



Temporal response dynamics of *Drosophila* olfactory sensory neurons depends on receptor type and response polarity

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Insect olfactory sensory neurons (OSN) express a diverse array of receptors from different protein families, i.e. ionotropic receptors (IR), gustatory receptors (GR) and odorant receptors (OR). It is well known that insects are exposed to a plethora of odor molecules that vary widely in both space and time under turbulent natural conditions. In addition to divergent ligand specificities, these different receptors might also provide an increased range of temporal dynamics and sensitivities for the olfactory system. To test this, we challenged different *Drosophila* OSNs with both varying stimulus durations (10–2000 ms), and repeated stimulus pulses of key ligands at various frequencies (1–10 Hz). Our results show that OR-expressing OSNs responded faster and with higher sensitivity to short stimulations as compared to IR- and Gr21a-expressing OSNs. In addition, OR-expressing OSNs could respond to repeated stimulations of excitatory ligands up to 5 Hz, while IR-expressing OSNs required ~5x longer stimulations and/or higher concentrations to respond to similar stimulus durations and frequencies. Nevertheless, IR-expressing OSNs did not exhibit adaptation to longer stimulations, unlike OR- and Gr21a-OSNs. Both OR- and IR-expressing OSNs were also unable to resolve repeated pulses of inhibitory ligands as fast as excitatory ligands. These differences were independent of the peri-receptor environment in which the receptors were expressed and suggest that the receptor expressed by a given OSN affects both its sensitivity and its response to transient, intermittent chemical stimuli. OR-expressing OSNs are better at resolving low dose, intermittent stimuli, while IR-expressing OSNs respond more accurately to long-lasting odor pulses. This diversity increases the capacity of the insect olfactory system to respond to the diverse spatiotemporal signals in the natural environment.

Keywords: odorant receptors, ionotropic receptors, pulse resolution, single sensillum recording

INTRODUCTION

Insect olfactory sensory neurons (OSN) express a large number of receptor proteins of different types. These receptor types include ionotropic receptors (IR), gustatory receptors (GR), and odorant receptors (OR) (Clyne et al., 1999; Vosshall et al., 1999; Benton et al., 2009). IRs are composed of three trans-membrane proteins and co-receptors, while GRs and ORs are seven trans-membrane proteins (Vosshall et al., 1999; Benton et al., 2006, 2009). ORs are co-expressed with the ubiquitous co-receptor Orco, while Gr21a, a CO₂ sensor, is co-expressed with Gr63a (Benton et al., 2006; Jones et al., 2007). All OSNs are housed within different morphological types of olfactory hairs, known as sensilla. There appear to be important organizational differences between OSNs that express IRs, GRs, or ORs. Multiple IRs and GRs can be co-expressed per neuron, while OR expression generally follows a one neuron-one receptor rule (Thorne et al., 2004; Wang et al., 2004; Couto et al., 2005; Benton et al., 2009). Receptors from different protein families can also be co-localized in the same sensillum (Couto et al., 2005; Song et al., 2012). For example, in *Drosophila*, the ab1 sensillum houses four OSNs, three expressing

ORs and one expressing Gr21a. Also, in the *Drosophila* coelomic sensillum ac3 an OSN expressing Or35a is co-localized with an OSN expressing Ir75abc (Yao et al., 2005; Silbering et al., 2011).

These diverse receptors have evolved at different points in evolutionary time (Robertson et al., 2003; Croset et al., 2010). Recent research also suggests that many have broad affinity to different chemical classes (Hallem et al., 2004; Yao et al., 2005; Benton et al., 2009; Ai et al., 2010). Yet specificity might not be the only reason for receptor diversification. In the natural environment, insects are constantly challenged with odors not only of diverse molecular types, but with diverse spatio-temporal dynamics. At some distances, odor plumes can present brief and intermittent stimuli (Kaissling et al., 1987; Vickers et al., 2001) with low molecular flux, while at close range or high molecular flux, odors could present a nearly continuous stimulus (Murlis et al., 2000; Louis et al., 2008; Gomez-Marin et al., 2011). These spatiotemporal factors could also be a significant driving force for diversification. The behavior of an insect is a result of the integration of responses from several OSNs expressing a variety of receptor

types (Silbering et al., 2011). Thus it is worthwhile to characterize the response dynamics across the OSN repertoire.

To address whether these different receptor types exhibit differences in temporal response kinetics, we assess the response dynamics of *Drosophila* OSNs expressing various receptor types to both different stimulus durations and frequencies. We evaluate the temporal dynamics of antennal OSNs expressing ORs (Or59b and Or35a), IRs (Ir84a, Ir75abc, and Ir41a), and GRs (Gr21a). Or59b-OSNs and Ir41a-OSNs respond with either excitation or inhibition to different ligands, and were chosen to assess the effect of response polarity on temporal kinetics. Or35a- and Ir75abc-OSNs are housed in the same sensillum, and are tested to control for the effects of the perireceptor environment on the temporal response. Finally, Gr21a-expressing OSNs are the only GR-expressing OSNs found on the antenna. Here we show that sensory neurons expressing receptors from different protein families also exhibit different dynamics to brief and intermittent stimuli.

MATERIALS AND METHODS

Both male and female flies at 2–6 days of age were used. Stocks were maintained on conventional cornmeal agar medium under a 12 h light: 12 h dark cycle at 25°C.

ELECTROPHYSIOLOGY

A fly was mounted in a cut pipette tip with the head protruding and small amount of wax placed into the tip end to prevent movement. The pipette was then fixed onto a microscope slide with wax and the antennae fixed on a cover slip with a sharpened glass micropipette, similar to (Hallem et al., 2004; Yao et al., 2005; Pellegrino et al., 2010). An electrolytically sharpened tungsten electrode was placed in the eye for grounding and a sharpened tungsten recording electrode was brought into contact with the base of the sensillum using a Luigs and Neumann, SM-59 manipulator (Ratingen, Germany) at 1000× magnification with an Olympus BX-51 microscope (Olympus Corporation, Tokyo, Japan).

