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# Proportion of Odorants Impacts the Configural versus Elemental Perception of a Binary Blending Mixture in Newborn Rabbits

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## Abstract

Processing of odor mixtures by neonates is weakly understood. Previous studies showed that a binary mixture of ethyl isobutyrate/ethyl maltol (odorants A/B) blends in newborn rabbits at the 30/70 ratio: Pups would perceive a configural odor in addition to the components' odors. Here, we investigated whether the emergence of this additional odor in AB is determined by specific ratio(s) of A and B. To that goal, we tested whether pups discriminated between AB mixtures with lower ( $A^-B$ , 8/92 ratio) or higher ( $A^+B$ , 68/32) proportion of A. In Experiment 1, pups conditioned to A (or B) responded to  $A^-B$  and  $A^+B$  but not to AB. In Experiment 2, pups responded to  $A^-B$  after learning of  $A^-$  (and to  $A^+B$  after learning of  $A^+$ ) but not to AB. In Experiment 3, after conditioning to  $A^-B$  pups responded to  $A^-$  and B (and to  $A^+$  and B after learning of  $A^+B$ ) but not or less to AB. In Experiment 4, pups responded to  $A^-B$  and  $A^+B$  after conditioning to AB. These results confirm the configural perception of certain odor mixtures by young organisms and reveal that the proportion of components is a key factor influencing their coding, recognition, and discrimination of complex stimuli.

**Key words:** configural perception, neonates, odorant proportion, odor mixture, olfaction, rabbit (*Oryctolagus cuniculus*)

## Introduction

Studies investigating the nature of odor cues involved in the behavior and adaptation of organisms are often looking for key-odor cues. However, mixtures by themselves may also constitute efficient stimuli, that is, signals active on behavior (Gottfried 2009). The perception of such mixtures by animals, including humans, is usually suggested to be elemental (analytical) or configural (synthetical) (Kay et al. 2005; Harris 2006; McNamara et al. 2007; van Wijk et al. 2010). The perception is considered as elemental when the organism perceives the specific odor of each odorant included in the mixture, the mixture smelling then like its constituents (Laing and Francis 1989; Laska and Hudson 1993; Wiltrout et al. 2003; Linster and Cleland 2004). On the opposite, configural perception occurs when the mixture presents an odor quality distinct from the respective odor of each component (Derby et al. 1996; Smith 1996; Valentincic et al. 2000; Kay et al. 2005): The odorants in the mixture lose their individual odor identity and their association gives rise to a novel quality, that is, to a configuration specific to the mixture (Rescorla and Wagner 1972; McBurney 1986; Jinks and Laing 1999; Thomas-Danguin et al. 2007; Riffell et al.

2009). This configuration may be complete or weak, depending on the mixture smelling completely distinctively or at least in part different, as compared with its constituents (Kay et al. 2005). For instance, results obtained in adult rats suggest a perception of certain binary odor mixtures that differs from the perception of the components (Linster and Smith 1999). Similarly, in human adults, some mixtures of 2 or 3 odorants smell partially or totally different as compared with their constituents. For example, a mixture of ethyl isobutyrate (odorant A; strawberry like odor) and ethyl maltol (odorant B; caramel like odor) generates at the 30/70 v/v ratio of A/B, the perception of a new configural odor (pineapple odor; Le Berre, Thomas-Danguin, et al. 2008). Interestingly, recent studies highlighted that newborn rabbits also process this AB mixture in a partial configural way at the same ratio: After learning of AB, rabbit pups respond both to the component A and to the component B, but after learning of one component they do not respond to AB. This result, which is not a consequence of overshadowing, strongly suggests that the newborns perceive in AB, not only the odor of A and the odor of B but also a third odor

different from the odor of each component (Coureaud et al. 2008, 2009). Here, we pursue the exploration of this neonatal processing of odor mixture, in examining whether it strictly depends on the concentrations of the odorants that compose the mixture.

In human adults, Laing and Willcox (1983) and Laing et al. (1984) observed that the perceived quality of a binary mixture elementally processed can change dramatically from the quality of one odorant to the quality of both components or from the quality of one to the quality of the other odorant, after very small changes in the odorants' intensity (concentration). Differences in the ratio of components' concentration can also produce distinct perceptual changes that affect the recognition of the odor mixture (Laska and Hudson 1993; Olson and Cain 2000). With regard to mixtures eliciting novel odor percepts, Le Berre, Beno, et al. (2008) evidenced that very small variations (just noticeable differences), in the concentration of one component of the AB mixture (ethyl isobutyrate/ethyl maltol 30/70 ratio), are sufficient to modify the typicality of the mixture perceived by human subjects, that is, to lessen significantly the perception of the configuration.

