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1 **Neonatal representation of odour objects: distinct memories of the whole and its**
2 **parts**

3
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15 Short title for website: Neonatal memory of complexity

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18 **SUMMARY**

19 Extraction of relevant information from highly complex environments is a prerequisite to survival.
20 Within odour mixtures, such information is contained in the odours of specific elements or in the
21 mixture configuration perceived as a whole unique odour. For instance, an AB mixture of the
22 element A (ethyl isobutyrate) and the element B (ethyl maltol) generates a configural AB percept in
23 humans and apparently in another species, the rabbit. Here, we examined whether the memory of
24 such a configuration is distinct from the memory of the individual odorants. Taking advantage of
25 the newborn rabbit abilities to learn odour mixtures, we combined behavioural and pharmacological
26 tools to specifically eliminate elemental memory of A and B after conditioning to the AB mixture,
27 and evaluate consequences on configural memory of AB. The amnesic treatment suppressed
28 responsiveness to A and B but not to AB. Two other experiments confirmed the specific perception
29 and particular memory of the AB mixture. These data demonstrate the existence of configurations
30 in certain odour mixtures and their representation as unique objects: after learning, animals form a
31 configural memory of these mixtures, which coexists with, but is relatively dissociated from,
32 memory of their elements. This capability emerges very early in life.

33

34 **Keywords:** *Oryctolagus cuniculus*, newborn, odour mixture, configural perception, memory,
35 representation

36

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38

39 **1. INTRODUCTION**

40 Animals constantly interact with the environment. Specifically, they must discriminate the sensory
41 information available in the surroundings and extract those which are the most relevant for survival
42 and development. Indeed, the environment is highly complex in terms of the number and diversity
43 of stimuli. For instance, mammals must process composite visual and auditory stimuli, or mixtures
44 of chemical cues, to respectively recognize the face, the voice or the odour of conspecifics [1-4].
45 However, this processing raises a question currently debated in the scientific literature, namely do
46 the face, the voice and the odour each constitute for the receiver a sum of elements or individual
47 unique cues? Here, we consider this problem within olfaction.

48 In some cases, mixtures of odorants are perceived as a collection of independent, identifiable
49 elements; the perception is then elemental [e.g., 5-7]. However, some mixtures induce a configural
50 processing, meaning that the mixture gives rise to either a unique and novel perceptual odour
51 quality, different from the odour qualities of the elements, or to a novel quality perceived in
52 addition to the qualities of the odorants [8]. Configural odour processing has been described in a
53 variety of species (e.g., bee, catfish, human, spiny lobster, moth, rat), with different approaches
54 [e.g., 9-16]. For instance, data in human adults revealed that a mixture of two odorants (AB), one
55 smelling like strawberry (A: ethyl isobutyrate) and the other like caramel (B: ethyl maltol),
56 generates the configural perception of a pineapple odour at a specific ratio of A/B [17-18].

57 Interestingly, recent results in a young mammal, the newborn rabbit, showed similar configural
58 processing abilities with the same AB mixture. After the learning of one element (A or B), rabbit
59 pups do not respond to the AB mixture (while they respond very well to an AC mixture; odorant C:
60 guaiacol), suggesting that they perceive in AB something more than the odours of A and B, i.e. an
61 AB configuration. Conversely, after the learning of the AB mixture, they respond both to A, B and
62 to AB, suggesting that they acquire both the elements and the configuration during the conditioning
63 [19-21]. Therefore, newborn rabbits are strongly suspected to perceive the AB mixture in a weak
64 configural way, i.e. to perceive three distinct odours in the mixture: the odour of the AB

65 configuration in addition to the odours of the elements A and B. When the ratio of A/B is modified,
66 the pups strongly respond to this A'B' mixture after the learning of one element (A or B),
67 suggesting a shift from the configural representation to the elemental one [22]. However, a clear
68 demonstration that configural representation is distinct from representation of each element was still
69 lacking in the literature for either young or adult organisms. Here, we combined a behavioural
70 approach with pharmacological tools to assess the perception and retention of complex odour
71 stimuli (mixtures) in the form of entities (configurations) or through their constituting elements.