ODOR STIMULI

Methyl acetate (>98%), citral (>95%), phenyl acetaldehyde (>90%), butyric acid (>99%), 1, 4-diaminobutane and isoamylamine (>98%), 1-hexanol (>99%), and ethyl hexanoate (>99%) were purchased from Sigma Aldrich Germany. Phenyl acetaldehyde, methyl acetate, 1-hexanol and ethyl hexanoate were diluted in mineral oil (BioChemika Ultra, Fluka), and butyric acid, 1, 4-diaminobutane, and isoamylamine were dissolved in water. Citral was dissolved in hexane (>99%, Fluka Analytical, Buchs, Switzerland). We chose odor concentrations within the linear portion of the dose response curve and the tested concentrations are indicated with circles (Figure 1). All concentrations are reported as log [odor] v/v. For Gr21a stimulation, a 1.5 ml glass vial was filled with pure CO₂ and placed into the stimulus system similar to the other stimuli. After each frequency set (1–10 Hz), the CO₂ was refilled.

For frequency stimulation, we used a custom-built multi-component stimulus system similar to (Olsson et al., 2011). Briefly, 400 µl of appropriate dilutions of each odorant was added

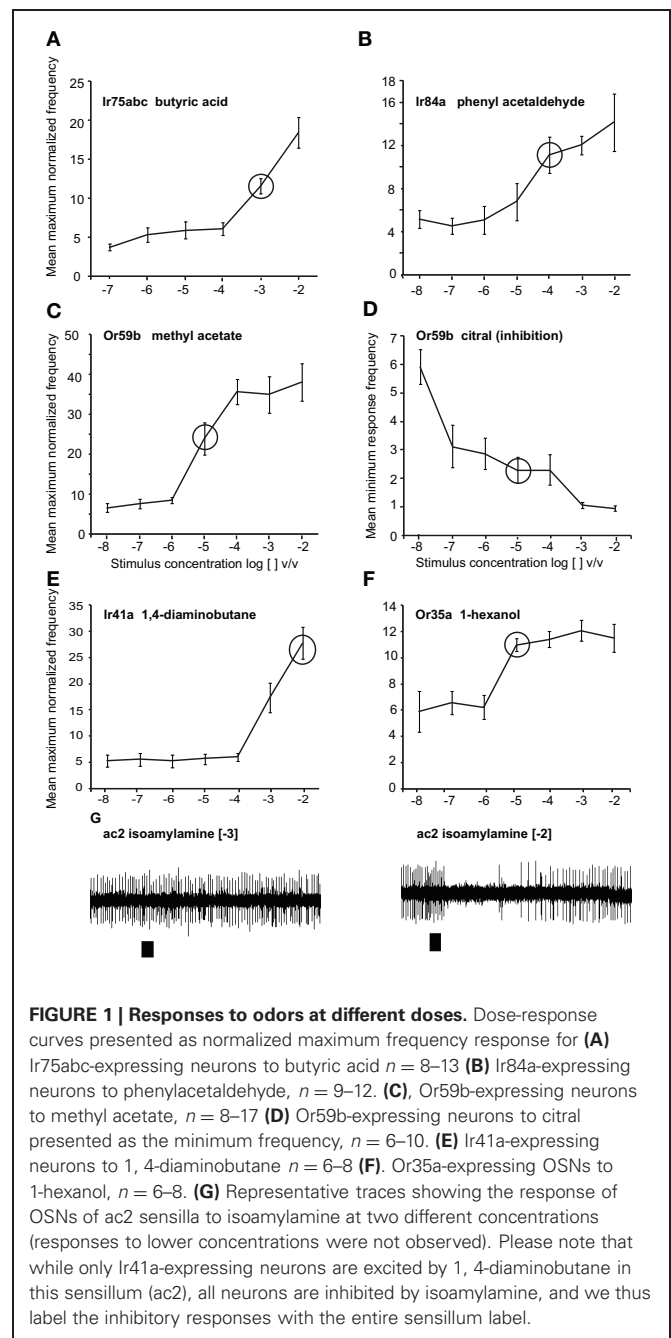


FIGURE 1 | Responses to odors at different doses. Dose-response curves presented as normalized maximum frequency response for (A) Ir75abc-expressing neurons to butyric acid $n = 8-13$ (B) Ir84a-expressing neurons to phenylacetaldehyde, $n = 9-12$. (C), Or59b-expressing neurons to methyl acetate, $n = 8-17$ (D) Or59b-expressing neurons to citral presented as the minimum frequency, $n = 6-10$. (E) Ir41a-expressing neurons to 1, 4-diaminobutane $n = 6-8$ (F). Or35a-expressing OSNs to 1-hexanol, $n = 6-8$. (G) Representative traces showing the response of OSNs of ac2 sensilla to isoamylamine at two different concentrations (responses to lower concentrations were not observed). Please note that while only Ir41a-expressing neurons are excited by 1, 4-diaminobutane in this sensillum (ac2), all neurons are inhibited by isoamylamine, and we thus label the inhibitory responses with the entire sensillum label.

to an Eppendorf tube and placed in the bottom of a PEEK vial (4.6 cm × 2.5 cm × 2.5 cm dimensions). Each vial was sealed with a stainless steel plug (Olsson et al., 2011). The pulse duration, inter-stimulus interval and number of pulses were adjusted through a custom built Labview program (Olsson et al., 2011). The odors were delivered from the headspace via Teflon tubing 150 cm long with an inner diameter of 1 mm and positioned as close as possible (~1.5 cm) to the antennae. The flow rate of air was 0.5 L/min. For stimulation, the stimulus system was connected to the IDAC (Syntech, Ockenfels, Germany) and through USB connection to a PC. Stimulation was controlled by an OEM (EDP 0504, thinXXS) pump control system and DAQ (USB 6008

data acquisition hardware, National Instruments, Austin, TX, USA) with custom-built Labview 8.5 software (built by Daniel Veit; National Instruments). For frequency stimulation the on time was 50 ms for OR- and Gr21a-OSNs and the off time was adjusted from 950 ms or 50 ms for 1–10 Hz, respectively. For IR-OSNs stimulated with [−4] and [−3] stimulus concentrations, the pump on time was 200 ms and off time 800, 300, or 50 ms for 1–4 Hz, respectively. At [−2], the protocol was identical to the OR-OSNs and Gr21a-OSNs. The consistency of odor delivery for different pulse durations and frequencies was confirmed using PID (200a, Aurora Scientific Ontario, Canada).