In the present series of 4 experiments, we investigated whether newborn rabbits detect changes in the proportion of odorants that compose the AB mixture and whether these changes alter their responsiveness to the mixture. The newborn rabbit model offers the relevant opportunity to induce the rapid learning of an odorant or of a mixture including it (with the help of the reinforcing mammary pheromone [MP]; Coureaud, Moncomble, et al. 2006), before to test the responsiveness of the pups to the odorants and the mixture. Experiment 1 explored whether pups who usually do not respond to the AB mixture after learning of odorant A (or B), respond to the mixture including a lower or higher proportion of component A. Experiment 2 evaluated whether a change in the concentration at which the component A is learned during the conditioning impacts the perception of A and B at 3 different ratios. Finally, Experiments 3 and 4 explored the ability of newborn rabbits to extract information contained in the AB mixture and to generalize this information to the same mixture but with distinct ratios of components A and B.

All these experiments were designed to probe the singular perceptual properties that certain odor mixtures present according to their composition, both in terms of chemical nature and concentration of components, leading to configural processing in the neonatal brain.

## Materials and methods

### Animals and housing conditions

New Zealand rabbit females and males (Charles River strain) from the Centre de Zootechnie (Université de Bourgogne) were kept in individual cages, and a nest box ( $0.39 \times 0.25$

$\times 0.32$  m) was added on the outside of the pregnant females' cages 2 days before delivery (the day of delivery was considered as day 0). To equalize the nursing experience of the pups, all the females had access to their nest between 11:30 and 11:45 AM. This procedure allowed observing the brief (3–4 min) usually daily nursing of the species (Zarrow et al. 1965). The animals were kept under a constant 12:12 light:dark cycle (light on at 7:00 AM) with ambient air temperature maintained at 21–22 °C. Water and pelleted food (Lapin Elevage 110) were provided ad libitum. A total of 170 newborns born from 24 females were used in the study.

We strictly followed the local, institutional, and national rules (French Ministries of Agriculture and of Research & Technology) regarding the care and experimental use of the animals. Thus, all experiments were carried out in accordance with ethical rules enforced by French law and were approved by the Ethical Committee for Animal Experimentation under no. 5305.

### Odorants

The odorants consisted in the MP (2-methylbut-2-enal; CAS# 497-03-0), ethyl isobutyrate (odorant A; CAS# 97-62-1), ethyl maltol (odorant B; CAS# 4940-11-8), and their mixtures (all the components were purchased from Sigma-Aldrich). In the following, superscript symbols “<sup>-</sup>” and “<sup>+</sup>” refer, respectively, to lower or higher concentration of odorant A as compared with its standard concentration (no symbol) and to lower or higher proportion of A in AB mixtures. Table 1 lists the concentrations of the components used in the different experiments.

To induce the associative learning of unknown odorants or odor mixtures, the MP was used as unconditioned stimulus, always at a concentration of  $10^{-5}$  g/mL (previously shown to be highly efficient; Coureaud, Moncomble, et al. 2006; Coureaud et al. 2008, 2009). The MP-A and MP-B blends were prepared in a hydroalcoholic solution (0.2% ethanol in water; ethanol anhydrous, Carlo Erba; purified water, MilliQ system, Millipore) with a final concentration of  $10^{-5}$  g/mL of each constituent. The MP-AB blend contained  $0.3 \times 10^{-5}$  g/mL of odorant A and  $0.7 \times 10^{-5}$  g/mL of odorant B. This 30/70 v/v ratio of A/B was chosen because it generates the perception of a configuration (pineapple odor) in human adults due to odor-blending (Thomas-Danguin et al. 2007; Le Berre, Thomas-Danguin, et al. 2008) and because it partially blends also in newborn rabbits, who perceive it following a weak configural processing (Coureaud et al. 2008, 2009). In Experiment 2, we modified the concentration of odorant A in the MP-A blend as follows: A<sup>-</sup> included  $0.2 \times 10^{-5}$  g/mL of odorant (i.e., 5× less than in A), A<sup>+</sup> included  $5 \times 10^{-5}$  g/mL of odorant (i.e., 5× more than in A). In mixtures of A and B, due to mixing chemical constraints, modifying the concentration of one component led to a modification

**Table 1** Concentrations of single odorants A and B and of AB mixtures during the conditioning session and the behavioral test of the 4 experiments (concentration unit:  $10^{-5}$  g/mL)

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
MP-induced conditioning	A: 1 B: 1	A <sup>-</sup> : 0.2 A <sup>+</sup> : 5	A <sup>-</sup> B: A <sup>-</sup> (0.06), B (0.7) A <sup>+</sup> B: A <sup>+</sup> (1.5), B (0.7)	AB: A (0.3), B (0.7)
Test	A <sup>-</sup> B: A <sup>-</sup> (0.06), B (0.7) AB: A (0.3), B (0.7) A <sup>+</sup> B: A <sup>+</sup> (1.5), B (0.7)	A <sup>-</sup> : 0.2 A <sup>+</sup> : 5 A <sup>-</sup> B: A <sup>-</sup> (0.06), B (0.7) AB: A (0.3), B (0.7) A <sup>+</sup> B: A <sup>+</sup> (1.5), B (0.7)	A <sup>-</sup> : 0.2 A <sup>+</sup> : 5 B: 1 A <sup>-</sup> B: A <sup>-</sup> (0.06), B (0.7) AB: A (0.3), B (0.7) A <sup>+</sup> B: A <sup>+</sup> (1.5), B (0.7)	A <sup>-</sup> B: A <sup>-</sup> (0.06), B (0.7) A <sup>+</sup> B: A <sup>+</sup> (1.5), B (0.7)

