Temporal coding of odor mixtures in an olfactory receptor neuron

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Most natural odors are mixtures and often elicit percepts distinct from those elicited by their constituents. This emergence of a unique odor quality has long been attributed to central processing. Here we show that sophisticated integration of olfactory information begins in olfactory receptor neurons (ORNs) in *Drosophila*. Odor mixtures are encoded in the temporal dynamics as well as in the magnitudes of ORN responses. ORNs can respond to an inhibitory odorant with different durations depending on the level of background excitation. ORNs respond to mixtures with distinctive temporal dynamics that reflect the physicochemical properties of the constituent odorants. The insect repellent DEET (*N*,*N*-diethyl-*m*-toluamide), which attenuates odor responses of multiple ORNs, differs from an ORN-specific inhibitor in its effects on temporal dynamics. Our analysis reveals a means by which integration of information from odor mixtures begins in ORNs and provides insight into the contribution of inhibitory stimuli to sensory coding.

A fascinating aspect of the sense of smell is that odor mixtures often have distinctive emergent qualities, and their individual constituents are difficult to identify (1). How the emergent quality of an odor mixture arises is unknown. It is believed to originate mainly from information processing in the central nervous system, as in the insect antennal lobe (2–4) and the vertebrate olfactory bulb (5, 6). However, olfactory receptor neurons (ORNs) also contribute to the integration of olfactory information. For example, some individual rat ORNs respond to a binary odor mixture with response magnitudes, i.e., firing frequencies, that cannot be predicted simply from the responses to its components (7, 8). In addition, studies in moths (9–14) and beetles (15) have provided evidence that information about odorants in a mixture can be integrated in the periphery. However, little is known about how information is processed in ORNs. It is unclear, for example, whether individual ORNs are capable of integrating information via means other than simple alterations of their response magnitudes.

Equally intriguing in sensory coding is the role of inhibitory stimuli. In taste, certain stimuli inhibit vertebrate sweet receptors (16, 17). In olfaction, inhibitory odorants have been identified for olfactory receptors of vertebrates (18–21) and invertebrates (13, 22–26). When delivered as a monomolecular odor stimulus, inhibitors reduce the spontaneous activity of ORNs. However, the low spontaneous activities of most ORNs (13, 22, 23, 27) limit the dynamic range of inhibition, and inhibitory odorants may have greater functional significance when paired with excitatory odorants. Indeed, an inhibitory odorant reduced the intensity of a concurrent excitatory odor stimulus in a human psychophysical study (28), and odorants that inhibited CO_2-sensing neurons prevented CO_2-mediated avoidance behaviors in *Drosophila* (24). However, it is not clear whether a binary mixture of an excitatory stimulus and an inhibitory stimulus can be distinguished from an excitatory stimulus alone at a lower intensity. How is a mixture encoded by the primary sensory neuron?

To analyze how ORNs integrate excitatory and inhibitory odor information, we have carried out in vivo single-unit recordings in the relatively simple and well-defined *Drosophila* olfactory system. Our studies reveal an unexpected capacity of individual ORNs to encode odor mixtures. The results indicate how ORNs may contribute to the generation of unique percepts for odor mixtures. More generally, our findings reveal means by which inhibitory stimuli can shape sensory responses. In addition to reducing the magnitudes of excitatory responses, inhibitors may alter their temporal dynamics. Our results identify degrees of freedom by which primary sensory neurons may encode complex sensory information.

Results and Discussion

Excitatory and Inhibitory Responses to Single Odorants. We first compared the responses to single excitatory and inhibitory odorants in a well-characterized class of ORN, ab2A, which expresses the Or59b receptor (23, 29, 30). Excitatory responses are elicited in ab2A by several structurally similar esters and ketones (31). The magnitude of the excitatory response increased with increasing odorant concentrations (Fig. S1, *Left* panels). However, the width of the response, defined as the time interval between the half-maximal points, i.e., between the time points in the rising and falling phases at which the response magnitudes are half of the peak response, remained largely constant (Fig. 1*A* and Fig. S1). At all concentrations, the excitatory response terminated shortly after the end of the stimulus.