72 After learning, memories become stabilized within hours following a consolidation phase
73 involving protein synthesis [23,24]. The use of protein synthesis inhibitors, such as anisomycin,
74 during this time-limited period disrupts memory consolidation and consequently erases memory.
75 Retrieval can return memories to a labile, protein synthesis-dependent state, a process referred to as
76 memory reconsolidation [25,26]. Again, disruption of memory reconsolidation, by the use of
77 protein synthesis inhibitors, erases the reactivated memory. This process of reconsolidation has
78 been demonstrated across species, memory paradigms, and in particular in rabbit pups after odour
79 conditioning [27]. Moreover, this process is selective to the reactivated memory. For instance, in
80 newborn rabbits initially conditioned to two odorants, injection of anisomycin after reactivating
81 only one of the elements abolishes the response to this odorant without interfering with the response
82 to the other non-reactivated odorant [28,29].

83 Here, in three successive experiments, we systematically used a similar 3 steps procedure
84 including a) a *conditioning phase* inducing olfactory learning of a mixture (AB or A'B'),
85 b) a *reactivation phase* to reactivate some (A and B) or all (A, B and AB) of the previously
86 conditioned information in order to induce amnesia of the reactivated information using
87 pharmacological treatment (see [28,29]), and c) a *testing phase* to assess behavioural responsiveness
88 towards the mixture and its elements. First, in rabbit neonates previously conditioned to the
89 configural AB mixture, we injected anisomycin after separate reactivation of its elements (odorants
90 A and B) in order to specifically erase the memory of A and the memory of B; then, we evaluated

91 the consequences on the response to the AB mixture (Experiment 1). An absence of response to AB
92 will indicate that responsiveness to the mixture critically depends on the representation of the
93 elements. Conversely, response to AB after amnesia of A and B will indicate that the configural
94 memory of the mixture is clearly dissociated from the memories of its elements. As a second step,
95 we made a control experiment with the A'B' mixture (Experiment 2). Since this mixture is
96 perceived in an elemental way by rabbit pups, i.e. without a configural odour but as the sum of the
97 element odours, pharmacological disruption of A and B memories after reactivation should be
98 followed by amnesia of A'B'. Finally, after neonatal conditioning to the configural AB mixture, we
99 determined the effect of preventing AB memory reconsolidation on the memory of the AB mixture
100 and of its components. Based on previous work, rabbit pups were strongly suspected to perceive
101 both the elements (A and B) and the AB configuration during exposure to the AB mixture. As a
102 consequence, the reactivation of the whole mixture should reactivate the memory of its three
103 representations (A, B and AB) and amnesic treatment should induce forgetting of all these
104 memories (Experiment 3). Taken together, these experiments aimed to uncover chemosensory
105 perceptual and memory mechanisms available to promote initial decisions and actions critical for
106 social relationships and feeding behaviour in mammals.

107

108

109 **2. MATERIAL AND METHODS**

110 *(a) Animals and housing conditions*

111 Males and females New-Zealand rabbits *Oryctolagus cuniculus* (Charles River strain, L'Arbresle,
112 France) from the Centre de Zootechnie (University of Burgundy, Dijon) were kept in individual
113 cages. A nest box (0.39 x 0.25 x 0.32 m) was added on the outside of the pregnant females' cages 2
114 days before delivery (day of delivery was day 0; d0). To equalize pups' nursing experience, all
115 females had access to their nest between 11:30-11:45 a.m. This procedure allowed females to
116 follow the brief (3-4 min) daily nursing of the species [30]. Animals were kept under a constant

117 12:12 light:dark cycle (light on at 7:00 a.m.) with ambient air temperature maintained at 21-22°C.
118 Water and pelleted food (Lapin Elevage 110, Safe, France) were provided *ad libitum*. In the study,
119 68 newborns (from 18 litters) were used.

120

121 **(b) Odorants**

122 The odorants consisted of 2-methylbut-2-enal (the Mammary Pheromone, MP, CAS# 497-03-0)
123 [27,31], ethyl isobutyrate (odorant A, CAS# 97-62-1), ethyl maltol (odorant B, CAS# 4940-11-8)
124 for pure components, and of AB and A'B' mixtures. The AB mixture included 0.3×10^{-5} and
125 0.7×10^{-5} g/ml of components A/B; this 30/70 v/v ratio elicits configural perception of a pineapple
126 odour in human adults due to blending properties [17,32], and seems to induce weak configural
127 perception in newborn rabbits (i.e. perception of three distinct odours in the mixture, the odours of
128 A, B and AB) [19-22]. The A'B' mixture included 1.5×10^{-5} and 0.7×10^{-5} g/ml of components A/B;
129 this 68/32 v/v ratio elicits elemental perception of the mixture in newborn rabbits (i.e. perception of
130 two odours only, those of the element A and the element B) [22]. Contrary to the MP, none of the
131 A, B, AB and A'B' stimuli triggered spontaneous sucking behaviour of rabbit pups; they were
132 therefore considered as initially neutral [19-22].

