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# Infants' hedonic responsiveness to food odours: a longitudinal study during and after weaning (8, 12 and 22 months)

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## Abstract

**Background:** Olfaction is a highly salient sensory modality in early human life. Neonates show keen olfactory sensitivity and hedonic responsiveness. However, little is known about hedonic olfactory responsiveness between the neonatal period and 2 years of age. In an attempt to fill this gap, this longitudinal follow-up study aimed at investigating hedonic responses to food odours in infants during the first 2 years of life. The second objective was to evaluate whether gender has an influence on hedonic responses during this early period. Four control stimuli and eight odours (four rated by adults as a priori pleasant and four a priori unpleasant) were presented in bottles to 235 infants at 8, 12 and 22 months of age. The infant's exploratory behaviour towards odorized and control bottles was measured in terms of mouthing defined as direct contact with perioral and/or perinasal areas. For each odorized bottle, duration proportions of mouthing were calculated relative to the control bottles.

**Results:** For the three ages, shorter duration of mouthing was found for unpleasantly scented bottles compared to pleasantly scented bottles. This contrast between pleasant and unpleasant odours was similar for girls and boys. Correlations of responses between ages were modest in number and level, and concerned mostly unpleasant odours.

**Conclusion:** During the first two years of life, infants discriminate the hedonic valence of odours. They avoid most of the food odours considered as unpleasant by adults, but their attraction towards food-odours judged pleasant by adults does not appear to be fully shaped at this early age. Taken as a whole, the present results highlight both the plasticity of hedonic responses to food odours, and relatively stable avoidance behaviours towards some unpleasant odours.

**Keywords:** Human infant, Olfaction, Food odours, Preference, Development

## Background

Olfaction is a highly salient sensory modality in early human life. Shortly after birth, neonates can detect and discriminate odorants that differ in quality or intensity [1]. For example, 4-day-old neonates differentiate odour cues carried in their own amniotic fluid or in their mother's milk, when presented against control stimuli [2], and they can also olfactorily differentiate their own amniotic fluid

or their mother's milk from amniotic fluid or milk from another mother [3,4]. Neonates can also discriminate various artificial odorants [5], as shown by their directional head responses [6] or by heart- and respiratory-rate changes [5,7]. For example, full-term neonates display significantly greater respiratory changes when they are exposed to either vanillin or butyric acid compared to an odourless control [7]. Besides, in the very first hours of life, differential facial responses discriminate odours classified a priori by an adult panel as pleasant (that is, banana and vanilla odours) or as unpleasant (that is, rotten egg and shrimp odours) [8]. Pleasant odours elicit facial expressions read by adult coders as denoting enjoyment

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while unpleasant odours elicit facial expression interpreted as evoking disgust. Nevertheless, in a later study, the assessment of 3-day-old infants' facial expressions to highly diluted vanillin and butyric acid odours reveals that butyric acid elicits more negatively valenced facial expressions, while vanillin elicits as often negative and positive responses [7].

Beyond the neonatal period, infants do also exhibit hedonically specific behaviours to odours. For example, 9-month-old infants respond differentially to objects as a function of their odour; while butyric acid odour induces rejection of the object, the odour of methyl salicylate (which is locally considered pleasant by adults and children) elicits exploratory responses to it [9]. Nevertheless, infants from 7 to 15 months were found to exhibit less mouthing and handling for an object bearing an odour that was unfamiliar to them (violet) over an unscented object, even if violet was considered as neutral to pleasant by older children and adults [10]. In older children, hedonic responsiveness to odours can be assessed using a forced-choice categorization procedure. With this method, it was demonstrated that 2- to 3-year-old children exhibit adult-like preference patterns [11]. Beyond 3 years, hedonic assessment becomes easier and more reliable as children can then be asked to verbally report their odour likes and dislikes [12].

This quick survey of the literature indicates that most published results on the development of hedonic responsiveness to odours derive from studies run with neonates or with children older than 2 years, leaving almost blank the period in between. The main objective of the present study was thus to contribute to fill this gap in assessing the hedonic responsiveness to food odours along the first 2 years of life, a period during which the eating pattern of infants shifts from an exclusively milk-based diet until about 6 months to the typical local diet of adults at about 2 years. During this period of food diversification, infants are thus directly exposed to an extended range of flavours and odours. This period includes three ages corresponding to key steps in the establishment of the food repertoire: 8, 12 and 22 months. In France, at the age of 8 months, 100% of infants have consumed foods other than human or formula milk [13]. Then, around the age of 12 months, their food repertoire is progressively changing from baby foods to table foods, which provide a wider range of chemosensory stimuli [14,15]. By about 2 years of age, infants increasingly exhibit food neophobia, which is defined as the reluctance of trying foods that are novel or unknown to the child [16,17]. Based on these three periods of progressive changes in infant feeding and chemosensory experience in the culture described above, olfactory tests were followed up longitudinally when the participants were aged 8, 12 and 22 months. The goal of this study was to

assess how olfactory responses develop along this period of marked changes in the ways food-related stimuli are experienced. As suggested by Schmidt and Beauchamp [11], it was expected that infants would exhibit olfactory preferences that increasingly resemble those of adults during the period when they change from the mixed diet, including milk and baby-foods, to the local diet of adults. Thus, food odours locally considered pleasant by adults are expected to increasingly elicit attraction in growing infants, whereas food odours considered unpleasant by adults are expected to increasingly induce avoidance in infants.

Another relevant issue that relates to hedonic responsiveness is the influence of gender. Since the first psychophysical tests at the end of the nineteenth century, women are considered to be better in odour detection and discrimination as compared to men, and this gender difference was already noted in prepubertal children [18,19]. It was hypothesised that this gender difference derived either from lower thresholds or from higher cognitive abilities in women than in men (especially in tasks involving language or semantic performance) [19-21]. In older children in the 6 to 12 year range, girls were found to pay more attention than boys to a variety of odour contexts in everyday settings [22]. However, in the study of Durand *et al.* [10] on infants aged 7 to 15 months, no such gender effect was noted. Thus, gender differences in olfactory abilities remain controversial, and this study aimed to assess their development in the context of hedonically contrasted food-related odours.