Inhibitory responses are elicited in ab2A by linalool, a terpene that lowers the spontaneous activity of the ab2A ORN (22) (Fig. 1*B*). This inhibition depends on the receptor of the ab2A cell; it is observed following ablation of the neighboring ab2B ORN, and we verified that ectopic expression of the ab2A receptor (Or59b) in a mutant "empty neuron" (23, 32) conferred sensitivity to inhibition by linalool (Fig. S2). In measurements from the endogenous ab2A cell, we observed robust inhibition with linalool at 5×10^{-3} dilutions or at higher concentrations, although not at lower concentrations. In contrast to the ab2A excitatory responses, the width of inhibitory responses increased with higher odorant concentrations (Fig. 1*B*, *Right*). We also observed prolonged inhibition by a brief pulse of another ab2A inhibitory odorant, α-terpineol (Fig. S3*A*). Thus, the inhibitory responses of ab2A lasted much longer than the excitatory responses. We note that when linalool was used as an excitatory odorant, against a different ORN (ab7A), the responses did not extend beyond the length of the odor stimulus (Fig. 1*C*). Taken together, these results suggest a role for response duration in the coding of inhibitory stimuli in ab2A. In summary, responses of an individual ORN to individual excitatory and inhibitory odorants can differ not only in polarity but also in temporal dynamics.

Inhibition in an Excitatory Background. Having examined the responses to excitatory and inhibitory odorants individually, we next asked how ORNs encode a pulse of inhibitory odorant in

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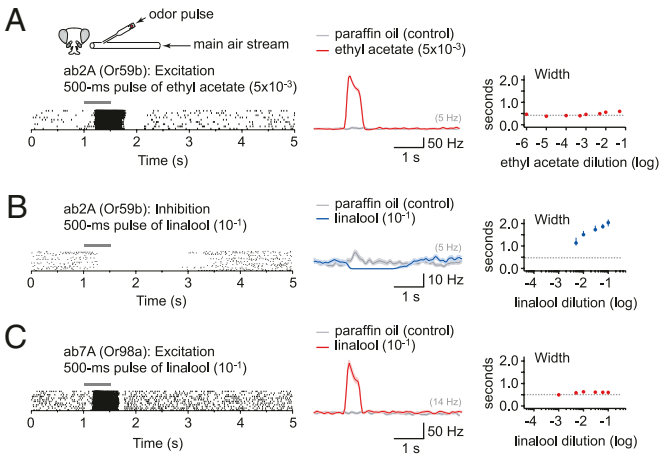

Fig. 1. Comparison of excitatory and inhibitory olfactory responses to single odorants. Single-unit recordings of ab2A ORNs in response to a single excitatory odorant, ethyl acetate (*A*) ($n = 9$) or an inhibitory odorant, linalool (*B*) ($n = 24$). (*C*) Excitatory responses of ab7A ORNs to linalool ($n = 12$). (*Left*) Raster plots of individual ORN responses to a 500-ms pulse of odorant (gray bar). Each short vertical line represents one spike. (*Center*) Corresponding peri-stimulus time histograms (PSTHs), spikes binned at 50 ms (shaded area represents SEM but, for ethyl acetate, is too small to be visible). (*Right*) Response width, defined as the time interval between half-maximal responses. Linalool at 10^{-3} or lower concentrations did not elicit any inhibitory response. Error bars indicate variations in width due to SEM (*Materials and Methods*), which are too small to be visible in *A* and *C*. Gray dotted lines (*Right* panels) denote the 500-ms stimulus duration.

the course of a response to an excitatory background odorant. We examined the response of ab2A to a 500-ms pulse of linalool, superimposed on a constant stream of background excitatory odorant, methyl acetate. Higher concentrations of the background excitatory odorant produced higher levels of background firing rates (Fig. 2*A*). Interestingly, the duration of inhibition decreased with increasing background excitation. At the maximum background firing levels tested, the duration of the period of inhibition was similar to the duration of the inhibitory stimulus [Fig. 2*A*, 1ac (5×10^{-5}), and Fig. 2*B*, *Upper*]. Similar results were observed with another background excitatory odorant, ethyl acetate, suggesting that changes in the duration of inhibition are determined primarily by the degree of background activation, rather than by the identity of the excitatory odorant. One interpretation of the decline in duration of inhibition is that higher levels of the background excitatory odorant may accelerate the recovery from inhibition.

