

André, V., Henry, S., Vuillemin, A. *et al.* A novel, short and easy-to-perform method to evaluate newborns' social olfactory preferences. *Animal Cognition* (2020).

<https://doi.org/10.1007/s10071-020-01397-w>

Author's pre-print

Editor's version available at the following: <https://rdcu.be/b4vyt>

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1 **Title:** A novel, short and easy-to-perform method to evaluate newborns' social olfactory  
2 preferences

3 **Authors:** Vanessa André<sup>a</sup>, Séverine Henry<sup>a</sup>, Adelyne Vuillemin<sup>a</sup>, Alain Beuchée<sup>b,c</sup>, Jacques  
4 Sizun<sup>d,e</sup>, Jean-Michel Roué<sup>d,e</sup>, Alban Lemasson<sup>a</sup>, Laurent Misery<sup>e,f</sup>, Martine Hausberger<sup>g</sup> &  
5 Virginie Durier<sup>g\*</sup>

6 **Affiliations:**

7 <sup>a</sup> Université de Rennes, Ethologie animale et humaine - EthoS, UMR 6552 - CNRS -  
8 Université Caen Normandie, France.

9 <sup>b</sup> Unité de Réanimation Néonatale et Pédiatrique, Centre Hospitalier Universitaire de Rennes,  
10 France

11 <sup>c</sup> Laboratoire Traitement du Signal et de l'Image, Université de Rennes, France

12 <sup>d</sup> Pôle de la Femme, de la Mère et de l'Enfant, Centre Hospitalier Régional Universitaire,  
13 Brest, France.

14 <sup>e</sup> Laboratoire de Neurosciences de Brest, Faculté de Médecine et des Sciences de la Santé,  
15 Université de Bretagne Occidentale, EA 4685, Brest, France

16 <sup>f</sup> Département de Dermatologie, Centre Hospitalier Régional Universitaire, Brest, France.

17 <sup>g</sup> CNRS, Ethologie animale et humaine - EthoS, UMR 6552 - Université de Rennes 1 -  
18 Université de Caen Normandie, France

19

20 \* **Corresponding author:** [virginie.durier@univ-rennes1.fr](mailto:virginie.durier@univ-rennes1.fr), + 33(0)223235145

21 ORCID identifier: 0000-0002-7323-8779

22

23 **Abstract**

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

36

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

38

## 1        **Introduction**

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

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

26 complex odors bearing information concerning individual characteristics. More precisely,  
27 apocrine glands, present in limited skin areas, such as armpits and chest, are particularly  
28 odorous and the major source of body odors. On the contrary, sebaceous glands, mainly  
29 present on the forehead, face and scalp, are only weakly odorant (for a review, see Doty,  
30 1981). Adults' axillary odors carry information about sex (Penn et al., 2007; Troccaz et al.,  
31 2009), age (Kippenberger et al., 2012), emotions (Chen and Haviland-Jones, 2000; Ackerl et  
32 al., 2002; Prehn et al., 2006) and even identity (Schleidt et al., 1981).

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

44 Furthermore, very long (a whole night) gauze impregnation durations appear to have been  
45 used for most studies on newborns, whereas odors collected by expositions of only a few  
46 minutes (i.e. emotion recognition) were sufficient for adults (Chen and Haviland-Jones,  
47 2000). Sullivan and Toubas (1998) obtained significant results for newborns with odor  
48 impregnations lasting 2 hours. Thus gauze-impregnation duration could be a major factor  
49 influencing studies of body odor discrimination, as it could be a constraint and, in any case,  
50 the question of the best impregnation time remains unsolved.

