-44.
Balkenius, A., Rosen, W. and Kelber, A. (2006). The relative importance of olfaction and vision in a diurnal and a nocturnal hawkmoth. *J. Comp. Physiol. A* **192**, 431-437.
Brown, E. S. and Dewhurst, C. F. (1975). The genus *Spodoptera* (Lepidoptera, Noctuidae) in Africa and the Near East. *Bull. Entomol. Res.* **65**, 221-262.
Carey, A. F., Wang, G. R., Su, C. Y., Zwiebel, L. J. and Carlson, J. R. (2010). Odorant reception in the malaria mosquito *Anopheles gambiae*. *Nature* **464**, 66-77.
Carlsson, M. A., Galizia, C. G. and Hansson, B. S. (2002). Spatial representation of odours in the antennal lobe of the moth *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Chem. Senses* **27**, 231-244.
Carlsson, M. A., Bisch-Knaden, S., Schaepers, A., Mozuraitis, R., Hansson, B. S. and Janz, N. (2011). Odour maps in the brain of butterflies with divergent host-plant preferences. *PLoS ONE* **6**, e24025.
Christensen, T. A. and Hildebrand, J. G. (1987). Male-specific, sex pheromone-selective projection neurons in the antennal lobes of the moth *Manduca sexta*. *J. Comp. Physiol. A* **160**, 553-569.
Couton, L., Minoli, S., Kieu, K., Anton, S. and Rospars, J.-P. (2009). Constancy and variability of identified glomeruli in antennal lobes: computational approach in *Spodoptera littoralis*. *Cell Tissue Res.* **337**, 491-511.
Daly, K. C., Chandra, S., Durtschi, M. L. and Smith, B. H. (2001). The generalization of an olfactory-based conditioned response reveals unique but overlapping odour representations in the moth *Manduca sexta*. *J. Exp. Biol.* **204**, 3085-3095.
Daly, K. C., Carrell, L. A. and Mwilaria, E. (2007). Detection versus perception: physiological and behavioral analysis of olfactory sensitivity in the moth (*Manduca sexta*). *Behav. Neurosci.* **121**, 794-807.
de Bruyne, M., Smart, R., Zammit, E. and Warr, C. G. (2010). Functional and molecular evolution of olfactory neurons and receptors for aliphatic esters across the *Drosophila* genus. *J. Comp. Physiol. A* **196**, 97-109.
Dupuy, F., Josens, R., Giurfa, M. and Sandoz, J. C. (2010). Calcium imaging in the ant *Camponotus fellah* reveals a conserved odour-similarity space in insects and mammals. *BMC Neuroscience* **11**, 28.
Eisthen, H. L. (2002). Why are olfactory systems of different animals so similar? *Brain Behav. Evol.* **59**, 273-293.
El Jundi, B., Huetteroth, W., Kurylas, A. E. and Schachtner, J. (2009). Anisometric brain dimorphism revisited: Implementation of a volumetric 3D standard brain in *Manduca sexta*. *J. Comp. Neurol.* **517**, 210-225.
Fan, R.-J. and Hansson, B. S. (2001). Olfactory discrimination conditioning in the moth *Spodoptera littoralis*. *Physiol. Behav.* **72**, 159-165.
Fraser, A. M., Mechaber, W. L. and Hildebrand, J. G. (2003). Electroantennographic and behavioral responses of the sphinx moth *Manduca sexta* to host plant headspace volatiles. *J. Chem. Ecol.* **29**, 1813-1833.
Galizia, C. G., Nagler, K., Holldobler, B. and Menzel, R. (1998). Odour coding is bilaterally symmetrical in the antennal lobes of honeybees (*Apis mellifera*). *Eur. J. Neurosci.* **10**, 2964-2974.
Galizia, C. G., Sachse, S., Rappert, A. and Menzel, R. (1999). The glomerular code for odor representation is species specific in the honeybee *Apis mellifera*. *Nat. Neurosci.* **2**, 473-478.
Gao, Q., Yuan, B. B. and Chess, A. (2000). Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe. *Nat. Neurosci.* **3**, 780-785.
Goyret, J., Markwell, P. M. and Raguso, R. A. (2007). The effect of decoupling olfactory and visual stimuli on the foraging behavior of *Manduca sexta*. *J. Exp. Biol.* **210**, 1398-1405.
Grosse-Wilde, E., Kuebler, L. S., Bucks, S., Vogel, H., Wicher, D. and Hansson, B. S. (2011). Antennal transcriptome of *Manduca sexta*. *Proc. Natl. Acad. Sci. USA* **108**, 7449-7454.
Guerrieri, F., Schubert, M., Sandoz, J.-C. and Giurfa, M. (2005). Perceptual and neural olfactory similarity in honeybees. *PLoS Biol.* **3**, 718-732.
Haddad, R., Khan, R., Takahashi, Y. K., Mori, K., Harel, D. and Sobel, N. (2008). A metric for odorant comparison. *Nat. Methods* **5**, 425-429.
Hallem, E. A. and Carlson, J. R. (2006). Coding of odors by a receptor repertoire. *Cell* **125**, 143-160.
Heil, J. E., Oland, L. A. and Lohr, C. (2007). Acetylcholine-mediated axon-glia signaling in the developing insect olfactory system. *Eur. J. Neurosci.* **26**, 1227-1241.
Hildebrand, J. G. and Shepherd, G. M. (1997). Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. *Ann. Rev. Neurosci.* **20**, 595-631.
Johnson, B. A., Xu, Z., Ali, S. S. and Leon, M. (2009). Spatial representations of odorants in olfactory bulbs of rats and mice: similarities and differences in chemotopic organization. *J. Comp. Neurol.* **514**, 658-673.
