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1 **Brief olfactory learning drives perceptive sensitivity in newborn rabbits: new insights in**
2 **peripheral processing of odor mixtures and induction**

3

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16

17

18 **Abstract**

19 Perception of the wide, complex and moving odor world requires that the olfactory system
20 engages processing mechanisms ensuring detection, discrimination and environment
21 adaptation, as early as the peripheral stages. Odor items are mainly elicited by odorant mixtures
22 which give rise to either elemental or configural perceptions. Here, we first explored the
23 contribution of the peripheral olfactory system to configural and elemental perception through
24 odorant interactions at the olfactory receptor (OR) level. This was done in newborn rabbits,
25 which offer the opportunity to pair peripheral electrophysiology and well characterized
26 behavioral responses to two binary mixtures, AB and A'B', which differ in their component
27 ratio (A: ethyl isobutyrate, B: ethyl maltol), and that rabbit pups respectively perceived
28 configurally and elementally. Second, we studied the influence on peripheral reactivity of the
29 brief but powerful learning of one mixture component (odorant B), conditioned by association
30 with the mammary pheromone (MP), which allowed us to assess the possible implication of the
31 phenomenon called induction in neonatal odor learning. Induction is a plasticity mechanism
32 expected to alter both the peripheral electrophysiological responses to, and perceptual detection
33 threshold of, the conditioned stimulus. The results reveal that perceptual modes are partly
34 rooted in differential peripheral processes, the AB configurally perceived mixture mirroring
35 odorant antagonist interactions at OR level to a lesser extent than the A'B' elementally
36 perceived mixture. Further, the results highlight that a single and brief MP-induced odor
37 learning episode is sufficient to alter peripheral responses to the conditioned stimulus and
38 mixtures including it, and shifts the conditioned stimulus detection threshold towards lower
39 concentrations. Thus, MP-induced odor learning relies on induction phenomenon in newborn
40 rabbits.

41 **Keywords**

42 odor mixtures; configural perception; learning; peripheral processing; induction; newborn

43 **1. Introduction**

44 The world of scents is defined by a quasi-limitless number of odor molecules (odorants) and
45 their combinations in mixtures organisms have to deal with. Through behavioral and
46 psychophysical experiments, odor mixtures have been revealed as perceived in a non-linear
47 way since interactions between odorants such as synergy, overshadowing or masking may occur
48 [1, 2] and find their origin, at least in part, at the olfactory system peripheral level [3, 4]. Indeed,
49 in the olfactory mucosa lie olfactory receptor neurons (ORNs) expressing olfactory receptors
50 (ORs), which are mainly weakly selective [5-7]. As a consequence, odorants in mixture
51 combine their simultaneous actions on multiple ORs, resulting in antagonist or agonist
52 interactions through competitive or non-competitive (allosteric) effects [8-12]. At the end of
53 the integration process, certain of or all of the odorants that compose a mixture may be
54 perceived (elemental perception) or a new odor quality may arise, i.e., a configural odor quality
55 specific to the whole mixture and distinct from the element qualities (configural perception)
56 [13, 14]. These two modes may be shown as two poles defining the olfactory perception axis
57 along which there is a perceptive continuum, which goes through a dual perception of both
58 elements and configuration named weak configural perception [15].

59 Strikingly, elemental and configural perceptions are both functional from early in life. Thus,
60 newborn rabbits perceive weak configurally a binary AB mixture of element A (ethyl
61 isobutyrate) and B (ethyl maltol) [16-18], which is also perceived configurally in human adults
62 [e.g., 19] and adult mice [20]. The ratio of components is a crucial factor in this perception.
63 Indeed, the AB configural perception requires a 30/70 ratio of A/B (AB mixture) while a 68/32
64 ratio (A'B' mixture) elicits the AB elemental perception in newborn rabbits [21]; see [19] and
65 [20] for convergent results in humans and mice. While some neurophysiological corollaries of
66 AB and A'B' differentiated perceptions have been found in the newborn rabbit brain [22], the
67 question of the processing of these mixtures at the peripheral level and the contribution of this

68 level to elemental vs. configural perception has remained unexplored so far. The first aim of
69 the present study was to fill that gap in the newborn rabbit model, which offers the advantage
70 of being able to pair peripheral electrophysiology (e.g. [23, 24]) and well characterized
71 behavioral responses.

72 In olfaction, a major process that implements and modulates perception and contributes to
73 the immediate and long-term adaptation of organisms to their environment is plasticity, which
74 occurs from the peripheral captors. However, much attention has been paid to plasticity
75 mechanisms occurring in higher brain areas (i.e. [25-28]) and much less is known about
76 peripheral mechanisms. Perceptual olfactory plasticity, dealing with peripheral changes and
77 driven by experience, has been first described in human adults. Indeed, Wysocki and colleagues
78 have observed that when patients displaying androstenone specific anosmia were exposed to
79 androstenone for some minutes, three times a day during six weeks, perception of this odorant
80 was restored [29]. These changes have been termed “experienced incidental induction” and
81 because anosmia was suspected to result from a genetic deficit in peripheral captors, they have
82 been assigned to the olfactory system periphery. Repeated exposure to androstenone would
83 result in sensitization of some olfactory receptor neurons (ORNs) equipped with androstenone-
84 binding ORs. Such ORs would be initially at subthreshold density in anosmic patients and the
85 sensitization would increase their expression [29]. The periphery involvement has been
86 supported in experiments reporting that chronic exposures to odorants increased the peripheral
87 olfactory responses (measured through electro-olfactograms, EOG) in anosmic mice and
88 humans [30, 31]. These results concerned with anosmic patients and animals have been then
89 extended to normosmic ones. Subsequently, a body of experimental data, collected throughout
90 the animal kingdom (from insects to various vertebrates, including in humans) confirmed that
91 changes in peripheral reactivity can happened following odorant learning or exposure protocols;
92 such changes could result mainly in an increase [32-42] or sometimes in a decrease in responses

93 to target stimuli [43] (or even in no change [44,45]). In parallel with these changes, regulation
94 in the expression rate of some ORs [43, 46] or proteins involved in peripheral transduction [47]
95 has been reported.

96 Thus, the analysis of the literature currently leads to consider induction as a generic
97 phenomenon, with a "positive side" (enhancement of olfactory abilities) that would mainly
98 occur after discontinuous exposure and/or conditioning, whereas passive and continuous
99 exposure to odor cues would rather reveal a "negative side" (loss in olfactory reactivity or
100 habituation). From the mechanistic perspective, induction would quantitatively and/or
101 qualitatively alter the expression of peripheral captors (ORs) leading to an optimized peripheral
102 tuning, without ceasing to favor the detection of new odors [48, 49]. Importantly, in conjunction
103 with the peripheral effect, induction is considered to impact the olfactory perceptual detection
104 threshold [29]. This aspect is unfortunately weakly documented, although it directly links
105 peripheral processing and perceptual/behavioral performances.

106 In the context of induction, newborn rabbits constitute a relevant model to study to what
107 extent the induction phenomenon is involved in learning-induced plasticity in a newborn
108 mammal, an issue that has never been addressed to date. This constituted the second aim of our
109 study. Rabbit neonates can indeed learn a new stimulus (odorant or odor mixture) after a
110 remarkably powerful (unique, extremely rapid < 5 min) associative conditioning promoted by
111 the mammary pheromone (MP) (e.g., [16, 22, 50]), a signal naturally emitted by all lactating
112 females in their milk and used spontaneously by pups to localize the nipples and suck (e.g., [50,
113 51]). Twenty-four hours after the conditioning, rabbit pups display in response to the CS the
114 typical orocephalic behavior usually triggered by the MP itself. Thus, in close conjunction with
115 our analysis of the peripheral responses to mixtures vs. mixture components, we therefore tested
116 induction by exploring in naive vs. conditioned pups the two criteria changes observed in
117 humans [29], i.e., the consequences of MP-induced conditioning for odorant B (contained in

118 the AB and A'B' mixtures) on CS electrophysiological responses (EOG) and detection
119 threshold of this odorant.

120

121 **2. Methods**

122 *2.1. Animals*

123 New-Zealand rabbit pups (Charles River strain, France) originated from the breeding
124 facility of IUT Feyssine (Université Lyon 1, France). Adult males and females were housed
125 individually in cages under constant 12:12 light-dark cycle (light on at 7 am), temperature (21-
126 22°C) and *ad libitum* access to food and water. Nest boxes were fixed to the housing cages of
127 pregnant does two days prior to parturition, to allow nest building. The day of birth was
128 designated as postnatal day 0. To equalize the time of mother-young interaction, females were
129 allowed access to the nest box only once a day for 15 min at 11:30 am. This short daily period
130 of nursing respected the natural rhythm of maternal visits [52]. A total of 239 pups aged 2-4
131 days from 56 litters were used in this study, 84 for electrophysiological recordings (38 naive,
132 46 conditioned) and 155 for behavioral procedures. All experiments were performed in
133 accordance with ethic rules enforced by French law and were approved by the ethical committee
134 of Lyon 1 University (CEEA-55) and the French Ministry of Higher Education and Research
135 under the no. 9745.

136

137 *2. Stimuli*

138 The stimuli were the mammary pheromone (MP; 2-methylbut-2-enal; CAS# 497-03-0),
139 ethyl isobutyrate (odorant A; CAS# 97-62-1), ethyl maltol (odorant B; CAS# 4940-11-8), and
140 their mixtures (all the odorants were purchased from Sigma-Aldrich; Saint-Quentin-Fallavier,
141 France). The latter were AB (30/70 ratio), which was reported as generating a configural
142 perception of a pineapple odor in human adults (e.g., [19, 53]), adult mice [20], and weak

143 configural perception in newborn rabbits (e.g., [16-18, 22]), and A'B' (68/32 ratio), which
144 generates elemental perception in both human adults [19, 53], mice [20] and newborn rabbits
145 (e.g., [16-18, 22]).

146 For each odorant, stock solutions were prepared at 10^{-2} v/v, first in ethanol and then in
147 successive dilutions in distilled water (ethanol: 0.01% in stock solutions; this solvent has been
148 demonstrated as behaviorally neutral for rabbit pups, see [16]). To induce the associative
149 learning of odorant B, MP was used as unconditioned stimulus at 10^{-5} g/ml (as previously
150 validated (e.g., [16, 17, 22, 50])). The MP-B blend was prepared from 10^{-2} g/ml stock-solutions
151 of each component in distilled water, with a final concentration of 10^{-5} g/ml of MP and B at a
152 50/50 ratio.

