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► **To cite this version:**

Gérard Coureaud, Chloé Letagneaux, Thierry Thomas-Danguin, Sébastien Romagny. Developmental changes in elemental and configural perception of odor mixtures in young rabbits. *Developmental Psychobiology*, 2020, 62 (4), pp.471-483. 10.1002/dev.21929 . hal-02335139

HAL Id: hal-02335139

<https://hal.science/hal-02335139v1>

Submitted on 25 Nov 2020

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1 **Developmental changes in elemental and configural perception of odor mixtures in**
2 **young rabbits**

3

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16 *Running title: Odor mixture perception in young rabbits*

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19

1 **Abstract**

2 Development can change the way organisms represent their environment and affect their
3 behavior. In vision, complex stimuli are treated as the sum of their elements (elemental
4 perception) in children or as a whole (configural perception) in adults. However, the influence
5 of development in elemental/configural perception has never been tested in olfaction. Here we
6 explored this issue in young rabbits, which are known to perceive during the neonatal period
7 certain binary odor mixtures elementally and others weak configurally. Using conditioning
8 and behavioral testing procedures, we set out 6 experiments evaluating the putative evolution
9 of their odor perception between birth and weaning. Results highlighted that between
10 postnatal days 2 and 9 the perception of an initially weak configural mixture became robust
11 configural while that of two elemental mixtures did not. Additional switches from elemental
12 to configural perception were observed at postnatal day 24. The use of a chemically more
13 complex senary mixture resulted also in a shift from weak to robust configural perception
14 between postnatal days 2 and 9. Thus, the perception of certain odor mixtures may rapidly
15 evolve toward a more holistic mode in young rabbits, which may help simplifying their
16 representation of the environment once out of the nest.

17

18 **Keywords:** European rabbit (*Oryctolagus cuniculus*); newborn; weaning; olfaction; odor
19 mixture; configural perception; behavior.

20

1. INTRODUCTION

Animals are surrounded by an incommensurable amount of sensory information, from which they have to extract the most relevant cues to survive. This is true as soon as the sensory systems become functional. Regarding chemosensation, invertebrates and vertebrates (including humans) start using their olfactory system before birth (Gottlieb, 1971), which allows to anticipate the processing of, and behavioral responsiveness to neonatal cues, in particular thanks to perinatal chemosensory continuity (Schaal, 2000; Coureaud et al., 2002; Romagny et al., 2012). They can also be predisposed to respond to certain biological cues (Morrow-Tesch and McGlone, 1990; Smotherman and Robinson, 1992; Schaal et al., 2003; Coureaud et al., 2010).

Relevant odor stimuli are rarely constituted by single odorants emitted alone, but by more or less complex mixtures of odorants. It is commonly considered that organisms perceive odor mixtures in the elemental or configural ways. When organisms can extract the odors of the mixture elements and respond to them separately, the perception of the mixture is elemental (Kay et al., 2005; Bos et al., 2013; Rokni et al., 2014). Conversely, when a new percept emerges from the mixture and prevents the perception of the element odors, the perception is configural (Livermore et al., 1997; Menzel et al., 1999; Avarguès-Weber et al., 2010; Howard and Gottfried, 2014). In between, the perception is weak configural when the odor of the configuration is detected in addition to the odors of the elements (Kay et al., 2005). These perceptual considerations result from experimental studies mainly conducted in adults. Much scarce are experiments conducted in very young organisms and, to our knowledge, no study has tested the evolution of the elemental and configural olfactory perception over the development. Nevertheless, there might be an advantage being able to process and perceive highly complex chemical signals differently at birth compared to later over the development, in order to display adaptive behaviors crucial for survival at these successive periods of life.

1 The present study deals with this issue. It examines the influence of development on odor
2 mixture perception between birth and weaning in a model of young mammals, the rabbit.

3 In the European rabbit (*Oryctolagus cuniculus*), elemental and configural processing of
4 odor mixtures are functional in neonates. For instance, it has been observed that 24h after
5 conditioning to ethyl isobutyrate (odorant A) or guaiacol (odorant C) by a single and brief
6 pairing with a natural reinforcer, the mammary pheromone, 2-day-old rabbit pups responded
7 to the binary AC mixture; in addition, after single conditioning to this mixture, they later
8 responded not only to AC but also to A and to C (Coureaud et al., 2009). These results
9 highlighted the elemental perception of the AC mixture by rabbit pups, i.e., their ability to
10 perceive the odor quality of each element in that mixture. Interestingly, such elemental
11 processing was not observed with all mixtures. Indeed, after conditioning to odorant A or to
12 ethyl maltol (odorant B), rabbit neonates did not respond to the binary AB mixture, whereas
13 they responded to A and B after conditioning to AB; this mixture was actually perceived in
14 the weak configural way, meaning that the pups perceived not two, but three information in
15 the mixture, i.e., a specific odor quality for the AB configuration in addition to the odor
16 quality of each element (Coureaud et al., 2008a, 2009). Therefore, it has been proposed that
17 rabbit neonates did not respond to the AB mixture after learning only one of its components
18 (A or B) because the mixture contained too much (two) unfamiliar information compared to
19 the single one that became familiar through learning. The weak configural perception of AB
20 has been definitely confirmed by the fact that after conditioning to AB then reactivation of the
21 elements A and B (but not of the AB configuration), followed by a pharmacological treatment
22 that disturbed the memories of A and B, the pups became amnesic to the elements but still
23 responded to the AB configuration (Coureaud et al., 2014a; see also Coureaud et al., 2014b
24 for convergent results). Another study showed that in this binary AB mixture, the ratio of
25 components is a critical factor influencing neonatal perception: whereas the AB mixture

1 (30/70 v/v ratio of A/B) was weak configurally perceived, the A'B' mixture (68/32 ratio of
2 A/B) was perceived elementally since after learning a single odorant, pups responded to A'B'
3 (Coureaud et al., 2011). Similar results have been observed with a chemically more complex
4 mixture of six odorants (RC mixture), which was found to be perceived weak configurally at a
5 particular ratio of the components (Sinding et al., 2013; Romagny et al., 2014, 2015). Thus, in
6 certain binary and senary mixtures, newborn rabbits perceive more than the simple odor
7 qualities of their elements, while in other mixtures of the same chemical complexity, they
8 perceive only the element odors.

9 Based on studies showing that the perception of complex stimuli changes over the
10 development in another sensory modality, i.e., human vision, in favor of configural perception
11 (e.g., Mondloch et al., 2002; Cohen Kadosh et al., 2013; Wakui et al., 2013), one may
12 hypothesize that configural perception of certain odor mixtures becomes more important in
13 older rabbits than in neonates. We addressed this hypothesis experimentally in the present
14 study by comparing the perception of the AB, A'B' and AC mixtures in rabbits at postnatal
15 (PND) days 2, 9 and 24, and of the RC mixture at PND 2 vs. 9. In this altricial mammal, PND
16 2 is a neonatal stage during which audition and vision are not yet functional; PND 9 is shortly
17 after audition becomes functional (around PND 6-7) and eyelids start to open up (full opening
18 around PND 12); at PND 24 young rabbits have left the nest, they start eating solid food in
19 addition to milk and are getting close to the weaning (which occurs between PND 28-35 in
20 domestic breeds) (Xiccato et al., 2005; Coureaud et al., 2008b).

21 In order to run age-dependent comparisons, keeping constant the procedures of
22 conditioning and testing at the different periods was required to strictly assess the
23 developmental-induced effects. For that reason, it was not possible to use the mammary
24 pheromone as a promoter of associative conditioning since its reinforcing action disappears
25 from PND 5 (Coureaud et al., 2010). Therefore, we tried to gain advantage of the maternal-

1 nursing environment, which is an ecological context known to be associative for neonates and
2 supposed to remain positive for pups throughout the first postnatal weeks. Nursing-induced
3 odor conditioning was previously demonstrated in newborn rabbits (e.g., Ivanistkii, 1962;
4 Hudson, 1985; Kindermann et al., 1991) including by our group (Coureaud et al., 2006;
5 Jouhanneau, 2016). During nursing, several reinforcing factors are assumed to act, e.g. the
6 perception of the mammary pheromone (Coureaud et al., 2006), the contact with the maternal
7 abdomen, the warmth of the mother, the sucking action and/or milk intake (Hudson et al.,
8 2002). Therefore, we hypothesized that at least some of these factors maintain their
9 reinforcing action from PND 2 to 24, and we ran experiments using this conditioning
10 procedure to evaluate the perception of the binary and senary aforementioned mixtures on this
11 developmental window.

12 The hypothesis raised was tackled owing to a series of six complementary experiments.
13 Experiment 1 aimed to confirm the weak configural perception of AB by pups at PND 2 and
14 their elemental perception of A'B' and AC after nursing-induced conditioning to a single
15 element or to a mixture. In Experiment 2, the same procedure was repeated but at PND 9. In
16 Experiment 3, the perception of the AB mixture was compared in pups at PND 9 vs. 2 after
17 the successive learning of the two elemental cues that compose the mixture (A and B). In
18 Experiment 4, a day-by-day approach allowed to evaluate precisely the evolution of the AB
19 mixture perception between PND 2 and 9. Experiment 5 aimed to assess the outcome of the
20 AB, A'B' and AC perception close to the weaning, at PND 24. Finally, Experiment 6
21 investigated if a shift in perception is observed between PND 2 and 9 for another and more
22 complex mixture, the 6-component RC mixture.