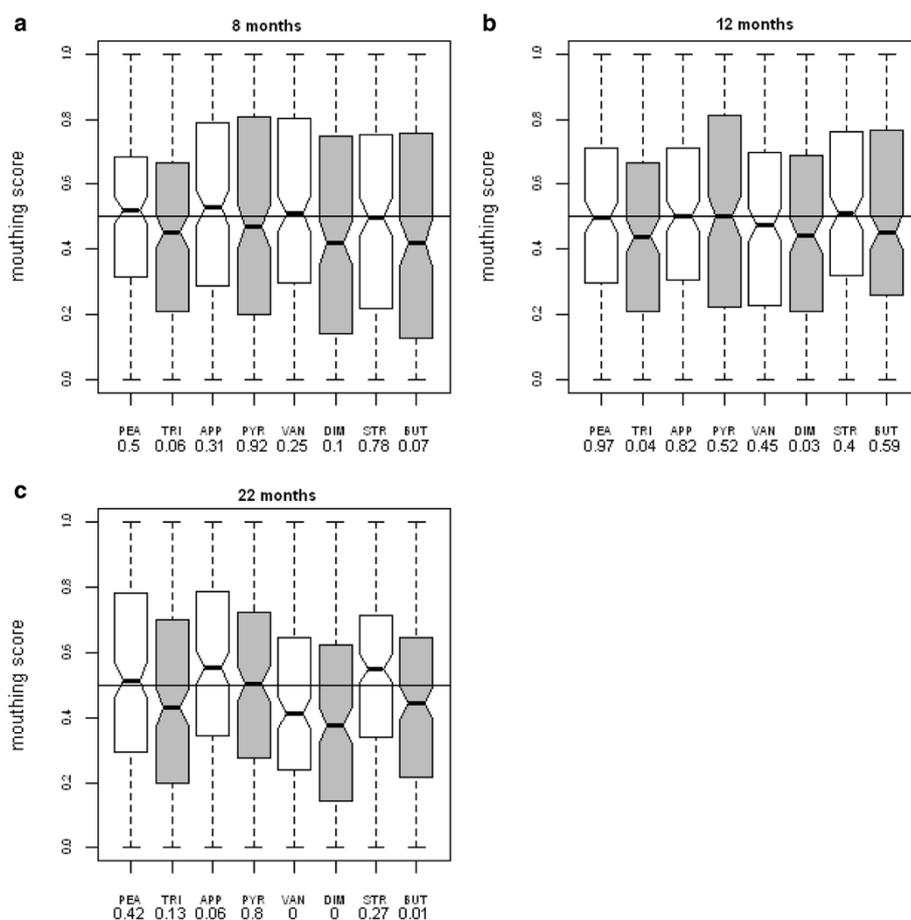
## Results

### Exploratory behaviour

The infants' exploratory activity was assessed by focusing on the duration of mouthing, which is considered as an index of interest for, and attraction to, a given odorant (see the Methods section). For each odorant, a mouthing score was computed based on the duration of mouthing. These mouthing scores were expected to be significantly higher than 0.5 for pleasant odours, indicating attraction for these odorants over the control. By contrast, mouthing scores lower than 0.5 were expected for unpleasant odours, indicating avoidance.

A global analysis (see Methods) run on the four pleasant versus the four unpleasant odour stimuli revealed a significantly lower mouthing score for the unpleasant than for the pleasant stimuli for the three age groups ( $P = 0.001$ ,  $0.006$ , and  $0.04$  at 8, 12 and 22 months, respectively).

Specific analyses run on each odorant showed that at 8 months, the mouthing scores were not significantly different from 0.5 for any of the tested odorants (Figure 1a). At 12 months, the mouthing score for trimethylamine and dimethyl disulphide became significantly lower than 0.5, suggesting a lower oro-tactile exploration compared to the



**Figure 1 Score of mouthing behaviour.** Scores are represented for 8 (a), 12 (b) and 22 (c) month-old infants in response to pleasant odours (PEA, peach/apricot; APP, apple; VAN, vanillin; STR, strawberry) in white, and in response to unpleasant odours (TRI, trimethylamine; PYR, 2-isobutyl-3-methoxypyrazine; DIM, dimethyl disulphide; BUT, butyric acid) in grey. *P*-values are from the Wilcoxon test comparing the median value to 0.5. The score is a ratio between the odorant and the sum of the odorant and the control, and 0.5 represents the value where no differences are observed between the odorant and the control.

control stimulus (Figure 1b). Finally, at 22 months, the mouthing scores induced by dimethyl disulphide and butyric acid odours remained significantly lower than 0.5, and the odour of vanillin also elicited mouthing scores lower than 0.5 (Figure 1c). In contrast, the mouthing score for the apple odour tended to be higher than 0.5, but without reaching statistical significance (Figure 1c).

These results suggest that from 8 months infants exhibit a differential mouthing behaviour towards pleasant and unpleasant odours. This difference in mouthing was mostly due to a shorter duration of mouthing for trimethylamine and dimethyl disulphide compared to the control at 12 months, and for dimethyl disulphide and butyric acid at 22 months. Pleasant odours did not elicit longer mouthing durations than did controls. Unexpectedly, at 22 months, vanilla - one of the pleasant odours - elicited a shorter mouthing duration than the control.

### Correlations between age groups

Kendall correlations between the mouthing scores (of each odorant) at the different age points are given in Table 1. Correlations were rather modest, and were intermittently significant across age groups. Mouthing scores were significantly linked between the ages of 8 and 12 months for dimethyl disulphide, and between 12 and 22 months for butyric acid.

