J Chem Ecol (2017) 43:106–117
DOI 10.1007/s10886-016-0804-x

Responses of Human Neonates to Highly Diluted Odorants from Sweat

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Received: 8 April 2016 / Revised: 16 October 2016 / Accepted: 15 December 2016 / Published online: 6 January 2017

Abstract Conjugated forms of odorants contributing to sweat odor occur not only in human sweat but also in amniotic fluid, colostrum, and milk. However, it is unclear whether the released odorants are detected and hedonically discriminated by human newborns. To investigate this issue, we administered highly diluted solutions of (*R*)/(*S*)-3-methyl-3-sulfanylhexan-1-ol (MSH), (*R*)/(*S*)-3-sulfanylhexan-1-ol (SH), (*E*)/(*Z*)-3-methylhex-2-enoic acid (3M2H), and (*R*)/(*S*)-3-hydroxy-3-methylhexanoic acid (HMHA) to 3-d-old infants while their respiratory rate and oro-facial movements were recorded. Adult sensitivity to these odorants was assessed via triangle tests. Whereas no neonatal stimulus-specific response was found for respiratory rate, oro-facial reactivity indicated orthonasal detection of MSH and SH by male neonates, and of HMHA by the whole group of neonates. Dependent on the dilution of odorants, newborns evinced neutral responses or longer negative oro-facial expressions compared with the reference stimuli. Finally, newborns appeared to be more sensitive to the target odorants than did adults.

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Keywords Olfaction · Human newborn · 3-methyl-2-hexenoic acid · 3-hydroxy-3-methylhexanoic acid · 3-sulfanyl-1-hexanol · 3-methyl-3-sulfanylhexan-1-ol

Introduction

Release of volatile organic compounds (VOCs) by living organisms is non-random temporally (e.g., Dufa et al. 2004; Pause et al. 2004; Steingass et al. 2014; Turlings et al. 1990), and hence is eligible to transmit time-triggered chemosensory cues. In plants, an event-related release of VOCs is, among others, afforded by conjugates, which set free VOCs when adequate enzymatic activity is present, e.g., upon cell damage, bringing together substrates and enzymes from different cellular compartments. A well-known example is in the genus *Allium* with its S-alk(en)yl-L-cysteine sulfoxides and γ-glutamyl-cysteine-S-conjugates (Block 1992; Starkenmann et al. 2011), although occurrence of cysteine-S-conjugates has been described more generally [e.g., *Vitis* (Tominaga et al. 1998); *Capsicum* (Starkenmann and Niclass 2011); *Passiflora* (Fedrizzi et al. 2012)].

Mammals also use conjugates as one of manifold ways (c.f., Flower 1996) to store or transport small hydrophobic molecules throughout the organism, and these conjugates may be cleaved to release volatile cues (e.g., Brewington et al. 1973; Fabregat et al. 2013; Lopez and Lindsay 1993; Starkenmann et al. 2014; Wagenstaller and Buettner 2013). For instance, in human sweat, conjugates provide an odor release that gains variability, not only related to an individual's secretory characteristics but also to an individual's axillary microbiota, and these factors can be interdependent (Harker et al. 2014; Kuhn and Natsch 2009; Leyden et al. 1981; Martin et al. 2010; Troccaz et al. 2004). Indeed, human sweat odor has been shown to emerge from originally odorless apocrine

sweat upon enzymatic cleavage of cysteinylglycine-S-conjugates, glutamine-N-α-conjugates, and glucuronides (Natsch et al. 2003, 2006; Shelley et al. 1953; Starkenmann et al. 2005, 2013). To date, however, it remains disputed whether these conjugates are formed during (from glutathionyl conjugates transported by ABCC11; Baumann et al. 2014) or after the apocrine gland secretion process (via opening of odorant-carrying proteins and enzymatic action; Preti and Leyden 2010; Zeng et al. 1996). Odorant-carrying proteins have also been suggested to occur in sweat from other regions (e.g., areolar sweat; Spielman et al. 1995). In contrast, part of the afore-mentioned conjugates, amongst others N-α-3-hydroxy-3-methylhexanoyl-L-glutamine and N-α-3-methylhex-2-enoyl-L-glutamine, are thought to be specific to axillary sweat since they have not been detected in sweat originating from other body regions, nor in plasma or urine (Natsch et al. 2006). However, this notion is challenged by the detection of the above-mentioned glutamine-N-α -conjugates in human amniotic fluid, colostrum, and milk (Hartmann et al. 2012). Further, cysteinylglycine-S-conjugates of 3-methyl-3-sulfanylhexan-1-ol and 3-sulfanylhexan-1-ol occur in human colostrum and milk (Hartmann et al. 2012). Thus, perinatal fluids appear to contain conjugated forms of odorants known to contribute to sweat odor. Yet, while efforts have been made to understand conditions, temporal dynamics, and communicative value of odor formed in the axilla (reviewed by James et al. 2013), it is unknown whether conjugates occurring in amniotic fluid and milk release odorants into a newborn's environment, and whether these odorants are involved in interpersonal communication.

In adults, cysteine-S-conjugates are cleaved by oral microbiota, leading to a delayed but prolonged olfactory percept compared with consumption of the free odorant itself (Starkenmann et al. 2008). An analogous, temporally defined, odor release during mother-infant interaction would offer opportunities for odor perception in the "perinate" (i.e., the transitional organism 2–3 d before, during, and 2–3 d after birth), especially when occurring concurrently with states of high neonatal learning susceptibility. Highly efficient odor acquisition is thought to happen during and/or shortly after birth due to high neonatal brain norepinephrine levels caused by the effect of uterine contractions on an infant's skull (Alberts and Ronca 2012; Varendi et al. 2002). In fact, 30 min exposure to cherry or mango odor leads neonates to prefer these odors when exposure occurs during the first hour after delivery, but not when it occurs 12 h after delivery (Romantshik et al. 2007). Further, breastfeeding offers highly reinforcing conditions for odor learning, generating odor preferences persisting as long as weeks (Schleidt and Genzel 1990) or months (Delaunay-El Allam et al. 2010). During both delivery and breastfeeding, enzymes able to cleave the aforementioned amino acid conjugates might be provided by a mother's or a newborn's microbiota, which occur within the amnion (e.g., DiGiulio et al. 2008), vagina (e.g., Tannock et al. 1990), milk and breast (e.g., Urbaniak et al. 2014), and in the mouth and skin (e.g., Rotimi and Duerden 1981).

From the above evidence, we hypothesized human perinates familiarize themselves with human sweat odorants through postnatal, and possibly perinatal, exposure. Whereas maternal sweat odor has been shown to be acquired by 12–18-d-old breast-fed newborns (Cernoch and Porter 1985), so far no studies have investigated neonatal responses to pure sweat odorants. Neonatal perception of these compounds is not only highly interesting in view of a possible chemocommunicative function of these odorants in perinatal fluids, such as colostrum and milk (e.g., Macfarlane 1975; Marlier and Schaal 2005; Russell 1976; Schaal 2005, 2015) but, also, in view of developmental aspects of human sweat odor perception: even if several authors have investigated pleasantness/unpleasantness of axillary and body odors [(e.g., adults (Doty et al. 1978; Troccaz et al. 2009), children (Ferdenzi et al. 2010)], the molecular bases of human sweat odor hedonicity are not yet completely understood.

In regard to this line of thought, we examined neonatal responses to (*R*)/(*S*)-3-methyl-3-sulfanylhexan-1-ol (MSH), (*R*)/(*S*)-3-sulfanylhexan-1-ol (SH), (*E*)/(*Z*)-3-methylhex-2-enoic acid (3M2H), and (*R*)/(*S*)-3-hydroxy-3-methylhexanoic acid (HMHA). Neonatal detection of and hedonic response to these odorants were assessed by administering aqueous solutions to 3-d-old human infants. Further, detection thresholds of adults were obtained by using the same presentation technique but with a different response measure, so as to compare adult and neonatal sensitivity to these odorants. These odorants were chosen because they: i) are impactful sweat odor contributors, ii) have, so far, not been detected in animal milk, and iii) might result from enzymatic cleavage of conjugates detected in amniotic fluid and milk. Since we were especially interested in a possible chemocommunicative function of these substances during breastfeeding, we used very low concentrations that were supposed to match the natural concentrations reported for amniotic fluid and milk.