153 In animals dedicated to the behavioral paired with electrophysiological recordings, two
154 groups were formed, each of which was stimulated with one or the other set of stimuli detailed
155 in Table 2. The two series of stimuli included A, B, AB and MP (AB series) or A', B', A'B'
156 and MP (A'B' series).

157 In rabbit pups devoted to the behavioral threshold determination, the conditioned
158 odorant B was tested at concentrations ranging from 10^{-5} to 10^{-22} g/ml in 9 distinct groups
159 (successive dilutions in distilled water; Table 1).

160

161 *3. Odor conditioning*

162 Conditioning sessions were run on postnatal days 1, 2 or 3 in an experimental room
163 close to the breeding room of the animal unit. They occurred 1h before the daily nursing (10:30
164 a.m.) to equalize the pups' motivational state and limit the impact of satiation on responses [54].
165 The pups were transferred by groups of 5/litter into a box maintained at room temperature. The
166 MP-induced conditioning was then run following a procedure previously validated (e.g. [16,
167 17, 22, 50])). Briefly, 6 ml of the B + MP blend were pipetted on a 10 x 15 cm cotton pad, which

168 was held 2 cm above the pups for 5 min. One minute after the end of conditioning, the pups
169 were individually marked and returned to their nest. Each procedure was run on 3 or 4
170 pups/litter; to avoid litter effect, only 1 or 2 of these pups were finally used for testing.

171

172 *4. Behavioral assay*

173 The behavioral assay consisted of an individual oral activation test previously validated
174 (e.g. [16, 17, 21, 22, 50, 51]). Briefly, each pup was gently maintained in one gloved hand of
175 the experimenter, allowing only head movements; with the other hand, the experimenter
176 presented the olfactory stimulus for 10 sec on the tip of a glass rod 0.5 cm in front of the nares.
177 The test was positive when the stimulus induced the on/off typical orocephalic response, i.e.,
178 head-searching usually followed by oral grasping movements. Spontaneously, this response is
179 highly selective, meaning that naive animals respond only to the MP. In our three procedures
180 involving behavior, each pup was tested with a maximum of 6 stimuli with an inter-trial interval
181 of 60 sec. The order of stimuli presentation was counterbalanced from one to another pup
182 (except for the MP presentation which always occurred last, as a control). If a pup responded
183 to a stimulus, its nose was softly dried before the next stimulation. The pups were immediately
184 reintroduced to their nest after testing. To minimize litter effects, each group was drawn from
185 several litters, with a maximum of 5 pups tested per litter.

186 *4.1. Procedure 1 - Behavioral test before electrophysiology: pre-recording test*

187 The naive animals were all tested with the 3 odor stimuli from either the AB or A'B'
188 series (Table 2), and systematically to the MP in order to assess that they did not respond
189 spontaneously to unfamiliar stimuli but to the MP. The conditioned animals were tested to the
190 same stimuli as the naive pups, 24h after odor learning, to determine the effectiveness of the
191 conditioning to B. All the animals were tested at 8:00 a.m., i.e., at least 30 min before the

192 beginning of electrophysiological recordings. Importantly, the same series was then used for
193 the electrophysiological recordings.

194 *4.2. Procedures 2 and 3 - Behavioral test for detection threshold determination*

195 We distinguished two procedures that used the same oral activation test in order to evaluate
196 first the spontaneous detection threshold (ST) of naive animals to odorant B, and second the
197 detection threshold to B after conditioning (CT) to that odorant.

198 Determining the spontaneous threshold to B was challenging due to the absence of
199 spontaneous behavioral response to that odorant in rabbit pups. Our strategy consisted in a
200 “learning failure” approach meaning that we determined the concentration level at which
201 odorant B could not be learned anymore. Thus, 7 groups of 1- or 2-day-old pups (15
202 newborns/group; n total = 105) were conditioned with the MP at 7 decreasing concentrations
203 of B from 10^{-10} to 10^{-21} g/ml (one concentration/group). All the pups were then orally tested the
204 day after at B 10^{-5} g/ml.

205 Regarding the post-conditioning threshold to B, 2 groups of 2-day-old pups (n = 25/group)
206 were conditioned to B at 10^{-5} g/ml by pairing with the MP, then tested 24h later either to B at
207 10^{-5} , 10^{-10} , 10^{-15} , 10^{-19} and 10^{-20} g/ml or at 10^{-5} , 10^{-17} , 10^{-21} and 10^{-22} g/ml, and systematically to
208 MP 10^{-5} g/ml.

209

210 *5. Electrophysiology*

211 The electrodes were homemade silver wire ended with a silver ball plated with AgCl
212 (200-500 μ m diameter, impedance \leq 100 k Ω). Each electrode was connected to an amplifier
213 (WPI, DAM80, amplifier, DC, gain 1000), the output signal of which was connected to a
214 computer via an acquisition card (analog to digital interface). The three electrodes were placed
215 on the surface of the mucosa using micromanipulators, each on a turbinate. The electrodes were
216 always placed in the distal part of the turbinates (Figure 1a). However, because the precision of

217 these locations cannot be based on any coordinate and animals naturally presented variations in
218 morphology (size, inclination and proximity of the turbinates to a greater or lesser extent), this
219 choice was only intended to standardize the measurements without any chemotopic information
220 being derived from them; knowing that the existence of chemotopy has not yet been proven.
221 Recording visualization and acquisition were made on-line using homemade Neurolabscope©
222 software. Data analysis was made off-line through a python script designed by S. Garcia
223 (CRNL, Lyon). Recordings are illustrated in Figure 1, which shows three simultaneously
224 recorded EOGs (raw signals) from the three turbinates and details the amplitude measure.

225

226 *6. Olfactory stimulation*

227 The olfactory stimuli were delivered in gas phase through an olfactometer derived from
228 a previous version [3]. The gaseous phase of odorants was however sucked off from vial by
229 bubbling 200 ml of odorized solution instead of U-shaped tubes filled with absorbent pellets.
230 This ensured that electrophysiological recordings were done by using exactly the same stimulus
231 concentrations as those used in previously published and the present behavioral experiments.
232 The stimulation consisted of 0.35 sec square pulses at an 80 ml/sec flow rate. The protocol
233 consisted of delivering the same stimulus 3 times successively at 1-min intervals; the 3
234 measures being averaged.

235

236 *7. Statistical analyses*

237 *7.1. Behavioral data*

238 Proportions of pups responding behaviorally were compared using the Cochran's Q test
239 when the data were dependent (i.e., pups from the same group tested for their response to the
240 three stimuli) or the χ^2 test of Pearson (with Yates correction when necessary) when the data
241 were independent (i.e., distinct groups tested for their response to the same stimulus). When the
242 Cochran's Q test was significant, proportions of responding pups were compared 2x2 by the χ^2

243 test of McNemar. Degrees of freedom are indicated when > 1 . Data were considered as
244 significant when the two-tailed tests yielded $p < 0.05$. Analyses were made with Statistica
245 software (StatSoft, Tulsa, OK, USA).

246 *7.2. Electrophysiological data*

247 A total of four sets of data were gathered: two sets from naive and two sets from
248 conditioned animals, each of which was stimulated by either the AB or the A'B' series. Mean
249 EOG amplitudes were compared inside each data set using non parametric paired-Friedman
250 test. Then, a Mann-Whitney *post hoc* paired test was used to compare stimuli by pair. For the
251 comparison between naive and conditioned groups, the same tests were used but for unpaired
252 data. The frequency of occurrence of each stimulus as being the least and the most effective
253 within the naive and conditioned animals were tested with the χ^2 test (comparison of k
254 proportions) and Monte Carlo method (distribution of the χ^2 distance based on simulations with
255 the total number of observations for the k groups, followed by the Marascuilo procedure, which
256 carries out to the pairwise comparisons (XLSTAT software, Microsoft, Redmond, USA). The
257 same tests were used to compare, for each stimulus, its occurrence as being the least and the
258 most effective stimulus in naive vs. conditioned pups.

259

260 **3. Results**

261 *3.1. Peripheral processing of odorants and mixtures*

262 *3.1.1. Naive animals: spontaneous behavior and peripheral processing*

263 From the 38 pups assigned to the naive group, 31 gave exploitable records, 5 did not and 2
264 were discarded because they did not respond to MP in the pre-recording behavioral test. Among
265 the 31 recorded pups, 15 were stimulated with the stimulus series AB comprising A, B, AB and
266 MP, while the remaining 16 pups were stimulated with the A'B' series including A', B', A'B'
267 and MP. The behavioral pre-recording test showed that all pups responded positively to the MP,

268 and none to the other odorants (in each series $Q > 40$, $p < 0.001$ and $\chi^2 > 13$, $p < 0.001$ for
269 global and 2x2 comparisons) (Figure 2; for simplification, results of the two series have been
270 pooled in the Figure).

271 The mean EOG amplitudes recorded in naive pups in response to the two-stimulus series are
272 shown in Figure 3. For the AB series, regardless of the stimulus, EOGs recorded in turbinate 1
273 (T1) were statistically smaller than those recorded in T2 and T3 (Friedman test, $p = 0.007$, T1
274 vs. T2: $p < 0.0001$, T1 vs. T3: $p = 0.002$). For the A'B' series, no statistical differences were
275 observed between the three turbinates.

276 In the AB series (Figure 3a), the MP induced the largest response in T2 (Wilcoxon: $0.04 \leq$
277 $p \leq 0.06$) and one of the largest in T1 and T3. Responses to the AB configural mixture were not
278 different from those of its components in T2-T3, lower than responses to A and MP in T1
279 (Wilcoxon: $p = 0.042$ and 0.037) and lower than response to MP in T2.

280 In the A'B' series (Figure 3b), the MP elicited one of the largest responses in T1 and the
281 largest one in T2 and T3 (Wilcoxon: $0.0001 \leq p \leq 0.05$). By contrast, the elemental mixture
282 A'B' elicited one of the lowest responses in T1 and T2. In particular, response to A'B' was
283 lower than that to the single A' component (A'B' vs. A' in T1 and T2: $p=0.002$ and <0.001),
284 despite the mixture concentration being very close to that of A'.