Kendall correlations between responses to all odours and two consecutive age points were also performed per infant in order to assess individual stability of the olfactory responses. The medians of the distribution of the Kendall correlation coefficients were 0.07 between 8 and 12 months, and 0 between 12 and 22 months. Wilcoxon tests revealed that the medians of the distributions were not significantly different from 0 (all *P*-values >0.39). Thus, we noted as many positive as negative correlations, and only 6% were significant (*P* <0.05). Therefore,

**Table 1 Kendall correlations (unilateral tests) between mouthing scores at two ages**

Hedonic value	Odorants	8 to 12 months		12 to 22 months	
		Kendall $\tau$	<i>P</i>	Kendall $\tau$	<i>P</i>
Pleasant odours	PEA	0.05	0.19	0.06	0.13
	APP	0.06	0.12	-0.05	0.80
	VAN	-0.06	0.87	-0.04	0.82
	STR	-0.03	0.73	0.07	0.12
Unpleasant odours	TRI	0.05	0.20	<0.01	0.48
	PYR	-0.02	0.62	-0.02	0.63
	DIM	0.12*	0.01*	0.02	0.36
	BUT	-0.01	0.55	0.11*	0.03*

The odorants were peach/apricot (PEA), apple (APP), vanillin (VAN), strawberry (STR), trimethylamine (TRI), 2-isobutyl-3-methoxypyrazine (PYR), dimethyl disulphide (DIM), butyric acid (BUT). The number of participants varied between 140 and 175 because in some cases, toddlers did not complete the session, thus data for some odorants were missing. \*Significant correlation ( $P < 0.05$ ).

very few infants exhibited similar exploratory mouthing behaviours towards the present set of odorants at two different ages.

#### Gender effects

Wilcoxon tests performed on the individual differences between the median of mouthing scores obtained for pleasant odours and the median of mouthing scores obtained for unpleasant odours did not reveal any gender effect ( $P = 0.77, 0.36, \text{ and } 0.62$  at 8, 12 and 22 months, respectively).

#### Breast feeding effects

Additional Wilcoxon tests were performed on the differences between the median of mouthing scores obtained for pleasant odours and the median of mouthing scores for unpleasant odours to compare breast-fed and bottle-fed infants at 8 months. No effect of breast feeding reached significance for mouthing behaviour ( $P = 0.44$ ). Moreover, no difference was noted between infants who were still breast-fed at 8 months and infants who were no longer breast-fed ( $P = 0.17$ ).

## Discussion

### Mouthing behaviour

The results of the present study indicate that infants aged from 8 to 22 months exhibit differential mouthing responses to food odours that were classified as pleasant or unpleasant by an adult panel. A first finding was that infants' responses considered to express avoidance were clearer than responses considered to express attraction. In our conditions, infants could show negative appreciation for odours by manifesting less mouthing responses than the control, and conversely they could show positive appreciation by expressing more mouthing responses than

the control. It came out that most of the odours that were selected because they were unpleasant for adults of the same culture, and because they corresponded to foodstuffs known to be avoided by children and infants, elicited reduced mouthing responses (that is, trimethylamine in 12-month-old, dimethyl disulphide in 12- and 22-month-old, and butyric acid in 22-month-old infants). However, the same analyses on the odours chosen because they were pleasant to adults and represented foodstuffs generally liked by children resulted in the absence of strong positive responsiveness at any age (in comparison to control stimuli). While not obviously attractive to the infants, these pleasant odours were however not repulsive, as indicated by the fact they did not elicit decreased mouthing responses, with one notable exception (vanilla) that will be developed below. Thus, in the present experimental conditions, most odours that are pleasant for adults appeared to be treated by 8- to 22-month-old infants as hedonically neutral (that is, not different from the control stimuli). It is worth noting that our study was carried out when the infants were not hungry, at least as reported by their mothers, and deduced from the time of the last feed prior to the test. Thus, their motivation to investigate food-related stimuli may not be maximal, and even more so as these stimuli were presented by the means of bottles. It cannot be excluded that the hedonic responses to the pleasant food odorants might have been exacerbated if infants had been in hunger state, but this is a point of future enquiry. As expected, participants' hedonic responses indicated avoidance for most of the unpleasant odorants tested here, and this might reflect the dislike ratings for these odours by the adult panel. However, the participants' hedonic responses were clearly not aligned with those of adults for the pleasant odours. Multiple explanations can be advanced to figure out this asymmetric hedonic response pattern of infants to the present set of stimuli.

First, although previous studies showed that neonates and children older than 3 years of age express an adult-like pattern of olfactory preferences [8,11], their results must be carefully examined. In Steiner's work assessing neonates' facial responses while exposing them to highly concentrated odour stimuli, the most unambiguous negative facial actions were released by the stimuli that were unpleasant to adults. The neonates' facial responses to the pleasant odour were not as clear-cut, and, accordingly, the corresponding between-observer agreement was medium to low. For example, the infant's facial responses to the fruity odour (banana) was rated as expressing acceptance, rejection, and indifference in 55, 20 and in 25% of the participants, respectively; similarly, 46% acceptance, 46% indifference and 8% rejection ratings were assigned to infants' facial reactions elicited by the vanilla odour. Thus, infants and adults did not appear to attribute equivalent hedonic value to odours,

and this is clearer on the side of odours considered pleasant to adults. Steiner [8] himself noted, but without further elaboration, a difference in neonates' responses to pleasant versus unpleasant odours in terms of hedonic clarity of their facial reactions ('...the appearance and the course of the reaction to "pleasant odours" was more hesitant or sluggish [than those to "unpleasant odours"]'; p. 274). A later study on neonatal hedonic responses using highly diluted, intensity-matched pleasant and unpleasant odour stimuli supported the notion that neonates do not appraise odour hedonics as adults do, in that they react positively to some odours that adults find aversive, and conversely [7]. Finally, although they demonstrated an overall higher convergence between children and adults, studies on older participants also highlighted age-related discrepant hedonic responses to pleasant odours, while unpleasant odours generated more unanimous responses. For example, in Schmidt and Beauchamp's study [11] in 31- to 38-month-old infants, the participants responded differently from adults to odours among both pleasant and unpleasant representatives in the odour series. Thus, the results of the present study, not only corroborate previous studies in younger and older participants in showing different hedonic evaluation of odours by infants and adults, but they highlight that this age-dependent difference is more pronounced for odours that are not aversive to adults. In other words, food odours that are unpleasant to adults - at least those tested in our study - can be predicted with some reliability as also unpleasant for infants, while it is more difficult to predict how infants will perceive food odours that are pleasant to adults. A possible sensory basis of the differential responses induced in infants by the pleasant versus unpleasant odours in the present study will be further developed below.

