

Brief olfactory learning drives perceptive sensitivity in newborn rabbits: New insights in peripheral processing of odor mixtures and induction

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

3

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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
22 mixtures which give rise to either elemental or configural perceptions. Here, we first explored
23 the contribution of the peripheral olfactory system to configural and elemental perception
24 through odorant interactions at the olfactory receptor (OR) level. This was done in newborn
25 rabbits, which offer the opportunity to pair peripheral electrophysiology and well
26 characterized behavioral responses to two binary mixtures, AB and A'B', which differ in their
27 component ratio (A: ethyl isobutyrate, B: ethyl maltol), and that rabbit pups respectively
28 perceived configurally and elementally. Second, we studied the influence on peripheral
29 reactivity of the brief but powerful learning of one mixture component (odorant B),
30 conditioned by association with the mammary pheromone (MP), which allowed us to assess
31 the possible implication of the phenomenon called induction in neonatal odor learning.
32 Induction is a plasticity mechanism expected to alter both the peripheral electrophysiological
33 responses to, and perceptual detection threshold of, the conditioned stimulus. The results
34 reveal that perceptual modes are partly rooted in differential peripheral processes, the AB
35 configurally perceived mixture mirroring odorant antagonist interactions at OR level to a
36 lesser extent than the A'B' elementally perceived mixture. Further, the results highlight that a
37 single and brief MP-induced odor learning episode is sufficient to alter peripheral responses to
38 the conditioned stimulus and mixtures including it, and shifts the conditioned stimulus
39 detection threshold towards lower concentrations. Thus, MP-induced odor learning relies on
40 induction phenomenon in newborn 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)
45 and 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
48 occur [1, 2] and find their origin, at least in part, at the olfactory system peripheral level [3, 4].
49 Indeed, in the olfactory mucosa lie olfactory receptor neurons (ORNs) expressing olfactory
50 receptors (ORs), which are mainly weakly selective [5-7]. As a consequence, odorants in
51 mixture combine their simultaneous actions on multiple ORs, resulting in antagonist or
52 agonist interactions through competitive or non-competitive (allosteric) effects [8-12]. At the
53 end of 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
55 quality specific to the whole mixture and distinct from the element qualities (configural
56 perception) [13, 14]. These two modes may be shown as two poles defining the olfactory
57 perception axis along which there is a perceptive continuum, which goes through a dual
58 perception of both elements and configuration named weak configural perception [15].

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

68 peripheral level and the contribution of this level to elemental vs. configural perception has
69 remained unexplored so far. The first aim of the present study was to fill that gap in the
70 newborn rabbit model, which offers the advantage of being able to pair peripheral
71 electrophysiology (e.g. [23, 24]) and well characterized 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
78 colleagues have observed that when patients displaying androstenone specific anosmia were
79 exposed to androstenone for some minutes, three times a day during six weeks, perception of
80 this odorant was restored [29]. These changes have been termed “experienced incidental
81 induction” and because anosmia was suspected to result from a genetic deficit in peripheral
82 captors, they have been assigned to the olfactory system periphery. Repeated exposure to
83 androstenone would result in sensitization of some olfactory receptor neurons (ORNs)
84 equipped with androstenone-binding ORs. Such ORs would be initially at subthreshold
85 density in anosmic patients and the sensitization would increase their expression [29]. The
86 periphery involvement has been supported in experiments reporting that chronic exposures to
87 odorants increased the peripheral olfactory responses (measured through electro-olfactograms,
88 EOG) in anosmic mice and humans [30, 31]. These results concerned with anosmic patients
89 and animals have been then extended to normosmic ones. Subsequently, a body of
90 experimental data, collected throughout the animal kingdom (from insects to various
91 vertebrates, including in humans) confirmed that changes in peripheral reactivity can
92 happened following odorant learning or exposure protocols; such changes could result mainly

93 in an increase [32-42] or sometimes in a decrease in responses to target stimuli [43] (or even
94 in no change [44,45]). In parallel with these changes, regulation in the expression rate of some
95 ORs [43, 46] or proteins involved in peripheral transduction [47] 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
102 peripheral tuning, without ceasing to favor the detection of new odors [48, 49]. Importantly,
103 in conjunction with the peripheral effect, induction is considered to impact the olfactory
104 perceptual detection threshold [29]. This aspect is unfortunately weakly documented,
105 although it directly links 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
109 our 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.,
113 [50, 51]). Twenty-four hours after the conditioning, rabbit pups display in response to the CS
114 the typical orocephalic behavior usually triggered by the MP itself. Thus, in close conjunction
115 with our analysis of the peripheral responses to mixtures vs. mixture components, we
116 therefore tested induction by exploring in naive vs. conditioned pups the two criteria changes
117 observed in humans [29], i.e., the consequences of MP-induced conditioning for odorant B