DATA ANALYSIS

All raw spike data were acquired and converted to digital spikes using Autospike 3.7 (Syntech). Co-localized neurons were identified based on spike amplitude. Peri-stimulus time histograms (PSTHs) were obtained by averaging spike activities in 25 ms bins from the start of the stimulation and normalized to the average frequency for 2 s before stimulation (Olsson et al., 2011; Sargsyan et al., 2011). The OSN responses between consecutive pulses were compared using repeated measure ANOVA by assessing the normalized mean of area under curve (AUC) spike frequency per each stimulus duration, i.e. pump on time + off time. Consecutive pulses were normalized to the response of 1st pulse. Between treatments, a Mann-Whitney U test or *t*-test was used depending on the normality of the data. To evaluate the capacity of receptors to resolve pulsed stimuli, we visualized the response using normalized peri-stimulus histograms and quantified the % return to the spontaneous activity (baseline), using the ratio between the first value in the 2nd pulse and the maximum peak value of the first (previous) pulse converted to a percentage: Percent return to baseline = $1 - (\text{1st value of the 2nd pulse} / \text{maximum frequency of the 1st pulse}) \times 100$ (Bau et al., 2002). A One-Way ANOVA followed by a Tukey *post-hoc* test was performed to determine if the return to baseline was significantly reduced between the different stimulation frequencies. Latency was measured as the time from the onset of the odor stimulus to the maximum response frequency (mechanical delay was not considered). Response width was calculated as the time between half-maximal response for excitation and half-minimal response in the case of inhibition. Spearman's correlation was used to assess the relationship between repeated pulses and latency as well as between response width and intensity with stimulus duration. All analyses were performed using SPSS version 17 (IBM Corporation, Armonk, New York, US).

RESULTS

RESPONSE DYNAMICS OF DIFFERENT SENSORY NEURONS TO VARYING STIMULUS DURATIONS

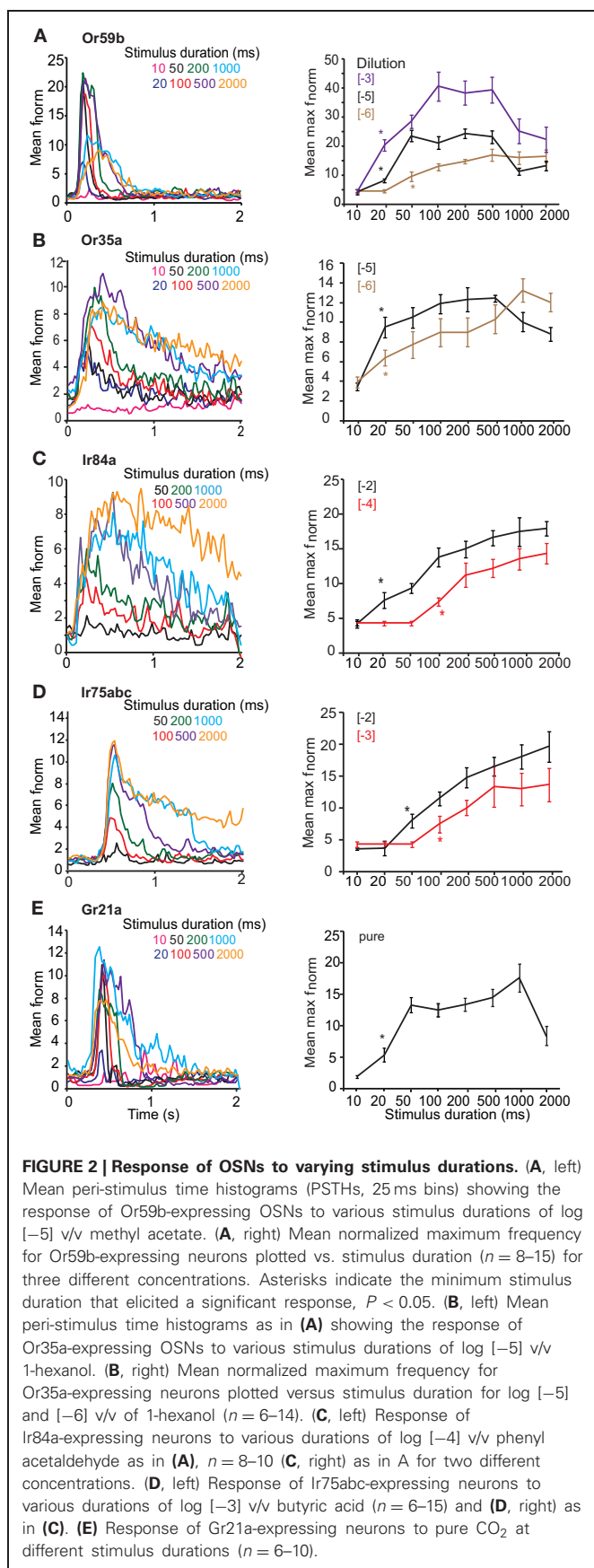
We first assessed the response of OSNs carrying ORs, Gr21a, or IRs to key ligands presented with varying stimulus durations at concentrations found in the linear portion of the dose-response curve for each OSN (Figure 1). OSNs expressing Or59b housed in basiconic sensillum type ab2 were stimulated with methyl acetate at [−5] concentration, with stimulus durations varying from 10 ms to 2 s. At 20 ms, the mean normalized frequency of Or59b-expressing OSNs was greater than the spontaneous

activity ($t = 3.482$, $P = 0.005$), indicating that a 20 ms stimulation was sufficient to elicit a response (Figure 2A asterisk right). A maximal stimulus response was obtained with a 50 ms stimulation ($P < 0.05$), however, stimulations of 1 s or more significantly reduced the OSN response maximum ($t = 3.482$, $P = 0.005$, mean normalized maximum frequency for 500 ms vs. 1 s stimulation and $t = 5.047$, $P < 0.001$ for 500 ms vs. 2 s, Student's *t*-test). Similar response dynamics were observed in Or35a-expressing OSNs ($t = 5.007$, $P < 0.001$ mean normalized maximum frequency for 500 ms vs. 2 s stimulation; Figure 2B). Adaptation to long stimulus durations (>1 s) was also apparent for Or22a-OSNs (data not shown). There was also a positive and significant correlation between response width at half-maximal response and stimulus duration for both OR-expressing OSNs ($r = 0.853$, $P < 0.001$ for Or59b-OSNs and $r = 0.93$, $P < 0.001$ for Or35a-OSNs; both Spearman's correlation Figures 2A,B left panels).