Several hypotheses can be advanced regarding the direction of an infant's responsiveness to these separate sweat odorants after presumed prenatal exposure through amniotic fluid, and/or postnatal exposure through colostrum, milk, or maternal sweat. If these compounds elicit positive reactions (e.g., oro-facial responses indicating attraction or appetence), they may be inferred to be somehow reminiscent of the original substrate's smell, an individual compound's odor being potentially representative of the odor of the whole mixture. In contrast, neutral or even negative responses may indicate that newborns do not attend to, or even avoid, one or several of these isolated compounds; in this case, the odor percepts conveyed by isolated compounds may differ from the percept of the original substrates from which they stem.

Methods and Materials

Participants The study was conducted at the maternity department of the Dijon University Hospital. It was approved by the local Committee for the protection of persons submitted to experimentation and complied with the Declaration of Helsinki for medical research involving human subjects. The parents were informed about the aims and methods of the research. They all gave written consent to let their infants participate, and were physically present during the experiment. All infants were in optimal health at birth (Apgar score > 8 at 1 min and =10 at 5 and 10 min) and at the time of olfactory testing.

Experiments Two experiments were conducted to assess neonatal responsiveness to thiols (Exp. 1) or to acids (Exp. 2). In Experiment 1, 52 newborns participated. As 9 newborns woke up before the test was finished, they were excluded from further analyses, and the final sample consisted of 43 infants (40 Caucasian, 2 African, 1 Asian). In Experiment 2, 18 newborns participated, although complete testing could not be achieved for 2 newborns. The final sample consisted of 16 infants (15 Caucasian, 1 African). Characteristics of the participants having undergone the whole test session are described in Table 1.

Stimuli The target odorants, 3-sulfanylhexan-1-ol (SH; 98%; Acros Organics, Geel, Belgium), 3-methyl-3-sulfanylhexan-1-ol (MSH), 3-methyl-2-hexenoic acid (3M2H; 90% *E*), and 3-hydroxy-3-methylhexanoic acid (HMHA; all from aromaLab, Freising, Germany), were checked for odorous contaminants by gas chromatography-olfactometry (Trace Ultra GC, Thermo Finnigan, Dreieich, Germany; capillary column DB-FFAP, 30 m × 0.32 mm, film thickness 0.25 μm, J&W Scientific, Agilent Technologies, Santa Clara, US). Solutions in dichloromethane were injected on-column at 40 °C. After 2 min, the oven temperature was raised by 12 °C/min to 230 °C and held for 5 min. The flow rate of the helium carrier gas was 2.2 ml/min. One to two contaminants were detected in the SH, MSH, and HMHA. Since no contaminant was perceived with higher intensity than the target odorant, we proceeded without further purification.

Based on quantitative data reported in Hartmann et al. (2012; thiol conjugates: < 100 ng/kg human milk, acid conjugates: < 400 μg/kg human milk), maximum hypothetical concentrations of the free thiols/acids in human milk (assuming 100% cleavage of the respective precursors) were calculated for testing in Experiments 1 and 2.

Experiment 1 The SH was diluted in distilled water to 1 and 10 ng/l, while MSH was taken from a stock solution (990 μg/ml in propylene glycol; 99.5%, Sigma Aldrich, Steinheim, Germany) and diluted in distilled water to 1, 10, and 50 ng/l. Odorless references were distilled water and distilled water containing propylene glycol ("control"; 52 μg/l). To compare target odorants with stimuli of hedonic values known from previous studies (Bingham et al. 2003; Marlier and Schaal 2005; Soussignan et al. 1997), the stimulus set included vanillin (0.01% in distilled water; > 98%, Sigma-Aldrich, Steinheim, Germany) and familiar milk [for a breast-fed infant, its mother's milk expressed between 1 and 5 min before the test; for a bottle-fed infant, the formula milk consumed since birth (brands: Gallia, Nidal, Modilac, Guigoz)].

Experiment 2 The acids were diluted in distilled water to 30 ng/l and 80 μg/l for 3M2H, and 0.5 ng/l, 50 ng/l, and 200 μg/l for HMHA, corresponding to the maximal and minimal hypothetical concentrations of the acids in colostrum and milk, assuming complete cleavage of the conjugates (see Hartmann et al. 2012). Since the target odorants occur in human sweat, familiar sweat was included as a reference stimulus in addition to distilled water, vanillin, and familiar milk (see above). The sweat stimulus was obtained a few minutes before being tested by turning a glass rod 2–6 times in the maternal axilla and adding 10 μl of distilled water. The mothers were asked previously not to use odorous soap, shower gel, or deodorants on the day of the test.

Table 1 Descriptive Characteristics Of The Newborn Samples Assessed In Experiments 1 And 2

	Experiment 1	Experiment 2
Number	43	16
Females/males	23/20	10/6
Exclusively breast-/bottle-fed	26/17	13/3
Birth weight ± SD (range) (in g)	3360 ± 376 (2630–4290)	3332 ± 411 (2710–4150)
Gestational term ± SD (range) (in weeks)	40 ± 1 (38–42)	39 ± 1 (37–42)
Vaginal/cesarean section	36/7	12/4
Multi-/Primiparae	25/18	11/5
Age at testing ± SD (range) (in h)	73 ± 16 (48–118)	79 ± 15 (63–118)
Time since last feed ± SD (range) (in min)	123 ± 30 (70–260)	131 ± 19 (110–180)

Procedure and Behavioral State of Newborns Testing took place in a quiet, dedicated "Baby-lab" (temperature: 23–27 °C) and was conducted by two experimenters. A newborn was seated in a semi-reclining chair surrounded by draperies equilibrating light on each side. Experimenter 1 prepared the stimuli in random order by pipetting 10 μl of a solution on the tip of a 20-cm long glass rod. Experimenter 2, blind to the nature of the stimuli, stood behind the infant and administered the stimuli approximately 1 cm under the nostrils. Following online psychophysiological responses and the infant's behavior through a TV monitor, Experimenter 1 silently (visually) paced the onset/offset of stimulus administration. Stimulus onset followed a period of at least 10 s, during which an infant had no visible facial movements. Stimulus offset was 10 s after the onset, and inter-stimulus intervals were at least 50 s. The test was considered complete only in the case of all 9 stimuli being applied. Newborns display higher facial reactivity to odors during irregular than during regular sleep (Soussignan et al. 1997). Therefore, infants were required to be in irregular sleep at the beginning of the test, as determined by respiratory and behavioral cues according to Prechtl's (1974) classification of neonatal behavioral states. Some infants, however, changed to regular sleep during the test (in Exp. 1, 22 newborns, 11 male and 11 female, while in Exp. 2, 4 newborns). Testing was continued in these cases. However, before conducting statistical analyses on these data, we verified that the proportion of newborns having changed to regular sleep was similar for each stimulus (Exp. 1, 5 newborns with water, 6 newborns with MSH at 10 ng/l, SH at 1 ng/l, vanillin, and the control, and 7 newborns with familiar milk, SH at 10 ng/l, MSH at 1 ng/l, and MSH at 50 ng/l; $Q_8 = 0.9$; $P = 0.999$; in Exp. 2, 0 newborns with vanillin and HMHA at 200 μg/l, 1 newborn with HMHA at 50 ng/l, 3M2H at 80 μg/l, maternal sweat, and water, and 2 newborns with HMHA at 0.5 ng/l, 3M2H at 30 ng/l, and familiar milk; $Q_8 = 6.5$; $P = 0.59$). For Experiment 1, this was verified for both females and males ($Q_8 = 4.2$, $P = 0.84$ and $Q_8 = 8.0$, $P = 0.43$, respectively), as well as for breast-fed and bottle-fed infants ($Q_8 = 6.2$, $P = 0.62$ and $Q_8 = 3.7$, $P = 0.88$, respectively).

Autonomic Responses Breathing was recorded using an 8-channel MacLab data-recording system (ADInstruments Pty Ltd., Castle Hill, Australia) by means of a pneumobelt (Model 1132, Pneumotrace, UFI Instruments, Morro Bay, CA, USA) secured around an infant's abdomen. The respiratory rate (in beats per minute, bpm) was measured online using the Chart software (version 3.5.2). The dependent variable was computed offline by calculating the mean of data during the 5-s pre-stimulus block (baseline condition), and the subsequent 5-s blocks of the stimulus period (2 × 5 s) and the post-stimulus period (2 × 5 s).