118 (contained in the AB and A'B' mixtures) on CS electrophysiological responses (EOG) and
119 detection 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
126 (21-22°C) and *ad libitum* access to food and water. Nest boxes were fixed to the housing
127 cages of pregnant does two days prior to parturition, to allow nest building. The day of birth
128 was designated as postnatal day 0. To equalize the time of mother-young interaction, females
129 were allowed access to the nest box only once a day for 15 min at 11:30 am. This short daily
130 period of nursing respected the natural rhythm of maternal visits [52]. A total of 239 pups
131 aged 2-4 days from 56 litters were used in this study, 84 for electrophysiological recordings
132 (38 naive, 46 conditioned) and 155 for behavioral procedures. All experiments were
133 performed in accordance with ethic rules enforced by French law and were approved by the
134 ethical committee of Lyon 1 University (CEEA-55) and the French Ministry of Higher
135 Education and Research under the no. 9745.

136

137 *2. Stimuli*

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

143 weak configural perception in newborn rabbits (e.g., [16-18, 22]), and A'B' (68/32 ratio),
144 which generates elemental perception in both human adults [19, 53], mice [20] and newborn
145 rabbits (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-
151 solutions of each component in distilled water, with a final concentration of 10^{-5} g/ml of MP
152 and B at a 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
155 detailed in Table 2. The two series of stimuli included A, B, AB and MP (AB series) or A',
156 B', A'B' 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
164 (10:30 a.m.) to equalize the pups' motivational state and limit the impact of satiation on
165 responses [54]. The pups were transferred by groups of 5/litter into a box maintained at room
166 temperature. The MP-induced conditioning was then run following a procedure previously
167 validated (e.g. [16, 17, 22, 50])). Briefly, 6 ml of the B + MP blend were pipetted on a 10 x 15

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

172

173 *4. Behavioral assay*

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

188 *4.1. Procedure 1 - Behavioral test before electrophysiology: pre-recording test*

189 The naive animals were all tested with the 3 odor stimuli from either the AB or A'B'
190 series (Table 2), and systematically to the MP in order to assess that they did not respond
191 spontaneously to unfamiliar stimuli but to the MP. The conditioned animals were tested to the
192 same stimuli as the naive pups, 24h after odor learning, to determine the effectiveness of the

193 conditioning to B. All the animals were tested at 8:00 a.m., i.e., at least 30 min before the
194 beginning of electrophysiological recordings. Importantly, the same series was then used for
195 the electrophysiological recordings.

196 *4.2. Procedures 2 and 3 - Behavioral test for detection threshold determination*

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

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

207 Regarding the post-conditioning threshold to B, 2 groups of 2-day-old pups (n = 25/group)
208 were conditioned to B at 10^{-5} g/ml by pairing with the MP, then tested 24h later either to B at
209 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
210 to MP 10^{-5} g/ml.

211

212 *5. Electrophysiology*

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

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

228

229 *6. Olfactory stimulation*

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

238

239 *7. Statistical analyses*

240 *7.1. Behavioral data*

241 Proportions of pups responding behaviorally were compared using the Cochran's Q
242 test when the data were dependent (i.e., pups from the same group tested for their response to
243 the three stimuli) or the χ^2 test of Pearson (with Yates correction when necessary) when the

244 data were independent (i.e., distinct groups tested for their response to the same stimulus).
245 When the Cochran's Q test was significant, proportions of responding pups were compared
246 2x2 by the χ^2 test of McNemar. Degrees of freedom are indicated when > 1 . Data were
247 considered as significant when the two-tailed tests yielded $p < 0.05$. Analyses were made with
248 Statistica software (StatSoft, Tulsa, OK, USA).

249 *7.2. Electrophysiological data*

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

262

263 **3. Results**

264 *3.1. Peripheral processing of odorants and mixtures*

265 *3.1.1. Naive animals: spontaneous behavior and peripheral processing*

266 From the 38 pups assigned to the naive group, 31 gave exploitable records, 5 did not and 2
267 were discarded because they did not respond to MP in the pre-recording behavioral test.
268 Among the 31 recorded pups, 15 were stimulated with the stimulus series AB comprising A,

269 B, AB and MP, while the remaining 16 pups were stimulated with the A'B' series including
270 A', B', A'B' and MP. The behavioral pre-recording test showed that all pups responded
271 positively to the MP, and none to the other odorants (in each series $Q > 40$, $p < 0.001$ and $\chi^2 >$
272 13 , $p < 0.001$ for global and 2x2 comparisons) (Figure 2; for simplification, results of the two
273 series have been pooled in the Figure).

