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Charlotte Sinding, Thierry Thomas-Danguin, Guillemette Crépeaux, Benoist Schaal, Gérard Coureaud. Experience influences elemental and configural perception of certain binary odour mixtures in newborn rabbits.. *Journal of Experimental Biology*, 2011, 214 (24), pp.4171-8. 10.1242/jeb.063610 . hal-00788004

**HAL Id: hal-00788004**

**<https://hal.science/hal-00788004v1>**

Submitted on 29 May 2020

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## RESEARCH ARTICLE

# Experience influences elemental and configural perception of certain binary odour mixtures in newborn rabbits

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Accepted 23 September 2011

### SUMMARY

**Elemental and configural olfactory perception allows interaction with the environment from very early in life. To evaluate how newborn rabbits can extract and respond to information from the highly complex chemical surroundings, and how experience acts on this sensory, cognitive and behavioural capability, we ran a study in four steps including a total of eight experiments. We mainly used a binary AB mixture comprising ethyl isobutyrate (component A) and ethyl maltol (component B), previously shown as a bearer of blending properties; in rabbit pups (as in human adults), the mixture elicits a weak configural perception, i.e. the perception of a configural odour different from the odours of the components. First, a repeated exposure to one component of AB led to a more elemental perception of this mixture; conversely, a repeated exposure to AB facilitated its configural processing. Second, similar impact of experience did not appear with a non-blending AC mixture (ethyl isobutyrate-guaiacol). Third, repeated exposure to AB impacted not only the perception of AB, but also and in the same way the perception of the AC mixture sharing one component, and reciprocally. However, facilitation to perceive one mixture in one mode (configural/elemental) was not generalized to a mixture sharing no components with the experienced mixture [AB *versus* DE (damascenone and vanillin)]. Thus, experience contributes to the neonatal perception of odour mixtures and adds plasticity to the perceptual system. However, this impact remains dependent on the chemical composition of the mixtures.**

Key words: neonatal perception, olfaction, mixture, experience, mammary pheromone, rabbit.

### INTRODUCTION

Newborns need to interact with their highly complex environment to survive. This pressure shapes abilities to extract relevant information from the background and to discriminate between a plethora of information. Independently of the sensory modality, two processes may contribute to the neonatal perception of surroundings: the perception of single elements among other elements, and/or the perception of mixtures/patterns as configurations [combinations of elements eliciting the perceptual emergence of a new single cue (e.g. Spelke, 1990; Tovee et al., 1996)].

In olfaction, elemental perception occurs when organisms perceive the specific quality of each odorant in a mixture, which then smells like its constituents (Laska and Hudson, 1993; Livermore et al., 1997; Linster and Cleland, 2004; Eschbach et al., 2011). In contrast, configural perception refers to the perception of a single odour mixture quality (configuration) distinct from the respective odour of each component (Smith, 1998; Derby, 2000; Valentincic et al., 2000; Jinks and Laing, 2001). Such configural perception has been increasingly investigated over the past few years, with distinct approaches and in various models including humans (e.g. Boyle et al., 2009; Riffell et al., 2009; Deisig et al., 2010; van Wijk et al., 2010; Kuebler et al., 2011; Su et al., 2011). The perceived configuration may be weak or complete, depending on the mixture smelling in part similar to or completely different from its constituents (Kay et al., 2005). Initially, configural processing has been described in adult organisms as in rats (Linster and Smith, 1999). In human adults, an AB mixture composed of ethyl

isobutyrate (A) and ethyl maltol (B), smelling like strawberry and caramel, respectively, induces the configural perception of 'pineapple' (Le Berre et al., 2008; Barkat et al., 2011). Such a mixture has been called a blending mixture (Dreumont-Boudreau et al., 2006; Le Berre et al., 2008; Le Berre et al., 2010; Barkat et al., 2011). Recently, configural perception of the same AB mixture has also been reported in newborn rabbits: after a single conditioning to AB, pups respond to odorants A and B, showing abilities to extract the odour of each element during conditioning. However, after learning only one component (A or B), they do not respond to AB, whereas they respond perfectly to another mixture including odorant A (AC; C is guaiacol). Thus, the AB but not the AC mixture seems to generate almost spontaneously the perception of a weak configuration in newborn rabbits, i.e. the perception of an AB configural odour in addition to the odours of A and B (Coureaud et al., 2008; Coureaud et al., 2009; Coureaud et al., 2011).

A theoretical question regarding the elemental *versus* configural modes of perception concerns their relative plasticity. Indeed, among factors that modulate the processing of odour mixtures [e.g. chemical composition (Uchida et al., 2000; Daly et al., 2004); proportion of components (Wright et al., 2005; Uchida and Mainen, 2008; Coureaud et al., 2011); and complexity (Jinks and Laing, 1999)], experience appears as a dominant one. In adult humans or animals, experience with components improves the elemental perception of mixtures including them. For instance, pre-exposure to each odorant of the weak configural AB mixture (cited above) during two 30 min sessions favoured the elemental perception of

this mixture (Le Berre et al., 2008; Le Berre et al., 2010). Configural processing of an odour mixture is also improved after repeated exposure to it (Livermore et al., 1997; Stevenson, 2001). Similar results have been observed in face recognition studies (Dolan et al., 1997; Van Belle et al., 2010). From a developmental point of view, one may wonder about this impact of experience on the perception of odour mixtures early in life. Elemental perception could be favoured in neonates because picking up relevant information from the surroundings is crucial for survival, such as the perception of key odorants emitted for instance from the body of the mother (Blass and Teicher, 1980; Schaal et al., 2009; Coureaud et al., 2010). However, responsiveness to configurations could also constitute a strategy allowing newborns to reduce complexity and to quickly find their way in the multi-odorant neonatal niche.

