Chapter I

Odor concentration coding in the antennal lobe of *Drosophila melanogaster*

Summary

We have analyzed the processing of odor and concentration information in the antennal lobe (AL) of *Drosophila melanogaster*. Olfactory information encoded in the combinatorial pattern of activated olfactory sensory neurons (OSN) is integrated and processed within the insect antennal lobe (AL), analogue to the vertebrate olfactory bulb. Here, several networks of local neurons (LN) interact with OSNs and with output neurons - insect projection neurons (PN), analogue to vertebrate mitral cells - which in turn project to higher order brain centers. The extent and form of information processing taking place in the AL of *Drosophila* is far from being understood.

We have therefore performed *in vivo* recordings of odor-evoked spatio-temporal activity patterns in four neuron populations of the fly AL. Using a genetically encoded calcium sensor (G-CaMP), we have optically recorded odor induced activity from OSNs, two distinct LN populations and PNs. We have found that (1) the spatio-temporal patterns of odor responses were odor and concentration specific across animals in all neuron types. (2) Increasing odor concentration generally lead to stronger glomerular responses, both excitatory and inhibitory, and (3) increasing odor concentration also evoked responses in more glomeruli. (4) Spatial response patterns in OSNs and PNs were clearly glomerular. (5) LN responses were also spatially structured but non-glomerular, and were broadly distributed over the AL. (6) Responses in the two LN subpopulations differed, suggesting that they posses diverse functional roles. (7) The activity transfer from OSNs to PNs was odor and glomerulus dependent, showing that interglomerular interactions shape PN responses.

Introduction

Olfactory stimuli provide vital information for most animals, which rely on odor cues to find food sources or mating partners but also to avoid dangers. The environment of an animal is populated with thousands of substances which need to be detected, categorized and identified

for appropriate behavior to be produced. These tasks are fulfilled by the olfactory system, which receives chemical information and translates it into a neural code that allows the brain to produce adequate behavioral responses.

The first olfactory structure in the insect brain is the antennal lobe (AL), analogue to the vertebrate olfactory bulb (OB). In *Drosophila*, ~1320 olfactory sensory neurons (OSN) project from the third antennal segment and the maxillary palps into olfactory glomeruli of the AL (Stocker, 1994; Stocker, 2001). Each of the ~50 glomeruli receives input from OSNs expressing the same odorant receptor protein (OR) (Vosshall, 2000; Fishilevich and Vosshall, 2005; Couto et al., 2005). In each glomerulus, OSNs make synaptic contact with local interneurons (LN) and with projection neurons (PN). PNs send axons to higher-order brain areas, such as the mushroom bodies and the lateral protocerebrum. An estimated total of 150 PNs innervates the AL and most of them project their dendrites in only one glomerulus (Wong et al., 2002; Marin et al., 2002), where they make both input and output synapses (Malun et al., 1993; Distler and Boeckh, 1996; Distler et al., 1998; Ng et al., 2002). The total number of LNs innervating the AL has been estimated in ~100 (Stocker et al., 1997; Ng et al., 2002). Most of the characterized LNs arborize in many glomeruli homogeneously (Stocker et al., 1990). GABAergic (Ng et al., 2002) and cholinergic (Shang et al., 2007) LNs have been described.

Odors elicit characteristic spatio-temporal activity patterns across olfactory glomeruli both in insects (Galizia and Menzel, 2001) and in vertebrates (Korsching, 2001). These patterns are driven by the odor-response profiles of OSNs, given the orderly separation of OSN axons of the same class into individual glomeruli. Since the AL is not structured in layers as the vertebrate OB, most synaptic interactions take place within the AL glomeruli in insects (Sachse and Galizia, 2006). To a first approximation, odor response profiles of insect PNs innervating a given glomerulus correspond to the odor response profiles of the corresponding OSNs (Ng et al., 2002; Wang et al., 2003; Sachse and Galizia, 2003). However, a more detailed analysis shows that the AL network modulates odor responses: inhibitory interactions among glomeruli shape PN responses (Sachse and Galizia, 2002), and these connections are strongest among glomeruli with similar physiological properties (Linster et al., 2005). Also, excitatory interactions broaden the response profiles of some glomeruli (Wilson et al., 2004; Olsen et al., 2007), possibly through a mechanism mediated by excitatory interneurons (Shang et al., 2007).

In the natural environment of animals, odors occur in a wide range of concentrations. For example, a fruit fly might smell a banana from some distance in a strongly diluted odor

plume, and later sit on the banana mush. Insects are capable of extracting both odor-quality and odor-quantity (concentration) information from an olfactory stimulus (Borst, 1983; Ditzen et al., 2003). Therefore, an understanding of odor concentration coding is central to the understanding of odor coding in the AL. We have investigated how odor information is transferred across identified neuron populations (OSNs, LNs and PNs) in the fly AL over a wide range of odor concentrations. Our results confirm that odor information contained in the spatio-temporal activity patterns is mostly conserved between input and output of the AL network. However, odor specific modulations occur in some glomeruli, bringing further evidence of interglomerular interactions in the AL of *Drosophila*.

Materials and Methods

Flies

Adult female *Drosophila melanogaster* (age: 1-3 weeks) were used. Flies were reared in standard medium (100ml contained: 91.8 ml water, 11.8 g sugar beet syrup, 1.08 g yeast, 0.406 g agar, 9.4 g cornmeal, 0.24 ml propionic acid), and kept at 25°C, 50-60% relative humidity with a 12h:12h light:darkness regime.

All experimental flies contained the UAS:G-CaMP 1.3 insertion in the first chromosome (crossed from *w*P[UAS:G-CaMP];CyO/Sp;+ flies provided by Jing Wang, New York), and a promoter:Gal4 insertion to direct expression of the calcium sensor to the desired neuron subpopulation. OSN flies were of the following genotype: *yw*P[UAS:G-CaMP];P[OR83b:Gal4] (provided by Leslie Vosshall, New York). PN flies were of the genotype: *yw*P[UAS:G-CaMP];P[GH146:Gal4]/CyO (stable line crossed from +; P[GH146:Gal4] flies provided by Reinhard Stocker, Fribourg). LN1 flies were of the genotype *yw*P[UAS:G-CaMP];P[NP1227:Gal4] (crossed from +;P[NP1227:Gal4] provided by Kei Ito, Tokio]. LN2 flies were of the genotype *yw*P[UAS:G-CaMP]/P[NP2426:Gal4];+/CyO or *yw*P[UAS:G-CaMP]/P[NP2426:Gal4];+/Sp (crossed from P[NP2426:Gal4] flies provided by Kei Ito, Tokio). Since P[NP2426:Gal4] and P[UAS:G-CaMP] are located in the same chromosome we used the F1 generation for experiments. No differences were found in the performance of the two genotypes (Sp or CyO). P[NP1227:Gal4] and P[NP2426:Gal4] drive expression of Gal4 in two non-overlapping subpopulations of GABAergic LNs (Okada and Ito, unpublished observations).

Fly preparation

Flies were prepared as previously described (Pelz et al., 2006). Briefly, flies were immobilized on ice for ten minutes and then fixed to a Plexiglas stage by the head and neck, with thorax and abdomen hanging (Fig. 1.1A). Legs and abdomen were fixed to the stage with n-eicosane (Sigma Aldrich, Germany) to reduce movement. Antennae were pulled forward using fine wire (Rediohm-800, HP Reid Inc, USA) in order to allow visual access to the AL. Then, a thin polyethylene foil supported by a plastic coverslip was sealed to the head with two-component silicon (Kwik Sil, WPI, Germany) and a hole was cut to expose the cuticle. After placing a drop of Ringer´s (in mM: 130 NaCl, 5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 36 Saccharose, 5 Hepes, pH 7,3 (Estes et al., 1996)) on top of the head, a window in the cuticle was cut. Glands and airbags were removed, and saline was exchanged several times. Flies were now ready for imaging.

Odor preparation and application

50 µl of the pure odor substance were diluted in 5 ml mineral oil for the highest concentration tested (10^{-2} v/v). 10-fold serial dilutions were made, down to a concentration of 10^{-7} v/v. Throughout the text concentrations are expressed as potency of 10 and the indication v/v is omitted. All dilutions were prepared in 20 ml glass vials which were sealed with aluminum ring caps with a silicon-Teflon septum. All odors were purchased from Sigma-Aldrich, with maximum purity level available. New odor dilutions were prepared every 3-4 weeks. Odors used were isopentyl acetate, 1-butanol and propionic acid. 1-butanol 10^{-2} or 10^{-3} was applied repeatedly to monitor the quality of the signals during the experiment. Control measurements were done with clean air and mineral oil.

A constant air stream (60 ml/min) generated with a vibrating armature air compressor (Reischle Thomas, Switzerland) was directed to the fly antennae at a distance of ~1cm through a Teflon tube (1mm ID, VWR, Germany). Odors were applied with a computer controlled auto-sampler originally designed for gas chromatography (CombiPAL, CTC analytics, Switzerland) which injected 1 ml (1 ml/s) of odor-loaded headspace from the 20 ml vials into the Teflon tube 38 cm away from the tube exit. During odor application the constant air stream was interrupted to avoid mechanical artifacts due to increased air flow.