The concentration of linalool is encoded not only by the temporal dynamics of inhibition, but also by the magnitude of the inhibitory effect. Even at high levels of background excitation, linalool inhibited ab2A firing nearly completely (Fig. 2*A*). Thus, the magnitude of inhibition, defined here as the reduction in number of spikes, increased proportionally with the background firing rate (Fig. 2*B*, *Lower*). An important corollary of these results is that the response to a mixture of an inhibitory odorant and an excitatory odorant cannot be determined simply by summing the effects each has individually on the magnitude of the ORN firing rate.

We note finally that other receptor-odorant combinations, one including a mosquito olfactory receptor, AgOr8 (33–35), also showed a reduction in the duration of inhibition and an increase in the magnitude of inhibition following increases in the level of background excitation (Fig. S3). These results suggest that the use of these parameters to represent odor mixtures in insects may be a common principle.

Sharpened ORN Response to an Odor Mixture. Above we have analyzed the effects of superimposing a pulse of one odor upon a prolonged stimulus of another odor. Next we considered a pulse of a binary mixture of two odorants intended to simulate a blend of components in a food source. We compared the response to a pulse of a mixture of an excitatory odorant (methyl butyrate) and an inhibitory odorant (linalool) to the response to a pulse of the excitatory odorant and the diluent (paraffin oil). At lower concentrations, methyl butyrate elicited small excitatory responses from ab2A (peak response <50 spikes), which could be completely abolished by the inhibitory odorant, linalool (Fig. S4*A*). These results are consistent with a previous report that inhibition can override excitation in a binary odor mixture (36).

With higher methyl butyrate concentrations, however, linalool could only attenuate, not abolish, methyl butyrate-elicited excitatory responses (Fig. S4*A*). Interestingly, the attenuation was more pronounced during the falling phase of the response to the

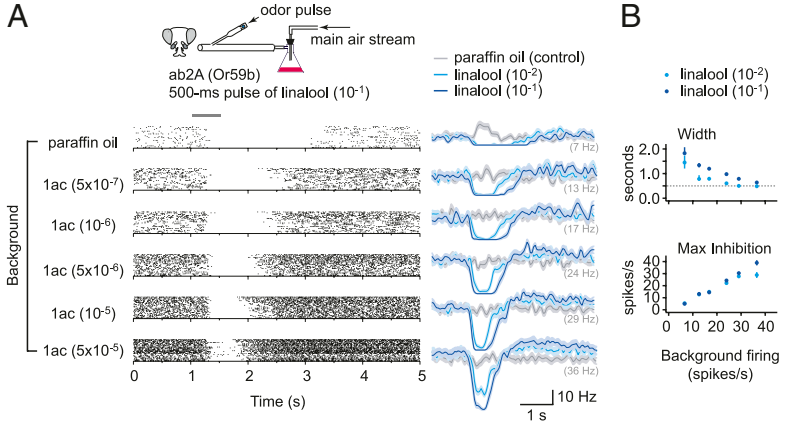

Fig. 2. Changes in the duration of inhibition with background excitation. (*A*) (*Left*) Raster plots and the corresponding PSTHs of ab2A spike activity to a 500-ms pulse of linalool (10^{-1}) (gray bar), with increasing concentrations of a background excitatory odorant, methyl acetate (1ac). Recordings were made when excitation was at steady state. (*Right*) Gray traces represent solvent control, a pulse of paraffin oil; blue traces represent a pulse of linalool (10^{-1}). The mean prestimulus background firing rates are indicated in parentheses ($n = 24$). As a control, we confirmed, using a photoionization detector, that the presence of the background excitatory odorant had little if any effect on the time course of individual linalool stimulus. (*B*) Average width of the inhibitory responses and magnitude of inhibition, plotted as a function of average background firing rate. Error bars: ±SEM except for "Width," which indicates variations in width due to SEM.

mixture, and the resulting response appeared "sharpened" (Fig. 3 *A* and *B* and Fig. S4*A*). In fact, linalool nearly halved the width of methyl butyrate-elicited responses to ~200 ms (Fig. 3*C*, *Upper Left*).