285 To sum up, regardless of the turbinates, the AB and A'B' mixtures spontaneously induced
286 differential responses compared to their components: the AB mixture tended to trigger
287 responses closer to its components than the A'B' mixture, which mostly induced lower
288 responses than A'. Strikingly, the MP appeared in the two series as the most, or one of the most,
289 powerful stimuli (despite a concentration lower than those of A, A', AB and A'B').

290

291 *3.1.2. Conditioned animals: post-learning behavior and peripheral processing*

292 From the 46 pups assigned to the conditioned group, 36 gave exploitable records, 3 did not
293 and 7 were discarded because they failed to give clear behavioral responses to B (CS). Among
294 these 36 newborn rabbits, 17 pups were stimulated with the AB series and 19 pups with the
295 A'B' series. In terms of behavior, within the AB series, pups strongly responded to B (94.1%)
296 and to the MP (100%) but not to A and AB (whole comparison: $Q = 47$, $df = 3$, $p < 0.001$; 2x2
297 comparisons between B or MP vs. A or AB: $\chi^2 > 14.06$, $p < 0.001$; B vs. MP: $\chi^2 < 0.5$, $p > 0.05$)
298 (Figure 2; to simplify reading, responses of the two series were pooled in the Figure).

299 Within the A'B' series, a strong proportion of pups responded behaviorally to B (89.5%)
300 and to the MP (100%) as for the AB series, but also to A'B' (84.2%); however, as for the AB
301 series, the pups did not respond to A' (0%) (whole comparison: $Q = 46$, $df = 3$, $p < 0.001$; 2x2
302 comparisons between B or MP or A'B' vs. A': $\chi^2 > 14.06$, $p < 0.001$; B vs. MP or AB and MP
303 vs. AB: $\chi^2 < 0.5$, $p > 0.05$) (Figure 2). Thus, as expected, conditioned pups responded
304 behaviorally more to B and to A'B' than naive animals ($\chi^2 > 20.5$, $p < 0.001$ in the 2x2
305 comparisons between conditioned vs. naive pups) and as much to the MP (100%) (Figure 2).

306 The mean EOG amplitudes recorded in conditioned pups in response to the two-stimulus
307 series are shown in Figure 4. In the AB series (Figure 4a), EOGs' amplitudes recorded in T1
308 differed from those recorded in T2 and T3 (Friedman test, $p < 0.0001$, T1 vs. T2 and T1 vs. T3,
309 $p < 0.001$); T1 EOGs being lower regardless of the stimulus. In addition, T2 and T3 responses
310 differed too (Friedman, $p = 0.024$); noticeably in T2, B induced larger EOGs than in T3
311 (Wilcoxon, $p = 0.041$). In T2, A and B both elicited larger responses than AB (Wilcoxon, $B >$
312 AB , $p = 0.008$; $A > AB$, $p = 0.001$). In T3, odorant A, the odorant with the highest concentration,
313 induced larger responses than AB (Wilcoxon, $p = 0.003$) and MP (Wilcoxon, $p = 0.029$).

314 In the A'B' series (Figure 4b), EOGs' amplitudes recorded in T1 differed from those recorded
315 in T2 and T3 (Friedman, $p < 0.001$, T1 vs. T2 $p < 0.0001$; T1 vs. T3 $p = 0.0002$); T1 EOGs
316 being lower regardless the stimulus. In T2, B' induced larger responses than A'B' and MP (B'

317 > A'B'; Wilcoxon, $p = 0.028$, $B' > MP$; Wilcoxon, $p = 0.031$). In T3, B' induced larger
318 responses than A'B' (Wilcoxon $p = 0.037$).

319 To sum up, regardless of the turbinates, after conditioning to the odorant B, the AB mixture
320 triggered lower responses compared to A, while the A'B' mixture triggered responses that were
321 close to those induced by its components. Strikingly, although the conditioned stimulus (B/B')
322 was the lowest concentration stimulus, it triggered the largest or one of the largest responses.

323

324 *3.1.3. Comparison of EOG amplitudes in naive vs. conditioned animals*

325 Regarding the AB series (Figure 5a), in conditioned pups the response to B increased in T2
326 ($p = 0.033$) while the response to MP decreased in the three turbinates ($p < 0.045$). Regarding
327 the A'B' series (Figure 5b), in conditioned pups the response to B' increased in T2 and T3 (p
328 < 0.05) as well as the responses to A' and A'B' in T2 ($p < 0.05$ and < 0.01). In T1, the response
329 to MP decreased in conditioned pups ($p = 0.037$).

330 Thus, the conditioning mainly impacted the responses to MP and B (B'): responses to MP
331 decreased while those to B (B') increased, regardless of the stimulus series. However, the
332 conditioning to B appeared to be more impacting on the responses to A'B' than to AB.

333

334 *3.1.4. Comparison of stimulus relative effectiveness in naive vs. conditioned animals*

335 Here, we addressed the question of the high variability in EOG amplitude for a same stimulus
336 (from one recording site to another, from one nasal cavity to another and from one animal to
337 another), by analyzing the differences in response between different stimuli in relative terms.
338 To that end, based on their amplitude, the EOG responses were ranked from the lowest to the
339 largest and the corresponding stimulus sequence was used to determine the least or the most
340 effective stimuli; only the sets of recording comprising exploitable responses to the 5 stimuli
341 (i.e., complete stimulus sequence) were taken into account. Doing so, we compared the

342 occurrence of each stimulus as being the least and the most effective one, first, within the naive
343 and conditioned groups for the AB and A'B' series, respectively, and then between the two
344 groups.

345 *3.1.4.1. Occurrence of stimuli as least effective (Figure 6a)*

346 Within the AB-series naive animals (Figure 6a-left, grey bars), all stimuli occurred as the
347 least effective with the same probability ($p > 0.5$). By contrast, within the A'B'-series naive
348 animals (Figure 6a-right, grey bars) statistical differences appeared between stimuli ($p <$
349 0.0001 , χ^2 and Monte Carlo method): the occurrence of A'B' differed from that of A' and MP,
350 A'B' being most frequently the least effective stimulus ($p < 0.05$, pairwise comparisons by the
351 Marascuilo procedure).

352 In conditioned animals, statistical differences existed in both stimulus series (χ^2 and Monte
353 Carlo method; $p < 0.0001$). In the AB series (Figure 6a-left, black bars), the stimulus pairwise
354 comparison (Marascuilo procedure) showed that AB and MP occurred more frequently as the
355 least effective stimuli compared to A. In the A'B' series (Figure 6a-right, black bars), A' was
356 most rarely observed as the least effective stimulus in comparison with B', A'B' and MP
357 ($p < 0.05$).

358 The comparisons between naive and conditioned groups showed that the occurrence of MP
359 as the least effective stimulus increased from 28% to 43% ($p < 0.05$, in the AB series) and from
360 18% to 34% ($p = 0.02$; in A'B' series) in conditioned neonates. Comparatively, the occurrence
361 of A'B' as the least effective stimulus decreased from 43% in naive pups to 29% in conditioned
362 ones ($p < 0.05$, bilateral test of two proportions, confirmed with Monte-Carlo method).

363 *3.1.4.2. Occurrence of stimuli as most effective (Figure 6b)*

364 Within both naive and conditioned groups, and in both AB and A'B' series, stimuli differed
365 in their occurrence as the most effective stimulus ($p < 0.0001$, χ^2 and Monte Carlo method). In
366 the AB-series naive animals (Figure 6b-left, grey bars), MP and A significantly differed from

367 B and AB ($p < 0.05$), and were most often the most effective stimuli. In the A'B'-series naive
368 animals (Figure 6b-right, grey bars), MP and A' differed from B and A'B', and B differed from
369 A'B' ($p < 0.05$). MP and A' were most often the most efficient stimuli especially compared to
370 A'B', which never induced the largest EOGs.

371 In the AB-series conditioned animals (Figure 6b-left, black bars), A and B differed from AB
372 ($p < 0.05$) and were the most effective stimuli. In the A'B'-series conditioned animals (Figure
373 6b-right, black bars), the odorants B, A' and MP were more frequently the most effective
374 stimulus compared to A'B' ($p < 0.05$).

375 Comparisons between naive and conditioned pups showed that the occurrence of B as the
376 most effective stimulus increased in conditioned vs. naive pups for both the AB and A'B' series
377 (14 vs. 34 % and 13 vs. 33% respectively; $p \leq 0.002$ for the two groups). Furthermore, the
378 occurrence of A'B' as the most effective stimulus increased from 1% to 15%, ($p = 0.001$). The
379 opposite result was observed for MP after conditioning: its occurrence as the most effective
380 stimulus decreased from 46 to 24% ($p = 0.001$).

381

382 To sum up, regardless of the AB and A'B' series, the comparison of relative effectiveness
383 between the stimuli showed that MP, the most effective stimulus in naive animals, lost this
384 status in conditioned pups: since, after conditioning its occurrence increased as the least
385 effective stimulus, while simultaneously decreased as the most effective one. At the same time,
386 the frequency of odorant B (i.e., the conditioned stimulus) as the most effective stimulus, alone
387 (for the AB series) or in the elemental mixture A'B' (for the A'B' series), increased in
388 conditioned animals, whereas the occurrence of B as the lowest effective cue did not change in
389 the two series.

390

391 *3.2. Detection threshold in naive and conditioned animals*

392 Our original assumption considered that MP-induced conditioning is based on induction.
393 This assumption requires theoretically that some plasticity happens at the periphery of the
394 olfactory system during/after the conditioning (see above), and also that the detection threshold
395 of the conditioned stimulus is influenced by the conditioning. Here, we therefore determined
396 the detection threshold of the odorant B, both before and after it has been learned by pairing
397 with the MP.