A second explanation of the asymmetry in hedonic responses to pleasant/unpleasant odours may be related to the design of the present study, which might have accentuated contrasts between the stimuli presented within a same triplet. The within-triplet presentation order of the stimuli was intended to limit the infants' loss of compliance and attention, so unpleasant stimuli were systematically administered last (first, scentless control; second, pleasant odour; third, unpleasant odour; see Methods section). In this way, we could have created contrast effects (that is, control-pleasant and pleasant-unpleasant), as well as affective carry-over effects from the pleasant odour on the unpleasant odour. Thus, control-pleasant contrasts might have increased the sensory salience of pleasant odours, while pleasant-unpleasant contrasts might have either magnified perception of unpleasant odours due to a quality contrast, or attenuated it due to a carry-over effect of pleasant appraisals onto unpleasant appraisals. As these effects were not systematically manipulated so that all contrasts are

represented, any final statement is unwarranted. What can be noted, however, is that the control-pleasant contrasts did not enhance the infants' attraction as indexed by the mouthing response to the stimulus bottles containing the pleasant odours. Regarding the pleasant-unpleasant odour contrasts, it cannot be decided whether they magnified or attenuated avoidance responses to unpleasant stimuli, but such avoidance responses were high anyway.

It can also be suggested that the consecutive presentation of stimuli can lead to a boredom effect magnifying avoidance responses to unpleasant odours. These stimuli were always presented third and last in the sequence, and are compared to the controls, which were presented first. If a systematic boredom effect had occurred, the scores calculated for the unpleasant odours would have been significantly lower than 0.5. However, the present results did not systematically indicate differences between control and unpleasant stimuli (scores are significantly lower than 0.5 for trimethylamine and dimethyl disulphide at 12 months, and for dimethyl disulphide and butyric acid at 22 months). Thus, the avoidance responses observed towards the unpleasant odours mentioned above are more likely due to the perception of hedonic valence than to a potential boredom effect.

A third explanation of the asymmetry in hedonic responses to pleasant/unpleasant odours may be that the pleasant stimuli were unfamiliar, whereas the unpleasant stimuli were both unfamiliar and conveyed trigeminal potency. Several studies showed indeed that unfamiliar odours are treated as either hedonically neutral [23] or aversive [10]. In our conditions, the stimuli considered pleasant evoked neither attraction, nor avoidance responses (with the exception of vanilla; see below) in 8-, 12- and 22-month-old infants. Regarding unpleasant stimuli, their unfamiliar quality is obviously confounded with irritant properties as reported by adults (see below, Methods section). Thus, the infants' avoidance reaction towards unpleasant odours could be explained in part by the trigeminal component of the odours. This hypothesis is backed by adult data on these odours, showing that irritation ratings and pleasantness ratings are negatively correlated ( $\tau = -0.40$ ,  $P < 0.001$ ). However, trigeminal side effects do not explain avoidance responses to all odours. For example, whereas the odours of strawberry and butyric acid did not differ significantly in terms of irritation ratings by adults, strawberry odour did not induce avoidance while butyric acid odour did. Finally, vanillin elicited avoidance behaviour (reduced mouthing at 22 months), despite the fact that this compound is typically regarded devoid of trigeminal properties [24,25], and was the least irritating in the present odour series. Thus, the negative impact of unpleasant odours in our study cannot be exclusively attributed to confounded trigeminal features.

Although the various explanations offered above may have contributed separately or in combination to the present pattern of findings (that is, an asymmetry in hedonic responses to pleasant/unpleasant odours), our data cannot fully tell them apart. Nonetheless, the main results of a differential hedonic responsiveness of 8- to 22-month-old infants to pleasant and unpleasant odours are in line with studies conducted on earlier and later ages (see references in the Introduction). Taking the present findings together with earlier published data, it may be generally concluded that infants and children appear to be more reliable in their negative responses than in their positive responses to odours. For example, while the facial actions expressing disgust do accurately differentiate butyric acid from vanilla odours, those expressing smiles are not discriminant [7,26]. In sum, during early development, odour-related hedonic processes may be better integrated on the negative pole than on the positive pole of the hedonic space [27].

The finding on vanilla odour was unexpected: despite vanilla being rated as highly pleasant by adults, it induced avoidance in 22-month-old infants. Vanilla odour is assumed to be one of the most familiar odorants in the present stimulus series as it is a regular aroma component of formula milk and infant foods. Two processes can be proposed to explain infants' avoidance of this particular odorant in the present conditions. First, it is known from previous infant studies that frequent and/or recent exposures to a specific flavour lead to a boredom effect, thus altering an infant's responsiveness to it [28,29]. For example, an increase in acceptance for carrot-flavoured cereals after exposure to carrot flavour through mother's milk was noted when the delay between last exposure and acceptance testing was from 4 to 6 months [30], but not when it was only 3 days [28]. Second, an alliesthesia effect may have operated, infants responding rather negatively to odours and flavours that dominated in their food. Satiation-related factors were indeed shown to reduce liking of food odour in neonates [31] and older children [32], and there is no reason why such motivational factors should not also affect infants of intermediate ages although age differences in alliesthesia effects were shown in later development [32]. Finally, and in line with the previous effect, it cannot be excluded that the test-bottle used in the present study could be reminiscent of the bottle from which the infants drank beverages. Since most formula milk for older infants are vanilla-flavoured, infants may have expected a reward when presented a vanilla-scented bottle. This expectation not being satisfied in the test, infants may have exhibited less mouthing.