274 The mean EOG amplitudes recorded in naive pups in response to the two-stimulus series
275 are shown in Figure 3. For the AB series, regardless of the stimulus, EOGs recorded in
276 turbinate 1 (T1) were statistically smaller than those recorded in T2 and T3 (Friedman test, p
277 $= 0.007$, T1 vs. T2: $p < 0.0001$, T1 vs. T3: $p = 0.002$). For the A'B' series, no statistical
278 differences were observed between the three turbinates.

279 In the AB series (Figure 3a), the MP induced the largest response in T2 (Wilcoxon: $0.04 \leq$
280 $p \leq 0.06$) and one of the largest in T1 and T3. Responses to the AB configural mixture were
281 not different from those of its components in T2-T3, lower than responses to A and MP in T1
282 (Wilcoxon: $p = 0.042$ and 0.037) and lower than response to MP in T2.

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

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

293

294 *3.1.2. Conditioned animals: post-learning behavior and peripheral processing*

295 From the 46 pups assigned to the conditioned group, 36 gave exploitable records, 3 did not
296 and 7 were discarded because they failed to give clear behavioral responses to B (CS). Among
297 these 36 newborn rabbits, 17 pups were stimulated with the AB series and 19 pups with the
298 A'B' series. In terms of behavior, within the AB series, pups strongly responded to B (94.1%)
299 and to the MP (100%) but not to A and AB (whole comparison: $Q = 47$, $df = 3$, $p < 0.001$; 2x2
300 comparisons between B or MP vs. A or AB: $\chi^2 > 14.06$, $p < 0.001$; B vs. MP: $\chi^2 < 0.5$, $p >$
301 0.05) (Figure 2; to simplify reading, responses of the two series were pooled in the Figure).

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

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

318 In the A'B' series (Figure 4b), EOGs' amplitudes recorded in T1 differed from those recorded
319 in T2 and T3 (Friedman, $p < 0.001$, T1 vs. T2 $p < 0.0001$; T1 vs. T3 $p = 0.0002$); T1 EOGs
320 being lower regardless the stimulus. In T2, B' induced larger responses than A'B' and MP ($B' > A'B'$;
321 Wilcoxon, $p = 0.028$, $B' > MP$; Wilcoxon, $p = 0.031$). In T3, B' induced larger
322 responses than A'B' (Wilcoxon $p = 0.037$).

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

328

329 *3.1.3. Comparison of EOG amplitudes in naive vs. conditioned animals*

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

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

338

339 *3.1.4. Comparison of stimulus relative effectiveness in naive vs. conditioned animals*

340 Here, we addressed the question of the high variability in EOG amplitude for a same
341 stimulus (from one recording site to another, from one nasal cavity to another and from one
342 animal to another), by analyzing the differences in response between different stimuli in

343 relative terms. To that end, based on their amplitude, the EOG responses were ranked from
344 the lowest to the largest and the corresponding stimulus sequence was used to determine the
345 least or the most effective stimuli; only the sets of recording comprising exploitable responses
346 to the 5 stimuli (i.e., complete stimulus sequence) were taken into account. Doing so, we
347 compared the occurrence of each stimulus as being the least and the most effective one, first,
348 within the naive and conditioned groups for the AB and A'B' series, respectively, and then
349 between the two groups.

350 *3.1.4.1. Occurrence of stimuli as least effective (Figure 6a)*

351 Within the AB-series naive animals (Figure 6a-left, grey bars), all stimuli occurred as the
352 least effective with the same probability ($p > 0.5$). By contrast, within the A'B'-series naive
353 animals (Figure 6a-right, grey bars) statistical differences appeared between stimuli ($p <$
354 0.0001 , χ^2 and Monte Carlo method): the occurrence of A'B' differed from that of A' and MP,
355 A'B' being most frequently the least effective stimulus ($p < 0.05$, pairwise comparisons by
356 the Marascuilo procedure).

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

363 The comparisons between naive and conditioned groups showed that the occurrence of MP
364 as the least effective stimulus increased from 28% to 43% ($p < 0.05$, in the AB series) and
365 from 18% to 34% ($p = 0.02$; in A'B' series) in conditioned neonates. Comparatively, the
366 occurrence of A'B' as the least effective stimulus decreased from 43% in naive pups to 29%

367 in conditioned ones ($p < 0.05$, bilateral test of two proportions, confirmed with Monte-Carlo
368 method).

