A Technique for Characterizing the Time Course of Odor Adaptation in Mice

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Abstract

Although numerous studies have analyzed the temporal characteristics underlying olfactory adaptation at the level of the olfactory receptor neuron, to date, there have been no comparable behavioral measures in an animal model. In this study, odor adaptation was estimated in a group of mice employing a psychophysical technique recently developed for use in humans. The premise of this technique is that extended presentation of an odorant will produce odor adaptation, decreasing the sensitivity of the receptors and increasing thresholds for a brief, simultaneous target odorant presented at different time points on the adaptation contour; adaptation is estimated as the increase in threshold for a target odorant presented simultaneously with an adapting odorant, across varying adapting-to-target odorant onset delays. Previous research from our laboratory suggests that this method provides a reliable estimate of the onset time course of rapid adaptation in human subjects. Consistent with physiological and behavioral data from human subjects, the present findings demonstrate that measurable olfactory adaptive effects can be observed for odorant exposures as brief as 50–100 ms, with asymptotic levels evident 400–600 ms following adapting odorant onset. When compared with the adaptation contour in humans using the same odorant and stimulus paradigm, some differences in the onset characteristics are evident and may be related to sniffing behavior and to relative differences in thresholds. These data show that this psychophysical paradigm can be adapted for use in animal models, where experimental and genetic manipulations can be used to characterize the different mechanisms underlying odor adaptation.

Key words: animal behavioral model, animal psychophysics, mouse, odor adaptation, olfactometer

Introduction

Olfactory adaptation is a complex, time-dependent process that functions to limit receptor saturation, suppress neural responses to sustained odorant stimulation, and enhance the detection of new chemical stimuli (Kelliher et al. 2003; Best et al. 2005; Nagel and Wilson 2011). Adaptation and the associated process of disadaptation (or the recovery of sensitivity from adaptation) are fundamental properties of olfactory receptor function and play critical roles in chemotaxis (Yadon and Wilson 2005; Kostal et al. 2008; Rao et al. 2008; Rao and Ordal 2009) and in differentiating target olfactory stimuli from odorant backgrounds (cf. Best and Wilson 2004; Kadohisa and Wilson 2006; Linster et al. 2007, 2009).

From a biophysical perspective, much is already known about the mechanisms underlying odor adaptation. In vivo calcium imaging studies have identified at least 3 calcium-dependent, negative-feedback loops in olfactory receptor neuron (ORN) cilia that reduce responding to sustained or repeated olfactory stimulation (Getchell and Shepherd 1978a, 1978b; Kurahashi and Shibuya 1990; Kurahashi and Menini 1997; Leinders-Zufall et al. 1997, 1998; Reisert and Matthews 1999; Reisert and Matthews 2000; Zufall and Leinders-Zufall 2000; Munger et al. 2001; Kelliher et al. 2003; Boccaccio et al. 2006; Lecq et al. 2009), with each process distinguishable by its respective recovery time course and molecular mechanisms (cf. Zufall and Leinders-Zufall 2000). Most typically, the odor...
adaptation time course has been characterized by comparing the responses to 2 brief 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). Using this paradigm, adaptation is observed as a reduced response to the second pulse, relative to the first, which decreases in magnitude with temporal separation of the 2 stimuli. Onset time constants range from ~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). These mechanisms are concentration dependent and at least one of these processes can be activated, independent of cellular firing, at subthreshold levels of odor stimulation (Leinders-Zufall et al. 1999; Zufall and Leinders-Zufall 2000).

Importantly, these physiological mechanisms evolved to subserve olfactory behavior. Several behavioral studies have shown that central mechanisms also play a role in the behavioral process of odor habituation (Best and Wilson 2004; Linster et al. 2007, 2009). To date, however, few animal psychophysical studies have characterized the role peripheral mechanisms play in the behavioral response of odor adaptation. Kelliher et al. (2003) demonstrated that deletion of the CNGA4 gene in mice, which modulates cyclic nucleotide-gated membrane channels, significantly reduced odor adaptation and increased the effects of self-adapting background odors.