Conditioning was promoted by the MP used at  $1 \times 10^{-5}$  g/mL.

of the ratio for both components. Therefore, our strategy was to modify the concentration of component A only, following the same range as in monomolecular odorant solutions: A<sup>-</sup>B contained  $0.06 \times 10^{-5}$  g/mL of odorant A (i.e., 5 $\times$  less than in AB) and  $0.7 \times 10^{-5}$  g/mL of odorant B (i.e., 8/92 v/v ratio), A<sup>+</sup>B contained  $1.5 \times 10^{-5}$  g/mL of odorant A (i.e., 5 $\times$  more than in AB) and  $0.7 \times 10^{-5}$  g/mL of odorant B (i.e., 68/32 v/v ratio). Behavioral assays were run with the same solutions than those prepared for the conditioning but without MP. For instance, conditioning to A<sup>-</sup>B means for pups to be exposed to MP-A<sup>-</sup>B (MP:  $10^{-5}$  g/mL; A<sup>-</sup>:  $0.06 \times 10^{-5}$  g/mL; B:  $0.7 \times 10^{-5}$  g/mL), whereas behavioral testing to A<sup>-</sup>B consisted in an exposure to a mixture including A<sup>-</sup> ( $0.06 \times 10^{-5}$  g/mL) and B ( $0.7 \times 10^{-5}$  g/mL).

The MP was also used as a control to ensure that the pups were awake and responsive at the time of behavioral testing. It was then diluted in a solvent constituted by hydroalcoholic solution (0.1% ethanol [anhydrous, Carlo Erba] in purified water [MilliQ System, Millipore]) (the solvent is behaviorally neutral for pups; see Coureaud et al. 2008), at a concentration of  $10^{-5}$  g/mL, a level known to release high orocephalic responses in rabbit neonates (Coureaud et al. 2004).

### Odor conditioning and behavioral assay

The conditioning sessions and behavioral (retention) assays were run in a room isolated from the breeding room. The pups from a same litter were transferred into a box lined with nest materials and maintained at room temperature for the duration of the conditioning or the assay (10 min maximum).

The MP-induced conditioning was carried out with 2-day-old pups following a procedure previously described (Coureaud, Moncomble, et al. 2006; Coureaud et al. 2008, 2009). Two milliliters of the MP-single odorant or MP-binary mixture blends were pipetted on a pad (19  $\times$  14 cm, 100% cotton), then held 2 cm above the pups for 5 min. Five minutes after the end of the conditioning, the pups were individually marked (with scentless ink) and returned to their nest for 24 h. The box containing the litter was rinsed with alcohol and distilled water after each conditioning.

The behavioral assay consisted in an oral activation test (Schaal et al. 2003; Coureaud, Moncomble, et al. 2006, Coureaud, Langlois, et al. 2006; Coureaud et al. 2008, 2009). During this test, the pup was immobilized in one hand of the experimenter, its head being left free. The test odor was presented for 10 s with a glass-stick 0.5 cm in front of the nares. A test was considered positive when the stimulus elicited head searching movements (vigorous, low amplitude horizontal, and vertical scanning movements of the head, displayed after stretching of the neck toward the stick) eventually followed by oral grasping movements (labial seizing of the stick's extremity). Nonresponding pups displayed no response but sniffing to the stimulus. Each pup participated in only one experiment but was successively tested for its responses to 2 or 3 stimuli and systematically to the MP used as control. The successive testing consisted in the presentation of a first stimulus to all the pups from a same litter, then a second stimulus, and so on, with an intertrial interval of 120 s. The order of stimuli presentation was counterbalanced from one to another pup, and the MP was always presented at last. If a pup responded to a stimulus, its nose was softly dried with absorbing paper before the next stimulation.

To minimize litter effects, each experimental group was drawn from 4 to 6 litters, with half a litter as a maximum (i.e., 5 pups) in a given group; the pups remaining in the litter were usually included in another group. The conditioning and testing were always run in the morning, 1–2 h before the daily nursing, to equalize the pups' motivational state and limit the impact of satiation on the response (Montigny et al. 2006).