The present study aimed to address this issue assuming that some plasticity should exist in the young animals' perception of odour mixtures, as plasticity allows adaptation throughout development (e.g. Gottlieb, 1983). Following a more or less intensive protocol, we exposed rabbit pups to the AB mixture cited above, initially perceived in a weak configural mode (Coureaud et al., 2008; Coureaud et al., 2009; Coureaud et al., 2011; Le Berre et al., 2008; Le Berre et al., 2010; Barkat et al., 2011), or to one of its components, before testing their responsiveness to the mixture and to the single odorants. We hypothesized that variations in experience may modulate the perception of AB by neonates (Expt 1). We also tested this hypothesis with a mixture initially perceived in an elemental manner (AC; Expt 2) and in the more ecological situation of between-mixtures generalization, using mixtures that shared one component (AB, AC; Expt 3). Finally, using completely distinct AB and DE mixtures, we assessed whether rabbit neonates may be trained to process odour mixtures in an elemental or configural manner, depending not on the chemical composition of the mixtures but on the training to the perceptual process by itself (Expt 4).

## MATERIALS AND METHODS

### Animals and housing conditions

Male and female New Zealand rabbits *Oryctolagus cuniculus* (Linnaeus 1758) (Charles River strain, L'Arbresle, France) from the Centre de Zootechnie (Université de Bourgogne, Dijon, France) were kept in individual cages. A nest box (0.39×0.25×0.32 m) was added to the outside of the pregnant females' cages 2 days before delivery (day of delivery was day 0). Females had access to their nest between 11:30 and 11:45 h. This procedure allowed females to follow the brief (3–4 min) daily nursing typical of the species (Zarrow et al., 1965). It also allowed us to assess whether females nursed effectively and whether pups used in the experiments sucked efficiently. Animals were kept under a constant 12 h:12 h light:dark cycle (lights on at 07:00 h), with ambient air temperature maintained at 21–22°C. Water and pelleted food (Lapin Elevage 110, Safe, Augy, France) were provided *ad libitum*. In the study, 424 newborns (from 107 litters) were used.

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

### Odorants

The following odorants were used: 2-methylbut-2-enal (the mammary pheromone, MP) (Coureaud et al., 2010), ethyl isobutyrate (odorant A), ethyl maltol (odorant B), guaïacol (odorant C), damascenone

(odorant D) and vanillin (odorant E) as unmixed components, and the AB, AC and DE mixtures as complex stimuli. The AB mixture included  $0.3 \times 10^{-5}$  and  $0.7 \times 10^{-5}$  g ml<sup>-1</sup> of components A and B, respectively; this 30/70 v/v ratio elicits the perception of a pineapple odour in human adults because of blending properties (Thomas-Danguin et al., 2007; Le Berre et al., 2008; Le Berre et al., 2010; Barkat et al., 2011), and weak configural perception in newborn rabbits (Coureaud et al., 2008; Coureaud et al., 2009; Coureaud et al., 2011). The AC mixture contained a 50/50 ratio of each component ( $0.5 \times 10^{-5}$  g ml<sup>-1</sup> of each odorant) and the DE mixture contained a 40/60 ratio of D/E ( $0.4 \times 10^{-5}/0.6 \times 10^{-5}$  g ml<sup>-1</sup>, a ratio chosen on the basis of a preliminary assay in humans). AC has been shown (Coureaud et al., 2009) and DE was suspected to trigger elemental perception in rabbit pups.

The MP allowed us to induce the learning of odorants A or B, and of the AB, AC or DE mixtures through associative conditioning (see 'Odour conditioning' below). It was used at  $10^{-5}$  g ml<sup>-1</sup>, a concentration known to be highly efficient (Coureaud et al., 2006). Thus, the AB-MP, AC-MP and DE-MP blends included  $1 \times 10^{-5}$  g ml<sup>-1</sup> of MP and  $0.3$  and  $0.7 \times 10^{-5}$  g ml<sup>-1</sup> of A and B,  $0.5$  and  $0.5 \times 10^{-5}$  g ml<sup>-1</sup> of A and C, and  $0.4$  and  $0.6 \times 10^{-5}$  g ml<sup>-1</sup> of D and E, respectively.

During behavioural testing, as during conditioning, the odorants alone were used at a concentration of  $10^{-5}$  g ml<sup>-1</sup>. During behavioural testing, mixtures were used at the same ratios as during conditioning.

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

### Odour conditioning

Conditioning sessions were run in an experimental room close to the breeding room. For a given litter, four or five pups were transferred into a box lined with nest materials and maintained at room temperature. The MP-induced conditioning was run following a procedure described previously (Coureaud et al., 2008; Coureaud et al., 2009; Coureaud et al., 2011). Just before the conditioning session, 2 ml of the MP+single component or MP+binary mixture were pipetted on a pad (19×14 cm, 100% cotton), which was then held 2 cm above the pups for 5 min. Two minutes after the end of the conditioning, the pups were individually marked with scentless ink and returned to their nest. The box containing the pups was rinsed with alcohol and distilled water after each conditioning session. If other pups from the same litter were used for another group (when permitted by the litter size), these operations were repeated with these pups before continuing with another litter.

Single conditioning of the pups occurred on the third day after birth, 1 h before the daily nursing (10:30 h), to equalize the pups' motivational state and limit the impact of satiation on responses (Montigny et al., 2006). When the pups were submitted to three conditioning episodes, each episode occurred between 08:30 and 10:30 h on three consecutive days (days 1–3). When they were submitted to nine conditionings, three episodes occurred per day at 08:30, 09:30 and 10:30 h (days 1–3). Finally, the nine conditionings+one extra (Expt 4) consisted of three conditionings per day from 08:30 to 10:30 h (days 1–3) and one extra conditioning at 11:00 h (day 3).

