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Developmental Fine-tuning of Human Olfactory Discriminability

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Abstract

Unlike vision or audition, human olfaction is generally considered evolutionarily ancient and well-functioning at birth, yet there have been few empirical data on the development of olfactory acuity. The current study has assessed olfactory discriminability in children aged 3 to 6 years with 16 pairs of single-compound odorants that differ in various degrees in structure and smell. We report a significant improvement over age in young children's overall olfactory discriminability. Critically, such improvement is modulated by the degree of structural similarity between odorants independent of odor familiarity. Our findings indicate that odor representations in the olfactory system are fine-tuned during early childhood (3–6 years of age) to allow refined discrimination. Moreover, they suggest the need to take molecular similarity into consideration in the evaluation of olfactory discrimination in pediatric populations.

Key words: development, early childhood, molecular similarity, odor discrimination

Introduction

The human olfactory system is constantly bombarded with various odorants. Resolving the differences in the dynamic olfactory inputs is naturally at the core of human olfactory functionality, which relies upon the spatiotemporal representations of the chemical environment (i.e. molecules) generated at distinct levels of olfactory processing, with complex feed-forward and feedback circuitries (Lledo et al. 2005; Barnes et al. 2008; Howard et al. 2009). A healthy young adult can discriminate over a trillion odors (Wolfe et al. 2008; Bushdid et al. 2014), yet how such ability is shaped through development remains unclear. Nor is it known at what age olfactory acuity reaches adult level. Unlike vision and audition, where synaptic "blooming and pruning" as well as myelination accompany postnatal sensory developments (Thompson and Nelson 2001), neurogenesis and synaptogenesis in the olfactory system persist through lifespan (Altman 1969; Kaplan and Hinds 1977; Lois and Alvarez-Buylla 1994; Mizrahi and Katz 2003), and the axons of olfactory sensory neurons (OSNs) are unmyelinated (Doucette 1984). Mapping out the developmental trajectory of olfactory acuity will thus provide fresh insights into the maturation process of human perception.

As one of the oldest senses in the course of evolution, olfaction is commonly considered well-functioning at birth (Schaal 1988). Observational studies have shown that newborn infants utilize maternal breast odors to locate nipples (Varendi et al. 1994), and neonates exhibit different degrees of body activities and physiological responses to various household smells (Engen et al. 1963). More fine-grained comparisons between the olfactory discrimination performances in school-aged children and adults seem to have yielded inconsistent results regarding whether an age-related difference exists (Thomas and Murray 1980; Richman et al. 1995; Stevenson et al. 2007a, 2007b), with part of the discrepancies potentially

explained by odor familiarity (Stevenson et al. 2007a, Stevenson et al. 2007b), trigeminality (Doty et al. 1978; Laska et al. 1997), and experimental methods (Schaal 1988). Familiar odorants are generally easier to discriminate than unfamiliar ones for both adults and school-aged children (Rabin 1988; Stevenson et al. 2007a). For certain odorants, discrimination could be based on trigeminal cues (degrees to which different odorants activate the trigeminal nerve) aside from differences in olfactory quality. Moreover, procedures insensitive to children's cognitive development (Fritzley and Lee 2003), including those that tax working memory (Hitch et al. 2001) or language processing (Doty et al. 1984; Cain et al. 1995), may conceal their olfactory ability. Attempts have been made to quantify children's olfactory function in manners that rely less on cognitive abilities. For instance, Richman et al. developed a match-to-sample odor discrimination task (Richman et al. 1995). Hummel et al. asked children to smell two odorants presented one after the other and report whether the two smelled the same or different (Hummel et al. 2007a). These tasks minimally involved linguistic abilities yet inevitably recruited working memory: as odors were presented sequentially, one had to compare a newly smelled odor with the target odor or the last smelled odor held in working memory to decide whether they matched or not. As such, use of these tasks in children below the age of 5 years was not successful (Richman et al. 1995; Hummel et al. 2007a).

To date, there have been few empirical data on the development of olfactory discrimination during early childhood (3–6 years), when the brain develops rapidly not only in the neural activities in the cortex but also in the interconnections among neurons (Berk 2009). Critically, whereas the primary neural representations of odorants, namely "odor maps", are intrinsically reflections of chemical structures (Buck and Axel 1991), it remains unknown whether molecular similarity plays a role in the developmental course of human olfactory discrimination.

