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# A Novel Psychophysical Method for Estimating the Time Course of Olfactory Rapid Adaptation in Humans

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## Abstract

In this presentation, we describe a novel method for estimating the onset time course of psychophysical odor adaptation in human observers. The method employs stimulus conditions derived from an analogous stimulus paradigm in audition. To test this procedure, we used liquid-dilution olfactometry to estimate 2-bottle discrimination thresholds for brief (600 ms) presentations of vanilla odor; 17 volunteers (14 females; ages 18–24) served as participants. The adapting odorant concentration for each participant was set relative to baseline threshold for the 600-ms target alone (i.e., the same level relative to each participant's threshold). To characterize the adaptation-onset time course, we compared thresholds for targets presented simultaneously with the adapting stimulus as a function of the relative delay between the onset of the adapting stimulus and onset of the target. As predicted from the analogous auditory studies, thresholds for the target stimulus increased in an orderly manner with increases in adaptation-to-target onset delay (i.e., as the adaptation process progressively decreased sensitivity). Initial increases in threshold were consistently observed for the briefest onset delays of 50–100 ms. An onset time constant was estimated at 319 ms by fitting a 2-component exponential to the mean group function. Adaptation magnitude was dependent on the level of adapting odorant, relative to threshold. When thresholds were measured in one participant with a different, unrelated target odorant, cineole, there was no effect of the vanilla-adapting stimulus on threshold. The results suggest that olfactory rapid adaptation is measurable psychophysically within 50–200 ms after odor onset, values consistent with physiological measures of adaptation in olfactory receptor neurons. This novel stimulus paradigm offers a powerful psychophysical tool to study both odor adaptation and stimulus interactions at the olfactory periphery.

**Key words:** human psychophysics, odor adaptation, self-adapting

## Introduction

In the presence of continuous or repeated stimulation, sensory receptors exhibit a marked reduction in responsiveness. Perceptual adaptation, a fundamental property of all sensory systems, functions to attenuate neural and perceptual responses to sustained or redundant stimulation as a means of enhancing the detection of new, transient stimuli. Physiologically, olfactory adaptation is accomplished by shifting the receptor sensitivity function to higher stimulus concentrations, thereby increasing stimulus intensity saturation levels (cf. Munger et al. 2001). Olfactory adaptation likely plays an important role in chemotaxis (Yadon and Wilson 2005; Kostal et al. 2008; Rao et al. 2008; Rao and Ordal 2009) and the differentiation of target olfactory stimuli from odorant backgrounds (cf. Best and Wilson 2004; Kadohisa and Wilson 2006; Linster et al. 2007; Linster et al. 2009).

In all senses, adaptation is a complex, time-dependent process and, in olfaction, is composed of both peripheral (Getchell and Shepherd 1978a, 1987b; Kurahashi and Shibuya 1990; Kurahashi and Menini 1997; Zufall and Leinders-Zufall 1997, 2000; Leinders-Zufall et al. 1999; Reisert and Matthews 1999, 2000; Munger et al. 2001; Kelliher et al. 2003; Boccaccio et al. 2006; Lecoq et al. 2009) and central mechanisms (Best and Wilson 2004; Linster et al. 2007, 2009). The effects of adaptation can be observed in the physiological response of individual olfactory receptor neurons (ORNs) to single odorant pulses (Getchell and Shepherd

1978a, 1978b; Kurahashi and Shibuya 1990; Kurahashi and Menini 1997; Reisert and Matthews 2000; Zufall and Leinders-Zufall 2000) and includes as many as 3 processes, each distinguishable by its respective ''recovery'' time course and molecular mechanisms (cf. Zufall and Leinders-Zufall 2000).

Most typically, the time course of odor adaptation has been characterized by comparing the responses to 2 brief, identical odorant pulses separated by a short interval (Kurahashi and Shibuya 1990; Kurahashi and Menini 1997; Leinders-Zufall et al. 1998; Reisert and Matthews 1999, 2000; Zufall and Leinders-Zufall 2000). In general, using a paired-pulse paradigm, adaptation is observed as an attenuation of the response to the second pulse, relative to the first, and decreases in magnitude with temporal separation of the 2 stimuli. Onset time constants range from approximately 100 ms for short-term adaptation (Kurahashi and Shibuya 1990; Kurahashi and Menini 1997; Leinders-Zufall et al. 1998) to tens of seconds for long-term adaptation (Leinders-Zufall et al. 1998). Disadaptation, the restoration of sensitivity following adaptation, similarly varies from several seconds in the case of short-term adaptation to minutes for long-term adaptation (Zufall and Leinders-Zufall 2000).

