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1 **Brief Communication**

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4 Higher-order trace conditioning in newborn rabbits

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24 second-order conditioning.

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28 **Abstract**

29 Temporal contingency is a key factor in associative learning but remains weakly investigated  
30 early in life. Few data suggest simultaneous presentation is required for young to associate  
31 different stimuli, whereas adults can learn them sequentially. Here, we investigated the ability  
32 of newborn rabbits to perform sensory preconditioning and second-order conditioning using  
33 trace intervals between odor presentations. Strikingly, pups are able to associate odor stimuli  
34 with 10- and 30-sec intervals in sensory preconditioning and second-order conditioning,  
35 respectively. The effectiveness of higher-order trace conditioning in newborn rabbits reveals  
36 that very young animals can display complex learning despite their relative immaturity.

37

38 **Text**

39 Temporal contiguity between stimuli is usually considered essential for associative learning in  
40 adult individuals (Pavlov 1927). However, first-order conditioning remains effective when a  
41 trace interval (TI), usually 10 to 30 sec, is inserted between the conditioned stimulus (CS) and  
42 unconditioned stimulus (US) (e.g., Kamin 1954; Ost et al. 1968; Kirkpatrick and Balsam  
43 2016). In higher-order conditioning such as sensory preconditioning and second-order  
44 conditioning, two CSs, CS1 and CS2, are paired before or after the CS1 is paired with a US,  
45 respectively (Brogden 1939; Rescorla 1980a). Here again, conditioning is possible in adult  
46 rats when the CS1 and CS2 are separated by a short TI, and this is true for both sensory  
47 preconditioning (Silver and Meyer 1954; Lavin 1976; Lyn and Capaldi 1994; Holmes and  
48 Westbrook 2017) and second-order conditioning (Rescorla 1982, Stout et al. 2004).

49 Very young individuals exhibit robust first- and higher-order conditioning when pairings  
50 involved simultaneous presentations of the to-be-conditioned stimuli (Cheslock et al. 2003;  
51 for review: Cuevas and Giles 2016). However, a few studies suggest that they might be  
52 particularly sensitive to the insertion of a TI between stimulus presentation in training (e.g.,

53 first-order conditioning: Moye and Rudy 1987; Ivkovich et al. 2000; Barnet and Hunt 2005;  
54 higher-order conditioning: Cheatle and Rudy 1979; Cheslock et al. 2003). Indeed, sequential  
55 conditioning becomes more efficient with age in human infants (Cuevas and Giles 2016) and  
56 young animals (Chen et al. 1991).

57 In the European rabbit, newborns can learn new odorants through first- and higher-order  
58 conditioning. The mammary pheromone (MP) emitted by lactating rabbit females in their  
59 milk not only triggers stereotyped orocephalic movements allowing for pups to search and  
60 grasp the maternal nipples (Coureaud 2001; Schaal et al. 2003) but also remarkably promotes  
61 associative odor learning. Indeed, the MP acts as a US inducing appetitive conditioning to an  
62 odorant (CS1) by simultaneous and brief pairing: 24h later, the CS1 triggers a conditioned  
63 orocephalic searching-grasping response similar to the response triggered by the US  
64 (Coureaud et al. 2006, 2009). Rabbit pups are also able to respond to a second odorant (CS2)  
65 when CS1+MP pairing is followed by simultaneous CS1+CS2 pairing, demonstrating second-  
66 order conditioning (Coureaud et al. 2011), but also when simultaneous exposure to CS1+CS2  
67 preceded CS1+MP pairing, demonstrating sensory preconditioning (Coureaud et al. 2013).  
68 These higher-order conditionings seem to be associative in nature as unpaired presentations of  
69 either CS1 and US or CS1 and CS2 impede CS2 responding (Coureaud et al. 2011, 2013). In  
70 particular, sensory preconditioning and second-order conditioning do not occur if CS1 and  
71 CS2 are separated by a 1-min TI (Coureaud et al. 2011, 2013). However, the effectiveness of  
72 shorter TIs in supporting higher-order conditioning remains to be evaluated in newborn  
73 rabbits. The present study provides this evaluation.

74 To that goal we used 305 newborn rabbits (from 64 litters) and odorants A, B and MP (see  
75 below) purchased from Sigma-Aldrich (France). Only 4 or 5 pups per litter were used per  
76 experiment (they were included in a single group, i.e. not used in more than one condition).  
77 To avoid interference with the pups' prandial state (Montigny et al. 2006) experiments always

78 occurred at 10:30 a.m., one hour before the daily nursing. Statistics consisted in comparison  
79 of proportion of responding pups to the stimuli by the Cochran Q test and  $\chi^2$  test of McNemar  
80 when pups from a same group are tested to distinct stimuli, and  $\chi^2$  test of Pearson when pups  
81 from distinct groups are tested to the same stimulus.

