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Abstract

Humans' early olfactory perception has been studied mainly within the framework of mother-offspring interactions and only a few studies have focused on newborns' abilities to discriminate body odors *per se*. The aim of this study was to develop a method to evaluate olfactory social preferences of infants at term-equivalent age. Twenty dyads of infants (10 born preterm and 10 born at term) at term-equivalent age and their mothers were included. We analyzed the behavioral reactions of infants to their mother's upper-chest odor (that bears social, non-food related information). The two impregnated gauzes and a control gauze were presented to the infants for 10 seconds each, in a random order. We compared two durations of gauze impregnation: 30 minutes and 12 hours. This study reveals that mothers' upper chest emits sufficient olfactory information to induce reactions in infants born full-term or born preterm and that a short impregnation is preferable to evaluate their perception of body odors, notably for those born preterm.

Key words: methodology, odor perception, behavior, full-term, preterm, infants.

Introduction

From birth, newborns are exposed to several persons, among whom some of them will become familiar, in particular fathers and siblings. They will develop preferences for familiar people beyond the well-studied one for the mother, on a more or less short scale. Cues, be they visual, acoustic or olfactory, will trigger the expression of behaviors reflecting an attraction or a preference for the emitter of these cues (voice: DeCasper and Fifer, 1980; faces: Pascalis et al., 1995; Quinn et al., 2002). Olfactory cues may allow the emergence of social preference from birth. Indeed, the ability of newborns to perceive, discriminate and recognize natural or artificial odors has been demonstrated extensively by Schaal and collaborators (1995, 1998, 2000). Through prenatal exposure, newborns are able to discriminate odors of their own amniotic fluid or some of its olfactory components (related to maternal diet during gestation) from amniotic fluids of other newborns, and they prefer familiar over unfamiliar odors (Schaal et al., 1995, 1998, 2000). After a brief post-natal familiarization, full-term but also preterm newborns are able to discriminate between familiar and novel odors (Balogh and Porter, 1986; Goubet et al., 2002).

However, overall, few studies have investigated infants' perception of body odor *per se*. Most studies focus on reactions to feeding-related odors, even when maternal odor is concerned (Macfarlane, 1975; Makin and Porter, 1989; Doucet et al., 2009). Attempts to test newborns' reactions to maternal odor from body areas not associated with feeding have been made, but they yield ambiguous results. Maternal forehead odors did not elicit any clear reactions in newborns (Doucet et al., 2009), but neck odors could help newborns to discriminate their mother from an unfamiliar woman (Schaal, 1986a), as do axillary odors but only for breast-fed (and not bottle-fed) newborns (Cernoch and Porter, 1985). One explanation for these discrepancies is that corporal secretions vary between body areas. Various glands are spread heterogeneously all over the skin, but only some of them emit rich

complex odors bearing information concerning individual characteristics. More precisely, apocrine glands, present in limited skin areas, such as armpits and chest, are particularly odorous and the major source of body odors. On the contrary, sebaceous glands, mainly present on the forehead, face and scalp, are only weakly odorant (for a review, see Doty, 1981). Adults' axillary odors carry information about sex (Penn et al., 2007; Troccaz et al., 2009), age (Kippenberger et al., 2012), emotions (Chen and Haviland-Jones, 2000; Ackerl et al., 2002; Prehn et al., 2006) and even identity (Schleidt et al., 1981).

A reliable method to test newborns' reactions to body odors would provide a major contribution to facilitate assessment of their social, non-maternal, olfactory preferences. Indeed, divergences in methods prevent us from drawing solid conclusions concerning newborns' olfactory abilities to perceive social non-feeding odors. Only a few authors questioned whether newborns were able to discriminate between non-maternal odors. Cernoch and Porter (1985) concluded that newborns did not discriminate their father's armpit odor from that of another man. However, whether this was due to a lack of recognition *per se* or whether odors from this area are not relevant for infants remains unanswered. Indeed, the axillary area, although it is odorous, is not an area to which infants are the most exposed. Chest or neck may be far better candidates for emitting olfactory information an infant could perceive and recognize.

Furthermore, very long (a whole night) gauze impregnation durations appear to have been used for most studies on newborns, whereas odors collected by expositions of only a few minutes (i.e. emotion recognition) were sufficient for adults (Chen and Haviland-Jones, 2000). Sullivan and Toubas (1998) obtained significant results for newborns with odor impregnations lasting 2 hours. Thus gauze-impregnation duration could be a major factor influencing studies of body odor discrimination, as it could be a constraint and, in any case, the question of the best impregnation time remains unsolved.