Psychophysical studies of odor adaptation in humans have likewise depended on procedures employing intermittent stimulation (Cain and Polak 1992; Hummel et al. 1996; Pierce et al. 1996; Dalton 2000; Sobel et al. 2000; Wang et al. 2002; Jacob et al. 2003; Goyert et al. 2007). Usually, these studies ask observers to inhale odor pulses, singly or in trains, before removing the conditioning or adapting odorant to permit observer estimation of odor intensity or of changes in odor threshold for a comparison odorant. Employing these stimulus conditions, as in the physiological studies, evidence of adaptation can be found following a single conditioning stimulus as brief as 1 s (Hummel et al. 1996) and trains of 200-ms odor pulses (Kobayashi et al. 2008). Recovery from perceptual odor adaptation, disadaptation, follows a similar time course to physiological desensitization and long-term adaptation and can last, depending on stimulus concentration and exposure duration, from tens of seconds to minutes or longer (Dalton 2000).

A significant issue concerning use of discontinuous or intermittent odorants in characterizing the time course and magnitude of olfactory adaptation is the process of disadaptation, where presentation of the adapting odorant is terminated for some interval prior to onset of the target or comparison stimulus, permitting receptors to regain sensitivity (Zufall and Leinders-Zufall 2000). As discussed above, the extant adaptation literatures, both physiological and psychophysical, are derived primarily from such studies. The degree to which disadaptation influences a given measure, of course, is dependent on the precise nature and time course of the underlying physiological adaptation process (e.g., short-term, desensitization or long-lasting adaptation) (Zufall and Leinders-Zufall 2000). In the situation where short-term physiological processes are involved, an interval of 2 s or greater between the offset of the conditioning stimulus and onset of the target would reduce or preclude measurement of the effects of short-term adaptation (cf. Hummel et al. 1996; Sobel et al. 2000; Wang et al. 2002; Jacob et al. 2003). Evidence supporting this interpretation can be seen in continuous stimuli being judged less intense because of greater adaptation effects, relative to intermittent, pulsed stimulus trains of equal duration (Kobayashi et al. 2008).

In this study, we describe use of a novel psychophysical technique for estimating the onset time course of perceptual odor adaptation in humans. The premise of the technique is that extended presentation of an odorant will produce adaptation, decreasing the sensitivity of the receptor and increasing thresholds for a brief, ''simultaneous'' target odorant presented at various time points after the adapting stimulus onset. In the present study, both the adapting odorant and the target odorant are the same (i.e., self-adapting). Using this simultaneous stimulation technique allows estimation of the onset time course of the adaptation process in the absence of the confounding disadaptation processes.

## Materials and methods

### Subjects

College-aged participants were recruited for this study. Prior to testing, a brief history was taken from each participant to document a history of nasal/olfactory-related complaints, smoking, respiratory conditions, allergies, and colds. Additionally, participants who exhibited elevated thresholds for the vanilla target odorant alone, ≥20% v/v, were excluded from further study because there was an inadequate range of target odor concentrations available over which the adapting odorant concentration could be set and increases in threshold measured. Ultimately, 17 volunteers (14 females; ages 18–24) served as participants in this study.

All experiments were approved by the Institutional Review Board of the University of Florida.

### Olfactometer

An automated, liquid-dilution olfactometer was designed for use in this study. A schematic of the olfactometer is shown in Figure 1. The olfactometer was controlled by a PC-based program written in BASIC. The experimental conditions, indicated by a series of 3 light emitting diodes (LEDs), and all participant responses were communicated to and from the participant via a hand-held response box.

The participants sampled the odorant by placing their nose in a vented nasal mask. Odorants were delivered to the sampling mask through 3/16″ internal diameter (ID) tubing (C-flex; Cole Parmer) inserted through a small hole, just below the nares, and were evacuated from the front of the