82 First, in order to confirm that simultaneous pairing of CS1+CS2 promotes sensory  
83 preconditioning, 1-day old pups ( $n = 15$ ) were exposed in a behavioral room (adjacent to the  
84 animal housing room) by sub-group of 5 neonates as a maximum (in order to optimize their  
85 exposure) to a mixture of odorants A (ethyl-isobutyrate,  $10^{-5}$  g/ml) and B (ethyl maltol,  $10^{-5}$   
86 g/ml). The exposure happened in a plastic basin through a scented cotton glove positioned 1  
87 cm above the litter for 5 min (timer-controlled by a second experimenter) then immediately  
88 retired. On day 2, the pups were conditioned to odorant A by simultaneous pairing with MP  
89 (2-methylbut-2-enal,  $10^{-5}$  g/ml) for 5 min in a new basin and through a new glove (the basins  
90 were rinsed with alcohol, water and then dried after each use; the gloves were used only once  
91 per experiment). On day 3, the pups were individually tested in an oral activation test (e.g.  
92 Schaal et al. 2003; Coureaud et al. 2009, 2011, 2013) during which each pup was immobilized  
93 in one hand of the experimenter, its head being left free, and the test odor was presented for  
94 10 s with a glass-rod 0.5 cm in front of the nares. The response was positive when the  
95 stimulus elicited vigorous scanning movements of the head during stretching toward the rod  
96 followed by oral grasping of the rod; non-responding pups displayed no response but sniffing.  
97 Under the present conditions, the pups responded to both odorants A and B as well as to MP  
98 (A vs. B vs. MP: Cochran's Q test = 2,  $P = 0.37$ ). For second-order conditioning, 1-day-old  
99 pups ( $n = 9$ ) were conditioned to odorant A (by simultaneous pairing with MP for 5 min) and  
100 exposed on day 2 to A+B for 5 min (here and hereafter: same procedure of exposure as  
101 above). On day 3, pups responded in a strong and similar manner to A, B and MP (A vs. B vs.  
102 MP:  $Q = 2$ ,  $P = 0.37$ ).

103 To determine if an TI between presentations of neutral odorants would affect sensory  
104 preconditioning, 1-day-old pups were exposed to odorants A then B for 2.5 min each with a  
105 10-sec (n = 15) or 30-sec TI (n = 15); the exposure to each odorant was made in distinct  
106 basins separated by more than 1 meter in a permanently ventilated room, to avoid any  
107 contamination between the different odorants. On day 2, they were conditioned to odorant A  
108 (by simultaneous pairing with MP for 5 min). Strikingly, pups responded strongly and  
109 similarly on day 3 to A, B and MP after 10-sec TI (A vs. B vs. MP:  $Q = 3$ ,  $P = 0.22$ ).  
110 However, pups responded to A and MP but not to B after 30-sec TI (A vs. B vs. MP:  $Q = 18$ ,  
111  $P < 0.001$ ; 2 x 2 comparisons by  $\chi^2$  test of McNemar: B vs. MP or A:  $\chi^2 \geq 7.1$ ,  $P < 0.01$ ) (Fig.  
112 1A-B). These results support the presence of sensory preconditioning in the 10- but not the  
113 30-sec condition. Indeed, the proportions of responders to A after 10- and 30-sec TI were  
114 strong and similar ( $\chi^2 < 0.5$ ,  $P > 0.05$ ) while they dropped dramatically to B after 30-  
115 compared to 10-sec TI ( $\chi^2 = 13.5$ ,  $P < 0.001$ ).

116 Similar results were obtained when preconditioning to A then B was followed by  
117 conditioning to B (Fig. 1C-D) and after reverse exposure to the odorants (B then A) during  
118 preconditioning then conditioning to odorant A or B (Fig. 1E-H): pups responded to A, B and  
119 MP after 10-sec TI but only to the conditioned odorant after 30-sec TI (n = 10 per group;  $Q =$   
120  $2$ ,  $P > 0.05$  with 10 sec of TI;  $Q > 12$ ,  $\chi^2 \geq 7.1$ , and  $P < 0.05$  in comparisons between A and B  
121 or MP with 30 sec of TI; drop in responsiveness to A in Fig. C vs. D and G vs. H:  $\chi^2 \geq 7.2$ ,  $P$   
122  $< 0.05$ , and in responsiveness to B between the 10- and 30-sec condition in Fig. E-F:  $\chi^2 = 9.8$ ,  
123  $P < 0.01$ ). Therefore, two main results emerged from these data: 1) sensory preconditioning  
124 was still effective when a short TI (10-sec) takes place between the odorants during initial  
125 exposure, and 2) it was present and equivalent in the forward (CS2//CS1, CS1+US) and  
126 backward (CS1//CS2, CS1+US) protocols.