Oro-Facial Responses The oro-facial reactivity of newborns was recorded by a silent digital video camera (DCR-TRV22E, Sony Corporation, Tokyo, Japan). Oro-facial responses were coded using Observer software (Version 8, Noldus, Wageningen, Netherlands), and immediately classified into positive and negative responses, as follows. Rooting, munching, tongue or lip protrusions, licking, and sucking were considered positive mouthing actions (as defined in Doucet et al. 2007). Facial responses considered as conveying a negative emotion were conservatively coded into three different exclusive clusters of items (items of a cluster that occurred concurrently with items of another cluster were not coded). The first cluster was composed of the following actions: lowered, oblique, or raised and drawn together brow movements [action units (AUs) 1, 4, 1 + 4, 1 + 2 + 4 of the infant version of Ekman and Friesen's Facial Action Coding System (Oster 2007)]. Nose wrinkling and upper lip rising, associated or not with cheek raising and lids tightening (AUs 9, 10, 9 + 10, 6/7 + 9 + 10), formed the second cluster. The third cluster was composed of 8 AUs: nasolabial furrow deepening (AU 11), lip corner tightening (AU 14), lip corner depressing (AU 15), lower lip depressing (AU 16), chin raising (AU 17), lateral lip stretching (AU 20), and lips tightening and pressing (AUs 23, 24).

The selected items were viewed on a TV screen in slow motion and frame-by-frame to determine onset and offset times with a precision of ±1 video frame (±0.04 s). For each stimulus, the relative duration of positive/negative responses was computed by dividing the value during the 20-s block, comprising the stimulus and post-stimulus period, by the exact duration of the coded video. Inter-observer reliability was assessed between the main coder (not blind to the olfactory stimuli) and a second coder who independently scored randomly selected video clips (Exp. 1, 27 videos; Exp. 2, 42 videos). The second coder was blind to the hypotheses/aims of the study and to the identity of the olfactory stimuli. Spearman correlation coefficients were computed between the two coders for the duration of positive and negative oro-facial actions (Exp. 1, 0.85, and 0.67, respectively; Exp. 2, 0.85, and 0.69, respectively).

Olfactory Testing with Adults Twenty healthy subjects (age 19–31 years; 10 females) without history of olfactory dysfunction were recruited for each target odorant. The composition of the panel was not identical for each odorant, due to restricted availability of some participants who could participate only in the threshold determination of one of the odorants. A triangle test was carried out with aqueous solutions of the target odorants presented in ascending order of 3-fold dilution steps on 20 cm-long glass rods (SH at 8 ng/l to 160 μg/l, MSH at 52 ng/l to 113 μg/l, 3M2H at 55 μg/l to 360 mg/l, HMHA at 29 ng/l to 5.19 mg/l) against two control stimuli (distilled water). Participants had to choose one of the stimuli as the deviating one. The choice of the odorous glass rod was noted "1" and the choice of a water stimulus as "0".

Pleasantness then was rated for defined supraliminal concentrations of the odorants (SH at 500 μg/l, MSH at 383 μg/l, 3M2H at 40 mg/l, HMHA at 1.7 mg/l) on a

nine point Likert scale (ranging from 1 = extremely unpleasant to 9 = extremely pleasant). These concentrations were chosen as they were readily perceivable by participants. The solutions (10 ml) were presented in brown glass jars (volume = 30 ml, ø ext. = 34 mm).

Statistical Analyses - Respiratory Data A logarithmic transformation was performed on raw data since they were not normally distributed (Shapiro-Wilk test). Neither age, nor birth weight, nor time elapsed between the last feeding and the test, were consistently correlated with the dependent variables (Pearson test). Analyses of variance (ANOVA) were calculated with the mode of feeding (breast- vs. bottle-fed) and an infant's sex (male vs. female) as between-subject factors, and olfactory stimulus and test period [5-s interval(s) before, during, and after stimulus administration] as within-subject factors. Fisher's least significant difference (LSD) test was used for *post-hoc* multiple comparisons between means.

Statistical Analyses - Oro-Facial Activity Data Proportions of oro-facially reactive newborns were compared by Cochran's Q tests on the whole group and, for Exp. 1, on the feeding mode (breast- vs. bottle-fed) and an infant's sex (male vs. female). McNemar's tests were calculated *post-hoc* to compare responsiveness between stimuli.

Durations of facial activity were not normally distributed (Shapiro-Wilk test). Since no transformation resulted in normally distributed data, further analyses were conducted on the raw data. Friedman's test was used as an omnibus test on the whole group and, for Exp. 1, on the feeding mode and sex (breast- vs. bottle-fed, and male vs. female, respectively). *Post-hoc*, durations of facial activity in response to different stimuli were compared using Wilcoxon tests.

Results

Experiment 1: Neonatal Reactivity to the Odors of the Thiols (MSH and SH)

Respiratory Rate A main effect was found for the stimulus period (Fig. 1; $F_{4,156} = 4.5$; $P < 0.01$). The mean respiratory rate during the 5-s interval prior to stimulus administration was higher compared to the respiratory rate during the fourth 5-s interval after stimulus onset (LSD; $P < 0.01$). The mean respiratory rate during the 5-s interval following stimulus onset was higher compared with the third and fourth 5-s interval (LSD; $P < 0.05$ and $P < 0.001$, respectively). In sum, neonates did not appear to change their respiratory rate in a stimulus-dependent way. Averaged over all stimuli, the respiratory rate decreased after stimulus offset compared with the baseline level and the 5-s interval following stimulus onset.

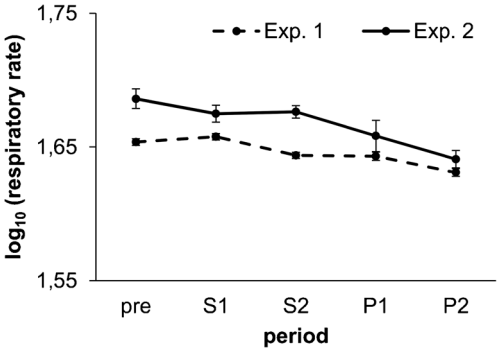

Fig. 1 Mean respiratory rate (± s.e.m.) of neonates over five consecutive 5-s periods in Experiments 1 and 2 (N = 43 and 16, respectively; see Results for significant differences between periods). Pre = baseline period 5 s prior to stimulus administration, S1 and S2 = consecutive 5-s periods during stimulus administration, P1 and P2 = consecutive 5-s periods after stimulus administration

Positive Oral Actions

Proportion of Responding Newborns Between 19 and 37% of newborns evinced positive oral actions, depending on the stimulus (familiar milk, MSH at 10 ng/l: 19%, control, SH at 10 ng/l, MSH at 1 ng/l, MSH at 50 ng/l: 26%, water: 35%, vanillin, SH at 1 ng/l: 37%). Cochran's Q test did not reach significance for these odorants in the whole group, and also not for feeding mode or sex of newborns.

Duration of Positive Oral Actions The results are depicted in Fig. 2. Friedman's test was not significant for the whole group or for feeding mode. Segregated by sex, Friedman's test was marginally significant for male newborns only (Friedman $\chi^2_8 = 14.3$; $P = 0.07$). Wilcoxon tests indicated that males showed shorter oral responses to SH at 10 ng/l compared to water ($P < 0.01$; Fig. 3), vanillin and MSH at 1 ng/l ($P < 0.05$ in both cases). Further, MSH at 10 ng/l elicited shorter ($P < 0.05$) oral activity than did water. In summary, stimulus-dependent responses were evident in males only, with MSH at 10 ng/l eliciting shorter oral activity compared to water, and with SH at 10 ng/l eliciting shorter oral activity compared to water, vanillin, and MSH at 1 ng/l.

Negative Oro-Facial Actions

Proportion of Responding Newborns Depending on the stimulus, 16–35% of newborns displayed negative oro-facial actions (vanillin: 16%, familiar milk, MSH at 10 ng/l: 19%, control, MSH at 1 ng/l: 21%, water, SH at 10 ng/l: 23%, SH at 1 ng/l: 28%, MSH at 50 ng/l: 35%). Cochran's Q test did not reach significance for these odorants in the whole group, and there was no impact of feeding mode. Segregated by sex, Cochran's Q test indicated a trend for the effect of the stimulus in males ($Q_8 = 12.8$, $P = 0.12$). McNemar's test indicated that more male newborns showed negative oro-facial actions in