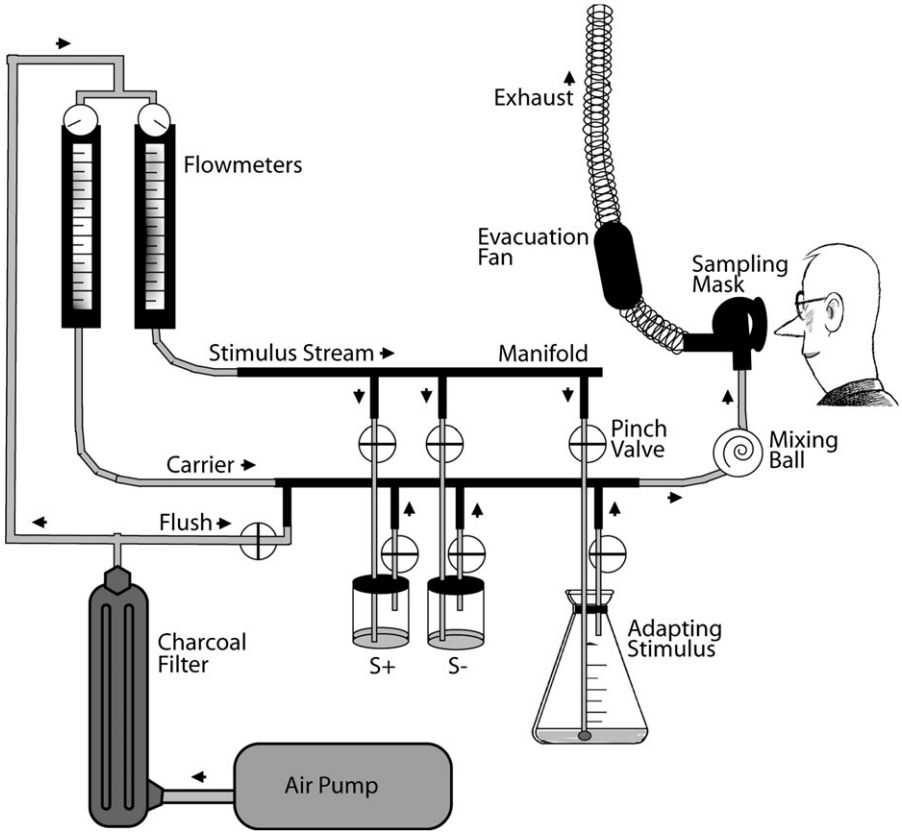


**Figure 1** Schematic depiction of olfactometer. Ambient room air was pumped through a charcoal filter and divided into separately controlled stimulus and carrier air streams. Presentation and relative timing of adapting and target stimulus presentation was controlled by a series of pinch valves that delivered the stimuli into the carrier stream, through a mixing ball and to the sampling mask. The odorants were evacuated by an inline fan. To ensure consistent, relative stimulus timing, the S+ (target + diluent) and S− (diluent alone) saturation bottles were fixed in position on the manifold upstream from the adapting odorant.

mask via 1″ ID silastic respiratory tubing fitted with a 2″ DC-powered fan.

A series of solenoid pinch valves produced 2 separate, charcoal-filtered, manually regulated air streams, one each for the stimulus and carrier. The stimulus stream flow rate was 4.1 L/min and the carrier stream was 0.27 L/min. These flow rates were chosen to optimize the onset slope of the adapting and target stimuli at the nose. A separate charcoal-filtered air stream (approximately 6.0 L/min) was manifolded upstream of the saturation bottles and was activated following each stimulus presentation to flush any residual odorants from the stimulus delivery system and sampling mask. Because the ''relative'' travel time of the odorants to the nose was critical to the implementation of this simultaneous stimulus paradigm, the position along the manifold of the specific saturation bottles for each stimulus (target, control, and adapting stimulus) was fixed.

The sampling mask was fabricated from carbon fiber and was coated with a fluoropolymer to prevent odor absorption (Swift Nasal Mask; St Croix Sensory). The mask was wiped with ethanol between participants and all glassware (manifolds and mixing ball) and tubing was removed, washed in Alconox, rinsed in ethanol and de-ionized water, and then placed in a stainless steel convection oven (50 °C) for drying and storage. Tubing was replaced daily to avoid possible residual odors from accumulating over time. The saturation jars were cleaned in an automated laboratory dishwasher with unscented detergent, rinsed in ethanol and deionized water, and stored between uses in the convection oven.

### Odorants

Bulk pure vanilla extract was purchased from Gordon Food Service. To prevent oxidation, the pure extract was stored in a refrigerator under inert gas (nitrogen). Serial dilutions of the odorant in deionized water ($DH_2O$) were made fresh daily to generate the target and adapting stimuli. Ten milliliters of the liquid-phase vanilla odorant was placed in a 500-ml glass saturation jar for creation of the target stimulus and 100 ml of the liquid-phase vanilla odorant was placed in a glass 2000-ml Erlenmeyer flask in order to produce the adapting stimulus. To generate the control stimulus, 10 ml of the diluent alone, $DH_2O$, was placed in a 500-ml glass saturation jar.

Target (S+; vanilla in $DH_2O$ diluent) and control stimuli (S–; $DH_2O$ alone) were single, 600-ms duration odor pulses. The adapting odorant, also vanilla diluted in $DH_2O$, was 3000 ms in duration. The target and control odorants were created by drawing off the headspace of the respective saturation bottles. The adapting odorant was created by sparging the stimulus air stream through an inert air diffuser placed in the liquid-phase stimulus and drawing off saturated air from the headspace. The target, control, and adapting odorants were manifolded into the carrier air stream for delivery to the sampling nasal mask.

To verify that any observed changes in threshold were related to adaptation (in this case, self-adaptation), thresholds for one participant were also estimated using a target odorant, cineole (Sigma Aldrich), unrelated to the vanilla-adapting stimulus. In this case, thresholds for cineole alone and in the presence of the vanilla-adapting stimulus were estimated using the same experimental procedures as described for vanilla target stimuli, with all other stimulus and experimental conditions being identical.

The actual concentration of all odorants at the participant's nose was unknown and concentration here refers here to the v/v concentration of the odorant in liquid phase in the saturation bottle. For the purposes of this study, the actual concentration of the odorant at the nose is relatively unimportant as our concern in this study is the "relative change" in threshold produced by the adaptation process, not the "absolute" threshold.