OSN expressing Ir84a (Figure 2C) were stimulated with [−4] phenyl acetaldehyde and a significant response was obtained at 100 ms ($P < 0.001$, Mann-Whitney *U*-test; Figure 2B). A maximal response was reached at 500 ms ($P = 0.001$, Mann-Whitney *U*-test, as compared to 100 ms), and the maximum response intensity did not decrease at longer stimulation durations ($t = 0.605$, $P = 0.554$ at 500 ms stimulation vs. 1 s, and $t = 0.394$, $P = 0.699$ for 500 ms vs. 2 s; Student's *t*-test). The response of Ir75abc-expressing neurons was similar when stimulated with [−3] butyric acid, (significant response at 100 ms; Mann-Whitney *U*, $P = 0.016$, Figure 2D), and reached a maximum response at 500 ms ($t = 2.286$, $P = 0.036$ compared to 100 ms). Furthermore, the response did not change at longer stimulus durations ($t = 0.096$, $P = 0.924$, 500 ms vs. 1 s, $t = 0.068$, $P = 0.946$, 500 ms vs. 2 s; Figure 2D right panel). There was also a positive and significant correlation between stimulus duration and response width at half maximal response ($r = 0.905$, $P < 0.001$ for Ir84a-OSNs, and $r = 0.917$, $P < 0.001$ for Ir75abc-OSNs, Spearman's correlation; Figures 2C and D left panel). Similarly, the Ir41a-OSN response to 1,4-diaminobutane at [−2] did not show adaptation at longer stimulus durations ($t = 0.073$, $P = 0.944$ for 500 ms vs. 1 s stimulations; $t = 0.01$, $P = 0.992$ for 500 ms vs. 2 s stimulations).

OSNs expressing Gr21a, which are housed in ab1 sensilla on the *Drosophila* antenna, respond to pure CO₂ beginning at a 20 ms stimulation (Mann-Whitney *U*-test, $P = 0.009$ Figure 2E). Peak response was obtained at 1 s ($t = 4.641$, $P = 0.002$, Student's *t*-test compared to 20 ms), while at a 2 s stimulation the maximum response frequency decreased significantly ($t = 2.63$, $P = 0.02$, Student's *t*-test, 1 s vs. 2 s). However, the response latency also became shorter with stimulus duration, decreasing from the 20 ms duration (with a mean half-maximal response on set time of 400 ± 26.35 ms), to 1 s (with a mean half maximal response on set time 300 ± 17.67 ms, $t = 3.028$, $P = 0.016$, Student's *t*-test; Figure 2E left panel). This is opposite to both OR- and IR-expressing OSNs, where there was no difference (Figures 2A–D). Similarly, the response width also increased with stimulus duration ($r = 0.781$, $P < 0.001$, Spearman's correlation, Figure 2E left panel).

Increasing stimulus concentrations reduced the duration required to elicit a response regardless of the receptor expressed.



For example, Or59b-OSNs required 50 ms at [−6] to elicit a significant response ($t = 2.486$, $P = 0.025$; **Figure 1A** right), but only 20 ms at [−3] ($P < 0.001$, Mann–Whitney U -test, asterisk in **Figure 2A** right). Similarly, Ir84a-expressing OSNs stimulated with phenyl acetaldehyde at [−2] required only 20 ms to elicit a significant response (Mann–Whitney U , $P = 0.02$, **Figure 2C**), while Ir75abc-expressing OSNs required a 50 ms stimulation when the concentration of butyric acid increased by $10\times$ [−2] (Mann–Whitney U , $P = 0.002$, **Figure 2D**, asterisk right).

However, the dose-dependency of OSN adaptation to long stimulus durations was dependent on the receptor expressed. At [−6] long stimulus durations did not reduce the response of Or59b-expressing OSNs ($t = 0.292$, $P = 0.776$ for 500 ms vs. 1 s; $t = 0.33$, $P = 0.745$ for 500 ms vs. 2 s) or Or35a-expressing OSNs ($t = 1.151$, $P = 0.147$ for 500 ms vs. 1 s; $t = 0.948$, $P = 0.356$ for 500 ms vs. 2 s; **Figures 2A,B** right). However, at [−3] concentration, stimulations of 1 s or more significantly reduced the Or59b-expressing OSN response maximum ($t = 2.235$, $P = 0.045$ for 500 ms vs. 1 s; $t = 2.658$, $P = 0.021$ for 500 ms vs. 2 s, **Figure 2A**). In contrast, longer stimulus durations did not reduce the response of IR-expressing OSNs regardless of concentration (Ir84a-expressing OSNs at [−2]: Mann–Whitney U , $P = 0.847$ for 500 ms vs. 1 s; Ir75abc-expressing OSNs at [−2]: $t = 0.644$, $P = 0.531$ for 500 ms vs. 1 s; Ir41a-expressing OSNs at [−2], $t = 0.073$, $P = 0.944$ for 500 ms vs. 1 s; **Figures 2C,D** right panels).

PULSE RESOLUTION OF DIFFERENT SENSORY NEURONS

After investigating the response of OSNs to various stimulus durations, we presented the neurons with repeated stimulations of varying frequency. The latency to repeated stimulations at 1 Hz increased for all OSN types ($r = 0.742$, $P < 0.001$ for Or59b-OSNs; $r = 0.94$, $P < 0.001$ for Gr21a-OSNs; $r = 0.787$, $P < 0.001$ for Ir75abc-OSNs; $r = 0.652$, $P < 0.001$ for Ir84a-OSNs; Spearman's correlation; **Figures 3A–E**). However, a variability in latency was observed between the tested OSNs; e.g., Ir75abc-OSNs showed more delayed time to maximum than all other neurons tested, $P < 0.001$, ANOVA followed by Tukey *post-hoc* test (**Figure 3E**). At $100\times$ stimulus concentrations or a 5 s interstimulus interval, the latency for Or59b-expressing OSNs did not change with repeated stimulation ($r = 0.09$, $P = 0.475$; $r = -0.006$, $P = 0.952$, respectively, Spearman's correlation; **Figure 3F**). Similarly, Ir75abc-expressing OSN response onset recovered with a higher concentration ($r = 0.01$, $P = 0.90$, Spearman's correlation). However, at 5 s interstimulus intervals the response onset became significantly faster for the later pulses ($r = -0.885$, $P < 0.001$, Spearman's correlation; **Figure 3G**). The latency also decreased with subsequent stimulations of CO₂ for Gr21a-expressing OSNs at 5 s interstimulus intervals ($r = -0.976$, $P < 0.001$, Spearman's correlation; **Figure 3H**). In summary, this shows that changes in response onset kinetics to repeated stimuli are similar across all tested OSNs and response latencies can be regulated either by altering stimulus concentrations or inter-stimulus intervals.