133 The MP allowed us to induce the learning of the AB or A'B' mixtures through associative
134 conditioning [see section (c)]. MP served as the unconditioned stimulus and was used at 10^{-5} g/ml, a
135 concentration known to be highly efficient to promote conditioning [31], while the AB or A'B'
136 mixtures served as the conditioned stimuli. Thus, the AB-MP and A'B'-MP blends included 1×10^{-5}
137 g/ml of MP and respectively 0.3 and 0.7×10^{-5} g/ml, or 1.5×10^{-5} and 0.7×10^{-5} of A and B.

138 Single odorants A and B (10^{-5} g/ml), or the AB mixture were also used in the reactivation
139 procedure, and the same stimuli plus the A'B' mixture were used during behavioural testing.

140 All the odorants were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France) and all
141 the final solutions were prepared in a solvent composed of 0.1% of ethanol (anhydrous, Carlo Erba,
142 Val de Reuil, France) and 99.9% of MilliQ water (Millipore, Molsheim, France).

143

144 ***(c) Phase 1: Odour conditioning***

145 Conditioning sessions were run on day 1 in an experimental room close to the breeding room. The
146 pups were transferred by groups of 4 (2/litter) into a box lined with nest materials and maintained at
147 room temperature. The MP-induced conditioning was run following a procedure previously
148 described, which consisted in a single, brief and simultaneous exposure both to the unconditioned
149 MP and the conditioned stimulus. Thus, for the conditioning session, 4 ml of the MP-AB mixture
150 (Experiments 1 and 3) or MP-A'B' mixture (Experiment 2) were pipetted on a pad (19x14 cm,
151 100% cotton) then held 2 cm above the pups for 5 min. This exposure is known to induce the very
152 rapid learning of the stimulus paired with the MP, here an odour mixture [e.g., 19,27,28,31]. The
153 conditioning session occurred 1h before the daily nursing (10:30 a.m.), to equalize the pups'
154 motivational state and limit the impact of satiation on responses [33]. Two minutes after the end of
155 the conditioning, the pups were individually marked with weakly odorous ink and returned to their
156 nest. The box containing the pups was rinsed with alcohol and distilled water after each
157 conditioning session.

158

159 ***(d) Phase 2: Reactivation and Pharmacological treatment***

160 On day 2, twenty-four hours after the conditioning, the memory of pups was reactivated in
161 Experiment 1 and 2 by successive exposure to each element A and B (the order of presentation of A
162 and B was counter-balanced between pups from a same group). The exposure consisted in
163 stimulation with each odorant A and B during 2.5 min (inter-stimulation delay: 1 min) following the
164 same procedure as for conditioning (odorised cotton pad held above the litter). In Experiment 3, the
165 AB mixture itself was used during a 5-min long reactivating exposure.

166 In each experiment, immediately after reactivation, anisomycin (AN; Aldrich) was injected to
167 half of the pups (42 mg/kg, i.p.) after dilution in 0.9% NaCl solution and adjustment of pH 7.2 with
168 1N HCl [27-28]. Control for the effect of AN injection was realized with the other half of animals

169 receiving saline 0.9%. As in other studies with other newborn or adult mammals [e.g., 23,34-36] we
170 considered that AN in newborn rabbits may induce a real amnesia and not a perturbation in
171 responsiveness due to an aversive effect [27,28]. Pups were returned to the nest just after AN or
172 saline injection.