127 To assess whether second-order conditioning would also tolerate TIs, 1-day-old pups were  
128 conditioned to odorant A (by simultaneous pairing with MP for 5 min) and exposed the day  
129 after to odorants A then B for 2.5 min each with a 10-sec ( $n = 10$ ) or 30-sec TI ( $n = 10$ ). On  
130 day 3, pups responded highly and similarly to A, B and MP after the 10-sec TI (A vs. B vs.  
131 MP:  $Q = 2$ ,  $P = 0.37$ ) but also after the 30-sec TI (A vs. B vs. MP:  $Q = 4$ ,  $P = 0.14$ ) (Fig. 2A-  
132 B). When the same experiment was conducted with a 60-sec TI between A and B, pups  
133 significantly responded to A and MP but not to B ( $n = 10$ ; A vs. B vs. MP:  $Q = 11$ ,  $P < 0.01$ ;  
134 B vs. MP:  $\chi^2 = 5.1$ ,  $P < 0.05$ ; A vs. MP:  $\chi^2 = 1.3$ ,  $P = 0.25$ ; drop in responsiveness to B  
135 between 30- and 60-sec:  $\chi^2 = 7.9$ ,  $P < 0.01$ ) (Fig. 2C). Similar results were obtained after  
136 conditioning to odorant A and reverse exposure to the odorants (B then A) (Fig. D-F) and  
137 conditioning to B followed by exposure to B then A (Fig. G-H): pups responded to A, B and  
138 MP after the 10- and 30-sec TI but only to the conditioned stimulus after 60-sec TI ( $n = 10$   
139 per group;  $Q \leq 4$ ,  $P > 0.05$  with 10- and 30-sec TI;  $Q > 15$ ,  $\chi^2 \geq 5.1$  and  $P < 0.05$  in  
140 comparisons between A and B or MP with 60-sec TI; responsiveness to odorant B in Fig. E  
141 vs. F:  $\chi^2 = 7.9$ ,  $P < 0.01$ , and to odorant A in Fig. G vs. H:  $\chi^2 = 5.2$ ,  $P < 0.05$ ). Thus, second-  
142 order trace conditioning was functional in the forward (CS1+US, CS2//CS1) and backward  
143 (CS1+US, CS1//CS2) protocols in newborn rabbits with longer TI (30-sec instead of 10-sec)  
144 than sensory preconditioning.

145 To determine if this difference was related to the age of the pups at A and B exposure, i.e.  
146 day 1 for sensory preconditioning *but* day 2 for second-order conditioning, 12 pups were  
147 exposed on day 2 to odorants A then B with 30-sec TI, and conditioned to A on day 3. On day  
148 4, pups responded to A and MP but not to B (A vs. B vs. MP:  $Q = 17$ ,  $P < 0.001$ ; B vs. MP or  
149 A:  $\chi^2 \geq 6.1$ ,  $P < 0.05$ ; data not shown) indicating sensory preconditioning was ineffective with  
150 a 30-sec TI. Thus, the difference of TI tolerated by the two procedures does not depend on the  
151 age of the newborns.

152 Natural environment of animals is extremely complex chemically: animals are exposed to  
153 numerous odor molecules and mixtures of molecules. We thus investigated higher-order trace  
154 conditioning in newborn rabbits using more than two odorants, i.e. three odorants. We first  
155 demonstrated that pups responded strongly and similarly to A, B and C (ethyl acetoacetate;  
156 Sigma-Aldrich) after simultaneous exposure to the 3 odorants in both preconditioning and  
157 second-order conditioning procedures (n = 10 per group; A vs. B vs. C vs. MP:  $Q < 2$ ,  $P >$   
158 0.05) (Fig. 3A,D). Thus, rabbit pups exhibit robust higher-order conditioning to three odorants  
159 after simultaneous presentation.