### Statistics

The frequencies of pups responding in the behavioral test were compared using the  $\chi^2$  test of Pearson (with Yates correction when necessary) when the groups were independent (i.e., distinct groups of pups tested for their response to a same stimulus) or the  $\chi^2$  test of McNemar when the groups were dependent (i.e., pups from a same group tested for their response to several stimuli). Data were deemed significant when the 2-tailed tests ended with  $P < 0.05$ .

## Results

### Experiment 1—preexposure to A or to B and comparative perception of AB, A<sup>-</sup>B, and A<sup>+</sup>B

This first experiment aimed to evaluate the behavioral responsiveness of newborn rabbits to 3 mixtures of odorants (AB, A<sup>-</sup>B, and A<sup>+</sup>B) varying in their proportions of A and B, after learning of the component A or of the component B. The hypothesis was that the pups should respond to A<sup>-</sup>B and A<sup>+</sup>B in contrast to the AB blending mixture because the configural perception of AB may be dependent of a highly bound ranged ratio of A/B in the mixture (i.e., the 30/70 ratio) as noted in humans (Le Berre, Thomas-Danguin, et al. 2008). To test this hypothesis, 15 and 17 pups (each group from 4 litters) were, respectively, exposed to the MP-A or MP-B blends on day 2 and tested the day after for their response to AB, A<sup>-</sup>B, and A<sup>+</sup>B.

As expected, pups conditioned to A displayed a low responsiveness to AB (6.7%); on the opposite, they highly and indistinctly responded to A<sup>-</sup>B and A<sup>+</sup>B (86.7%) (AB vs. A<sup>-</sup>B or A<sup>+</sup>B:  $\chi^2 = 10.1$ ,  $P < 0.01$  for both comparisons) (Figure 1a). The pattern was nearly the same for the pups conditioned to B (proportions of responding pups to AB, A<sup>-</sup>B, and A<sup>+</sup>B, respectively: 5.8%, 47.1%, and 64.7%; AB vs. A<sup>-</sup>B or A<sup>+</sup>B:  $\chi^2 > 6.1$ ,  $P < 0.05$ ; A<sup>-</sup>B vs. A<sup>+</sup>B:  $\chi^2 = 1.3$ ,  $P > 0.05$ ) (Figure 1b).

Thus, after having learned the component A or B of the AB mixture, rabbit pups detected and responded to this component when exposed to A<sup>-</sup>B and A<sup>+</sup>B. This is verified both when the pups have learned the component that remains quite similar in terms of concentration between the learning session (B was at  $1 \times 10^{-5}$  g/mL) and the behavioral test (B was at  $0.7 \times 10^{-5}$  g/mL in A<sup>-</sup>B and A<sup>+</sup>B) and also for the component which concentration mainly varied (A). In contrast, having learned the odorant A or B, rabbit pups

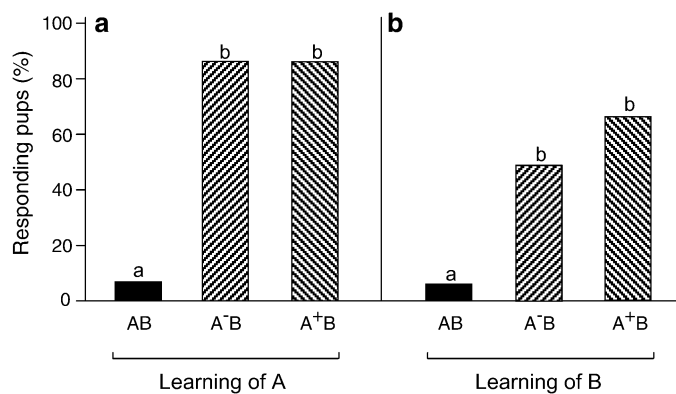
did not respond to the AB mixture. These results confirmed the very particular perception of the AB mixture at the 30/70 A/B ratio as compared with the 2 other ratios forming the A<sup>-</sup>B and A<sup>+</sup>B mixtures, which could be considered as non-blending mixtures.

### Experiment 2—preexposure to A<sup>-</sup> or A<sup>+</sup> and subsequent perception of A<sup>-</sup>B or A<sup>+</sup>B