Ir84a and Ir75abc-OSNs, housed in ac4 and ac3 sensilla respectively, could resolve repeated 200 ms pulses of [−4] and [−3] stimulus concentrations, respectively, up to 4 Hz (the maximum testable frequency due to stimulation length). The mean

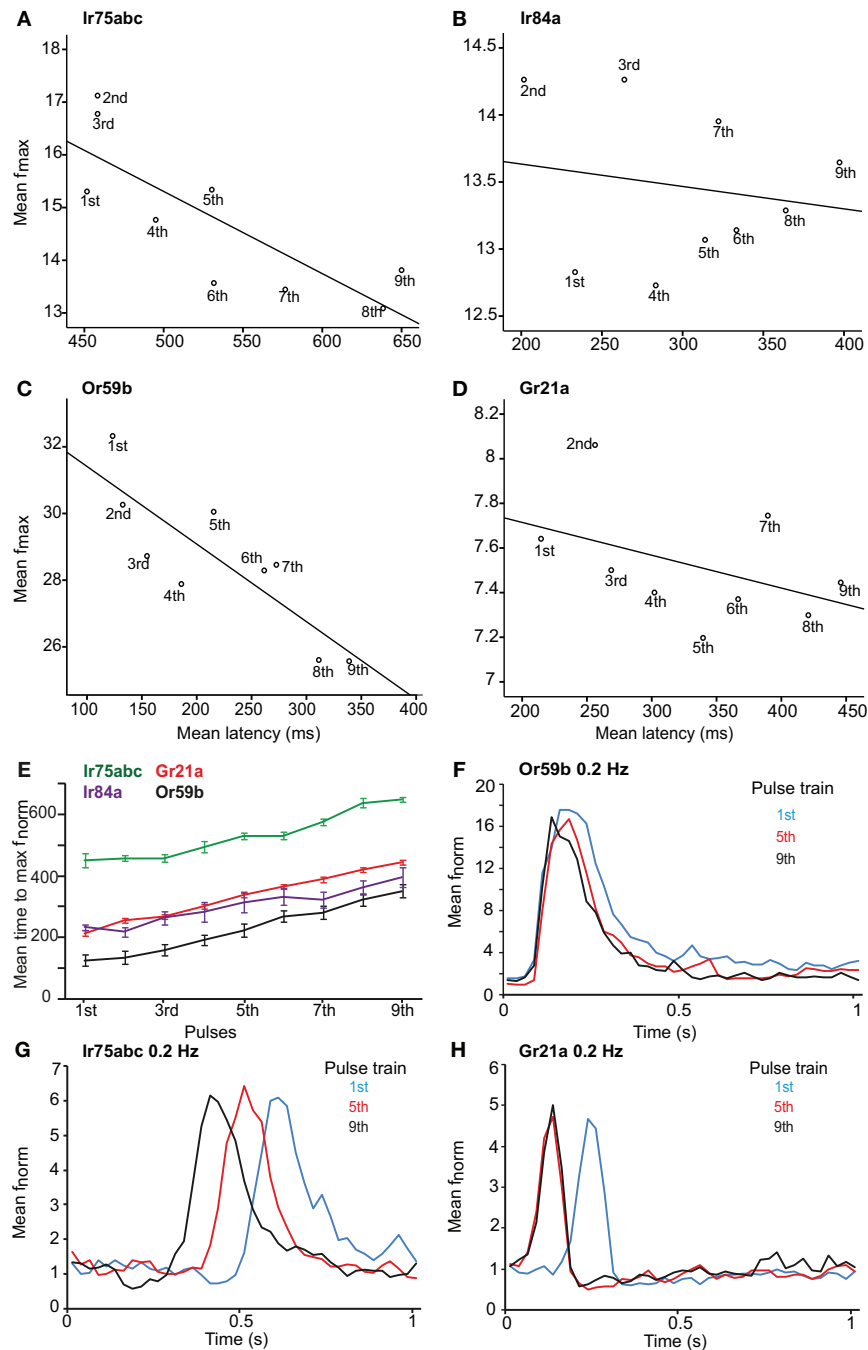
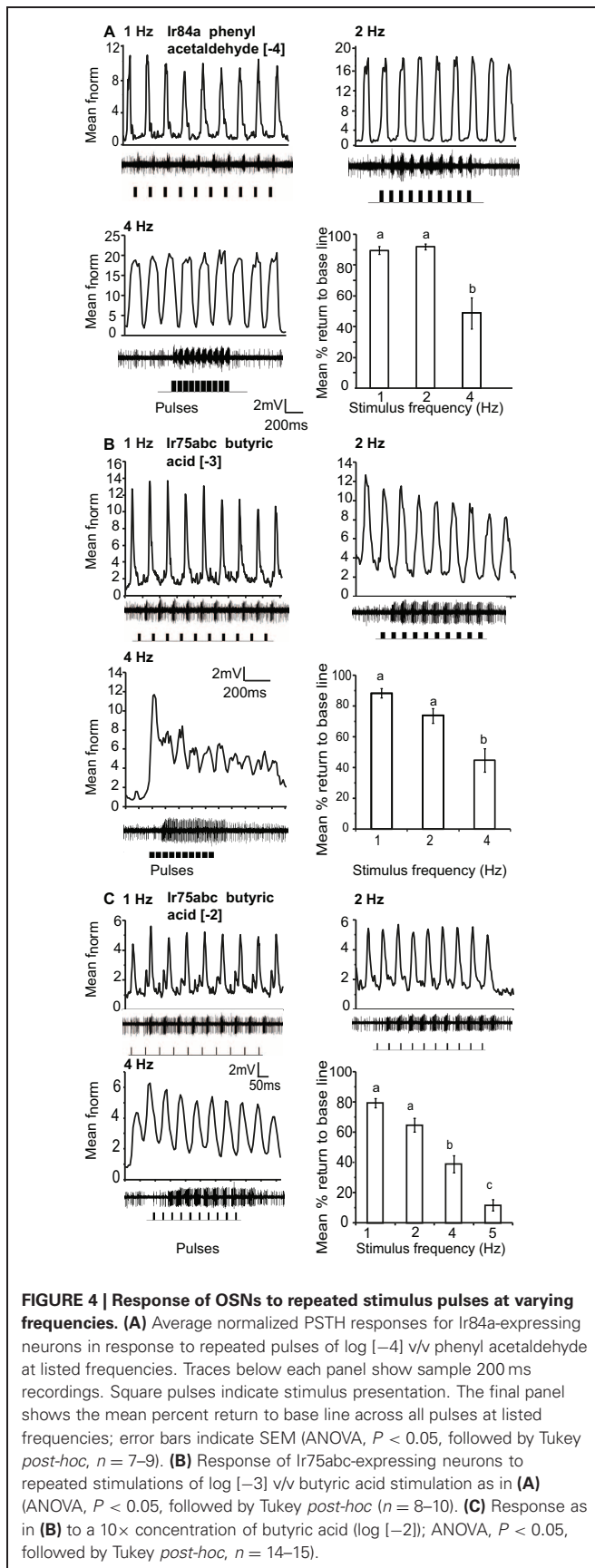