### Psychophysical procedures

During the experimental session, the response box was placed on the counter in front of the participant. The beginning of a trial sequence was indicated by the flashing of all 3 LEDs. The participant initiated a trial by inserting their nose into the nasal mask carrier air stream and depressing and holding the response key. Once depressed, the 3 LEDs were extinguished and a yellow LED was illuminated, indicating that the participant was to exhale through their nose slowly and continuously for 3 s. Following the 3-s exhalation period, the yellow LED was extinguished and a green LED was illuminated, cuing the participant to inhale steadily and continuously for 3 s. All odorants were presented during this 3-s inhalation period.

Odorant detection thresholds were estimated using a 2-odorant discrimination paradigm similar to that described by Laska and colleagues (Laska and Seibt 2002; Hernandez Salazar et al. 2003) and Slotnick and colleagues (Bodyak and Slotnick 1999). The participants were asked to discriminate dilutions of the target vanilla odorant in a diluent (S+; vanilla extract in $DH_2O$) from the diluent alone (S–; $DH_2O$). Following lighting of the green (sample) LED, either the S+ or S– was introduced into the carrier stream with an onset delay of 1000 ms. Immediately following the 3-s sampling period, the green LED was extinguished and the participant was required to release the response key and then was given a 3-s period (decision interval) to report detection of the S+ odorant by pressing the left response key or failure to detect the S+ odorant (i.e., "detection" of the S– odorant) by pressing the right response key. Feedback was provided immediately to the participant for correct (green flashing LED) and incorrect (red flashing LED) responses.

Trials were presented in quasi-random blocks of 20 (10 S+ and 10 S–), and the percent correct was calculated (for both correct detection and correct rejection) individually for each block. The initial odorant concentration presented to each participant was 100% v/v, and when the percent correct for a specific dilution reached 85% or greater for one block, the concentration of the S+ stimulus was decreased in 10% serial dilutions (v/v), for the following block. If a participant was unable to detect a concentration, they were presented a concentration half-way between the last passed concentration and the failed concentration. Threshold was estimated as the last dilution at which the participant achieved 85% or higher on 2 consecutive blocks. Additionally, to determine whether or not thresholds were affected by repeated exposure to the adapting and target odorants, thresholds for the target stimulus alone were remeasured intermittently during the experiment. If threshold increases were observed over time, testing of that participant was stopped. However, no evidence of systematic changes in threshold was observed in any participant.

Following determination of threshold for the 600-ms vanilla target odorant in a null background (i.e., for the target-alone), the adapting odorant concentration for each participant was set at twice their threshold concentration. Setting the adapting stimulus level in this manner, whereas the absolute concentration of the adapting stimulus varied across participants, ensured that the relative level was consistent across participants. Additionally, to characterize the effects of absolute adapting odorant level on adaptation, in one group of participants, the adapting stimulus level was set at 0.1%, 10.0%, and 30.0% v/v, irrespective of baseline threshold.

For adaptation trials, when the green LED was lighted, the 3000-ms adapting odorant would be presented in 100% of the trials (both S+ and S–), beginning 500 ms after the green LED was illuminated; this interval served to ensure that the participants were inhaling at the onset of the adapting odorant presentation. The stimulus conditions employed in estimating the time course of adaptation are shown in Figure 2. The effect of adaptation was measured as a change in olfactory sensitivity (i.e., a change in threshold) produced by the "simultaneous" 3000-ms adapting odorant. To estimate the adaptation time course, target stimulus thresholds were estimated for different adapting-to-target stimulus-onset delays, ranging from 50 to 1500 ms. The order of the onset delays presented during each session was randomized within and across participants.