This study assessed the development of hedonic responses to odours at three time points in the first 2 years of life. When considering the 8 odours separately,

no significant difference in mouthing score was noted at 8 months, whereas two significant differences were observed at 12 months (for trimethylamine and dimethyl disulphide). Finally, three significant differences were observed at 22 months (dimethyl disulphide, butyric acid and vanillin). All but one of these differential odour-based mouthing responses concerned unpleasant odours. One could argue that infants might exhibit increasingly sharper avoidance behaviour when they grow older. The progressive emergence of neophobia [17] could explain this behavioural change.

As regards the correlations between mouthing scores for the same odorant at two different ages, some significant correlations were noted only for unpleasant odours. Moreover, if we look at the individual correlations calculated between ages, only a few were significant (about 6% of all correlations tested). Thus, very few infants display the same pattern of mouthing behaviour towards the odours between two different age points. These results suggest both inter- and intra-individual differences in the development of the hedonic perception of the odours. Given that the organization of the human olfactory epithelium may reflect key dimensions of olfactory perception (odorant pleasantness) [33], one may think that this organization is stable and inflexible. Nevertheless, this mapping of odour perception is malleable by context and experience [33]. Thus, either positive or negative context of previous exposures can contribute to the uniqueness of each individual's development of the hedonic appraisal of odours or flavours [34,35]. Alternatively, the emergence of food neophobia could also explain individual variability in the development of hedonic perception of odours. This phenomenon could happen more or less early depending on infants, and its strength could differ as a function of an infant's temperament [17,36]. Individual variability from one age to the other suggests plasticity of olfactory responses across time, which is particularly important in the formation of positive responsiveness to odours. This assumption is backed by a follow up study which indicated a significant increase in liking of food odours between the ages of 3 and 5 years [12]. By contrast, the present results indicate that infants' responses to the unpleasant odours are partially stable across ages. Moreover, the follow-up study mentioned above on 3- to 5-year-olds showed that there is no significant change of dislike for odours classified as toxic [12]. It seems that odours related to potential toxic or harmful foods are considered as unpleasant - and are actually avoided in laboratory studies - early in life, and remain unpleasant and avoided when infants grow up (at least when presented only as chemosensory stimuli). This response might constitute an olfactory alarm system protecting against potentially toxic food. Finally, it has been shown that 6- to 12-year-old children from

different ethnic backgrounds (French Canadians, Sudanese Indonesian, and Syrian) agreed on the odours they judged as being unpleasant but not on those judged as being pleasant [37], highlighting the relative consensus of children's responses towards unpleasant odours relative to pleasant ones.

#### Gender effect

No gender effect reached significance concerning differential mouthing responses between pleasant and unpleasant odours. Thus, the present result supports the studies in olfactory development that do not report any gender difference [10]. As semantic representations were shown to already influence olfactory perception in young children [38], and as female individuals early outperform male individuals in olfactory identification tasks [20], gender differences in olfaction might appear mostly when verbal abilities reach some maturity.

#### Breast feeding effect

No breast feeding effect was noted on the mouthing behaviours studied at 8 months. This result raises two hypotheses. Either breast-feeding has no effect on olfactory responses from the age of 8 months, or complementary feeding already well engaged at 8 months has equalized flavour and odour experience in breast-fed and bottle-fed infants. Consequently, complementary feeding may have masked the effects of breast feeding. This last hypothesis is in line with a previous finding showing that breast-fed infants express higher initial acceptance of a novel flavour than bottle-fed infants, and that this difference disappears after repeated exposure to that flavour [39].

#### Conclusions

The present study longitudinally assessed the hedonic responses of infants aged 8, 12 and 22 months to odour stimuli chosen to represent typical local foods that are pleasant and unpleasant to adults. The infants' hedonic responsiveness to the distinct odorants was discriminative between these stimuli, but they were more obvious toward the unpleasant odours. Some correlations reached significance between age points, but they were noted only for a few unpleasant odours, suggesting that, in the first two years of life, olfactory preferences undergo a phase of developmental plasticity. During this extended period of early life when infants shift from lacteal to solid foods carrying diverse qualities, their likes/dislikes for odours are certainly fine-tuned by exposure and learning effects in the feeding context. Nevertheless, from the earliest age point, infants also manifested avoidance responses that appeared to be stable across ages, suggesting a pattern of early olfactory responsiveness that is plastic on the pleasant side and both

predisposed and plastic on the unpleasant side of the perceptual space.

#### Methods

##### Context and ethical conditions

The present data were collected in the context of a longitudinal investigation of food preferences from birth up to 2 years of age within an Observatory of Food Preferences in Infants and Children (*Observatoire des Préférences Alimentaires du Nourisson et de l'Enfant*, OPALINE). Participating mothers were recruited before the last trimester of pregnancy, using leaflets and posters affixed in health professionals' practices and in day-care centres. To be included in the cohort, both parents had to have reached 18 years of age (legal majority), and infants had to be in good health. The aims and methods of the study were explained to both parents in great detail. For the part of the programme intended to investigate longitudinal changes in infants' reactions to food odours, the parents were extensively informed about the methods and timing of the olfactory tests. Written informed consent was obtained from the parents to bring their infant to the laboratory when she or he was 8, 12 and 22 months of age ( $\pm 2$  weeks) to participate in olfactory testing. The study was conducted according to the Declaration of Helsinki, and was approved by the local ethical committee (*Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Bourgogne*).

##### Participants

The infants ( $n = 235$ , 112 girls and 123 boys) participated in the experiment at each time point, at about 8, 12, and 22 months (mean age  $\pm$  SD  $239 \pm 13$  days,  $372 \pm 12$  days, and  $670 \pm 10$  days, respectively). They were born without medical complications, with an average birth weight of  $3.30 \pm 0.48$  kg. At the time of the visits to the laboratory, they were in optimal health, did not present any eating disorders or oro-nasal infection or allergies, and had all begun complementary feeding (on average at  $167.3 \pm 32.6$  days of age). Among the participants, 89 and 11% of the participants were breast- and bottle-fed at birth, respectively, and at the 8-, 12- and 22-month visit, 23, 10, and 4% of the infants were still partly breast-fed, respectively.