FIGURE 3 | Latency and maximum response of OSNs to repeated stimuli. Maximum response frequency vs. time to peak (latency), with best fit line, for OSNs carrying various receptors in response to repeated 1 Hz stimulations. **(A)** Ir75abc, **(B)** Ir84a, **(C)** Or59b and **(D)** Gr21a. Pulse number (1–9) indicated below each point. **(E)** Mean time

to maximum response frequency for neurons in **(A–D)** at a 1 Hz repeated stimulation. **(F)** The response onset recovery of Or59b-expressing OSNs when stimulated at 0.2 Hz, $n = 10$ **(G)** as in **(F)** for Ir75abc-expressing OSNs, $n = 7$ and **(H)** as in **(F)** for Gr21a-expressing OSNs, $n = 10$.

return to base line during repeated stimulation was significantly reduced at 4 Hz as compared to 1 and 2 Hz stimulation, ($P < 0.05$ ANOVA followed by Tukey *post-hoc* test; **Figures 4A,B**). At an increased concentration of $[-2]$, Ir75abc OSNs could resolve pulsed stimuli up to 5 Hz at a 50 ms pulse duration (**Figure 4C**).

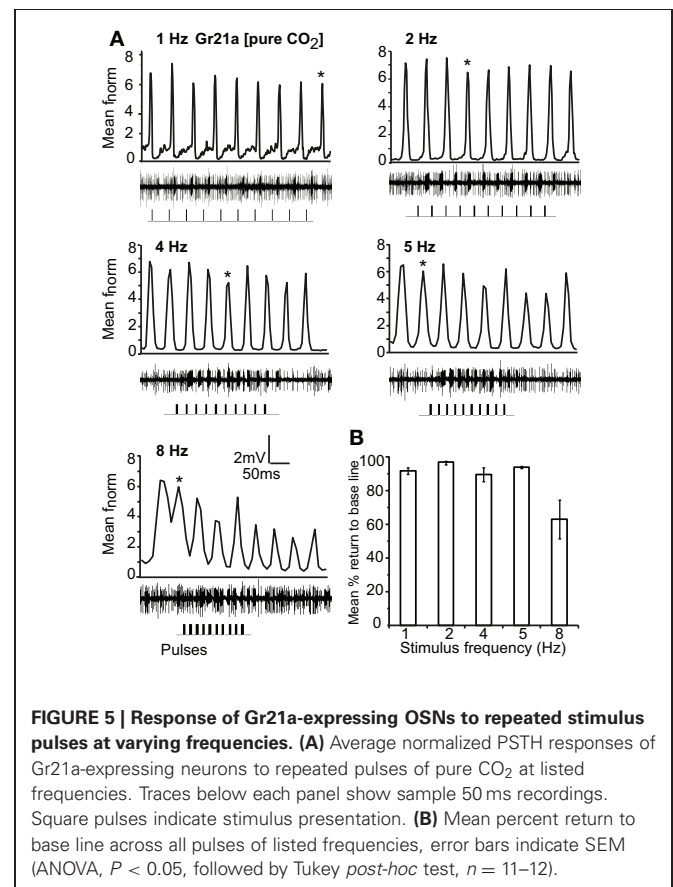
Gr21a-expressing OSNs housed in ab1 sensilla resolved intermittent pulses of CO_2 as fast as 8 Hz with no significant difference in return to baseline between 1 Hz and 5 Hz stimulations. At 8 Hz, the mean return to base line was significantly reduced, and at 10 Hz only 2.4% recovery to the base line occurred



($P < 0.001$, ANOVA followed by Tukey *post-hoc* test; **Figures 5A and B**). Gr21a-expressing OSNs also exhibited short term adaptation based on AUC (see “Materials and methods”) that was frequency dependent, i.e. at 1 Hz stimulation the 9th pulse resulted in a significantly reduced response compared to the 1st pulse (repeated measure ANOVA, $P < 0.001$), while at 2 Hz the 4th pulse was reduced ($P = 0.039$), at 4 Hz the 5th ($P = 0.001$), and at 5 and 8 Hz the 2nd ($P < 0.01$, repeated measure ANOVA; **Figure 5** asterisks).

PULSE RESOLUTION OF STIMULI ELICITING OPPOSITE RESPONSE POLARITY

We also tested the pulse following capacity to single excitatory and inhibitory odor ligands in Or59b-expressing OSNs. We applied [−5] methyl acetate as an excitatory and [−5] citral as an inhibitory ligand. Or59b-expressing OSNs could resolve the excitatory stimulus up to 5 Hz (**Figure 6A**). The mean return to base line was significantly reduced at 5 Hz stimulation as compared to 1 and 2 Hz ($P < 0.05$, ANOVA followed by Tukey *post-hoc* test; **Figure 6C**). However, the pulse resolution was also affected by concentration, as a 100× increase in concentration reduced the pulse resolution to 2 Hz ($P < 0.05$). In contrast to the excitatory responses, Or59b-cells were able to resolve pulses of the inhibitory ligand citral only up to 2 Hz, and at 4 and 5 Hz the OSNs showed total inhibition and did not recover when stimulated repeatedly with the inhibitory ligand ($P < 0.05$ ANOVA followed by