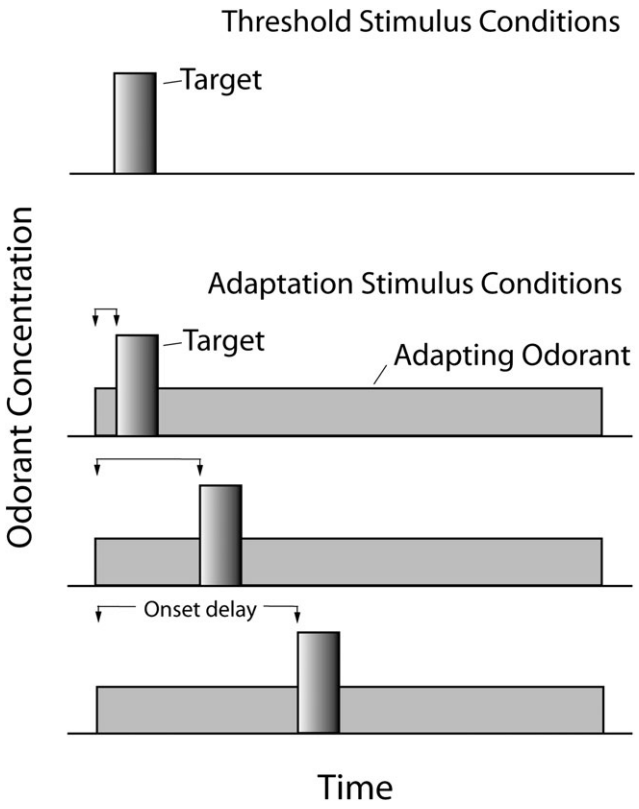


**Figure 2** Schematic presentation of stimulus conditions. Except where noted, during the adaptation measurements, adapting odorant level was set relative to threshold. To determine the level for the adapting stimulus, threshold for the target alone was initially estimated for each participant (top line). Adapting stimulus concentration was set at a concentration of 2 times the estimated threshold for the target stimulus alone. The physiological adaptation process was activated by the onset of the adapting odorant and the time course estimated by placing the target stimulus at different temporal points within the adaptation process (i.e., at different delays relative to adapting odorant onset) (bottom 3 lines). Target onset delays of 50–1500 ms were presented.

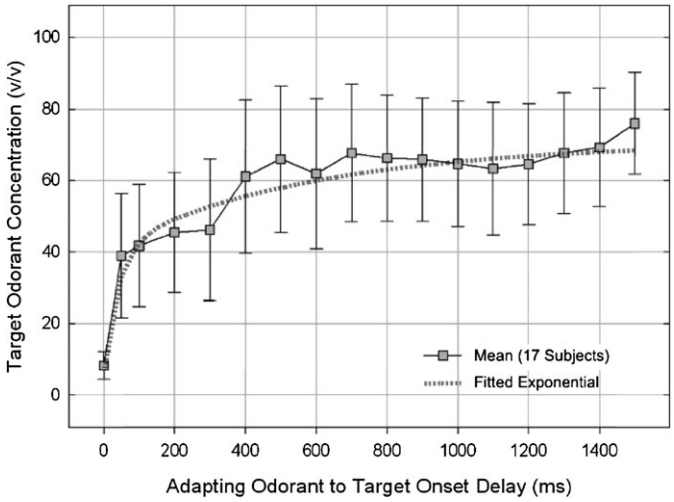


**Figure 3** Mean change in threshold as a function of adapting odorant to target odorant onset delay. The threshold data point at 0 ms delay represents the mean group threshold for the target stimulus alone (i.e., target stimulus threshold in the absence of adaptation). Increases in threshold stimulus concentration were evident at the briefest onset delays of 50 and 100 ms and increased to an asymptotic level of 80–90% (v/v) with onset delays longer than 700 ms (solid line with square symbols). To estimate the adaptation onset time course, a 2-component exponential (dashed line) was fit to the mean participant data and a time constant of 319 ms was estimated.

## Results

Thresholds for the vanilla target alone varied across participants from 0.1% to 10% v/v, with an across participant mean of 6.1% v/v (plotted in Figure 3 as 0 ms), for the 17 participants reported below. As such, the concentration of the vanilla-adapting stimulus, set at twice each participant's threshold, ranged from 0.2% to 20% v/v. Thresholds, averaged across participants, are plotted as a function of adapting stimulus to target stimulus-onset delay in Figure 3 (standard deviations are given by error bars). Increases in threshold were evident for adapting to target onsets of as brief as 50–100 ms. Thresholds increased in an orderly manner with onset delay and reached a maximum mean asymptotic value of 70% v/v at a 1400-ms onset delay. To calculate an onset time constant, a 2-component exponential curve was fitted to the mean threshold plot (superimposed dashed function; MatLAB, MathWorks) and a value of 319 ms was calculated.

When the vanilla-adapting odorant level was fixed, irrespective of participant threshold, at 0.1%, 10%, or 30% v/v, the magnitude of threshold increase with adaptor to target delay was level dependent (Figure 4). Thresholds measured with the 30% adapting odorant level increased rapidly, reaching an asymptotic level of 100% v/v at a 200-ms adaptor-to-target onset delay. With the adapting odorant set to 10% v/v, average threshold increases with onset delay were comparable to those obtained with a relative adaptor level set to twice threshold or 6.1% v/v (Figure 3); mean threshold increases were evident at onset delays as short as 50–100 ms, increasing systematically to asymptotic levels at delays of 300–400 ms and longer. At the lowest absolute adaptor level of 0.1% v/v, mean thresholds increased to approximately 30% v/v at the shortest onset delay of 50 ms, then remained relatively flat until delays reached approximately 500 ms, whereafter they increased progressively to an asymptotic level of 50% v/v.