The present study set out to examine these issues by comparing the discrimination performances in children aged 3.5, 4.5, and 5.5 years across 16 pairs of single-compound odorants, which differed in various degrees in both molecular structure and the corresponding olfactory percept, namely smell (Haddad et al. 2008) (Supplementary Table S1). To this end, we modified the standard testing procedure of olfactory discrimination for young children with the intention to maximally reduce cognitive load (see Materials and methods). We also collected data from young adults, which served as a reference. Confounding factors, including odor familiarity, trigeminality, children's comprehension of the task as well as their performance stability, were also assessed.

Materials and methods

Participants

A total of 96 participants from 4 age groups of 24 each—3.5-year-olds (11 boys, mean age ± SEM = 3.63 ± 0.02 years), 4.5-year-olds (13 boys, 4.49 ± 0.05 years), 5.5-year-olds (11 boys, 5.52 ± 0.04 years), and young adults (11 males, 23.68 ± 1.18 years)—took part in the odor discrimination task. All children tested were within 4 months (or 0.3 years) of 3.5, 4.5, or 5.5 years of age. An additional 24 young adults (15 males, 22.79 ± 0.59 years) judged the familiarity of each odorant as well as the perceptual similarity between the 2 odorants in each pair. Furthermore, 60 parents (10 males, 35.4 ± 0.51 years) and 36 kindergarten teachers (all females, 27.1 ± 1.04 years) provided familiarity ratings for each odorant based on the olfactory experience of their children and students, respectively. Another group of 20 young adults (7 males, 22.15 ± 0.42 years) was tested for odor trigeminality. The children were recruited from three local kindergartens and were reported by their teachers and parents to have no respiratory allergy or upper respiratory infection at the time of testing and no documented history of speech, language, or hearing difficulties. The adult participants were healthy nonsmokers who self-reported to have a normal sense of smell and no respiratory allergy or upper respiratory infection at the time of testing. Oral assent and parental written informed consent were obtained for each child. Written informed consents were obtained from all adult participants. The complete study was approved by the Institutional Review Board of the Institute of Psychology at Chinese Academy of Sciences (H13003).

Olfactory stimuli

Olfactory discrimination was assessed with the odor discrimination module of Sniffin' Sticks Extended Test (Hummel et al. 1997) (Burghart Medical Technology, Wedel, Germany). It consisted of 16 triplets of single-compound odorants contained in felt-tip pens. In each triplet, 2 pens contained the same odorant and one pen contained a different odorant (Supplementary Table S1). Odorants within the same triplet were similar in intensity and hedonic tone (Hummel et al. 1997).

Procedure

The children were tested individually in a quiet and well-ventilated concrete room with no detectable ambient odor at each kindergarten. The adult participants were tested individually in a designated room in our laboratory equipped with an air purifier and a high-volume ventilation system.

Assessment of task comprehension

As a preparatory step for the assessment of olfactory discrimination, we designed a visual discrimination task to test whether children could understand the concepts of "same" and "different". Each item in the 12-item visual discrimination test (Supplementary Figure S1) consisted of 3 shapes, out of which 2 were identical and one differed from the other 2 in either form (3 items), color (3 items), size (3 items), or orientation (3 items). The order of the items was pseudo-randomized. For each item, children were asked to point out the one that differed from the other 2. No feedback was provided.

Assessment of olfactory discrimination

The pens in each triplet of the odor discrimination module of Sniffin' Sticks Extended Test were presented in a random order, whereas the order of the triplets was fixed, namely from No.1 to No.16. Participants were instructed to pick out the one that smelled different from the other 2 (see below for procedural details). There was at least a 30-s break in between the presentations of two triplets to eliminate olfactory habituation. No feedback was provided. The total number of correct discriminations out of the16 triplets indexed one's overall olfactory discriminability. For each child, the difference between the numbers of correct discriminations for the first 8 and the last 8 triplets was used to approximately index his/her performance stability.