Yet, so far as we are aware, no animal studies have explicitly measured the time course of odor adaptation. In this study, odor adaptation was estimated in a group of mice employing a psychophysical technique recently developed for human testing. Using this technique in humans, adaptation was estimated as an increase in threshold for a relatively brief, target odorant presented simultaneously with an adapting odorant (AO), across varying adapting-to-target onset delays (Smith et al. 2010). Based on this stimulus paradigm, extended presentation of an odorant will produce odor adaptation, decreasing the sensitivity of the receptors and increasing detection threshold for a target odorant presented at different time points on the adaptation contour. Previous research from our laboratory suggests that this method provides a reliable temporal estimate of rapid adaptation in human subjects (Smith et al. 2010; Keith and Smith 2011; Yoder et al. 2013). Using this approach in mice, the time course of adaptation-induced changes in odor sensitivity was measured for a vanilla extract odorant. Vanilla extract was chosen to facilitate comparisons with existing human data collected employing the same odorant, stimulus paradigm, and olfactometer.

Materials and methods

Animals

Four C57Bl/6J mice were used in this study. Mice were obtained from Jackson Laboratories and from an in-house breeding colony maintained at the McKnight Brain Institute, University of Florida. The mice were maintained on a 12:12 h light:dark cycle, and behavioral testing was conducted during the light cycle. Mice had ad libitum access to dry LabDiet chow (Purina Mills) and restricted access to water. This regimen resulted in the mice stabilizing at 85–90% of their free-feeding body weight, which facilitated use of a nutritional liquid food reinforcer during training and testing procedures (Ensure, Abbott Laboratories). During a typical session, mice received ~3 mL of Ensure per day, followed by 2 h of unrestricted access to water after daily testing. Mice were tested once daily, 5–7 days per week.

All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication no. 86-23, revised 1985) and were approved by the University of Florida Animal Care and Use Committee.

Olfactometer

A custom, 8-channel liquid-dilution rodent olfactometer was employed in this study to characterize odor adaptation. The olfactometer, adapted for use with mice by addition of an operant chamber, and methods employed in this study are comparable to those used in our previous work and detailed discussions of the training and testing techniques can be found in those previous publications (Smith et al. 2008; Gamble and Smith 2009; Yoder, Setlow, et al. 2014). The mouse olfactometer operant box consists of a 15-cm deep, 20-cm wide, and 13-cm tall, ventilated Plexiglas chamber. The chamber is fitted with a conductive stainless steel floor and a PVC sniffing port containing a metal licking tube. The ventilation system provides a steady stream of fresh room air in the chamber, maintaining positive pressure and ensuring that the odorant remains within the sniffing port air stream.

A photo beam was broken when the mouse inserted its nose into the sniffing port, initiating a trial sequence. Mice were initially required to keep their noses within the port and sample the stimulus air stream for a minimum of 200 ms, at which time the AO and a stimulus, either the S+ (target stimulus) or S− (control stimulus) (as defined below), were introduced through the bottom of the sampling port. The air stream and odorant were drawn through the sampling port in which the mouse positioned its nose, then exhausted out of the top of the port by an in-line, DC-powered exhaust fan. Stimulus delivery and behavioral responses were controlled and monitored by a computer running custom-designed software.

Stimuli

In the present study, vanilla extract (35% ethanol; Gordon Food Service) was used as the target/self-AO. DH2O was used as the odorless diluent for all conditions. The rationale for choosing vanilla extract was to facilitate comparisons of mouse data with comparable data using the same odorant,
olfactometer, and stimulus paradigm in humans (Smith et al. 2010; Keith and Smith 2011). Odorant concentrations are described in terms of volume/volume concentrations of the liquid-phase stimuli, though, given the air flow rates through the olfactometer, the odor concentration experienced by the mice was approximately 2.5% of the saturated headspace vapor in the saturation bottles.

The stock odorants, once opened, were stored under inert gas (nitrogen) in glass and refrigerated to prevent oxidation. Serial dilutions of the target odorant were prepared using DH$_2$O as a diluent. Ten milliliters of the liquid-phase (vanilla) odorant, placed in a 500-mL glass saturation jar, served as either the target or control stimulus. The olfactometer functioned by use of digitally controlled solenoid pinch valves. These valves sparged the stimulus air stream through a tube submerged in the liquid-phase odorant to produce a volatilized stimulus that filled the headspace before introduction into the carrier stream and subsequent presentation to the animal.