369 *3.1.4.2. Occurrence of stimuli as most effective (Figure 6b)*

370 Within both naive and conditioned groups, and in both AB and A'B' series, stimuli
371 differed in their occurrence as the most effective stimulus ($p < 0.0001$, χ^2 and Monte Carlo
372 method). In the AB-series naive animals (Figure 6b-left, grey bars), MP and A significantly
373 differed from B and AB ($p < 0.05$), and were most often the most effective stimuli. In the
374 A'B'-series naive animals (Figure 6b-right, grey bars), MP and A' differed from B and A'B',
375 and B differed from A'B' ($p < 0.05$). MP and A' were most often the most efficient stimuli
376 especially compared to A'B', which never induced the largest EOGs.

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

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

387

388 To sum up, regardless of the AB and A'B' series, the comparison of relative effectiveness
389 between the stimuli showed that MP, the most effective stimulus in naive animals, lost this
390 status in conditioned pups: since, after conditioning its occurrence increased as the least
391 effective stimulus, while simultaneously decreased as the most effective one. At the same

392 time, the frequency of odorant B (i.e., the conditioned stimulus) as the most effective
393 stimulus, alone (for the AB series) or in the elemental mixture A'B' (for the A'B' series),
394 increased in conditioned animals, whereas the occurrence of B as the lowest effective cue did
395 not change in the two series.

396

397 *3.2. Detection threshold in naive and conditioned animals*

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

404 *3.2.1 Spontaneous detection threshold of the odorant B*

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

414 The results showed that differences existed in pup responsiveness to B depending on the
415 concentration of the odorant used during the conditioning ($Q = 81.2$, $df = 7$, $p < 0.001$)
416 (Figure 7a). A strong and similar proportion of pups responded to B after conditioning at 10^{-5} ,

417 10^{-10} and 10^{-15} g/ml ($> 80\%$; $\chi^2 < 0.7$, $p > 0.05$ in all 2x2 comparisons) attesting that B was
418 effectively learned, i.e., that it was spontaneously perceived at these concentrations. The level
419 of responsiveness dropped dramatically between 10^{-15} and 10^{-17} g/ml (80 vs. 20%; $\chi^2 = 7.1$, p
420 < 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
421 2x2 comparisons).

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

424 3.2.2. *Post-conditioning detection threshold of the odorant B*

425 To determine the detection threshold of odorant B after MP-induced conditioning
426 (“conditioned threshold”, CT), two groups of 25 pups were conditioned to B 10^{-5} g/ml (paired
427 with MP 10^{-5} g/ml), then each tested 24h later to several concentration steps of B over a range
428 from 10^{-5} and 10^{-22} g/ml, and systematically to MP 10^{-5} g/ml in the end. In the two groups, the
429 responsiveness to B varied according to its concentration ($Q < 73.1$, $df = 3$ and 4 , $p < 0.001$).
430 The proportion of responding pups was strong and similar to B from 10^{-5} to 10^{-19} g/ml
431 ($> 80\%$; $\chi^2 < 3.2$, $p > 0.05$ in all 2x2 comparisons) but it strongly decreased at 10^{-20} g/ml
432 (20%; $\chi^2 > 16.05$, $p < 0.001$ in all 2x2 comparisons between 10^{-20} vs. stronger concentration
433 steps) and then remained low or null until 10^{-22} g/ml ($< 20\%$; $\chi^2 < 3.5$, $p > 0.05$ in all 2x2
434 comparisons) (Figure 7b).

435 Between the ST and CT determination procedures, no difference contrasted the pups’
436 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
437 comparisons). Conversely, in the CT determination procedure, rabbit pups responded clearly
438 more to B at 10^{-17} and 10^{-19} g/ml than in the ST determination procedure ($\chi^2 > 20.6$, $p < 0.001$
439 in the two comparisons).

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

443

444 **4. Discussion**

445 The present study provides original insights into the peripheral contribution to odor
446 mixtures' perception, by focusing on the AB and A'B' mixtures initially characterized as
447 perceived configurally and elementally, respectively, in both human adults and newborn
448 rabbits, and also more recently in adult rodents [20]; this by using here the same
449 concentrations and ratios of components for acquisition of electrophysiological and
450 behavioral data. At the same time, the use of MP as a reference stimulus led us to observe the
451 peripheral processing of this pheromone. In addition, by assessing in parallel potential
452 alterations of peripheral electrophysiological activity and behavioral detection threshold, we
453 tested whether the induction phenomenon contributes to MP-induced odor learning in the
454 newborn rabbit.