Children were not blindfolded during this task, as they were generally afraid of darkness. Instead, the color-coded bottom part of each pen was covered from being seen. Sixty-seven children (out of 72, 93%) were tested using a modified triangle procedure that posed less burden on their limited working memory span (Berk 2009) as compared to a standard triangle procedure (employed for adult participants). Specifically, the 3 pens in each triplet were

uncapped, aligned in a row (there was a ~10 cm gap in between 2 adjacent tips to eliminate odor crossover), and simultaneously presented to the children, who could sequentially sample all three smells within about 3 s (as opposed 15–20 s in a typical triangle procedure that involves uncapping a pen, presenting it to the subject, recapping it, and repeating the process for each of the remaining 2 pens), re-smell any one of them if they would like to, and point to the one they found different. The remaining 5 children (3 in the 3.5 years group and 2 in the 4.5 years group) were tested using a duo-trio method, in which the children smelled 2 pens in a trial and decided whether they contained the same or different smells. Three such pairwise comparisons were made for each triplet, with at least a 30 s break in between the trials. We found no indication that the cognitively easier but more time-consuming duo-trio procedure improved olfactory discrimination as compared with the modified triangle procedure. A total of 25 children (35%) re-smelled one or more triplets.

Assessment of odor familiarity and similarity

A separate panel of 24 young adults sampled each odorant used in the odor discrimination task, one at a time, and rated on a 100-unit visual analogue scale how familiar each odorant was, and how similar the two odorants in each pair smelled, in an order balanced across participants, with a 30-s break in between the samplings. It was difficult in practice to obtain reliable olfactory familiarity evaluations from young children, as they could not properly assign ratings on a rating scale (Cycowicz et al. 1997). We thus followed common practice in developmental studies (Tardif et al. 1999; Yoshida and Smith 2001) and recruited 60 parents and 36 kindergarten teachers (20 parents and 12 kindergarten teachers per children's age group) to rate the familiarity of each odorant based on the olfactory experience of his/her child or that of students in her class, respectively. Their ratings collectively reflected children's exposures to the odorants at home and in kindergartens.

Assessment of odor trigeminality

The trigeminality of each odorant was assessed in another group of 20 young adults using a lateralization test (Wysocki et al. 2003). This was done to rule out the possibility that discrimination was based on trigeminality rather than odor quality. Here, we did not test young children as their trigeminal function had been shown to be generally poorer than that of adults (Hummel et al. 2007b) and thus were less likely to base their discrimination on the trigeminality of odorants. Each pen containing an odorant was paired with a control pen containing only the solvent propylene glycol. The 2 pens, respectively fitted with a Teflon nosepiece, were simultaneously presented to both sides of the nose, one to each nostril, such that they would be independently sniffed in during the same inspiration. The blindfolded participants made a forced choice which nostril smelled an odor. This task was repeated 4 times per odorant per participant, with a 30-s break in between the trials. The side of odorant presentation was randomized.

Analyses

Analyses were conducted in IBM SPSS Statistics (Version 22). Overall olfactory discriminability, as indicated by the total number of correct discriminations in the odor discrimination task, was analyzed using univariate ANOVA, with age group (4 levels: 3.5-year-olds, 4.5-year-olds, 5.5-year-olds, and young adults) and gender (males vs. females) as the fixed factors. We observed no gender-related effect (see Results), and thus excluded gender as a factor in subsequent analyses. Next, bivariate Pearson correlation was performed between children's actual age and their olfactory discriminability to estimate the proportion of variance in children's odor discrimination that was attributable to age. To assess if the observed age-related changes were due to younger children's poorer ability to maintain attention, and thereby greater performance instability, we employed a repeated measures ANOVA and compared children's performances in the first half and the second half of the olfactory discrimination test (number of correct discriminations for the first 8 and the last 8 triplets, respectively; within-subjects variable) across the 3 age groups (between-subjects variable, 3.5-year-olds, 4.5-year-olds, and 5.5-year-olds).