The present study aims to develop an easy-to-use procedure for evaluating newborns' reaction to social olfactory cues, *i.e.* whether they express behavioral responses to particular odors from their social environment. The first step involved identifying a body area where odors, even after only a short impregnation duration, carried social, non-feeding-related information that can elicit an infant's behavioral reaction. The body odor of the upper chest (between the base of the neck and the breasts, away from nipples) appeared to be a good candidate, for two reasons: 1) it is an area to which adults naturally guide a baby's head when cuddling or during skin-to-skin interactions; and 2) it is an area rich in apocrine glands, particularly known to produce odorous components, relevant for body odor recognition (Schleidt et al., 1981). Although apocrine glands are present in several body areas including breasts, their odorous components differ from those of the areolar glands known to trigger breast-fed newborns' appetitive behaviors (Doucet et al. 2009). Here, we used maternal odors to test the pertinence of the targeted skin area, as cues from the mother are the most likely to trigger infants' behavioral responses (odors: Marlier et al, 1998a,b; voice: DeCasper & Fifer, 1980; face: Pascalis et al, 1995). If the upper chest brings enough olfactory components, infants should respond differently to a gauze impregnated with their mother's odor than to a control gauze. We also compared two durations of gauze impregnation: 30 minutes and 12 hours to estimate the possibility of using shorter impregnation times than those used in earlier studies. Finally, we observed the responses of infants born preterm or born full term when they were at term age. Preterm and full-term infants receive different sensory experiences after birth, in particular concerning the amount and frequency of contacts with their mother's skin and also due to the possible overload of olfactory stimulations in the neonatal intensive care unit (Bartocci et al., 2001). Infants born preterm also have smaller reaction thresholds to sensory stimulations than those born full-term (André et al., in press). Therefore, to test the

impact of a premature birth on the response to maternal olfactory cues we observed 2-day-old infants born at term and several-week-old infants born preterm.

Materials and Methods

Recruiting and testing protocols followed the Declaration of Helsinki, and were approved by the ethical committees of Brest and Rennes Regional and University Hospital Centres.

The adults and infants' parents gave written agreement to participate to the study and for video-recording. Informed consent was obtained for each experimentation. They could (but were not obliged) sign an additional document which allows the diffusion of their images or videos for scientific communication. Participant anonymity was insured by identifying individuals by a number.

1. Participants

Our subjects were 20 infants at term-equivalent age:

i) 10 infants born preterm (6 girls, 4 boys) at 30.93 ± 2.54 s.d. gestational weeks and tested when they had reached 37 - 39 weeks post-conception age (postnatal ages 51 ± 19.44 s.d. days).

ii) 10 infants born at term (4 girls, 6 boys) at 40.87 ± 0.69 s.d. weeks and tested when 2 days old.

Infants with major known congenital, neurological and sensory perception disorders and/or analgesic or sedative treatment were excluded. All participants had an AGPAR score above 7 at 5'. They were tested at two university hospitals (Brest and Rennes, France) in the neonatal intensive care units (NICU) and maternity wards. At both sites, NICU followed developmental care guidelines, which recommend in particular that, as much as possible, lights and sounds are reduced and infants' activity rhythm respected. These guidelines encourage parents to visit whenever they want (day or night), and for as long as they wish.

Parents are strongly encouraged to perform skin-to-skin and routine care when present. All preterm infants were fed maternal milk (7 were partly breast-fed, 1 was bottle-fed, 2 received food through a nasogastric tube). Six infants born at term were breast-fed and 4 were bottle-fed with milk formula. All infants born preterm had skin-to-skin experiences with their mother at least one hour per day, each day, for several weeks following birth (variable according to the gestational age at birth and health). All infants born at term had at least one skin-to-skin experience with their mother for at least one hour during their first days of life. However, the exact amount of skin-to-skin received by each infant in either group could not be assessed.

2. Procedure

During the experiment, three different gauzes (5*5 cm) were presented to the infants. Two gauzes were impregnated with their mother odor: a) one for 30 minutes (Gshort), b) the other for 12 hours (Glong). The third one was not impregnated and served as control (Gcontrol).

2.1. Body odor collection

We collected maternal odor by fastening gauze swabs (Gazin®, 5 x 5cm, 100% cotton, Lohmann & Rauscher) with cloth surgical tape (Medipore™, 3M) on mothers' upper chest (between the base of neck and breasts) (Fig. 1).

Before fixing each gauze, a mother was asked to apply a disinfectant gel on her chest to remove any odor of perfume, laundry or shower gel and thus avoid differences due to environmental odors other than body odor.