51 The present study aims to develop an easy-to-use procedure for evaluating newborns'  
52 reaction to social olfactory cues, *i.e.* whether they express behavioral responses to particular  
53 odors from their social environment. The first step involved identifying a body area where  
54 odors, even after only a short impregnation duration, carried social, non-feeding-related  
55 information that can elicit an infant's behavioral reaction. The body odor of the upper chest  
56 (between the base of the neck and the breasts, away from nipples) appeared to be a good  
57 candidate, for two reasons: 1) it is an area to which adults naturally guide a baby's head when  
58 cuddling or during skin-to-skin interactions; and 2) it is an area rich in apocrine glands,  
59 particularly known to produce odorous components, relevant for body odor recognition  
60 (Schleidt et al., 1981). Although apocrine glands are present in several body areas including  
61 breasts, their odorous components differ from those of the areolar glands known to trigger  
62 breast-fed newborns' appetitive behaviors (Doucet et al. 2009). Here, we used maternal odors  
63 to test the pertinence of the targeted skin area, as cues from the mother are the most likely to  
64 trigger infants' behavioral responses (odors: Marlier et al, 1998a,b; voice: DeCasper & Fifer,  
65 1980; face: Pascalis et al, 1995). If the upper chest brings enough olfactory components,  
66 infants should respond differently to a gauze impregnated with their mother's odor than to a  
67 control gauze. We also compared two durations of gauze impregnation: 30 minutes and 12  
68 hours to estimate the possibility of using shorter impregnation times than those used in earlier  
69 studies. Finally, we observed the responses of infants born preterm or born full term when  
70 they were at term age. Preterm and full-term infants receive different sensory experiences  
71 after birth, in particular concerning the amount and frequency of contacts with their mother's  
72 skin and also due to the possible overload of olfactory stimulations in the neonatal intensive  
73 care unit (Bartocci et al., 2001). Infants born preterm also have smaller reaction thresholds to  
74 sensory stimulations than those born full-term (André et al., in press). Therefore, to test the

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

## 77 **Materials and Methods**

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

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

85

### 86 **1. Participants**

87 Our subjects were 20 infants at term-equivalent age:

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

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

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

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

109

## 110 **2. Procedure**

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

### 115 **2.1. Body odor collection**

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

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

122 Glong was fixed in the evening before the day of the experiment and was left there for  
123 12 hours, *i.e.* a whole night. Gshort was fixed the following morning, for the last 30 minutes  
124 before both gauzes were removed simultaneously (example: Glong positioned at 9 p.m.,



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

## 135 **2.2. Body odor presentation**

136 The infants were tested in their crib in their hospital room. A test began when the  
137 infant was awake and alert, rated stage 3 (eyes open, no brisk movements) on Prechtl's scale  
138 (1974), a stage commonly used to investigate infants' sensory perception (e.g. Andrews and  
139 Fitzgerald, 1994; Soussignan et al., 1997; Barbu-Roth et al., 2009). Infants were only tested  
140 when they had woken up spontaneously (not by parents or experimenter) and mostly after  
141 their meal or during a short break during a meal. All infants were lying on their back with  
142 their head sometimes turned naturally to one side. The experimenter presented the gauzes  
143 about 5 cm away from his/her nose with medical tweezers and for 10 seconds (so that several  
144 respiratory cycles were covered (Marlier et al., 2001)). These distance and duration of gauze  
145 presentation have been validated by several studies of newborns' odor perception (Cernoch  
146 and Porter, 1985; Soussignan et al., 1997; Schaal et al., 2000; Goubet et al., 2002; Delaunay-  
147 El Allam et al., 2006; Doucet et al., 2009). The gauzes were presented one after the other,  
148 with a minimum interval of 20 seconds. A gauze was presented either directly (*i.e.* 20 seconds  
149 later) after removing the previous gauze (when the infant was still calm) or later (after the

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

159

### 160 **3. Data recording and analyses**

#### 161 **3.1. Behavior recording**

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

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

175 1997; Faas et al., 2000; Goubet et al., 2002) and movements indicating attraction or avoidance  
176 (Schaal et al., 1995, 1998, 2000; Marlier et al., 1998a, 1998b). We considered here that an  
177 infant reacted when a behavioral change (movement or facial expression) occurred within the  
178 10-second gauze presentation. These reactions were characterized as attraction (*e.g.* head  
179 approaching, turning head or eyes towards the stimulus) or avoidance (*e.g.* moving head  
180 backwards, turning head or eyes away) based on the literature (see Table 1 for more details).  
181 We noted the numbers of occurrences of all behaviors.