Explaining the observed threshold shifts with increasing adapting to target onset delay as resulting from an adaptation process assumes that the adapting odorant is acting on and producing adaptation in the same receptors responsible for detecting the target stimulus (i.e., are self-adapting odorants). To test this assumption, we compared the effects of the vanilla-adapting odorant on thresholds for both the standard vanilla target and an unrelated target odorant, cineole, presented separately, in one test participant. Figure 5 plots changes in threshold for both vanilla and cineole target

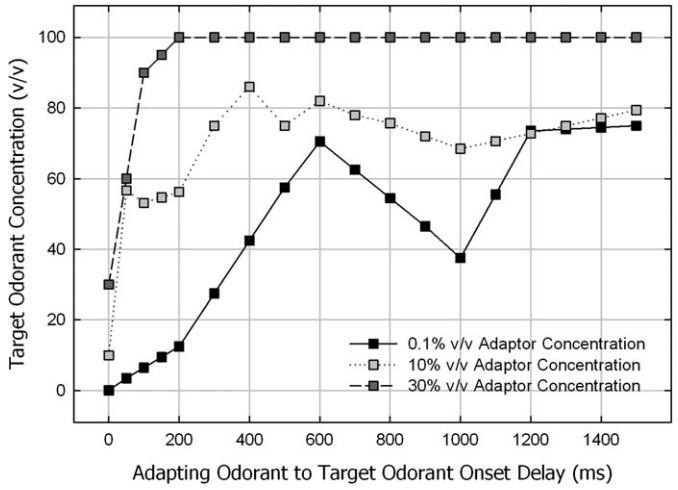


**Figure 4** Effects of absolute adapting odor concentration on perceptual threshold as a function of adapting to target odorant onset delay. Adapting odorant level was fixed at 0.1%, 10%, and 30% v/v. Increases in threshold with adapting to target onset delay were adapting odorant concentration dependent.

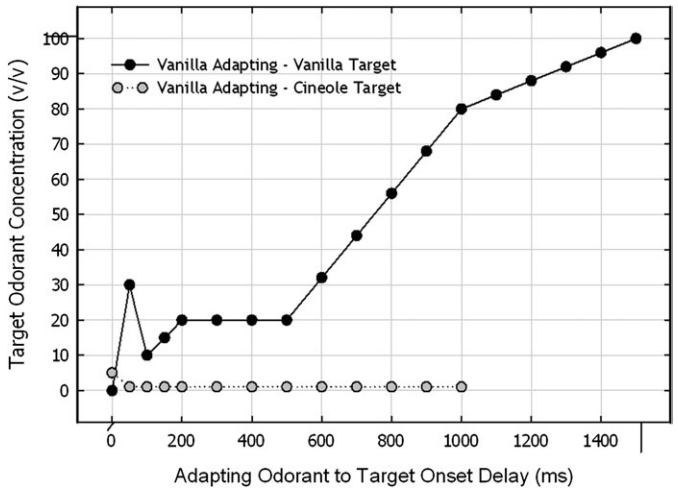


**Figure 5** Comparison of vanilla extract (i.e., self-adapting) and cineole (i.e., unrelated) adapting odorants on threshold for a vanilla target as a function of adapting odorant to target odorant onset delay. For the vanilla-adapting odorant, thresholds increased with increases in onset delay (filled circles) but were unaffected by the unrelated cineole adaptor (open circles).

odorants as a function of the adapting stimulus to target onset. For this participant, as expected, thresholds for the vanilla target increased with onset delay, whereas thresholds for the cineole target were unaffected, at all onset delays tested, by the vanilla-adapting odorant.

## Discussion

Our objective was to develop a new psychophysical paradigm to measure perceptual odor adaptation in humans. More specifically, we sought to measure the "time course" of the "peripheral" contribution to odor adaptation in human observers without the confounding effects of disadaptation, which is inherent in more typical adaptation paradigms employing paired odorant pulses (cf. Gagnon et al. 1994; Pierce et al. 1996, Leinders-Zufall et al. 1998, 1999; Reisert and Matthews 1999, 2000). Although adaptation is demonstrably a complex temporal process with both peripheral and central components, work by several investigators has shown that adaptation occurs at the most peripheral aspects of the olfactory system, beginning in the ORN cilia (cf. Getchell and Shepherd 1978a, 1978b; Leinders-Zufall et al. 1998; Kelliher et al. 2003; Lecoq et al. 2009), where the various mechanisms can be differentiated by their time constants (Zufall and Leinders-Zufall 2000).

Kelliher et al. (2003) showed that deletion of the gene encoding for the ORN CNGA4 channel significantly reduces, or eliminates, olfactory perceptual adaptation in behaviorally trained mice, demonstrating that these peripheral processes can be characterized using simple psychophysical measures. Because our interest was in the development of a method to characterize the "onset time course" of the peripheral adaptation process, we modified the odorant conditions employed by Kelliher et al. (2003) to, instead of using a continuous adapting odorant, employ brief, intermittent but simultaneous stimuli where precise control over the relative onsets of the adapting odorant and target odorants was possible. Our stimulus paradigm (see Figure 2) presents a relatively long-duration–adapting odorant to the observer, and changes in sensitivity for detection of a brief target stimulus (produced by the simultaneous self-adapting odorant) were estimated as a change in sensitivity as a function of the adaptor-to-target onset delay. This modified stimulus paradigm, adapted from an analogous psychophysical procedure from audition, termed "overshoot" (cf. Bacon and Smith 1991; Overson et al. 1996; Bacon and Liu 2000), is known to measure the effects of peripheral adaptation on target stimulus detection (cf. Zeng et al. 2000; Smith et al. 2005).