23

24 **2. MATERIALS AND METHODS**

25 *2.1 Animals and housing conditions*

1 Males and females New-Zealand rabbits *Oryctolagus cuniculus* (Charles River strain,
2 L'Arbresle, France) were kept in individual cages at the Centre de Zootechnie (University of
3 Burgundy, Dijon). A nest box (0.39 x 0.25 x 0.32 m) was added on the outside of the pregnant
4 females' cages 2 days before delivery (day of delivery was day 0; PND 0). To equalize pups'
5 nursing experience, all females had access to their nest between 11:30-11:45 a.m. from PND 0
6 to 10. This procedure allowed females to follow the natural brief daily nursing of the species
7 (< 5 min; Zarrow et al., 1965). After PND 10, the females had free access to the nest, except
8 during the late experimental period: the access was then controlled again from the day before
9 the conditioning to the day of behavioral testing (i.e., PND 22 to PND 24), in order to
10 homogenize the conditions between all the experiments. Animals were kept under a constant
11 12:12 light:dark cycle (light on at 7:00 a.m.) with ambient air temperature maintained at 21-
12 22°C. Water and pelleted food (Lapin Elevage 110, Safe, France) were provided *ad libitum*.
13 In the study, 506 newborns (from 104 litters) were used. We strictly followed the local,
14 institutional and national rules (French Ministries of Agriculture, and of Higher Education and
15 Research) regarding the care and experimental use of the animals. All experiments were thus
16 carried out in accordance with ethical rules enforced by French law and approved by the
17 Ethical Committee of the University of Burgundy (Dijon, France; no. 5305).

18

19 **2.2 Odorants**

20 All the odorants were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). The
21 final solutions were prepared in a solvent composed of 0.1% of ethanol (anhydrous, Carlo
22 Erba, Val de Reuil, France) and 99.9% of MilliQ water (Millipore, Molsheim, France). At this
23 concentration level, ethanol does not carry significant odor for rabbit pups and is likely not
24 detectable (it does not induce any behavior; Coureaud et al., 2008a, 2009). The single, binary

1 and senary stimuli did not spontaneously trigger sucking-related response in pups, and can be
2 considered as behaviorally neutral before conditioning.

3 Concerning binary mixtures, the odorants were ethyl isobutyrate (odorant A, CAS# 97-62-
4 1), ethyl maltol (odorant B, CAS# 4940-11-8), guaiacol (odorant C, CAS# 90-05-1) and their
5 AB, A'B' and AC mixtures (naming and abbreviations are the same as in our previous
6 studies). The AB mixture included 0.3×10^{-5} and 0.7×10^{-5} g/ml of odorants A and B
7 respectively; this 30/70 v/v ratio elicits the perception of a pineapple odor in human adults
8 due to blending properties (Le Berre et al., 2008) and weak configural perception in newborn
9 rabbits in experimental situation of pheromone-induced conditioning (Coureaud et al., 2008a,
10 2009, 2011, 2014a,b; Schneider et al., 2016). The A'B' mixture included 1.5×10^{-5} and
11 0.7×10^{-5} g/ml of components A/B; this 68/32 v/v ratio triggers the elemental perception of the
12 mixture in rabbit pups (Coureaud et al., 2011, 2014a,b). The AC mixture included 0.5×10^{-5}
13 and 0.5×10^{-5} g/ml of odorant A and C, respectively; this 50/50 v/v ratio promotes pure
14 elemental perception of the mixture in rabbit neonates (Coureaud et al., 2009).

15 The senary RC mixture was composed of vanillin (odorant V; CAS # 8014-42-4),
16 frambinone (F; CAS # 5471-51-2), isoamyl acetate (IA; CAS # 123-92-2), β -ionone (B; CAS
17 # 79-77-6), ethyl acetate (EA; CAS # 141-78-6) and damascenone (D, CAS # 23696-85-7) at
18 the 41.8/41.8/5.0/4.3/4.3/2.8 ratio (i.e., $3.3/3.3/0.39/0.34/0.34/0.22 \times 10^{-6}$ g/ml) for
19 V/F/IA/B/EA/D. At these proportions, the mixture elicits configural perception of a red
20 cordial odor (i.e., grenadine) in human adults and weak configural perception in rabbit
21 neonates (Sinding et al., 2013; Romagny et al., 2014, 2015, 2018).

22 All the AB, A'B', AC and RC components were also used as single stimuli. Then, the
23 single odorant concentrations were the same as the concentrations of each odorant in the
24 mixture.

25

1 **2.3 Odor conditioning**

2 Depending on the experiments, the conditioning of pups was run either on a single day
3 between PND 1 and 8 (Exp. 1, 2, 4, 6), on PND 23 (Exp. 5) or on two consecutive days, i.e.,
4 PND 1+2 or 7+8 (Exp. 3). The conditioning was made according to a nursing-induced
5 procedure adapted from previous studies (Ivanistkii, 1962; Hudson, 1985; Kindermann et al.,
6 1991) and validated by our group (Coureaud et al., 2006; Patris et al., 2008; Jouhanneau et al.,
7 2016): the pups from a given litter were exposed during the daily nursing to the initially
8 neutral stimulus (odorant or mixture), which was applied immediately before nursing along
9 the mammary lines (nipples + surrounding fur) of their mother. Application was made with a
10 cotton pad (19 x 14 cm, 100% cotton) scented with 6 ml of solution for 2 x 30 s (interval
11 between applications: 10 s). After treatment, the doe was reintroduced into its cage and the
12 nest box opened. Almost all the females entered the nest in less than 15 s and nursed for 3-4
13 min (as usual for the species). On very rare occasions, when they did not come into the nest in
14 less than 15 s, they were gently introduced into it manually.

15 Usually, between 15 and 20 pups were used per group. However, in order to reduce the
16 number of animals used in the whole study (with regard to ethical constraints), it was limited
17 to 10 in certain experiments which aimed to confirm the results obtained in other experiments.

18

19 **2.4 Behavioral testing**

20 Testing occurred 24h after the conditioning (or last conditioning when the procedure was
21 repeated twice) in an experimental room adjacent to the breeding room, and 1h before the
22 daily nursing to limit the impact of satiation on motivation and behavioral responsiveness
23 (Montigny et al., 2006). The pups from a same litter were transferred from their nest to the
24 testing room in a box maintained at ambient temperature; they were marked with scentless ink
25 to become identifiable by the experimenter. To minimize litter effects, a maximum of 5

1 conditioned pups were tested per litter and per group. The behavioral assay consisted in an
2 oral activation test during which a pup was immobilized in one gloved hand of the
3 experimenter, its head being left free. The odor stimulus was presented for 10 s with a glass
4 rod, 0.5 cm in front of the nares (e.g., Coureaud et al., 2006, 2008a, 2014a; Romagny et al.,
5 2014, 2015; Schneider et al., 2016). A test was positive when the stimulus elicited (on/off
6 response) head-searching movements (vigorous, low amplitude horizontal and vertical
7 scanning movements displayed after stretching towards the rod) usually followed by grasping
8 movements (labial seizing of the rod extremity). Non-responding pups displayed no response
9 but sniffing. Pups were tested only once to the set of stimuli, litters after litters (i.e., different
10 groups were tested on each day). During an assay, they were successively tested with 3
11 stimuli for the binary mixtures (two single odorants and their mixture) or 4 stimuli for the
12 senary RC mixture (three single components of RC and the RC mixture). Successive testing
13 involved the presentation of a first stimulus to a pup, then a second stimulus to another pup,
14 and so on with an inter-trial interval of 60 s. Therefore, in a given litter, the order of stimuli
15 presentation was systematically counterbalanced from one pup to another. If a pup responded
16 to a stimulus, its nose was softly dried before the next stimulation. The pups were
17 immediately reintroduced in their nest after testing.

18

19 *2.5 Statistics*

20 The proportions of responding pups were compared using the χ^2 test of Pearson (with Yates
21 correction when necessary) when the data were independent (i.e., distinct groups tested for
22 their response to a same stimulus) or the Cochran's Q test when the data were dependent
23 (i.e., pups from a same group tested for their response to the three stimuli). When the
24 Cochran's Q test was significant, proportions of responding pups were compared 2x2 by the
25 χ^2 test of McNemar. Degrees of freedom are indicated when > 1 . Data were considered as

1 significant when the two-tailed tests yielded $p < 0.05$. Analyses were made with the Statistica
2 software (StatSoft, Tulsa, OK, USA).

3

4 **3. RESULTS**

5 *Experiment 1: Perception of binary odor mixtures in 2-day-old rabbits*

6 The goal of this first experiment was to check whether nursing-induced odor learning led to
7 the weak configural and pure elemental perception of the AB and A'B' mixtures, as
8 previously shown in newborn rabbits after pheromone-induced odor learning.