Notably, the fast-falling dynamics of the response to a 500-ms pulse of the mixture also differed from those of the response to a 200-ms pulse of methyl butyrate alone over a variety of concentrations (Fig. 3*D* and Fig. S4*B*). Thus, in the periphery, an ORN is capable of encoding a binary odor mixture with unique temporal dynamics that distinguish the mixture from the excitatory odorant alone, even when the excitatory odorant is presented at different concentrations or with different stimulus durations.

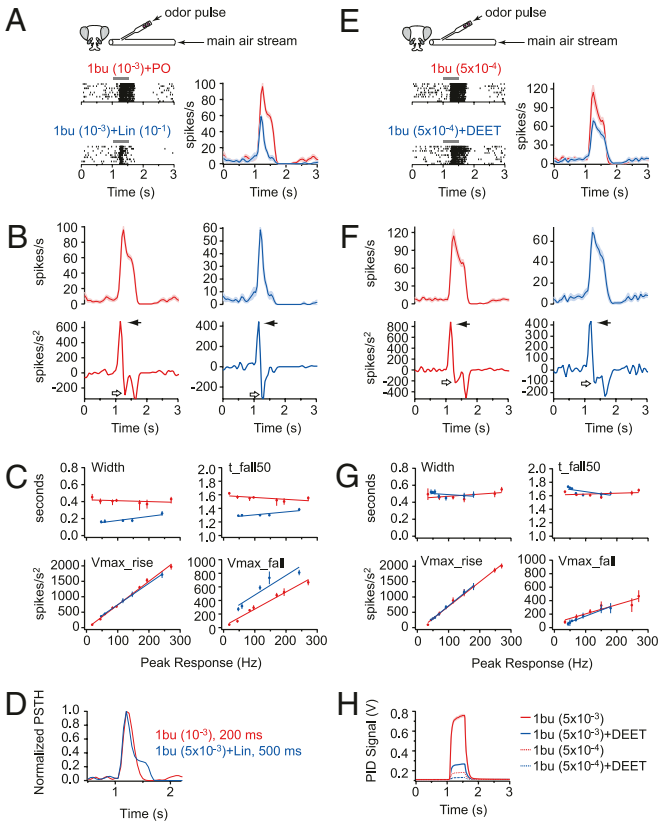

Fig. 3. Sharpening of the excitatory response by an inhibitory odorant in a mixture. (*A*) Responses of ab2A ORNs to an odor mixture containing equal parts of an excitatory odorant, methyl butyrate (1bu), and either an inhibitory odorant, linalool (Lin, 10^{-1}), or a solvent control, paraffin oil (PO) (n = 9). (*B*) PSTHs (*Upper Left* and *Right*) shown in *A* and the respective derivatives (*Lower Left* and *Right*), showing the maximum rising (solid arrows, Vmax_rise) and first maximum falling speed (open arrows, Vmax_fall). (*C*) Comparison of the excitatory response dynamics of methyl butyrate (10^{-5} to 5×10^{-2}) with either paraffin oil (red) or an inhibitory odorant, linalool (10^{-1}) (blue). Width: the time interval of the half-maximal responses; t_fall50: the time point when the response falls to its half-maximal magnitude. Linalool at a lower concentration (10^{-2}) caused a similar but smaller transformation in response dynamics. Recordings in *A*–*C* were conducted in the same ORNs. Values are plotted as a function of peak response. (*D*) Normalized PSTHs of ab2A to a 500-ms pulse of a methyl butyrate/linalool mixture and a 200-ms pulse of methyl butyrate (n = 9). (*E*–*G*) Similar to *A*–*C*, showing ab2A responses to methyl butyrate alone or as a mixture containing equal parts of methyl butyrate and DEET (100%, 50 μL), which is not soluble in paraffin oil (n = 9). (*H*) Photoionization detector (PID) measurements of odor stimuli, presented as 500-ms puffs of methyl butyrate alone or methyl butyrate mixed with DEET (n = 12). The photoionization potential of DEET is outside the detection range of the PID, and thus the PID signals represent only methyl butyrate concentrations in the gaseous phase.