455

456 *4.1. Spontaneous peripheral processing of odor mixtures*

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

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

469 Compared to what happened with AB, the interactions between components seem to
470 operate differently regarding the A'B' mixture. In naive animals, A'B' responses were
471 smaller than those to A' in T1 and to the components in T2. When considering the relative
472 effectiveness of stimuli, A'B' was the least effective stimulus in 44% of cases and the most
473 effective stimulus in only 1%; such frequencies ranked it as the least effective of all the
474 stimuli.

475 Mixture processing at the peripheral level is reported to as involving mainly suppressive
476 interactions between odorants, whether in binary [3, 4, 11, 55-57] or more complex mixtures
477 [58-60]. Moreover, such a predominance of suppressive interactions seems true whatever the
478 overlapping, or absence of overlapping ORs' assemblies activated by the odorants [60]. In
479 line with this, the responses to AB and A'B' both appeared as mainly resulting from
480 suppressive interactions between the components. However, such interactions seem to be
481 consistently weaker in AB, which would involve either hypoadditive or at least less marked
482 suppressive interactions than A'B'. This result fits with some others gathered in adult rats,
483 suggesting that AB involves hypoaddition, synergy and only then suppression, in descending
484 order of importance [61, 62 and personal observations]. Comparatively, the elemental A'B'
485 mixture would engage stronger suppressive interactions, as shown in rats for the elemental
486 isoamyl acetate (ISO)/whiskey lactone (WL) mixture [4, 61]. Strikingly, both A'B' and
487 ISO/WL combine a high vapor pressure component (A' and ISO, respectively) with a low
488 vapor pressure one (B' and WL). Regarding the AB and A'B' concentrations, the high vapor

489 pressure of A means that in both AB (A/B ratio: 30/70) and A'B' (68/32), the odorants A and
490 A' are largely dominant in concentration: 1.23×10^{-6} and 16.16×10^{-6} mol/l, respectively, vs.
491 2.38×10^{-12} mol/l for B (and B'). Therefore, the theoretical concentrations of the mixtures are
492 close to those of A and A', while A'B' is about 5 times more concentrated than AB; thus, the
493 higher the concentration of A in mixture (A'B'), the stronger the suppressive interaction with
494 B (and B').

495 Taken together, the results obtained in rabbit neonates here, and previous ones obtained in
496 adult rats, argue that the peripheral responsiveness to a mixture mainly results from
497 suppressive or antagonistic interactions, whatever the elemental or configural perceptual
498 attributes of the mixture. Additionally, our observations lead us to go further by suggesting
499 that if A and B in mixture mainly show negative interactions, these interactions are less
500 marked in AB than A'B' depending on the concentration of A/A' (which impacts *de facto* the
501 ratio with B). The observation that AB is perceived configurally, (even weakly) in rabbit
502 pups, may lie in the fact that A and B at the 30/70 ratio and current concentrations set in
503 motion suppressive interactions to a lesser extent than A' and B' do in the A'B' mixture. The
504 reason why A'B' peripheral responses would induce stronger inhibitory interaction than AB
505 remains unknown. It could rely on the higher concentration of A' and negative action of B/B'
506 on OR responding to A' [57].

507

508 4.2. Conditioning impacts the peripheral processing of odorant B

509 In naive animals, as expected because it was the weakest concentrated stimulus in the AB
510 and A'B' series, odorant B induced the smallest or one of the smallest EOG responses.
511 However, B gained in effectiveness after conditioning, in T2 in the AB series' recordings and
512 in T2-T3 in the A'B' series. This gain was confirmed when considering the relative
513 effectiveness of B over the recorded sites: its occurrence as the most effective stimulus
514 increased from 13 to 34%. Noteworthy here is that B learning contemporarily impacted the

515 relative effectiveness of MP in conditioned animals: indeed, the gain in B effectiveness led to
516 a loss of MP peripheral stimulating hegemony in favor of B.

517 The increase of peripheral responses to a conditioned stimulus, observed here in newborn
518 rabbits, is in agreement with previous studies in adult rodents including peripheral recordings
519 [30, 31, 35-37, 42] and optical imaging of olfactory bulb inputs [38, 63]. However, in rabbit
520 neonates, it is remarkable that this gain observed at the periphery appeared after only a single
521 and extremely brief conditioning episode (5 min), a result that highlights the rapidity of the
522 physiological (i.e., cellular, molecular) mechanisms engaged in peripheral plasticity.