Imaging

Images were acquired with a CCD camera (TILL Photonics, Germany) mounted on a fluorescence microscope (Olympus BX51WI, Japan) equipped with a 20x water dip objective (NA 0.95, X LUM Plan FI, Olympus, Japan). Excitation light of 465 nm was produced with a monochromator (Polychrome II, TILL Photonics, Germany). A 490 nm dichroic mirror was used together with a 506-547 nm band pass emission filter. Binned image size was 153 x 106 pixels on chip, corresponding to 245 µm x 170 µm at the preparation. The exposure time was varied between 70 and 120 ms to adjust for different basal fluorescence values across preparations. Eleven-second films were recorded with an acquisition rate of 3 Hz. Odors were presented 3 s into each measurement. Inter-stimulus interval (ISI) was 2 minutes.
Because odor-responses could be influenced by previous experience, in particular due to adaptation, we made control experiments where we measured the responses to the odors in two concentration sequences: starting at low concentrations and going to higher ones ("increasing run"), and then starting at high concentrations and going down ("decreasing run"), with an ISI of 2 minutes. There were no differences between the "increasing" and the "decreasing" runs. We only found a weak adaptation effect for isopentyl acetate in glomerulus DM2 for OSNs and in glomeruli DM2 and X2 for PNs (slightly left-shifted concentration-response curve between increasing and decreasing runs). Thus, adaptation did not play an important role under our experimental conditions with an ISI of 2 minutes, and results shown correspond to the "increasing run".

Data analysis

Flies with reliable calcium responses to the reference odor 1-butanol [10^{-3} or 10^{-2}] throughout the protocol and no or negligible responses to air were considered for data analysis. Data were processed with custom made routines written in IDL (Research Systems, USA). First, films were corrected for movement artifacts using anatomical landmarks. Then a bleach correction was performed by fitting a logarithmic function of the form $F(x) = a \times e^{(bx)} + c$ to the average frame light intensity change over time. Frames covering 7 s after stimulus onset were excluded from the bleach correction calculation. After bleach correction the relative calcium change was calculated for each frame as $\Delta F/F_i$ (%) = $[(F_i-F_0)/F_0] \times 100$, where F_i is the

absolute fluorescence of the *i*th frame and F_0 the average fluorescence of the five frames before stimulus onset (background fluorescence).

To visualize signals, color coded images were calculated as the average fluorescence of 3 frames during stimulation minus the average fluorescence of the 3 frames before stimulus. A Gaussian filter was applied to these images (kernel = 3 pixels).

In order to identify glomeruli, a set of odors was presented at a concentration that only elicits responses in one or few known classes of OSN (information extracted from (de Bruyne et al., 2001; Hallem et al., 2004; Fishilevich and Vosshall, 2005; Couto et al., 2005). Glomerulus identification was also performed based on anatomical cues. Some glomeruli could be reliably identified in all flies, but not assigned to a glomerulus in the *Drosophila* AL atlas (Laissue et al., 1999) due to the lack in spatial resolution. The following landmark odors and concentrations were used for glomerulus identification: ethyl-hexanoate 10^{-6} for **DM2**, which is the glomerulus innervated by OSNs expressing OR22a and OR22b; pentyl acetate 10^{-6} for **DM3** (OR47a and OR33b) and E2-hexenal 10^{-4} for **DL5** (OR7a). Glomerulus **DL1** (OR10a) was identified due to its vicinity to DL5 and its response to isoamyl acetate (Wang et al., 2003). **VA1lm** (OR47b) was identified due to its shape and its location at the point where the antennal nerve enters the AL. Glomerulus **X1** was characterized by its response to 1-butanol and 1-hexanol, and might correspond to glomerulus DA2 or DA4 due to its position. Glomerulus **X2** was activated by 1-butanol and was located next to glomerulus DM5, which was identified by stimulation with ethyl-3-hydroxybutyrate 10^{-6}. Glomerulus DM5 was not included in the analysis because it did not respond to any of the three tested odors. Glomerulus X2 might correspond to VM2 due to its position. Glomerulus **X3** was characterized by its negative response to propionic acid in OSNs and the vicinity to a glomerulus activated by 1-butanol (X1). An additional glomerulus (X4) responded consistently to mineral oil in OSNs, but not in PNs. Although no tentative assignment can be made, it is likely that this glomerulus is not labeled by the GH146:Gal4 line and was therefore excluded from the analysis. Since LNs have multiglomerular arborizations and show non-glomerular activity patterns, the coarse region corresponding to a particular glomerulus was chosen due to its geometrical localization in the AL. Since responses in the LNs show no clear glomerular pattern, this coarse assignment should give a good approximation to the activity of the local neurons in the corresponding glomeruli.

For time traces, the average light intensity of a 7×7 pixel square was calculated for each glomerulus. Maximum responses shown in Figure 1.3 correspond to the normalized response value at the peak within 3 seconds after stimulus onset. In all other cases response amplitude

was calculated as the average of 3 frames during stimulation minus the average of 3 frames before stimulation. Since the initial responses (positive or negative) began within the stimulation time in all cases, this calculation was found to best reflect the changes in response amplitude.

For quantitative analysis and most figures, data were normalized to the response to 1-butanol 10^{-3} in glomerulus DM2 in each fly (Response[glomerulus$_i$, odor$_j$, conc$_x$, fly] = $\Delta F/F$[glomerulus$_i$, odor$_j$, conc$_x$, fly] / $\Delta F/F$[DM2, 1-butanol, 10^{-3}, fly]). Throughout the paper normalized calcium changes will be referred to as odor responses. Median responses and traces correspond to the median of n animals (one measurement per animal).

Responses were considered for fall time calculation if: 1) the initial response (first second after stimulus onset) was higher than 3*SD of the mean fluorescence measured in the first two seconds of the recording (before stimulus onset); and 2) if fluorescence decayed at least to 66% of the maximum value by the end of the measurement. Fall time was calculated as the time elapsed between 90% and 66% of the maximum value in the decaying phase of the response.

In Figure 1.4B a glomerulus was counted as activated if its response to odor stimulation was significantly different from the response to mineral oil (Wilcoxon Rank Sum Test).

Data analysis was performed with IDL (Research Systems, USA), Excel (Microsoft Office 2003), R (http://www.R-project.org), SigmaStat (SPSS Inc., USA) and ImageJ (http://rsb.info.nih.gov/ij/).

Controls

Air and mineral oil were presented as control stimuli. Air stimulation induced either no responses at all or very small responses (≤2.5% $\Delta F/F$). These responses were possibly due to a mechano-sensory stimulus artifact. Small responses to air were most frequently found in LN2 and PNs. Mineral oil elicited small responses in all neuron types, suggesting that in *Drosophila* some receptors are tuned to long-chain hydrocarbons. In OSNs mineral oil elicited moderate responses in glomeruli X4 (up to 18% $\Delta F/F$) and DL5 (up to 6% $\Delta F/F$), and negligible responses in all other glomeruli (up to 1% $\Delta F/F$). In PNs and LNs, mineral oil elicited small responses in several glomeruli, with calcium changes reaching maximum amplitudes of 3% (DM3), 4.3% (DL5) and 2% (DL5) $\Delta F/F$, in PN, LN1 and LN2, respectively.

Results

Calcium responses to 1-butanol, isopentyl acetate and propionic acid at six concentrations ranging from 10^{-7} to 10^{-2} were measured in the AL of female *Drosophila*. We imaged OSNs, two genetically defined types of LNs, and PNs using the Gal4-UAS system to drive expression of the calcium reporter G-CaMP (Nakai et al., 2001). The promoting region of the Or83b gene was used to stain ~60% of all OSNs (Larsson et al., 2004); the enhancer trap line GH146 was used to drive Gal4 expression in ~80-90 PNs (Stocker et al., 1997; Wong et al., 2002) and two non-overlapping GABAergic subpopulations of multiglomerular LNs were labeled with the enhancer trap lines NP1227 (LN1) and NP2426 (LN2). These enhancer-trap lines drive Gal4 expression in ~18 (LN1) and ~37 (LN2) non-overlapping GABAergic LNs that branch in all AL glomeruli. The arborization patterns within glomeruli differ: LN1 neurons make blebs in the glomerulus core while LN2 neurons arborize homogeneously within each glomerulus (Okada and Ito, unpublished observations).

Odor responses were measured in glomeruli located on the dorsal-anterior part of the AL. Eight out of the ~16 visible glomeruli responded reliably to our odor set across animals, and were selected for analysis (one additional glomerulus responded only to mineral oil and was excluded). For OSNs and PNs five glomeruli could be identified as DM2, DM3, DL5, DL1 and VA1lm (Laissue et al., 1999), while the remaining three glomeruli could be determined only tentatively. We therefore refer to these glomeruli as X1, X2, and X3 (see Materials and Methods). In the case of LNs, the approximate positions corresponding to these eight glomeruli were determined based on morphological cues.