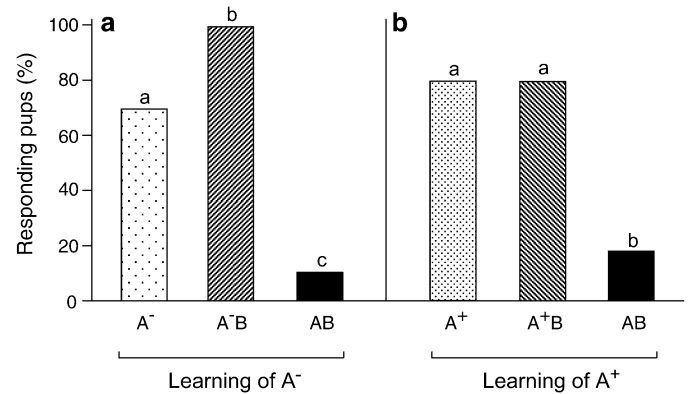
In this second experiment, we aimed to assess the ability of newborn rabbits to generalize the information acquired from a component at 2 different concentrations (A<sup>-</sup> or A<sup>+</sup>) to a mixture containing this component at slightly different concentrations (A<sup>-</sup>B or A<sup>+</sup>B, respectively) or at the particular concentration of A in the AB mixture ( $0.3 \times 10^{-5}$  g/m). Such a generalization was not found to be possible for AB when A or B were learned (see Experiment 1). We hypothesized that the learning of A<sup>-</sup> should allow the detection of this component in A<sup>-</sup>B, and similarly for A<sup>+</sup> in A<sup>+</sup>B because these mixtures are not suspected to blend. Here, we therefore evaluated the responsiveness to A<sup>-</sup>, A<sup>-</sup>B, and AB of pups conditioned to A<sup>-</sup> ( $n = 20$  pups from 4 litters) and to A<sup>+</sup>, A<sup>+</sup>B, and AB of pups conditioned to A<sup>+</sup> ( $n = 20$ , 4 litters).

Under these conditions, the pups conditioned to A<sup>-</sup> highly responded to A<sup>-</sup> and to A<sup>-</sup>B (70% and 100%) but very weakly to AB (10%; comparisons A<sup>-</sup> or A<sup>-</sup>B vs. AB:  $\chi^2 > 5.7$ ,  $P < 0.001$ ; comparison A<sup>-</sup> vs. A<sup>-</sup>B:  $\chi^2 = 4.9$ ,  $P = 0.027$ ) (Figure 2a). Similarly, the pups conditioned to A<sup>+</sup> highly responded to A<sup>+</sup> and A<sup>+</sup>B (80%) but weakly to AB (20%; comparisons A<sup>+</sup> or A<sup>+</sup>B vs. AB:  $\chi^2 > 4$ ,  $P < 0.05$ ) (Figure 2b).

Thus, as expected, A<sup>-</sup>B and A<sup>+</sup>B did not appear to blend. Moreover, despite their ability to detect the familiar (previously learned) component A in mixtures, here in mixtures that did not blend, the pups did not generalize the learned information to the AB mixture. This suggested that the



**Figure 1** Frequency (%) of 3-day-old rabbit pups responding in an oral activation test to the AB, A<sup>-</sup>B, and A<sup>+</sup>B mixtures (i.e., 3 mixtures including the same components but in different proportions) 24 h after their MP-induced conditioning to (a) the odorant A ( $n$  pups tested = 15) or (b) the odorant B ( $n = 17$ ). Distinct letters above the bars indicate statistical differences at the  $P < 0.05$  level.



**Figure 2** Frequency (%) of 3-day-old rabbit pups responding in an oral activation test to (a) the odorant A<sup>-</sup> and the A<sup>-</sup>B and AB mixtures ( $n$  pups tested = 20) or (b) the odorant A<sup>+</sup> and the A<sup>+</sup>B and AB mixtures ( $n = 20$ ) 24 h after their MP-induced conditioning to the component A<sup>-</sup> or the component A<sup>+</sup>, respectively. Distinct letters above the bars indicate statistical differences at the  $P < 0.05$  level.

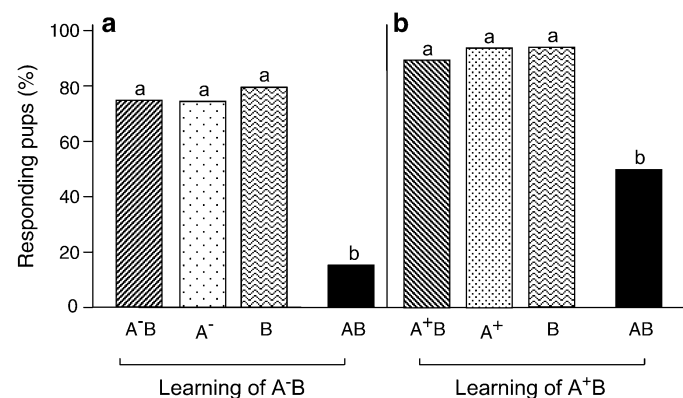


nonblending mixtures were perceived by the pups in a elemental way, while something different occurred with AB, making its perception particular, as soon as A and B are in 30/70 ratio.