398 *3.2.1 Spontaneous detection threshold of the odorant B*

399 Because rabbit pups did not respond spontaneously to B, the determination of B spontaneous
400 threshold (ST) could not be evidenced in really naive animals but in animals which failed to
401 learn. This issue was addressed by using decreasing concentrations of B paired with a constant
402 concentration of MP, expecting that at a given concentration of B this odorant would cease to
403 be perceived and, therefore, would not be learned anymore. The ST was then set between the
404 last concentration of B supporting learning and the first concentration of B failing to induce it.
405 We tested 105 pups divided in 7 groups. Each group was conditioned by pairing B at 10^{-5} , 10^{-10} ,
406 10^{-15} , 10^{-17} , 10^{-20} , 10^{-21} or 10^{-22} g/ml with MP at 10^{-5} g/ml. All the pups were tested 24h later
407 for their orocephalic response to B alone at 10^{-5} g/ml.

408 The results showed that differences existed in pup responsiveness to B depending on the
409 concentration of the odorant used during the conditioning ($Q = 81.2$, $df = 7$, $p < 0.001$) (Figure
410 7a). A strong and similar proportion of pups responded to B after conditioning at 10^{-5} , 10^{-10} and
411 10^{-15} g/ml ($> 80\%$; $\chi^2 < 0.7$, $p > 0.05$ in all 2x2 comparisons) attesting that B was effectively
412 learned, i.e., that it was spontaneously perceived at these concentrations. The level of
413 responsiveness dropped dramatically between 10^{-15} and 10^{-17} g/ml (80 vs. 20%; $\chi^2 = 7.1$, $p <$
414 0.01) and then remained very low from 10^{-17} to 10^{-22} g/ml ($< 20\%$; $\chi^2 < 0.7$, $p > 0.05$ in all 2x2
415 comparisons).

416 Thus, rabbit pups could not learn the odorant B when its concentration fell below 10^{-15} g/ml,
417 setting ST for this odorant between 10^{-15} and 10^{-17} g/ml in our conditions.

418 3.2.2. *Post-conditioning detection threshold of the odorant B*

419 To determine the detection threshold of odorant B after MP-induced conditioning
420 (“conditioned threshold”, CT), two groups of 25 pups were conditioned to B 10^{-5} g/ml (paired
421 with MP 10^{-5} g/ml), then each tested 24h later to several concentration steps of B over a range
422 from 10^{-5} and 10^{-22} g/ml, and systematically to MP 10^{-5} g/ml in the end. In the two groups, the
423 responsiveness to B varied according to its concentration ($Q < 73.1$, $df = 3$ and 4 , $p < 0.001$).
424 The proportion of responding pups was strong and similar to B from 10^{-5} to 10^{-19} g/ml
425 ($> 80\%$; $\chi^2 < 3.2$, $p > 0.05$ in all 2x2 comparisons) but it strongly decreased at 10^{-20} g/ml (20%;
426 $\chi^2 > 16.05$, $p < 0.001$ in all 2x2 comparisons between 10^{-20} vs. stronger concentration steps)
427 and then remained low or null until 10^{-22} g/ml ($< 20\%$; $\chi^2 < 3.5$, $p > 0.05$ in all 2x2 comparisons)
428 (Figure 7b).

429 Between the ST and CT determination procedures, no difference contrasted the pups’
430 responsiveness to B at 10^{-5} , 10^{-10} , 10^{-15} , 10^{-20} , 10^{-21} and 10^{-22} g/ml ($\chi^2 < 0.5$, $p > 0.05$ in all
431 comparisons). Conversely, in the CT determination procedure, rabbit pups responded clearly
432 more to B at 10^{-17} and 10^{-19} g/ml than in the ST determination procedure ($\chi^2 > 20.6$, $p < 0.001$
433 in the two comparisons).

434 Thus, after a single and brief conditioning to B at 10^{-5} g/ml, the conditioned threshold
435 appeared to be set between 10^{-19} and 10^{-20} g/ml, i.e., 100 times lower than the spontaneous
436 threshold.

437

438 4. Discussion

439 The present study provides original insights into the peripheral contribution to odor
440 mixtures’ perception, by focusing on the AB and A’B’ mixtures initially characterized as

441 perceived configurally and elementally, respectively, in both human adults and newborn
442 rabbits, and also more recently in adult rodents [20]; this by using here the same concentrations
443 and ratios of components for acquisition of electrophysiological and behavioral data. At the
444 same time, the use of MP as a reference stimulus led us to observe the peripheral processing of
445 this pheromone. In addition, by assessing in parallel potential alterations of peripheral
446 electrophysiological activity and behavioral detection threshold, we tested whether the
447 induction phenomenon contributes to MP-induced odor learning in the newborn rabbit.

448

449 *4.1. Spontaneous peripheral processing of odor mixtures*

450 In this work, EOG measures have been done simultaneously from 3 turbinates and
451 successively from the two nasal cavities of pups. This strategy primarily intended to optimize
452 the number of measures and, as expected, no clear-cut chemotopic reactivity between turbinates
453 and stimuli was evidenced; all stimuli induced EOG in the three turbinates. However, this does
454 not mean that there is no differential sensitivity over the turbinates for the used stimuli, but
455 establishing such a difference would require the use of ranges of concentrations for each. This
456 was not the purpose of our work.

457 Regarding the AB mixture, the mean EOG response to AB was lower than to A in turbinate
458 T1, but not different than those to A and B in T2 and T3, and close to MP, the most effective
459 stimulus in T3. According to the relative comparison of effectiveness, AB was the least
460 effective stimulus in 30% of cases (which is similar to its components, A and B), whereas it
461 was the most powerful in 12% of cases (which is similar to B, only).

462 Compared to what happened with AB, the interactions between components seem to operate
463 differently regarding the A'B' mixture. In naive animals, A'B' responses were smaller than
464 those to A' in T1 and to the components in T2. When considering the relative effectiveness of

465 stimuli, A'B' was the least effective stimulus in 44% of cases and the most effective stimulus
466 in only 1%; such frequencies ranked it as the least effective of all the stimuli.

467 Mixture processing at the peripheral level is reported to as involving mainly suppressive
468 interactions between odorants, whether in binary [3, 4, 11, 55-57] or more complex mixtures
469 [58-60]. Moreover, such a predominance of suppressive interactions seems true whatever the
470 overlapping, or absence of overlapping ORs' assemblies activated by the odorants [60]. In line
471 with this, the responses to AB and A'B' both appeared as mainly resulting from suppressive
472 interactions between the components. However, such interactions seem to be consistently
473 weaker in AB, which would involve either hypoadditive or at least less marked suppressive
474 interactions than A'B'. This result fits with some others gathered in adult rats, suggesting that
475 AB involves hypoaddition, synergy and only then suppression, in descending order of
476 importance [61, 62 and personal observations]. Comparatively, the elemental A'B' mixture
477 would engage stronger suppressive interactions, as shown in rats for the elemental isoamyl
478 acetate (ISO)/whiskey lactone (WL) mixture [4, 61]. Strikingly, both A'B' and ISO/WL
479 combine a high vapor pressure component (A' and ISO, respectively) with a low vapor pressure
480 one (B' and WL). Regarding the AB and A'B' concentrations, the high vapor pressure of A
481 means that in both AB (A/B ratio: 30/70) and A'B' (68/32), the odorants A and A' are largely
482 dominant in concentration: 1.23×10^{-6} and 16.16×10^{-6} mol/l, respectively, vs. 2.38×10^{-12}
483 mol/l for B (and B'). Therefore, the theoretical concentrations of the mixtures are close to those
484 of A and A', while A'B' is about 5 times more concentrated than AB; thus, the higher the
485 concentration of A in mixture (A'B'), the stronger the suppressive interaction with B (and B').

486 Taken together, the results obtained in rabbit neonates here, and previous ones obtained in
487 adult rats, argue that the peripheral responsiveness to a mixture mainly results from suppressive
488 or antagonistic interactions, whatever the elemental or configural perceptual attributes of the
489 mixture. Additionally, our observations lead us to go further by suggesting that if A and B in

490 mixture mainly show negative interactions, these interactions are less marked in AB than A'B'
491 depending on the concentration of A/A' (which impacts *de facto* the ratio with B). The
492 observation that AB is perceived configurally, (even weakly) in rabbit pups, may lie in the fact
493 that A and B at the 30/70 ratio and current concentrations set in motion suppressive interactions
494 to a lesser extent than A' and B' do in the A'B' mixture. The reason why A'B' peripheral
495 responses would induce stronger inhibitory interaction than AB remains unknown. It could rely
496 on the higher concentration of A' and negative action of B/B' on OR responding to A' [57].

497

498 *4.2. Conditioning impacts the peripheral processing of odorant B*

499 In naive animals, as expected because it was the weakest concentrated stimulus in the AB
500 and A'B' series, odorant B induced the smallest or one of the smallest EOG responses.
501 However, B gained in effectiveness after conditioning, in T2 in the AB series' recordings and
502 in T2-T3 in the A'B' series. This gain was confirmed when considering the relative
503 effectiveness of B over the recorded sites: its occurrence as the most effective stimulus
504 increased from 13 to 34%. Noteworthy here is that B learning contemporarily impacted the
505 relative effectiveness of MP in conditioned animals: indeed, the gain in B effectiveness led to
506 a loss of MP peripheral stimulating hegemony in favor of B.

507 The increase of peripheral responses to a conditioned stimulus, observed here in newborn
508 rabbits, is in agreement with previous studies in adult rodents including peripheral recordings
509 [30, 31, 35-37, 42] and optical imaging of olfactory bulb inputs [38, 63]. However, in rabbit
510 neonates, it is remarkable that this gain observed at the periphery appeared after only a single
511 and extremely brief conditioning episode (5 min), a result that highlights the rapidity of the
512 physiological (i.e., cellular, molecular) mechanisms engaged in peripheral plasticity.

513

514 *4.3. Influence of odorant B learning on peripheral processing of mixtures including it*

515 The conditioning to B enhanced the contrast between the AB and A'B' mixtures' peripheral
516 processing. Indeed, the mean response amplitude to A'B' was clearly increased in conditioned
517 vs. naive pups, which may mirror weaker suppressive interactions occurring between A' and
518 B' after the conditioning. In parallel, in terms of relative efficacy, A'B' elicited less frequently
519 the lowest response (decreasing from 44 to 29%) and more the highest one (rising from 1 to
520 15%) in conditioned vs. naive animals. By contrast, responses to AB did not appear to be
521 influenced by B conditioning, neither through the comparison of the absolute mean amplitudes
522 nor the relative effectiveness. Such a differential impact of B learning on peripheral
523 responsiveness to A'B' and AB further supports the different processing of these mixtures and
524 the particular (configural) perception of AB. It can be proposed that after learning, AB and
525 A'B' mixtures faced a differently tuned population of ORs, at least in part. Indeed, odor learning
526 could have increased the expression of ORs presenting high affinity for B [29, 46]; this might
527 contribute to improving the peripheral processing of the elemental A'B' mixture by increasing
528 the perceptual weight of B' (i.e., B) component.