We found highly stereotypical spatial odor responses across flies for each of the neuron types analyzed. In OSN and PN responses, activated glomeruli could be easily segmented (Fig. 1.1C). This is in correspondence with the uniglomerular arborizations of OSNs and PNs, as seen in Figure 1.1B. In contrast, odor induced activity in LNs was broader and not confined to glomerular boundaries (Fig. 1.1C), consistent with their multiglomerular morphology (Fig. 1.1B). Furthermore, we also found that the time courses of odor responses were conserved across animals, and calcium changes followed a temporal dynamic that was characteristic for each glomerulus and odor (Fig. 1.1D). Calcium changes reached up to 80% in OSNs and up to 50% in PNs for the highest concentrations in the strongest glomeruli. In LNs, calcium changes were smaller, reaching up to ~25% and ~10% $\Delta F/F$ in LN1 and LN2, respectively.

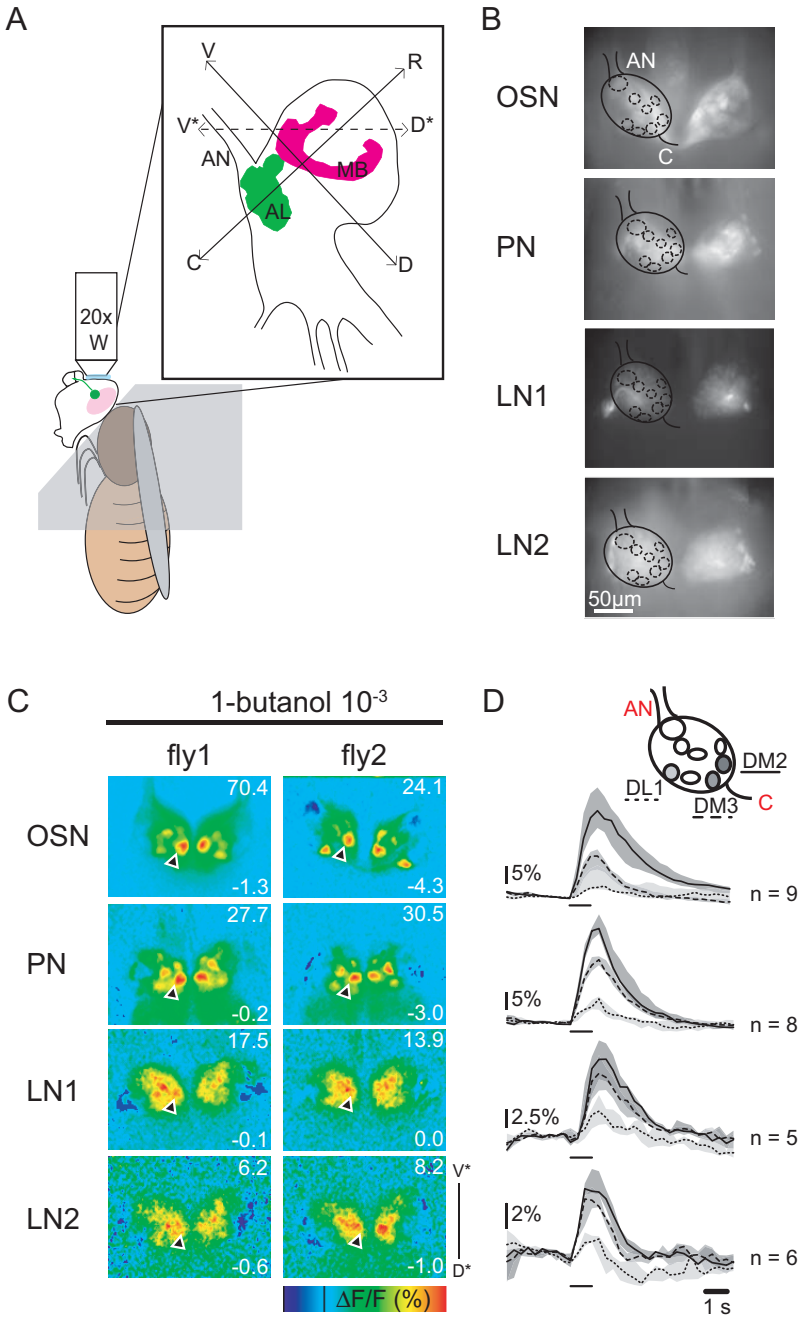

Figure 1.1 - Odor responses in the antennal lobe of *Drosophila*

(A) Lateral view of the preparation. The antennae are pulled forward to allow optical access to the antennal lobes. The frontal view of the antennal lobes obtained with this preparation is tilted with respect to the neural axis. Inset: AN: antennal nerve; AL: antennal lobe; MB: mushroom body; V* and D* stand for the ventral and dorsal direction tilted with respect to the neural axis. **(B)** Raw fluorescence view of the AL for the four measured neuron types (OSN, PN, LN1 and LN2). The left AL is overlaid with a schematic view of the AL (see Fig. 1.2 for details). These views correspond to fly1 in C. **(C)** Color-coded activity patterns evoked by 1-butanol 10^{-3}. Frontal view of the ALs of two flies per neuron population. The numbers on the top and bottom right corners indicate the maximum and minimum values used for scaling. The black line indicates the color corresponding to no relative fluorescence change. Arrowheads point to the position of glomerulus DM2. This glomerulus could be easily localized and identified in OSNs and PNs, its approximate location is marked for LN1 and LN2. **(D)** Median time traces of the odor responses of three glomeruli measured in n animals. Shadowed areas indicate the range between Q_{25} and Q_{75} (1^{st} and 3^{rd} quartiles). The time of odor stimulation (1 s) is indicated by the black bar below the traces. On the top right corner a schematic representation of the left AL shows the approximate position of all glomeruli measured in this study (filled glomeruli are DM2, DM3 and DL1, AN: antennal nerve, C: commissure).

Odor responses: Spatial aspects

Both in OSNs and PNs, isopentyl acetate, 1-butanol and propionic acid elicited activity patterns that included common and distinct glomeruli (Fig. 1.2). The strongest glomeruli in the response to isopentyl acetate were DM2, DM3 and X2. The strongest glomeruli in the response to 1-butanol were DM2, X1 and DL5. The response to propionic acid was characterized by calcium increase in DM2 and - in OSNs only – a calcium decrease in glomerulus X3 (Fig. 1.2).

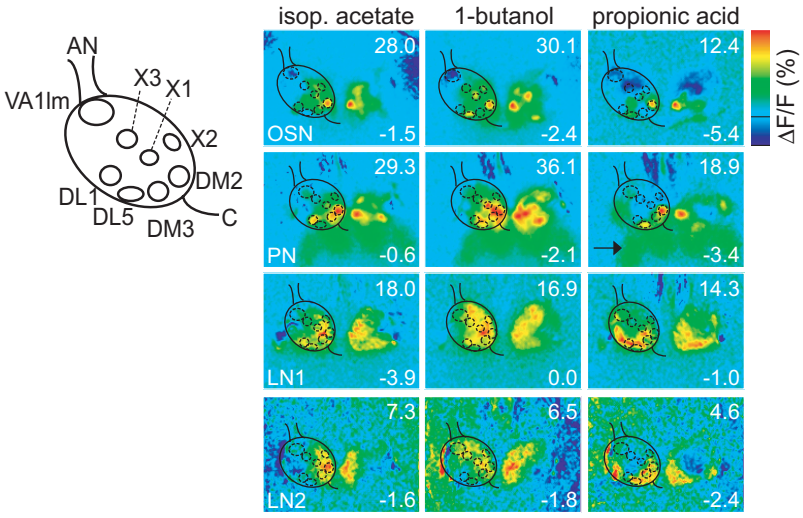

Figure 1.2 - Spatial response patterns are odor and neuron-type specific

Left: Diagram shows the position of the measured glomeruli in the AL. AN: Antennal nerve; C: commissure. Right: Responses to isopentyl acetate, 1-butanol and propionic acid in one fly for each neuron type. Numbers in the upper and lower right corners of each color-coded image correspond to the maximum and minimum of the scale for that picture. All odors were presented at a concentration of 10^{-3} in the OSNs, and 10^{-2} for the other neuron types. The black arrow in the PN response to propionic acid points to the activity in the γ/β lobe.

Spatial response patterns in LNs also differed among odors and among the two lines tested. Most importantly, the lack of a glomerular organization in LNs (see above) did not imply a lack of spatial organization. LN1 responses were spatially broader than LN2 responses for all three odors. Furthermore, while in some cases the location of LN activity corresponded with that of active glomeruli in OSNs and/or PNs, this was not the case for the whole AL. For example, the responses to propionic acid 10^{-2} in LN2 were located laterally (in

correspondence of glomerulus DL1), while the activity in LN1, OSNs and PNs was mostly focused in the medial region of the AL (glomerulus DM2, see Fig. 1.2).
In this study, we focused on the AL. However, enhancer-trap lines often label more neurons than those of specific interest. Apart from PNs, GH146:Gal4 also labels neurons outside the AL (Stocker et al., 1997). In addition to odor induced activity in the AL and the PN target areas in the mushroom body calyxes (Fiala et al., 2002) we found responses in two curved areas in the protocerebrum, which correspond to the γ/β lobe (see arrow in Fig. 1.2, propionic acid). These responses might originate from neurons that arborize in the γ/β lobe. We did not further analyze these signals.