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

186

### 187 **3.2. Statistical analyses**

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

### 195 **Results**

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

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

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

### 213 **Discussion and conclusion**

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

222 This study confirms that infants at term-equivalent age are able to react to body odors.  
223 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.

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

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

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

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

#### 289 **Acknowledgements**

290 We are grateful to the hospital staff and especially Dr A. de La Pintièrre, the nursing staff S. le  
291 Gall and I. Paire, the team members of the NIDCAP program S. Bleunven, I. Olivard, B.  
292 Kerleroux, N. Ratynski and the research nurse S. Roudaut, for their help during this research.

293 We are also very grateful to all the participants, and their parents, for volunteering to be part  
294 of this study. We thank also Ann Cloarec for correcting the English. The study resulted from  
295 collaborations within the interdisciplinary Group of Scientific Interest GIS "Cerveau-  
296 Comportement-Société".

298

299 **Compliance with ethical standards**

300 *Funding:* This work was supported by the University of Rennes 1 and the CNRS and a  
301 doctoral fellowship from the French research ministry to V. André.

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

303 *Ethical approval:* All procedures performed in studies involving human participants were in  
304 accordance with the ethical standards of the institutional research committee and with the  
305 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

308

309 **References**

310 Ackerl, K., Atzmueller, M., and Grammer, K. 2002. The scent of fear. *Neuroendocrinol Lett.*  
311 23:79–84.

312 André, V., Durier, V., Beuchée, A., Roué, J.-M., Lemasson, A., Hausberger, M., Sizun, J.,  
313 and Henry, S. in press. Higher tactile sensitivity in preterm infants at term-equivalent age: a  
314 pilot study. *PLoS One*.

315 Andrews, K., and Fitzgerald, M. 1994. The cutaneous withdrawal reflex in human neonates:  
316 sensitization, receptive fields, and the effects of contralateral stimulation. *Pain.* 56:95–101.

317 Balogh, RenéD., and Porter, R.H. 1986. Olfactory preferences resulting from mere exposure  
318 in human neonates. *Infant Behav Dev.* 9:395–401.

319 Barbu-Roth, M., Anderson, D.I., Desprès, A., Provasi, J., Cabrol, D., and Campos, J.J. 2009.  
320 Neonatal Stepping in Relation to Terrestrial Optic Flow. *Child Dev.* 80:8–14.

321 Bartocci, M., Winberg, J., Papendieck, G., Mustica, T., Serra, G., and Lagercrantz, H. 2001.  
322 Cerebral Hemodynamic Response to Unpleasant Odors in the Preterm Newborn Measured by  
323 Near-Infrared Spectroscopy. *Pediatr Res.* 50:324–330.

324 Cernoch, J.M., and Porter, R.H. 1985. Recognition of Maternal Axillary Odors by Infants.  
325 *Child Dev.* 56:1593–1598.

326 Chen, D., and Haviland-Jones, J. 2000. Human olfactory communication of emotion. *Percept*  
327 *Mot Skills.* 91:771–781.

328 Chen, E.-M., Gau, M.-L., Liu, C.-Y., and Lee, T.-Y. 2017. Effects of Father-Neonate Skin-to-  
329 Skin Contact on Attachment: A Randomized Controlled Trial. *Nurs Res Pract.* 2017:8612024.