523

524 *4.3. Influence of odorant B learning on peripheral processing of mixtures including it*

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

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

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

549

550 *4.4. Molecular and cellular mechanisms underlying peripheral plasticity and EOG alterations*

551 The current observations of EOG alterations in response to B and to mixtures including B,
552 after the brief B-learning could be supported by OR expression regulation. ORs are expressed
553 in a monogenic, monoallelic and seemingly stochastic location [65, 66]. Furthermore, it has
554 been reported that OR transcription (and then expression) can be induced by odorant
555 stimulation, especially during ORN differentiation, but also in mature neurons, thanks to
556 *de novo* DNA methyltransferase [67]; this enzyme would be responsible for both repressive
557 and active transcriptional states within neurons, which generate the full set of gene responses
558 associated with neuronal plasticity. Regulation of specific ORs' expression have been
559 observed in honeybees in correlation with change in EAG (i.e., EOG in vertebrates; [43]) and
560 with more central plasticity like long term memory [68, 69]. A role of down and up-
561 regulations of ORs would be to ensure that new floral scents are detected and would allow the
562 honeybees to adapt to their ever-changing scent environment [48, 49]. From a broader view,
563 OR plasticity linked to olfactory environment influence appears a widespread phenomenon

564 [46, 48, 49, 70, 71]. Here, the increase of peripheral response to B supports a parallel increase
565 of either the density of ORs dedicated to B processing or of the number of ORNs expressing
566 such ORs (mainly through an activity dependent orientation in OR selection in newly mature
567 ORNs, or both). The reported results [63] that odorant exposure did not alter spatial pattern,
568 peak magnitude, or odorant-selectivity of aldehyde-evoked OSN input to olfactory bulb
569 glomeruli, but did alter the temporal dynamics of that input, plead in favor of an increase of
570 density of B sensitive ORs expressed by ORNs spontaneously responsive to B.

571

572 *4.5. MP peripheral processing is spontaneously prioritized in rabbit neonates*

573 In a previous *ex vivo* study in rabbit pups, high concentrations of MP (1.17×10^{-5} and 10^{-6}
574 mol/l) induced large EOGs (up to 8 mV; [24]). Strikingly, here, despite the MP delivery at a
575 lower concentration (7.18×10^{-7} mol/l), which was ranked in the optimal concentration range
576 known to elicit behavioral responses [72], MP induced one of the largest, even the largest
577 EOG in naive pups. This observation supports the conclusion that spontaneous peripheral
578 processing of MP is favored in rabbit neonates' olfactory mucosa, a result consistent with the
579 crucial function of this pheromonal signal in newborn rabbits [e.g., 24, 50, 51, 73]. This raises
580 the question of the origin of MP prioritization at the olfactory system periphery. Since MP is
581 absent in the uterine environment (no trace of the molecule has been found in the amniotic
582 fluid or blood of pregnant rabbit females; [51]), the hypothesis of prenatal MP learning can be
583 rejected. Moreover, the releasing activity of MP is immediately displayed at birth, even before
584 any postnatal exposure to the natural MP contained in the rabbit milk [50, 51]. Therefore,
585 even if in our conditions the peripheral sensitivity to MP could be putatively enhanced thanks
586 to the sucking experience of the first postnatal days, it would only complement a predisposed
587 MP-OR tuning effective immediately at birth. Our results give the first demonstration of the

588 strong and direct link that may exist between peripheral and behavioral processing for a
589 pheromone in mammals as soon as early in life.

590

591 *4.6. Impact of learning on odorant B detection threshold*

592 MP-induced learning of B resulted in an increase of the B sensitivity of newborn rabbits, in
593 our conditions by a factor of at least 100. This validated the second criteria of induction [29].
594 Strikingly, in both naive and conditioned pups, the B threshold concentrations were extremely
595 low, i.e., around 3.40×10^{-22} mol/l (1×10^{-15} g/ml) for the spontaneous threshold and $3.40 \times$
596 10^{-26} mol/l (1×10^{-19} g/ml) for the conditioned threshold. Thus, the spontaneous and
597 conditioned thresholds were respectively 5.3×10^{11} and 5.3×10^{15} times lower than the
598 detection threshold observed for MP in a previous study (1.79×10^{-10} mol/l gas estimated
599 concentration; [72]). It may seem surprising that the MP threshold was higher than that of B, a
600 spontaneously non-relevant odorant. However, in ecological conditions, the MP threshold in
601 newborn rabbits must fit with an optimal detection, discrimination and recognition of that
602 signal in the mother milk. Furthermore, B concentration (at saturated vapor pressure value) is
603 about 7.35×10^6 lower than the MP one. Thus, it can be proposed that the olfactory system
604 adapted to the reality of odor molecule concentrations in order to detect low volatile and (or)
605 concentrated odorants; this may explain, at least partly, why the B threshold value is very low
606 even spontaneously. In line with this hypothesis, the sexual Bombykol pheromone in *Bombyx*
607 *Mori* (Lepidoptera) is perceived at around 2.82×10^{-22} mol/l (gas estimated concentration from
608 [74]), thus far lower than the MP one but close to the ST observed for odorant B in our
609 conditions (3.40×10^{-22} mol/l). However, although sharing pheromonal status, MP is
610 contained in the milk and detected by rabbit pups in the near proximity of the nipples,
611 whereas Bombykol must be detected in the air during the flying of moths, thus at very low
612 concentrations, requiring highly sensitive and specialized OR(s). The olfactory system of this