##### Stimuli

Eight odorants representing diverse foods were used (Table 2). These stimuli were selected to form a set comprising four odours that were considered a priori pleasant (apple, peach/apricot, strawberry and vanillin) and four odours that were considered a priori unpleasant (dimethyl disulphide, trimethylamine, butyric acid and 2-isobutyl-3-methoxypyrazine). The rationale for choosing these odour qualities is that they represent foodstuffs

**Table 2 Characteristics of odorants**

A priori pleasant odours			A priori unpleasant odours		
Odorants	Associated foods	Concentrations	Odorants	Associated foods	Concentrations
Apple <sup>c</sup> (mixture)	Apple	0.6 mL/L <sup>a</sup>	Dimethyl disulphide <sup>d</sup>	Garlic, cruciferous	0.075 mL/L <sup>b</sup>
Strawberry <sup>d</sup> (mixture)	Strawberry	0.7 mL/L <sup>b</sup>	Trimethylamine <sup>d</sup>	Fish	0.025 mL/L <sup>a</sup>
Peach/apricot <sup>e</sup> (mixture)	Peach/apricot	6 mL/L <sup>a</sup>	Butyric acid <sup>f</sup>	Cheese, rancid butter	0.0025 mL/L <sup>b</sup>
Vanillin <sup>f</sup>	Vanilla	1 g/L <sup>a</sup>	2-isobutyl-3-methoxypyrazine <sup>f</sup>	Green vegetables	0.0005 mL/L <sup>b</sup>

<sup>a</sup>diluted in water (Evian, France); <sup>b</sup>diluted in mineral oil (Sigma-Aldrich, Saint Quentin Fallavier, France); <sup>c</sup>provided by Firmenich, Geneva, Switzerland; <sup>d</sup>provided by Symrise, Clichy la Garenne, France; <sup>e</sup>provided by IFF, Dijon, France; <sup>f</sup>bought from Sigma-Aldrich, Saint Quentin Fallavier, France.

that evoke contrastive liking responses in young participants. A large proportion of French children have declared to strongly like strawberries (85.4%), apricots (68.5%), and apples (67.3%), and quite a large proportion declared to dislike garlic (35.8%), strong cheese (30.7%), and green pepper (25.2%) [40]. Further, mature cheese and fish were scarcely chosen by infants in a free-choice situation (11 and 9%, respectively) [41], and fish odour is generally known to be rejected in young children [42] and neonates [8]. Butyric acid and vanillin were chosen since previous studies showed contrasted hedonic responses in infants and young children [7,43]. In the present study, four odours were thus associated with foods generally liked by children, and four odours were associated with foods quite often disliked. The control stimuli consisted of mineral oil.

The providers of the odorants, their dilution grade and solvents are given in Table 2. Each stimulus was presented in nipple-less, transparent infant-ergonomic bottles (12 × 6 cm, opening diameter of 2.3 cm; Tex, Carrefour, France). Odorant solutions (10 ml) were soaked in a scentless absorbent (3 M, Lièges, Belgium), a strip of which (11 × 5 cm) was placed in the bottom of the bottles to optimize evaporation and avoid spilling. During the tests, no visual differences between the control and odorized bottles were accessible to the infant or the mother.

The hedonic valence, subjective intensity, irritation from the eight odorants, or their typicality to represent a given foodstuff was checked by an adult panel. Naïve and non-smoking participants (n = 35, 22 women and 13 men, mean age ± SD 34.5 ± 7.7 years, range 19 to 48 years) devoid of respiratory allergies and/or nasal pathologies were asked to rate pleasantness, intensity, irritation and food typicality of the eight odorants on four different visual analogue scales ranging from 'highly unpleasant/not at all intense/not at all irritating/not at all typical' to 'highly pleasant/very intense/very irritating/very typical'. The responses were converted into scores varying from 0 to 10. To mimic infants who do not spontaneously express sniffing [11], the panellists were asked to smell by merely inhaling the odours. The presentation order of the odorants was balanced between

subjects, with a 1-minute inter-stimulus time. As expected, the odorants were clumped by the adults into two categories (Table 3), one pleasant (that is, apple, peach/apricot, strawberry and vanillin) and another unpleasant (that is, 2-isobutyl-3-methoxypyrazine, butyric acid, dimethyl disulphide and trimethylamine). The control stimulus was rated hedonically neutral. All odorants were rated as equivalently intense, except vanillin and butyric acid, which were rated as significantly less intense, with vanillin rated as less intense than butyric acid. The stimuli were different regarding ratings of irritation. All unpleasant stimuli except butyric acid were rated as significantly more irritant than the pleasant stimuli. Finally, all odorants were judged to be typical of their associated foodstuff.

#### Procedure

The experiment took place in a quiet, ventilated room especially dedicated to run experiments with young participants. All tests were completed in the presence of one parent, usually the mother. To control the infants' hunger state, parents were asked not to feed them for at least 1.5 hours before the test session. Compliance with this instruction was checked before the test by asking when the infant's last meal had occurred and was confirmed. Parents were also asked not to apply any scented care products on their infant or on themselves the day of the test, and not to disturb the infants' sleeping rhythm.