Glong was fixed in the evening before the day of the experiment and was left there for 12 hours, *i.e.* a whole night. Gshort was fixed the following morning, for the last 30 minutes before both gauzes were removed simultaneously (example: Glong positioned at 9 p.m.,

Gshort positioned at 8.30 a.m., both gauzes removed at 9 a.m.). Gcontrol was prepared on the morning of the experiment, when the second gauze was fixed on the mother's chest. A drop of disinfectant gel was applied under the lid of the Petri dish so that all three gauzes had been in contact with the disinfectant gel and could have absorbed this odor. The three gauzes were manipulated with medical tweezers and kept in closed sterile Petri dishes at ambient temperature in the infant's room, until the session began. As odors are preserved under these conditions for several hours (e.g. 11.5 jours: Cernoch and Porter, 1985), we decided that a session could occur at any time during the 12 hours following gauze removal. This allowed us to be opportunistic so that we could test infants in similar conditions when they were awake and there were no environmental disturbances.

2.2. Body odor presentation

The infants were tested in their crib in their hospital room. A test began when the infant was awake and alert, rated stage 3 (eyes open, no brisk movements) on Prechtl's scale (1974), a stage commonly used to investigate infants' sensory perception (e.g. Andrews and Fitzgerald, 1994; Soussignan et al., 1997; Barbu-Roth et al., 2009). Infants were only tested when they had woken up spontaneously (not by parents or experimenter) and mostly after their meal or during a short break during a meal. All infants were lying on their back with their head sometimes turned naturally to one side. The experimenter presented the gauzes about 5 cm away from his/her nose with medical tweezers and for 10 seconds (so that several respiratory cycles were covered (Marlier et al., 2001)). These distance and duration of gauze presentation have been validated by several studies of newborns' odor perception (Cernoch and Porter, 1985; Soussignan et al., 1997; Schaal et al., 2000; Goubet et al., 2002; Delaunay-El Allam et al., 2006; Doucet et al., 2009). The gauzes were presented one after the other, with a minimum interval of 20 seconds. A gauze was presented either directly (*i.e.* 20 seconds later) after removing the previous gauze (when the infant was still calm) or later (after the

infant had calmed down if he/she had moved after the previous gauze). The gauze was placed according to the infants' head orientation so that it was as much as possible in front of their nose (gauzes were always at 5 cm of the nose but sometimes not directly in front of it due to clutter within or around the crib preventing proper access to the infant); thus 36.67 % of the infants were tested when on their right side, 51.67% on their left side and 11.66% when they were lying on their back and facing forward. When the infant turned his/her head away from the gauze, the experimenter moved the stimulus accordingly so the gauze stayed at 5 cm from the nose for the whole presentation. The order of presentation of the three gauzes varied randomly among subjects.

3. Data recording and analyses

3.1. Behavior recording

All sessions were video recorded using a Sony HDR-PJ350E camera, placed on a tripod facing the subject (approximately 1 meter from the infant). The videos were analyzed later, data were analyzed using 0/1 scan sampling (absence/presence of a behavior), with a scan every 0.2 second for 10 seconds from the time a gauze was placed in front of the infant. The experiments were all realized by the same experimenter (V.A.) whereas the videos were analyzed using a Solomon Coder© by a naive observer (A.V. who was blind to the type of gauze presented). V.A. analyzed one video randomly to allow us to assess inter-observer agreement for several categories of behaviors with a Cohen's kappa. All coefficients were above 0.93.

In literature, infants' olfactory perception has often been evaluated by measuring sucking and arousal/withdrawal responses, such as opening of the eyes, retraction of the head, facial grimacing or arm/leg movements (Sarnat, 1978; Gauthaman et al., 1984). More recently, authors have focused on facial expressions (Pihet et al., 1997; Soussignan et al.,

1997; Faas et al., 2000; Goubet et al., 2002) and movements indicating attraction or avoidance (Schaal et al., 1995, 1998, 2000; Marlier et al., 1998a, 1998b). We considered here that an infant reacted when a behavioral change (movement or facial expression) occurred within the 10-second gauze presentation. These reactions were characterized as attraction (*e.g.* head approaching, turning head or eyes towards the stimulus) or avoidance (*e.g.* moving head backwards, turning head or eyes away) based on the literature (see Table 1 for more details). We noted the numbers of occurrences of all behaviors.

As some parts of an infant's body were not always visible on the video (e.g. a gauze could hide part of his/her face), we adjusted the number of occurrences of each behavior by dividing this number by the exact duration during which it was observed and multiplying the result by 10, the total duration of a presentation.