Kawahara, A. Y., Mignault, A. A., Regier, J. C., Kitching, I. J. and Mitter, C. (2009). Phylogeny and biogeography of hawkmoths (Lepidoptera: Sphingidae): evidence from five nuclear genes. *PLoS ONE* **4**, e5719.
Kazawa, T., Namiki, S., Fukushima, R., Terada, M., Soo, K. and Kanzaki, R. (2009). Constancy and variability of glomerular organization in the antennal lobe of the silkmoth. *Cell Tissue Res.* **336**, 119-136.
Klagges, B. R. E., Heimbeck, G., Godenschwege, T. A., Hofbauer, A., Pflugfelder, G. O., Reifegerste, R., Reisch, D., Schaupp, M., Buchner, S. and Buchner, E.

(1996). Invertebrate synapsins: A single gene codes for several isoforms in *Drosophila*. *J. Neurosci.* **16**, 3154-3165.
Knudsen, J. T. and Tollsten, L. (1993). Trends in floral scent chemistry in pollination syndromes – floral scent composition in moth-pollinated taxa. *Bot. J. Linn. Soc.* **113**, 263-284.
Kristensen, N. P. and Skalski, A. W. (1999). Phylogeny and palaeontology. In *Handbook of Zoology IV/35: Lepidoptera, Moths and Butterflies*, Vol. 4 (ed. M. Fischer), pp. 7-25. Berlin, New York: Walter de Gruyter.
Kuebler, L. S., Kelber, C. and Kleineidam, C. J. (2010). Distinct antennal lobe phenotypes in the leaf-cutting ant (*Atta vollenweideri*). *J. Comp. Neurol.* **518**, 352-365.
Leppla, N. C., Hamilton, E. W., Guy, R. H. and Lee, F. L. (1979). Circadian rhythms of locomotion in six noctuid species. *Ann. Entomol. Soc. Am.* **72**, 209-215.
Malnic, B., Hirono, J., Sato, T. and Buck, L. B. (1999). Combinatorial receptor codes for odors. *Cell* **96**, 713-723.
Manzini, I. and Schild, D. (2003). Multidrug resistance transporters in the olfactory receptor neurons of *Xenopus laevis* tadpoles. *J. Physiol.* **546**, 375-385.
Ômura, H. and Honda, K. (2009). Behavioral and electroantennographic responsiveness of adult butterflies of six nymphalid species to food-derived volatiles. *Chemoecology* **19**, 227-234.
Pittaway, A. R. (1993). *The Hawkmoths of the Western Palaearctic*. Colchester: Harley Books.
Raguso, R. A. and Willis, M. A. (2005). Synergy between visual and olfactory cues in nectar feeding by wild hawkmoths, *Manduca sexta*. *Anim. Behav.* **69**, 407-418.
Riffell, J. A., Lei, H., Christensen, T. A. and Hildebrand, J. G. (2009). Characterization and coding of behaviorally significant odor mixtures. *Curr. Biol.* **19**, 335-340.
Robinson, G. S., Ackery, P. R., Kitching, I. J., Beccaloni, G. W. and Hernández, L. M. (2010). *HOSTS – A Database of the World's Lepidopteran Hostplants*. London: Natural History Museum. http://www.nhm.ac.uk/research-curation/research/projects/hostplants/.
Rostelien, T., Stranden, M., Borg-Karlson, A. K. and Mustaparta, H. (2005). Olfactory receptor neurons in two heliothine moth species responding selectively to aliphatic green leaf volatiles, aromatic compounds, monoterpenes and sesquiterpenes of plant origin. *Chem. Senses* **30**, 443-461.
Shields, V. D. C. and Hildebrand, J. G. (2000). Responses of a population of antennal olfactory receptor cells in the female moth *Manduca sexta* to plant-associated volatile organic compounds. *J. Comp. Physiol. A* **186**, 1135-1151.
Soucy, E. R., Albeanu, D. F., Fantana, A. L., Murthy, V. N. and Meister, M. (2009). Precision and diversity in an odor map on the olfactory bulb. *Nat. Neurosci.* **12**, 210-220.
Stensmyr, M. C., Dekker, T. and Hansson, B. S. (2003). Evolution of the olfactory code in the *Drosophila* melanogaster subgroup. *Proc. R. Soc. Lond. B* **270**, 2333-2340.
Stranden, M., Rostelien, T., Liblikas, I., Almaas, T. J., Borg-Karlson, A. K. and Mustaparta, H. (2003). Receptor neurones in three heliothine moths responding to floral and inducible plant volatiles. *Chemoecology* **13**, 143-154.
Theobald, J. C., Warrant, E. J. and O'Carroll, D. C. (2010). Wide-field motion tuning in nocturnal hawkmoths. *Proc. R. Soc. Lond. B* **277**, 853-860.
Valentincic, T., Miklavc, P., Dolensek, J. and Plibersek, K. (2005). Correlations between olfactory discrimination, olfactory receptor neuron responses and chemotopy of amino acids in fishes. *Chem. Senses* **30**, I312-I314.
Visser, J. H. (1986). Host odor perception in phytophagous insects. *Ann. Rev. Entomol.* **31**, 121-144.
Vosshall, L. B., Wong, A. M. and Axel, R. (2000). An olfactory sensory map in the fly brain. *Cell* **102**, 147-159.
Youngentob, S. L., Johnson, B. A., Leon, M., Sheehe, P. R. and Kent, P. F. (2006). Predicting odorant quality perceptions from multidimensional scaling of olfactory bulb glomerular activity patterns. *Behav. Neurosci.* **120**, 1337-1345.