529 These results from EOGs are fully consistent with those obtained in behavioral tests during
530 which the pups conditioned to B responded to A'B' but not to AB. Interestingly, if a single
531 conditioning to B does not alter the peripheral processing and configural perception of AB, it
532 may already modulate the processing of A'B'. Along the same line, the observation that three
533 or nine repeated conditionings to A or B alters the AB perception and leads rabbit pups to
534 perceive the AB mixture more elementally [64] might also involve OR expression changes.

535 Altogether, the results highlighting that AB and A'B' are spontaneously processed
536 differently at the periphery and differently influenced by the learning of B are fully in line with
537 previous ones, which indicated the distinct processing of these mixtures both before and after
538 learning at the olfactory bulb and piriform cortex brain levels in newborn rabbits [22].

539

540 *4.4. Molecular and cellular mechanisms underlying peripheral plasticity and EOG alterations*

541 The current observations of EOG alterations in response to B and to mixtures including B, after
542 the brief B-learning could be supported by OR expression regulation. ORs are expressed in a
543 monogenic, monoallelic and seemingly stochastic location [65, 66]. Furthermore, it has been
544 reported that OR transcription (and then expression) can be induced by odorant stimulation,
545 especially during ORN differentiation, but also in mature neurons, thanks to
546 *de novo* DNA methyltransferase [67]; this enzyme would be responsible for both repressive and
547 active transcriptional states within neurons, which generate the full set of gene responses
548 associated with neuronal plasticity. Regulation of specific ORs' expression have been observed
549 in honeybees in correlation with change in EAG (i.e., EOG in vertebrates; [43]) and with more
550 central plasticity like long term memory [68, 69]. A role of down and up-regulations of ORs
551 would be to ensure that new floral scents are detected and would allow the honeybees to adapt
552 to their ever-changing scent environment [48, 49]. From a broader view, OR plasticity linked
553 to olfactory environment influence appears a widespread phenomenon [46, 48, 49, 70, 71].
554 Here, the increase of peripheral response to B supports a parallel increase of either the density
555 of ORs dedicated to B processing or of the number of ORNs expressing such ORs (mainly
556 through an activity dependent orientation in OR selection in newly mature ORNs, or both). The
557 reported results [63] that odorant exposure did not alter spatial pattern, peak magnitude, or
558 odorant-selectivity of aldehyde-evoked OSN input to olfactory bulb glomeruli, but did alter the
559 temporal dynamics of that input, plead in favor of an increase of density of B sensitive ORs
560 expressed by ORNs spontaneously responsive to B.

561

562 *4.5. MP peripheral processing is spontaneously prioritized in rabbit neonates*

563 In a previous *ex vivo* study in rabbit pups, high concentrations of MP (1.17×10^{-5} and 10^{-6} mol/l)
564 induced large EOGs (up to 8 mV; [24]). Strikingly, here, despite the MP delivery at a lower

565 concentration (7.18×10^{-7} mol/l), which was ranked in the optimal concentration range known
566 to elicit behavioral responses [72], MP induced one of the largest, even the largest EOG in naive
567 pups. This observation supports the conclusion that spontaneous peripheral processing of MP
568 is favored in rabbit neonates' olfactory mucosa, a result consistent with the crucial function of
569 this pheromonal signal in newborn rabbits [e.g., 24, 50, 51, 73]. This raises the question of the
570 origin of MP prioritization at the olfactory system periphery. Since MP is absent in the uterine
571 environment (no trace of the molecule has been found in the amniotic fluid or blood of pregnant
572 rabbit females; [51]), the hypothesis of prenatal MP learning can be rejected. Moreover, the
573 releasing activity of MP is immediately displayed at birth, even before any postnatal exposure
574 to the natural MP contained in the rabbit milk [50, 51]. Therefore, even if in our conditions the
575 peripheral sensitivity to MP could be putatively enhanced thanks to the sucking experience of
576 the first postnatal days, it would only complement a predisposed MP-OR tuning effective
577 immediately at birth. Our results give the first demonstration of the strong and direct link that
578 may exist between peripheral and behavioral processing for a pheromone in mammals as soon
579 as early in life.

580

581 *4.6. Impact of learning on odorant B detection threshold*

582 MP-induced learning of B resulted in an increase of the B sensitivity of newborn rabbits, in our
583 conditions by a factor of at least 100. This validated the second criteria of induction [29].
584 Strikingly, in both naive and conditioned pups, the B threshold concentrations were extremely
585 low, i.e., around 3.40×10^{-22} mol/l (1×10^{-15} g/ml) for the spontaneous threshold and $3.40 \times 10^{-$
586 26 mol/l (1×10^{-19} g/ml) for the conditioned threshold. Thus, the spontaneous and conditioned
587 thresholds were respectively 5.3×10^{11} and 5.3×10^{15} times lower than the detection threshold
588 observed for MP in a previous study (1.79×10^{-10} mol/l gas estimated concentration; [72]). It may
589 seem surprising that the MP threshold was higher than that of B, a spontaneously non-relevant

590 odorant. However, in ecological conditions, the MP threshold in newborn rabbits must fit with
591 an optimal detection, discrimination and recognition of that signal in the mother milk.
592 Furthermore, B concentration (at saturated vapor pressure value) is about 7.35×10^6 lower than
593 the MP one. Thus, it can be proposed that the olfactory system adapted to the reality of odor
594 molecule concentrations in order to detect low volatile and (or) concentrated odorants; this may
595 explain, at least partly, why the B threshold value is very low even spontaneously. In line with
596 this hypothesis, the sexual Bombykol pheromone in *Bombyx Mori* (Lepidoptera) is perceived
597 at around 2.82×10^{-22} mol/l (gas estimated concentration from [74]), thus far lower than the MP
598 one but close to the ST observed for odorant B in our conditions (3.40×10^{-22} mol/l). However,
599 although sharing pheromonal status, MP is contained in the milk and detected by rabbit pups in
600 the near proximity of the nipples, whereas Bombykol must be detected in the air during the
601 flying of moths, thus at very low concentrations, requiring highly sensitive and specialized
602 OR(s). The olfactory system of this invertebrate is adapted for such an extreme sensitivity since
603 "one pheromone molecule is sufficient to elicit a nerve impulse" [75]. In rabbit neonates,
604 according to our results, B presents a spontaneous threshold quite close to that of Bombykol.

605 Thus, it can be summed up first that the olfactory system of rabbit neonate is amazingly
606 potent to spontaneously detect low vapor pressure compounds at very low concentration, and
607 second, that by promoting the rapid learning of the odorant B, MP would further increase such
608 exceptional detection abilities to the odorant that became relevant through conditioning. This
609 may be adaptive in the sense that, in nature, some odorants present in the nest or carried by the
610 maternal body may be learned by MP co-perception, and then become key information for the
611 neonates in their immediate environment, or later on when the pups leave the nest and are
612 autonomous [76].

613

614 *4.7. Brain plasticity mechanisms underlying post-learning perception of odorant B*

615 In rodents, the anatomical connection between the first two stages of the olfactory system,
616 namely ORNs and olfactory bulb glomeruli takes place very early in life and for instance ORNs
617 respond to odorants immediately at birth ([77] and personal observations). It has been proposed
618 that ORN/OR activity is a prerequisite to the basal expression of ORs [78, 79] and to the correct
619 projection of the axons defining the initial glomerular map [80]. Indeed, the selective
620 suppression of activity in a set of ORNs prevents the formation of the targeted glomeruli [81].
621 Conversely, chronic exposition to heptaldehyd and acetophenone in adult mice induced the
622 formation of supernumerary glomeruli receiving I7 and M72 axons (these two OR types
623 interacting with the two odor ligands [81]), while exposition to acetophenone and isopropyl
624 tiglate around birth enlarged the size of glomeruli receiving M72 and M71 inputs [82]. Not only
625 simple exposure but also and mainly odor associative learning can induce alteration of the
626 glomerular map [36, 83, 84]. Thus, the literature converges towards the idea that olfactory
627 experience not only influences OR expression and OR signaling (i.e., ORN activity) but governs
628 the development and plasticity of the glomerular map with sensory experience, resulting in the
629 permanent formation or reorganization of glomeruli through a “constructivist” process [85].

630 Our results suggest that B paired to MP during the conditioning episode triggers or (and) up-
631 regulates the expression of ORs having high affinity to B. The weight of B inputs at the
632 olfactory bulb would be thus increased leading the pups to have a lower perceptual threshold.
633 Because we are testing neonates here, and are using a powerful and crucial pheromone for them
634 as unconditioned stimulus during the associative conditioning procedure, olfactory bulb and
635 targeted central neuroanatomic structures may quickly adapt in less than 24h (see also [22]). In
636 addition, the co-input of B and MP confers a high level of biological pertinence to B, likely
637 involving the amygdala, which mediates emotionally arousing experiences by reinforcing
638 memorization (e.g., [22]).

639

640 **5. Conclusions**

641 First, our results highlight that, as soon as the periphery, the olfactory system differentially
642 and spontaneously processes two mixtures containing the same elements, making more or less
643 salient antagonistic interactions between odorants, and leading to their distinct perception in the
644 same animals (i.e., AB and A'B' mixtures in newborn rabbits here). Such a differential process
645 probably results from the concentration (dependent on the volatility) and ratio of the
646 components; the periphery being adapted to deal with low saturated vapor pressure components,
647 such as odorant B, allowing them to largely influence the mixture processing, with respect to
648 one component with high saturated vapor pressure (as for example odorant A). Thus, peripheral
649 processes may contribute, at least in part, to the configural vs. elemental perception of odor
650 mixtures. Moreover, MP-induced odor learning of B altered the peripheral processing of the
651 A'B' mixture by decreasing suppressive interaction between the components, which may
652 facilitate the detection of the learned component in that elemental mixture and explain the
653 behavioral responsiveness of the pups to A'B' (although the mixture is initially inactive on the
654 behavior). Conversely, the configural perception of AB would be favored by less suppressive
655 interactions between its odorants.