### Behavioural assay

The behavioural assay occurred on day 4 in the experimental room previously used for the conditioning. After transfer of animals (the complete litter to avoid repeated interventions in a same nest), we

ran an oral activation test during which a pup was immobilized in one gloved hand of the experimenter, its head being left free. The odour stimulus was presented for 10 s with a glass rod 0.5 cm in front of the nares (Coureaud et al., 2008; Coureaud et al., 2009; Coureaud et al., 2011). A test was positive when the stimulus elicited head-searching movements (vigorous, low amplitude horizontal and vertical scanning movements displayed after stretching towards the rod) followed occasionally by grasping movements (labial seizing of the tip of the glass rod). Non-responding pups displayed no response but sniffing. Each pup participated in only one experiment but was successively tested with two to four stimuli depending on the experiment (no more than four to avoid tiredness or habituation). Successive testing involved the presentation of the first stimulus to pups from the same litter, then a second stimulus and so on, with an inter-trial interval of 120 s. The order of stimulus presentation was systematically counterbalanced from one pup to another. If a pup responded to a stimulus, its nose was softly dried before the next stimulation. The pups were immediately reintroduced to their nest after testing. To minimize litter effects, each experimental group was drawn from four or five litters, with four or a maximum of five pups conditioned and/or tested per litter; the pups remaining in the litter were sometimes included in another group (depending on the size of the litter). No litter effect appeared in each group exposed to the different stimuli ( $\chi^2 < 2.95$ , d.f.=3 or 4,  $P > 0.39$  in all comparisons by generalized estimating equations modelling of binomial data; SAS v. 9, SAS Institute Inc., Cary, NC, USA).

**Statistics**

The pups that did not respond to any of the stimuli tested were considered as not conditioned and therefore were not included in the analysis ( $N=29$ ; their distribution was consistent between litters). The frequencies of responding pups were compared using Pearson's  $\chi^2$  test (with Yates correction when necessary) when the groups were independent (i.e. distinct groups tested for their response to a same stimulus) or Cochran's  $Q$  test when the groups were dependent (i.e. pups from a same group tested for their response to several stimuli). When the Cochran's  $Q$  test was significant (multiple comparisons), proportions of responding pups were compared  $2 \times 2$  using McNemar's  $\chi^2$  test. Degrees of freedom are indicated when  $> 1$ . Data were considered as significant when the two-tailed test ended with  $P < 0.05$ . Except for the litter effect, all analyses were conducted using R release 2.10.1 (The R Foundation for Statistical Computing, Vienna, Austria).

**RESULTS**

**Impact of experience on the perception of a blending mixture**

These experiments aimed to determine whether the perception of the AB mixture by newborn rabbits, initially weakly configural

(Coureaud et al., 2008; Coureaud et al., 2009; Coureaud et al., 2011), changes according to the repeated experience of A, B or AB. We hypothesised that the perception of AB might become more elemental after repeated conditionings to one of the components, and more configural after repeated exposures to the mixture.

**Expt 1.1 – impact of experience on elemental processing of AB**

To assess whether repeated exposure to odorant A influences the perception of AB, three groups of 3-day-old pups were conditioned one, three or nine times to A by MP-induced conditioning between day 1 and day 3 ( $N=16, 18$  and  $19$  pups from five, six and six litters, respectively). On day 4, they were tested for their responsiveness to A, B and AB.

As a result, 100% of the pups conditioned once to odorant A responded to A, 6.2% to B and 0% to AB ( $Q=30.1$ , d.f.=2,  $P < 0.001$ ), as shown in previous studies (Coureaud et al., 2008; Coureaud et al., 2009; Coureaud et al., 2011). Pups from the  $3 \times A$  group distinctively responded to the three stimuli ( $Q=25.7$ , d.f.=2,  $P < 0.001$ ), i.e. more to A than to B and AB (100 vs 5.6 and 61.1%, respectively; A vs B or AB:  $\chi^2 > 4.17$ ,  $P < 0.05$ ), as after only  $1 \times A$  conditioning. Interestingly,  $3 \times A$  conditioned pups displayed a higher rate of response to AB than to B ( $\chi^2=6.12$ ,  $P < 0.05$ ). Clearly, the proportion of pups responding to AB was higher after  $3 \times$  than after  $1 \times$  conditioning ( $\chi^2=11.8$ ,  $P < 0.001$ ). Similarly, after nine conditionings to odorant A, the pups did not respond equally to A, B and AB (94.7 vs 10.5 vs 47.4%;  $Q=22.71$ , d.f.=2,  $P < 0.001$ ), but more to A and AB compared with B ( $\chi^2 > 5.14$ ,  $P < 0.05$ ). Finally, the pups' responsiveness to AB was similar after  $9 \times A$  conditionings compared with  $3 \times A$  conditionings ( $\chi^2=0.26$ ,  $P > 0.5$ ; Fig. 1A).

Thus, three repeated conditionings to A, compared with one conditioning only, modified the way rabbit pups perceived the AB mixture: an increased conditioning to A induced an increased responsiveness to AB. A stronger conditioning ( $9 \times A$ ) had a similar effect. However, despite the increase in responsiveness to AB, more pups responded to the learned odorant (A) than to the AB mixture.

These effects were also observed after MP-induced conditioning to odorant B. Although 20 pups (five litters) selectively responded to B after  $1 \times B$  conditioning (100, 0 and 0% of responsiveness to B, A and AB, respectively;  $Q=32$ , d.f.=2,  $P < 0.001$ ), a  $3 \times B$  reinforced exposure ( $N=19$  pups, five litters) also triggered distinct responsiveness between stimuli (100, 10.5 and 73.7% of pups responding to B, A and AB, respectively;  $Q=27$ , d.f.=2,  $P < 0.001$ ) and improved responsiveness to AB ( $3 \times B$  vs  $1 \times B$ :  $\chi^2=19.1$ ,  $P < 0.001$ ). Results were similar after  $9 \times B$  conditionings ( $N=21$  pups, six litters; 100, 4.8 and 52.4% of pups responding to B, A and AB, respectively;  $Q=30$ , d.f.=2,  $P < 0.001$ ; responsiveness to AB after  $9 \times B$  vs  $1 \times B$  and  $9 \times B$  vs  $3 \times B$  conditionings:  $\chi^2=11.2$ ,  $P < 0.001$  and  $\chi^2=1.13$ ,  $P > 0.05$ , respectively). Moreover, the proportion of