173

174 ***(e) Phase 3: Behavioural assay***

175 The behavioural assay occurred on day 3 (i.e., 24h after Phase 2 of reactivation) in the experimental
176 room previously used for conditioning and reactivation. It happened also 1h before the daily nursing
177 to limit the impact of satiation on motivation and behavioural responsiveness [33]. The assay
178 consisted of an oral activation test during which a pup was immobilized in one gloved hand of the
179 experimenter, its head being left free. Each odour stimulus (odorant A, odorant B, and the AB or
180 A'B' mixture) was presented for 10 s. with a glass rod 0.5 cm in front of the nares [e.g.,
181 19,27,28,31]. A test was positive when the conditioned stimulus elicited (on/off response) head-
182 searching movements (vigorous, low amplitude horizontal and vertical scanning movements
183 displayed after stretching towards the rod) usually followed by grasping movements (labial seizing
184 of the rod extremity). Non-responding pups displayed no response except sniffing. Pups were tested
185 in groups of 4 or 6, mixing AN- and saline-treated newborns. The experimenters did not know if the
186 currently tested pups belonged to one or the other treatment group. Each pup participated in only
187 one experiment but was successively tested with 3 stimuli, i.e. odorant A, odorant B and the AB
188 mixture in Experiments 1 and 3, and odorant A, odorant B and the A'B' mixture in Experiment 2.
189 Successive testing involved the presentation of a first stimulus to a pup, then a second stimulus to
190 another pup, and so on with an inter-trial interval of 60 s. The order of stimuli presentation was
191 systematically counterbalanced from one to another pup. If a pup responded to a stimulus, its nose
192 was softly dried before the next stimulation. The pups were immediately reintroduced in their nest
193 after testing.

194

195 *(f) Statistics*

196 Due to the death of 4 pups (2 AN, 2 saline), the analyses focused on 64 newborns. The frequencies
197 of responding pups were compared using the χ^2 test of Pearson when the groups were independent
198 (i.e., distinct groups tested for their response to a same stimulus) or the Cochran's Q test when the
199 groups were dependent (i.e., pups from a same group tested for their response to three stimuli).
200 When the Cochran's Q test was significant, proportions of responding pups were compared 2 x 2 by
201 the χ^2 test of McNemar. Degrees of freedom are indicated when > 1 . Data were considered as
202 significant when the two-tailed test ended with $p < 0.05$.

203

204

205 **3. RESULTS**

206 **1. Learning of the AB configural mixture, amnesia of A and B and resulting memory of AB**

207 The AB mixture is hypothesized to be weakly configurally perceived by rabbit neonates, meaning
208 that pups should perceive the odour of AB in addition to the odours of the element A and the
209 element B in the mixture. If this is true, after conditioning to the whole mixture, the memory of the
210 AB configural odour might be distinct from the memory of the element odours. To assess whether
211 conditioning to the AB mixture induced separate memories of the suspected AB configural odour
212 compared to the odours of the A and B elements, 24 rabbit pups were conditioned to AB by pairing
213 with the MP on day 1, reactivated by successive exposure to the A and B elements on day 2
214 (without MP) then immediately injected with saline ($n = 10$, control group) or AN ($n = 14$,
215 experimental group), and tested for their behavioural responsiveness to A, B and AB on day 3. The
216 AN treatment should induce amnesia of the element odours, but not necessarily of the AB
217 configuration. In the control group, which did not receive AN treatment, the pups should respond
218 strongly and equally to the odours of A, B and AB.

219 One AN-treated pup died between days 2 and 3; the results therefore concerned 10 vs 13
220 neonates. On day 3, the saline-treated pups highly responded both to the AB mixture and to the

221 components ($> 80\%$, $Q = 2$, $ddl = 2$, $p > 0.38$). Conversely, AN-treated pups displayed distinct
222 responsiveness to the stimuli ($Q = 12.3$, $ddl = 2$, $p = 0.006$): they did not respond to the odorants
223 presented separately ($< 8\%$), but were still robustly responsive to the AB mixture ($> 60\%$, AB vs A
224 or B: $\chi^2 > 5.6$, $p < 0.018$). While AN-treated pups responded clearly less to the A and B odorants
225 than saline-treated neonates ($\chi^2 > 9.5$, $p < 0.01$ for each odorant), they maintained a similar level of
226 responsiveness to AB ($\chi^2 = 1.12$, $p = 0.29$) (Figure 1).

227 Thus, after conditioning to the AB mixture and separated reactivation of odorants A and B, AN-
228 injection was followed by retrograde amnesia of the odours of the two components. This amnesia
229 was however not sufficient to prevent responsiveness to the whole mixture; the memory of the
230 mixture, and only of the mixture, remained intact.