To accustom the participants to the experimental room and to the experimenters, a familiarization phase took place before the test itself. The 8- and 12-month-olds were seated on their parent's lap, whereas the 22-month-olds were seated in a baby-seat next to the parent. All participants were seated facing a remote-controlled video camera placed unhidden at a distance of 3.5 meters (no experimenter was operating the camera in front of the participants). A white game board (45 × 25 cm) was placed on the table in front of them to delineate the area of exploration. Parents were asked not to interact with the infant during the test, and not to handle the bottles. The test was introduced to the infant as the "game of odours". In an attempt to

**Table 3 Mean ± standard error of pleasantness, intensity, irritation and typicality for each odorant rated by an adult panel on continuous scales of 0 to 10**

Odorants	Odour source	Pleasantness	Intensity	Irritation	Typicality
Strawberry	Strawberry	8.69 ± 1.46 <sup>a</sup>	7.55 ± 1.72 <sup>a</sup>	2.65 ± 2.99 <sup>b</sup>	8.38 ± 2.20
Peach/apricot	Peach/apricot	8.17 ± 1.86 <sup>ab</sup>	7.61 ± 2.11 <sup>a</sup>	2.01 ± 2.53 <sup>bc</sup>	8.28 ± 2.03
Apple	Apple	8.08 ± 1.92 <sup>ab</sup>	7.61 ± 2.09 <sup>a</sup>	2.19 ± 2.86 <sup>bc</sup>	7.84 ± 2.37
Vanillin	Vanilla	7.34 ± 1.24 <sup>b</sup>	1.63 ± 2.08 <sup>c</sup>	0.59 ± 1.21 <sup>c</sup>	6.11 ± 3.37
2-isobutyl-3-methoxypyrazine	Green vegetables	2.85 ± 2.08 <sup>d</sup>	6.40 ± 2.56 <sup>a</sup>	5.12 ± 3.24 <sup>a</sup>	7.14 ± 2.80
Dimethyl disulphide	Cruciferous or bulb vegetables	2.17 ± 2.09 <sup>de</sup>	6.75 ± 2.97 <sup>a</sup>	6.27 ± 3.09 <sup>a</sup>	6.91 ± 2.93
Butyric acid	Cheese	2.11 ± 1.96 <sup>de</sup>	5.17 ± 3.37 <sup>b</sup>	3.57 ± 3.22 <sup>b</sup>	7.87 ± 2.31
Trimethylamine	Fish	1.22 ± 1.54 <sup>e</sup>	7.79 ± 2.23 <sup>a</sup>	5.48 ± 3.62 <sup>a</sup>	7.84 ± 3.06
Mineral oil	Scentless	4.44 ± 1.21 <sup>c</sup>	0.86 ± 1.04 <sup>c</sup>	0.92 ± 1.33 <sup>c</sup>	-

Typicality scoring refers to the name of odour sources. Participants were asked to rate how odorant is typical of odour source. For Pleasantness, Intensity and Irritation, values with different letters are significantly different according to Newman-Keuls test ( $P < 0.05$ ). Examples of cruciferous vegetables are cabbage and cauliflower; examples of bulb vegetables are garlic, onion and shallot.

control and standardize parent-infant interactions during the tests themselves, a first experimenter questioned the parent about domestic habits involving smell (data not shown), while a second experimenter handed the bottles to the infant. The odorized and control bottles were presented one by one in sequences of three bottles: a control stimulus, followed by a pleasant odour, and an unpleasant odour. This order of presentation was chosen to avoid the infant refusing to pursue the test after smelling an unpleasant odour first (as was noted in previous studies [11] and in our own pilot tests). To limit the number of stimuli, and, hence, session duration, no control stimulus was included between pleasant and unpleasant odours. A typical test session included four sequences, that is, four stimulus triplets (each composed of one control stimulus, one pleasant and one unpleasant stimuli). The presentation order of these stimulus triplets was balanced between subjects (Figure 2), but was maintained constant within

subjects across the three ages. The following instructions were given to the participants: 'Here, [name of the infant], I give you this bottle and you can do anything you want with it'. The experimenter presented the bottle under the nose of the infant during 5 s to cover several breathing cycles, placed it in front of her/him, and let her/him free to investigate the bottle during 60 s at 8 and 12 months. Preliminary tests revealed that signs of disinterest for the test were expressed more rapidly in 22-month-old infants than at the other ages. Thus, the duration of stimulus presentation was shortened to 30 s at this age. At the end of each odour presentation, the bottle was gently removed by the experimenter, and the next bottle was presented approximately 15 s later. A break varying from 5 to 15 minutes was managed after the presentation of the first two stimulus triplets. If the infant looked tired, angry or bored with testing, the session was ended after the presentation of two triplets, and the parent was asked to bring the infant

Order A				
Sequence 1	Sequence 2	Break	Sequence 3	Sequence 4
C1 PEA TRI	/ C2 APP PYR	//	C3 VAN DIM	/ C4 STR BUT
Order B				
Sequence 1	Sequence 2	Break	Sequence 3	Sequence 4
C3 VAN DIM	/ C4 STR BUT	//	C1 PEA TRI	/ C2 APP PYR
Order C				
Sequence 1	Sequence 2	Break	Sequence 3	Sequence 4
C2 APP PYR	/ C3 VAN DIM	//	C4 STR BUT	/ C1 PEA TRI
Order D				
Sequence 1	Sequence 2	Break	Sequence 3	Sequence 4
C4 STR BUT	/ C1 PEA TRI	//	C2 APP PYR	/ C3 VAN DIM

**Figure 2 Presentation orders of odours.** A sequence is composed of three odours (control, a priori pleasant odour and a priori unpleasant odour). C, control. A priori pleasant odours were apple (APP), peach/apricot (PEA), strawberry (STR) and vanillin (VAN). A priori unpleasant odours were butyric acid (BUT), dimethyl disulphide (DIM), 2-isobutyl-3-methoxypyrazine (PYR) and trimethylamine (TRI).

again on another day (within a maximum of two weeks) to complete the test (13, 39, and 25% of the infants had to come twice to the laboratory at the ages of 8, 12 and 22 months, respectively). However, in some cases the impossibility of return within this delay period led to missing values (1, 2, and 7% of missing values at 8, 12 and 22 months respectively).