160 To assess whether sensory preconditioning was effective with sequential exposure to 3  
161 odorants, 1-day-old pups were exposed to odorants A then B then C for 2.5 min each with a  
162 10-sec or 30-sec TI (n = 10 per group). Pups were then conditioned to A on day 2 and tested  
163 for their responsiveness to the distinct odorants on day 3. After the 10-sec TI, pups responded  
164 highly and similarly to A, B and MP, but not to C (A vs. B vs. C vs. MP:  $Q = 23$ ,  $P < 0.001$ ; C  
165 vs. MP, A or B:  $\chi^2 \geq 6.1$ ,  $P < 0.05$ ) (Fig. 3B), whereas pups responded only to A and MP after  
166 the 30-sec TI (A vs. B vs. C vs. MP:  $Q = 26$ ,  $P < 0.001$ ; B or C vs. A or MP:  $\chi^2 \geq 7.1$ ,  $P <$   
167 0.01; drop in responsiveness to B between 30- and 60-sec:  $\chi^2 = 12.9$ ,  $P < 0.001$ ) (Fig. 3C).  
168 Thus, backward sensory preconditioning tolerated the same TI with two or three odorants in  
169 newborn rabbits, i.e. 10-sec but not 30-sec. However, the effect was only partial after 10-sec  
170 TI as behavioral response extended only to the odorant (B here) that directly followed the  
171 odorant that was conditioned (odorant A).

172 Regarding second-order conditioning with sequential exposure to three odorants, pups  
173 were conditioned to odorant A on day 1 before being exposed on day 2 to odorants A then B  
174 then C for 2.5 min each with a 10-sec, 30-sec or 60-sec TI (n = 10 per group). On day 3, pups  
175 responded strongly and similarly to A, B and MP but not to C with 10-sec (A vs. B vs. C vs.  
176 MP:  $Q = 18$ ,  $P < 0.001$ ; C vs. MP or B:  $\chi^2 = 6.1$ ,  $P < 0.05$ ; C vs. A:  $\chi^2 = 3.1$ ,  $P = 0.08$ ) and 30-

177 sec TI (A vs. B vs. C vs. MP:  $Q = 25$ ,  $P < 0.001$ ; C vs. MP, A or B:  $\chi^2 \geq 7.1$ ,  $P < 0.01$ ) (Fig.  
178 E-F) whereas pups only responded to A and MP with the 60-sec TI (A vs. B vs. C vs. MP:  $Q$   
179 = 30,  $P < 0.001$ ; B or C vs. A or MP:  $\chi^2 \geq 10$ ,  $P < 0.005$ , drop in responsiveness to B between  
180 30- and 60-sec:  $\chi^2 = 12.9$ ,  $P < 0.001$ ) (Fig. 3G). Thus, backward second-order conditioning  
181 tolerated the same 30-sec TI with two or three odorants in rabbit pups but, as for sensory  
182 preconditioning, with a partial effect as soon as 10-sec TI with three odorants; pups indeed  
183 responded only to the odorant that directly followed the presentation of the conditioned one  
184 but not to the third odorant (odorant C here).

185 According to previous literature in rat pups and human babies, trace conditioning would be  
186 non-functional in early life, as newborns do not tolerate any delay between stimuli to be  
187 associated, whereas older animals do (Cheatle and Rudy 1979; Moye and Rudy 1987; Chen et  
188 al. 1991; Ivkovich et al. 2000; Barnet and Hunt 2005; Cuevas and Giles 2016). Similarly,  
189 previous studies indicate rabbit pups cannot display first-order trace conditioning with 0-sec  
190 TI (Coureaud et al. 2006, 2011, 2013). Regarding the importance of hippocampus in first-  
191 order trace conditioning with discrete cues (Goldsberry et al. 2015; Kitamura et al. 2015), the  
192 failure of this first-order trace conditioning in rabbit neonates might result from the late  
193 maturation of the hippocampus.

194 The present study strikingly shows that rabbit pups are however able to form higher-order  
195 trace conditioning, and that different TI between odor presentations are tolerated: 10-sec TI  
196 for sensory preconditioning and 30-sec TI for second-order conditioning, regardless of the  
197 order of CSs presentation (forward or backward CS1-CS2 pairing). Similar greater tolerance  
198 for longer TI in second-order conditioning over sensory preconditioning was previously  
199 demonstrated in newborn rats, as immediate-sequential (0-sec TI) presentation of odor CSs  
200 impedes sensory preconditioning but not second-order conditioning; the latter being not  
201 effective with a 30-sec TI (Cheslock et al. 2003).

