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► To cite this version:

Vanessa André, Séverine Henry, Adelyne Vuillemin, Alain Beuchee, Jacques Sizun, et al.. A novel, short and easy-to-perform method to evaluate newborns' social olfactory preferences. *Animal Cognition*, 2020, 23 (5), pp.843-850. <10.1007/s10071-020-01397-w>. <hal-02732989>

HAL Id: hal-02732989

<https://univ-rennes.hal.science/hal-02732989v1>

Submitted on 9 Jun 2020

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HAL Authorization

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

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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 ± 2.54 s.d. gestational weeks and
89 tested when they had reached 37 - 39 weeks post-conception age (postnatal ages 51 ± 19.44
90 s.d. days).

91 ii) 10 infants born at term (4 girls, 6 boys) at 40.87 ± 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[®], 5 x 5cm, 100% cotton,
117 Lohmann & Rauscher) with cloth surgical tape (Medipore[™], 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

290 **Acknowledgements**

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

294 We are also very grateful to all the participants, and their parents, for volunteering to be part
295 of this study. We thank also Ann Cloarec for correcting the English. The study resulted from
296 collaborations within the interdisciplinary Group of Scientific Interest GIS "Cerveau-
297 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

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



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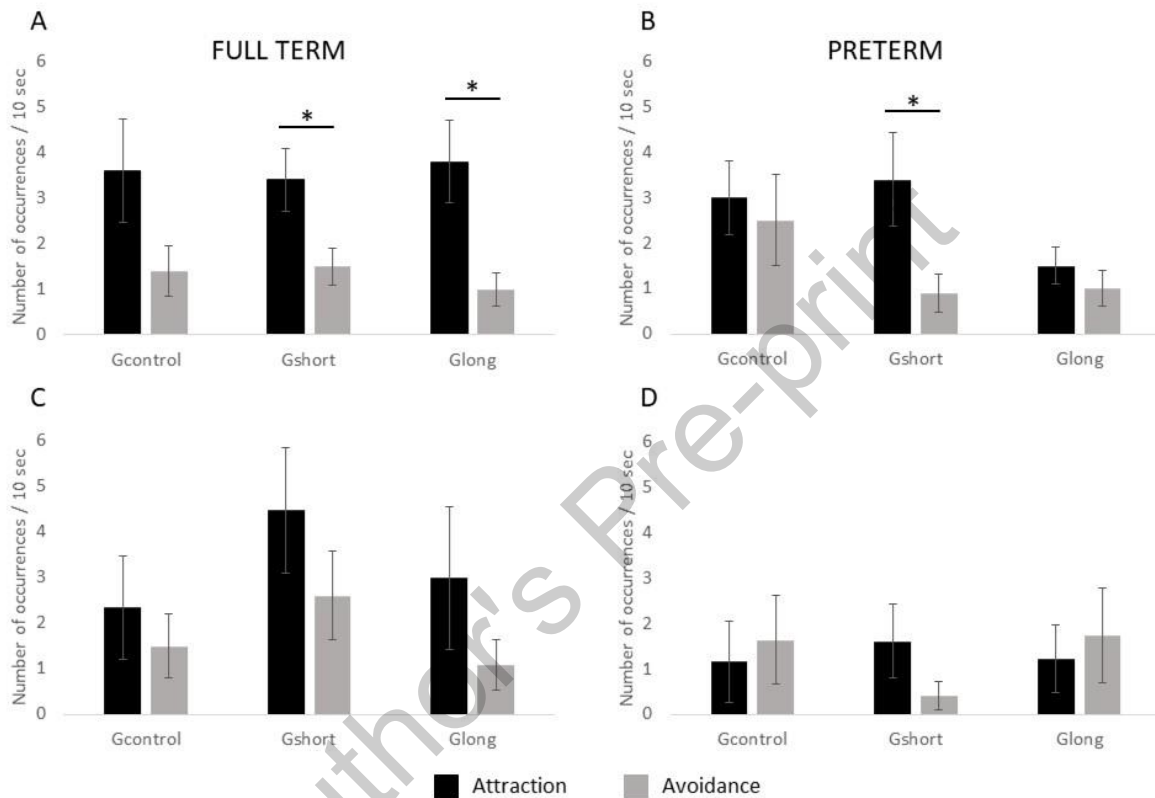
436 Table. 1. Infants' reactions to odor-impregnated gauzes. Attraction and avoidance categories
 437 are based on Young and Décarie's (1977), Steiner's (1979), Ganchrow et al.'s (1983),
 438 Soussignan et al.'s (1997) and Schaal et al.'s (2000) reports.

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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