- 330 DeCasper, A.J., and Fifer, W.P. 1980. Of human bonding: newborns prefer their mothers'  
331 voices. *Science*. 208:1174–1176.
- 332 Delaunay-El Allam, M., Marlier, L., and Schaal, B. 2006. Learning at the breast: preference  
333 formation for an artificial scent and its attraction against the odor of maternal milk. *Infant*  
334 *Behav Dev*. 29:308–321.
- 335 Dormont, L., Bessièrè, J.-M., and Cohuet, A. 2013. Human Skin Volatiles: A Review. *J Chem*  
336 *Ecol*. 39:569–578.
- 337 Doty, R.L. 1981. Olfactory communication in humans. *Chem Senses*. 6:351–376.
- 338 Doucet, S., Soussignan, R., Sagot, P., and Schaal, B. 2009. The secretion of areolar  
339 (Montgomery's) glands from lactating women elicits selective, unconditional responses in  
340 neonates. *PLoS One*. 4:e7579.
- 341 Erlandsson, K., Dsilna, A., Fagerberg, I., and Christensson, K. 2007. Skin-to-Skin Care with  
342 the Father after Cesarean Birth and Its Effect on Newborn Crying and Prefeeding Behavior.  
343 *Birth*. 34:105–114.
- 344 Faas, A.E., Spontón, E.D., Moya, P.R., and Molina, J.C. 2000. Differential responsiveness to  
345 alcohol odor in human neonates: Effects of maternal consumption during gestation. *Alcohol*.  
346 22:7–17.
- 347 Frie, J., Bartocci, M., Lagercrantz, H., and Kuhn, P. 2018. Cortical Responses to Alien Odors  
348 in Newborns: An fNIRS Study. *Cereb Cortex*. 28:3229–3240.
- 349 Ganchrow, J.R., Steiner, J.E., and Daher, M. 1983. Neonatal facial expressions in response to  
350 different qualities and intensities of gustatory stimuli. *Infant Behav Dev*. 6:189–200.
- 351 Gauthaman, G., Jayachandran, L., and Prabhakar, K. 1984. Olfactory reflexes in newborn  
352 infants. *Indian J Pediatr*. 51:397–399.
- 353 Goubet, N., Rattaz, C., Pierrat, V., Allémann, E., Bullinger, A., and Lequien, P. 2002.  
354 Olfactory Familiarization and Discrimination in Preterm and Full-Term Newborns. *Infancy*.  
355 3:53–75.
- 356 Goubet, N., Rattaz, C., Pierrat, V., Bullinger, A., and Lequien, P. 2003. Olfactory experience  
357 mediates response to pain in preterm newborns. *Dev Psychobiol*. 42:171–180.
- 358 Goubet, N., Strasbaugh, K., and Chesney, J. 2007. Familiarity Breeds Content? Soothing  
359 Effect of a Familiar Odor on Full-Term Newborns. *J Dev Behav Pediatr*. 28:189–194.
- 360 Grunau, R.E., Holsti, L., Haley, D.W., Oberlander, T., Weinberg, J., Solimano, A., Whitfield,  
361 M.F., Fitzgerald, C., and Yu, W. 2005. Neonatal procedural pain exposure predicts lower  
362 cortisol and behavioral reactivity in preterm infants in the NICU. *Pain*. 113:293–300.
- 363 Havlicek, J., and Lenochova, P. 2008. Environmental Effects on Human Body Odour. In: J.L.  
364 Hurst, R.J. Beynon, S.C. Roberts, and T.D. Wyatt, eds. *Chemical Signals in Vertebrates* 11.  
365 Springer New York. pp. 199–210.