We reasoned that the unique response dynamics elicited by linalool may arise from a different effect on the rising and falling phases of the response. Indeed, the time required for the response to fall from its peak to its half-maximal magnitude (t_fall50) was much shorter in the presence of linalool (Fig. 3*C*, *Upper Right*), whereas no difference was observed in the time required for the response to rise to its half-maximal magnitude. We examined the derivative of the response to quantify the rate at which responses rise and fall (Fig. 3*B*, *Lower Left* and *Right*). The analysis showed that linalool did not affect the rising process, but accelerated the falling process (Fig. 3*C*, *Lower Left* and *Right*), as measured by the maximum rising rate and the first maximum falling rate (solid arrowheads and open arrowheads, respectively, in Fig. 3*B*, *Lower Left* and *Right*).

In addition, to visualize the "net effect" of the inhibitory odorant in a mixture, we subtracted the control responses (methyl butyrate mixed with paraffin oil) from the responses to the binary odor mixture containing methyl butyrate and linalool (Fig. S5). Consistent with the notion that the response to a mixture of an inhibitory odorant and an excitatory odorant cannot be determined simply by summing the effects each has individually on the magnitude of the ORN firing rate (Fig. 2), the net inhibitory effect of linalool in a binary mixture varied depending on the concentration of the concurrent excitatory odorant, methyl butyrate (Fig. S5).

DEET Attenuation Does Not Change Response Dynamics. Having found that linalool, an ORN-specific inhibitor, affects the temporal dynamics of the response to a concurrent excitatory stimulus, we next asked whether a putative inhibitor of special interest, DEET (*N*,*N*-diethyl-*m*-toluamide), has similar effects on response dynamics. DEET attenuates odor responses in multiple ORN classes by blocking the common coreceptor Or83b (37) and/or by reducing the level of the concurrent excitatory odorant vapor (38), referred to as a fixative effect. We compared methyl butyrate-elicited responses with or without DEET. Indeed, DEET attenuated the responses elicited by methyl butyrate at higher concentrations (Fig. 3*E*). However, such attenuation resulted in little if any change in the response dynamics (Fig. 3 *F* and *G*). These results suggested that DEET differs from linalool in the mechanism by which it attenuates the activation of ab2A by methyl butyrate. Photoionization detector (PID) measurements showed that DEET reduced methyl butyrate concentration in the gaseous phase (Fig. 3*H*), supporting the notion that DEET has a fixative effect on other odorants (38). Our results do not exclude the possibility of additional inhibitory effects of DEET.

Odor Stimuli Differ in Their Temporal Dynamics. Why does linalool predominantly affect the falling phase of the excitatory response? PID measurements suggested that, although linalool and methyl butyrate were premixed in solution and carried via the same puff of air, their vapors may reach the fly's antenna at different rates (Fig. 4*A*). At all concentrations tested, levels of methyl butyrate vapor rose faster than linalool, as reflected by the smaller rising time constants (Fig. 4*B*; for τ_{on}, see *Materials and Methods*). Consequently, methyl butyrate-elicited excitatory responses also rose faster than linalool-elicited inhibitory responses, which may explain why the falling phase of methyl butyrate-elicited excitatory response was preferentially inhibited by linalool.

To verify this interpretation, we paired linalool with three other ab2A excitatory odorants (methyl acetate, ethyl acetate, and 2,3-butanedione), all of which demonstrate fast-rising PID profiles like that of methyl butyrate (Fig. S6). The physicochemical similarity shared by these strong ab2A excitatory odorants may contribute to the similarity of their PID profiles. As expected, we observed comparable asymmetric sharpening of the response dynamics by linalool in all three combinations (Fig. S6). Thus, in an odor mixture, a slow-arriving inhibitory odorant can sharpen the response elicited by a fast-arriving excitatory odorant. Our findings