Odor responses: Temporal aspects

Responses to 1-butanol and isopentyl acetate followed a monophasic time course with calcium concentration rising immediately after stimulus onset and decaying at or shortly after the end of the 1 s stimulus in OSNs and PNs (Fig. 1.3A, compare with Fig. 1.5). LN responses to 1-butanol and isopentyl acetate also showed a stimulus-correlated upstroke, but in some cases a calcium decrease below baseline was observed after stimulus offset (e.g. glomerulus DL1 in LN2, Fig. 1.3A).
In contrast, responses to propionic acid 10^{-2} showed more complex time courses in OSNs and PNs (Fig. 1.3B): there was a second response peak ~3 s after stimulus offset in glomerulus DL5; glomerulus X2 exhibited a delayed (1 s) long lasting response in OSNs, while a weak response in PNs was followed by a delayed long-lasting response. An initial calcium decrease in glomerulus VA1lm was followed by a slight overshoot in OSNs, and a strong response peak in PNs. Delayed response peaks in PNs were also found in the absence of complex ORN time courses (e.g. glomerulus DM3, Fig. 1.3B). The time course of LN1 responses to propionic acid did not follow the dynamic of OSN and PN responses and was monophasic across glomeruli (Figure 1.3AB). In contrast, responses of LN2 were multi-phasic, and showed an initial calcium increase upon stimulus onset, followed by a calcium concentration decrease 1-2 s after stimulus offset (Fig. 1.3AB, glomeruli X1 and X2). These undershoots in responses to propionic acid in LN2 were in temporal and sometimes in spatial correspondence with the second calcium increase peaks in PNs.

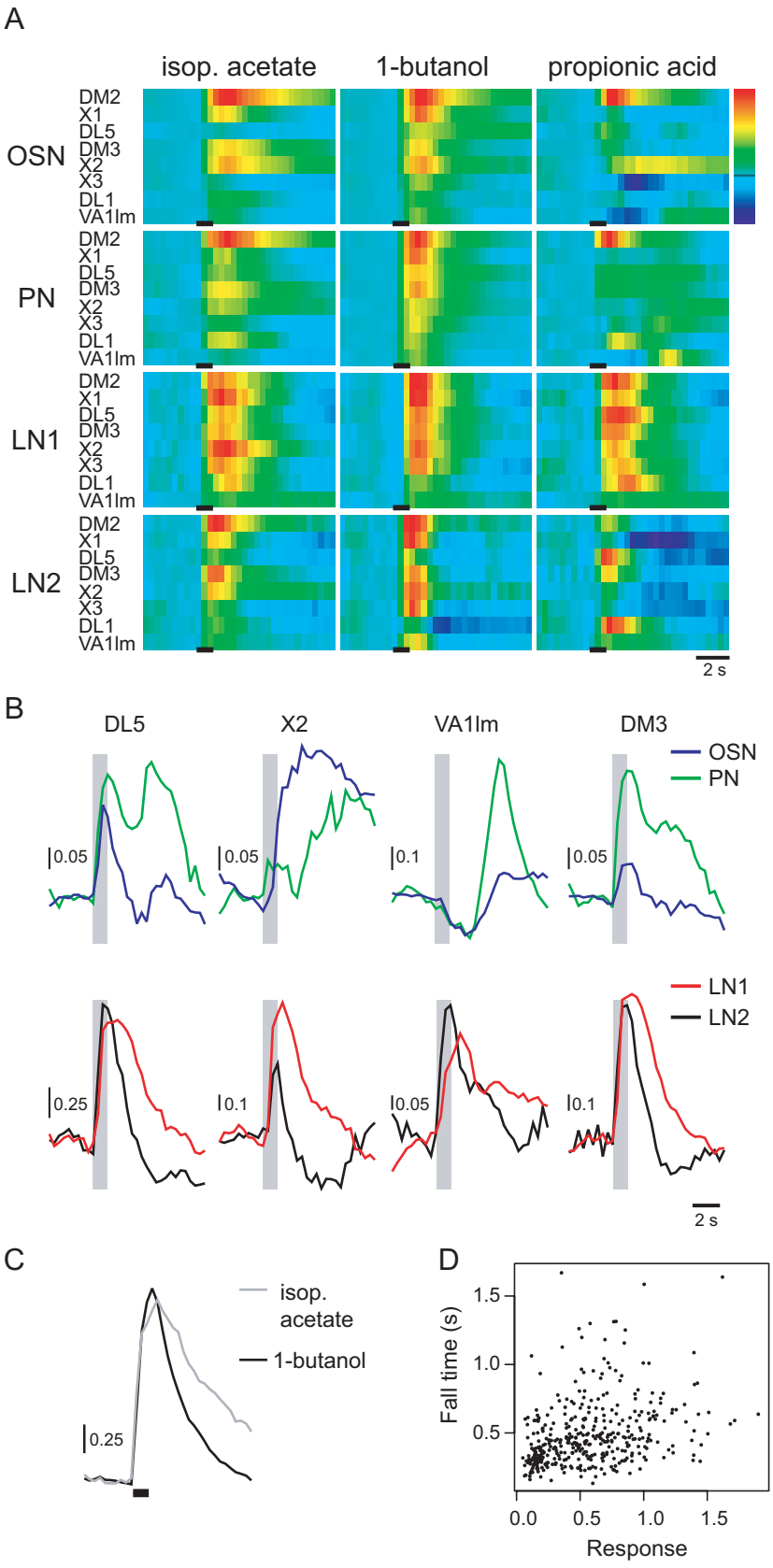

Figure 1.3 - Temporal response patterns are odor and neuron type specific

(A) Evolution of the odor responses over time. Median odor responses over all animals are shown. OSN: n = 8-9; PN: n = 7-8; LN1: n = 4-5; LN2: n = 5-6). Colors are scaled from blue to red with blue ≅ -0.5 × max and red ≅ max, where max is the maximum response amplitude measured for each odor in

each neuron type (over all glomeruli and time points). Stimulus (1 s) is indicated as a black line at the bottom of each response ensemble. Odor concentration was 10^{-2}. **(B)** Comparison of the responses to propionic acid 10^{-2} in different glomeruli. Upper row: OSN and PN. Lower Row: LN1 and LN2. Grey bars indicate the stimulus (1 s). Vertical bars indicate the scale of the response amplitude. **(C)** Median responses to isopentyl acetate 10^{-2} and 1-butanol 10^{-2} in the PNs of glomerulus DM2. Fall time (calculated as the time elapsed during signal decay from 90% to 66% of maximum amplitude) was 1.65 s (Q_{25}=1.29 s, Q_{75}=1.92 s) for isopentyl acetate and 0.57s (Q_{25}=0.48 s, Q_{75}=0.67 s) for 1-butanol. Maximum responses were 1.63 (Q_{25}=1.42, Q_{75}=1.85) and 1.69 (Q_{25}=1.48, Q_{75}=1.72) for isopentyl acetate and 1-butanol respectively. The black line below the traces indicates the time of stimulus (1 s). **(D)** Relationship between the response maximum and fall time. Each point represents the median response for one odor-neuron type-glomerulus-concentration combination (3 odors, 4 neuron types, 8 glomeruli and 6 concentrations). A given combination was included only if the fall time could be calculated for at least two responses (403 out of 576 possible combinations. See Materials and Methods for inclusion criteria). The correlation between response maximum and fall time accounts for 11% fall time change (linear model, $p<10^{-11}$, R^2= 0.11), indicating that the response fall time depends mostly on the glomerulus-stimulus combination.

The rate of signal decay (fall time) varied and in some cases, mostly at high concentrations, calcium concentration did not recover to baseline by the end of the measurement (e.g. 1-butanol and isopentyl acetate in DM2 in the OSNs, see Fig. 1.3A). The responses to 1-butanol 10^{-2} and isopentyl acetate 10^{-2} in glomerulus DM2 (PNs) were comparable in amplitude, but fall time was longer for isopentyl acetate, showing that different fall times were not just a consequence of the differences in response amplitude (Fig. 1.3C). A correlation analysis between fall time and response amplitude showed that response amplitude accounted for only 11% of fall time variation (Fig. 1.3D, Linear Model fit, $p<10^{-11}$, R^2= 0.11).

Responses to increasing odor concentrations: Spatial aspects

We measured concentration-response curves at six concentrations covering five log orders (10^{-7} to 10^{-2}). No responses were found at 10^{-7}, either because this concentration was below the sensitivity threshold for these odors, or because the range of the calcium changes was below the sensitivity threshold of G-CaMP. Since at least in the OSNs some odors induced decreases in calcium concentration, the resting calcium level in these neurons must be in the dynamic range of G-CaMP, and it is thus unlikely that the lack of responses at the concentration of 10^{-7} is due to the sensitivity threshold of the reporter. Figure 1.4A shows spatial activity patterns elicited by increasing concentrations of isopentyl acetate, 1-butanol and propionic acid from 10^{-6} to 10^{-2}.