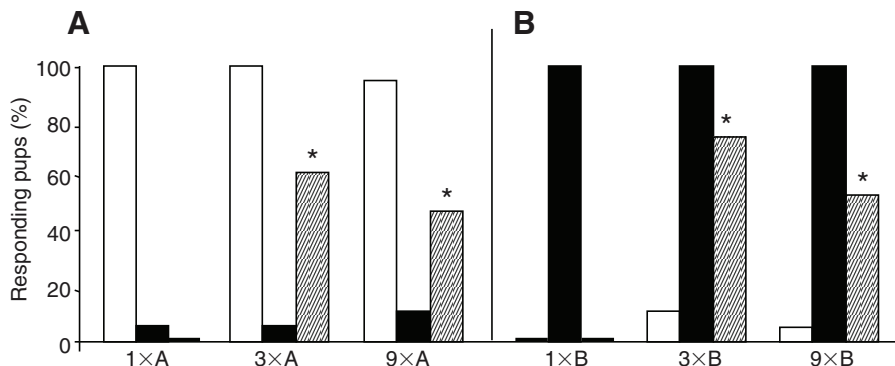


Fig. 1. Proportions of 4-day-old rabbit pups responding in an oral activation test to component A (white bars), component B (black bars) and the AB blending mixture (hatched bars), after one, three and nine conditionings to (A) component A (ethyl isobutyrate;  $N=16, 18$  and  $19$  pups, respectively) or (B) component B (ethyl maltol;  $N=20, 19$  and  $21$  pups, respectively). \*Significantly different ( $P < 0.05$ ) proportion of pups responding to this stimulus according to the level of conditioning.

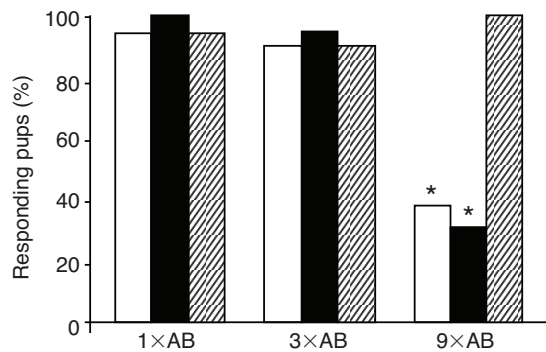


Fig. 2. Proportions of 4-day-old rabbit pups responding in an oral activation test to component A (white bars), component B (black bars) and the AB blending mixture (hatched bars) after one, three and nine conditionings to AB ( $N=18$ , 20 and 16 pups, respectively). \*Significantly different ( $P<0.05$ ) proportion of pups responding to this stimulus according to the level of conditioning.

responding pups was higher during the presentation of B alone compared with the presentation of AB, after 3xAB and 9xAB conditionings ( $\chi^2=3.2$ ,  $P=0.07$  and  $\chi^2=8.1$ ,  $P<0.01$ , respectively; Fig. 1B).

Thus, the repeated learning of one component of the AB mixture seemed sufficient to improve the elemental perception of this component in the mixture, whichever the component learned.

#### Expt 1.2 – impact of experience on configural processing of AB

To assess whether a repeated experience of the AB mixture may improve its configural perception, 18, 20 and 16 pups (from five, six and five litters, respectively) were MP-conditioned 1x, 3x or 9x to AB before testing their responsiveness to A, B and AB on day 4.

After one conditioning, as expected (Coureaud et al., 2008; Coureaud et al., 2009; Coureaud et al., 2011), the pups strongly and similarly responded both to AB, A and B (94.7, 100 and 94.7%, respectively;  $Q=1$ , d.f.=2,  $P=0.6$ ). After 3xAB conditionings, rates of response to AB, A and B were similar (90, 90 and 95%, respectively;  $Q=0.5$ , d.f.=2,  $P=0.78$ ). Regarding AB, the responsiveness was not altered by the repeated exposure (3xAB vs 1xAB:  $\chi^2=2.2$ ,  $P>0.05$ ). Moreover, the rate of pups responding to odorants A or B after three conditionings compared with after one conditioning to AB was similar ( $\chi^2<0.09$ ,  $P>0.05$  for both comparisons; Fig. 2). In contrast, the pups responded differently to the stimuli in the 9xAB group: they responded more frequently to AB than to A and B (100 vs 37.5 and 31.2%, respectively;  $Q=18.5$ , d.f.=2,  $P<0.001$ ; AB vs A or B:  $\chi^2>8.1$ ,  $P<0.01$ ). Clearly, the 9xAB conditioning decreased the responsiveness to the mixture's

components (9xAB vs 1xAB or 3xAB:  $\chi^2>11$ ,  $P<0.01$  for comparisons concerning A or B). However, the repeated exposure to AB did not alter the responsiveness to the mixture, which remained high (9xAB vs 1xAB or 3xAB:  $\chi^2<0.35$ ,  $P>0.05$ ; Fig. 2).

Thus, the processing of the AB mixture was affected by experience in newborn rabbits. After sufficient exposure to the mixture, their perception could be oriented towards the perception of the configural AB odour to the detriment of each component's odour.

#### Impact of experience on the perception of a non-blending mixture

The previous experiments demonstrated that the perception of a partially blending odour mixture may change according to the neonates' experience of the mixture or of its components. Here, we investigated whether this impact may also affect the perception of a non-blending mixture, initially processed elementally.

##### Expt 2.1 – experience of a component and impact on the perception of a non-blending mixture

After a single conditioning to odorant A, rabbit pups did not respond to AB, suggesting a distinct perception of the mixture and its components. This was not the case with the non-blending AC mixture: 2-day-old pups MP-conditioned once to A have been shown to respond later to A and AC, but not to C, showing an elemental processing of AC (Coureaud et al., 2009). Here, we evaluated the impact of the experience of A alone on the perception of this AC mixture with the hypothesis that it could further increase the recognition of A in the mixture. To that goal, 14, 17 and 14 pups (four litters per group) were conditioned 1x, 3x or 9x, respectively, to the component A before testing their responsiveness to A, C and AC.