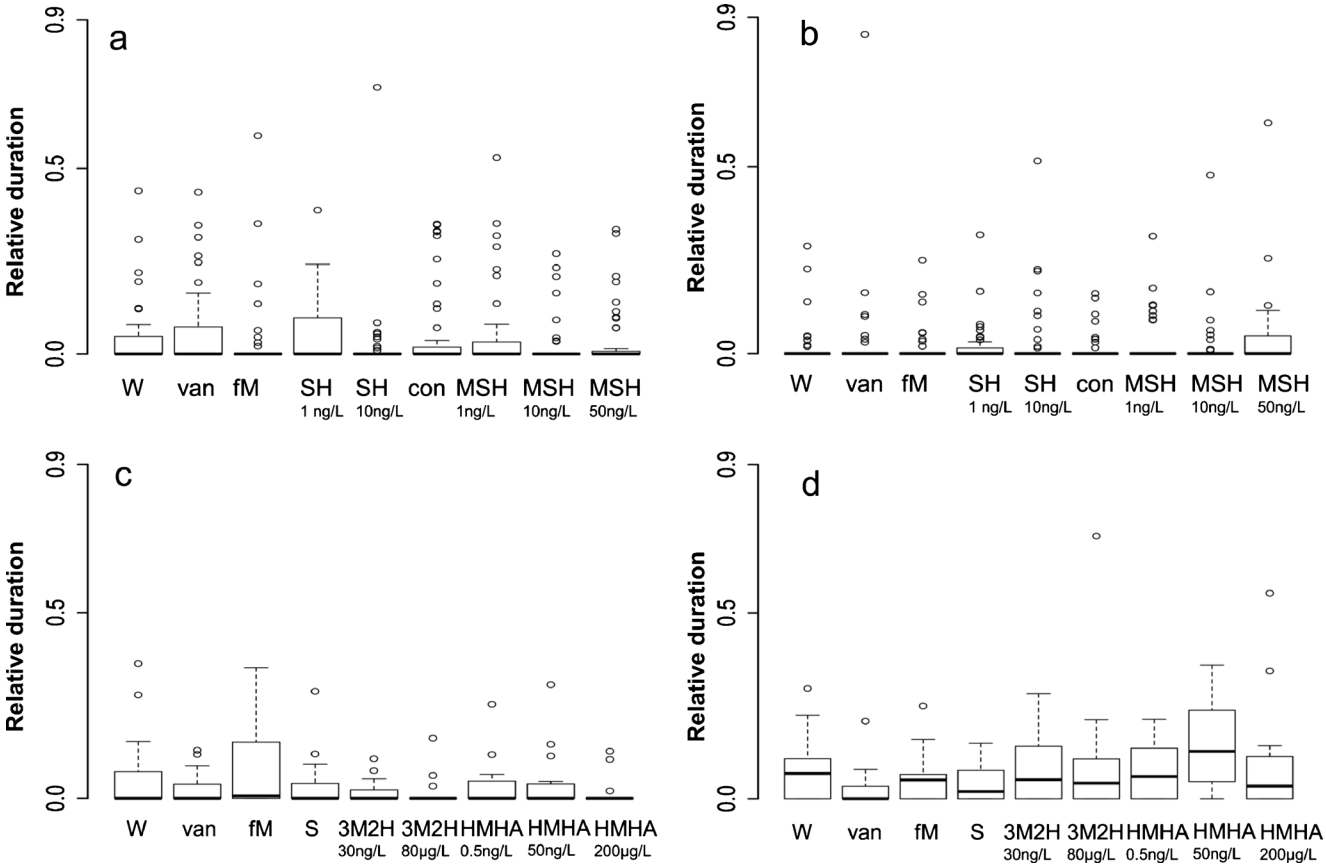

Fig. 2 Medians and quartiles of the relative duration of positive oral (**a**,**c**) and negative oro-facial (**b**,**d**) movements of neonates in response to stimuli of Experiment 1 (**a**, **b**; $N = 43$) and Experiment 2 (**c**, **d**; $N = 16$). Values >1.5 times the interquartile range are denoted by a circle. See Results for significant differences. W = water, fM = familiar milk, van = vanillin, S = sweat, con = odorless control containing propylene glycol, SH = 3-sulfanylhexan-1-ol, MSH = 3-methyl-3-sulfanylhexan-1-ol, 3M2H = 3-methyl-2-hexenoic acid, HMHA =3-hydroxy-3-methylhexanoic acid

response to MSH at 50 ng/l compared to familiar milk (50% vs. 20%; $P = 0.08$), vanillin, SH at 1 ng/l and MSH at 1 ng/l (50% vs. 15%, $P < 0.05$ in all cases).

Duration of Negative Oro-Facial Actions Results are presented in Fig. 2. Friedman's test was not significant for the tested odorants and also not for feeding mode. Segregated by sex, Friedman's test indicated a trend for an effect of the stimulus in male newborns ($\chi^2_8 = 12.9$, $P = 0.12$). Wilcoxon tests indicated that male newborns showed longer negative oro-facial responses to MSH at 50 ng/l, compared to water, MSH at 1 ng/l, and the propylene glycol-containing odorless control ($P < 0.05$ in all cases; Fig. 3).

In summary, stimulus-specific responsiveness was apparent in male newborns only, with the longest facial activity occurring in response to MSH at 50 ng/l. MSH at 50 ng/l elicited longer negative responses in males compared with the odorless reference and MSH at 1 ng/l.

Adults' Sensitivity and Hedonic Ratings Adults chose the odorous stimulus with higher than chance level at concentrations ≥ 1 µg/l and ≥ 0.7 µg/l for MSH and SH, respectively (χ^2, $P < 0.05$ and $P < 0.001$). Overall, women performed better than men (Fig. 4). Thus, MSH was detected by women at 1 µg/l (χ^2; $P < 0.01$), whereas a concentration of 38 µg/l was necessary for this compound for olfactory detection by men (χ^2; $P < 0.05$). For SH, 0.7 µg/l was detected by both men and women (χ^2; $P < 0.05$ and $P < 0.01$, respectively) but, with increasing concentrations, men again chose the correct sample only when 55 µg/l was reached (χ^2; $P < 0.01$). Supra-liminal concentrations of the odorants were rated to be close to hedonic neutrality [mean ± SD = 5.4 ± 1.7 (MSH), 4.6 ± 1.7 (SH), on a 9-point Likert scale].

Experiment 2: Neonatal Reactivity to the Odors of the Acids (3M2H and HMHA)

Respiratory Rate The main effect of the stimulus period was significant (Fig. 1; $F_{4,60} = 3.7$; $P < 0.01$). The mean respiratory rate was lower during the fourth than during the first and second 5-s interval after stimulus onset (LSD; $P < 0.05$ and 0.01, respectively). Baseline respiratory rate was higher compared with respiratory rate during the third and fourth 5-s

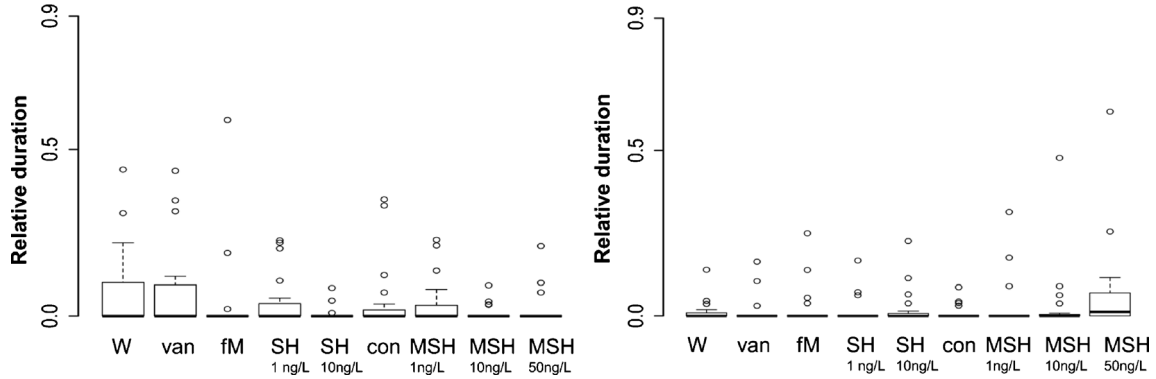

Fig. 3 Medians and quartiles of the relative duration of positive oral (left) and negative oro-facial (right) movements of neonates in response to stimuli of Exp. 1 for males only (N = 20). Values >1.5 times the interquartile range are denoted by a circle. See Results for significant differences. W = water, fM = familiar milk, van = vanillin, SH = 3-sulfanylhexan-1-ol, con = odorless control containing propylene glycol, MSH = 3-methyl-3-sulfanylhexan-1-ol

interval after stimulus onset (LSD; $P < 0.05$ and 0.01, respectively). In summary, an overall decrease of respiratory rate after stimulus onset was observed, but no stimulus-specific respiratory pattern was apparent.

Positive Oral Actions

Proportion of Responding Newborns Depending on the stimulus, between 19 and 50% of newborns evinced positive oral actions (3M2H at 80 μg/l, HMHA at 200 μg/l: 19%, water, vanillin, 3M2H at 30 ng/l: 31%, maternal sweat, HMHA at 0.5 and 50 ng/l: 38%, familiar milk: 50%). Cochran's Q test did not reach significance on these percentages ($Q_8 = 5.5$; $P = 0.7$).