9 Concerning the AB mixture (Figure 1a), a first group of pups ($n = 20$) was nursed by
10 females scented with the whole mixture at PND 1. The day after, their responsiveness was
11 strong and similar to all the stimuli, i.e., to AB, A and B ($> 85\%$; $Q = 2$, d.f. = 2, $p > 0.05$). On
12 the contrary, after nursing by females scented with the odorant A only, a second group of
13 newborns ($n = 20$) responded differently to the stimuli ($Q = 34.1$, d.f. = 2, $p < 0.001$), that is
14 strongly to A (90%) but neither to B (5%; comparison B vs. A: $\chi^2 = 15.1$, $p < 0.001$) nor to
15 AB (0%; AB vs. A: $\chi^2 = 16.1$, $p < 0.001$; AB vs. B: $\chi^2 < 0.5$, $p > 0.05$). Similarly, a last group
16 of pups ($n = 20$) nursed by females scented with the odorant B responded distinctively ($Q =$
17 36.1 , d.f. = 2, $p < 0.001$) to B (95%) compared to A and AB ($< 5\%$; comparison B vs. A or
18 AB: $\chi^2 > 16.1$, $p < 0.001$; A vs. AB: $\chi^2 < 0.5$, $p > 0.05$). Thus, the pups generalized their
19 conditioned response to the mixture and to its components after conditioning to the mixture,
20 but only to the conditioned stimulus after conditioning to a single odorant.

21 Regarding the A'B' mixture (Figure 1b), three other groups of pups were tested
22 ($n = 10$ /group). After nursing-induced conditioning to the mixture, all pups responded to both
23 the mixture and to its components. However, after conditioning to A or to B, the
24 responsiveness varied according to the stimuli ($Q > 14.8$, d.f. = 2, $p < 0.001$): after
25 conditioning to A, the responsiveness was strong for A and A'B' ($> 70\%$; $\chi^2 < 0.5$, $p > 0.05$)

1 while none of the pups responded to B (A or A'B' vs. B: $\chi^2 > 5.1$, $p < 0.05$); after
2 conditioning to B, the pups responded strongly to B and A'B' (80%) but not to A (B or A'B'
3 vs. A: $\chi^2 = 6.1$, $p < 0.05$). Thus, as for the AB mixture, the pups generalized their response to
4 A'B' and to its elements after conditioning to the mixture but, in contrast to AB, they also
5 generalized their response to both the conditioned stimulus and the A'B' mixture after
6 conditioning to a single odorant.

7 Concerning the AC mixture (Figure 1c; 3 groups, $n = 10/\text{group}$), the results were similar to
8 those obtained with A'B'. After conditioning to the mixture, the newborns responded
9 similarly to AC, A and C (90%), while after conditioning to A or C their responsiveness
10 differed depending on the stimuli ($Q > 18$, $d.f. = 2$, $p < 0.001$): it was always strong to both
11 the conditioned odorant and the AC mixture (100%) but null for the non-conditioned odorant
12 (conditioned odorant or AC vs. non-conditioned odorant: $\chi^2 > 7.1$, $p < 0.01$ for the two
13 comparisons).

14 Thus, since pups generalized their conditioned response from an odorant to the A'B' and
15 AC mixtures, and from the mixtures to the odorants, their perception of these mixtures was
16 elemental at PND 2. Conversely, the absence of response to AB after conditioning to A or B
17 but the response to the elements after conditioning to the mixture pinpointed its weak
18 configural perception. Therefore, the nursing-induced procedure led to results similar to those
19 obtained after mammary pheromone-induced conditioning (e.g., Coureaud et al., 2008a, 2009,
20 2014a,b; Sinding et al., 2011; Schneider et al., 2016). In the following, this nursing-induced
21 procedure was used to evaluate the outcome of the perception of the mixtures in rabbits older
22 than newborns.

23

24

25

1 *Experiment 2: Perception of binary odor mixtures in 9-day-old rabbits*

2 To compare the perception of the AB, A'B' and AC mixtures at postnatal days 9 vs. 2, the
3 same conditionings as in Exp. 1 were performed at PND 8 and behavioral testing at PND 9.

4 Regarding the AB mixture (Figure 1d), after conditioning to A or to B (n = 19/group), the
5 pups' responsiveness varied according to the stimuli ($Q > 34$, d.f. = 2, $p < 0.001$): it was
6 strong to the conditioned odorant (> 95%) but weak to both the unfamiliar odorant and the AB
7 mixture (< 16%; conditioned vs. unfamiliar odorant or AB: $\chi^2 > 13.1$, $p < 0.001$). Therefore,
8 after conditioning to a single element, the results were similar to those obtained at PND 2
9 (comparisons PND 9 vs. 2 for each stimulus: $\chi^2 < 0.6$, $p > 0.05$). However, after conditioning
10 to the AB mixture (n = 19), the pups discriminated the stimuli ($Q > 26.5$, d.f. = 2, $p < 0.001$)
11 and strongly responded to the mixture (89%) but not to A and B (11%; AB vs. A or B: $\chi^2 =$
12 13.1, $p < 0.001$). In that case, the responsiveness to the odorants was lower at PND 9
13 compared to PND 2 ($\chi^2 > 18.7$, $p < 0.001$), while it remained high and similar to AB ($\chi^2 < 0.5$,
14 $p > 0.05$).

15 Concerning the A'B' and AC mixtures (Figure 1e and 1f, respectively; n = 10/group), the
16 pups always strongly and similarly responded to the mixture and to its components after
17 conditioning to the mixture (> 70%; $Q < 2$, d.f. = 2, $p > 0.05$), while their responsiveness
18 depended on the stimuli after conditioning to one odorant only ($Q > 14$, d.f. = 2, $p < 0.001$): it
19 was strong to both the conditioned stimulus and to the mixture (> 80%; $\chi^2 < 0.5$, $p > 0.05$) and
20 null to the unfamiliar odorant ($\chi^2 > 6.1$, $p < 0.05$). For the A'B' and AC mixtures, the results
21 were thus the same at PND 9 compared to PND 2 ($\chi^2 < 0.5$, $p > 0.05$ for all PND 9 vs. 2
22 comparisons).

23 Taken together, the results of the first two experiments showed that the pups still perceived
24 the elements in the A'B' and AC mixtures at PND 9, as they did at PND 2. However,
25 concerning the AB mixture, the pups still not responded to the mixture after conditioning to

1 one element at PND 9, but at this age they responded only faintly to the elements after
2 conditioning to the mixture, while in the same situation they strongly responded to the
3 elements at PND 2. This suggested that at PND 9 the pups perceived more the odor of the AB
4 configuration than the odors of the elements A and B in the AB mixture, while their
5 perception of the A'B' and AC mixtures remained elemental.

6

7

PLEASE, INSERT FIGURE 1 ABOUT HERE

8

9 ***Experiment 3: Configural perception of the AB mixture in 9- vs. 3-day-old rabbits after***
10 ***successive learning of A and B***

11 To confirm and extend the results of Exp. 2, we assessed whether the perception of AB in
12 young rabbits would become more configural at the beginning of the 2nd postnatal week than
13 early after birth. To this end, the pups were conditioned to the odorant A then the odorant B
14 on two consecutive days. In a previous study using pheromone-induced odor learning,
15 newborn rabbits became able to respond to the AB mixture at PND 3 after conditioning to A
16 at PND 1 then B at PND 2 (or *vice versa*), showing that their perception of AB shifted from
17 weak configural to elemental (Coureaud et al., 2008a). Here, we promoted the successive
18 learning of A and B but with nursing-induced conditioning, first at PND 1 to 3 and second at
19 PND 7 to 9. The assumption was that if the perception of AB becomes more configural over
20 the development, the previous learning of A then B at PND 7-8 should be insufficient to
21 induce the response to the mixture.

22 A first group of pups was conditioned to one odorant during nursing at PND 1 then to the
23 other odorant at PND 2 (A then B, or B then A; n = 5/sub-group) before behavioral testing at
24 PND 3. Since the results were comparable between the two sub-groups, they were pooled.
25 Pups' responsiveness was strong and similar for A, B and the AB mixture (> 85%; Q = 2, d.f.

1 = 2, $p > 0.05$) (Figure 2a). Thus, the successive nursing-induced learning of the two odorants
2 of the AB mixture favored neonatal responsiveness to the mixture, as previously observed
3 after pheromone-induced learning of each odorant.

4 The procedure was repeated on a second group of pups conditioned at PND 7 then 8
5 ($n = 10$, two sub-groups of 5 were pooled). At PND 9, responsiveness differed according to
6 the stimuli ($Q = 12$, d.f. = 2, $p < 0.01$): the pups responded more to A and B (90%) than to the
7 AB mixture (30%; AB vs. A or B: $\chi^2 = 4.2$, $p < 0.05$) (Figure 2b). While the responsiveness to
8 A and B was strong and similar at both PND 3 and 9 ($\chi^2 < 0.5$, $p > 0.05$), it was significantly
9 lower to AB at PND 9 compared to PND 3 ($\chi^2 = 7.9$, $p < 0.01$).