In order to examine the role of molecular similarity between odorants in the development of olfactory discriminability, we adopted 2 complementary approaches. The first approach was data-driven. We carried out an omnibus full-factorial ANOVA with odorant pairs as the within-subjects variable (16 levels, Supplementary Table S1) and children's age group as the between-subjects variable. This was followed by 16 univariate ANOVAs, one for each of the 16 odorant pairs, with children's age group as the fixed factor. The second approach was based on our classification of the odorant pairs. We loosely divided the 16 pairs of odorants into 3 groups of comparable sizes based on the physicochemical distance values between their constituting odorants in a multidimensional odor metric space (Haddad et al. 2008), namely high (pairs 2, 8, 11, 13; physicochemical distance < 6.0), medium (pairs 4, 5, 7, 10, 15, 16; lower half of the remaining 12 odorant pairs; 6.0 < physicochemical distance < 6.85), and low (pairs 1, 3, 6, 9, 12, 14 in Supplementary Table S1; upper half of the remaining 12 odorant pairs; physicochemical distance ≥ 6.85) molecular similarity. We then performed a repeated measures ANOVA with the degree of molecular similarity as the within-subjects variable (3 levels: low vs. medium vs. high molecular similarity) and children's age group as the between-subjects variable. Admittedly, the classification of molecular similarity was approximate. It served as a supplementary approach to characterize the relationships between molecular similarity and the development of children's olfactory discrimination. We note that using slightly different cut-off values did not significantly alter the results. For young adults, repeated measures ANOVAs were also performed to compare the olfactory similarity ratings and odor discrimination accuracies (dependent variables), respectively, for the odorant pairs with low, medium, and high structural similarity (within-subjects factor).

With regard to odor familiarity, we first assessed in 2 separate univariate ANOVAs the influence of age group on overall odor familiarity (all target and non-target odorants combined) and familiarity with the target odorants, respectively. We then specifically examined whether age differentially affected children's familiarities (based on ratings provided by their parents and kindergarten teachers on behalf of them) with the odorant pairs of low, medium, and high molecular similarity, or the target odorants in these 3 categories, in 2 repeated measures ANOVAs where molecular similarity served as the within-subject factor, and children's age group served as the between-subjects factor.

For odor trigeminality, we calculated for each odorant the participants' accuracies in the lateralization test, and compared those with chance level (0.5) in a series of one-sample T tests.

Multiple comparisons were corrected with Bonferroni method where appropriate. All statistical tests were 2 tailed.

Results

General developmental improvement in odor discrimination during early childhood

In general, performances on the odor discrimination task showed a main effect of age group ($F_{3, 88}$ = 45.38, P < 0.001, partial η^2 = 0.61) and no significant role of gender (main effect: $F_{1, 88}$ = 0.94, P = 0.34; gender × age group: $F_{3, 88}$ = 1.41, P = 0.25), with 3.5-year-olds falling below 4.5-year-olds (mean number of correct discriminations ± SEM: 9.6 ± 0.31 vs. 11.1 ± 0.27, P = 0.002, corrected), 4.5-year-olds falling below 5.5-year-olds (11.1 ± 0.27 vs. 13.0 ± 0.28, P < 0.001, corrected), and 5.5-year-olds comparable to young adults (13.0 ± 0.28 vs. 13.9 ± 0.30, P = 0.13, corrected) (Figure 1a). Children's age strongly correlated with their overall olfactory discriminability (r_{72} = 0.69, P < 0.001, Figure 1b) and explained 47% of the data variance. This appeared not because younger children simply failed to understand the task requirement (all got 100% correct in the initial assessment of task comprehension, Supplementary Figure S1), or to maintain their attention in the latter part of the testing, which in turn led to worse odor discrimination and poorer performance stability (Spitzer et al. 1988) ($F_{2, 29}$ = 0.005, P = 0.99, Figure 1c).

Role of molecular similarity in the developmental change of odor discrimination during early childhood

Whereas overall odor discrimination improved with age during early childhood, this was not the case for every odorant pair, as indicated by a significant interaction between odorant pair and children's age group (Phillai's trace $F_{30, 112}$ = 1.80, P = 0.015, partial η^2 = 0.33). A closer look at the discrimination performances for individual odorant pairs (see Supplementary Table S2 for details) revealed three distinct patterns that by eyeballing corresponded well with the levels of molecular similarity between the constituting odorants:

(1) Relatively high discrimination accuracy across the 3 children's age groups with little improvement with age, as in the case of octyl acetate and cinnamaldehyde (pair 1 in Supplementary Tables S1 and S2, $F_{2, 69}$ = 0.069, P = 0.93, Figure 2a).
(2) Significantly improved discrimination accuracy with age, as in the case of geraniol and octyl acetate (pair 5 in Supplementary Tables S1 and S2, $F_{2, 69}$ = 8.27, P = 0.001 uncorrected, P < 0.05 corrected, partial η^2 = 0.19, Figure 2b).
(3) Poor performance across the three age groups with little improvement with age, as in the case of citronellal and linalool (pair 13 in Supplementary Tables S1 and S2, $F_{2, 69}$ = 0.49, P = 0.62, Figure 2c).