- 366 Keller, W.D., Hildebrandt, K.A., and Richards, M.E. 1985. Effects of extended father-infant  
367 contact during the newborn period. *Infant Behav Dev.* 8:337–350.
- 368 Kippenberger, S., Havlíček, J., Bernd, A., Thaçi, D., Kaufmann, R., and Meissner, M. 2012.  
369 'Nosing Around' the human skin: What information is concealed in skin odour? *Exp*  
370 *Dermatol.* 21:655–659.
- 371 Macfarlane, A. 1975. Olfaction in the development of social preferences in the human  
372 neonate. *Parent-Infant Interact.* 33:103–113.
- 373 Makin, J.W., and Porter, R.H. 1989. Attractiveness of lactating females' breast odors to  
374 neonates. *Child Dev.* 803–810.
- 375 Marlier, L., Schaal, B., Gaugler, C., and Messer, J. 2001. Olfaction in premature human  
376 newborns: detection and discrimination abilities two months before gestational term. In:  
377 *Chemical Signals in Vertebrates 9.* Springer. pp. 205–209.
- 378 Marlier, L., Schaal, B., and Soussignan, R. 1998a. Bottle-fed neonates prefer an odor  
379 experienced in utero to an odor experienced postnatally in the feeding context. *Dev*  
380 *Psychobiol.* 33:133–145.
- 381 Marlier, L., Schaal, B., and Soussignan, R. 1998b. Neonatal responsiveness to the odor of  
382 amniotic and lacteal fluids: a test of perinatal chemosensory continuity. *Child Dev.* 69:611–  
383 623.
- 384 Mitchell, A., and Boss, B.J. 2002. Adverse effects of pain on the nervous systems of  
385 newborns and young children: a review of the literature. *J Neurosci Nurs.* 34:228–236.
- 386 Pascalis, O., Schonen, S. de, Morton, J., Deruelle, C., and Fabre-Grenet, M. 1995. Mother's  
387 face recognition by neonates: A replication and an extension. *Infant Behav Dev.* 18:79–85.
- 388 Penn, D.J., Oberzaucher, E., Grammer, K., Fischer, G., Soini, H.A., Wiesler, D., Novotny,  
389 M.V., Dixon, S.J., Xu, Y., and Brereton, R.G. 2007. Individual and gender fingerprints in  
390 human body odour. *J R Soc Interface.* 4:331–340.
- 391 Pihet, S., Mellier, D., Bullinger, A., and Schaal, B. 1997. Réponses comportementales aux  
392 odeurs chez le nouveau-né prématuré: étude préliminaire. *Enfance.* 50:33–46.
- 393 Prechtl, H.F.R. 1974. The behavioural states of the newborn infant (a review). *Brain Res.*  
394 76:185–212.
- 395 Prehn, A., Ohrt, A., Sojka, B., Ferstl, R., and Pause, B.M. 2006. Chemosensory anxiety  
396 signals augment the startle reflex in humans. *Neurosci Lett.* 394:127–130.
- 397 Quinn, P.C., Yahr, J., Kuhn, A., Slater, A.M., and Pascalis, O. 2002. Representation of the  
398 gender of human faces by infants: a preference for female. *Perception.* 31:1109–1121.
- 399 Rattaz, C., Goubet, N., and Bullinger, A. 2005. The calming effect of a familiar odor on full-  
400 term newborns. *J Dev Behav Pediatr.* 26:86–92.
- 401 Sarnat, H.B. 1978. Olfactory reflexes in the newborn infant. *J Pediatr.* 92:624–626.

- 402 Schaal, B. 1986a. Presumed olfactory exchanges between mother and neonate in humans.  
403 Ethol Psychol Priv IEC Toulouse. 101–110.
- 404 Schaal, B. 1986b. Presumed olfactory exchanges between mother and neonate in humans.  
405 Ethol Psychol. 101–110.
- 406 Schaal, B., Hummel, T., and Soussignan, R. 2004. Olfaction in the fetal and premature infant:  
407 functional status and clinical implications. Clin Perinatol. 31:261–285.
- 408 Schaal, B., Marlier, L., and Soussignan, R. 1995. Responsiveness to the odour of amniotic  
409 fluid in the human neonate. Neonatology. 67:397–406.
- 410 Schaal, B., Marlier, L., and Soussignan, R. 1998. Olfactory function in the human fetus:  
411 Evidence from selective neonatal responsiveness to the odor of amniotic fluid. Behav  
412 Neurosci. 112:1438–1449.
- 413 Schaal, B., Marlier, L., and Soussignan, R. 2000. Human foetuses learn odours from their  
414 pregnant mother's diet. Chem Senses. 25:729–737.
- 415 Schleidt, M., Hold, B., and Attili, G. 1981. A cross-cultural study on the attitude towards  
416 personal odors. J Chem Ecol. 7:19–31.
- 417 Soussignan, R., Schaal, B., Marlier, L., and Jiang, T. 1997. Facial and Autonomic Responses  
418 to Biological and Artificial Olfactory Stimuli in Human Neonates: Re-Examining Early  
419 Hedonic Discrimination of Odors. Physiol Behav. 62:745–758.
- 420 Steiner, J.E. 1979. Human facial expressions in response to taste and smell stimulation. Adv  
421 Child Dev Behav. 13:257–295.
- 422 Sullivan, R.M., and Toubas, P. 1998. Clinical usefulness of maternal odor in newborns:  
423 soothing and feeding preparatory responses. Neonatology. 74:402–408.
- 424 Troccaz, M., Borchard, G., Vuilleumier, C., Raviot-Derrien, S., Niclass, Y., Beccucci, S., and  
425 Starkenmann, C. 2009. Gender-specific differences between the concentrations of nonvolatile  
426 (R)/(S)-3-methyl-3-sulfanylhexan-1-ol and (R)/(S)-3-hydroxy-3-methyl-hexanoic acid odor  
427 precursors in axillary secretions. Chem Senses. 34:203–210.
- 428 Wallace, P. 1977. Individual discrimination of humans by odor. Physiol Behav. 19:577–579.
- 429 Young, G., and Décarie, T.G. 1977. An ethology-based catalogue of facial/vocal behaviour in  
430 infancy. Anim Behav. 25, Part 1:95–107.
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433 Fig. 1. Position of gauzes on a mother's upper chest to collect body odors