As expected, after 1xA conditioning, the pups responded more to A and AC than to C (92.8, 92.8 and 0%, respectively;  $Q=24.14$ , d.f.=2,  $P<0.001$ ). This was also true after three and nine conditionings (3x: 94.1, 70.6 and 0%; 9x: 100, 85.7 and 0%;  $\chi^2<0.5$ ,  $P>0.4$  for all intra-group comparisons). There was no statistical difference between the rates of pups responding to A and to AC (1x, 3x or 9x groups:  $\chi^2<0.33$ ,  $P>0.05$ ; Fig. 3A).

Thus, repeated conditioning to A did not modulate the perception of A in a mixture including another component (C), and therefore did not change the responsiveness to the AC mixture.

##### Expt 2.2 – experience of a non-blending mixture and impact on the perception of its components

After a single conditioning to AC when 2 days old, rabbit pups have been shown to display a high occurrence of responses to A, C and AC (Coureaud et al., 2009). In the present study, 19 pups (five litters) were conditioned once to AC on day 3 before testing on day 4. Their responsiveness to A, C and AC was compared with that of pups conditioned 3x or 9x to AC between days 1 and 3 (20 and 19 pups, from six and five litters, respectively).

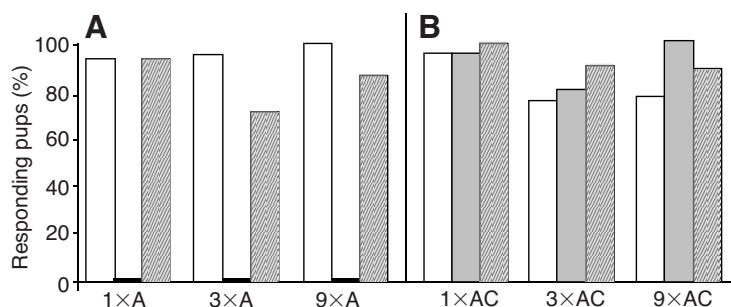


Fig. 3. Proportions of 4-day-old rabbit pups responding in an oral activation test to component A (white bars), component C (grey bars) and the non-blending AC mixture (grey hatched bars) after one, three and nine conditionings to (A) component A ( $N=14$ , 17 and 14 pups, respectively) or (B) AC ( $N=19$ , 20 and 19 pups, respectively). No difference in responsiveness to the same stimulus appears in pups from the different groups.

The pups strongly and similarly responded to A, C and AC in the 1×, 3× and 9× groups (94.7/94.7/100, 75/80/90 and 73.7/94.7/84.2%, respectively;  $Q < 5$ , d.f.=2,  $P > 0.05$  for the three intra-group comparisons). No difference appeared between the groups for each stimulus ( $\chi^2 < 2.8$ ,  $P > 0.05$ ; Fig. 3B).

Thus, in contrast to what happened with the AB mixture (Expt 1.2), the repeated conditioning to AC did not reduce the responsiveness to the components: in our three conditions, pups always responded to each component presented alone, independently of their experience of AC.

**Impact of experience of one mixture on the processing of another mixture**

Because in nature organisms are never exposed to odorants alone but among other odorants, it should be adaptive to discriminate or generalize between mixtures of odorants. For instance, it may be advantageous to generalize to a context Y information previously learned in a context X, i.e. to respond to a mixture containing an odorant learned in another mixture. Rabbit neonates can do that: after a single conditioning of the currently used AB mixture, they later respond to the present AC mixture (the opposite is not true, because of the perceptual blending properties of AB) (Coureaud et al., 2009). Here, we investigated whether the effect of experience noted in Expts 1.1 and 1.2 may also occur after repeated exposure to one of these mixtures before testing to the other mixture, which shares one component.

**Expt 3.1 – experience of a non-blending mixture and perception of a blending mixture**

We first hypothesized that repeated exposure to AC could improve the subsequent perception of A in the AB mixture (as did repeated exposure to A alone; Expt 1.1). Thus, the rates of pups responding to AB were compared after one, three or nine MP-induced conditionings to AC ( $N=19, 20$  and  $19$  pups, from five, six and nine litters, respectively) and compared with the rates displayed after conditionings to A alone (Expt 1.1).

As after repeated conditioning to A, various levels of conditioning to AC induced distinct responsiveness to AB: null after one conditioning, it increased after three conditionings and was highest after nine conditionings (45 and 73.7%, respectively; 1× vs 3× or 9×:  $\chi^2 > 8.72$ ,  $P < 0.01$ ). The proportion of pups responding to AB did not differ after three or nine conditionings to AC compared with conditionings to A (3×: 45 vs 61.1%; 9×: 73.7 vs 47.4%;  $\chi^2 = 2.11$ ,  $P > 0.05$ ; Fig. 4A).

Therefore, depending on their experience, rabbit pups generalized to a blending mixture (AB) the information (A) previously learned in a non-blending mixture (AC).

**Expt 3.2 – experience of a blending mixture and perception of a non-blending mixture**

We examined whether repeated exposure to the AB mixture, which decreased the responsiveness to the odorant A (and B) as compared with AB (Expt 1.2), also impacted the perception of the AC mixture. Thus, the proportions of pups responding to AC were compared between groups of newborns MP-conditioned 1×, 3× or 9× to AB ( $N=18, 20$  and  $16$ , from five, six and five litters, respectively) and compared with the levels of responsiveness to A alone (Expt 1.2).