To better capture the relationship between odorants' molecular similarities and developmental patterns of odor discrimination performances, we followed previous work and classified the 16 odorant pairs into 3 groups of high, medium, and low molecular similarity based on the physicochemical distance values between their constituting odorants in a multidimensional odor metric space representing over 1,600 chemical features (see Materials and methods) (Haddad et al. 2008). In young adults, the degrees of molecular similarity (high, medium and low) largely paralleled subjective olfactory similarity ratings ($F_{1.58, 36.41}$ = 17.63, P < 0.001, partial η^2 = 0.43) and odor discrimination accuracies ($F_{2, 46}$ = 10.94, P < 0.001, partial η^2 = 0.32).They rated the odorant pairs with higher molecular similarity as perceptually more similar (high vs. medium vs. low: 37.3 ± 3.7 vs. 29.1 ± 3.2

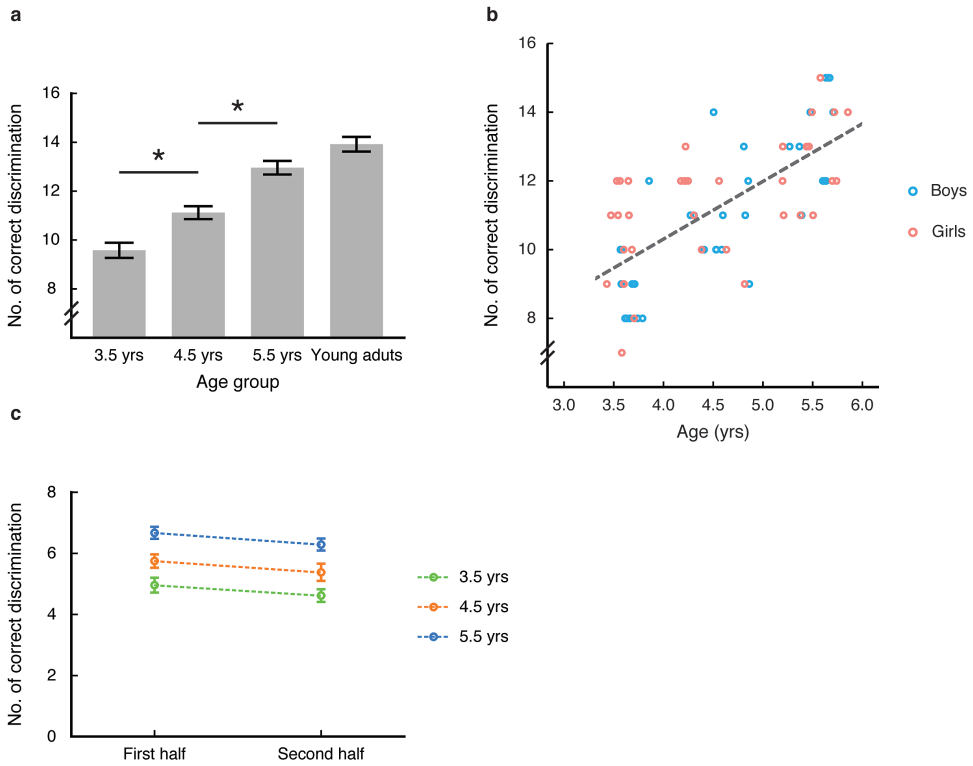

Figure 1. General developmental changes in olfactory discrimination during early childhood. (a) Developmental improvement in overall olfactory discriminability. (b) In both boys and girls, age strongly correlated with odor discrimination. (c) Children of all three age groups showed comparable performance stabilities in the odor discrimination test. Error bars represent the standard errors of the means. Asterisks indicate significant differences between groups, P < 0.05, corrected.