10 Thus, unlike newborns, the successive learning of each component of the AB mixture was
11 insufficient to promote responsiveness to the mixture in 9-day-old pups. This confirmed that a
12 change in the perception of AB happened during the pups' development between PND 2 and
13 9.

14
15 *PLEASE, INSERT FIGURE 2 ABOUT HERE*

16
17 ***Experiment 4: Developmental change in rabbit pups' perception of the AB mixture between***
18 ***PND 2 and PND 9***

19 Experiments 1 to 3 showed that the AB mixture was perceived differently by 9- compared to
20 2-day-old rabbits. Here, we evaluated more precisely when such a change occurs over this
21 period, by implementing a daily follow-up of the pups' perception. Hence, added to the two
22 groups conditioned at PND 1 or 8 and tested at PND 2 or 9 (Exp. 1 and 2), six other groups
23 were conditioned to the AB mixture during a single episode of nursing that occurred between
24 PND 2 and 7 before testing of their responsiveness 24h later, i.e., between PND 3 and 8 ($n =$
25 $20/\text{group}/\text{testing day}$ except at d7, $n = 19$).

1 The responsiveness to the AB mixture (Figure 3a) remained strong and relatively constant
2 between PND 2 and 9 (> 85%; $\chi^2 = 5.2$, d.f. = 7, $p > 0.05$). Conversely, the responsiveness to
3 the odorant A and the odorant B decreased with increasing age ($\chi^2 > 73.2$, d.f. = 7, $p < 0.001$).
4 Concerning the responsiveness to A (Figure 3b), it was strong between PND 2 and 4 (> 85%;
5 $\chi^2 = 1.3$, d.f. = 2, $p > 0.05$), then dropped between PND 4 and 5-6 (60%; $\chi^2 = 5.1$, $p < 0.05$),
6 decreased again between PND 6 and 7 (21%; $\chi^2 = 4.6$, $p < 0.05$) and finally became weak or
7 null at PND 7 and later ($\chi^2 = 4.7$, d.f. = 2, $p > 0.05$). The pattern was quite similar concerning
8 the responsiveness to B (Figure 3c): the proportion of responding pups was very strong at
9 PND 2-4 (> 90%; $\chi^2 = 0.4$, d.f. = 2, $p > 0.05$), it decreased between PND 4 and 5-6 (55%; $\chi^2 =$
10 5.6, $p < 0.05$) then not significantly between PND 6 and 7 but between PND 6 and 8 (5%; $\chi^2 =$
11 9.6, $p < 0.01$), and remained weak from PND 7 to 9 (< 26%; $\chi^2 = 4.1$, d.f. = 2, $p > 0.05$).
12 These changes were confirmed by intra-group comparisons: after conditioning to the mixture,
13 the pups' responsiveness to AB, A and B was strong and similar in 2-, 3- and 4-day-old pups
14 ($Q < 2$, d.f. = 2, $p > 0.05$), while it was higher to the mixture compared to its components in 5-
15 to 9-day-old pups ($Q > 6.2$, d.f. = 2, $p < 0.05$).

16 Thus, whereas most of the pups perceived the odorants A and B in the AB mixture
17 between PND 2 and 4 (i.e., responded to the odorants after conditioning to the mixture), only
18 almost half of them displayed such capability at PND 5, and even less after PND 6.

19

20 *PLEASE, INSERT FIGURE 3 ABOUT HERE*

21

22 ***Experiment 5: Perception of the AB, A'B' and AC mixture in 24-day-old rabbits***

23 According to Experiments 1-4, development induced a shift toward a more configural
24 perception of the AB mixture between PND 2 and 9, a phenomenon that did not occur for
25 the A'B' and AC mixtures (still elementally perceived at PND 9). However, perceptual

1 changes could also concern A'B' and AC but at a more advanced age. To evaluate this
2 assumption, we conditioned young rabbits at PND 23 either to the AB, A'B' or AC mixtures,
3 or to the odorant A only, before testing them at PND 24 for responsiveness to the mixtures
4 and the components.

5 First, two groups (each of $n = 15$ pups) were conditioned to AB or to the odorant A before
6 testing to AB, A and B (Figure 4a). After conditioning to AB, the pups responded differently
7 to the three stimuli ($Q = 14.2$, d.f. = 2, $p < 0.001$), that is more to AB (60%) than to A and B
8 (13.3 and 6.6%; AB vs. A or B: $\chi^2 > 5.1$, $p < 0.05$); the responsiveness to each odorant was
9 weak and similar ($\chi^2 < 0.5$, $p > 0.05$). This pattern of responses was close to the pattern
10 observed at PND 9 (Figure 1d) with a stronger response to AB than to A or B, while at PND 2
11 the pups strongly responded to all the stimuli (Figure 1a). The only difference between PND
12 24 and 9 was that the pups responded less to AB at PND 24 (60 vs. 89%; $\chi^2 = 4.0$, $p < 0.05$).
13 Regarding the pups conditioned to A, some differences also appeared in their response to the
14 three stimuli ($Q = 16$, d.f. = 2, $p < 0.001$) with a stronger response to A (53.3%) compared to
15 AB and B (0%; A vs. AB or B: $\chi^2 = 6.1$, $p < 0.05$) (Figure 4a), as observed at PND 2 and 9
16 after conditioning to A (Figure 1b,e). Between the three periods, responsiveness to the
17 conditioned stimulus A was lower at PND 24 compared to PND 2 or 9 ($\chi^2 > 6.0$, $p < 0.01$),
18 while it was consistently low to AB and B.

19 Two other groups ($n = 15$ and 15, respectively) were conditioned either to the A'B'
20 mixture or to the component A (Figure 4b). After conditioning to A'B', the proportion of pups
21 responding to the mixture and to the A and B odorants did not vary significantly between the
22 stimuli (around 50%; $Q = 3$, d.f. = 2, $p > 0.05$), as observed at PND 2 and 9. However, the
23 level of responsiveness to these stimuli at PND 24 (range: 46.7 - 60% depending on the
24 stimulus) tended to be or was lower than at PND 2 ($\chi^2 > 3.3$, $p < 0.07$); this was not the case
25 compared to PND 9 ($\chi^2 < 0.6$, $p > 0.05$). After conditioning to A, the pups responded more to

1 A (53.3%) than to B (6.7%) but interestingly also more than to A'B' (13.3%; $Q = 12.3$, d.f. =
2 2, $p < 0.01$; A vs. B or A'B': $\chi^2 > 4.1$, $p < 0.05$; A'B' vs. B: $\chi^2 < 0.5$, $p > 0.05$). The
3 responsiveness to A was similar than at PND 2 and 9 ($\chi^2 < 2.3$, $p > 0.05$), but the
4 responsiveness to A'B' was much lower at PND 24 than at PND 2 and 9 ($\chi^2 > 6.1$, $p < 0.01$).

5 Finally, two last groups ($n = 15$ /group) were conditioned to AC or A at PND 23, before
6 being tested to AC, A and C at PND 24 (Figure 4c). After conditioning to the mixture, the
7 responsiveness to the three stimuli was equivalent (range: 40-53.3%; $Q = 2.7$, d.f. = 2, $p <$
8 0.05), as observed at PND 2 and 9. It was also equivalent for AC at the three periods ($\chi^2 < 2.2$,
9 $p > 0.05$) and lower for A and C at PND 24 vs. 2 ($\chi^2 = 4.3$, $p < 0.05$) but not at PND 24 vs. 9
10 ($\chi^2 < 2.4$, $p > 0.05$). After conditioning to A, pups' responsiveness differed according to the
11 stimuli ($Q = 12.3$, d.f. = 2, $p > 0.05$) with a relatively high and similar rate of response to AC
12 and A (53.3 vs. 60%; $\chi^2 < 0.5$, $p > 0.05$) but no response to C (AC or A vs. C: $\chi^2 > 6.1$, $p <$
13 0.05). The pattern of response to the three stimuli was similar to that observed at PND 2 and
14 PND 9 ($\chi^2 < 2.21$, $p > 0.05$ for each stimulus and all the comparisons).

15 Thus, while a decrease in pups' responsiveness to certain conditioned stimuli appeared at
16 PND 24 compared to earlier in life, 24-day-old pups responded to both the mixtures and to
17 their components after conditioning to A'B' or to AC, as the pups did at PND 2 and 9.
18 However, after conditioning to A the pups did not respond significantly to the A'B' mixture
19 anymore, in stark contrast with earlier ages.

20

21

PLEASE, INSERT FIGURE 4 ABOUT HERE

22

23 ***Experiment 6: Perception of the senary RC mixture in 2- vs. 9-day-old rabbits***

24 The aim of this last experiment was to determine if the shift of perception observed for the
25 binary AB mixture between PND 2 and 9 might be also observed for a more complex mixture.

1 To that goal, 20 pups at PND 1 (two sub-groups of $n = 10$) and 20 other pups at PND 8 (two
2 sub-groups of $n = 10$) were conditioned to the RC mixture, known to be perceived weak
3 configurally by rabbit neonates (Sinding et al., 2013; Romagny et al., 2014, 2015, 2018). The
4 day after this single conditioning, the pups were tested to the RC mixture and to three of its
5 elements (IA, D and B for one sub-group, V, F and EA for the other sub-group, at each age).