Responsiveness to AC differed according to the experience of AB. After one and three conditionings, responsiveness was high and not statistically different (100 and 80%,  $\chi^2 = 2.18$ ,  $P = 0.14$ ); it decreased significantly after nine conditionings (37.5%; 1× or 3× vs 9×:  $\chi^2 > 5.08$ ,  $P < 0.05$ ). The responsiveness to AC and A (Expt

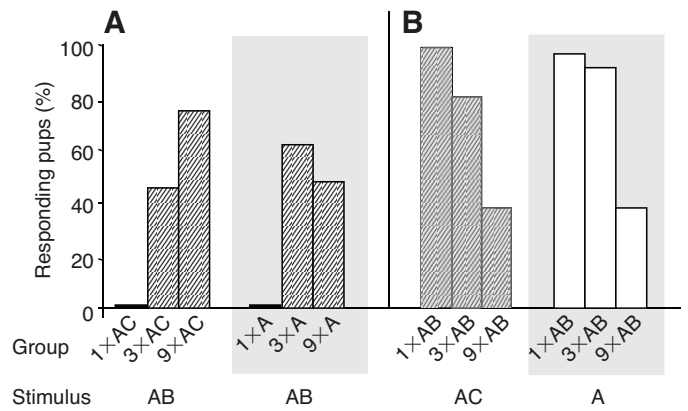


Fig. 4. Proportions of 4-day-old rabbit pups conditioned 1×, 3× or 9× to (A) the non-blending AC mixture ( $N=19, 20$  and  $19$  pups, respectively) or the component A alone (grey-shaded part of the graph, same results as in Fig. 1) and responding in an oral activation test to the AB blending mixture; or (B) the AB blending mixture ( $N=18, 20$  and  $16$  pups, respectively) and responding in the activation test to the AC non-blending mixture and to the component A alone (grey-shaded part of the graph, same results as in Fig. 2). No difference in responsiveness appears between the groups presenting a similar level of conditioning (groups 1×, 3× or 9×).

1.2) was similar after three and nine conditionings to AB (80 vs 90%, and 37.5 vs 37.5%;  $\chi^2 < 2.11$ ,  $P > 0.15$ ; Fig. 4B).

Thus, repeated exposure to a blending mixture impacted the perception of a non-blending mixture sharing one component.

**Generalization of the processing mode**

Here, we investigated whether the between-mixtures effect of experience strictly depended on the chemical overlap of the mixtures, as suggested by the results of previous experiments, or whether this effect could also be due to a general facilitation in perceptual abilities. We assessed whether newborns trained with a non-blending mixture (elemental processing) could later tend to elementally process any other mixture, independently of its chemical composition. Reciprocally, we evaluated whether training with a blending mixture (configural processing) could promote the configural perception of unfamiliar mixtures.

**Expt 4.1 – training to elemental processing and perception of a blending mixture**

To evaluate the effect of experience on the general facilitation of elemental processing, pups were exposed to a putatively non-blending DE mixture sharing no component with the AB mixture. To assess the elemental perception of DE in a preliminary assay, 20 pups were MP-conditioned 1× to D and 19 other pups were MP-conditioned 1× to E on day 3 (five litters per group). On day 4, 100% of the pups responded to D and DE after conditioning to D (0% to E), and 100 and 94.7% responded to E and DE after conditioning to E (5.3% to D). Therefore, as for the AC mixture, most of the pups responded to the DE mixture after conditioning to one of its components, confirming the elemental perception of this non-blending mixture. Then, 12 other pups (from four litters) were conditioned 9× to DE between days 1 and 3 before they were MP-conditioned once to odorant A. On day 4, they were tested for their responsiveness to A, B and AB. According to the present hypothesis, facilitation to perceive elements in the DE mixture could improve the subsequent perception of A in the AB mixture and therefore promote responsiveness to AB. In a control group, 18 pups

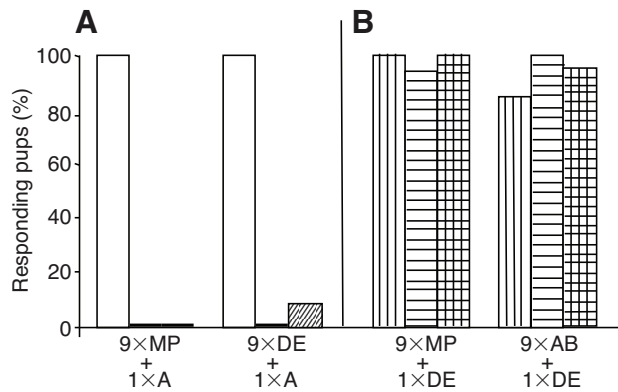


Fig. 5. Proportions of 4-day-old rabbit pups (A) exposed 9× to the MP and conditioned 1× to component A ( $N=18$ ), or conditioned 9× to the DE mixture and then 1× to component A ( $N=12$ ), that respond in an oral activation test to the components A, B and to the AB mixture (white, black and striped bars, respectively); and (B) exposed 9× to the MP and conditioned 1× to the non-blending DE mixture ( $N=16$ ), or conditioned 9× to the blending AB mixture and then 1× to the non-blending DE mixture ( $N=20$ ), that respond in an oral activation test to the components D, E and to the DE mixture (vertical, horizontal and vertical + horizontal hatched bars, respectively). No difference in responsiveness to a given stimulus appears between control and experienced pups ( $9\times\text{MP}+1\times\text{A}$  vs  $9\times\text{DE}+1\times\text{A}$ , and  $9\times\text{MP}+1\times\text{DE}$  vs  $9\times\text{AB}+1\times\text{DE}$ ).

(five litters) were exposed 9× to MP alone between days 1 and 3, before being conditioned once to component A on day 3. We expected no response of these pups to AB on day 4 (as in Expt 1.1 without repeated exposure to MP) (Coureaud et al., 2008; Coureaud et al., 2009; Coureaud et al., 2011).

After nine reinforced exposures to DE and a single conditioning to A, 100% of the pups responded to A. However, they did not respond to B or AB (0 and 8.3%;  $Q=22.2$ , d.f.=2,  $P<0.001$ ). Their responsiveness to A, B and AB was then similar compared with that of the pups only conditioned to A ( $Q=36$ , d.f.=2,  $P<0.001$ ;  $9\times\text{DE}+1\times\text{A}$  vs  $9\times\text{MP}+1\times\text{A}$ :  $\chi^2<0.22$ ,  $P>0.5$  for rates of responses to A, B and AB; Fig. 5A).