656 Second, our results demonstrate that MP-induced single trial odor learning involves (at least
657 partly) an induction phenomenon in newborn rabbits. Indeed, the conditioning promoted by MP
658 increased extremely rapidly (24h) and in parallel the peripheral reactivity to the learned
659 stimulus and its detection abilities by the animal. These changes totally agree with the seminal
660 results pinpointing induction in anosmic human patients [29] and extend the notion of induction
661 to the neonatal period in normosmic animals. After learning, MP loses its spontaneous and
662 unique privileged peripheral processing to the shared benefit of the conditioned odorant. As a
663 consequence, some new elements (odorants) from the external chemical environment appears
664 to become relevant for the young organism, the olfactory system favoring their detection from

665 the OR level, while remaining reactive to spontaneously valuable stimuli (as the MP). In
666 ecological conditions of mother-young interactions, MP-induced odor learning is functional
667 when the mother comes into the nest to nurse her litter and newborns are exposed to odor cues
668 carried by her body during nursing, allowing them to acquire odors contemporarily present in
669 the living environment of the mother and social group [18, 76].

670 Induction is consistent with the idea that the olfactory system structure and functioning
671 operates according to constructivist theory, perfectly adapted to a sensory system facing a quasi-
672 infinite number of stimuli. Thus, even if over phylogenetic evolution, neocortex, auditory and
673 visual information apparently take the place of the chemosensory systems primarily
674 contributing to decision-making in most mammals, olfaction supports detection and perception
675 performances even more remarkable than visual and acoustic ones [86]. This is highlighted here
676 very early in life, in organisms still partly immature.

677

678

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690

691 **Conflict of Interest**

692 The authors declare that they have no conflict of interest.

693

694 **Ethical approval**

695 All applicable international, national, and/or institutional guidelines for the care and use of
696 animals were followed. All procedures performed were approved by the ethical committee of
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699

700 **Informed consent**

701 This article does not contain any studies with human participants performed by any of the
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703

704 **7. References**

- 705 1. Livermore, A. and Laing, D.G. (1998) The influence of chemical complexity on the
706 perception of multicomponent odor mixtures. *Percept Psychophys*, 60:650-61.
- 707 2. Kay, L.M., Lowry, C.A., and Jacobs, H.A. (2003) Receptor contributions to configural
708 and elemental odor mixture perception. *Behav Neurosci*, 117:1108-14.
- 709 3. Duchamp-Viret, P., Duchamp, A., and Chaput, M.A. (2003) Single olfactory sensory
710 neurons simultaneously integrate the components of an odor mixture. *Eur J Neurosci*,
711 18:2690-2696.
- 712 4. Chaput, M.A., El Mountassir, F., Atanasova, B., Thomas-Danguin, T., Le Bon, A.M.,
713 Perrut, A., Ferry, B., and Duchamp-Viret, P. (2012) Interactions of odorants with
714 olfactory receptors and receptor neurons match the perceptual dynamics observed for

- 715 woody and fruity odorant mixtures. *Eur J Neurosci*, 35:584-97.
- 716 5. Duchamp-Viret, P., Chaput, M.A., and Duchamp, A. (1999) Odor response properties
717 of rat olfactory receptor neurons. *Science*, 284:2171-2174.
- 718 6. Zwicker, D., Murugan, A. and Brenner, M.P. (2016) Receptor arrays optimized for
719 natural odor statistic. *Proc Natl Acad Sci U S A*, 113:5570-5575.
- 720 7. Kepchia, D., Sherman, B., Haddad, R. and Luetje, C.V. (2017) Mammalian odorant
721 receptor tuning breadth persists across distinct odorant panels. *PLoS One*, 12:
722 e0185329.
- 723 8. Oka, Y., Nakamura, A., Watanabe, H., and Touhara, K. (2004) An odorant derivative
724 as an antagonist for an olfactory receptor. *Chem Senses*, 29:815-22.
- 725 9. Rospars, J.P., Lansky, P., Chaput, M., and Duchamp-Viret, P. (2008) Competitive and
726 noncompetitive odorant interactions in the early neural coding of odorant mixtures.
727 *J Neurosci*, 28:2659-66.
- 728 10. Ukhanov, K., Corey, E.A., Brunert, D., Klasen, K., and Ache, B.W. (2010) Inhibitory
729 odorant signaling in Mammalian olfactory receptor neurons. *J Neurophysiol*, 103:1114-
730 22.
- 731 11. De March, C.A., Titlow, W.B., Sengoku, T., Breheny, P., Matsunami, H., and
732 McClintock, T.S. (2020) Modulation of the combinatorial code of odorant receptor
733 response patterns in odorant mixtures. *Mol Cell Neurosci*, 104:103469.
- 734 12. Zak, J.D., Reddy, G., Vergassola, M., and Murthy, V.N. (2020) Antagonistic odor
735 interactions in olfactory sensory neurons are widespread in freely breathing mice.
736 *Nat Comm*, 11:3350.
- 737 13. Kay, L.M., Crk, T., and Thorngate, J. (2005) A redefinition of odor mixture quality.
738 *Behav Neurosci*, 119:726-33.
- 739 14. Thomas-Danguin, T., Sinding, C., Romagny, S., El Mountassir, F., Atanasova, B., Le

- 740 Berre, E., Le Bon, A.M., and Coureaud, G. (2014) The perception of odor objects in
741 everyday life: a review on the processing of odor mixtures. *Front Psychol*, 5:504.
- 742 15. Romagny S., Coureaud G., Thomas-Danguin T. (2018) Key odorants or key
743 associations? Insights into elemental and configural odour processing in humans.
744 *Flavour Fragr J*, 33, 97-105.
- 745 16. Coureaud, G., Thomas-Danguin, T., Le Berre, E., and Schaal, B. (2008) Perception of
746 odor blending mixtures in the newborn rabbit. *Physiol Behav*, 95:194-9.
- 747 17. Coureaud, G., Thomas-Danguin, T., Wilson, D.A., and Ferreira, G. (2014) Neonatal
748 representation of odour objects: distinct memories of the whole and its parts.
749 *Proc R Soc B*, 281:20133319.
- 750 18. Coureaud, G., Letagneaux, C., Thomas-Danguin, T., and Romagny, S. (2020)
751 Developmental changes in elemental and configural perception of odor mixtures in
752 young rabbits. *Dev Psychobiol*, 62:471-83.
- 753 19. Le Berre, E., Beno, N., Ishii, A., Chabanet, C., Etievant, P., and Thomas-Danguin, T.
754 (2008) Just noticeable differences in component concentrations modify the odor quality
755 of a blending mixture. *Chem Senses*, 33:389-95.
- 756 20. Wilson, D.A., Fleming, G., Vervoordt, S., and Coureaud, G. (2020) Cortical processing
757 of configurally perceived odor mixtures. *Brain Res* 1729:146617.
- 758 21. Coureaud, G., Gibaud, D., Le Berre, E., Schaal, B., and Thomas-Danguin, T. (2011)
759 Proportion of odorants impacts the configural versus elemental perception of a binary
760 blending mixture in newborn rabbits. *Chem Senses*, 36:693-700.
- 761 22. Schneider, N.Y., Datiche, F., Wilson, D.A., Gigot, V., Thomas-Danguin, T., Ferreira,
762 G., and Coureaud, G. (2016) Brain processing of a configural vs elemental odor mixture
763 in the newborn rabbit. *Brain Struct Funct*, 221:2527-39.
- 764 23. Semke, E., Distel, H., and Hudson, R. (1995) Specific enhancement of olfactory

765 receptor sensitivity associated with foetal learning of food odors in the rabbit.
766 *Naturwissenschaften*, 82:148-9.

767 24. Hanser, H.I., Faure, P., Robert-Hazotte, A., Artur, Y., Duchamp-Viret, P., Coureaud,
768 G., and Heydel, J.M. (2017) Odorant-odorant metabolic interaction, a novel actor in
769 olfactory perception and behavioral responsiveness. *Sci Rep*, 7:10219.

770 25. Buonomano, D.V. and Merzenich, M.M. (1998) Net interaction between different forms
771 of short-term synaptic plasticity and slow-IPSPs in the hippocampus and auditory
772 cortex. *J Neurophysiol*, 80:1765-74.

773 26. Feldman, D.E. and Brecht, M. (2005) Map plasticity in somatosensory cortex. *Science*,
774 310:810-5.

775 27. Accolla, R. and Carleton, A. (2008) Internal body state influences topographical
776 plasticity of sensory representations in the rat gustatory cortex. *Proc Natl Acad Sci U S*
777 *A*, 105:4010-5.

778 28. Lai, C.S., Franke, T.F., and Gan, W.B. (2012) Opposite effects of fear conditioning and
779 extinction on dendritic spine remodelling. *Nature*, 483:87-91.

780 29. Wysocki, C.J., Dorries, K.M., and Beauchamp, G.K. (1989) Ability to perceive
781 androstenone can be acquired by ostensibly anosmic people. *Proc Natl Acad Sci USA*,
782 86:7976-8.

783 30. Wang, H.W., Wysocki, C.J., and Gold, G.H. (1993) Induction of olfactory receptor
784 sensitivity in mice. *Science*, 260:998-1000.

785 31. Wang, L., Chen, L., and Jacob, T. (2004) Evidence for peripheral plasticity in human
786 odour response. *J Physiol*, 554:236-44.

787 32. Nevitt, G.A., Dittman, A.H., Quinn, T.P., and Moody, W.J. Jr (1994) Evidence for a
788 peripheral olfactory memory in imprinted salmon. *Proc Natl Acad Sci USA*, 91:4288-
789 92.