202 Sequential higher-order conditioning with three odorants provided similar but also novel  
203 information compared to two odorants in newborn rabbits. As with two odorants, backward  
204 sensory preconditioning was effective with 10-sec TI and backward second-order  
205 conditioning with 30-sec TI. However, these procedures were only partially effective since  
206 the pups did only respond to the odorant that directly followed the conditioned one (and not to  
207 the third one in the sequence). It would now be interesting to investigate what would be the  
208 response of the newborns when the conditioned odorant is the second or the third of the  
209 sequence (allowing to compare forward and backward pairings) to further understand how  
210 rabbit pups efficiently learn associative information about their environment.

211 Higher-order trace conditioning is thus functional in newborn rabbits, highlighting their  
212 remarkable cognitive capabilities despite their relative immaturity. An important and  
213 unexpected result was the equivalent backward and forward higher-order trace conditioning.  
214 Indeed, it is classically reported that forward conditioning is more effective than backward  
215 conditioning, even for higher-order conditioning (see for instance Rescorla 1980b; Hussaini et  
216 al., 2007), highlighting that the manner in which stimuli are presented influences their  
217 integration (see for review Holmes et al., 2022). This effective backward conditioning in  
218 rabbit pups is unlikely to be related to odor contamination according to the well-controlled  
219 experimental conditions (distinct recipients separated by 1 meter for each odorant in  
220 ventilated room) and the fact that first-order trace conditioning was ineffective (excluding any  
221 overlap between odorants). This could rather reflect a rabbit singularity compared to rodent  
222 pups (or even babies) due to the ecology of the species (for review: Cuevas and Giles 2016).  
223 Notably, the rabbit is to date the only species in which a pheromone (MP) emitted by lactating  
224 females and involved in newborns adaptation (nipple location/grasping and milk intake) has  
225 been described (Schaal et al. 2003; Coureaud et al. 2010). The use of the MP to induce  
226 conditioning might promote specific learning abilities, since this molecule is an extremely

227 powerful biological signal and reinforcer. These suggestions would require further  
228 comparative studies with other species such as rats, mice or humans, to investigate neonatal  
229 learning abilities in different conditioning procedures and their potential species-specificity.  
230 The difference of TI - 10-sec versus 30-sec - tolerated between the two procedures used here  
231 could be related to distinct levels of arousal (Kikas et al. 2021) and/or types of association  
232 involved. Indeed, in sensory preconditioning the two odorants are completely neutral during  
233 their presentation, therefore triggering low levels of arousal, whereas one odorant has already  
234 been reinforced in second-order conditioning, which enhances levels of arousal during  
235 subsequent CS1 and CS2 presentations. Therefore, stimulus-stimulus association supports  
236 sensory preconditioning, whereas stimulus-reward and/or stimulus-response association  
237 participates in second-order conditioning (Sharpe et al. 2017). Interestingly, these different  
238 types of association may relate on different brain structures. Indeed, several experiments have  
239 shown in adult rodents that higher-order conditioning depends on a wide brain network and  
240 that neural circuits underlying sensory preconditioning and second-order conditioning differ.  
241 For instance, manipulation of the perirhinal or the retrosplenial cortices affects stimulus-  
242 stimulus association during sensory preconditioning but not second-order conditioning in rats  
243 (Nicholson and Freeman 2000; Holmes et al. 2013; Robinson et al. 2014; Todd et al. 2016).  
244 On the contrary, the basolateral amygdala is involved in second-order conditioning but not in  
245 sensory preconditioning (Hatfield et al. 1996; Blundell et al. 2003; Dwyer and Killcross 2006;  
246 Parkes and Westbrook 2010). The selective recruitment of the amygdala during stimulus-  
247 reward and/or stimulus-response association may therefore help tolerating longer TI  
248 compared to sensory preconditioning.

249 However, much less is known about neural mechanisms that mediate sensory  
250 preconditioning and second-order conditioning early in life, and how these mechanisms  
251 evolve during development. Different neural circuits have been shown to support first-order

252 aversive learning in young and adult rats, with gradual involvement of the amygdala when the  
253 animal is getting older (Shionoya et al. 2006; Raineki et al. 2009). Moreover, noradrenaline  
254 depletion in the forebrain alters sensory preconditioning but not first-order aversive  
255 conditioning in both preweanling and adult rats (Archer et al. 1986; Chen et al. 1993). Since  
256 only very limited data are available in newborns and according to age-related differences in  
257 higher-order conditioning, it would be valuable to investigate the neural mechanisms and in  
258 particular the neurotransmitters (using pharmacological and molecular approaches)  
259 underlying these higher-order conditioning procedures in rabbit pups.