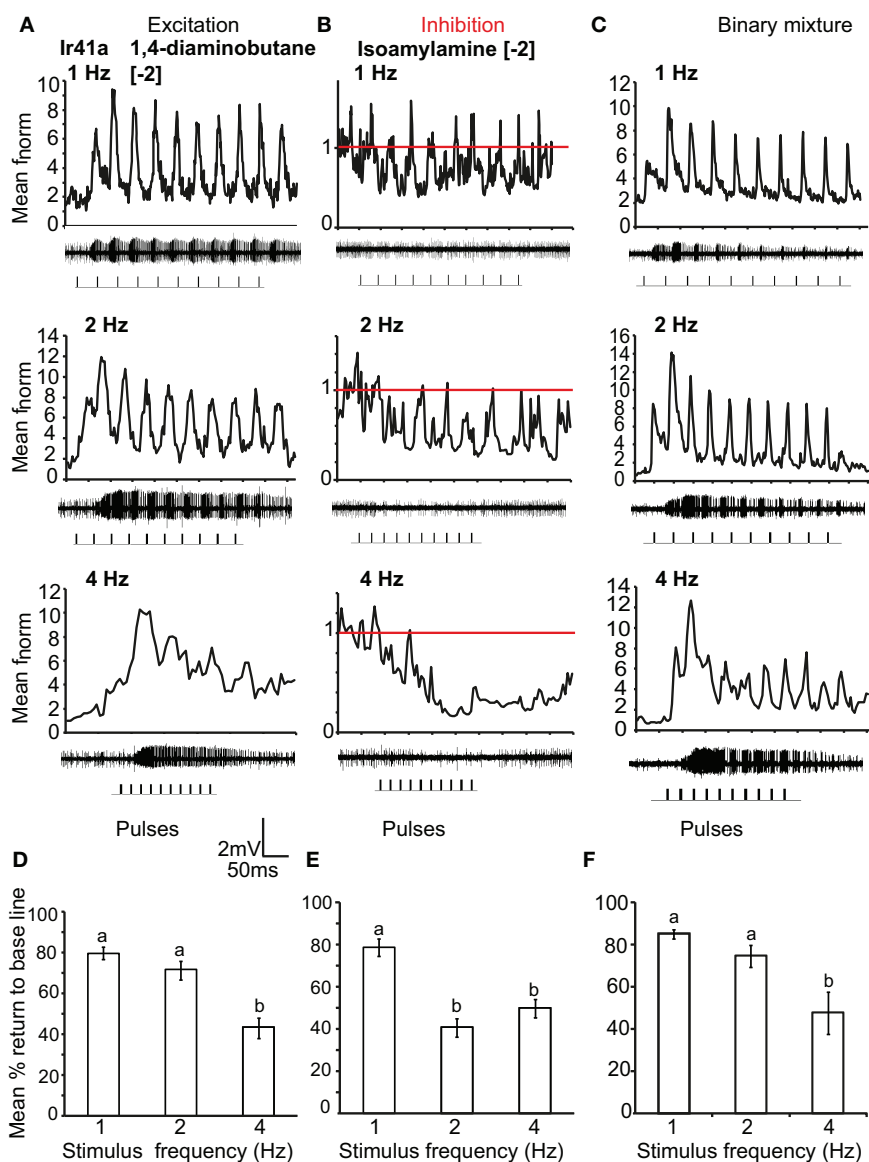


FIGURE 7 | IR-expressing OSN response polarity and pulse resolution.

(A) Mean normalized PSTH response of Ir41a-expressing OSNs to repeated pulses of log [-2] v/v 1, 4-diaminobutane at listed frequencies. Traces below each panel show sample 50 ms recordings. Square pulses indicate stimulus presentation. (B) As in (A) for log [-2] v/v of the inhibitory odor isoamyl amine. Red line indicates baseline frequency. (C) Mean normalized PSTH response of Ir41a-expressing OSNs to a binary

mixture of 1, 4-diaminobutane and isoamyl amine at [-2] v/v. (D) Mean percent return to base line to the excitatory ligand across all pulses at listed frequencies, error bars indicate SEM (ANOVA, $P < 0.05$, followed by Tukey *post-hoc* test, $n = 7-8$) (E), as in (D) for log [-2] v/v of the inhibitory odor isoamyl amine (ANOVA, $P < 0.05$, followed by Tukey *post-hoc* test; $n = 7-9$). (F) as in (D) for the binary mixture (ANOVA, $P < 0.05$, followed by Tukey *post-hoc* test, $n = 7-9$).

RESPONSE DYNAMICS TO DIFFERENT STIMULUS DURATIONS ARE A FUNCTION OF RECEPTOR TYPE

We found that the response of *Drosophila* OSNs to varying stimulus durations (Figure 2) depends on the type of receptor expressed in that neuron. OR-expressing OSNs showed adaptation to higher concentrations of long stimulus pulses (>1 s), both in maximum frequency and latency. This response feature was also independent of ligand (data not shown). In contrast, when IR-expressing OSNs were tested with the same

protocol, they required longer stimulus durations to respond, and there was no desensitization even up to 2 s stimulation either in response intensity or latency regardless of stimulus concentration. As a consequence, OSNs that express IRs are able to transmit information concerning the presence of long-lasting odors in their environment better than OR-expressing OSNs. However, this could also present a trade off, because the signal transduction in these OSNs appears to be slower, as seen in Figure 3E, where the time to maximum frequency was

longer in IR-expressing OSNs as compared to OR-expressing OSNs.

The difference in response between IR- and OR-expressing OSNs to longer pulses was not a function of stimulus presentation, which was assessed by PID (see “Materials and methods”). It is therefore a property of the OSNs themselves. Are these differences a function of the peri-receptor environment, or rather a property of internal OSN kinetics? To test this, we assessed the response of Or35a-OSNs, which are housed in coeloconic sensillum ac3 together with Ir75abc-OSNs. As with other OR-expressing OSNs, Or35a-OSNs also responded to stimulations as brief as 20 ms and showed desensitization at longer pulses (2 s) in maximum response frequency (**Figure 2B**). The response kinetics of these OSNs is therefore less influenced by the environment where they are expressed and rather by intrinsic properties of the neurons themselves.

The broad protostome conservation of IRs contrasts sharply with the restriction of ORs to insect genomes. This phylogenetic evidence suggests that IRs were the first olfactory receptor repertoire in insects (Robertson et al., 2003; Croset et al., 2010). IRs are also restricted to coeloconic sensilla, whereas ORs are found in several morphological sensillum types (Gupta and Rodrigues, 1997; Goulding et al., 2000; zur Lage et al., 2003; Benton et al., 2009). Our results show that IR-expressing OSNs required longer stimulation times to respond to key odorants, and responded with lower response intensities. This could imply that IRs are less efficient and less sensitive in detecting and transducing a chemical signal. OR activation results in both ionotropic and metabotropic signaling (Wicher et al., 2008; Deng et al., 2011), while IRs are thought to be purely ionotropic (Benton et al., 2009). Ionotropic signaling is also known to be less sensitive (Sato et al., 2008, 2011; Wicher et al., 2008). The requirement for higher concentrations in IR-expressing OSNs has been also shown in Yao et al. (2005). The signal transduction in Gr21a has been shown to involve $G\alpha_q$ protein, but not $G\alpha_s$ (Yao and Carlson, 2010; Deng et al., 2011). Thus, it is possible that the transduction cascade itself leads to these differences in response to varying stimulus durations.