3.2. Statistical analyses

Friedman test and post-hoc Wilcoxon tests were used to compare the number of behavioral occurrences between the three presentations (Gcontrol, Gshort, Glong) for both groups of participants (infants born preterm or full-term). Categories of behaviors were compared by Wilcoxon tests for each gauze presentation, data for the three gauzes were analyzed separately. Mann Whitney tests were used to compare data between infants born preterm and full-term. Bonferroni corrections were used for multiple comparisons. All statistics were computed with R© and Statistica©.

Results

Even if they didn't show any preferences for one of the gauzes (Friedman tests for body movements or facial expressions either for attraction or for avoidance, $p>0.1$), infants expressed significantly more "attraction" (approach head, turn head or eyes towards the stimulus) than "avoidance" (move head backwards, turn head or eyes away) when either

Glong or Gshort was presented (Wilcoxon, Glong: Z=2.605, P=0.009; Gshort: Z=3.124, P=0.002). When Gcontrol was presented, infants showed as much "attraction" as they did "avoidance" (Z=1.695, P=0.090). Only the full-term newborns followed this pattern (Glong: Z=2.521, P=0.012; Gshort: Z=2.073, P=0.038; Gcontrol: Z=1.836, P=0.066). Infants born preterm expressed more "attraction" when exposed to Gshort but not when exposed to Glong or Gcontrol (Glong: Z=0.839, P=0.402; Gshort: Z=2.310, P=0.021; Gcontrol: Z=0.539, P=0.590).

These results were supported by analyses of facial expressions: infants expressed more attraction than avoidance when they were presented Gshort (Wilcoxon, N=20, Z=2.197, p=0.028, Fig. 2b), but not when they were presented Glong or Gcontrol (Wilcoxon Glong: N=20, Z=0.815, p=0.415 / Gcontrol: N=20, Z=0.153, p=0.878). No significant differences could be evidenced when data from preterm and full-term infants were considered separately (Wilcoxon, $0.270<Z<1.604$, $p>0.05$ in all cases).

Discussion and conclusion

Our results validate a simple novel methodological tool for evaluating odor perception in infants at term-equivalent age. The novelty lies in two aspects: 1) one easy-to-access body part, *i.e.* the upper chest, emits sufficient olfactory information; 2) a short duration (only 30 min) of impregnation is sufficient to induce significant reactions. Full-term newborns were significantly attracted to their mother’s body odor whatever the duration of impregnation (30 minutes or 12 hours), while infants born preterm were only attracted by 30-minute impregnated gauzes and not by longer-impregnated gauzes. Furthermore, this method is not invasive, so it can be used with minimum perturbation of the infants.

This study confirms that infants at term-equivalent age are able to react to body odors. Indeed, whether the infants had already had a long and repeated experience with various

224 odors, including their mother's odors through skin-to-skin sessions (infants born preterm and
225 tested when 50 days old on average), or a short exposure to their mother's skin (infants born
226 at term and tested when 2 days old), they expressed attraction behaviors towards the
227 impregnated gauzes. Thus, even limited exposure (Delaunay-El Allam et al., 2006) and/or
228 prenatal experience (Wallace, 1977; Havlicek and Lenochova, 2008) are sufficient to elicit a
229 reaction to maternal body odors.

230 Furthermore, the fact that reactions were elicited by natural non-feeding-related odors,
231 that is by odors collected somewhere else than at the mother's breast, and mainly related to
232 movements and not facial expressions shows that here the responses were most probably
233 socially driven rather than feeding related. The procedure proposed in this paper could then be
234 extended to study further on infants' reaction to social odors beyond their mothers' ones, such
235 as odors from the father or siblings. As human body odors encode important social and
236 emotional information, it would be interesting to investigate infants' abilities to use this
237 information.

238 Indeed, many cues, among which odors are major at birth, guide the newborn toward
239 the main caregivers to whom she/he will bond. In mammals, the main caregiver is obviously
240 the mother. Still, in humans, other family members, most notably the father, can also play an
241 important role in caregiving and later in the child's development. As familiar odors have a
242 soothing effect on distressed newborns, even when they are not related to feeding activities
243 (Goubet et al., 2003, 2007; Rattaz et al., 2005), the father's body odors could well play this
244 role and be involved in father-infant bonding (Erlandsson et al., 2007). Still, few studies
245 investigated the possible attachment between father and infant and when they exist, they
246 mainly focused on the paternal point of view (Keller et al., 1985; Chen et al., 2017). To
247 investigate this relationship more thoroughly, we felt the need to develop this easy-to-use
248 method to test social olfactory discrimination.