Thus, even after repeated exposure to a binary mixture elementally processed, rabbit pups still processed the AB mixture in a configural way.

#### Expt 4.2 – training to configural processing and perception of a non-blending mixture

After nine conditionings to AB, the pups process the mixture in a configural way (Expt 1.2). Here, we investigated whether this enhancement could be generalised to a mixture that did not carry blending properties (DE). To that goal, 20 pups (five litters) were conditioned 9× to AB between days 1 and 3, and then conditioned 1× to DE on day 3. A control group of 18 pups (five litters) was exposed 9× to the MP alone between days 1 and 3 before being MP-conditioned 1× to DE on day 3. On day 4, we suspected that the pups conditioned 9× to AB before conditioning to DE would respond less to D and E than to DE compared with control pups.

Almost all of the control pups responded to the three stimuli (100, 93.7 and 100% to D, E and DE, respectively;  $Q=2$ , d.f.=2,  $P>0.05$ ). Results were similar in pups conditioned 9× to AB and then 1× to DE (85, 100 and 95% responsiveness to D, E and DE, respectively;  $Q=4.7$ , d.f.=2,  $P>0.5$ ). No reduction in responsiveness to D and E nor improvement of the responsiveness to DE appeared compared with the control group ( $\chi^2<1.33$ ,  $P>0.1$  for each stimulus; Fig. 5B).

Thus, if the processing of a mixture changed according to the experience of this mixture, one of its components or another mixture including a common component, the effect was not generalized to a mixture including distinct components.

## DISCUSSION

The present study aimed to assess whether neonatal perception of a binary odour mixture can change from the elemental to the configural mode, or the reverse, according to experience. First, under our experimental conditions, experience with a single component of the AB (weak configural) blending mixture improved the elemental perception of that mixture, whereas intense exposure to the AB mixture itself favoured its configural perception. Second, similar results were observed after exposure to AB and testing to an AC mixture, or the opposite, i.e. from mixture to mixture sharing a component, a condition closer to natural conditions. Third, chemical composition seemed to remain the first driver of perception: there was no effect of experience on the perception of the non-blending (elemental) binary mixture used here (AC), or on the general facilitation of odour mixture perception in one mode (i.e. during testing to AB after exposure to DE, or the opposite).

Regarding the elemental perception of binary odour mixtures, the present study reveals that after nine and even three conditionings to component A only (or B), the perception of the initially weak configural AB mixture becomes more elemental, as if familiarization to one component makes it predominantly salient in the mixture. Thus, the experience of a component could fine-tune the perception of its quality and result in a better discrimination of it among the other components of the mixture [for theoretical considerations or related results obtained in the spiny lobster, see Derby, Livermore, Rescorla and others (Derby et al., 1996; Livermore et al., 1997; Rescorla, 1997)]. Neurobiological findings lend credit to this hypothesis: elements of a mixture become more salient after having been experienced because learning decreases the overlap between bulbar patterns of each component (which reflect odour identity), which is caused by the action of inhibitory neurons (Linster and Cleland, 2004). This ability to extract distinct information from a complex stimulus according to experience could positively impact survival. For instance, although it remains to be tested, this ability may contribute to the detection of slight changes in the proportion or nature of specific odorants between chemically overlapping odour sources (Getz and Smith, 1987) and thus to the discrimination/recognition of conspecifics or the recognition/avoidance of life-threatening components redundant in food.

Another adaptive process suitable for extracting significance from complex chemosensory stimuli rests on the capacity to attribute biological value to configurations, i.e. to give more significance to mixtures than to their components (Rescorla et al., 1985; van Wijk et al., 2011). In previous studies, we showed that newborn rabbits initially process the present AB mixture in part configurally, perceiving the AB configural odour and the odours of A and B in the mixture (Coureaud et al., 2008; Coureaud et al., 2009; Coureaud et al., 2011). Interestingly, results from the present study demonstrate that increased experience of this mixture can modulate and favour the perception of the configuration at the expense of that of the elements. Indeed, pups responded less to the components than to the mixture after nine conditionings to AB. Thus, repeated exposure to AB could trigger the specific processing of AB as a unique percept. For a relatively immature brain, such a phenomenon may allow the pups to extract crucial information from their complex surroundings through the perceptual canalization of mixtures as unique cues. As odorants constitute elements at the level of a

mixture, mixtures may also represent elements at a larger scale. This could be valuable for the organism when odorants alone do not support a sufficient meaning, i.e. when one odorant included in a mixture has been reinforced in some but not in other contexts so that the memorization of the whole mixture as a meaningful object is more useful than that of the single odorant [e.g. in the visual modality (Sutherland and Rudy, 1989)].

The results from the present study show that experience of a mixture or its components may rapidly affect the mode of perception in newborn rabbits, as previously shown in human adults (Stevenson, 2001; Le Berre et al., 2008; Le Berre et al., 2010). At least for certain mixtures, processing might operate on a continuum from strictly elemental to strictly configural perception: the initial perception of a mixture (free of any experience) would be located somewhere in between, depending on the mixture, whereas experience with either the mixture or the components would shift the perception to one or another mode. However, concerning the AB mixture, it seems harder to improve the configural perception with experience as compared with the elemental perception (nine vs three exposures are required). This difference may result from the 'risk' incurred of storing only a piece of information (configural cue) when initially several elements are detectable and known in that stimulus. Thus, it would be easier to deconstruct than to restructure complex stimuli, at least for the neonatal brain.