In order to measure the response threshold, we defined odor evoked calcium changes as odor responses if they were significantly different from the response to mineral oil in that

glomerulus (Wilcoxon Rank Sum Test, $p<0.05$). The threshold for isoamyl acetate was 10^{-6} for OSNs (glomerulus X1) and 10^{-5} for PNs (DM2 and DM3). The threshold for 1-butanol was 10^{-6} for OSNs (X1) and 10^{-4} for PNs (DM2, X1, DL5, DM3 and X2). The threshold for propionic acid was higher: 10^{-4} for OSNs (DM2 and X3, the response in X3 was negative) and 10^{-4} for PNs (DM2 and X3, the response in X3 was also negative). OSNs had a lower threshold than PNs in 9 out of 24 odor-glomerulus combinations, while PNs had a lower threshold than OSNs in only 1 out of 24 odor-glomerulus combinations. In the remaining 14 odor-glomerulus combinations the threshold was the same for both neuron types.

Given that LN responses were not glomerular, thresholds were assessed based on the strongest response across the AL. LN1 had a higher threshold to isopentyl acetate (10^{-4}) than either OSN or PN (10^{-6} and 10^{-5}, respectively), a threshold of 10^{-5} to 1-butanol that was intermediate between OSNs and PNs, and a lower threshold to propionic acid (10^{-5}) than either OSNs or PNs. LN2 responded to isopentyl acetate at concentrations as low as 10^{-5}, with a spatial pattern similar to those observed for OSNs and PNs at this concentration. LN2 responses to 1-butanol had a higher threshold (10^{-4}) than OSN responses but not than PN responses, whereas this threshold was lower (10^{-5}) for propionic acid. The fact that in some cases we found lower response thresholds in LNs than in OSNs cannot be explained by a lack in sensitivity to detect low activity levels in the OSNs (see above). Therefore, a more likely explanation is that the LNs might receive input in glomeruli outside our field of view which are activated by lower stimulus concentrations. Additionally, activity in the LNs below the OSN and PN threshold could reflect their capacity to sum inputs from many weakly activated glomeruli.

Higher odor concentrations led to more glomeruli being active in OSNs and PNs (Fig. 1.4AB), with a tonic concentration-response relationship for isopentyl acetate and 1-butanol. Concentration-response effects were more complex for propionic acid: increasing the concentration initially led to more glomeruli being activated but also more glomeruli in which the calcium concentration decreased in OSNs. This effect was reversed at the highest concentration: fewer glomeruli were inhibited with propionic acid 10^{-2} than 10^{-3}. In PNs, propionic acid only caused consistent calcium decreases at the concentrations 10^{-4} and 10^{-3} (Fig. 1.4B).

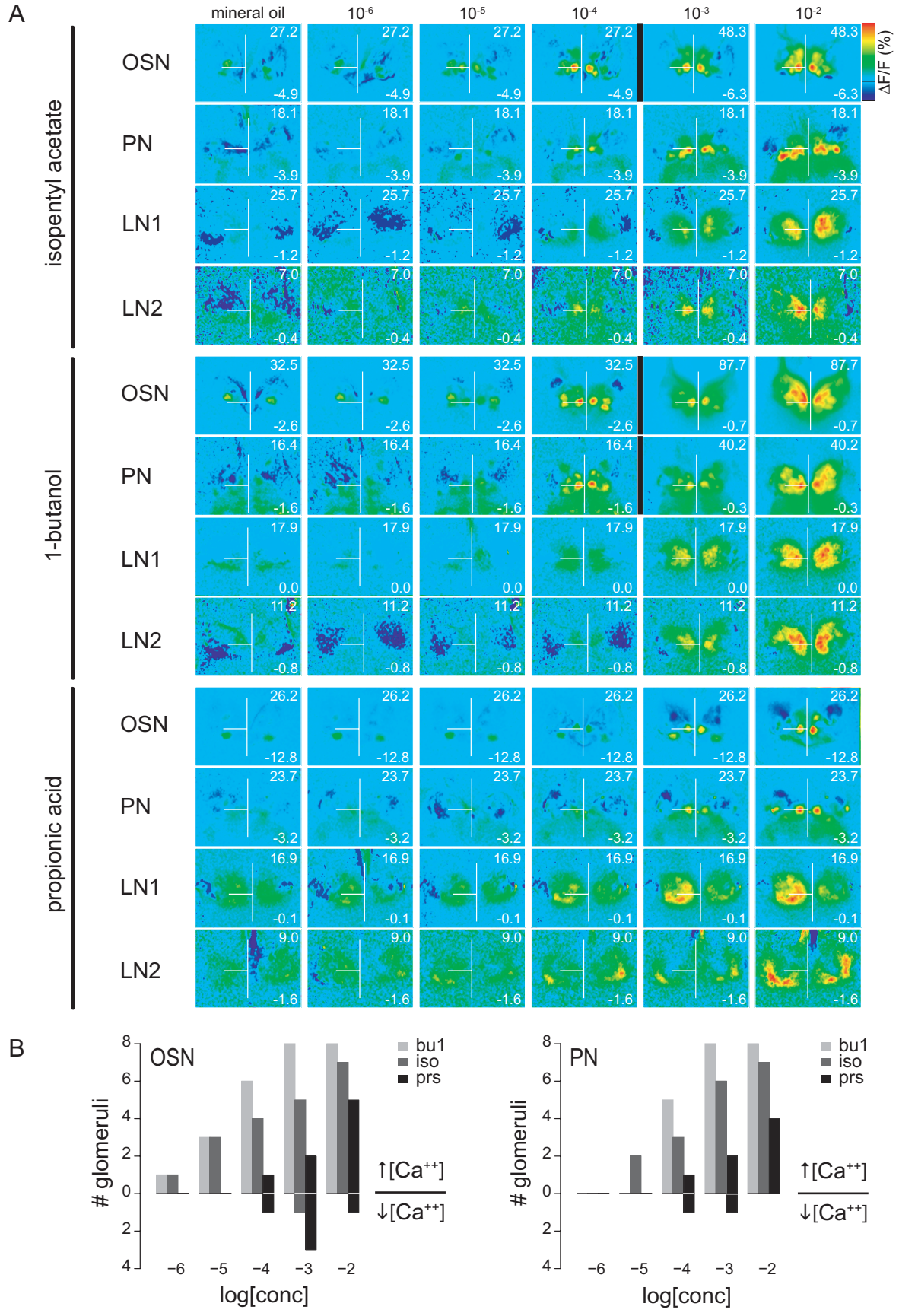

Figure 1.4 - Increasing concentrations activate more glomeruli and increase response amplitudes

(A) Color coded activity patterns in response to isopentyl acetate, 1-butanol and propionic acid in increasing concentrations. The first column shows the responses to the solvent mineral oil. The numbers in the upper and lower right corner of each picture indicate the max and min of the color scale. The black vertical lines point to the change in scaling between the pictures. The vertical white line indicates the midline and the horizontal white line the position of glomerulus DM2 in the left AL. Since none of the odors elicited significant responses at a concentration of 10^{-7} in any of the neuron types tested, these data were excluded from this figure (but see Fig. 1.6). **(B)** Number of responding glomeruli with each odor and each concentration (out of the 8 analyzed glomeruli) in the OSNs and PNs. Glomeruli were counted when the odor response at a given concentration was significantly different from the mineral oil-induced response (Wilcoxon Signed Rank Test). Upward bars show the number of glomeruli where the calcium concentration increased and downward bars the number of glomeruli where calcium concentration decreased.

Also in the LNs spatial activity patterns changed across concentrations. In all cases, an increase in stimulus concentration led to spatially broader activation. In both LN lines, stimulation with 1-butanol and isopentyl acetate induced responses in the medial region of the AL (in the area of DM2, DM3 and X2) at low concentrations (10^{-4}), and responses expanded to the ventral and lateral regions with higher concentrations. For propionic acid, however, the two LN subpopulations differed. LN1 responses were distributed in the dorsal (around glomerulus DL5) and medial areas for the lower concentrations and then expanded laterally, while in LN2 the responses were located laterally (around glomerulus DL1) at low concentrations (10^{-4}), and expanded to the medial area with higher concentrations. We never observed significant calcium decreases in the LNs upon stimulus onset, but there were calcium decreases after stimulus offset (see below).

Responses to increasing odor concentrations: Temporal aspects

We then looked at the relationship between odor concentration and temporal course of the responses. For most glomeruli and odors in all measured lines, increasing odor concentrations did not change response shapes (e.g. 1-butanol in glomerulus DM2 in Fig. 1.5, left panel).
However, the effect of concentration on response dynamic was more complex in some glomeruli in response to propionic acid: in glomerulus X1, for example, stimulation with propionic acid below 10^{-3} did not elicit responses in the OSNs. In the same neuron line, propionic acid 10^{-3} elicited a calcium decrease, which reversed to a calcium increase at a 10 fold higher concentration (Fig. 1.5, right panel). PN responses in X1 were not significantly different from mineral oil for concentrations from 10^{-7} to 10^{-3}, and propionic acid 10^{-2} elicited two response peaks. LN1 responses were monophasic and increased monotonically over the whole concentration range. In LN2, higher concentrations of propionic acid led to shorter

responses with a late calcium undershoot after stimulus offset at the high concentrations (10^{-4} to 10^{-2}) (Fig. 1.5).