A major aspect of our results shows that impregnation of gauzes with body odors can be as short as 30 minutes, which is much shorter than in all studies up to now (e.g. Cernoch and Porter, 1985). This is an important methodological issue that should facilitate acceptance of donors and experimentation. The significant responses only for short durations of impregnation show that longer durations may even hinder detection of the potentially higher sensitivity of infants, notably those born preterm. Why infants showed more attraction than avoidance mainly after a short duration of impregnation and not after a long one can be explained by a higher concentration of odorous components on the long duration gauze. Indeed, body odor components are volatile compounds (Dormont et al., 2013) and they were entrapped between the skin and the gauze for 12 hours. Their consequent high concentration may deter slightly the attraction they elicit at a lower concentration.

The slight difference in responses observed between infants born preterm and those born full-term can be surprising. In fact, comparative studies between full-term and preterm infants' capacities are scarce and contradictory (see review in Schaal et al., 2004), some studies report a lesser sensitivity or responsiveness to odors in preterm newborns than in full-term ones (Sarnat, 1978), others reporting similar results for both age groups (Goubet et al., 2002). At a cortical level, Frie *et al.* (2018) evidenced differences between preterm newborns, full term newborns and preterm infants tested at term-equivalent age while no behavioral differences could be observed between those groups. It is noteworthy that in all these studies, olfactory stimuli were artificial odors and not potentially relevant and socially connoted odors. To our knowledge, even fewer studies focus on the sensory response threshold of preterm infants at term-equivalent age. It is then difficult to state that our results corroborate previous ones or diverge completely. Still, in other sensory domains, infants born preterm are more sensitive to very subtle stimulations than infants born full-term. For instance, more preterm infants at term-equivalent age responded to a very subtle tactile stimulation than infants born at term

(André et al., in press). According to Schaal (1986b), the "range between the most pleasant and the most unpleasant odors is narrower" for newborns than for adults. In light of our results, it seems that the range may well be even narrower in infants born preterm, probably due to the numerous experiences they previously had with strong odors from detergents, disinfectants and so on. From 31 weeks postmenstrual age, these odors are perceived at a cortical level in olfactory, trigeminal and nociceptive processing areas and elicited pain-associated behaviors (Frie et al., 2018). We know that an over-load of nociceptive stimulations can impact the behavioral and cortisol responses of infants born preterm (Mitchell and Boss, 2002; Grunau et al., 2005). The sensitization to olfactory stimulations may then explain the difference we observed between infants born at term and those born preterm.

In conclusion, this study describes and validates an easy-to-use, non-invasive, tool to test infants' olfactory social preferences at term-equivalent age, including for infants born preterm. This should prove useful for future studies on discrimination of related and non-related human body odors, beyond maternal odors.

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Compliance with ethical standards

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Conflict of interest: The authors declare no conflicts of interest.

Ethical approval: All procedures performed in studies involving human participants 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.

Informed consent: Informed consent was obtained from the parents of all individual participants included in the study.

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433 Fig. 1. Position of gauzes on a mother's upper chest to collect body odors

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435

436 Table. 1. Infants' reactions to odor-impregnated gauzes. Attraction and avoidance categories
437 are based on Young and Décarie's (1977), Steiner's (1979), Ganchrow et al.'s (1983),
438 Soussignan et al.'s (1997) and Schaal et al.'s (2000) reports.

Categories	Types of behavior	Behaviors
Attraction	Facial expressions	Sticks tongue out
		Sucks
		Advances lips
		Puts lips commissures upward
		Opens mouth
	Body movements	Moves head nearer to the stimulus
		Turns head towards the stimulus
		Turns eyes towards the stimulus
Avoidance	Facial expressions	Places lips in an asymmetrical shape
		Puts lips commissures downwards
		Tightens lips
		Gapes
		Wrinkles nose
		Wrinkles eyes
	Body movements	Moves head backwards
		Turns head away
		Turns eyes away

439

440

Fig. 2. Full term (on the left, A & C) and preterm (on the right, B & D) infants' body movements (upper line, A & B) and facial expressions (lower line, C & D) of attraction and avoidance during the presentation of the three gauzes (Glong: gauze placed on maternal upper chest for 12 hours / Gshort: gauze placed on maternal upper chest for 30 minutes / Gcontrol: control gauze). Mean numbers (+/- standard error). Wilcoxon test, *: P<0.05.