In nature, most of the time odour stimuli comprise odour mixtures. It is therefore interesting to investigate how perception of certain mixtures affects perception of other mixtures. Here, we show that experience of A through conditioning to AC improved the elemental perception of AB, as did experience of A alone. Similarly, the decrease in responsiveness to A after repeated exposure to AB, highlighting the better configural perception of AB, was also observed when pups weakly responded to AC after repeated conditioning to AB. Experience of an element thus has consequences on the perception of distinct mixtures including this element. Similarly, experience of a mixture impacts the subsequent perception of another mixture containing a shared component. Thus, the impact of experience is not modified by the presence of other odorants, a result in favour of an experience's effect on generalization between overlapping mixtures. In nature, where, for instance, components are sometimes redundant in food (e.g. alkyl esters in fruits), exposure to a rewarded mixture allowing the perceptual extraction of some of the elements (non-blending mixture) could promote the recognition of these positive elements in other mixtures, insofar as the organism is able to detect them. Moreover, inter-individual recognition certainly depends on ability to detect common cues (elements and/or configurations) initially learned in contact with a conspecific (e.g. the mother for neonates) and to respond by attraction/avoidance when later encountered on the same and/or on another individual (e.g. social/sexual partner after weaning) (Halpin, 1986). Thus, the impact of experience with complex stimuli on the perception of other complex stimuli may critically contribute to the rapid acquisition of knowledge about the environment and the use of this knowledge throughout development. This will be tested in further dedicated experiments in the rabbit.

If experience clearly influences the way neonates perceive binary odour mixtures, this impact appears sometimes limited depending on individuals or on the initial perception of the mixture. Thus, after nine conditionings to odorant A (or B), only half of the pups responded to the AB mixture, i.e. one of every two pups still processed the mixture as different from its constituents (in the configural manner). Similarly, after nine conditionings to AB, approximately 60% of the pups showed a decrease in their elemental

processing of AB (lower responsiveness to A and B) whereas 40% of them did not present this perceptual shift. Therefore, if AB is a mixture from which a configuration may dominate in rabbit pups as a consequence of experience, this is not systematic. This inter-individual discrepancy could be related to factors such as motivation, vigilance state and neurophysiological maturity (Smith, 1996; Goldstone, 1998).

Moreover, if experience impacted the perception of AB in numerous pups, the AC mixture remained always elementally perceived. One cannot exclude that the perception of AC would become configural after more than nine repeated exposures. However, one may also suggest that an experience-dependent shift in perception occurs only for certain mixtures, i.e. for blending mixtures initially perceived in a weak configural manner (as the AB mixture), and not for non-blending mixtures initially perceived elementally (as the AC mixture).

Finally, between-mixtures generalization of experience appeared only when mixtures shared a component. Indeed, repeated exposures to the DE non-blending mixture, which could putatively facilitate elemental perception, did not affect the configural perception of AB; just as successive conditionings to AB (facilitation of configural perception) did not change the elemental perception of DE. Similarly, honeybees were found to generalize between floral scent mixtures only when the mixtures shared a minimum of components [at least three among 14 (Reinhard et al., 2010)]. In newborn rabbits, as in honeybees, between-mixtures generalisation would then remain highly dependent on the composition of the mixtures but not on the training by itself (i.e. training to perceive elementally a mixture is not followed by a similar processing of any mixture). However, results obtained in adult rats showed a better discrimination of novel odorants in a mixture after pre-exposure to another mixture, a behavioural effect that was supported by enrichment-associated neurogenesis (Mandairon et al., 2006; Mandairon et al., 2008). At least three reasons might explain why, in our study, learning associated with one configural or elemental stimulus did not translate to an unrelated odour mixture. First, mixtures used in adult rats by Mandairon and collaborators included pairs of odorants (+/-limonene, pentanol/butanol) that activate overlapping patterns at the olfactory bulb level. However, the effect was not observed for another pair of odorants, decanal/dodecanone. This latter pair elicited activation patterns of the olfactory bulb that were distinct from the +/-limonene pair (Mandairon et al., 2008). Here, odorants D and E (as A and C) have very different chemical structures and qualities (for humans); therefore, they could potentially compete for distinct olfactory receptor neurons. Indeed, it is conceivable that ligands of a receptor neuron bearing different structural features could also differ in their odour quality (Doszczak et al., 2007; Sanz et al., 2008). Second, the pre-exposure consisted of a long enrichment of the whole environment (1 h during 10 days) without any conditioning in rats, in contrast to a 5 min-long MP-induced conditioning to a given stimulus occurring for 3 days as a maximum in rabbit pups. If the period/intensity of exposure may influence the perception, the reinforcement may also induce a higher selectivity in stimulus-response pairing (rabbits) than the mere exposure (rats). Third, differences in maturity of the olfactory system and brain may explain why experience does not trigger exactly the same effect in newborn and adult mammals.

To conclude, the present study demonstrates that repeated conditioning to certain binary odour mixtures or components of these mixtures, promoted here by a maternal signal (the MP), may modulate the elemental/configural mode of perception of such stimuli in altricial newborn rabbits. In this way, the mother may favour the



acquisition by neonates of information about her and the surroundings, and the young may improve their ability to discriminate between more or less complex and overlapping stimuli. Results illustrate the plasticity of olfactory–cognitive–motor loops, crucial for neonatal adaptation to the changing environment. However, the present findings suggest that this effect of experience occurs only for binary mixtures initially processed in a weak configural way. Thus, under our experimental conditions, experience influences perception but is modulated by the molecular composition of the mixtures. Anyway, neonatal perception appears functional to process complex stimuli either elementally or configurally, and when experience influences one of these modes, it does not alter the ability of the system to function in the other mode. Regardless of experience, young rabbits could thus retain their ability to perceive some mixtures elementally or configurally, and so to respond optimally to the diversity of their chemical, social and feeding environment.

### ACKNOWLEDGEMENTS

We sincerely thank Valérie Saint-Giorgio, Nicolas Malaty, Jérôme Antoine and all the Centre de Zootechnie staff from Dijon for their cooperation.

### FUNDING

This study was supported by the National Center for Scientific Research (CNRS), the National Institute for Agricultural Research (INRA), grants from the Burgundy Regional Council [EUERDF and IFR 92 to G.C. and T.T.-D.] and a fellowship from the French Ministère Supérieur de la l'Enseignement Supérieur et de la Recherche [to C.S.].

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