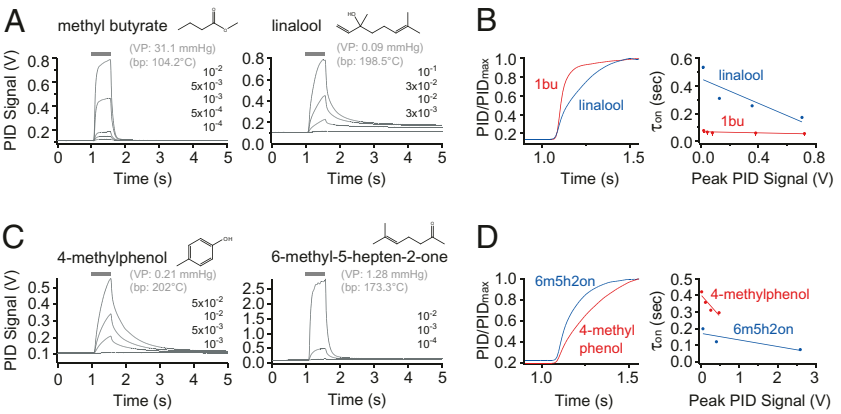

Fig. 4. Temporal dynamics of excitatory and inhibitory odor stimuli. (*A* and *C*) Photoionization detector (PID) measurements of odor stimuli, presented as 500-ms pulses (gray bars) (n = 12). Odor dilutions are indicated on the right in each panel. Information about saturated vapor pressure (VP, at 25 °C) and boiling point (bp, at 760 mmHg) of individual compounds was obtained from http://www.chemspider.com and is indicated in parentheses. (*B* and *D*) (*Left*) Normalized PID measurements of methyl butyrate (10^{-2}) and linalool (10^{-1}) (*B*) and of 4-methylphenol (5×10^{-2}) and 6-methyl-5-hepten-2-one (10^{-3}) (*D*). (*Right* in *B* and *D*) Comparisons of the odorant arrival kinetics. PID signals were normalized to the respective peak responses in *A* and *C*. The rising time constant (τ_{on}) was derived from PID measurements of each individual odorant.

support the notions that ORN responses can reflect the relative differences in stimulus dynamics and that insect olfactory systems can respond to odor stimuli with high temporal precision (39).

Biphasic Response to an Odor Mixture. We next considered the reciprocal situation: the pairing of a fast-arriving inhibitory odorant (6-methyl-5-hepten-2-one) with a slow-arriving excitatory odorant (4-methylphenol), which act on AgOr1 (34, 35, 40). For all concentrations tested, 6-methyl-5-hepten-2-one vapor rose faster than 4-methylphenol (Fig. 4 *C* and *D*). Interestingly, at a low concentration of the excitatory odorant 4-methylphenol, addition of the inhibitor 6-methyl-5-hepten-2-one resulted in a biphasic response (Fig. 5*A*). At a higher concentration of the excitatory odorant, a single, prolonged plateau was observed (Fig. 5*B*). At either concentration, the temporal dynamics differed from those observed for the excitatory odorant alone. We note that, in addition to rising slowly, 4-methylphenol vapor fell slowly (Fig. 4*B*), which may contribute to the long duration of the excitatory responses in AgOr1 (Fig. 5). Taken together, in addition to attenuating the excitatory responses, an inhibitory odorant in a mixture can generate distinctive response dynamics, depending on the properties and concentrations of the two odorants. Thus, an individual ORN may respond with distinctive dynamics that serve as a unique signature for a given blend of odorants.

Finally, we note that the relative differences in the PID profiles of fast and slow odorants were observed consistently with different odor delivery methods (Fig. S7 and *SI Text*). The relatively slow kinetics of linalool emission was also observed using a method in which odor delivery tubes were not used (41). Most "fast" odorants in our study have higher saturated vapor pressures and lower boiling points than the "slow" odorants, which is indicative of higher volatilities (Fig. 4).

Functional Implications for Higher-Order Neurons. As the temporal dynamics of the postsynaptic projection neurons (PNs) largely follow the temporal dynamics of the presynaptic ORNs (42), the unique temporal features of ORN responses to a given odor mixture are likely to be preserved in the PN responses. To address the effects of ORN inhibition on PN response, we used a formula that predicts the postsynaptic PN responses on the basis of the presynaptic ORN responses (43). We found a striking inhibitory effect of linalool on the PN responses (Fig. 6).