In this new paradigm, illustrated schematically in Figure 6, we rely on the relatively long-duration odorant to initiate the adaptation process and produce a progressive "decrease" in sensitivity for the vanilla extract odorant. Using this approach, the brief, simultaneous target odorant can be precisely placed within the adaptation time course by varying the adaptor-to-target onset delay. When thresholds are estimated for the self-adapting vanilla target odorant as a function of onset delays (i.e., at different points in the decreasing sensitivity curve), thresholds should increase progressively with adapting odorant-to-target odorant onset delay. As predicted, the present data show that we were able to measure a rapid increase in thresholds for a brief target odorant as a function of adaptor-to-target stimulus onset delay (Figures 3) in a group of 17 human observers. Although substantial across-participant variability was evident in the time course of the observed adaptation, all participants showed systematic increases in threshold with onset delay. Increases

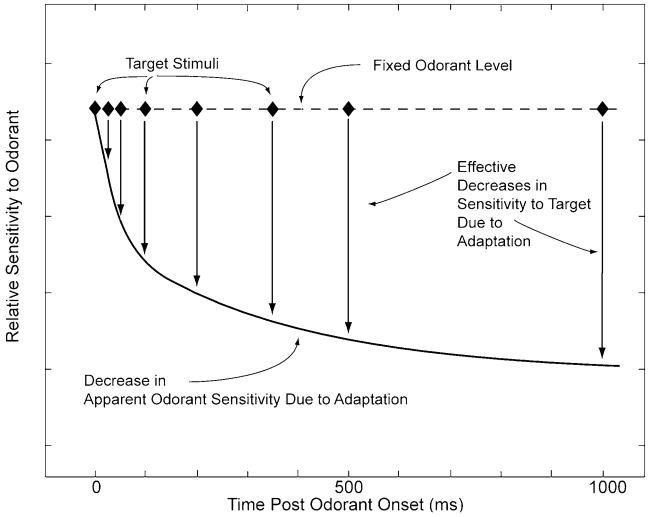


**Figure 6** Conceptual depiction of simultaneous stimulus paradigm for measuring rapid odorant adaptation. Dashed horizontal line shows actual stimulus level with time after adapting odorant onset. Solid line shows effective decrease in sensitivity with time following onset of adapting odorant. Vertical solid lines (with arrowheads) illustrate relative decreases in sensitivity, as evidenced by increases in threshold for target odorants, with adapting to target onset delay.

in threshold were evident in the mean data at the shortest onset delays of 50–100 ms and increased progressively with onset delay. When a 2-component exponential was fit to the data, an onset time constant of 319 ms was estimated.

Zufall and Leinders-Zufall (2000) argued that 3 different adaptation mechanisms can be differentiated by their respective time constants, labeling them short-term adaptation, desensitization, and long-term adaptation. Short-term adaptation can be evoked by very brief odor pulses, with a pulse of 100 ms being capable of producing a significant adaptive effect on subsequent stimulation (cf. Kurahashi and Shibuya 1990; Leinders-Zufall et al. 1999). Desensitization, evoked by a relatively longer duration odorant, has an onset time constant of 1–4 s (Kurahashi and Shibuya 1990; Zufall and Leinders-Zufall 2000). The third form of adaptation, long-term, is evoked by both sustained, long-term stimulation as well as brief, rapidly repeating odor pulses with an overall duration of 25 s or longer (Zufall and Leinders-Zufall 1997, 2000).

Using the analysis from Zufall and Leinders-Zufall (1997), 2 pieces of data suggest that our stimulation paradigm was measuring short-term or rapid adaption. First, adaptation was evident for very brief adapting odorant-to-target onset delays; increases in threshold were reliably observed at the briefest onset delays of 50-100 ms. When the mean adaptation contour was fitted with a 2-component exponential, a time constant of 319 ms was computed (Figure 3), well within the range of onset time constants reported for short-term adaptation (Kurahashi and Shibuya 1990; Leinders-Zufall et al. 1999) but also significantly shorter than for either desensitization (Getchell and Shepherd 1978b; Kurahashi and Shibuya 1990; Leinders-Zufall et al. 1999) or long-term adaptation (Zufall and Leinders-Zufall 1997, 2000).

The second piece of evidence comes from the relative offset time courses for the 3 forms of adaptation; although not systematically measured in the present study because we permitted our participants to set their own testing pace, we believe that the typical interval between trials was less than 3–5 s. If this is accurate, then the fact that we could easily, and repeatedly, measure onset delay-related threshold increases on a trial-by-trial basis suggests that any adaptation produced by the immediately preceding adapting odorant (i.e., the adapting odorant delivered in the preceding trial) had, at least partially, recovered by this time. Furthermore, we addressed this issue directly by remeasuring thresholds for the target stimulus alone during and at the end of each testing session; we found no systematic changes in thresholds for any participant. Again, this suggests our stimulus paradigm was measuring adaptation-induced increases in threshold produced by presentation of the simultaneous odorant. Each form of adaptation has a distinct offset or recovery time constant that is determined by the differing underlying mechanisms. Sensitivity recovers within ~5 s following short-term adaptation, desensitization within 25 s, and recovery of sensitivity following long-term adaptation requires minutes (cf. Zufall and Leinders-Zufall 2000). Future studies using a modification of this paradigm will explicitly study the offset time course of the perceptual adaptation process.