Training
Training methods followed those described by Bodyak and Slotnick (1999). Briefly, mice were initially rewarded for contacting the lick tube with their tongue, followed by nose pokes into the sampling port, and finally for remaining in the sampling port for odorant presentation. Prior to training on the odor adaptation paradigm, mice acquired an association between the target odorant and delivery of liquid reinforcement. Once the mice successfully completed the initial lick training, they were transferred to a 2-odorant discrimination program where they were trained to discriminate dilutions of the target (S+; vanilla extract diluted in DH$_2$O) odorant in a diluent from the diluent alone (S−; DH$_2$O alone). Reinforcement was contingent upon the mouse reporting detection of the S+ odorant by licking on the metal tube (correct detection), which completed an electrical circuit with the metal floor and registered the response with the computer-based olfactometer control program. Correct detection was followed by presentation of approximately 5-$\mu$L of Ensure through the lick tube. Failures to report the presence of the S+ (a miss) or licking the response tube during presentation of the S− stimulus (false alarm) were recorded as incorrect responses and required mice to withdraw their nose from the sampling port for 5 s before reinserting their nose to initiate a new trial. The mouse was not required to lick during the control (S−) trials and, therefore, was free to leave the odor port once the stimulus presence decision was made.

Baseline threshold
Threshold for the target (vanilla) stimulus was initially estimated for each animal; baseline thresholds were used to determine both the AO concentrations for each mouse and to establish a baseline, nonadapted sensitivity level against which adaptation-induced decreases in sensitivity could be compared. Prior to each testing session, mice were exposed to 40 shaping trials. Similar to the training sessions described above, animals were reinforced for licking in the presence of the target odorant. This initiating procedure enabled sufficient time for the animal to acclimate to the expected target odorant, as well as confirm appropriate motivation levels for the experimenter. Had an animal failed to achieve 85% during the introductory shaping session, testing would not proceed. However, no such instance was observed. Trials were presented in pseudorandom blocks of 20 (10 S+ and 10 S−). Within each block, the sequence of the 20 trials was quasirandom such that each stimulus was limited to 3 consecutive presentations. The percent correct was calculated for both correct detection and correct rejection for each block. When the percent correct reached 85% on 3 successive blocks, the concentration of the S+ stimulus was decreased 10-fold for the following block. During a given session, animals were allowed to remain in the testing chamber for as long as they continued to initiate trials. When an animal failed to reach 85% correct on 3 consecutive blocks during a given session, the same concentration was presented the following day. This was repeated for 2 days. If the animal failed to reach the 85% criterion for the next serial dilution, then the final value recorded (for that dilution) was the average of the last 5 blocks. “Threshold” was defined as the lowest concentration at which the animal achieved 85% or higher on 3 consecutive blocks. Baseline threshold estimates were obtained in the presence of a null, AO background (odorless DH$_2$O).

Adaptation testing
Our odor adaptation stimulus paradigm is based on extended presentation of a self-adapting (vanilla extract) odorant. The AO subsequently induces adaptation by decreasing receptor sensitivity. Consequently, detection thresholds for a simultaneous, target odorant (vanilla extract) increase. The onset time course of adaptation is then estimated by presenting the target odorant at different time points on the adaptation time contour (Smith et al. 2010). The AO onset began when the animal inserted its nose into the odor port, breaking the photo beam; onset delay refers to the temporal interval between the onset of the AO and the onset of the target odorant. Prior to measuring the adaptation time course, mice were trained to remain in the odor port without responding for hold intervals to 1000 ms. Beginning with a 50-ms onset delay, training sessions increased the hold to 100 ms, followed by 100-ms increments until reaching a 1000-ms hold interval. Once mice learned to maintain nose pokes without responding at a given interval, onset delays were systematically increased. During these trials, the animals were, in effect, presented with delayed S+ stimuli and lengthened nose poke durations. Mice required several weeks of training to maintain the nose poke and reach equivalent response performance (85% at threshold) on all the onset delays.
Following sequential training, the initial baseline thresholds were confirmed by randomizing the onset delays presented in each block. When DH₂O was used as the AO, mice consistently maintained 85% or higher accuracy, regardless of the onset delay. This design ensured that later experimental conditions varied only in terms of onset delays, rather than behavioral and/or motivational differences when the mice had to maintain a noise poke in the sniffing port for longer intervals.