231

232

233 **2. Learning of the A'B' elemental mixture, amnesia of A and B and resulting memory of A'B'**

234 Previous evidence suggests that, in contrast to the AB mixture, the A'B' mixture is olfactorily
235 perceived by rabbit pups as the sum of its elements but not as a configuration, i.e. that pups
236 perceived the odours of A and of B but not of an A'B' configuration in the A'B' mixture [22].
237 Therefore, we hypothesized that after conditioning to A'B', reactivation and amnesia of the
238 elements A and B should dramatically impair responsiveness to the A'B' mixture, in contrast to
239 what we observed in Exp. 1 with the AB mixture. To run this control experiment, 24 new rabbit
240 pups were conditioned to A'B' by association with the MP on day 1, exposed successively to A and
241 to B and immediately injected with saline ($n = 12$, control group) or AN ($n = 12$, experimental
242 group) on day 2, and tested for their behavioural responsiveness to A, B and A'B' on day 3. Pups
243 from the AN group were hypothesized to neither respond to A or to B nor to A'B', while saline-
244 injected control pups should respond both to the elements and to the mixture.

245 Three pups died between days 2 and 3 (respectively saline, $n = 2$ and AN, $n = 1$); thus analysis
246 concerned 10 vs 11 pups. On day 3, the saline-treated pups fully responded to A, B and AB (100%).

247 Comparatively, AN-treated neonates responded extremely weakly to the stimuli, not only to the
248 odorants but also the A'B' mixture (< 10%). For each stimulus, the responsiveness was therefore
249 higher in saline- than in AN-treated pups ($\chi^2 > 13.9$, $p < 0.001$ in all 2x2 comparisons) (Figure 2).

250 Thus, in rabbit pups initially conditioned to the A'B' mixture, reactivation with odorants A and
251 B followed by blockade of reconsolidation, impaired the memory both of the elements and of the
252 A'B' mixture. Responsiveness to the components seemed essential to respond to their mixture at
253 this ratio.

254

255

256 **3. Learning then amnesia of the AB configural mixture and resulting memory of AB, A and B**

257 Results of Exp. 1 showed that after MP-induced conditioning to the AB mixture, rabbit pups
258 responded to the mixture even after forgetting its elements A and B. This suggested distinct
259 memory of the AB configural odour versus memory of the A and B element odours, and accounted
260 for the weak configural perception of the AB mixture, i.e. the perception during conditioning of a
261 specific AB odour in addition to the odour of each element. To finally confirm the weak configural
262 perception of the AB mixture, we induced its conditioning, reactivated the memory of the whole
263 mixture (not of its elements only as in Exp. 1) before inducing amnesia, and tested the
264 responsiveness of the pups to AB, A and B. In this particular situation, if AB was perceived as the
265 sum of two elements plus one configuration, the presentation of AB during the reactivation phase
266 should reactivate both the memory of the configuration and of the elements which then should both
267 be erased by the pharmacological treatment. This should consequently impede the pups'
268 responsiveness to each of the three odours perceived in the mixture, A, B and AB. Thus, 20 pups
269 were conditioned to AB by association with the MP on day 1, reactivated with AB before injection
270 of saline (n= 10, control group) or AN (n = 10, experimental group) on day 2, and tested for their
271 responsiveness to A, B and AB on day 3. Pups from the AN group should not respond to any of the
272 stimuli, while those from the saline group should respond to all.

273 All saline-treated pups responded to the odorants A and B, and to the AB mixture. Conversely,
274 AN-treated pups displayed no or extremely weak responsiveness to any of the stimuli (< 10%;
275 $Q = 2$, $ddl = 2$, $p = 0.38$). As in Exp. 1, saline treated pups responded therefore more to the odorants
276 than AN-treated neonates ($\chi^2 = 12.9$, $p < 0.001$ for each odorant). However, here, injection of AN
277 was followed by a major drop in responsiveness to AB compared to injection of saline ($\chi^2 = 16.2$,
278 $p < 0.001$) (Figure 3).

279 Thus, after AB conditioning, when reactivation concerned the whole AB mixture, amnesia of AB
280 appeared suppressive of any response to either the mixture or to its elements.