6 At PND 2, the pups previously conditioned to the RC mixture responded strongly and
7 similarly to both the mixture and to its components ($> 80\%$; $Q < 3$, d.f. = 3, $p > 0.05$ in each
8 sub-group) (Figure 5). Conversely, their responsiveness differed according to the stimuli at
9 PND 9 ($Q > 24.4$, d.f. = 3, $p < 0.001$ in each sub-group) with a strong level of response to the
10 RC mixture ($> 90\%$) but a very low and similar level of response to all the elements ($< 20\%$;
11 RC vs. IA, D and B or vs. V, F and EA: $\chi^2 > 6.1$, $p < 0.05$ for all the 2x2 comparisons) (Figure
12 5). The responsiveness to RC remained thus high and similar at the two ages ($\chi^2 < 0.5$, $p >$
13 0.05 for all the sub-groups) while it dramatically decreased for each of the odorant ($\chi^2 > 7.2$, p
14 < 0.01 for all the comparisons).

15 Thus, as observed for the AB mixture, a change in the perception of the RC mixture vs. its
16 elements appeared in 9- compared to 2-day-old rabbit pups in the sense of a consistently
17 strong response to the mixture at the two periods of age but a response that becomes lower to
18 the elements at PND 9.

19

20 *PLEASE, INSERT FIGURE 5 ABOUT HERE*

21

22 **4. DISCUSSION**

23 The present study outlines an original evaluation of the influence of development on odor
24 mixture perception in a young mammal. In our conditions, before PND 2, it is unlikely that
25 pups had been exposed to the A, B, C odorants and/or AB, A'B' and AC mixtures that we

1 used, so that perinatal exposure cannot contribute to the strong variations in behavioral
2 responses which were recorded. Incidentally, all these odorants and mixtures were
3 behaviorally neutral before conditioning (they triggered sniffing response only). Moreover,
4 the same procedure of single conditioning was used all along the study at different PNDs,
5 except in the special case of Experiment 3 (successive conditionings). Therefore, the
6 contrasted responses displayed by young rabbits at different ages can be considered as
7 developmentally-induced rather than experience-induced.

8 The results confirm first that nursing-induced conditioning is an efficient way to promote
9 the rapid acquisition of odor cues in rabbit neonates (e.g., Ivanistkii, 1962; Hudson, 1985;
10 Kindermann et al., 1991; Hudson et al., 2002; Coureaud et al., 2006; Jouhanneau et al., 2016)
11 and demonstrate that this conditioning is functional in 1- as in 3-week-old animals for single
12 odorants as for odor mixtures. This feature is certainly due to the milk dependency that still
13 exists in 24-day-old domestic rabbits (Scapinello et al., 1999; Fortun-Lamothe and Gidenne,
14 2000). However, the proportion of pups responding to the conditioned stimulus at PND 24,
15 whatever the complexity of the stimulus (odorant or mixture), was lower than at PND 2 and
16 sometimes at PND 9, i.e., the conditioning procedure was then less efficient than early after
17 birth. This could be due to the fact that around PND 20, young rabbits start eating solid food
18 in addition to milk (Scapinello et al., 1999; Gidenne and Fortun-Lamothe, 2002; Coureaud et
19 al., 2008b), which may lead them to become more sensitive to reinforcing factors linked to
20 solid food intake than to milk intake. Nevertheless, the proportions of pups responding to the
21 conditioned stimuli at PND 24 were higher than to the control (not conditioned) stimuli, and
22 therefore sufficient to discuss the evolution of the AB, A'B' and AC mixtures perception
23 between PND 2 and 24.

24 The results of the six experiments reported here demonstrate that the ability to generalize
25 response to an odor mixture after learning its elements, or *vice versa*, is a function of both the

1 chemical nature of the mixture used and the age of the rabbit. At PND 2, after nursing-
2 induced conditioning to the AB, A'B' or AC mixtures, the neonates strongly responded to
3 both the mixtures and to their elements. However, after conditioning to a single odorant they
4 responded to A'B' and AC but not to AB (Exp. 1). These results, similar to those obtained in
5 previous studies after mammary pheromone induced-conditioning (Coureaud et al., 2008b,
6 2009, 2011, 2014a,b; Sinding et al., 2011), confirmed that 2-day-old rabbits perceived the
7 A'B' and AC mixtures elementally, but the AB mixture weak configurally. Thus, even in the
8 naturally reinforcing context of nursing, the acquisition of elements A or B is not sufficient to
9 promote a conditioned response to AB. This supports the assumption of a third odor quality
10 perceived specifically in the AB mixture and not in the odorants. This configural odor is
11 unfamiliar for the pups and therefore inhibits their response to the binary stimulus after
12 conditioning to only one of its components.

13 A week later, 9-day-old pups still perceived the A'B' and AC mixtures as the sum of their
14 elements since they responded to the odorants after conditioning to the mixture and *vice*
15 *versa*. Conversely, after conditioning to A or to B, they did not respond to AB. This result
16 suggests that the pups' perception of AB was still weak configural at PND 9, as it was at PND
17 2. However, a difference appeared between the two ages: while after conditioning to AB, 2-
18 day-old pups strongly responded to the mixture and to its components, 9-day-old pups did not
19 respond significantly to the components anymore (Exp. 2). This supports the idea that the
20 perception of the mixture shifted from weak configural to robust configural between PND 2
21 and 9, i.e., that the pups would perceive only one main bit of information in the AB mixture at
22 PND 9, namely the specific odor of the AB configuration. This is confirmed by the results
23 obtained after the successive learning of odorants (Exp. 3), which indeed promoted
24 responsiveness to the AB mixture in 3-day-old neonates but not in 9-day-old pups: being
25 familiar with more than 50% of the information that compose the whole mixture (i.e., A and B

1 gather 66% of the A, B and AB odor information) is sufficient to promote the pups'
2 responsiveness to the AB mixture at PND 2, but not a week later. At PND 9, a majority of
3 young rabbits no longer perceived the odorants A and B within the AB mixture.

4 According to the day-by-day follow up of Experiment 4, the switch from weak to robust
5 configural perception of the AB mixture seems first to be balanced between the odorants,
6 since the decrease in responsiveness to A and B followed the same time-course. This result
7 suggests the involvement of a general process that preserved and facilitated the perception of
8 the AB configuration, which remained high from PND 2 to 9, to the detriment of the two
9 elements, which declined over the same period. Second, the switch appeared progressively,
10 with no abrupt change from one day to another: most of the pups responded to the odorants up
11 to PND 4, half of them at PND 5 and 6, approximately a quarter at PND 7, and finally only a
12 few at PND 8-9. However, this evolution was restricted to a short developing period, i.e.,
13 PND 5 to 7. During this period, some anatomical, neurophysiological and/or maturational
14 phenomena are in progress in rabbit pups (Allingham et al., 1998; Caba et al., 2003;
15 Montúfar-Chaveznava et al., 2013; Wójcik et al., 2013; Olivo et al., 2017; Schneider et al.,
16 2018). They are associated with the enhanced synchrony of arousal, motor actions and
17 progressive acquisition of audition and vision, which are not functional at birth (Gottlieb,
18 1971; Rapisardi et al., 1975; Coureaud et al., 2008b). The emergence of non-olfactory sensory
19 systems could lead the young to perceive progressively a huge number and diversity of cues,
20 and to simplify this multitude of stimuli through the perceptual grouping of some of them
21 within intramodal configurations, or maybe also intermodal configurations. With regard to
22 olfaction, this could favor the perception of certain mixtures in a more configural way, in
23 particular for mixtures that are spontaneously perceived at birth in the weak configural way
24 (e.g., the AB mixture). This prioritization of configural treatment could favor cognitive
25 economy in developing rabbits compared to what would constitute the systematic processing

1 of all the elements contained in a mixture (Coureaud et al., 2014a; Thomas-Danguin et al.,
2 2014). Such assumption in olfaction is in line with results obtained in human vision and face
3 processing. Face processing can be considered as weak configural since individuals have
4 perceptual access to both the elements that compose a face (e.g., the eyes, eyebrows, nose and
5 mouth) and the configuration formed by the face itself, with configural processing of the
6 facial features in the upright orientation (1st order configuration) then of the spacing of the
7 features relative to each other (2nd order configuration); holistic processing refers then to
8 perceiving the individual features and their spatial relations as an integrated whole (Pascalis et
9 al., 2011). Several studies have reported that during long-term development, face perception
10 which is initially mainly elemental becomes more configural to the detriment of the face
11 features (Mondloch et al., 2002; Cohen Kadosh et al., 2013; Wakui et al., 2013), with a switch
12 generally established in middle childhood and/or adolescence (Jüttner et al., 2013; Joseph et
13 al., 2015) but with individual differences (Petraikova et al., 2018). This evolution could be
14 related to the maturation of general cognitive functioning such as focusing attention and
15 improvements in reasoning (Betts et al., 2013) and to age effect in the occipital and temporal
16 lobes as in the inferior frontal gyrus (Cohen Kadosh et al., 2013). Our results suggest that in
17 olfaction such evolution could appear earlier, at least in altricial newborns as rabbit pups.
18 Further investigations are required to give credit to this hypothesis. One could evaluate
19 whether this difference is linked to a species-specific effect or a developmental effect that
20 may differently influenced the time-course of configural facilitation in olfaction vs. vision,
21 and determine if anatomical/physiological changes in the olfactory system may account for
22 the perceptual switch observed here. For instance, olfactory bulb mitral/tufted cells and the
23 piriform cortex have been highlighted as being involved in configural vs. elemental
24 perception of the AB and A'B' mixtures in 4-day-old pups (Schneider et al., 2016). It would
25 be interesting to perform a comparative study in 9- and 24-day-old pups in order to assess

1 whether some differences appear at these levels according to the age in parallel with the
2 behavioral differences displayed in the work presented here.