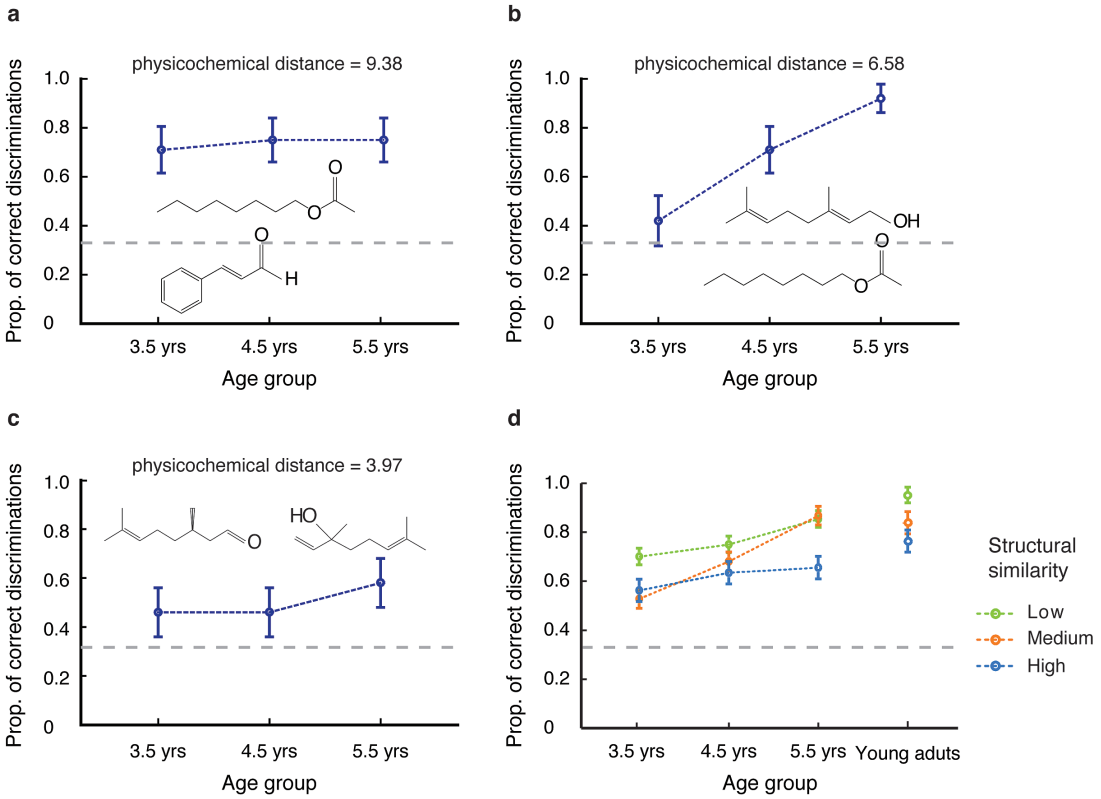

Figure 2. Interaction between molecular similarity and age in olfactory discrimination during early childhood. (a) Children of all age groups performed equally well in discriminating between octyl acetate and cinnamaldehyde. (b)The discrimination between geraniol and octyl acetate showed a significant improvement with age. (c) For citronellal and linalool, children of all age groups performed equally poorly. (d) In general, olfactory discrimination improved with age and was better for structurally dissimilar odorants. Nevertheless, the discrimination between odorants of medium structural similarity showed a steeper improvement with age as compared with those of high or low structural similarity. Error bars represent the standard errors of the means. Dashed lines represent chance level (0.33).

vs. 21.0 ± 3.0, $P < 0.007$, corrected), and showed better discrimination for the odorant pairs with low molecular similarity (discrimination accuracy: 0.96 ± 0.02) as compared to those with medium (0.85 ± 0.03) and high (0.77 ± 0.04) molecular similarities (*P*s < 0.015, corrected; no difference between the latter 2 groups, $P = 0.31$, corrected).

In children, repeated measures ANOVA on their odor discrimination accuracies yielded a significant interaction between the degree of molecular similarity and age group ($F_{4, 138} = 2.37$, $P = 0.05$, partial $\eta^2 = 0.064$), on top of significant main effects of both factors (children's age group: $F_{2, 69} = 28.8$, $P < 0.001$, partial $\eta^2 = 0.46$; degree of molecular similarity: $F_{2, 138} = 9.10$, $P < 0.001$, partial $\eta^2 = 0.12$). Although children's overall olfactory discriminability improved with age and was better for structurally less similar odorants, there was clearly a steeper improvement over age in discerning between odorants of medium (linear regression, beta = 0.60, $P < 0.001$) rather than low (beta = 0.36, $P = 0.002$) or high (beta = 0.17, $P = 0.15$) molecular similarity (Figure 2d). At 3.5 years old, children's discrimination accuracy for the odorant pairs with medium molecular similarity was as poor as that for the ones with high molecular similarity (medium vs. high: 0.53 ± 0.05 vs. 0.56 ± 0.05; $t_{23} = -0.43$, $P = 0.67$), and significantly worse compared to those with low molecular similarity (medium vs. low: 0.53 ± 0.05 vs. 0.70 ± 0.04; $t_{23} = -2.72$, $P = 0.012$). By the age of 5.5 years, however, it caught up with the discrimination accuracy for the odorant pairs with low molecular similarity (medium vs. low: 0.87 ± 0.02 vs. 0.85 ± 0.03; $t_{23} = 0.40$, $P = 0.69$) and became significantly better as compared with those with high molecular similarity (medium vs. high: 0.87 ± 0.02 vs. 0.66 ± 0.05; $t_{23} = 3.84$, $P = 0.001$), reaching the same level as that of young adults (5.5-year-olds vs. young adults: 0.87 ± 0.02 vs. 0.85 ± 0.03; $t_{46} = 0.52$, $P = 0.61$).