281

282

283 **4. DISCUSSION**

284 Natural odours are constituted by mixtures of distinct molecules which carry by themselves
285 particular odours. In that context, it is often considered that configural processing, i.e. perception of
286 some mixtures as odour objects, is an efficient way to reduce the complexity of the chemical
287 surroundings and optimize the detection, identification and discrimination between stimuli carrying
288 biological significance [4,37-39]. However, a clear demonstration of brain and cognitive processes
289 that could selectively differentiate a complex odour as a whole - unique representation - from its
290 odorant parts has been lacking. Here, we combined behavioural and pharmacological approaches to
291 evaluate whether the representation of AB, an apparent configural mixture for rabbit pups at a
292 specific ratio (30/70) [19-22], is relatively distinct from the representation of each component
293 odorant.

294 As main results, we provide evidence that after neonatal learning of the AB mixture, amnesia of
295 A and B did not propagate to AB: pups that did not respond to either A or to B still responded to
296 AB (Exp. 1). Thus, a particular memory of AB was created during conditioning to the mixture, in
297 parallel to the memories of odorant A and odorant B, and all these memories created together
298 become rapidly dissociated. However, after conditioning to the AB mixture, reexposure to the

299 whole mixture (Exp. 3) reactivated not only the AB configuration but also the elements A and B,
300 which were all sensitive to the post-reactivation pharmacological treatment: the pups became
301 amnesic to AB but also to A and to B. In terms of perception, this demonstrates something only
302 previously suggested: the AB mixture evokes a configural odour perceived by newborn rabbits in
303 addition to (no to the detriment of) the specific odours of A and B. In other words, the perception of
304 the AB mixture by rabbit pups is weak configural and not robust configural [for previous
305 suggestions of partial configural perception of AB, see 19-22]. The configural AB odour can be
306 processed on its own and is sufficient to trigger the behavioural response to the AB mixture. Thus,
307 in the context of neonatal odour perception, the results indicate that a complex stimulus can induce
308 different percepts which are simultaneously memorized but form rapidly, relatively separated
309 memory traces. The memories of the mixture and its components are not entirely independent
310 however. That is, while disruption of the component memory did not affect the configural AB
311 memory, disruption of the configural AB memory did impair the memory of the elements. This
312 asymmetrical relationship suggests complex interactions between these different, relatively distinct
313 representations. As further evidence of interactions between the representations of A, B and AB, we
314 have recently demonstrated competition between the elemental and configural long-term (several
315 days) memories [40].

316 The memory treatment observed here could be also at play later in life, since in human adults the
317 same AB mixture is known to evoke an odour (pineapple) different from those of its A and B
318 elements (strawberry and caramel, respectively) [17,18]. Comparatively, when rabbit pups are
319 conditioned to the elements A and B in mixture, but at a ratio known to trigger the elemental
320 perception of the mixture (A'B', ratio 68/32) [22], amnesia of A and of B abolished the response to
321 A'B'. This demonstrates that responsiveness to the A'B' mixture is based exclusively on perception
322 of elements A and B and that no mixture-specific memory is created at this ratio [see 41, for similar
323 results with an elementally processed compound composed of a tone and a light].

324 The present findings, when combined with previous work [19,20,29], demonstrate that memory
325 for odour mixtures may be encoded in a variety of ways, depending on the nature of the stimuli and
326 post-training events. The evidence that memories of certain odour mixtures (as the AB mixture
327 here) can be simultaneously, and relatively separately, configural and elemental suggests that even
328 mixtures perceived configurally have traces of their components somewhere in the brain. Analysis
329 of configural processing and the formation of odour objects suggest a strong role for the olfactory
330 (piriform) cortex and plasticity of intracortical association fibre synapses which can link distributed,
331 co-active cortical neurons [38]. Thus, while spatial coding of mixture-evoked activity within the
332 olfactory bulb did not discriminate configural mixtures from their components [42-44], piriform
333 cortical neural ensembles have been demonstrated to rapidly process co-occurring odorants into
334 distinct representations, different from the representations of their component parts [45]. This
335 cortical representation of configural odour objects is experience-dependent, promotes odour
336 discrimination, and can be impaired by disrupting normal synaptic plasticity selectively within the
337 piriform cortex [46-48].