A particularly dramatic effect is predicted at low odorant concentrations. The greater effect at lower concentrations may be explained by the nonlinear amplification observed at the ORN–PN

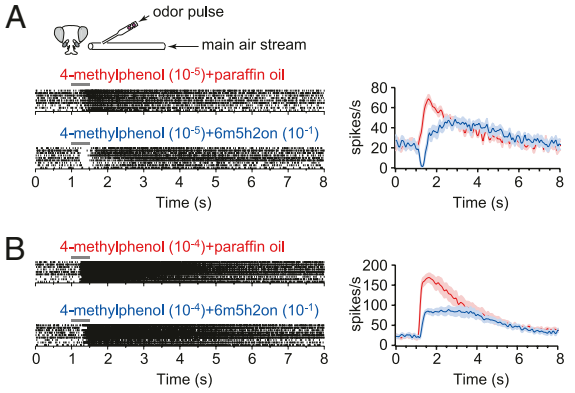

Fig. 5. Unique temporal dynamics elicited by an inhibitory odorant in a mixture of 4-methylphenol and 6-methyl-5-hepten-2-one are excitatory and inhibitory odorants for a mosquito receptor, AgOr1, respectively. Responses of AgOr1-expressing ORNs in response to a 500-ms pulse of an odor mixture containing equal parts of 4-methylphenol, 10^{-5} in *A* and 10^{-4} in *B*, and either 6-methyl-5-hepten-2-one (6m5h2on, 10^{-1}) or solvent control (paraffin oil). Recordings were conducted in the same ORNs (n = 9).

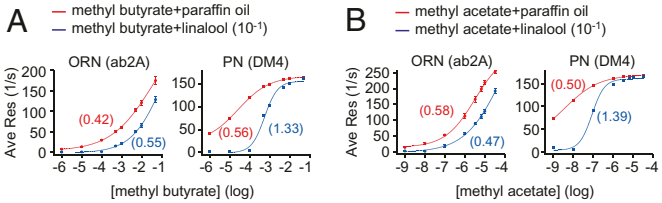

Fig. 6. Predicted responses of the postsynaptic projection neurons (PNs). The responses of the PNs receiving direct inputs from ab2A ORNs were estimated using the formula derived by Olsen et al. (43) using parameters for PNs in the DM4 glomerulus. ORN responses were average responses during the 500-ms odor stimulus. Dose–response relationships of two ab2A excitatory odorants are shown: methyl butyrate (*A*) and methyl acetate (*B*) (n = 9). Dose–response relationships were fitted with the Hill equation. Hill coefficients are indicated in parentheses.

synapse (42, 44): because weak ORN inputs are amplified more than strong ORN inputs in the PNs, inhibition of ORNs at lower concentrations caused greater inhibition in the corresponding range in the PN dose–response curve. Consequently, the PN dose–response curve appears steeper, characteristic of a curve with a greater Hill coefficient (PN panels). Thus, our analysis supports the notion that inhibition of ORNs is translated into inhibition of PNs; in fact, at low concentrations the inhibitory effects are likely to be even more pronounced in PNs than in ORNs.

Conclusions. We have found that ORNs are capable of sophisticated information processing. The emergent quality of an odor mixture has long been believed to arise from integration of olfactory information in the CNS. We have found that olfactory information is integrated in individual ORNs in the form of response dynamics as well as response magnitude.

We have analyzed how individual ORNs integrate olfactory information. We found that, when an inhibitory odorant is delivered in the presence of a background excitatory odorant, the duration and the magnitude of the inhibitory response reflect the concentrations of the inhibitory and excitatory odorants. ORNs also respond with distinctive temporal dynamics to premixed combinations of inhibitory and excitatory odorants. We show that the temporal dynamics of odor stimuli differ markedly, presumably due to differences in their physicochemical properties. The dynamics of the response to an odor mixture are likely to be shaped by the dynamics of its component stimuli. Taken together, our findings provide evidence that ORNs encode odor mixtures not only by altering their response magnitudes, but also by varying their response dynamics.