Odor familiarity and trigeminal response

Odor familiarity was previously shown to facilitate odor discrimination (Stevenson et al. 2007a; Stevenson et al. 2007b). To eliminate its influence, the current study employed single-compound odorants rather than common household smells. These single-compounds were rated as moderately unfamiliar to participants of all four age groups (parents and kindergarten teachers provided familiarity ratings on behalf of young children, see Materials and methods), with an average score of 51.5 (SEM = 1.29) on a 100-unit visual analogue scale where 100 marked "very familiar" (48.8 ± 2.42, 52.3 ± 2.52, 53.4 ± 2.48, 51.9 ± 3.07 for 3.5-year-olds, 4.5-year-olds, 5.5-year-olds, and young adults, respectively). There was no effect of age group on overall odor familiarity (target and non-target odorants combined, $F_{3, 116} = 0.62$, $P = 0.60$) or familiarity with the target odorants ($F_{3, 116} = 1.02$, $P = 0.39$).

Turning to individual odorant pairs, we found that odor familiarity could not explain the different developmental patterns of odor discrimination associated with different odorant pairs. For instance, as mentioned above, children of all three age groups discriminated

between octyl acetate and cinnamaldehyde with comparable high accuracy, whereas the discrimination between octyl acetate and geraniol showed significant improvement over age (Figure 2a and b). Both pairs contained octyl acetate, yet cinnamaldehyde was rated as less familiar than geraniol to children of all three age groups with no age-related difference (main effect of odorant: $F_{1, 93} = 110.8$, $P < 0.001$; main effect of age group: $F_{2, 93} = 0.48$, $P = 0.62$; odorant × age group: $F_{2, 93} = 0.29$, $P = 0.75$). There was also no interaction between the degree of molecular similarity (high, medium vs. low) and age group in children's familiarity with the odorant pairs (target and non-target odorants combined, $F_{4, 186} = 0.15$, $P = 0.96$) or just the target odorants ($F_{4, 186} = 1.49$, $P = 0.21$).

At the concentrations used, none of the odorants could be reliably localized above chance level when it was presented to one side of the nose (see Materials and methods; mean accuracy ranged from 38.75% to 57.5% vs. chance = 50%, $P > 0.05$, uncorrected), indicating an absence of significant trigeminal response (Wysocki et al. 2003).

Discussion

Taken together, our results outline the developmental trajectory of human olfactory discriminability in early childhood that diverges based on odorants' molecular similarity. In contrast to the visual system where adult-level acuity is acquired within the first year of life (Marg et al. 1976), the olfactory system seems to undergo steady refinements in early childhood and does not support adult-level discriminability until around 5.5 years of age (Figure 1a). Such developmental improvement appears to be largely independent of general cognitive advancement during this period on the basis of measures of task comprehension and performance stability (Figure 1c), as well as accurate discriminations of structurally dissimilar odorants in 3.5-year-olds. Rather, it reflects the intertwined contributions of innate, genetically programmed, chemical feature mappings and a dynamic, likely experience-dependent, fine-tuning process in the shaping of human olfactory perception. We expand on this inference below.