3 The perceptual change observed for the AB mixture between PND 2 and 9 did not exist for
4 the two other binary mixtures considered here, A'B' and AC, which remained perceived
5 elementally (Exp. 1 and 2). Therefore, the biological mechanisms that are susceptible to
6 induce a perceptual change for AB between PND 2 and 9 are not generalizable to any
7 mixture. A developmental influence was however observed on the same period for the senary
8 RC mixture: after conditioning to the mixture, a drop in responsiveness to each element
9 appeared at PND 9, while responsiveness to the 6 elements was strong at PND 2 (Exp. 6).
10 Strikingly, this happened for a mixture which has been shown to be perceived in the weak
11 configural way by rabbit neonates (Sinding et al., 2013; Romagny et al., 2014, 2015). Thus,
12 the switch of perception noted between PND 2 and 9 in our study for the AB and the RC
13 mixtures may be illustrative of perceptual changes and underlying mechanisms that concern
14 the processing of mixtures spontaneously perceived in the weak configural way by neonates,
15 namely of mixtures containing both elemental and configural information and for which the
16 perception may later evolve towards one of the two modes of perception.

17 Concerning the A'B' mixture, which differs from AB only in terms of component
18 proportions and is perceived elementally by newborn rabbits, a perceptual change was also
19 observed over the development but after a longer period of time. Indeed, at PND 24, after
20 conditioning to odorant A the young rabbits strongly responded to A but not to A'B' anymore
21 (Exp. 5), whereas 2- and 9-day-old pups responded to A'B' after the same conditioning (Exp.
22 1 and 2). Added to the fact that at PND 24 rabbit pups responded to both the elements and the
23 mixture after conditioning to the A'B' mixture, these results suggest that the perception of the
24 A'B' mixture became weak configural between PND 9 and 24. In contrast, the perception of
25 AB became robust configural as soon as PND 9. The perceptual evolution observed for AB

1 and A'B' did not occur for the AC mixture, which was perceived elementally from birth to
2 PND 24 (Exp. 1, 2, 5). Thus, the results suggest that mixtures of ethyl isobutyrate (A) and
3 ethyl maltol (B) carry some singularities that make them favorable to configural perception
4 either in part at birth then increasing over the first two postnatal weeks, or becoming weakly
5 configural as weaning approaches. Such evolution did not occur for other mixtures as the AC
6 mixture, even if it contains one element in common with AB, which confirms that the
7 chemical composition of a mixture is a critical factor supporting its configural or elemental
8 perception (e.g., Bult et al., 2002; Kay et al., 2003; Ferreira et al., 2012; Coureaud et al.,
9 2014a; Thomas-Danguin et al., 2014; Romagny et al., 2018). However, it cannot be excluded
10 that a perceptual evolution will also occur for the AC mixture but after the weaning.

11 In conclusion, during the postnatal development of young rabbits, some changes occur in
12 the perception of olfactory stimuli. Certain binary and senary mixtures become perceived
13 more as configurations than associations of distinct elements, which reduces their perceptual
14 complexity. Thus, from three distinct information perceived by neonates in the AB mixture
15 (the respective odors of A, B and AB) or seven in the RC mixture (the odors of the six
16 odorants and the odor of the RC configuration), the perception becomes limited to only one
17 information, respectively the AB and RC configurations. In altricial newborn rabbits, the
18 perception of elements in addition to configuration in complex odor stimuli, as for instance
19 the mammary pheromone within the complex rabbit milk odor, could optimize the
20 responsiveness to the mother's visit and adaptation to the restrained environment constituted
21 by the nest. When vision and audition become functional and that young rabbits are disposed
22 to leave the nest, explore the surroundings and feed independently, their representation of
23 mixtures as distinct odor objects could increase their ability to process rapidly and efficiently
24 a greater quantity of olfactory information and to extract relevant cues from the odor
25 background. Consequently, by favoring the configural representation of complex odor stimuli,

1 developmental processes could promote the efficient categorization and discrimination of
2 odor objects that carry strong biological values in the highly complex chemical environment,
3 and the rapid expression of adapted behaviors related to these objects (Wilson and Sullivan,
4 2011; Coureaud et al., 2014a,b). Finally, since newborn rabbits perceive the AB, A'B', AC
5 and RC mixtures used here in the same configural or elemental way as human adults do,
6 young rabbits constitute a relevant model to pursue investigations related to the evolution of
7 olfactory perception over the development, providing beneficial knowledge to other species
8 including our own species.

9

10

11 **ACKNOWLEDGEMENTS**

12 We sincerely thank Valérie Saint-Giorgio, Nicolas Malaty, Jérôme Antoine and the staff of
13 the Centre de Zootechnie from Dijon for their cooperation in this study. The work was
14 supported by grants from the Regional Council of Burgundy, the FEDER (European Regional
15 Development Fund) and the ANR-2010-JCJC-1410-1 MEMOLAP to GC and TTD. SR was
16 funded by the French Ministry of Higher Education and Research during his PhD.

17

18 **DATA AVAILABILITY STATEMENT**

19 The data that support the findings of this study are available from the corresponding author
20 upon reasonable request.

21

22 **REFERENCES**

23 Allingham, K., von Saldern, C., Brennan, P.A., Distel, H., & Hudson, R. (1998). Endogenous
24 expression of C-Fos in hypothalamic nuclei of neonatal rabbits coincides with their

1 circadian pattern of suckling-associated arousal. *Brain Research*, 783, 210-218.
2 [https://doi.org/10.1016/S0006-8993\(97\)01379-6](https://doi.org/10.1016/S0006-8993(97)01379-6).

3 Avarguès-Weber, A., Portelli, G., Benard, J., Dyer, A., & Giurfa, M. (2010). Configural
4 processing enables discrimination and categorization of face-like stimuli in honeybees.
5 *The Journal of Experimental Biology*, 213, 593-601. <http://dx.doi.org/10.1242/jeb.039263>.

6 Betts, J., Mckay, J., Maruff, P., & Anderson, V. (2013). The development of sustained
7 attention in children: The effect of age and task load. *Child Neuropsychology*, 12, 205-221.
8 <http://dx.doi.org/10.1080/09297040500488522>.

9 Bos, N., D'Ettoire, P., & Guerrieri, F.J. (2013). Chemical structure of odorants and perceptual
10 similarity in ants. *The Journal of Experimental Biology*, 216, 3314-3320.
11 <https://doi:10.1242/jeb.087007>.

12 Bult, J.H., Schifferstein, H.N., Roozen, J.P., Boronat, E.D., Voragen, A.G., Kroeze, J.H.
13 (2002). Sensory evaluation of character impact components in an apple model mixture.
14 *Chemical Senses*, 27, 485-494. <https://doi.org/10.1093/chemse/27.6.485>.

15 Caba, M., Rovirosa, M.J., & Silver, R. (2003b). Suckling and genital stroking induces Fos
16 expression in hypothalamic oxytocinergic neurons of rabbit pups. *Developmental Brain*
17 *Research* 143, 119-128. [https://doi.org/10.1016/S0165-3806\(03\)00064-6](https://doi.org/10.1016/S0165-3806(03)00064-6)

18 Cohen Kadosh, K., Johnson, M.H., Henson, R.N.A., Dick, F., & Blakemore, S-J. (2013).
19 Differential face-network adaptation in children, adolescents and adults. *Neuroimage*, 9,
20 11-20. <https://doi:10.1016/j.neuroimage.2012.11.060>.

21 Coureaud, G., Schaal, B., Hudson, R., Orgeur, P., & Coudert, P. (2002). Transnatal olfactory
22 continuity in the rabbit: behavioral evidence and short-term consequence of its disruption.
23 *Developmental Psychobiology*, 40, 372-390. <https://doi:10.1002/dev.10038>.