- 790 33. De Jong, R. and Pham-Delegue, M.H. (1991) Electroantennogram responses related to
791 olfactory conditioning in the honey bee (*Apis mellifera ligustica*). *J Insect Physiol*,
792 37:319-324.
- 793 34. Wadhams, L.J., Blight, M.M., Kerguelen, V., Le Metayer, M., Marion-Poll, F., Masson,
794 C., Pham-Delegue, M.H., and Woodcock, C.M. (1994) Discrimination of oilseed rape
795 volatiles by honey bee: Novel combined gas chromatographic-electrophysiological
796 behavioral assay. *J Chem Ecol*, 20:3221-31.
- 797 35. Youngentob, S.L. and Kent, P.F. (1995) Enhancement of odorant-induced mucosal
798 activity patterns in rats trained on an odorant identification task. *Brain Research*,
799 670:82-88.
- 800 36. Jones, S.V., Choi, D.C., Davis, M., and Ressler, K.J. (2008) Learning-dependent
801 structural plasticity in the adult olfactory pathway. *J Neurosci*, 28:13106-11.
- 802 37. Kass, M.D., Rosenthal, M.C., Pottackal, J., and McGann, J.P. (2013) Fear learning
803 enhances neural responses to threat-predictive sensory stimuli. *Science*, 342:1389-1392.
- 804 38. Abraham, N.M., Vincis, R., Lagier, S., Rodriguez, I., and Carleton, A. (2014) Long term
805 functional plasticity of sensory inputs mediated by olfactory learning. *Elife*, 3:e02109.
- 806 39. Pekala, K., Chandra, R.K., and Turner, J.H. (2016) Efficacy of olfactory training in
807 patients with olfactory loss: a systematic review and meta-analysis. *Int Forum Allergy*
808 *Rhinol*, 6:299-307.
- 809 40. Hummel, T., Stupka, G., Haehner, A., and Poletti, S.C. (2018) Olfactory training
810 changes electrophysiological responses at the level of the olfactory epithelium.
811 *Rhinology*, 56:330-335.
- 812 41. Jiang, R.S., Twu, C.W., and Liang, K.L. (2019) The effect of olfactory training on odor
813 identification in patients with traumatic anosmia. *Int Forum Allergy Rhinol*, 9:1244-51.
- 814 42. Cavazzana, A., Larsson, M., Munch, M., Hahner, A., and Hummel, T. (2018)

- 815 Postinfectious olfactory loss: A retrospective study on 791 patients. *Laryngoscope*,
816 128:10-15.
- 817 43. Claudianos, C., Lim, J., Young, M., Yan, S., Cristino, A.S., Newcomb, R.D.,
818 Gunasekaran, N., and Reinhard, J. (2014) Odor memories regulate olfactory receptor
819 expression in the sensory periphery. *Eur J Neurosci*, 39:1642-54.
- 820 44. Bhagavan, S. and Smith, B.H. (1997) Olfactory conditioning in the honey bee, *Apis*
821 *mellifera*: effects of odor intensity. *Physiol Behav*, 61:107-17.
- 822 45. Sandoz, J.C., Pham-Delegue, M.H., Renou, M., and Wadhams, L.J. (2001)
823 Asymmetrical generalisation between pheromonal and floral odours in appetitive
824 olfactory conditioning of the honey bee (*Apis mellifera* L.). *J Comp Physiol A*, 187:559-
825 68.
- 826 46. Cadiou, H., Aoude, I., Tazir, B., Molinas, A., Fenech, C., Meunier, N., and Grosmaître,
827 X. (2014) Postnatal odorant exposure induces peripheral olfactory plasticity at the
828 cellular level. *J Neurosci*, 34:4857-70.
- 829 47. Barbour, J., Neuhaus, E.M., Piechura, H., Stoepel, N., Mashukova, A., Brunert, D.,
830 Sitek, B., Stuhler, K., Meyer, H.E., Hatt, H., and Warscheid, B. (2008) New insight into
831 stimulus-induced plasticity of the olfactory epithelium in *Mus musculus* by quantitative
832 proteomics. *J Proteome Res*, 7:1594-605.
- 833 48. Menzel, R. and Müller, U. (1996) Learning and memory in honeybees: from behavior
834 to neural substrates. *Annu Rev Neurosci*, 19:379-404.
- 835 49. Eisenhardt, D. (2012) Extinction learning in honey bees. In *Honeybee Neurobiology*
836 *and Behavior*. Eds: Galizia, C.G., Eisenhardt, D. & Giurfa, M. Springer, New York, pp.
837 423-438.
- 838 50. Coureaud, G., Moncomble, A.S., Montigny, D., Dewas, M., Perrier, G., and Schaal, B.
839 (2006) A pheromone that rapidly promotes learning in the newborn. *Curr Biol*, 16:1956-

- 840 61.
- 841 51. Schaal, B., Coureaud, G., Langlois, D., Giniès, C., Sémon, E., and Perrier, G. (2003)
- 842 Chemical and behavioural characterisation of the rabbit mammary pheromone. *Nature*,
- 843 424:68-72
- 844 52. Zarrow, M.X., Denenberg, V.H., and Anderson, C.O. (1965) Rabbit: frequency of
- 845 suckling in the pup. *Science*, 150:1835-6.
- 846 53. Le Berre, E., Thomas-Danguin, T., Beno, N., Coureaud, G., Etievant, P., and Prescott,
- 847 J. (2008) Perceptual processing strategy and exposure influence the perception of odor
- 848 mixtures. *Chem Senses*, 33:193-9.
- 849 54. Montigny, D., Coureaud, G., and Schaal, B. (2006) Rabbit pup response to the
- 850 mammary pheromone: from automatism to prandial control. *Physiol Behav*, 89:742-9.
- 851 55. Atanasova, B., Thomas-Danguin, T., Chabanet, C., Langlois, D., Nicklaus, S., and
- 852 Etievant, P. (2005) Perceptual interactions in odour mixtures: odour quality in binary
- 853 mixtures of woody and fruity wine odorants. *Chem Senses*, 30:209-17.
- 854 56. Ishii, A., Roudnitzky, N., Beno, N., Bensafi, M., Hummel, T., Rouby, C., and Thomas-
- 855 Danguin, T. (2008) Synergy and masking in odor mixtures: an electrophysiological
- 856 study of orthonasal vs. retronasal perception. *Chem Senses*, 33:553-61.
- 857 57. Rioux, D., and Carlson, J.R. (2020) Olfaction: receptor antagonists. *Curr Biol*,
- 858 30:R815-R817.
- 859 58. Stierle, J.S., Galizia, C.G., and Szyszka, P. (2013) Millisecond stimulus onset-
- 860 asynchrony enhances information about components in an odor mixture. *J Neurosci*,
- 861 33:6060-9.
- 862 59. Reddy, G., Zak, J.D., Vergassola, M., and Murthy, V.N. (2018) Antagonism in olfactory
- 863 receptor neurons and its implications for the perception of odor mixtures. *Elife*, 7.
- 864 60. Zac, J. D., Reddy, G., Vergassola, M., and Murthy, V. N. (2020) Antagonistic odor

865 interactions in olfactory sensory neurons are widespread in freely breathing mice.
866 Nature Comm, 11:3350.

867 61. Duchamp-Viret, P. (2016) Odor mixture coding from the neuronal point of view. In: E.
868 Guichard, A.M. Le Bon, M. Mortzel & C. Salles (eds.) Flavour, from food to perception.
869 Wiley- Blackwell, pp: 341-372.

870 62. Duchamp-Viret, P., Baly, C., Lacroix, M. C. and Kuczewski, K. (2017) Olfactory
871 perception and integration. In Flavor: From food to behaviors, wellbeing and health,
872 Eds: Voilley, A., Salles, C., Guichard, E., Etiévant, P. and Sykes, R. Elsevier. pp: 57-
873 100.

874 63. Kass, M.D., Guang, S.A., Moberly, A.H., and McGann, J.P. (2016) Changes in
875 Olfactory Sensory Neuron Physiology and Olfactory Perceptual Learning After Odorant
876 Exposure in Adult Mice. Chem Senses, 41:123-33.

877 64. Sinding, C., Thomas-Danguin, T., Crepeaux, G., Schaal, B., and Coureaud, G. (2011)
878 Experience influences elemental and configural perception of certain binary odour
879 mixtures in newborn rabbits. J Exp Biol, 214:4171-8.

880 65. Chess, A., Simon, I., Cedar, H., and Axel, R. (1994) Allelic inactivation regulates
881 olfactory receptor gene expression. Cell, 78:823-34.

882 66. Monahan, K., Schieren, I., Cheung, J., Mumbey-Wafula, A., Monuki, E.S., and
883 Lomvardas, S. (2017) Cooperative interactions enable singular olfactory receptor
884 expression in mouse olfactory neurons. Elife, 6:e28620.

885 67. Colquitt, B.M., Markenscoff-Papadimitriou, E., Duffie, R., and Lomvardas, S. (2014)
886 Dnmt3a regulates global gene expression in olfactory sensory neurons and enables
887 odorant-induced transcription. Neuron, 83:823-38.

888 68. Biswas, S., Reinhard, J., Oakeshott, J., Russell, R., Srinivasan, M.V., and Claudianos,
889 C. (2010) Sensory regulation of neuroligins and neurexin I in the honeybee brain. PLoS

- 890 One, 5: e9133.
- 891 69. Reinhard, J. & Claudianos, C. (2012) Molecular insights into honeybee brain plasticity.
892 In Galizia, C.G., Eisenhardt, D. & Giurfa, M. (Eds), Honeybee Neurobiology and
893 Behavior. Springer, New York, pp. 359-372.
- 894 70. Zhou, S., Stone, E.A., Mackay, T.F., and Anholt, R.R. (2009) Plasticity of the
895 chemoreceptor repertoire in *Drosophila melanogaster*. PLoS Genet, 5: e1000681.
- 896 71. De Groof, G., Gwinner, H., Steiger, S., Kempnaers, B., and Van der Linden, A. (2010)
897 Neural correlates of behavioural olfactory sensitivity changes seasonally in European
898 starlings. PLoS One, 5:e14337.
- 899 72. Coureaud, G., Langlois, D., Sicard, G., and Schaal, B. (2004) Newborn rabbit
900 responsiveness to the mammary pheromone is concentration-dependent. Chem Senses,
901 29:341-50.
- 902 73. Coureaud, G., Fortun-Lamothe, L., Langlois, D., and Schaal, B. (2007) The reactivity
903 of neonatal rabbits to the mammary pheromone as a probe for viability. Animal, 1:1026-
904 1032.
- 905 74. Tabuchi, M., Sakurai, T., Mitsuno, H., Namiki, S., Minegishi, R., Shiotsuki, T., Uchino,
906 K., Sezutsu, H., Tamura, T., Haupt, S.S., Nakatani, K., and Kanzaki, R. (2013)
907 Pheromone responsiveness threshold depends on temporal integration by antennal lobe
908 projection neurons. Proc Natl Acad Sci USA, 110:15455-60.
- 909 75. Kaissling, K.E. (2014) Pheromone Reception in Insects: The Example of Silk Moths.
910 In: Mucignat-Caretta C, editor. Neurobiology of Chemical Communication. Boca Raton
911 (FL): CRC Press/Taylor & Francis. Chapter 4.
- 912 76. Jouhanneau, M., Schaal, B., and Coureaud, G. (2016) Mammary pheromone-induced
913 odour learning influences sucking behaviour and milk intake in the newborn rabbit.
914 Anim Behav, 111:1-11.