### Experiment 3—preexposure to the A<sup>-</sup>B and A<sup>+</sup>B nonblending mixtures and subsequent perception of the AB mixture

In this third experiment, we tried to determine whether the neonatal learning of the A<sup>-</sup>B nonblending mixture (and A<sup>+</sup>B mixture, respectively) would be followed by behavioral responses to this mixture and its components but not to the AB blending mixture. Our hypothesis was that newborn rabbits should be able to extract the components of the present mixtures (they can do it from a nonblending AC mixture; see Coureaud et al. 2009) as they do for the partially blending AB mixture (Coureaud et al. 2008) but that they should not generalize to AB the information learned in such other mixtures, due to the configural perceptual properties of AB. For this experiment, rabbit pups were MP conditioned to A<sup>-</sup>B and tested the day after either to A<sup>-</sup> and B ( $n = 20$  pups, 4 litters) or to A<sup>-</sup>B and AB ( $n = 20$  pups, 4 litters). Moreover, independent groups of pups were conditioned to A<sup>+</sup>B by association with the MP and then tested either to A<sup>+</sup> and B or to A<sup>+</sup>B and AB ( $n = 18$  and 20 pups, respectively, each group from 4 litters).

In the A<sup>-</sup>B conditioned group, the pups highly responded to this mixture (75%), confirming their learning of it. They also and similarly responded to each of the components (75% and 80% to A<sup>-</sup> and B, respectively). However, they were only a few to respond to AB (15%; comparisons AB vs. A<sup>-</sup>B, A<sup>-</sup> or B:  $\chi^2 > 10.1$ ,  $P < 0.01$ ) (Figure 3a). In the same way, the pups initially conditioned to A<sup>+</sup>B highly and similarly responded to this mixture and to its components (>90%;  $\chi^2 = 0.2$ ,  $P >$



**Figure 3** Frequency (%) of 3-day-old rabbit pups responding in an oral activation test (a) to the odorants A<sup>-</sup> and B ( $n$  pups tested = 20) or to the A<sup>-</sup>B and AB mixtures ( $n = 20$ ) 24 h after their MP-induced conditioning to the A<sup>-</sup>B mixture, or (b) to the odorants A<sup>+</sup> and B ( $n = 18$ ), or to the A<sup>+</sup>B and AB mixtures ( $n = 20$ ) 24 h after their conditioning to A<sup>+</sup>B. Distinct letters above the bars indicate statistical differences at the  $P < 0.05$  level.

0.05) but responded less to the AB mixture (50%; comparisons AB vs. A<sup>-</sup>B, A<sup>-</sup> or B:  $\chi^2 > 6.1$ ,  $P < 0.05$ ) (Figure 3b).

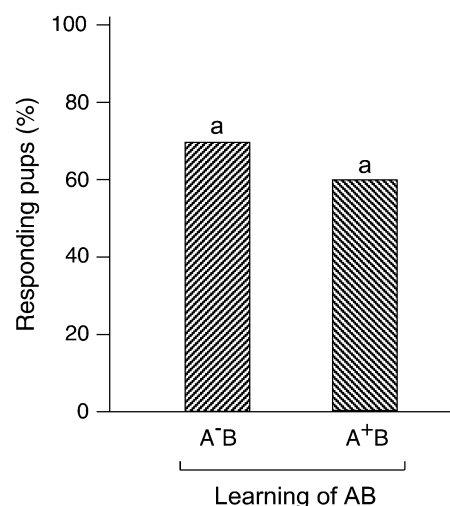
Thus, it clearly appeared that newborn rabbits extract the information corresponding to the odor of each constituent in the nonblending A<sup>-</sup>B and A<sup>+</sup>B mixtures (see also Coureaud et al. 2009). Moreover, one might note that this extraction of information was followed by a low generalization to the AB blending mixture but a significant one after conditioning to the A<sup>+</sup>B mixture (50% of the pups then responded to AB).

### Experiment 4—preexposure to the AB blending mixture and perception of A<sup>-</sup>B and A<sup>+</sup>B

The goal of this last experiment was to evaluate in another way the singularity of the AB mixture perception at the 30/70 ratio. We followed the opposite paradigm as compared with Experiment 3 and determined whether rabbit pups might generalize to the nonblending A<sup>-</sup>B and A<sup>+</sup>B mixtures, the odors of A and B learned after MP exposure to AB. As it was known that pups learned the odor of A and the odor of B after MP conditioning to AB, we hypothesized that they would generalize these information to A<sup>-</sup>B and A<sup>+</sup>B (B is at a constant concentration step in these mixtures and at a slightly variant one as compared with the conditioning) and respond to these nonblending mixtures. For this experiment, 20 pups (from 4 litters) were MP conditioned to AB and then tested to both A<sup>-</sup>B and A<sup>+</sup>B the day after.

Under these conditions, 70% and 60% of the pups responded to A<sup>-</sup>B and A<sup>+</sup>B, respectively ( $\chi^2 = 0.5$ ,  $P > 0.05$ ) (Figure 4).

Thus, the variations in the ratio of odorants A and B and in the concentration of A in the nonblending mixtures, as compared with the AB odorants' ratio, allowed the detection by the pups in A<sup>-</sup>B and A<sup>+</sup>B of the elements A and B



**Figure 4** Frequency (%) of 3-day-old rabbit pups responding in an oral activation test to the A<sup>-</sup>B and A<sup>+</sup>B mixtures 24 h after their MP-induced conditioning to the AB mixture ( $n$  pups tested = 20). The letter above the bars indicates no statistical difference at the  $P < 0.05$  level.

acquired from AB. This confirmed the nonblending status of the A<sup>-</sup>B and A<sup>+</sup>B mixtures and conversely the partial blending one of AB.