Duration of Positive Oral Actions Results are depicted in Fig. 2. Friedman's test did not reach significance (Friedman $\chi^2_8 = 7.9$; $P = 0.45$), indicating that the different stimuli were not discriminated in terms of positive oral movements.

Negative Oro-Facial Actions

Proportion of Responding Newborns Depending on the stimulus, between 38 and 81% of newborns showed negative oro-facial actions (vanillin: 38%, sweat: 50%, water, 3M2H at 80 μg/l, HMHA at 0.5 ng/l: 56%, familiar milk, 3M2H at 30 ng/l, HMHA at 200 μg/l: 63%, HMHA at 50 ng/l: 81%). Cochran's Q test did not yield significant differences among these response frequencies across stimuli ($Q_8 = 7.5$; $P = 0.49$).

Duration of Negative Oro-Facial Actions Friedman's test was marginally significant (Friedman $\chi^2_8 = 13.8$; $P = 0.09$). Newborns evinced longer negative oro-facial actions in response to HMHA at 50 ng/l compared with HMHA at 0.5 ng/l, familiar milk, maternal sweat, and vanillin (Wilcoxon; $P < 0.05$ for the former, $P < 0.01$ for the latter two stimuli; c.f., Fig. 2). Further, HMHA at 0.5 ng/l led to negative oro-facial actions of longer duration than did vanillin (Wilcoxon; $P < 0.05$).

In summary, up to 81% of neonates evinced negative oro-facial expressions. Stimuli were discriminated in terms of duration of negative reactivity. Thus, HMHA at 50 ng/l, but not at 200 μg/l, elicited higher responsiveness than HMHA at 0.5 ng/l, familiar milk, sweat, and vanillin, and HMHA at 0.5 ng/l led to higher responsiveness than vanillin.

Adults' Sensitivity and Hedonic Ratings Adults chose the odorous stimulus with higher than chance level at concentrations ≥ 40 mg/l and ≥ 576 μg/l for 3M2H and HMHA, respectively (χ^2; $P < 0.05$ and $P < 0.001$). For both compounds, women performed better than men (Fig. 4). Thus, for 3M2H, 120 mg/l was detected by women (χ^2; $P < 0.05$), whereas 360 mg/l was necessary for detection by men (χ^2; $P < 0.01$). For HMHA, a concentration of 576 μg/l was sufficient for above-chance detection by women, whereas 1.7 mg/l was necessary for detection by men (χ^2; $P < 0.001$ in both cases). Finally, supra-liminal concentrations of odorants were rated to be unpleasant [mean ± SD = 3.3 ± 1.8 (HMHA)] or close to neutral [mean ± SD = 4.2 ± 1.7 (3M2H)] on a 9-point Likert scale.

Discussion

The sensitivity and hedonic responsiveness to low concentrations of MSH and SH (Experiment 1), and HMHA and 3M2H (Experiment 2) were assessed in 3-d-old human infants. Upon delivering the odorants, as well as odorous and odorless reference stimuli, the infants' respiratory rates and facial responsiveness were recorded.

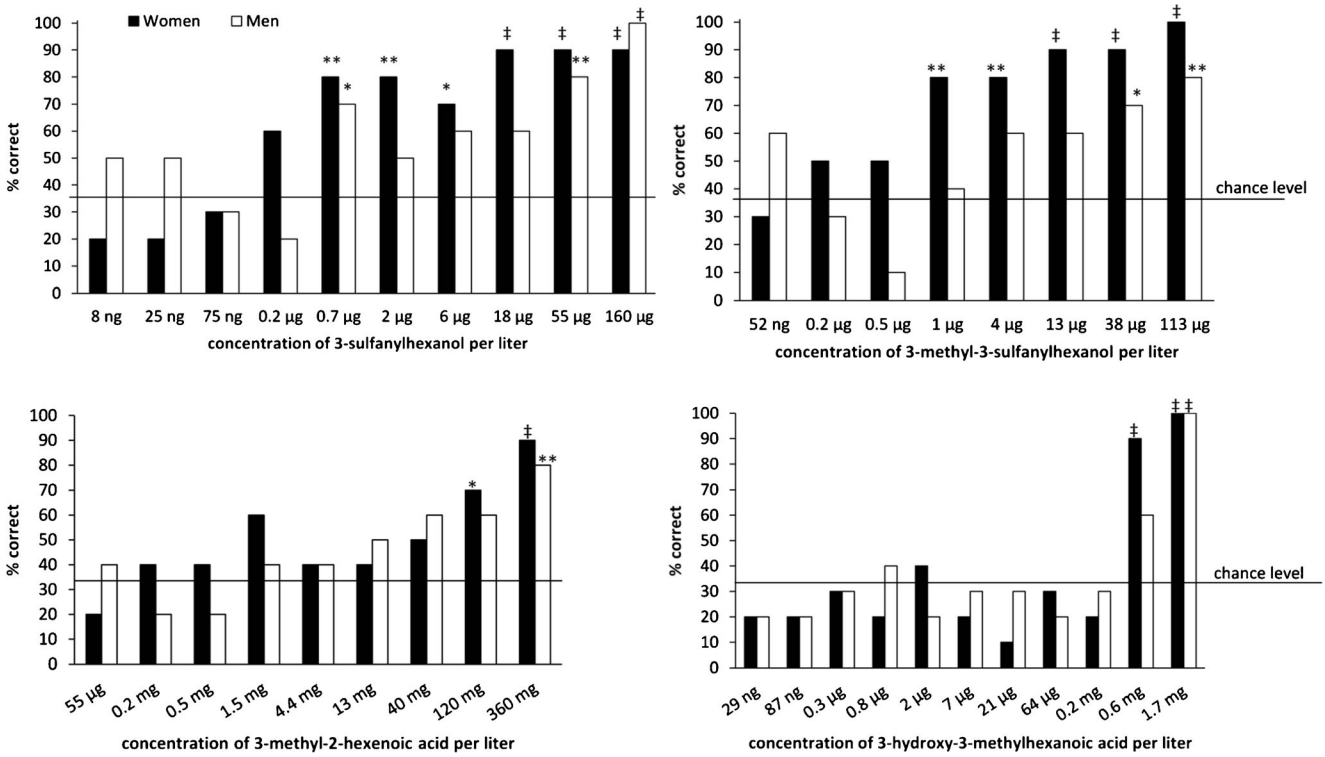

Fig. 4 Sensitivity of adults to 3-sulfanylhexan-1-ol, 3-methyl-3-sulfanylhexan-1-ol, 3-methyl-2-hexenoic acid, and 3-hydroxy-3-methylhexanoic acid. Percentage of women (N = 10; black bars) and men (N = 10; white bars) choosing the correct glass rod in a triangle test. (χ^2, comparison with chance level: ‡ = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$)

Detection of the Sweat-Related Odor Stimuli In line with the results obtained by Loos et al. (2014), respiratory rate was not discriminative for the olfactory stimuli. Oro-facial reactivity raised no strongly significant effects in the whole sample of newborns, with the odorous reference compounds (familiar milk, vanillin, and sweat) not being discriminated from the odorless stimulus. However, olfactory discrimination became apparent in male newborns exposed to MSH and SH. More precisely, males showed increased facial responsiveness to MSH at 10 and 50 ng/l, and to SH at 10 ng/l, relative to that to water. These results point to neonatal orthonasal detection of the odors of these thiols. In Experiment 2, HMHA at 50 ng/l led to increased facial responsiveness compared to HMHA at 0.5 ng/l, indicating neonatal detection of the acid. However, HMHA at 200 µg/l was not discriminated from HMHA at 0.5 ng/l. This finding, that the highest concentration induces a less intense behavioral response compared to intermediate concentrations, is reminiscent of a previous result with 5α-androst-16-en-3-one in newborns (Loos et al. 2014). The underlying reason is unclear. One may speculate about a non-linear concentration-response relation, as reported for several behavioral and perceptual brain processes (Burke et al. 2012; Coureaud et al. 2004; Nielsen et al. 2011).