A schematic depiction of the adaptation stimulus paradigm is shown in Figure 1. The conceptual model for estimating the onset time course of adaptation is shown in Figure 2. For adaptation testing, target (vanilla) odorant concentrations ranged from subthreshold level (one serial dilution below the estimated threshold for each mouse) to 100% v/v across a series of onset delays (0–1000 ms). The AO onset began with the mouse breaking the photo beam. Under the first condition, AO concentration was set for each mouse at its estimated, baseline threshold (i.e., 1× threshold); setting the AO level in this manner ensured that the relative level was consistent across mice. The physiological adaptation process was activated by the AO onset 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 AO onset). Target (S+; vanilla in DH₂O diluent) and control stimuli (S−; DH₂O alone) were single, 600-ms duration odor pulses. The AO, also vanilla diluted in DH₂O (at threshold concentration), was 2000 ms in duration. All odorants were created by sparging the stimulus air stream through an inert aquarium air diffuser placed in the liquid-phase stimulus and drawing off saturated air from the head-space. The target, control, and AOs were manifolded into the carrier air stream for delivery to the odor sniffing port. The effect of adaptation was measured as a change in olfactory sensitivity (i.e., a change in threshold) for the target odorant.
produced by the simultaneous 2000 ms AO. To estimate the adaptation time course, target stimulus thresholds were estimated for different onset delays, ranging from 50 to 1000 ms. The order of the onset delays and odorant concentrations presented during each session was randomized within and across animals for each block.

To assess the influence of AO concentration on the adaptation time course, changes in threshold were compared for 2 different AO concentrations (AO = 1× threshold, as described above, and AO = 2× threshold). With the exception of the AO concentration, procedures for the second AO condition (AO = 2× threshold) were identical to those described above. Mice completed a total of 40 trials (2 blocks) across a range of serial dilutions (from one dilution below estimated, baseline threshold through 100% v/v; 10-fold increments) for each onset delay (0, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 ms). Onset delays and % v/v target concentrations were randomized across blocks (both changed every 20 trials) and mice. Individual time course estimates were calculated from mean response accuracy across 3840–7200 trials per experimental condition (1× or 2× threshold). Thresholds for each onset delay were calculated as the lowest concentration the mouse averaged 85% or greater on 40 pseudorandomized trials.

Results

All mice readily learned to discriminate vanilla extract from DH2O. Individual thresholds for the vanilla target odorant, estimated in the presence of a null, DH2O AO, are plotted for each animal in Figure 3. Baseline thresholds were estimated in the presence of a null adapting background (odorless DH2O), and each data point represents the mean accuracy for the target odorant concentrations tested (% v/v). Thresholds are recorded as the lowest dilution at which the mouse received ≥85% on 3 consecutive blocks. Consistent with previous psychophysical studies in mice (Yougentob and Margolis 1999; Vedin et al. 2004; Pho et al. 2005; Clevenger and Restrepo 2006), baseline thresholds varied substantially between mice; estimated baseline thresholds for the target (vanilla) odorant alone ranged from $10^{-5}$ to $10^{-11}$% v/v, with a mean value of $2.50^{-6}$% v/v.

Figure 4 presents the estimated onset time course of adaptation, plotted as a function of AO concentration for individual mice. Baseline thresholds (black circles) from Figure 3 show the estimated, nonadapted thresholds for the target odorant alone. Mean detection thresholds estimated in the presence of AO concentrations (1× threshold concentration, gray squares; 2× threshold concentration, gray circles) are also shown. For both AO concentrations (1× threshold and 2× threshold), increases in target threshold with AO exposure indicate an adaptation-induced decrease in receptor odor sensitivity, observed as a duration-related increase in threshold. For onset delays of 50 and 100 ms, thresholds measured in the presence of the AO were unchanged for condition...
Threshold increases were evident for all subsequent onset delays (200 ms and higher). Beyond 200 ms, all mice exhibited orderly threshold increases, reaching asymptotic adaptation levels at ~700 ms onset delays. Under the second AO condition (AO = 2× threshold; gray circles), estimated thresholds were similarly unaffected by the 50-ms onset delay, but increased systematically after ~100 ms, reaching asymptotic adaptation levels at ~400–600 ms.

As noted above (Figure 3), there was substantial inter-animal variability in baseline thresholds. To account for the effects of variability across baseline thresholds on the time course or magnitude of adaptation, percent change from baseline was calculated for the individual mice (Figure 5). Percent change was calculated as (new threshold/baseline threshold) − 1. Using this approach, and normalizing baseline thresholds, it is apparent that the animals with the lowest initial baseline thresholds (10⁻¹¹ v/v) showed higher magnitudes of adaptation for both conditions.