## Discussion

The present study focused on the capacity of newborn rabbits to process odor mixtures elementally and/or configurally and aimed to investigate for the first time at this period of life whether the proportion of components in mixture influences these modes of perception. We evaluated whether the pups' perception of a configuration in the AB mixture (blending both in rabbits and humans) is dependent on specific proportions of A and B, that is, whether variations in components' proportion break the perception of the configuration.

In Experiment 1, rabbit pups do not respond to the AB mixture after learning of component A (as previously reported; Coureaud et al. 2008, 2009). However, they respond to A<sup>-</sup>B and A<sup>+</sup>B, that is, to mixtures of the same odorants including different proportions of component A. This result first indicates that the odorant A, learned at a certain concentration ( $10^{-5}$  g/mL), becomes significant for the pups even at another concentration in the mixture ( $0.06 \times 10^{-5}$  g/mL in A<sup>-</sup>B;  $1.5 \times 10^{-5}$  g/mL in A<sup>+</sup>B). Similar results are obtained in Experiment 2 after learning of A at a lower (A<sup>-</sup>:  $0.2 \times 10^{-5}$  g/mL) or higher concentration (A<sup>+</sup>:  $5 \times 10^{-5}$  g/mL). Second and strikingly, for instance after learning of A at the low concentration (A<sup>-</sup>:  $0.2 \times 10^{-5}$  g/mL), pups are unable to respond to this odorant at a close concentration in AB (A in AB:  $0.3 \times 10^{-5}$  g/mL). These results evidence that pups clearly discriminate AB from A<sup>-</sup>B and A<sup>+</sup>B, that is, that they detect the changes in concentration of A and that the variation in components' proportion directly affects their perception of the AB mixture. The detection of these differences is then followed by a contrast in the expression/nonexpression of the critical sucking behavior.

Interestingly, such contrast is not only observed after learning of A but also after learning of B and subsequent variations of component A concentration within the mixture. In other words, even when B constitutes the familiar odorant and when it remains at the same concentration in the 3 mixtures ( $0.7 \times 10^{-5}$  g/mL in AB, A<sup>-</sup>B, and A<sup>+</sup>B), pups differently process AB (nonresponse) as compared with A<sup>-</sup>B and A<sup>+</sup>B (response). Their discrimination of the mixtures demonstrates that even a variation in the concentration of the unknown component may seriously influence their perception of the mixture. Surprisingly, this influence does not follow a classical way. Indeed, after learning of component B 1) pups clearly respond to the A<sup>+</sup>B mixture, even if A is at a higher proportion than B (68/32 ratio). Thus, the presence of A does not mask the detection of B, in this context where no overlapping of B by A<sup>+</sup> occurs, as it is confirmed by the responsiveness to B after learning of A<sup>+</sup>B (Experiment 4); 2) pups also respond to the A<sup>-</sup>B mixture. This result appears less surprising because one may suggest that the pups weakly

perceive the odor of A as compared with the odor of B in A<sup>-</sup>B (8/92 ratio): odorant A could fall below its detection threshold or be overshadowed by odorant B. However, such hypothesis has to be rejected because after conditioning to A<sup>-</sup>B pups not only respond to B but also to A (Experiment 3). To summarize, pups do not respond to AB only when odorant A is at an intermediate concentration between A<sup>-</sup> and A<sup>+</sup>, that is, at the specific 30/70 ratio of A/B. It is worth noticing that this absence of response to AB is not due to an incapacity to detect A or B within the mixture because pups respond very well to A and to B after learning of AB (Coureaud et al. 2008, 2009).

It has been argued that variation in concentration of odor stimulus could affect the rate of generalization to subsequent odor stimuli, thus modulating the way animals perceive and attend to stimuli's features (McNamara et al. 2007). For instance, conditioning of honeybees to high-concentration stimuli decreases generalization to low-concentration test odorants (Wright and Smith 2004). Such results are only partly verified in our data because a variation in concentration between conditioning and behavioral testing modulates the generalization from components to mixture but 1) whatever the increase or decrease of concentration (at least in our experimental conditions) and 2) it appears much more driven by odorants ratio. In fact, the present results add further evidence to the configural processing of the AB mixture in 30/70 proportions (Coureaud et al. 2008, 2009) and especially underline the importance of the components' proportion in the perception of the mixture. Comparatively to the odors of A<sup>-</sup>B and A<sup>+</sup>B, the odor of AB would not be perceived as the sum of its 2 components but due to incomplete blending (for a similar effect in adult rats with another mixture, see Dreumont-Boudreau et al. 2006) as the odor of A, the odor of B, and a third odor inherent to the configuration (as humans perceive pineapple in the same mixture at similar proportions; Le Berre, Thomas-Danguin, et al. 2008a; Le Berre, Beno, et al. 2008). After learning of A, the pups would not respond to AB because too much unfamiliar information (2 of 3) would be perceived in the mixture (the odor of B and the odor of the configuration; see also Coureaud et al. 2008, 2009). However, when the A/B proportions are modified (distinctively from the 30/70 ratio), pups respond to the mixture (A<sup>-</sup>B or A<sup>+</sup>B; Experiments 1 and 2). This finding highlights that variations in the A/B ratio have for consequence a shift in neonatal perception: from configural when exposed to AB, the perception becomes elemental when the pups are stimulated with A<sup>-</sup>B or A<sup>+</sup>B. Thus, the proportion of components in mixture appears as a key feature for complex stimuli coding, recognition, and discrimination in rabbit neonates.