The current stimulus paradigm, as well as those employed by other investigators to study adaptation, assumes that the adapting and target odorants are activating the same ORN receptors and thus adaptation processes. Because olfactory receptors respond to a narrow range of similar odorants at threshold use of unrelated odorants to induce adaptation and to measure the changes in olfactory sensitivity should fail to result in measurable increases in threshold. Indeed, previous studies in humans (cf. Dalton 2000) and behaviorally trained mice (Kelliher et al. 2003) have shown that measurement of perceptual odor adaptation is dependent on the adapting odorant being the same or closely related structurally to the target odorant (Stone et al. 1972; Pierce et al. 1996; Dalton 2000). In the same manner as the previous studies, we took advantage of this dependency to verify that our paradigm was measuring an odor adaptation process. We compared changes in threshold produced by the vanilla extract–adapting odorant for vanilla extract (self-adapting) and cineole target (an unrelated odorant) stimuli in one participant. In that participant, as predicted, thresholds for the related, vanilla target odorant increased with increases of the onset delay between the adapting odorant and target stimulus, whereas the threshold for cineole was not affected by the constant background vanilla odorant (Figure 4). Although the precise time course of this participant's change in sensitivity to the cineole target with onset delay varies from that of the mean contour, it simply reflects the noted intersubject variability.

It should be noted, however, that previous work from our laboratory, as well as the work of others, has demonstrated that unrelated odorants can indeed elevate behavioral thresholds for unrelated odorants by "masking" (Laing et al. 1989; Smith et al. 2006). The underlying mechanisms differentiating masking from adaptation are not well understood (Takeuchi et al. 2009), but one clear difference in the adapting (using related odorants) and masking (using unrelated odorants) paradigms is the level of the adapting/masking odorant required to produce changes in detection sensitivity for a target stimulus. An earlier study with behaviorally trained mice in our laboratory showed that unrelated, simultaneous odorants could significantly increase thresholds once the masking odorants were increased to concentrations well above detection threshold (Smith et al. 2006). When the background odorant and target are self-adapting, using the same adaptation paradigm as in this study, we have recently demonstrated that detection thresholds in human observers are increased in an intensity-dependent manner when the adapting odorant is significantly "below its detection threshold" (Keith et al. 2009). Similarly, Zufall et al. (2000) have demonstrated that the calcium response of single ORNs show deceases in sensitivity in the presence of odorants that, when presented alone, fail to elicit an electrophysiological response.

Consistent with previous physiological and psychophysical studies of odorant adaptation, once adapting stimulus levels reached concentrations where increases in threshold for the target stimulus were observed, further increases in adaptor concentration produced systematic elevations in threshold that were adapting odorant concentration dependent (Figure 5). It is relevant to note that, although the adaptation contours in Figures 3 and 4 were generated by setting the adapting odorant to twice threshold for the target stimulus alone, the contours in Figure 5 were collected by fixing the adapting odorant at different "absolute" concentrations (0.1%, 10%, and 30%), irrespective of individual threshold. Unpublished observations from our laboratory suggest the only significant consequence of this difference in method is an increase in intersubject variability in thresholds as adaptation magnitude appears to be related to "threshold," and setting adaptor levels at "fixed concentrations" would result in adaptor levels varying relative to each participant's threshold. Future work will explicitly study this issue.

Data obtained from use of this simultaneous adaptation paradigm raises interesting practical and theoretical questions regarding previous estimates of onset time constants and the magnitude of adaptive effects. The extant adaptation literature, both physiological and perceptual, is derived primarily from use of a paired-pulse stimulus paradigm (cf. Pryor et al. 1970; Zufall and Leinders-Zufall 1997; Leinders-Zufall et al. 1998, 1999). This technique characterizes the effect of an adapting odorant on a second nonsimultaneous target odorant. The issue raised by use of intermittent stimuli, where a temporal gap is inserted between termination of the adapting stimulus and onset of the target, is the influence of disadaptation, the rapid, return of sensitivity, on the obtained measures. Because even the briefest interstimulus interval will produce some disadaptation, use of intermittent stimulus paradigms is likely to have underestimated both the onset time constant and magnitude of suppression. Use of a paired-pulse paradigm in human psychophysical studies, where the delay between the offset of the adapting odorant and the onset of the target can be seconds, suggests that these techniques cannot measure the faster forms of adaptation, where disadaptation would have returned sensitivity to normal before the onset of the target odorant (cf. Zufall and Leinders-Zufall 2000). Further studies comparing adaptation, physiological and perceptual, under conditions of simultaneous and intermittent stimulus paradigms will clarify these issues and provide a better understanding of the adaptation process in olfaction.

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