- 1 Coureaud, G., Moncomble, A-S., Montigny, D., Dewas, M., Perrier, G., & Schaal, B. (2006).
2 A pheromone that rapidly promotes learning in the newborn. *Current Biology*, *16*, 1956-
3 1961. <https://doi:10.1016/j.cub.2006.08.030>.
- 4 Coureaud, G., Thomas-Danguin, T., Le Berre, E., & Schaal, B. (2008a). Perception of odor
5 blending mixtures in the newborn rabbit. *Physiology & Behavior*, *95*, 194-199.
6 <https://doi:10.1016/j.physbeh.2008.05.018>.
- 7 Coureaud, G., Fortun-Lamothe, L., Rödel, H.G., Monclùs, R., & Schaal, B. (2008b).
8 Development of social and feeding behaviour in young rabbits. In: G. Xiccato, A. Trocino,
9 and S.D. Lukefahr, eds. *Proceedings of the 9th World Rabbit Congress*. Verona, Italy:
10 Ethology and Welfare. pp. 1131-1146.
- 11 Coureaud, G., Hamdani, Y., Schaal, B., & Thomas-Danguin, T. (2009). Elemental and
12 configural processing of odour mixtures in the newborn rabbit. *The Journal of*
13 *Experimental Biology*, *212*, 2525-2531. <https://doi:10.1242/jeb.032235>.
- 14 Coureaud, G., Charra, R., Datiche, F., Sinding, C., Thomas-Danguin, T., Languille, S., Hars,
15 B., & Schaal, B. (2010). A pheromone to behave, a pheromone to learn: the rabbit
16 mammary pheromone. *Journal of Comparative Physiology A*, *196*, 779-790.
17 <https://doi:10.1007/s00359-010-0548-y>.
- 18 Coureaud, G., Gibaud, D., Le Berre, E., Schaal, B., & Thomas-Danguin, T. (2011).
19 Proportion of odorants impacts the configural versus elemental perception of a binary
20 blending mixture in newborn rabbits. *Chemical Senses*, *36*, 693-700.
21 <https://doi:10.1093/chemse/bjr049>.
- 22 Coureaud, G., Thomas-Danguin, T., Wilson, D.A., & Ferreira, G. (2014a). Neonatal
23 representation of odour objects: distinct memories of the whole and its parts. *Proceedings*
24 *B of the Royal Society*, *281*, 20133319. <https://doi:10.1098/rspb.2013.3319>.

1 Coureaud, G., Thomas-Danguin, T., Datiche, F., Wilson, D.A., & Ferreira, G. (2014b).
2 Differential memory persistence of odor mixture and components in newborn rabbits:
3 competition between the whole and its parts. *Frontiers in Behavioral Neuroscience*, 8, 1-
4 10. <https://doi:10.3389/fnbeh.2014.00211>.

5 Ferreira, V. (2012). Revisiting psychophysical work on the quantitative and qualitative odour
6 properties of simple odour mixtures: a flavour chemistry view. Part 2: qualitative aspects.
7 A review. *Flavour and Fragrance Journal*, 27, 201-215. <https://doi.org/10.1002/ffj.2091>.

8 Fortun-Lamothe, L., & Gidenne, T. (2000). The effects of size of suckled litter on intake
9 behaviour, performance and health status of young and reproducing rabbits. *Annales De*
10 *Zootecnie*, 49, 517-529. <https://doi:10.1051/animres:2000142>.

11 Gidenne, T., & Fortun-Lamothe, L. (2002). Feeding strategy for young rabbits around
12 weaning : a review of digestive capacity and nutritional needs. *Animal Science*, 75, 169-
13 184.

14 Gottlieb, G. (1971). Ontogenesis of sensory function in birds and mammals. In Tobach, E.,
15 Aronson L.R., & Shaw E. (eds): *The Biopsychology of Development*. New York, US:
16 Academic Press. pp. 67-128.

17 Howard, J.D., & Gottfried, J.A. (2014). Configural and elemental coding of natural odor
18 mixture components in the human brain. *Neuron*, 84, 857-869.
19 <https://doi:10.1016/j.neuron.2014.10.012>.

20 Hudson, R. (1985). Do newborn rabbits learn the odor stimuli releasing nipple-search
21 behavior? *Developmental Psychobiology*, 18, 575-585. <https://doi:10.1002/dev.420180612>.

22 Hudson, R., Labra-Cardero, D., & Mendoza-Soylovna, A. (2002). Sucking, not milk, is
23 important for the rapid learning of nipple-search odors in newborn rabbits. *Developmental*
24 *Psychobiology*, 41, 226-235. <https://doi:10.1002/dev.10073>.

- 1 Ivanistkii, A.M. (1962). The morphological investigation of development of conditioned
2 alimentary reaction in rabbits during ontogenesis. In: *Experimental studies of higher*
3 *nervous activity in man and animals, Works of the Institute of Higher Nervous Activity.*
4 *Moscow, Russia: Physiological series.* pp. 126–141.
- 5 Joseph, J.E., DiBartolo, M.D., & Bhatt, R.S. (2015). Developmental changes in analytic and
6 holistic processes in face perception. *Frontiers in Psychology, 6,* 1165.
7 [https://doi:10.3389/fpsyg.2015.01165](https://doi.org/10.3389/fpsyg.2015.01165)
- 8 Jouhanneau, M., Schaal, B., & Coureaud, G. (2016). Mammary pheromone-induced odour
9 learning influences sucking behaviour and milk intake in the newborn rabbit. *Animal*
10 *Behaviour, 111,* 1-11. <http://dx.doi.org/10.1016/j.anbehav.2015.10.003>
- 11 Jüttner, M., Wakui, E., Petters, D., Kaur, S., & Davidoff, J. (2013). Developmental
12 trajectories of part-based and configural object recognition in adolescence. *Developmental*
13 *Psychology, 49,* 161-76. <http://doi:10.1037/a0027707>.
- 14 Kay, L.M., Crk, T., & Thorngate, J. (2005). A redefinition of odor mixture quality.
15 *Behavioral Neuroscience, 119,* 726-733. <http://doi:10.1037/0735-7044.119.3.726>.
- 16 Kindermann, U., Gervais, R., & Hudson, R. (1991). Rapid odor conditioning in newborn
17 rabbits: amnesic effect of hypothermia. *Physiology & Behavior, 50,* 457-460.
18 [http://dx.doi.org/10.1016/0031-9384\(91\)90094-5](http://dx.doi.org/10.1016/0031-9384(91)90094-5).
- 19 Le Berre, E., Thomas-Danguin, T., Béno, N., Coureaud, G., Etiévant, P., & Prescott, J.
20 (2008). Perceptual processing strategy and exposure influence the perception of odor
21 mixtures. *Chemical Senses, 33,* 193-199. <http://doi:10.1093/chemse/bjm080>.
- 22 Livermore, A., Hutson, M., Ngo, V., Hadjisimos, R., & Derby, C.D. (1997). Elemental and
23 configural learning and the perception of odorant mixtures by the spiny Lobster *Panulirus*
24 *argus.* *Physiology & Behavior, 62,* 169-174. [http://doi:10.1016/S0031-9384\(97\)00031-0](http://doi:10.1016/S0031-9384(97)00031-0).

- 1 Menzel, R., Giurfa, M., Gerber, B., & Hellstern, F. (1999). Elementary and configural forms
2 of memory in an insect: The honeybee. In: A.D. Friederici, and R. Menzel, eds. *Learning:
3 Rule Extraction and Representation*. Berlin: De Gruyter. p. 290.
- 4 Mondloch, C.J., Grand, R.L., & Maurer, D. (2002). Configural face processing develops more
5 slowly than featural face processing. *Perception*, *31*, 553-566. <http://doi:10.1068/p3339>.
- 6 Montigny, D., Coureaud, G., & Schaal, B. (2006). Rabbit pup response to the mammary
7 pheromone: from automatism to prandial control. *Physiology & Behavior*, *89*, 742-749.
8 <http://doi:10.1016/j.physbeh.2006.08.022>.
- 9 Montúfar-Chaveznava, R., Trejo-Múnoz, L., Hernandez-Campos, O., Navarrete, E., Caldelas,
10 I. (2013). Maternal olfactory cues synchronize the circadian system of artificially raised
11 newborn rabbits. *PLoS ONE* *8*, e74048. <http://doi:10.1371/journal.pone.0074048>.
- 12 Morrow-Tesch, J., & McGlone, J.J. (1990). Sensory systems and nipple attachment behavior
13 in neonatal pigs. *Physiology & Behavior*, *47*, 1-4. [http://doi:10.1016/0031-9384\(90\)90034-](http://doi:10.1016/0031-9384(90)90034-2)
14 [2.](http://doi:10.1016/0031-9384(90)90034-2)
- 15 Olivo, D., Caba, M., Gonzalez-Lima, F., Rodríguez-Landa, J.F., & Corona-Morales, A.A.
16 (2017). Metabolic activation of amygdala, lateral septum and accumbens circuits during
17 food anticipatory behavior. *Behavioural Brain Research*, *316*, 261-270. [https://doi:](https://doi:10.1016/j.bbr.2016.09.015)
18 [10.1016/j.bbr.2016.09.015](https://doi:10.1016/j.bbr.2016.09.015).
- 19 Pascalis, O., deVivies, X.D., Anzures, G., Quinn, P.C., Slater, A.M., Tanaka, J.W., & Lee, K.
20 (2011). Development of face processing. *Wiley Interdisciplinary Reviews – Cognitive
21 Science*, *2*, 666-675. <https://doi.org/10.1002/wcs.146>.
- 22 Patris, B., Perrier, G., Schaal, B., & Coureaud, G. (2008). Early development of filial
23 preferences in the rabbit: implications of nursing- and pheromone-induced odour learning?
24 *Animal Behaviour*, *76*, 305-314. <http://doi:10.1016/j.anbehav.2008.01.017>.