260

261

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269

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377 **Figure captions**

378

379 **Figure 1.** Sensory preconditioning with two odorants. *(A,B,E,F) Case of conditioning to the*  
380 *odorant A.* Newborn rabbits were sequentially exposed to *(A,B)* odorants A then B or *(E,F)*  
381 odorants B then A with a 10-sec *(A,E)* or 30-sec trace interval (TI) *(B,F)* on day 1 (d1), then  
382 conditioned to odorant A by association with the mammary pheromone (MP) on day 2 (d2).  
383 Their behavioral response to A and B, and to the MP as a control, was tested 24 h later (d3).  
384 Sensory preconditioning tolerated a 10-sec TI during the preexposure phase since pups  
385 responded later to A and B, but not a 30-sec TI. *(C,D,G,H) Case of conditioning to the*  
386 *odorant B.* Rabbit pups were sequentially exposed to *(C,D)* odorants A then B or *(G,H)*  
387 odorants B then A with a 10-sec *(C,G)* or 30-sec TI *(D,H)* on d1, then conditioned to odorant  
388 B by association with the MP on d2, and their behavioral response to B, A and MP was tested  
389 on d3. Again, sensory preconditioning tolerated a 10-sec but not a 30-sec TI during the  
390 preexposure phase. (\*)  $P < 0.05$ ; (\*\*\*)  $P < 0.001$ .

391

392 **Figure 2.** Second-order conditioning with two odorants. *(A,B,C) Case of conditioning to the*  
393 *odorant A and recall of A then B.* Newborn rabbits were conditioned to odorant A by pairing  
394 with the MP on d1, then sequentially exposed to A then B with *(A)* a 10-sec, *(B)* a 30-sec or  
395 *(C)* a 60-sec TI on d2, before testing of their behavior to A, B, MP on day 3 (d3). Second-  
396 order conditioning tolerated a 10- and 30-sec TI during the second conditioning phase since  
397 pups responded to B in addition to A, but not a 60-sec TI. *(D,E,F) Case of conditioning to the*  
398 *odorant A and recall of B then A.* Rabbit pups were conditioned to A by pairing with the MP  
399 on d1, then sequentially exposed to B then A with *(D)* a 10-sec, *(E)* a 30-sec or *(F)* a 60-sec  
400 TI on d2, and tested behaviorally to A, B, MP on d3. Second-order conditioning tolerated the  
401 10- and 30-sec TI but not the 60-sec TI during the second conditioning phase. *(G,H) Case of*

402 *conditioning to the odorant B and recall of B then A.* Rabbit pups were conditioned to B by  
403 pairing with the MP on d1, then sequentially exposed to B then A with (G) a 30-sec or (H) a  
404 60-sec TI on d2, and tested behaviorally to B, A, MP on d3. Second-order conditioning  
405 tolerated the 30-sec TI during the second conditioning phase since pups responded to A in  
406 addition to B, but not the 60-sec TI. (\*)  $P < 0.05$ ; (\*\*\*)  $P < 0.001$ .