With adults, concentrations in the µg/l range or higher were necessary for above chance olfactory detection of the acids and thiols in a triangle test. These values correspond approximately to threshold concentrations published so far for adults. MSH has been reported to be detected at 10 ppb in mineral oil (Hasegawa et al. 2004), SH retronasally at 22 ng/l in water (Starkenmann et al. 2008), HMHA, depending on the enantiomer, at 0.1 (*S*) or 10 ppm (*R*) in mineral oil (Hasegawa et al. 2004), and (*E*)-3M2H at about 1 ppm in mineral oil (Ferdenzi et al. 2015; Wysocki et al. 1993). In contrast with results reported by Troccaz et al. (2009), who found no gender variation of odor detection thresholds for MSH, HMHA, and 3M2H, women appeared to be more sensitive than men in the present study. Further investigations with a greater number of adult participants appear necessary to corroborate a possible difference in sensitivity between sexes.

Highly diluted odorants that elicited a behavioral response in neonates were not detected by adult participants in triangle tests, which could point to higher neonatal sensitivity. However, different response measures were used for infants and adults, which may account for divergent outcomes. Few investigators have assessed odor detection thresholds of neonates. Engen (1965) and Rovee (1969) determined infant detection thresholds to a homologous series of *n*-alcohols. Unfortunately, the threshold of adults was reported for propanol only, but was a factor of 10 lower compared to the average threshold for neonates. This lower sensitivity of neonates (at least to propanol) contrasts with the results obtained in the

present study. Clearly, studies are needed to elucidate the developmental course of human olfactory and nasal trigeminal sensitivities, not only in neonates, but also in young children whose olfactory detection thresholds have been assessed only for a few odorants, with varying outcomes [e.g., phenylethanol (Poncelet et al. 2010), tetrahydrothiophene and (R)-carvon (Monnery-Patris et al. 2009), butanol (Lehrner et al. 1999), trimethylamine (Solbu et al. 1990)].

In summary, the results indicate neonatal orthonasal detection of HMHA at 0.5 ng/l and 50 ng/l, and detection by male newborns of SH at 10 ng/l, and of MSH at 10 and 50 ng/l. Since neonatal responses to 3M2H were not different from those to other stimuli, detection of the odor of this acid at concentrations used here remains uncertain. Adults detected all four compounds at the μg/l level, indicating lower adult sensitivity to MSH, SH, and HMHA. However, to ascertain lower adult olfactory sensitivity to individual odorants more fully, methods are needed that determine, in a comparable manner, individual olfactory detection thresholds in neonates and adults.

Hedonic Response to Sweat-Related and Other Odor Stimuli The oral appetitive movements of newborns were not systematically increased by the odors of the thiols. Instead, male newborns evinced shorter oral movements to SH at 10 ng/l compared to water, vanillin, and SH at 1 ng/l. Further, longer oral movements were elicited by water compared to MSH at 10 ng/l. Thus, it appears that the odor of the thiols reduced the duration of appetitive oral movements in male newborns. MSH at 50 ng/l was even more efficient in eliciting negative oro-facial responses than several other stimuli, in terms of both percentage of responding male newborns and the duration of facial expressions, indicating a negative hedonic valence of its odor. No such response was evident in female newborns, suggesting a sex-dependent neonatal response to a human sweat odorant (c.f., Loos et al. 2014). Although female and male neonates behaved differently during certain double-choice tests (e.g., Delaunay-El Allam et al. 2006; Porter et al. 1991), we are not aware of a general sex-dependent difference in neonatal facial reactivity toward odors.

Female human sweat has been reported to contain, on average, a higher amount of MSH-conjugates than male sweat (Troccaz et al. 2009). However, no data are available exploring the influence of an infant's sex on the occurrence of thiols and their conjugates in amniotic fluid and human milk. Thus, one may only speculate whether the difference between male and female responses is due to different neonatal hormonal levels (Doty and Cameron 2009; Scott et al. 2009), or based on different rates of pre- and/or post-natal exposure to these compounds.

The odor of the acids also did not enhance appetitive oral movements in human newborns. Instead, longer negative oro-facial expressions were shown in response to HMHA at 50 ng/l compared to HMHA at 0.5 ng/l and familiar milk, and in response to HMHA at 0.5 ng/l compared to vanillin. It can thus be hypothesized that HMHA conveys a negative valence for infants, whereas 3M2H was not detected or of neutral valence under the present conditions.

Reference stimuli were not discriminated from each other. However, several authors have shown that the odor of familiar milk is perceived by newborns, and elicits orientation and increased mouthing toward the stimulus (e.g., Marlier and Schaal 1997; Mizuno et al. 2004). Vanillin, too, has been reported to elicit increased mouthing (Steiner 1979), although with high inter-individual variability (Soussignan et al. 1997). In contrast, Doucet et al. (2009) did not observe increased non-nutritive sucking with familiar milk and vanillin, in line with the present results. In view of this conflicting evidence, parameters that influence the mouthing-sucking response to familiar milk (e.g., duration and multimodal context of presentation or prandial state) should be considered in more detail in future experiments.

In summary, the results of the present study point to a neutral or negative hedonic valence of the target odorants, which is in accordance with hedonic ratings from adults. Further, in the present experimental conditions, no clear-cut hedonic valence of the odors of vanillin, familiar milk, and sweat could be inferred from neonatal responses.

Negative Responses to Human Sweat Odorants The present findings did not support the prediction (see Introduction) of a positive reactivity of newborns to the target stimuli due to olfactory learning *in* and/or *ex utero*. Possible explanations for the observed negative responses have been discussed already in a recent study on the detection and hedonic valence of the odor of androstenone in newborns (Loos et al. 2014), and only additional facets will be developed here.

First, in sweat, HMHA, MSH, and SH have mainly the *S*-configuration (*R*:*S* ratio = 28:72 for HMHA and MSH; Hasegawa et al. 2004; Starkenmann et al. 2005; Troccaz et al. 2004). Since odor quality and threshold can depend on the investigated enantiomer (Begnaud et al. 2006; Hasegawa et al. 2004; Starkenmann 2016; Troccaz et al. 2004), and no specific enantiomeric ratio was presented here, a novel olfactory percept might have been created in the human infant, leading to a neophobic response.

Second, release of considerable amounts of free odorants has been reported for human sweat [between 2 and 200 μg/l of *(E)*-3M2H in whole body sweat (Gordon et al. 1973), 100 μg/l of 3M2H in whole body sweat (Smith et al. 1969), and 2.2 μg/l of MSH in incubated underarm sweat (Troccaz et al. 2004)]. A neonate should thus detect the target odorants when smelling its mother's sweat. In fact, even if no specific response to familiar sweat was observed here, it has been demonstrated elsewhere that neonates perceive maternal sweat (e.g., Cernoch and Porter 1985). Bottle-fed and breast-fed infants preferentially orient toward breast odor rather than the axillary odor of an unfamiliar breastfeeding mother [Makin and Porter (1989); although this might not hold for

an infant's own mother, Porter et al. (1992)]. Thus, axillary odor appears to be less attractive to human infants than breast odor; the neutral or aversive response found here might be interpreted in the context of an association of the target compounds with sweat.

In summary, intrinsic trigeminal properties of the odorants, perceptual novelty of the stimuli, or learned association of the stimuli with human sweat odor may explain the neutral or even aversive responses of human neonates to MSH, SH, and HMHA observed here.

Limitations The interpretation of responses of preverbal organisms is not always unequivocal. For instance, it remains uncertain whether higher oral activity to MSH at 1 ng/l or lower activity to MSH at 10 ng/l underlies discriminative responsiveness to these two stimuli. This difficulty in interpreting results is exacerbated by the fact that no stimulus induced longer positive oral movements compared to the odorless reference. Thus, we cannot exclude the present experimental design having a low sensitivity for detection of positively valenced odorants.

Further, the neonates born by cesarean section were heterogeneous in terms of labor experience (cesarean sections with and without labor). Future studies should verify whether the experience of labor impacts the neonatal behavioral response to the investigated stimuli (c.f., Varendi et al. 2002). Finally, the coding procedure used in the present study does not allow a fine-grained analysis of the occurrence and duration of individual action units, nor of combinations thereof. It cannot be excluded that a more detailed analysis of individual facial action units might reveal further stimulus discrimination by neonates.

Outlook In the present study, target odorants were studied in concentrations corresponding to their presumed concentrations in amniotic fluid and milk. It came out, however, that adult detection thresholds were several magnitudes higher. In future studies, it would be of interest to investigate neonatal responses to target odorants at adult threshold concentrations. Further, future studies should consider measuring a mother's levels of odorants in order to account for individual differences in odor exposition of neonates, which might influence odor sensitivity to these compounds. Inter-individual differences could be maximized by a cross-cultural study design including different ethnicities and genotypes (Martin et al. 2010; Prokop-Prigge et al. 2016).