Figure 6 shows the mean (n = 4) adaptation contours for both the 1× AO (gray circles) condition and the 2× AO (gray squares) condition. To estimate the onset time constants for each AO condition, a best-fit curve was estimated for each function (dashed lines; SigmaPlot, Systat Software, Inc.). A sigmoidal curve was constructed to approximately fit the data for condition 1 (AO = 1× threshold), and an exponential curve was constructed to approximately fit the data for condition 2 (AO = 2× threshold). The adaptation onset time constants were estimated at 295 ms (1× AO) and 165 ms (2× AO), indicating that the onset time course was relatively faster for the higher AO concentration.

Changes in threshold as a function of onset delay were compared for each AO concentration. Repeated measures analysis of variance revealed a main effect of AO concentration, such that thresholds increased at a relatively faster rate for the 2× AO condition [F(2, 191) = 24.497, P < 0.001]. In addition, there was a main effect of onset delay, such that thresholds increased systematically as the delay between the onset of the AO and the target increased [F(2, 191) = 94.931,
Discussion

Recent work from our laboratory shows that the onset time course for odor adaptation in humans can be estimated using a new, simultaneous odorant paradigm (Smith et al. 2010; Keith and Smith 2011; Yoder et al. 2013). In this work, we sought to use the same psychophysical approach to estimate the time course of perceptual odor adaptation in the mouse. To facilitate comparisons with our earlier adaptation time course estimates in humans, the current work employed the same olfactometers, simultaneous stimulus paradigm, and vanilla extract odorant in 4 mice (Smith et al. 2010; Keith and Smith 2011). The primary objective was to demonstrate the utility of this behavioral technique in the mouse so that it may be used in future investigations to study the mechanisms underlying odor adaptation.

The present study evaluated threshold changes both as a function of time (onset delay) and concentration (AO = 1× threshold), AO concentration (2× threshold), and simultaneous odorant paradigm used in the present mouse study. As discussed above, the estimated onset time constant for the mouse is 165 ms, whereas the human time constant is a relatively slower 319 ms. Another important distinction between the human and animal data is a temporal difference in terms of when adaptation begins. For humans, measurable differences in threshold were evident following a 50-ms AO exposure, whereas observable differences in mice were not evident until 100 or 200 ms. Although the smaller group size (n = 4 mice) could potentially account for this disparity, systematic, adaptation-induced threshold increases were routinely observed for all mice. This suggests that although initial, baseline thresholds may vary across mice (and humans), the general adaptation trend remains relatively consistent.

One plausible explanation for these apparent differences at the briefest onset delays (50 ms, 100 ms) may be related to sniffing behaviors in rodents. For rodents, sniffing is thought to constitute an adaptive filter to the olfactory bulb (Verhagen et al. 2007; Wesson et al. 2008). Because sniff patterns were not recorded under the current conditions, inhalation rate
was not controlled in the mouse study. In contrast, human participants were instructed to inhale continuously for 3000 ms, during which time the odorants were presented. Though inhalation patterns were not directly quantified for the human study either, all participants received inhale/exhale instructions on the response box during each trial. Further studies are required to reconcile these differences in adaptation onset. In accordance with the effects described in the human study, mice also show measurable increases in both adaptation onset slope and overall magnitude.

Odor adaptation is a complex process with both peripheral and central components. Although more central habituation processes are considered basic elements of learning (Best and Wilson 2004), the simultaneous stimulus paradigm described here was devised to characterize more peripheral mechanisms (Smith et al. 2010). Although the present study evaluated the impact of adaptation on olfactory sensitivity, other laboratories have similarly shown changes in perceived intensity for a related phenomenon, masking (Hein et al. 2009; Vierriele et al. 2012). The underlying mechanisms differentiating masking from adaptation are not well understood, but the primary difference generally involves the level of adapting/masking odorant required to produce changes in sensitivity. An earlier rodent study in our laboratory showed that unrelated odorants could significantly increase thresholds once the masking odorant concentrations were increased well above threshold (Smith et al. 2006). Conversely, in adaptation paradigms, thresholds can systematically increase, even when the AO is significantly below threshold (Keith and Smith 2011).