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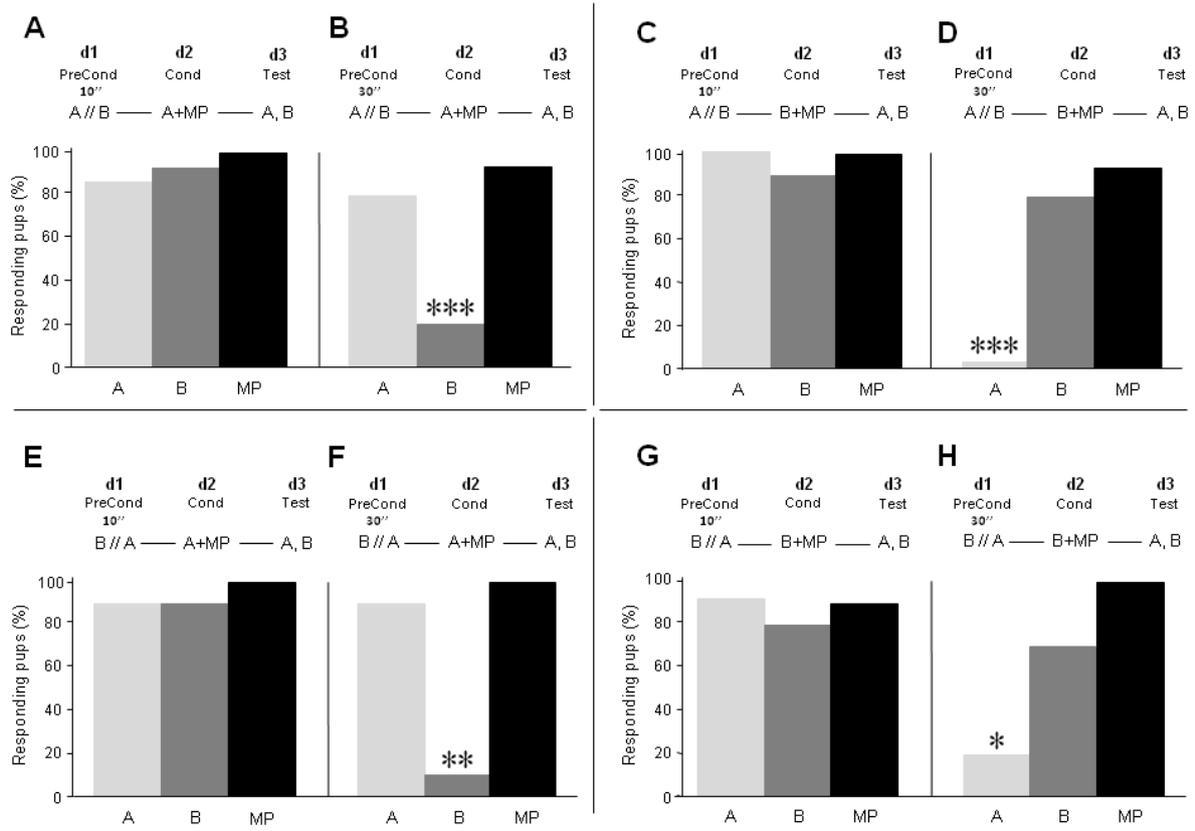
408 **Figure 3.** Sensory preconditioning and second-order conditioning with three odorants.  
409 (A,B,C) *Case of sensory preconditioning.* Newborn rabbits were exposed on d1 to the  
410 odorants A, B and C (A) simultaneously, or sequentially with (B) a 10-sec or (C) a 30-sec TI  
411 between the odorants, then conditioned to odorant A by pairing with the MP on d2, and tested  
412 behaviorally to A, B, C and MP on d3. Sensory preconditioning occurred simultaneously and  
413 with the 10-sec TI during the preexposure phase since the pups responded to B and C in  
414 addition to A, but not with the 30-sec TI. (D,E,F,G) *Case of second-order conditioning.* Pups  
415 were conditioned to A by pairing with the MP on d1, then exposed to A, B and C (D)  
416 simultaneously, or with (E) a 10-sec, (F) a 30-sec or (G) a 60-sec TI on d2, before behavioral  
417 testing to A, B, C, MP on d3. Second-order conditioning occurred simultaneously and with  
418 the 10-sec TI, but not with the 30-sec TI during the second conditioning phase. (\*)  $P < 0.05$ ;  
419 (\*\*)  $P < 0.01$ ; (\*\*\*)  $P < 0.001$ .

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**Figure 1 – Coureaud, Colombel, Duchamp-Viret, Ferreira**