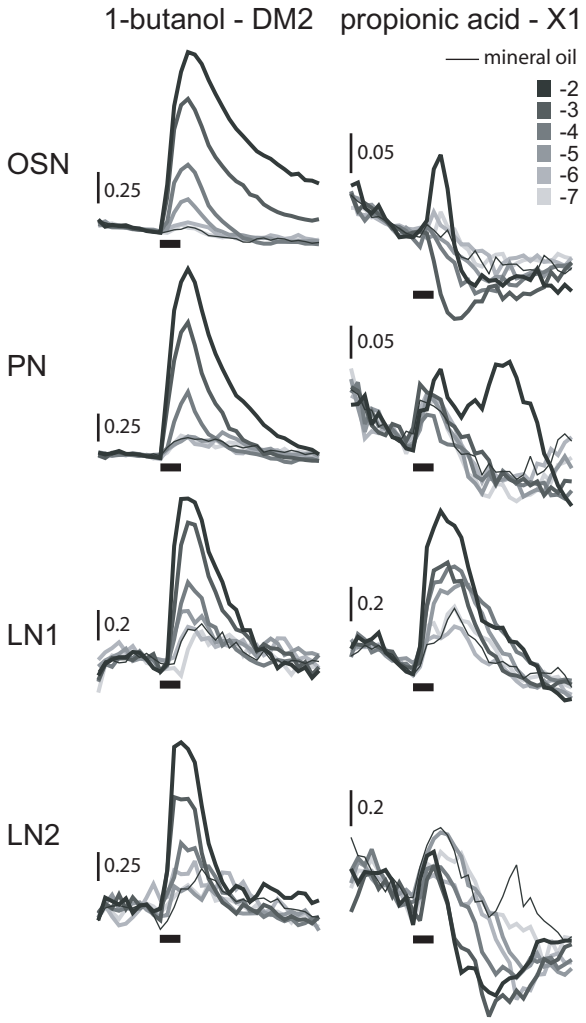

Figure 1.5 - Increasing concentration changes the response dynamics in some glomeruli

Median responses to increasing concentrations of 1-butanol in glomerulus DM2 (left) and propionic acid in glomerulus X1 (right) for OSNs, LNs and PNs. Responses to 1-butanol increased with increasing concentration. In OSN, propionic acid 10^{-3} induced a calcium decrease; however, propionic acid 10^{-2} induced a delayed calcium increase, which corresponds with stimulus offset. PNs responses were only significantly higher than mineral oil responses at 10^{-2}. Responses in LN2 decreased with increasing concentration. Vertical bars indicate the response amplitude scale. Black bars under the traces indicate the time of stimulus presentation (1 s).

Responses to increasing odor concentrations: Concentration-response functions

The response amplitude generally increased with concentration following a typical concentration-response relationship (Fig. 1.6). Exceptions were the responses to propionic

acid in some glomeruli (see above) and OSN responses to isopentyl acetate in glomerulus DL5, where responses decreased with increasing odor concentration (traces not shown, see Fig. 1.6).

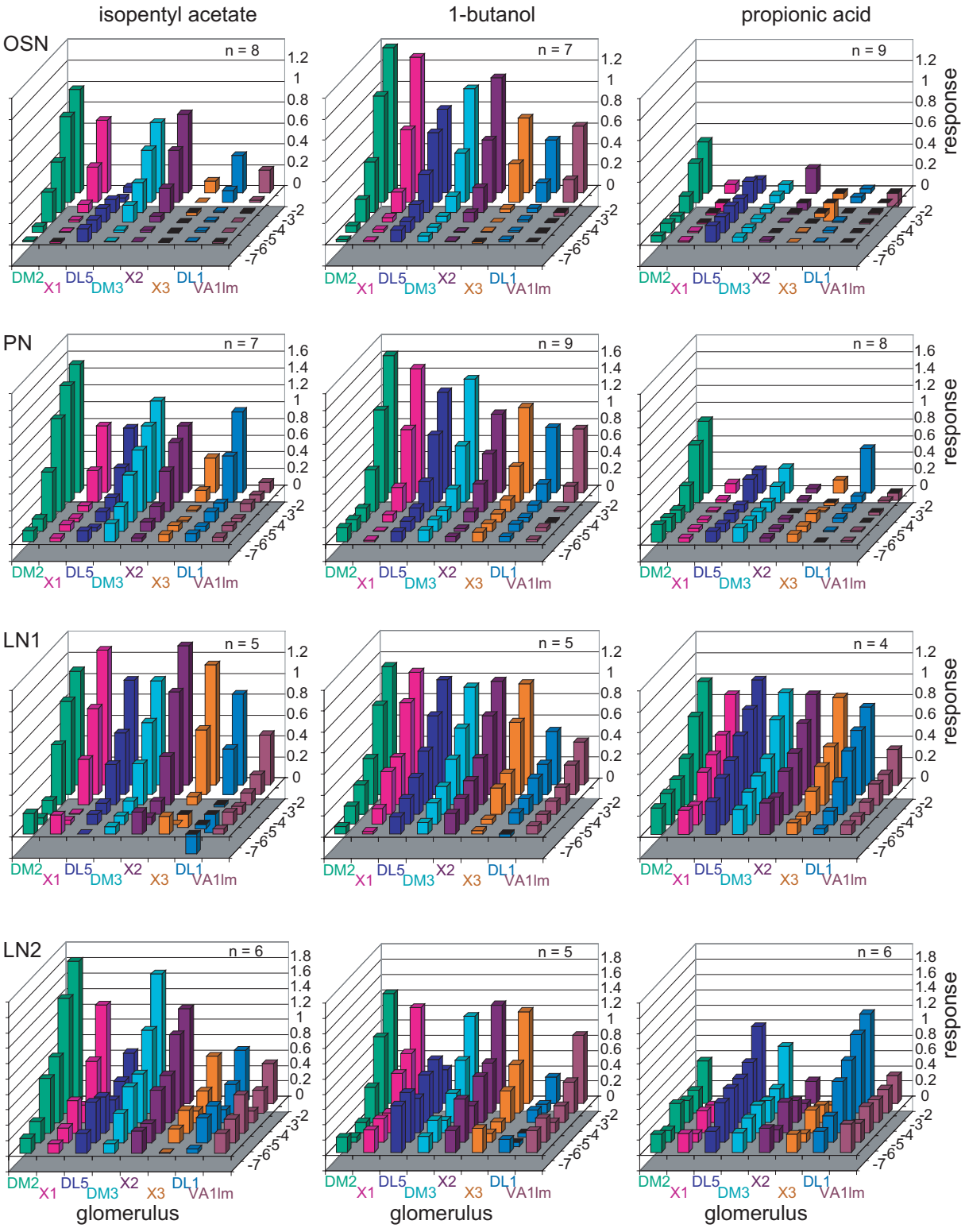

Figure 1.6 - Combinatorial odor responses across concentrations for the three tested odors

Median response amplitude of the responses to isopentyl acetate, 1-butanol and propionic acid are plotted for each neuron type in all analyzed glomeruli (in n animals). Note that some responses are negative.

Calcium responses did not saturate with the highest concentration tested for any odor in any glomerulus measured (Fig. 1.6). This implies that these concentrations were within the physiological dynamic range for the olfactory system of *Drosophila.* However, 10^{-2} is likely to be a high concentration compared to those found in the natural environment of a fly. For example, isopentyl acetate is one of the main components of banana aroma. The concentration of isopentyl acetate in banana mush is $\sim 2 \times 10^{-4}$ as estimated from published sources (Jordan et al., 2001; Pelz et al., 2006), and thus well within the range of concentrations tested here. It appears, therefore, that the olfactory system is not driven to its physiological upper limits in the natural environment of the flies.

Interglomerular interactions modify PN responses

Measuring responses in OSNs, different LN populations and PNs allows us to analyze how OSN input is modified within the AL network, leading to a different pattern of PN output activity. A direct quantitative comparison between the responses of OSNs and PNs is not possible. This is because measured calcium concentration changes are influenced not only by changes in firing patterns, but also by differences in resting calcium levels, calcium buffering properties, calcium sources (e.g. intracellular stores, or voltage-dependent calcium channels) and in the role that calcium plays within the monitored cell compartment (e.g. as a second messenger or as an exocytose signal). This means that the same relative calcium change might have a different biological significance in different neuron types, and possibly also for different glomeruli within a neuron type. To some extent, the same argument holds for any physiological activity monitor, including changes in action potential firing rate: while a single spike from one neuron may have a strong impact on the network, a train of spikes from another might be necessary to exert a similar influence, making a direct, quantitative comparison impossible across neurons. However, it is possible to compare physiological responses in a particular glomerulus across different odors in order to investigate network effects. If in a given glomerulus OSN responses to different odors are similar, but PN responses differ, then interglomerular interactions are the most plausible interpretation. The

differences in concentration-response relationship between OSNs and PNs for the three odors in glomerulus DM2 are shown in Figure 1.7A.