Conclusion

Neonatal facial responses to target odorants and reference stimuli led us to conclude that at least three of the four administered target odorants were detected by human newborns at the very low concentrations used here. This study lends further evidence that odorants occurring in complex mixtures in the perinatal environment are not automatically perceived as hedonically positive by an infant, especially when administered in pure form. Finally, our results point to a sex-dependent neonatal response to MSH and SH, and a higher olfactory sensitivity to these odorants in newborns compared to adults. These outcomes call for further investigations on developmental aspects of human sweat odor perception and on the quantitative occurrence of human sweat odorants in various body fluids.

Acknowledgments We express our gratitude to the parents who agreed to let their infants participate in this study, and to the midwives and staff at the maternity ward of Dijon University Hospital for their kind and invaluable support. Dr. Catherine Dacremont is thanked for continued discussion relating to this work. The constructive comments by two anonymous referees are gratefully acknowledged.

This work was supported by grants from the Bavarian Research Foundation (Bayerische Forschungsstiftung), the Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung), the Cusanuswerk Bischöfliche Studienförderung, the Regional Council of Burgundy (Conseil Régional de Bourgogne), the French National Research Agency (ANR Colostrum), and the National Center for Scientific Research (Centre National de la Recherche Scientifique).

Compliance with Ethical Standards All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of Interest Helene M. Loos, Sébastien Doucet, Fanny Védrines, Constanze Sharapa, Robert Soussignan, Karine Durand, Paul Sagot, Andrea Buettner, and Benoist Schaal declare that they have no conflict of interest.

References

Alberts JR, Ronca AE (2012) The experience of being born: a natural context for learning to suckle. Int J Pediatr 2012:129328

Baumann T, Bergmann S, Schmidt-Rose T et al (2014) Glutathione-conjugated sulfanylalkanols are substrates for ABCC11 and gamma-glutamyl transferase 1: a potential new pathway for the formation of odorant precursors in the apocrine sweat gland. Exp Dermatol 23:247–252

Begnaud F, Starkenmann C, Van de Waal M, Chaintreau A (2006) Chiral multidimensional gas chromatography (MDGC) and chiral GC-olfactometry with a double-cool-strand interface: application to malodors. Chem Biodiversity 3:150–160

Bingham PM, Abassi S, Sivieri E (2003) A pilot study of milk odor effect on nonnutritive sucking by premature newborns. Arch Pediatr Adolesc Med 157:72–75

Block E (1992) The organosulfur chemistry of the genus *Allium* – implications for the organic chemistry of sulfur. Angew Chem Int Ed Engl 31:1135–1178

Brewington CR, Parks OW, Schwartz DP (1973) Conjugated compounds in cow's milk. J Agric Food Chem 21:38–39

Burke SM, Veltman DJ, Gerber J, Hummel T, Bakker J (2012) Heterosexual men and women both show a hypothalamic response to the chemo-signal androstadienone. PLoS One 7:e40993

Cernoch JM, Porter RH (1985) Recognition of maternal axillary odors by infants. Child Dev 56:1593–1598

Coureaud G, Langlois D, Sicard G, Schaal B (2004) Newborn rabbit responsiveness to the mammary pheromone is concentration-dependent. Chem Senses 29:341–350

Delaunay-El Allam M, Marlier L, Schaal B (2006) Learning at the breast: preference formation for an artificial scent and its attraction against the odor of maternal milk. Infant Behav Dev 29:308–321

Delaunay-El Allam M, Soussignan R, Patris B, Marlier L, Schaal B (2010) Long-lasting memory for an odor acquired at the mother's breast. Dev Sci 13:849–863

DiGiulio DB, Romero R, Amogan HP et al (2008) Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. PLoS One 3:e3056

Doty RL, Cameron EL (2009) Sex differences and reproductive hormone influences on human odor perception. Physiol Behav 97:213–228

Doty RL, Orndorff MM, Leyden J, Kligman A (1978) Communication of gender from human axillary odors: relationship to perceived intensity and hedonicity. Behav Biol 23:373–380

Doucet S, Soussignan R, Sagot P, Schaal B (2007) The "smellscape" of mother's breast: effects of odor masking and selective unmasking on neonatal arousal, oral, and visual responses. Dev Psychobiol 49: 129–138

Doucet S, Soussignan R, Sagot P, Schaal B (2009) The secretion of areolar (Montgomery's) glands from lactating women elicits selective, unconditional responses in neonates. PLoS One 4:e7579

Dufa M, Hossaert-McKey M, Anstett MC (2004) Temporal and sexual variation of leaf-produced pollinator-attracting odours in the dwarf palm. Oecologia 139:392–398

Engen T (1965) Psychophysical analysis of the odor intensity of homologous alcohols. J Exp Psychol 70:611–616

Fabregat A, Kotronoulas A, Marcos J et al (2013) Detection, synthesis and characterization of metabolites of steroid hormones conjugated with cysteine. Steroids 78:327–336

Fedrizzi B, Guella G, Perenzoni D, Gasperotti M, Masuero D, Vrhovsek U, Mattivi F (2012) Identification of intermediates involved in the biosynthetic pathway of 3-mercaptohexan-1-ol conjugates in yellow passion fruit (*Passiflora edulis* F. Flavicarpa). Phytochemistry 77: 287–293

Ferdenzi C, Schaal B, Roberts SC (2010) Family scents: developmental changes in the perception of kin body odor? J Chem Ecol 36:847–854

Ferdenzi C, Roblin S, Baldovini N, Razafindrazaka H, Pierron D, Bensafi M (2015) Sensitivity to human body odor compounds. Chem Senses 40:271

Flower DR (1996) The lipocalin protein family: structure and function. Biochem J 318:1–14

Gordon SG, Smith K, Rabinowitz JL, Vagelos PR (1973) Studies of trans-3-methyl-2-hexenoic acid in normal and schizophrenic humans. J Lipid Res 14:495–503

Harker M, Carvell A-M, Marti VPJ, Riazanskaia S, Kelso H, Taylor D, Grimshaw S, Arnold DS, Zillmer R, Shaw J, Kirk JM (2014) Functional characterisation of a SNP in the ABCC11 allele - effects on axillary skin metabolism, odour generation and associated behaviours. J Dermatol Sci 73:23–30

Hartmann C, Doucet S, Niclass Y, Dittrich R, Cupisti S, Schaal B, Buettner A, Starkenmann C (2012) Human sweat odour conjugates in human milk, colostrum and amniotic fluid. Food Chem 135:228–233

Hasegawa Y, Yabuki M, Matsukane M (2004) Identification of new odoriferous compounds in human axillary sweat. Chem Biodivers 1: 2042–2050

James AG, Austin CJ, Cox DS, Taylor D, Calvert R (2013) Microbiological and biochemical origins of human axillary odour. FEMS Microbiol Ecol 83:527–540

Kuhn F, Natsch A (2009) Body odour of monozygotic human twins: a common pattern of odorant carboxylic acids released by a bacterial aminoacylase from axilla secretions contributing to an inherited body odour type. J R Soc Interface 6:377–392

Lehrner JP, Gluck J, Laska M (1999) Odor identification, consistency of label use, olfactory threshold and their relationships to odor memory over the human lifespan. Chem Senses 24:337–346

Leyden JJ, McGinley KJ, Holzle E, Labows JN, Kligman AM (1981) The microbiology of the human axilla and its relationship to axillary odor. J Invest Dermatol 77:413–416

Loos HM, Doucet S, Soussignan R, Hartmann C, Durand K, Dittrich R, Sagot P, Buettner A, Schaal B (2014) Responsiveness of human neonates to the odor of 5α-androst-16-en-3-one: a behavioral paradox? Chem Senses 39:693–703

Lopez V, Lindsay RC (1993) Metabolic conjugates as precursors for characterizing flavor compounds in ruminant milks. J Agric Food Chem 41:446–454

Macfarlane JA (1975) Olfactory discrimination in the neonate. Arch Dis Child 50:827–827

Makin JW, Porter RH (1989) Attractiveness of lactating females' breast odors to neonates. Child Dev 60:803–810

Marlier L, Schaal B (1997) Familiarité et discrimination olfactive chez le nouveau-né: Influence différentielle du mode d'alimentation? Enfance 1:47–61

Marlier L, Schaal B (2005) Human newborns prefer human milk: conspecific milk odor is attractive without postnatal exposure. Child Dev 76:155–168

Martin A, Saathoff M, Kuhn F, Max H, Terstegen L, Natsch A (2010) A functional ABCC11 allele is essential in the biochemical formation of human axillary odor. J Invest Dermatol 130:529–540

Mizuno K, Mizuno N, Shinohara T, Noda M (2004) Mother-infant skin-to-skin contact after delivery results in early recognition of own mother's milk odour. Acta Paediatr 93:1640–1645