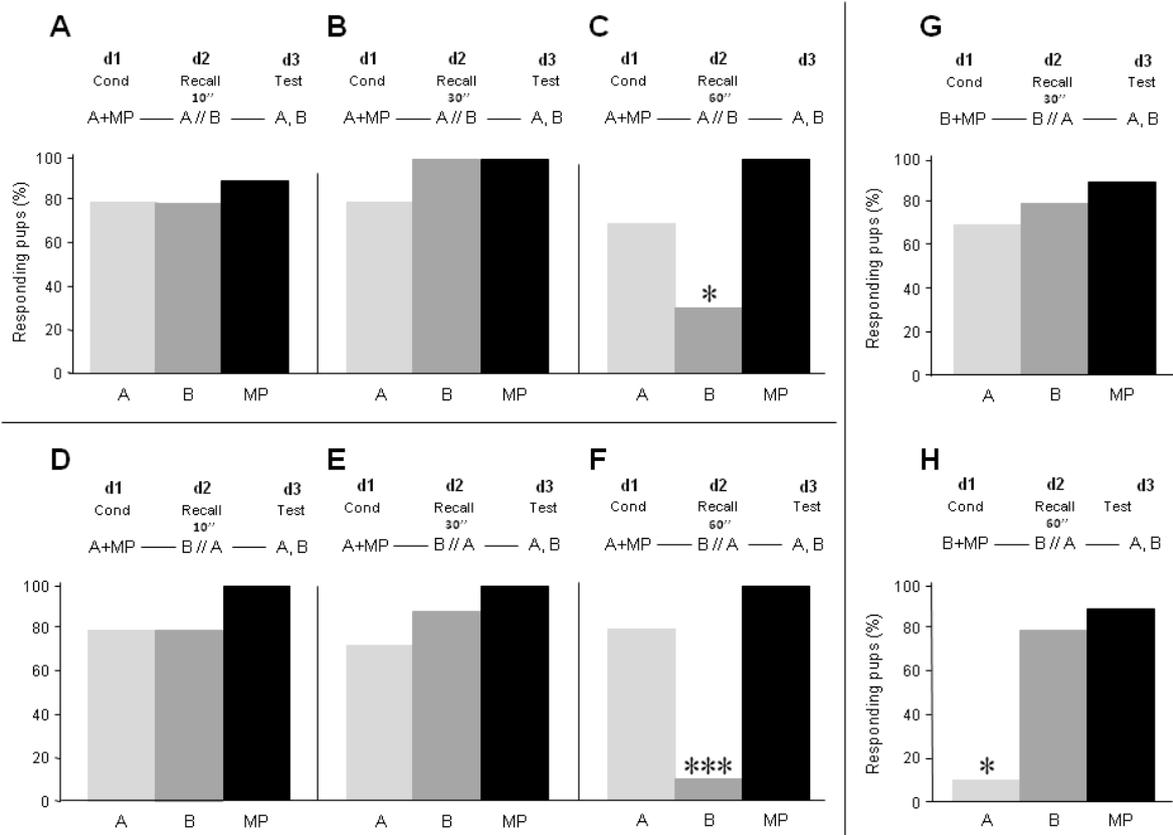
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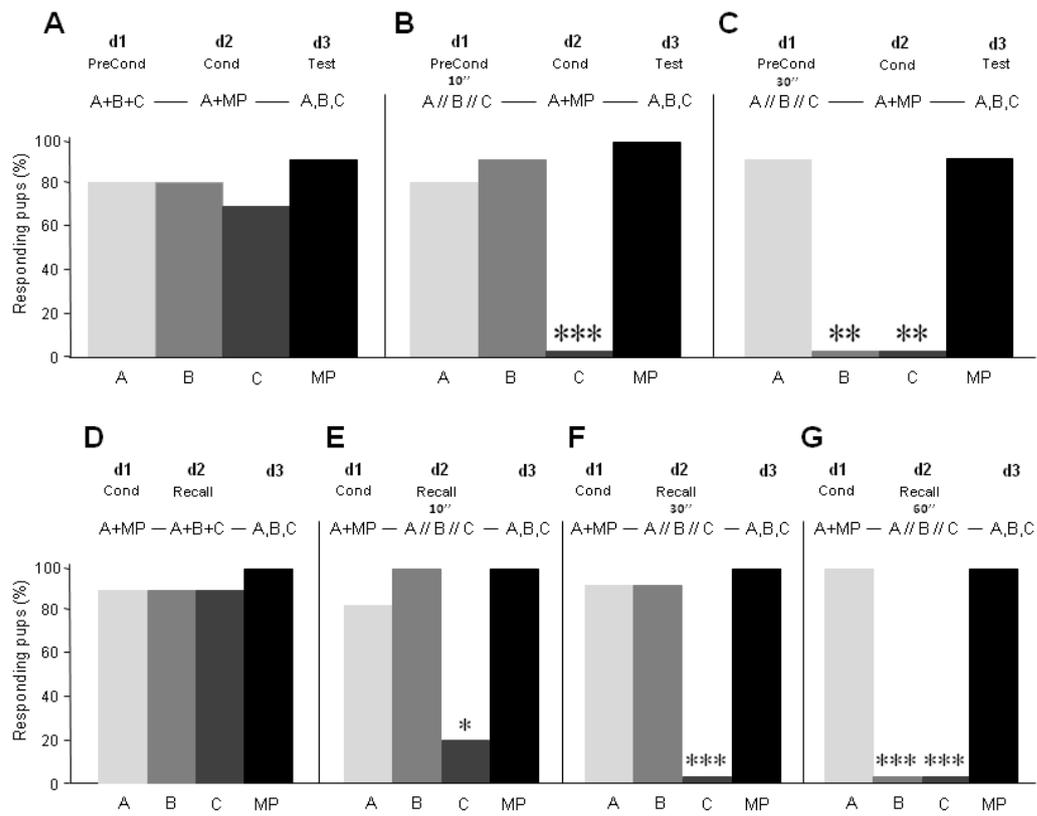
**Figure 2 – Coureaud, Colombel, Duchamp-Viret, Ferreira**



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**Figure 3 – Coureaud, Colombel, Duchamp-Viret, Ferreira**



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