Previous studies have shown that the initial adaptation mechanisms occur 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). Even in the periphery, multiple mechanisms are involved in the adaptation process (Zufall and Leinders-Zufall 2000; Reisert and Zhao 2011), with each dependent on unique, physiological mechanisms and exhibiting different temporal characteristics. In our initial study with humans, we argued that the simultaneous odorant paradigm measured the time course of rapid, peripheral adaptation mechanisms (Smith et al. 2010). This technique is based on using brief (600 ms), target odorants presented at well-controlled delays, relative to the onset of the 2000-ms AO. Because this approach provides mouse data that align closely with human data from our previous studies, we believe that the present findings reflect the same peripheral processes.

In a behavioral study of odor adaptation in mice, Kelliher et al. (2003) show that deleting the gene encoding the olfactory receptor CNGA4 channel significantly reduces, or eliminates, olfactory perceptual adaptation in the mouse. Importantly, their work also shows that these peripheral, intracellular mechanisms can be characterized using simple psychophysical measures in mice. Here, we extend the previous, psychophysical study by measuring the onset time course of peripheral adaptation in mice. Further, we demonstrate that our simultaneous paradigm, originally employed in humans, can be used to compare odor adaptation mechanisms in an animal model. Moreover, the technique described here can be combined with genetic or pharmacologic manipulations to investigate underlying odor adaptation mechanisms.

Indeed, although it is apparent that the CNGA4 gene plays a role in ORN adaptation (Kelliher et al. 2003), recent work has shown that other receptor mechanisms also play critical roles in the adaptation process. Specifically, Stephan et al. (2011) have shown that the potassium-dependent Na+/Ca2+ exchanger NCKX4 is not only necessary for response termination but also supports recovery from adaptation. Although adaptation and subsequent recovery must be temporally precise to maintain optimal, olfactory perception, current psychophysical techniques have been unable to measure these processes on timescales comparable to physiological studies. Therefore, the present paradigm may be useful for investigating how these underlying mechanisms are reflected perceptually and provide more precise temporal estimates. By extension, recent work from our laboratories has shown that recovery from adaptation (disadaptation)
can also be measured using a variation of the simultaneous stimulus paradigm described here (Yoder, LaRue, et al. 2014). Similarly, work is currently underway adapting that disadaptation protocol for use in rodents. These data not only establish a critical temporal link between perceptual adaptation and peripheral ORN mechanisms but also demonstrate the utility of this psychophysical paradigm for studying the mechanisms of odor adaptation in animal models.

It is worth noting that although the present study is the first to explicitly demonstrate the time course of peripheral adaptation using this specific, psychophysical technique, these findings align well with known physiological mechanisms. Stephan et al. (2011) previously indicated an adaptation time constant of ~200 ms in mice. Across species, time constants for rapid adaptation are routinely <500 ms. This temporal trend has been shown in Drosophila (de Bruyne et al. 2003), lobsters (Atema 1995), crabs (Reidenbach and Koehl 2011), newts (Kawai 2002), rats (Bradley et al. 2001; Verhagen et al. 2007), and salamanders (Firestein et al. 1990). Importantly, our laboratory has also repeatedly shown this effect at the perceptual level in humans (Smith et al. 2010; Yoder et al. 2013; Yoder, LaRue, et al. 2014).

Beyond understanding the basic temporal mechanisms of odor adaptation, the present study poses interesting questions regarding the translational utility of similar olfactory measures. A critical, ongoing challenge in clinical research has been the persistent disconnect between behavioral measures used in animal models and those used for human testing. Frequently, either the human analogs are sufficiently different to limit comparisons or the underlying mechanisms are too variable between rodents and humans to extract meaningful conclusions. Despite this incongruity, clinical phenotypes are still typically investigated in rodent models first. For instance, mice are routinely used to investigate the fundamental pathways involved in complex mechanisms, such as learning, memory, aging, and disease. An essential component of these investigations, however, rests upon identifying behavioral alterations that may not be directly translatable. The olfactory system is comparatively unique in this regard. Not only are olfactory mechanisms relatively preserved across species (Kay and Stopfer 2006) but essentially identical equipment, techniques, and stimuli can be used to evaluate odor perception in both humans and animals. If animal models are to have greater predictive validity and serve as tools for pharmacological discovery and development, then it will be necessary to identify physiological and behavioral changes that are relatively consistent across species. The technique described in this study may provide a way to integrate an objective, cross-species measure using odor adaptation as a key behavioral correlate.

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