Monnery-Patris S, Rouby C, Nicklaus S, Issanchou S (2009) Development of olfactory ability in children: sensitivity and identification. Dev Psychobiol 51:268–276

Natsch A, Gfeller H, Gygax P, Schmid J, Acuna G (2003) A specific bacterial aminoacylase cleaves odorant precursors secreted in the human axilla. J Biol Chem 278:5718–5727

Natsch A, Derrer S, Flachsmann F, Schmid J (2006) A broad diversity of volatile carboxylic acids, released by a bacterial aminoacylase from axilla secretions, as candidate molecules for the determination of human-body odor type. Chem Biodivers 3:1–20

Nielsen BL, Jerome N, Saint-Albin A, Thonat C, Briant C, Boué F, Rampin O, Maurin Y (2011) A mixture of odorant molecules potentially indicating oestrus in mammals elicits penile erections in male rats. Behav Brain Res 225:584–589

Oster H (2007) Baby FACS: Facial Action Coding System for infants and young children. New York University

Pause BM, Ohrt A, Prehn A, Ferstl R (2004) Positive emotional priming of facial affect perception in females is diminished by chemosensory anxiety signals. Chem Senses 29:797–805

Poncelet J, Rinck F, Ziessel A, Joussain P, Thevenet M, Rouby C, Bensafi M (2010) Semantic knowledge influences prewired hedonic responses to odors. PLoS One 5:e13878

Porter RH, Makin JW, Davis LB, Christensen KM (1991) An assessment of the salient olfactory environment of formula-fed infants. Physiol Behav 50:907–911

Porter RH, Makin JW, Davis LB, Christensen KM (1992) Breast-fed infants respond to olfactory cues from their own mother and unfamiliar lactating females. Infant Behav Dev 15:85–93

Prechtl HFR (1974) The behavioral states of the newborn infant (a review). Brain Res 76:185–212

Preti G, Leyden JJ (2010) Genetic influences on human body odor: from genes to the axillae. J Invest Dermatol 130:344–346

Prokop-Prigge KA, Greene K, Varallo L, Wysocki CJ, Preti G (2016) The effect of ethnicity on human axillary odorant production. J Chem Ecol 42:33–39

Romantshik O, Porter R, Tillmann V, Varendi H (2007) Preliminary evidence of a sensitive period for olfactory learning by human newborns. Acta Paediatr 96:372–376

Rotimi VO, Duerden BI (1981) The development of the bacterial flora in normal neonates. J Med Microbiol 14:51–62

Rovee CK (1969) Psychophysical scaling of olfactory response to the aliphatic alcohols in human neonates. J Exp Child Psychol 7:245–254

Russell MJ (1976) Human olfactory communication. Nature 260: 520–522

Schaal B (2005) From amnion to colostrum to milk: odor bridging in early developmental transitions. In: Hopkins B, Johnson SP (eds) Prenatal development of postnatal functions. Praeger, London, pp. 51–102

Schaal B (2015) Developing human olfaction and its functions in early cognition and behavior. In: Doty RL (ed) Handbook of olfaction and gustation. Wiley, New York, pp. 307–337

Schleidt M, Genzel C (1990) The significance of mother's perfume for infants in the first weeks of their life. Ethol and Sociobiol 11:145–154

Scott HM, Mason JI, Sharpe RM (2009) Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds. Endocr Rev 30:883–925

Shelley WB, Hurley HJ, Nichols AC (1953) Axillary odor: experimental study of the role of bacteria, apocrine sweat, and deodorants. AMA Arch Derm Syphilol 68:430–446

Smith K, Thompson GF, Koster HD (1969) Sweat in schizophrenic patients: identification of the odorous substance. Science 166:398–399

Solbu E, Jellestad F, Strætkvern K (1990) Children's sensitivity to odor of trimethylamine. J Chem Ecol 16:1829–1840

Soussignan R, Schaal B, Marlier L, Jiang T (1997) Facial and autonomic responses to biological and artificial olfactory stimuli in human neonates: Re-examining early hedonic discrimination of odors. Physiol Behav 62:745–758

Spielman AI, Zeng XN, Leyden J, Preti G (1995) Proteinaceous precursors of human axillary odor: isolation of two novel odor-binding proteins. Experientia 51:40–47

Starkenmann C (expected release 2016) The analysis and chemistry of human odors. In: Buettner A (ed) Springer handbook of odors. Springer, in press

Starkenmann C, Niclass Y (2011) New cysteine-S-conjugate precursors of volatile sulfur compounds in bell peppers (*Capsicum annuum* L. cultivar). J Agric Food Chem 59:3358–3365

Starkenmann C, Niclass Y, Troccaz M, Clark AJ (2005) Identification of the precursor of (S)-3-methyl-3-sulfanylhexan-1-ol, the sulfury malodour of human axilla sweat. Chem Biodivers 2:705–716

Starkenmann C, Le Calve B, Niclass Y, Cayeux I, Beccucci S, Troccaz M (2008) Olfactory perception of cysteine-S-conjugates from fruits and vegetables. J Agric Food Chem 56:9575–9580

Starkenmann C, Niclass Y, Cayeux I (2011) Occurrence of L-γ-glutamyl-S-(1-hydroxy-2-methyl-3-pentanyl)-L-cysteine and S-(1-ethyl-3-hydroxy-2-methylpropyl)-L-cysteine in fresh and processed *Allium caepa* L. cultivar. Flavour Fragrance J 26:378–384

Starkenmann C, Mayenzet F, Brauchli R, Troccaz M (2013) 5α-androst-16-en-3α-ol β-D-glucuronide, precursor of 5α-androst-16-en-3α-ol in human sweat. Chem Biodivers 10:2197–2208

Starkenmann C, Niclass Y, Cayeux I, Brauchli R, Gagnon A-C (2014) Odorant volatile sulfur compounds in cat urine: occurrence of (+/−)-3,7-dimethyloct-3-sulfanyl-6-en-1-ol and its cysteine conjugate precursor. Flavour Fragrance J 30:91–100

Steiner JE (1979) Human facial expressions in response to taste and smell stimulation. In: Reese HW, Lipsitt LP (eds) Adv Child Dev Behav, vol 13. Academic Press, New York, pp 257–293

Steingass CB, Grauwet T, Carle R (2014) Influence of harvest maturity and fruit logistics on pineapple (*Ananas comosus* L. Merr.) volatiles assessed by headspace solid phase microextraction and gas chromatography-mass spectrometry (HS-SPME-GC/MS). Food Chem 150:382–391

Tannock GW, Fuller R, Smith SL, Hall MA (1990) Plasmid profiling of members of the family *Enterobacteriaceae*, lactobacilli, and Bifidobacteria to study the transmission of bacteria from mother to infant. J Clin Microbiol 28:1225–1228

Tominaga T, Peyrot des Gachons C, Dubourdieu D (1998) A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon blanc: S-cysteine conjugates. J Agric Food Chem 46:5215–5219

Troccaz M, Starkenmann C, Niclass Y, van de Waal M, Clark AJ (2004) 3-methyl-3-sulfanylhexan-1-ol as a major descriptor for the human axilla-sweat odour profile. Chem Biodivers 1:1022–1035

Troccaz M, Borchard G, Vuilleumier C, Raviot-Derrien S, Niclass Y, Beccucci S, Starkenmann C (2009) Gender-specific differences between the concentrations of nonvolatile (R)/(S)-3-methyl-3-sulfanylhexan-1-ol and (R)/(S)-3-hydroxy-3-methylhexanoic acid odor precursors in axillary secretions. Chem Senses 34:203–210

Turlings TCJ, Tumlinson JH, Lewis WJ (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250: 1251–1253

Urbaniak C, Cummins J, Brackstone M et al (2014) Microbiota of human breast tissue. Appl Environ Microbiol 80:3007–3014

Varendi H, Porter RH, Winberg J (2002) The effect of labor on olfactory exposure learning within the first postnatal hour. Behav Neurosci 116:206–211

Wagenstaller M, Buettner A (2013) Quantitative determination of common urinary odorants and their glucuronide conjugates in human urine. Metabolites 3:637–657

Wysocki CJ, Zeng XN, Preti G (1993) Specific anosmia and olfactory sensitivity to 3-methyl-2-hexenoic acid: a major component of human axillary odor. Chem Senses 18:652

Zeng C, Spielman AI, Vowels BR, Leyden JJ, Biemann K, Preti G (1996) A human axillary odorant is carried by apolipoprotein D. Proc Natl Acad Sci U S A 93:6626–6630