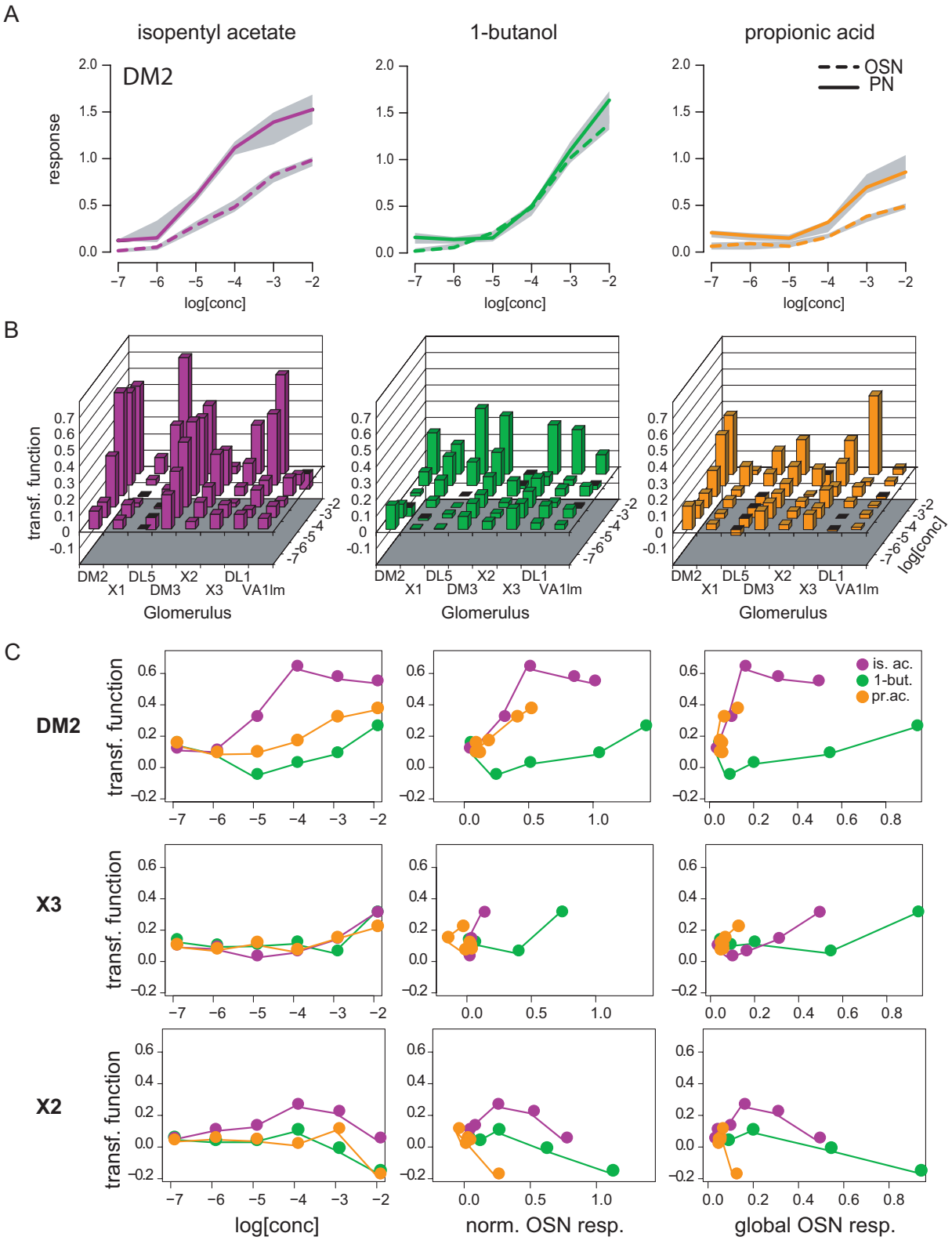

Figure 1.7 - Interglomerular interactions modify the combinatorial odor representation

(A) Concentration-response curves for the OSNs and PNs in glomerulus DM2. Curves show median responses. The grey areas behind the curves represent the range between the Q_{25} and Q_{75}. **(B)** Activity transfer function (PN response - OSN response) for each glomerulus and odor over all concentrations (from data in Fig. 1.6). **(C)** Activity transfer function as a function of concentration (left), OSN response in the same glomerulus (middle) or global OSN response (right) (global OSN response was calculated as the mean response of all glomeruli analyzed) for three exemplary glomeruli (DM2, X3 and X2). The segregation of the curves for the three odors in glomeruli DM2 and X2 shows that the modulation of the response profile in PNs cannot be accounted for by any of the three parameters, but is dependent on the odor, suggesting a network effect driven by odor-specific activity patterns in the AL.

We analyzed the input-output relationship for each odor in each glomerulus. We calculated the difference between PN and OSN responses across concentrations for each odor in each glomerulus (Fig. 1.7B, referred to as activity transfer function). For glomeruli VA1lm and X1 the activity transfer function remained around zero over the whole concentration range for all odors, indicating that PN responses correspond to OSN responses (Fig. 1.7B). The activity transfer function in glomerulus X3 increased with increasing concentration, i.e. PN responses increased more than OSN responses for the highest odor concentrations (Fig. 1.7BC). Since in these glomeruli the activity transfer functions is similar for the different odors, intraglomerular convergence ratio and synaptic strength are sufficient to explain the differences between OSN and PN activity. Thus, for glomeruli X1, X3 and VA1lm interactions among glomeruli need not be postulated to explain the activity transfer function (for the tested odors). In contrast, the activity transfer function in glomeruli DM2, DM3, X2 and DL1 was odor dependent. In DM2, responses to isopentyl acetate were higher in PNs than in OSNs over the whole concentration range, with a peak difference at 10^{-4}. PN responses to 1-butanol corresponded to OSN responses with only slight deviations at very low and very high concentrations. The difference between PNs and OSNs increased with concentration for propionic acid. Thus, in DM2, the AL network amplified responses to isopentyl acetate relative to the other two odors (Fig. 1.7AC). However, it is important to keep in mind that we cannot state that the responses to isopentyl acetate in DM2 are amplified in absolute terms, because the analysis of the response profiles between neuron types only makes sense relative to the profiles of other odors. If, for example, we consider the activity transfer function for propionic acid as the reference function for DM2 then the responses to isopentyl acetate are amplified in the PNs, and the responses to 1-butanol are inhibited. Modulation was also odor dependent in glomerulus DM3, where the activity transfer function was maximal at 10^{-4} for isopentyl acetate but was maximal at 10^{-2} for 1-butanol and propionic acid. In glomeruli DL5 and DL1 the difference between PNs and OSNs increased with concentration for all three odors, but the development was not parallel: PN responses were higher than OSN responses

for isopentyl acetate already at 10^{-3}, but only at 10^{-2} for 1-butanol and propionic acid. In glomerulus X2 the activity transfer function was around zero for most concentrations of 1-butanol and propionic acid and negative for 10^{-2}, but responses to isopentyl acetate were higher than OSN responses for concentrations 10^{-5} to 10^{-3}, indicating that both suppression and amplification can modulate PN responses in this glomerulus depending on the stimulus presented (Fig. 1.7C).

Do differences in activity transfer function depend on the global input strength to the AL? We found no correlation between the activity transfer function and the level of input to the glomerulus (measured as the activity in the corresponding OSNs), nor a correlation with the global input received by the AL (measured as the mean response in the OSNs over all analyzed glomeruli) (Fig. 1.7C). Since the activity transfer function was independent from the local and global input strength, the observed differences in activity transfer must be a consequence of glomerulus specific interactions which modulate PN responses. It is feasible that PNs receive inhibitory input from other glomeruli, or, alternatively that their activity is enhanced through disinhibition or lateral excitation.

Glomerulus specific interactions could not be unequivocally related to the responses of the two LN populations sampled here, measured either globally (data not shown) or at positions corresponding to the single glomeruli. For example, the activity transfer function was different for 1-butanol, isopentyl acetate and propionic acid in glomerulus DM2, however, LN1 responses were comparable for the three odors in that glomerulus, while LN2 responses were highest for isopentyl acetate (the odor with the highest amplification), and lowest for propionic acid (whose activity transfer function was intermediate) (Figs. 1.6 and 1.7AC). In contrast, the activity transfer function in glomerulus X3 was similar for the three odors, but both LN1 and LN2 activity differed across odors for that glomerulus (Figs. 1.6 and 1.7C)

Several possibilities might account for these inconsistencies: first, the observed calcium patterns in LNs could be a superposition of different calcium sources in different compartments, which might mar the picture about a possible spatial connectivity pattern. Second, it is conceivable that the genetically defined LN lines contain neurons with diverse physiological properties. Finally, the two LN lines measured here do not cover all LNs present in *Drosophila* and it is likely that further populations of inhibitory or excitatory LNs are involved in the glomerulus specific effect described here.