- 915 77. Lam, R. S. and Mombaerts, P. (2013) Odorant responsiveness of embryonic mouse
916 olfactory sensory neurons expressing the odorant receptors S1 or MOR23. *Eur J*
917 *Neurosci*, 38: 2210-7
- 918 78. Lewcock, J.W. and Reed, R.R. (2004) A feedback mechanism regulates monoallelic
919 odorant receptor expression. *Proc Natl Acad Sci USA*, 101:1069-74.
- 920 79. Serizawa, S., Miyamichi, K., Nakatani, H., Suzuki, M., Saito, M., Yoshihara, Y., and
921 Sakano, H. (2003) Negative feedback regulation ensures the one receptor-one olfactory
922 neuron rule in mouse. *Science*, 302:2088-94.
- 923 80. Valle-Leija, P. (2015) Odorant Receptors Signaling Instructs the Development and
924 Plasticity of the Glomerular Map. *Neural Plast*, 2015:975367.
- 925 81. Yu, C.R., Power, J., Barnea, G., O'Donnell, S., Brown, H.E., Osborne, J., Axel, R., and
926 Gogos, J.A. (2004) Spontaneous neural activity is required for the establishment and
927 maintenance of the olfactory sensory map. *Neuron*, 42:553-556.
- 928 82. Todrank, J., Heth, G., and Restrepo, D. (2011) Effects of in utero odorant exposure on
929 neuroanatomical development of the olfactory bulb and odour preferences. *Proc R Soc*
930 *B*, 278:1949-55.
- 931 83. Kerr, M.A. and Belluscio, L. (2006) Olfactory experience accelerates glomerular
932 refinement in the mammalian olfactory bulb. *Nat Neurosci*, 9:484-6.
- 933 84. Dias, B.G. and Ressler, K.J. (2014) Parental olfactory experience influences behavior
934 and neural structure in subsequent generations. *Nat Neurosci*, 17:89-96.
- 935 85. Valle-Leija, P., Blanco-Hernandez, E., Drucker-Colin, R., Gutierrez-Ospina, G., and
936 Vidaltamayo, R. (2012) Supernumerary formation of olfactory glomeruli induced by
937 chronic odorant exposure: a constructivist expression of neural plasticity. *PLoS One*,
938 7:e35358.
- 939 86. Bushdid, C., Magnasco, M.O., Vosshall, L.B., and Keller, A. (2014) Humans can

940 discriminate more than 1 trillion olfactory stimuli. *Science*, 343:1370-2.

941

942 Table 1. Concentrations of odorant B used in behavioral tests

Stimulus reference	Concentration in water (g/ml) in vials	Estimated gaseous concentration (mol/l)*	Estimated gaseous concentration (ppm v/v)
B ⁻⁵	10 ⁻⁵	3.40 x 10 ⁻¹²	7.61 x 10 ⁻⁵
B ⁻¹⁰	10 ⁻¹⁰	3.40 x 10 ⁻¹⁷	7.61 x 10 ⁻¹⁰
B ⁻¹⁵	10 ⁻¹⁵	3.40 x 10 ⁻²²	7.61 x 10 ⁻¹⁵
B ⁻¹⁷	10 ⁻¹⁷	3.40 x 10 ⁻²⁴	7.61 x 10 ⁻¹⁷
B ⁻¹⁹	10 ⁻¹⁹	3.40 x 10 ⁻²⁶	7.61 x 10 ⁻¹⁹
B ⁻²⁰	10 ⁻²⁰	3.40 x 10 ⁻²⁷	7.61 x 10 ⁻²⁰
B ⁻²¹	10 ⁻²¹	3.40 x 10 ⁻²⁸	7.61 x 10 ⁻²¹
B ⁻²²	10 ⁻²²	3.40 x 10 ⁻²⁹	7.61 x 10 ⁻²²

943 *: calculated by using Henry law constant [(Pi/Ci) Pa.m3/mol]

944

945

946 Table 2. Stimuli used in electrophysiological recordings

Stimuli	2-methylbut-2-enal	Ethyl Maltol	Ethyl Isobutyrate		Configural mixture	Elemental mixture
Abbreviation	MP	B and B'	A	A'	AB	A'B'
Concentration in liquid phase (g/ml)	1 x 10 ⁻⁵	7 x 10 ⁻⁶	3 x 10 ⁻⁶	1.5 x 10 ⁻⁵	1 x 10 ⁻⁵	2.2 x 10 ⁻⁵
Estimated gaseous concentration*: (mol/l)	7.18 x 10 ⁻⁷	2.38 x 10 ⁻¹²	1.23 x 10 ⁻⁶	6.16 x 10 ⁻⁶	1.23 x 10 ⁻⁶	6.16 x 10 ⁻⁶
Estimated gaseous concentration (ppm)	1.61 x 10 ¹	5.33 x 10 ⁻⁵	2.76 x 10 ¹	1.38 x 10 ²	2.76 x 10 ¹	1.38 x 10 ²

947 *: calculated by using Henry law constant [(Pi/Ci) Pa.m3/mol]

948

949

950

Figure Legends

951

952

953 **Figure 1: a.** Positioning of the three electrodes on the three turbinates (T1, T2, T3) on the hemi-
954 head of a rabbit neonate (the large arrow indicating an enlargement of the area). The olfactometer
955 nozzle is positioned so that the odorized puff (materialized in grey shadow) sweeps the 3
956 turbinates, as if it was naturally entering through the animal's nostril.
957 **b.** Illustration of raw recordings simultaneously obtained from the three turbinates. The EOG
958 amplitude (here in response to the odorant A) is measured between the baseline level sampled
959 within 100 ms prior to the start of olfactory stimulation (STIM) and the peak amplitude.

960

961 **Figure 2:** Proportions of newborn rabbits (in %) responding in the oral activation test either
962 spontaneously (naive pups in grey, n = 31) or 24h after MP-induced acquisition of the odorant
963 B (conditioned pups in black, n = 36) to the odorant B (B') and the stimulus series comprising
964 single odorants and mixtures (see Table 1 for stimuli concentrations). Stimuli are ordered from
965 the left to the right in ascending level of concentration except for MP, which was always tested
966 at the end as a control. Distinct geometrical symbols above the bars indicate significant
967 differences within naive or conditioned pups, respectively, at $p < 0.05$, and ** differences
968 between naive and conditioned pups at $p < 0.01$ (Cochran, McNemar and χ^2 tests).

969

970 **Figure 3:** Mean EOG amplitudes (\pm SD) recorded in the three nasal turbinates of naive rabbit
971 neonates in response to **a.** AB series (n = 15 pups); **b.** A'B' series (n = 16 pups). Stimuli are
972 ordered in ascending level of concentration from the left to the right except for MP, which was
973 always tested in the end as a control. Within each graph, distinct geometrical symbols indicate
974 statistical differences at $p \leq 0.05$ (Wilcoxon test for matched samples).

975 **Figure 4:** Mean EOG amplitudes (\pm SD) recorded in the three nasal turbinates of conditioned
976 rabbit neonates in response to **a.** the AB series (n =19 pups); **b.** the A'B' series (n = 17 pups).
977 Stimuli are ordered in ascending level of concentration from the left to the right except for MP,
978 which was always tested in the end as a control. Within each graph, distinct geometrical
979 symbols indicate statistical differences at $p \leq 0.05$ (Wilcoxon test for matched samples).

980

981 **Figure 5:** Comparisons of mean EOG amplitudes (\pm SD) in response to **a.** the AB series, and
982 **b.** the A'B' series, in nasal turbinates 1, 2 and 3 of naive and conditioned rabbit neonates.
983 Stimuli are ordered in ascending level of concentration from left to right except for MP, which
984 was always tested at the end as a control. *, **, *** indicate differences at $p < 0.05$, 0.01 and
985 0.001 (Wilcoxon test for non-matched samples).

986

987 **Figure 6:** Occurrences of each odor stimulus as being **a.** the least effective (i.e., inducing the
988 smallest EOGs) or **b.** the most effective (i.e., inducing the largest EOGs) in naive (grey bars)
989 and conditioned (black bars) rabbit neonates for the stimulus series AB (left) and A'B' (right).
990 Within the naive and conditioned conditions, χ^2 test and Monte Carlo method were applied on
991 the distributions. Then the Marascuilo procedure gives the statistical classification of stimuli.
992 Distinct 1 geometrical symbols above each bar (grey symbols for: naive pups; black symbols:
993 conditioned pups) indicate statistical differences at $p < 0.05$, and *, **, *** differences between
994 naive and conditioned pups at $p < 0.05$, 0.01 and 0.001, respectively; bilateral test of two
995 proportions, confirmed with Monte Carlo method.

996

997 **Figure 7: a.** Proportions of newborn rabbits responding to the odorant B at 10^{-5} g/ml, as a
998 function of the concentration of B used during the conditioning (ran 24h before). The
999 conditioning concentrations were ranged from 10^{-5} to 10^{-22} g/ml (grey bars). This experiment

1000 allowed to assess the spontaneous threshold (ST) of B in the animals (n = 50); **b.** Proportions
1001 of newborn rabbits responding to the odorant B at concentrations ranged from 10^{-5} to 10^{-22} g/ml
1002 24h after conditioning to B at 10^{-5} g/ml. This experiment pinpointed the conditioning threshold
1003 (CT) of B in the animals (n = 105).