Do the temporal dynamics of ORNs contribute to odor identification and discrimination? A recent study showed that temporal heterogeneity in ORN firing patterns is crucial for generating spatiotemporal neural codes for odors in the locust antennal lobe (45), which represents odor identity in a concentration-independent manner (46). In the antennal lobe of moths, precise temporal activation patterns unique to certain odor blends provide a means for triggering innate behaviors (47). Moreover, mutant flies with only one functional type of Or-expressing ORN can be trained to discriminate different odorants, suggesting that odors could be discriminated by virtue of the temporal dynamics that they elicited in the ORN (48). Finally, we note that an emerging body of evidence supports a role for temporal dynamics in the coding of individual odorants in vertebrates (49–51), Thus, ORN response dynamics distinctive for an odor mixture seem likely to make a major contribution to the emergent quality of the mixture, binding features of the individual constituents into a unifying odor percept.

In perfumery, odorants of high, moderate, and lower volatility are commonly blended together to create the top, middle, and base notes, respectively, of a perfume. The composition and ratio of these notes endow the perfume with a signature scent (52). Human psychophysical studies have shown that odorants in a mixture are processed and perceived in series (53). As shown by our PID measurements (Fig. 4), inhibitory and excitatory odorants may also arrive in series and may interact so as to give rise to unique, mixture-specific ORN response dynamics. In this manner, inhibitory odorants may influence not only the perception of odor intensity, but also the perception of odor identity. Inhibitory stimuli could influence the perception of stimulus identity in other sensory modalities as well.

Finally, we note that, for many years, analysis of odors that drive animal and human behavior has focused primarily on their excitatory constituents. Our results invite a greater emphasis on the identification and analysis of their inhibitory constituents.

Materials and Methods

***Drosophila* Stocks.** All experiments were performed on adult female flies, 5–7 d after eclosion, except for "empty neuron" recordings, in which both male and female flies were used. Flies were reared at 25 °C in an incubator with a 12-h light–dark cycle. Wild-type flies are Canton-S. "Empty neuron" recordings were from flies of genotype *w; Δhalo/Δhalo; Or22a-GAL4/UAS-Or*. The ab3A mutant flies and *Or22a-GAL4* and *UAS-Or* transgenic lines were described previously (23, 32, 34, 40). Table S1 lists genotypes of the flies used in all experiments.

Electrophysiology and Data Analysis. Extracellular single-unit recordings were performed essentially as described previously (23). All data were acquired using Axoscope 10.2 (Molecular Devices), and ORN spikes were detected using routines in Igor Pro-6.01 (Wavemetrics) (44). Peri-stimulus time histograms (PSTHs) were obtained by averaging spike activities in 50-ms bins (44) and smoothed using a binomial algorithm (Igor Pro-6.01, Wavemetrics). Response width was calculated on the basis of average PSTHs, as the time interval between half-maximal responses in the case of excitation (Max_{50}, excitation) or half-minimal responses in the case of inhibition (Min_{50}, inhibition). Two additional PSTH traces were generated as PSTH + SEM and PSTH − SEM, respectively. The time intervals of Max_{50} or Min_{50} for the two additional PSTH traces determined the ranges for error bars of the response widths.

Odor Stimuli. For short odor pulses, odor stimuli (50 μL) were delivered from a Pasteur pipette via a pulse of air (200 mL/min) into the main air stream (2,000 mL/min) as described previously (23, 34), except for binary odor mixture experiments (Figs. 3 and 5) in which 100 μL of a premixed odor dilution was added to two filter discs, placed adjacently along the long axis of the Pasteur pipette (54). We observed no difference in the temporal dynamics of odor stimuli using one or two filter discs in our PID measurements. Background odor stimuli were delivered from a 125-mL flask containing 3 mL of odor dilutions directly downstream of the main airflow (2,000 mL/min).

PID Measurements. A fast response mini-PID (200a, Aurora Scientific) was positioned between the head of a fly and the mouth of the odor delivery tube as described previously (55, 56). Fig. S7 and *SI Text* show the PID setup in relation to the odor delivery system. The rising phase of the PID response was fitted with an exponential curve $y(t) = A^*\exp(-t/\tau_{on})$. Parameters of the fits are listed in Table S2.

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