613 invertebrate is adapted for such an extreme sensitivity since "one pheromone molecule is
614 sufficient to elicit a nerve impulse" [75]. In rabbit neonates, according to our results, B
615 presents a spontaneous threshold quite close to that of Bombykol.

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

624

625 *4.7. Brain plasticity mechanisms underlying post-learning perception of odorant B*

626 In rodents, the anatomical connection between the first two stages of the olfactory system,
627 namely ORNs and olfactory bulb glomeruli takes place very early in life and for instance
628 ORNs respond to odorants immediately at birth ([77] and personal observations). It has been
629 proposed that ORN/OR activity is a prerequisite to the basal expression of ORs [78, 79] and
630 to the correct projection of the axons defining the initial glomerular map [80]. Indeed, the
631 selective suppression of activity in a set of ORNs prevents the formation of the targeted
632 glomeruli [81]. Conversely, chronic exposition to heptaldehyde and acetophenone in adult
633 mice induced the formation of supernumerary glomeruli receiving I7 and M72 axons (these
634 two OR types interacting with the two odor ligands [81]), while exposition to acetophenone
635 and isopropyl tiglate around birth enlarged the size of glomeruli receiving M72 and M71
636 inputs [82]. Not only simple exposure but also and mainly odor associative learning can
637 induce alteration of the glomerular map [36, 83, 84]. Thus, the literature converges towards

638 the idea that olfactory experience not only influences OR expression and OR signaling (i.e.,
639 ORN activity) but governs the development and plasticity of the glomerular map with sensory
640 experience, resulting in the permanent formation or reorganization of glomeruli through a
641 “constructivist” process [85].

642 Our results suggest that B paired to MP during the conditioning episode triggers or (and)
643 up-regulates the expression of ORs having high affinity to B. The weight of B inputs at the
644 olfactory bulb would be thus increased leading the pups to have a lower perceptual threshold.
645 Because we are testing neonates here, and are using a powerful and crucial pheromone for
646 them as unconditioned stimulus during the associative conditioning procedure, olfactory bulb
647 and targeted central neuroanatomic structures may quickly adapt in less than 24h (see also
648 [22]). In addition, the co-input of B and MP confers a high level of biological pertinence to B,
649 likely involving the amygdala, which mediates emotionally arousing experiences by
650 reinforcing memorization (e.g., [22]).

651

652 **5. Conclusions**

653 First, our results highlight that, as soon as the periphery, the olfactory system differentially
654 and spontaneously processes two mixtures containing the same elements, making more or less
655 salient antagonistic interactions between odorants, and leading to their distinct perception in
656 the same animals (i.e., AB and A'B' mixtures in newborn rabbits here). Such a differential
657 process probably results from the concentration (dependent on the volatility) and ratio of the
658 components; the periphery being adapted to deal with low saturated vapor pressure
659 components, such as odorant B, allowing them to largely influence the mixture processing,
660 with respect to one component with high saturated vapor pressure (as for example odorant A).
661 Thus, peripheral processes may contribute, at least in part, to the configural vs. elemental
662 perception of odor mixtures. Moreover, MP-induced odor learning of B altered the peripheral

663 processing of the A'B' mixture by decreasing suppressive interaction between the
664 components, which may facilitate the detection of the learned component in that elemental
665 mixture and explain the behavioral responsiveness of the pups to A'B' (although the mixture
666 is initially inactive on the behavior). Conversely, the configural perception of AB would be
667 favored by less suppressive interactions between its odorants.