338 It is unclear whether the simultaneous elemental representation of the components apparent here
339 is also dependent on the piriform cortex. Processing of odours occurs in a variety of regions beyond
340 the canonical olfactory pathway, including hippocampal formation, frontal areas and other limbic
341 structures [49-51], even during early development [52]. In humans, it has been demonstrated that
342 the brain can distinguish between single odorants and binary mixtures [53]. Moreover, combining
343 odours that differ in hedonic quality (e.g., pleasant and unpleasant) can create a configurally
344 pleasant odour percept [54]. However, when assessed with neural imaging, circuits normally
345 selectively activated in response to the unpleasant component are still activated, even though the
346 configural perception does not reflect this underlying component [54]. The present results suggest a
347 similar distributed network process may occur in newborn rabbits, with memory for individual
348 components occurring distinctively (at least in part) from the configural memory. Further work will

349 be required to identify neural mechanisms and locations of elemental versus configural processing,
350 including the roles of the olfactory bulb [7], olfactory cortex and elsewhere.

351 Finally and regarding adaptation, odour mixture perception has a major impact on animal
352 behaviour, both in aquatic and terrestrial species, and contributes to decision making related to food
353 searching, mate choice, spatial orientation, and to interspecies interactions as predator avoidance
354 and plant pollination [e.g., 11,12,55-58]. However, knowledge about the way odour mixtures are
355 precisely processed, retained and connected to behaviour remains scarce. Here, the findings
356 demonstrate the existence of configurations in certain odour mixtures, depending on odorants' ratio,
357 and their representation as unique objects. To date, these possibilities were only suggested at a
358 perceptual level (in young as in adults). Here, they are evidenced even in an incompletely mature,
359 neonatal organism, and strengthened by straightforward results related to a more integrative level,
360 memory: clearly, a specific memory of configural odour mixtures exists, and appears involved in
361 rapid processing and responsiveness to behaviourally significant chemically complex stimuli. In
362 newborn rabbits, odour learning occurs during the daily interaction with the mother devoted to
363 nursing [30,59-62]. Memory of single odorants but also of configural information is certainly at
364 work during this vital period of interaction. It could allow for neonates to acquire, represent and
365 successfully retain complex odour information carried by the maternal body, that may help
366 improving the relationships with the mother (attraction, sucking, recognition), as indirect
367 information linked to the social, physical and feeding environment useful later in life. More
368 generally, the reactivity to configural information contained in some complex sensory stimuli
369 certainly allows an animal to categorize efficiently the diversity of stimuli which constitute its own
370 world and to find familiarity in the most constant and crucial representations of this changing
371 environment.

372

373 The study was carried out under the local, institutional and national rules (French Ministries of
374 Agriculture, and of Research & Technology) regarding the care and experimental use of the

375 animals. All experiments were conducted in accordance with ethical rules enforced by French law,
376 and were approved by the Ethical Committee for Animal Experimentation (Dijon, France; no.
377 2406).

378

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539

540

Figure Legends

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543

544 **Figure 1:** Proportions of 3-day-old rabbit pups responding in an oral activation test to odorant A
545 (ethyl isobutyrate), odorant B (ethyl maltol) and the AB mixture (30/70 ratio of A/B), after
546 conditioning to AB on day 1 by pairing with the mammary pheromone (MP), and reactivation on
547 day 2 by successive exposure to A and to B followed by immediate injection of anisomycin (AN) or
548 NaCl (saline). *: $p < 0.05$.

549

550 **Figure 2:** Proportions of 3-day-old rabbit pups responding in an oral activation test to odorant A
551 (ethyl isobutyrate), odorant B (ethyl maltol) and the A'B' mixture (68/32 ratio of A/B), after
552 conditioning to A'B' on day 1 by pairing with the mammary pheromone (MP), and reactivation on
553 day 2 by successive exposure to A and to B followed by immediate injection of anisomycin (AN) or
554 NaCl (saline).

555

556 **Figure 3:** Proportions of 3-day-old rabbit pups responding in an oral activation test to odorant A
557 (ethyl isobutyrate), odorant B (ethyl maltol) and the AB mixture (30/70 ratio of A/B), after
558 conditioning to AB on day 1 by pairing with the mammary pheromone (MP), and reactivation on
559 day 2 by exposure to AB followed by immediate injection of anisomycin (AN) or NaCl (saline).

560