### Behavioural variables

The test sessions were video recorded to be later analysed frame-by-frame using the Observer software (Noldus, Wageningen, The Netherlands) to measure the duration of selected behaviours of infants toward the test bottles. Four variables were defined including: 1) handling, defined as any manual contact with the bottle using one or both hands (unless if mouthing occurred simultaneously; see below); 2) mouthing the bottle top (near the odour source), defined as direct contact between the infant's perioral and/or perinasal areas with the opening of the bottle (regardless of co-occurring handling; see below); 3) mouthing another part of the bottle, defined as direct contact between the infant's perioral and/or perinasal areas with any part of the bottle except the top (regardless of co-occurring handling; see below); and 4) no handling, defined as the absence of any physical (manual and oral) contact of the infant with the bottle. To render the different variables exclusive in the analytic scheme, mouthing actions were coded as mouthing only, despite the fact that infants were then also unavoidably handling the bottle. The coding of these behavioural variables was run by trained observers who were blind to the identity of the stimulus. Ten video sequences were randomly selected to check inter-observer reliability. The average percentage of agreement was  $>0.90$  for the durations of the selected behaviour responses.

Preliminary analyses indicated that mouthing directed to any other part of the bottle than the top decreased with age (that is, 20, 13 and 4% of the participants responded this way for half or more of the stimuli at 8, 12 and 22 months, respectively), while mouthing the top of bottle remained relatively stable and frequent (that is, 73, 72 and 78% displayed it for half or more of the stimuli at 8, 12 and 22 months, respectively). Thus, we decided to focus on the duration of mouthing directed to the top of the bottles. Handling, mouthing and no handling responses were previously used as variables to characterize infants' proximal behaviour with objects for example, as previously published [10]. For example, mouthing was reported by Delaunay-El Allam *et al.* [23], as being a most privileged mode of positive object exploration in infants aged 6 to 23 months based on the fact that these infants mouthed an object carrying a familiar odourant more than a visually similar object carrying an unfamiliar odourant. Moreover, mouthing is

related to other behavioural indicators highlighting infants' hedonic appreciation of odourants. For example, there is evidence for a link between mouthing and facial emotion expressions. Unpleasant odours that elicit negative facial expression also induce less mouthing movement than pleasant odours [7]. In our experimental design, it was not possible to precisely analyse the infants' facial expressions, as when infants handled and mouthed the bottle the bottle and infants' hand masked the mouth region. Mouthing can also be linked to stimulus seeking. For instance, infants respond by both increased head orientation and mouthing activation to human milk odour [44]. Moreover, a relationship between mouthing and familiarity has been established by Mennella and Beauchamp (1988) [45], and it is otherwise known that familiarity often correlates with pleasantness [38,46,47]. To sum up, mouthing appears to be related to three indicators of pleasantness and attraction (facial expressions, stimulus seeking, and familiarity), and we used it here as a reliable indicator of hedonic discrimination in young infants. As regards the modes of expressing negative or avoidant tendencies in their behaviour, infant studies have often focused on responses involving no handling of the target stimuli [10]. Initially, we intended to contrast the infants' responses in two opposite trends: on the one hand, mouthing considered as an index of interest and attraction and, on the other hand, no handling considered as an index of disinterest or avoidance. However, as the no handling response might also be considered as expressing an absence of noticeable response, it does not necessarily demonstrate avoidance. Taking this last possibility into account, all the present analyses were focused on the durations of mouthing as indicative of the participants' tendencies to explore the odour conveyed in the bottle.

If infants dropped the bottle on the floor, so that the bottle was then inaccessible for a while, we computed a duration of stimulus accessibility (accessibility duration = fixed duration of the test (that is, 60 s at 8 and 12 months, and 30 s at 22 months) minus duration of inaccessibility) for each test. Then, the durations of mouthing were divided by the duration of accessibility to obtain proportional durations of mouthing (called thereafter mouthing).

For each odourant, duration data were then transformed into mouthing scores defined as the proportion of time during which a target bottle was mouthed relatively to the added proportions of time this bottle and the matched control bottle were mouthed. For example, the mouthing score for the apple bottle was equal to proportion of mouthing duration to the apple bottle / (proportion of mouthing duration to apple odourant + proportion of mouthing duration to the control bottle). Mouthing scores equal to 0.5 indicate the same duration

of response to a given odorant bottle and the control bottle, and are interpreted as expressing indifference to the odour. A ratio  $>0.5$  indicates attraction, while a mouthing score  $<0.5$  indicates avoidance of the odour relative to the control. Thus, for each infant and at each age, eight mouthing scores (four for pleasant odours and four for unpleasant odours) were calculated.

### Statistical analyses

At each age, individual median scores for pleasant and for unpleasant odours were calculated. Then, a paired Wilcoxon test was performed at each age to test whether the median scores were significantly different in terms of hedonic valence of the odours. Moreover, for each odour, Wilcoxon tests were used to assess whether the score was different from the 0.5 level of neutrality. For each odour, Kolmogorov-Smirnov tests were performed to compare the distributions of scores at two consecutive age points. Kendall correlations were computed to assess whether the scores for a given odour at two age points were correlated (unilateral tests). Kendall correlations were also performed to assess whether the individual scores (for all odours) at two age points were correlated (unilateral tests). Moreover, for each age point, Wilcoxon tests were performed to assess the effect of gender on the differences between individual median scores for pleasant odours and individual median scores for unpleasant odours. Finally, Wilcoxon tests were performed to assess the effect of past and present breast feeding at 8 months on the differences between individual median scores for pleasant and individual median scores for unpleasant odours. Since very few infants were still breast fed at 12 and 22 months, analyses were not performed for these two age points.

All statistical analyses were carried out using the R software (version R2.11.1; Vienna, Austria) [48]. Results are reported as statistically significant if  $P < 0.05$ , and as marginally significant if  $P < 0.10$ .

### Competing interests

The authors declare that they have no competing interests. The OPALINE project was sponsored by both public and corporate funding, but these instances did in no way interfere with the tested hypotheses, methods, presentation and interpretation of results.

### Authors' contributions

SMP, SI, BS and LM designed the study. SMP and SW coded behaviour. CC and SW performed data analysis. SW, SI, SMP, CC, BS and LM were involved in writing the paper. All authors read and approved the final manuscript.

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