The desensitization/adaptation at longer stimulus durations could affect the temporal accuracy of OR-expressing OSNs in reporting long-lasting odor strands, but it may also enrich the coding possibilities for odor discrimination (DeBruyne and Baker, 2008; Nagel and Wilson, 2011) by allowing the neuron to return to its resting state more quickly. This could provide additional possibilities for odor discrimination such as under background odor, or for resolution of intermittent pulsed stimuli. Adaptation extends the operating range of sensory systems, in some cases over an enormous span of stimulus intensities (Torre et al., 1995). It may also play a role in complex functions of neuronal systems such as stimulus location (Kaissling et al., 1987). Similar results were reported in the locust where the electrophysiological response of projection neurons also depended on stimulus duration (Brown et al., 2005; Mazor and Laurent, 2005). In contrast, the long-lasting response of IR-expressing OSNs could allow for close range detection while on or very near the stimulus source where stimulus durations could persist for much longer periods of time

(Murlis et al., 2000; Louis et al., 2008; Gomez-Marin et al., 2011).

PULSE RESOLUTION IS RECEPTOR TYPE DEPENDENT

The different classes of OSNs also showed differences in their pulse resolution to repeated stimuli. Brief intermittent stimuli were not detected by IR-expressing OSNs, in contrast to those expressing ORs (which could respond up to 5 Hz). This response characteristic was mainly due to a difference in sensitivity, as increasing the stimulus concentration for IR-expressing OSNs improved the detection and resolution to 5 Hz. In contrast, a 100× increase in concentration actually reduced the OR-OSNs pulse resolution. The accuracy of encoding rapidly fluctuating intermittent odorant stimuli above 5 Hz was significantly reduced for all OSNs regardless of receptor type. Similarly, other insects resolved up to 5 Hz pulses of general odors or pheromones (e.g., Lemon and Getz, 1997; Barrozo and Kaissling, 2002; Bau et al., 2002), even at the antennal lobe (e.g., Christensen and Hildebrand, 1997; Lei and Hansson, 1999; Lei et al., 2009).

Short term adaptation and latency to peak response to repeated stimuli were independent of the receptor expressed in the OSN (**Figures 3A–E**). In addition, the time to peak response and the response intensity were recovered in all OSNs either by increasing the inter-stimulus interval to 5 s or by increasing the concentration. This suggests that adaptation to repeated stimulation is a general feature of all OSNs, regardless of the receptor expressed. Adaptation is assumed to be an early step in information processing and decision making (Kaissling et al., 1987; Baker et al., 1988; Dolzer et al., 2003; Theodoni et al., 2011), and appears to affect the response of all OSN types in a similar manner.

RESPONSE POLARITY AFFECTS PULSE RESOLUTION

Both OR- and IR-expressing OSNs were unable to resolve pulsed inhibitory ligands at frequencies as high as excitatory ligands (**Figures 6B** and **7B**). This could be because the response inhibition lasted longer than excitation (**Figure 6E**), even though the concentrations tested were at the same point in the dose response curve (**Figure 1**). According to Ghatpande and Reisert (2011), fast response termination improves pulse resolution. Similarly, Su et al. (2011) showed that the inhibitory responses of OSNs lasted much longer than their excitatory responses, but the reason for this difference is not clear. Interestingly, a mixture of both excitatory and inhibitory odors improved pulse resolution at high frequencies (**Figures 6F** and **7F**). As a consequence, OSNs may respond to intermittent blends at faster rates, which may increase their ability to track complex natural stimuli.

The fast-terminating biphasic response exhibited by Gr21a-OSNs in response to CO₂ stimulation could be the reason why Gr21a-OSNs resolved more rapid stimulations as compared to OR- and IR-expressing OSNs (**Figure 5**). A biphasic response improved pulse resolution in antennal lobe neurons (Lei and Hansson, 1999). Besides the OSN itself, the chemistry of CO₂ could also contribute to

better pulse resolution as it will readily hydrate to bicarbonate (Kwon et al., 2007), and the degree of odor clearing is one of the challenges for resolving rapidly fluctuating odorant stimuli (Ishida and Leal, 2005; Ghatpande and Reisert, 2011).

CONCLUSION

Terrestrial olfaction requires the tracking of brief, intermittent airborne stimuli in a turbulent and dynamic environment. Fast reaction times to pockets of clean air are suggested to be behaviorally important for successful and rapid source location; hence, the selection over evolutionary time for sensitive and high-fidelity odor strand detection and resolution in the insect olfactory system is crucial (Baker and Vickers, 1997). Equally, the temporal structure of olfactory information has been shown to be critical for odor coding in a variety of systems (Laurent et al., 2001). Here we show that IR-expressing OSNs are better in detecting long-lasting odor pulses, but they are less sensitive. That could suggest that they are better at close range odor detection where odor-OR interaction time is not a limiting factor (high molecular flux). In contrast OR-expressing neurons are more sensitive and better at resolving brief (low molecular flux) pulsed stimuli. This diversity in temporal characteristics could provide a broad palette of response kinetics for the insect olfactory system to respond to

the high-dimensional temporal input found in an insect's odor environment.

IRs are the only receptors found in basal insects and conserved between unicellular and multicellular organisms (Croset et al., 2010). ORs appear to have derived from the gustatory receptor family (Robertson et al., 2003; Nordström et al., 2011), which is present in insects as well as in aquatic arthropods such as water fleas (Peñalva-Arana et al., 2009). Besides increasing the diversity of chemicals that could be detected, OR-OSNs also allow the olfactory system to rapidly detect and transduce brief airborne odor information. This is especially important for flying insects, for which stimulus contact is brief and fast response in time is most critical. OR-expressing OSNs were indeed more sensitive to intermittent stimuli than IRs and Gr21a. The sensitive and fast neuronal response observed in OR-expressing OSNs could result from Orco-dependent transduction, which may have evolved through selective pressure to increase sensitivity and speed of odor detection while in flight.

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