Recognition through chemical ratios characterizes chemosensory communication (e.g., in insects, Baker et al. 1976; Tóth et al. 1992) and could be general for chemical sensing. Using psychophysical methods, Uchida and Mainen (2008) showed that rats classify binary mixtures according to the

components' molar ratios. It suggests that when encountering stimuli in mixture, rats base their decisions on information extracted from the ratio of components included in it (Kay et al. 2003, 2005). In our study, as soon as the A/B proportions are modified from the 30/70 ratio, the blending effect appears "broken" and the configural odor not perceived anymore. Then, the A<sup>-</sup>B and A<sup>+</sup>B mixtures smell like their components and the pups can respond to the AB mixture after having learned one of its components. This "break" in blending effect induced by a change in components' proportion, would be close to what happens in the auditory modality when variations in the frequency of 1 or 2 notes in a triad initially forming a chord, alter the perception of the chord (even if listeners can not say which notes are concerned; Acker and Pastore 1996).

Thus, the present results highlight in rabbit neonates the configural perception of certain odor mixtures and its dependence on components' proportion, as previously observed in human adults (Thomas-Danguin et al. 2007; Le Berre, Beno, et al. 2008). This proportion impacts the ability of rabbit neonates to generalize information learned in a simple context to a more complex one (from odorant to mixture; Experiments 1 and 2) but also to extract information from a mixture and generalize part of it to another mixture. For instance, rabbit pups perfectly learn both odorants in A<sup>-</sup>B but do not respond later to AB (Experiment 3a). This result, compared with previous findings showing that rabbit pups who have learned odorant A, then odorant B, respond to AB (Coureaud et al. 2008), adds credit to the idea of a configuration emerging during the perception of AB but not of A<sup>-</sup>B. Comparatively, after learning of A<sup>+</sup>B, 50% of the pups respond to AB (Experiment 3b). The higher concentration of A in A<sup>+</sup>B could facilitate the perception of A in addition to B, improve its saliency, retention, and therefore detection in AB or it could confer a higher value to A relatively to the AB configuration. The pups would then be able to clearly recognize 2 of 3 informations in AB, a relative amount sufficient to trigger their response to the mixture. In neurophysiological terms, the concentration impact on odor mixture perception could result from differentiated neural-activity patterns, for example, from inhibitory effects at the receptor (Duchamp-Viret et al. 2003), glomerular (McNamara et al. 2007; Cleland et al. 2007), or higher order areas (Frederick et al. 2009). Regarding the AB blending mixture, a change in the concentration of A would be sufficient to modify the neuronal pattern initially activated by AB at the 30/70 specific ratio and to suppress the perception of the AB configural odor.

To conclude, the present study discloses a new series of results confirming that the 30/70 mixture of ethyl isobutyrate and ethyl maltol is processed in a singular way by newborn rabbits, as it is by human adults. It generates the perception of a configuration in addition to the perception of the odors of A and B. Here, the components' proportion also appears to strongly matter on neonatal detection and behavioral responsiveness to information carried in mixtures, even if the

olfactory and cerebral systems are incompletely mature. In the feeding domain, it is essential for adult organisms to detect the palatability of natural products that compose the diet. The olfactory detection of variations in the quality of food, in particular the discrimination of mixtures similar in composition but distinct in components' proportion, may directly contribute to the rapid acceptance/rejection of that food (e.g., detection of ripeness). In newborns, such capacities may allow differentiating littermates and/or adult conspecifics (carrying overlapping odors but also variations in their "chemical signatures") but also to detect modification in the chemosignals emitted by the mother (e.g., in the rabbit: Hudson and Distel 1990; Coureaud, Langlois, et al. 2006). By detection of slight variations in related mixtures, young organisms could therefore better adjust their behavior to the chemosensory evolution of the mother (attraction to, and optimization of, the interaction with her during the lactation period, then decrease in this attraction when the young become autonomous) and more generally of the environment.

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