Discussion

We have characterized odor evoked responses in four subpopulations of AL neurons - OSNs, two genetically defined LN populations, and PNs. We found that in each of these neuron populations odors evoke stereotyped spatio-temporal activity patterns across animals. Increasing odor concentrations generally increased the number of activated glomeruli and their response strength. Moreover we found that PN output activity was modulated in an odor specific manner, suggesting that glomerulus-specific interglomerular interactions via LNs shape odor responses in the *Drosophila* AL.

Calcium signal source

Several calcium sources are likely to contribute to calcium signals. All AL neurons studied so far form both input and output synapses within the AL (Malun et al., 1993; Distler et al., 1998; Ng et al., 2002). Thus calcium influx caused by postsynaptic potentials as well as spike related depolarization should contribute to the calcium signals measured in all neuron populations in the AL. Another potential source of odor evoked calcium signals are ionotropic acetylcholine receptors, which are calcium permeable (Goldberg et al., 1999; Barbara et al., 2005) and mediate most excitatory connections in the insect brain. The exact contribution of the different calcium sources to the measured signals is not known, however, it has been shown that calcium influx through voltage activated calcium channels is more prevalent than calcium entry through ligand gated channels in insects (Oertner et al., 1999; Single and Borst, 2002). Furthermore, calcium concentration has been shown to follow the membrane potential changes and to correlate well with spiking activity (Svoboda et al., 1997; Charpak et al., 2001; Kurtz et al., 2001; Galizia and Kimmerle, 2004), suggesting that calcium signals are a good reporter for neuronal activity.

The sensitivity and dynamic range of G-CaMP cover the range between 0.1 and 1μM [Ca^{++}] (Nakai et al., 2001), and proved to be adequate for these neurons. At the lower end, we found odor induced calcium decreases in three out of four neuron types analyzed (OSNs, PNs, and LN2). Therefore, the intracellular calcium concentration at resting potential in these neurons must be already within the dynamic range of G-CaMP, at least in some glomeruli. We found no negative responses or undershoots in the LN1, but we also found almost no areas in the AL where the calcium did not increase upon stimulation, which indicates that it is unlikely that

we missed any negative responses due to the dynamic range of G-CaMP. At the upper end, we found no cases of response saturation of the concentration curves, which indicates that all responses were within the dynamic range of the calcium sensor. Even in those cases closest to saturation (e.g. isopentyl acetate in glomerulus DM2 in OSNs), calcium levels were not the highest measured for the given glomerulus in that neuron type, showing that in these cases saturation reflected a property of the neurons and not of the calcium sensor.

Spatial and temporal activity patterns

Our results show that different odors elicit responses in stereotyped and overlapping groups of glomeruli both in OSNs and PNs (Fig. 1.2), and that the time course of these responses is odor specific (Fig. 1.3). No study so far has investigated both the temporal and spatial structure of the odor responses in the AL for distinct neuron populations. While electrophysiological studies have a better temporal resolution, it is not possible to record from many neurons within identified glomeruli at the same time. Imaging studies, on the other hand, have good spatial resolution and the advantage of recording from many glomeruli simultaneously. However, previous studies in *Drosophila* have focused only on spatial aspects (Ng et al., 2002; Wang et al., 2003).

Contrary to recent studies (Wilson et al., 2004; Shang et al., 2007; Olsen et al., 2007), we found no broadening in the response profile of PNs as compared with those of OSNs. All glomeruli active in PN response patterns were also active in OSN patterns.

We found that the time course of OSN and PN responses was complex for some glomeruli and odors, and that the decay time of the signals was stimulus dependent, and only partially dependent on the amplitude of the calcium change (Fig. 1.3), in agreement with electrophysiological data (de Bruyne et al., 2001; Wilson et al., 2004; Hallem and Carlson, 2006). Among the odors tested, propionic acid had the most complex response dynamics: monophasic responses at stimulus onset (glomerulus DM2), prolonged delayed responses (glomerulus X2) and also negative responses (glomeruli X1 and X3) (Fig. 1.3). Part of the temporal complexities present in the responses to propionic acid might result from OR-independent interactions such as changes in the pH in the sensillar hemolymph. These non-canonical response components, however, probably contribute to the odor-specific signature of propionic acid in the brain. It remains an open question whether slow temporal response signatures are used in olfactory behavior. In mice and bees, olfactory decisions are taken

within a few hundreds of milliseconds (Ditzen et al., 2003; Uchida and Mainen, 2003; Abraham et al., 2004), making these slow patterns superfluous. However, they might have a role in some behavioral contexts, or be involved in plasticity within the olfactory system.

In contrast to our findings, Hallem and colleagues (2006) found no responsive OR for propionic acid, including OR22a and OR7a, corresponding to glomeruli DM2 and DL5, where we found strong responses. This discrepancy might be due to the fact that water was used as solvent in that study, which might change the protonation level of the molecule.

LN1 responses were monophasic, increased upon concentration increase, and their time courses were odor-independent (Figs. 1.3 and 1.5). LNs with these properties have been described in patch clamp experiments (Wilson et al., 2004). Thus, LN1 might integrate excitatory input across the AL network and give inhibitory output to large areas of the AL, maybe thereby controlling the global activity of the AL network (but other scenarios are possible, see below). Conversely, LN2 responses showed a brief calcium increase followed by a pronounced undershoot in calcium concentration in some odor-glomeruli combinations. The duration of the calcium increase and the amplitude of the undershoot were concentration dependent. This response type might reflect a complex computation including excitatory and inhibitory inputs to LN2.

The clear differences in spatial activity patterns between LN1 and LN2 in this study indicates that even with broadly arborizing LNs, a functional spatial organization can be achieved. Morphologically multiglomerular LNs might be functionally heterogeneous if we assume the existence of electrical sub-compartments within single neurons, or a non-uniform distribution of synaptic connections. Furthermore, several functional types of LNs might be contained in each of the genetic LN classes defined by our enhancer-trap lines.

Concentration dependency

Increasing odor stimulus concentration led to an increase in the number of active glomeruli and in the response amplitude both in OSNs and PNs. This finding is in correspondence with an increased firing rate of OSNs with increasing concentrations (de Bruyne et al., 2001; Hallem and Carlson, 2006), and has also been reported from honeybee and fly PNs (Wang et al., 2003; Sachse and Galizia, 2003).

The flies' olfactory system has a dynamic range of at least 3-4 log units of concentration (Fig. 1.6). Interestingly, most glomeruli were still in the dynamic range of responses for the highest

concentrations of the three odors tested, i.e. the concentration-response curves did not reach saturation. The concentrations tested are likely to correspond to those found by flies in natural situations, for example when sitting on a very strong smelling fruit. Therefore, it appears that flies do not reach olfactory saturation in their daily life.

Interglomerular interactions

Olfactory information from OSNs is processed within the AL by the interaction of several populations of LNs, thus transforming the odor and concentration-specific across-glomeruli pattern from OSNs to PNs. We show here that this transformation does not follow a single rule, but rather is the result of several superimposed and/or sequential steps. Specifically, by measuring both OSNs and PNs, we could calculate the glomerular activity transfer function between OSNs and PNs, and show that it is odor dependent. In other words, the PN response of a glomerulus depends not only on the OSN input to that glomerulus or the global input to the AL, but also on network activity between specific glomeruli. If we assume that the case where OSN and PN responses are comparable represents the intrinsic activity transfer function, then suppression and amplification take place in a glomerulus- and odor-dependent manner (Fig. 1.7C). Thus broadening and sharpening reshape the PN response profiles as compared to the OSN, confirming previous results in *Drosophila* (Wilson et al., 2004; Shang et al., 2007; Olsen et al., 2007) and the honeybee (Sachse and Galizia, 2003; Linster et al., 2005).

This effect across glomeruli cannot be mediated by the uniglomerular OSNs and PNs themselves, but must involve multiglomerular LNs and/or multiglomerular PNs. LN activity measured here was not restricted to glomerular boundaries, in accordance with the non-glomerular branching architecture of *Drosophila* LNs (Ng et al., 2002; Wilson et al., 2004; Wilson and Laurent, 2005; Shang et al., 2007). Although LN1 and LN2 responses were spatially structured and odor specific, activity patterns from the two LN lines imaged here cannot account for all interaction effects found (Fig. 1.6 and 1.7). The described interglomerular interactions could be mediated either by subpopulations of LN1 and LN2, or by LNs not measured in this study. Among the latter are: a recently published population of excitatory LNs (Shang et al., 2007) and previously described LNs that innervate only a few glomeruli (Wilson and Laurent, 2005). In honeybees, glomerulus-specific computations in the AL network are thought to be mediated by LNs with heterogeneous branching patterns,

innervating one glomerulus strongly and a few glomeruli weakly (Fonta et al., 1993; Linster et al., 2005; Sachse and Galizia, 2006).

Our results show, that the cellular network of the fruit fly AL performs the complex and multifaceted task of shaping odor responses. It would be interesting to investigate whether the ability of the AL network to amplify and suppress odor responses in a glomerulus specific manner is a stable property of the network or whether it can be subject to experience related plasticity.

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