- 1 Petrakova, A., Sommer, W., Junge, M., & Hildebrandt, A. (2018). Configural face perception
2 in childhood and adolescence: An individual differences approach. *Acta Psychologica*,
3 188, 148-176. <https://doi.org/10.1016/j.actpsy.2018.06.005>.
- 4 Rapisardi, S.C., Chow, KL, & Mathers, LH. (1975). Ontogenesis of receptive field
5 characteristics in the dorsal lateral geniculate nucleus of the rabbit. *Experimental Brain*
6 *Research*, 22, 295-305. <https://doi.org/10.1007/BF00234771>.
- 7 Rokni, D., Hemmelder, V., Kapoor, V., & Murthy, V.N. (2014). An olfactory cocktail party:
8 figure-ground segregation of odorants in rodents. *Nature Neuroscience*, 17, 1225-1232.
9 <http://doi:10.1038/nn.3775>.
- 10 Romagny, S., Darmaillacq, A-S., Guibé, M., Bellanger, C., & Dickel, L. (2012). Feel, smell
11 and see in an egg: emergence of perception and learning in an immature invertebrate, the
12 cuttlefish embryo. *The Journal of Experimental Biology*, 215, 4152-4130.
13 <http://doi:10.1242/jeb.078295>.
- 14 Romagny, S., Thomas-Danguin, T., & Coureaud, G. (2014). Newborn rabbit perception of 6-
15 odorant mixtures depends on configural processing and number of familiar elements. *PLoS*
16 *One*, 9, e107560. <http://doi:10.1371/journal.pone.0107560>.
- 17 Romagny, S., Thomas-Danguin, T., & Coureaud G. (2015). Configural processing of odor
18 mixture: does the learning of elements prevent the perception of configuration in the
19 newborn rabbit? *Physiology & Behavior*, 142, 161-169. [http://doi:](http://doi:10.1016/j.physbeh.2015.02.019)
20 [10.1016/j.physbeh.2015.02.019](http://doi:10.1016/j.physbeh.2015.02.019).
- 21 Romagny, S., Coureaud, G., & Thomas-Danguin, T. (2018). Key odorants or key
22 associations? Insights into elemental and configural odour processing in humans. *Flavour*
23 *and Fragrance Journal*, 33, 97-105. [http://doi: 10.1002/ffj.3429](http://doi:10.1002/ffj.3429).

1 Scapinello, C., Gidenne, T., & Fortun-Lamothe, L. (1999). Digestive capacity of the rabbit
2 during the post-weaning period, according to the milk/solid feed intake pattern before
3 weaning. *Reproduction Nutrition Development*, 39, 423-432.

4 Schaal, B. (2000). Human foetuses learn odours from their pregnant mother's diet. *Chemical*
5 *Senses*, 25, 729-737. <http://doi:10.1093/chemse/25.6.729>.

6 Schaal, B., Coureaud, G., Langlois, D., Giniès, C., Sémon, E., & Perrier, G. (2003). Chemical
7 and behavioural characterization of the rabbit mammary pheromone. *Nature*, 424, 68-72.
8 <https://doi.org/10.1038/nature01739>.

9 Schneider, N.Y., Piccin, C., Datiche, F., & Coureaud, G. (2016). Spontaneous brain
10 processing of the mammary pheromone in rabbit neonates prior to milk intake.
11 *Behavioural Brain Research*, 313, 191-200. <https://doi:10.1016/j.bbr.2016.07.014>.

12 Schneider N.Y., Datiche, F., Coureaud G. (2018). Brain anatomy of the 4-day-old European
13 rabbit. *Journal of Anatomy*, 232, 747-767. <https://doi:10.1111/joa.12789>.

14 Sinding, C., Thomas-Danguin, T., Crepeaux, G., Schaal, B., & Coureaud, G. (2011).
15 Experience influences elemental and configural perception of certain binary odour
16 mixtures in newborn rabbits. *The Journal of Experimental Biology*, 214, 4171-4178.
17 <https://doi:10.1242/jeb.063610>.

18 Sinding, C., Thomas-Danguin, T., Chambault, A., Béno, N., Dosne, T., Chabanet, C., Schaal,
19 B., & Coureaud, G. (2013) Rabbit neonates smell as well as human adults ... and even
20 better elementally. *PLoS ONE*, 8, e53534. <https://doi:10.1371/journal.pone.0053534>.

21 Smotherman, W.P., & Robinson, S.R. (1992). Dimethyl disulfide mimics the effects of milk
22 on fetal behavior and responsiveness to cutaneous stimuli. *Physiology & Behavior*, 52,
23 761-765. [https://doi.org/10.1016/0031-9384\(92\)90411-T](https://doi.org/10.1016/0031-9384(92)90411-T).

24 Thomas-Danguin, T., Sinding, C., Romagny, S., El Mountassir, F., Atanasova, B., Le Berre,
25 E., Le Bon, A-M., & Coureaud, G. (2014). The perception of odor objects in everyday life:

1 a review on the processing of odor mixtures. *Frontiers in Psychology*, 5, 1-18.
2 <https://doi:10.3389/fpsyg.2014.00504>.

3 Wakui, E., Jüttner, M., Petters, D., Kaur, S., Hummel, J.E., & Davidoff, J. (2013). Earlier
4 development of analytical than holistic object recognition in adolescence. *PLoS One*, 8,
5 e61041. doi:10.1371/journal.pone.0061041.

6 Wilson, D.A., & Sullivan, R.M. (2011). Cortical processing of odor objects. *Neuron*, 72, 506-
7 519. <https://doi:10.1016/j.neuron.2011.10.027>.

8 Wójcik, S., Łuczyńska, A., Dziwiątkowski, J., Spodnik, E., Ludkiewicz, B., & Moryś, J.
9 (2013). Expression of the calcium-binding proteins in the central, medial and cortical
10 nuclei of the rabbit amygdaloid complex during postnatal development. *Acta*
11 *Neurobiologiae Experimentalis*, 73, 260-279.

12 Xiccato, G., Trocino, A., Boiti, C., & Brecchia, G. (2005). Reproductive rhythm and litter
13 weaning age as they affect rabbit doe performance and body energy balance. *Animal*
14 *Science*, 81, 289-296. <https://doi:10.1079/ASC50270289>.

15 Zarrow, M.X., Denenberg, V.H., & Anderson, C.O. (1965). Rabbit: frequency of suckling in
16 the pup. *Science*, 150, 1835-1836. <https://doi:10.1126/science.150.3705.1835>.

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FIGURE CAPTIONS

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FIGURE 1: Proportions of 2- and 9-day-old rabbit pups responding in the oral activation test to the odorant A (ethyl isobutyrate), the odorant B (ethyl maltol), the odorant C (guaiacol) and the AB mixture (30/70 ratio of A/B), A'B' mixture (68/32 ratio) or AC mixture (50/50 ratio) 24h after nursing-induced conditioning to AB, A or B (graph a and d), A'B', A or B (b,e), and AC, A or C (c,f). The gray background highlights the results obtained after conditioning to the mixtures. Distinct digits indicate statistical differences within each group at the $p < 0.05$ level.

FIGURE 2: Proportions of 3- and 9-day-old rabbit pups responding in the oral activation test to the AB mixture (30/70 ratio), the odorant A (ethyl isobutyrate) and the odorant B (ethyl maltol) after nursing-induced conditioning to A then to B (or the reversal) realized at PND 1 then PND 2 (graph a) or at PND 7 then PND 8 (graph b). Distinct digits indicate statistical differences within each group at the $p < 0.05$ level.

FIGURE 3: Proportions of 2- to 9-day-old rabbit pups responding in the oral activation test to the AB mixture (30/70 ratio) (graph a), the odorant A (ethyl isobutyrate) (graph b) and the odorant B (ethyl maltol) (graph c), 24h after nursing-induced conditioning to the AB mixture (independent groups per age). Distinct digits indicate statistical differences within each group at the $p < 0.05$ level.

FIGURE 4: Proportions of 24-day-old rabbits responding in the oral activation test to the odorant A (ethyl isobutyrate), the odorant B (ethyl maltol), the odorant C (guaiacol) and the AB, A'B' or AC mixtures, 24h after nursing-induced conditioning to AB or A (graph a), A'B'

1 or A (graph b), and AC or A (graph c). Distinct digits indicate statistical differences within
2 each group at the $p < 0.05$ level.

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4 **FIGURE 5:** Proportions of 2- and 9-day-old rabbit pups responding in the oral activation test
5 to the senary RC mixture and each of its components, namely isoamyl acetate (IA; CAS #
6 123-92-2), damascenone (D, CAS # 23696-85-7), β -ionone (B; CAS # 79-77-6), vanillin
7 (odorant V; CAS # 8014-42-4), frambinone (F; CAS # 5471-51-2) and ethyl acetate (EA;
8 CAS # 141-78-6), 24h after their conditioning to the RC mixture. Distinct digits indicate
9 statistical differences within each group at the $p < 0.05$ level.

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