668 Second, our results demonstrate that MP-induced single trial odor learning involves (at
669 least partly) an induction phenomenon in newborn rabbits. Indeed, the conditioning promoted
670 by MP increased extremely rapidly (24h) and in parallel the peripheral reactivity to the
671 learned stimulus and its detection abilities by the animal. These changes totally agree with the
672 seminal results pinpointing induction in anosmic human patients [29] and extend the notion of
673 induction to the neonatal period in normosmic animals. After learning, MP loses its
674 spontaneous and unique privileged peripheral processing to the shared benefit of the
675 conditioned odorant. As a consequence, some new elements (odorants) from the external
676 chemical environment appears to become relevant for the young organism, the olfactory
677 system favoring their detection from the OR level, while remaining reactive to spontaneously
678 valuable stimuli (as the MP). In ecological conditions of mother-young interactions, MP-
679 induced odor learning is functional when the mother comes into the nest to nurse her litter and
680 newborns are exposed to odor cues carried by her body during nursing, allowing them to
681 acquire odors contemporarily present in the living environment of the mother and social group
682 [18, 76].

683 Induction is consistent with the idea that the olfactory system structure and functioning
684 operates according to constructivist theory, perfectly adapted to a sensory system facing a
685 quasi-infinite number of stimuli. Thus, even if over phylogenetic evolution, neocortex,
686 auditory and visual information apparently take the place of the chemosensory systems
687 primarily contributing to decision-making in most mammals, olfaction supports detection and

688 perception performances even more remarkable than visual and acoustic ones [86]. This is
689 highlighted here very early in life, in organisms still partly immature.

690

691

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703

704 **Conflict of Interest**

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

706

707 **Ethical approval**

708 All applicable international, national, and/or institutional guidelines for the care and use of
709 animals were followed. All procedures performed were approved by the ethical committee of
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712

713 **Informed consent**

714 This article does not contain any studies with human participants performed by any of the
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716

717 **7. References**

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960 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

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

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964 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

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

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Figure Legends

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Figure 1: a. Positioning of the three electrodes on the three turbinates (T1, T2, T3) on the hemi-head of a rabbit neonate (the large arrow indicating an enlargement of the area). The olfactomer nozzle is positioned so that the odorized puff (materialized in grey shadow) sweeps the 3 turbinates, as if it was naturally entering through the animal's nostril.

b. Illustration of raw recordings simultaneously obtained from the three turbinates. The EOG amplitude (here in response to the odorant A) is measured between the baseline level sampled within 100 ms prior to the start of olfactory stimulation (STIM) and the peak amplitude.

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

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

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

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999 **Figure 5:** Comparisons of mean EOG amplitudes (\pm SD) in response to **a.** the AB series, and
1000 **b.** the A'B' series, in nasal turbinates 1, 2 and 3 of naive and conditioned rabbit neonates.
1001 Stimuli are ordered in ascending level of concentration from left to right except for MP,
1002 which was always tested at the end as a control. *, **, *** indicate differences at $p < 0.05$,
1003 0.01 and 0.001 (Wilcoxon test for non-matched samples).

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1005 **Figure 6:** Occurrences of each odor stimulus as being **a.** the least effective (i.e., inducing the
1006 smallest EOGs) or **b.** the most effective (i.e., inducing the largest EOGs) in naive (grey bars)
1007 and conditioned (black bars) rabbit neonates for the stimulus series AB (left) and A'B' (right).
1008 Within the naive and conditioned conditions, χ^2 test and Monte Carlo method were applied on
1009 the distributions. Then the Marascuilo procedure gives the statistical classification of stimuli.
1010 Distinct I geometrical symbols above each bar (grey symbols for: naive pups; black symbols:
1011 conditioned pups) indicate statistical differences at $p < 0.05$, and *, **, *** differences
1012 between naive and conditioned pups at $p < 0.05$, 0.01 and 0.001, respectively; bilateral test of
1013 two proportions, confirmed with Monte Carlo method.

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1015 **Figure 7:** **a.** Proportions of newborn rabbits responding to the odorant B at 10^{-5} g/ml, as a
1016 function of the concentration of B used during the conditioning (ran 24h before). The
1017 conditioning concentrations were ranged from 10^{-5} to 10^{-22} g/ml (grey bars). This experiment

1018 allowed to assess the spontaneous threshold (ST) of B in the animals (n = 50); **b.** Proportions
1019 of newborn rabbits responding to the odorant B at concentrations ranged from 10^{-5} to 10^{-22}
1020 g/ml 24h after conditioning to B at 10^{-5} g/ml. This experiment pinpointed the conditioning
1021 threshold (CT) of B in the animals (n = 105).