Although odor coding is combinational such that each olfactory receptor recognizes multiple odorants and different odorants are encoded by different combinations of olfactory receptors (Malnic et al. 1999), the representations of structurally similar odorants are highly correlated whereas those of structurally dissimilar ones tend to be spatially segregated in the antennal lobe of Drosophila (Couto et al. 2005), as well as the main olfactory bulbs of rats (Rubin and Katz 1999; Takahashi et al. 2004) and rabbits (Imamura et al. 1992; Katoh et al. 1993). We infer that a comparable genetically programmed segregation between the "odor maps" of structurally dissimilar compounds may underlie children's ready discrimination of them at an early age (Figure 2a and d). Whereas the odorant pairs used in our study (range of physicochemical distance: 0 to 11.1) by no means cover the entire spectrum of physicochemical distances, we extrapolate that odorants of a greater physicochemical distance than that between pyridine and (−)-limonene (Pair 14 in Supplementary Tables S1 and S2, physicochemical distance = 11.1), namely lower structural similarities, would be well discriminated by children aged 3.5 years and above. Conversely, odorants with high molecular similarity likely induce highly similar response patterns that are difficult to resolve even for older children (Figure 2c and d) and young adults. For instance, the enantiomers of carvone (Pair 8 in Supplementary Tables S1 and S2) has a physicochemical distance of 0 and was the odorant pair with the poorest (albeit above chance) discrimination performance for all age groups, despite that (−)-carvone is typically described as smell like spearmint and (+)-carvone caraway seeds. There was also little improvement in odor discrimination over age.

Critically, when it comes to compounds of medium structural similarity, a different age-dependent mechanism seems to take effect (Figure 2b and d), driving the overall developmental enhancement of odor discrimination during early childhood (Figure 1a and b). In keeping with existing work on the plasticity of olfactory circuitry (Wilson and Stevenson 2003), this suggests that on top of the innate and initially coarse chemical feature mappings (Malnic et al. 2004), a dynamic fine-tuning process gradually enables fine-grained odor discrimination.

At the initial stage of olfactory processing, the chemical features of odors are encoded by diverse odorant receptors and segregated glomeruli in the main olfactory bulb. Based on animal models, these structures are available by birth (Mombaerts et al. 1996), but the projections between the axon terminals of OSNs and their postsynaptic targets are not fully refined until much later, and odor-driven neuronal activities in OSNs is essential in this process (Zou et al. 2004). In parallel, the functional organization in the olfactory bulb progresses through a series of postnatal changes (Greer et al. 1982), such that lateral and feedback inhibition and excitation dynamically combine to enhance contrast between similar chemical features (Yokoi et al. 1995; Luo and Katz 2001;). Further down the olfactory hierarchy, the piriform cortex serves as a site of odor object synthesis and has highly plastic and experience-dependent response patterns (Wilson 2003; Li et al. 2008). Given the importance of odor exposure in olfactory functioning in both rodents (Guthrie et al. 1990) and humans (Wu et al. 2012), it is plausible that olfactory experience gained in early childhood refines olfactory representations to enable improved acuity over age. In the meantime, an innate maturation process could also be taking place (Lin et al. 2000). The exact neurobiological and neurophysiologic mechanisms still await future studies to clarify.

Whereas the current study focuses on the role of molecular similarity in the development of olfactory discrimination during early childhood, it by no means negates the influences of odor familiarity and odor naming ability (literacy), which we were unable to adequately assess due to the children's young age and the use of single compounds that were rarely encountered in isolation in daily life and were not familiar even to young adults. Although we obtained odor familiarity ratings for participants of all age groups, it is possible that the ratings provided by parents and kindergarten teachers on behalf of young children might not be sensitive enough to developmental effects. We emphasize, however, that odor familiarity is unlikely associated with an odorant's chemical structure and hence unlikely to account for the observed interaction between molecular similarity and age in children's olfactory discriminability.

In summary, the current study lays out the developmental trajectory of olfactory discriminability in early childhood, which diverges based on odorants' molecular similarity. In doing so, it represents the first attempt to characterize the correspondence between olfactory perception and physicochemical properties of odorants in children, and hints at intertwined contributions of genetically programmed chemical feature mappings and a dynamic, possibly experience-dependent, fine-tuning process of odor representations in the human olfactory system. The findings complement the known developmental courses of vision (Boothe et al. 1985) and audition (Jensen and Neff 1993), and enrich our understandings of the maturation process

of human perception. Moreover, they suggest the need to take molecular similarity into consideration in the evaluation of olfactory discrimination in pediatric populations (Dalton et al. 2011).

Supplementary Material

Supplementary data are available at *Chemical Senses* online.

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Conflict of Interest

The authors declare no conflict of interest.

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