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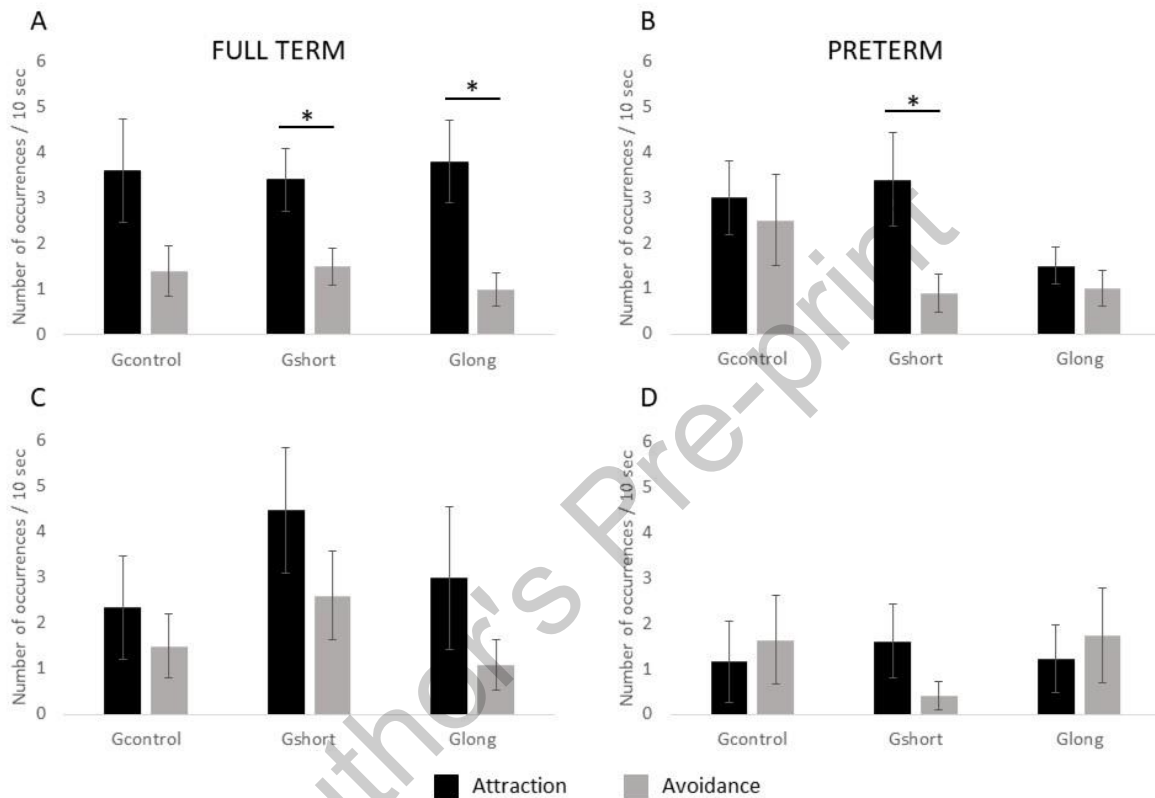
436 Table. 1. Infants' reactions to odor-impregnated gauzes. Attraction and avoidance categories  
 437 are based on Young and Décarie's (1977), Steiner's (1979), Ganchrow et al.'s (1983),  
 438 Soussignan et al.'s (1997) and Schaal et al.'s (2000) reports.

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

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



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