



Title	Combined predisposed preferences for colour and biological motion make robust development of social attachment through imprinting
Author(s)	Miura, Momoko; Nishi, Daisuke; Matsushima, Toshiya
Citation	Animal cognition <a href="https://doi.org/10.1007/s10071-019-01327-5">https://doi.org/10.1007/s10071-019-01327-5</a>
Issue Date	2020-11-11
Doc URL	<a href="http://hdl.handle.net/2115/79730">http://hdl.handle.net/2115/79730</a>
Rights	This is a pre-print of an article published in Animal cognition. The final authenticated version is available online at: <a href="https://doi.org/10.1007/s10071-019-01327-5">https://doi.org/10.1007/s10071-019-01327-5</a>
Type	article (author version)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Animal cognition 2019 11 Nov with figures1_11.pdf (本文)



[Instructions for use](#)

1 **Title:** Combined predisposed preferences for colour and biological motion make robust  
2 development of social attachment through imprinting

3  
4 **Authors:** Momoko Miura, Daisuke Nishi, Toshiya Matsushima\*

5 **Affiliations:**

6 Department of Biology, Faculty of Science, Hokkaido University, Sapporo, Japan.

7  
8 **\*Correspondence:** T. Matsushima, Department of Biology, Faculty of Science,  
9 Hokkaido University, N10-W8, Kita-ku, Sapporo, Hokkaido 060-0810, Japan.

10 ORCID: 0000-0001-9404-647X

11 E-mail address: matusima@sci.hokudai.ac.jp

12 Telephone: +81-11-706-3523

13  
14 **Abstract**

15 To study how predisposed preferences shape the formation of social attachment through  
16 imprinting, newly-hatched domestic chicks (*Gallus gallus domesticus*) were  
17 simultaneously exposed to two animations composed of comparable light points in  
18 different colours (red and yellow), one for a walking motion and another for a linear  
19 motion. When a walking animation in red was combined with a linear one in yellow,  
20 chicks formed a learned preference for the former that represented biological motion  
21 (BM). When the motion-colour association was swapped, chicks failed to form a  
22 preference for a walking in yellow, indicating a bias to a specific association of motion  
23 and colour. Accordingly, experiments using realistic walking chicken videos revealed a  
24 preference for a red video over a yellow one, when the whole body or the head was  
25 coloured. On the other hand, when the BM preference had been pre-induced by using an  
26 artefact moving rigidly (non-BM), a clear preference for a yellow walking animation  
27 emerged after training by the swapped association. Even if the first-seen moving object  
28 was a nonbiological artefact such as the toy, the visual experience would induce a  
29 predisposed BM preference, making chicks selectively memorise the object with natural  
30 features. Imprinting causes a rapid inflow of thyroid hormone in the telencephalon  
31 leading to the induction of the BM preference, which would make the robust formation  
32 of social attachment selectively to the BM-associated object such as the mother hen.

33

34 **Keywords**

35 early social deprivation; sensitive period; thyroid hormone; developmental homeostasis;  
36 Conspec-Conlern mechanism; domestic chicks

37

38 **Acknowledgements**

39 We thank Dr. Giorgio Vallortigara (University of Trento, Italy) for his critical  
40 comments and discussions on our manuscript. We also thank the editor and anonymous  
41 referees for instructive suggestions. Contribution of Mr. Yasutaka Sasaki (Machine  
42 Department of the Faculty of Science, Hokkaido University) must be acknowledged for  
43 production of the imprinting apparatus.

44

45 **Funding and Contributions of the authors**

46 The present study was supported by grants funded to T.M. by the Japan Society for  
47 Promotion of Science (JSPS, Kakenhi; Grants-in-aid for Scientific Research  
48 #25291071, #26650114, #18K07351). T.M. and M.M. conceived the study and  
49 designed the experiments. M.M. developed the animations, designed the experimental  
50 procedures, analysed the data, and prepared the figures. M.M. and D.N. carried out the  
51 experiments. T.M. developed the apparatus and the computer programmes. T.M. and  
52 M.M. wrote the manuscript and supplementary materials. All the authors gave final  
53 approval for publication.

54 **Introduction**

55       How could cognitive processes develop toward adaptive socialization? The  
56 importance of early post-natal experiences has been recognized since the studies by M.  
57 and H. Harlow on isolated neonatal babies of the rhesus monkey (Harlow et al., 1965,  
58 Harlow and Suomi 1971). Lasting and irreversible behavioural and neurobiological  
59 effects of early social deprivation have been shown in rats (Tóth et al., 2008), mice  
60 (Makinodan et al., 2012) and humans (Carlson and Earls, 1995; Moulson et al., 2015;  
61 also see the recent epigenome study by Naumova et al., 2019). Early isolation escalates  
62 maladaptive aggressiveness in various fish species (blue gourami, Tooker and Miller,  
63 1980; cichlid fish, Barki and Volpato, 1998; Siamese fighting fish, Ichihashi et al.,  
64 2004), indicating that the importance of social experiences in early life may be common  
65 among vertebrates.

66       Furthermore, these studies suggest that specific experiences are required and the  
67 way these experiences work is predisposed. In the case of the isolated monkey babies  
68 (Harlow and Suomi 1971) for example, even a limited physical contact with other infant  
69 monkeys and the subsequent predisposed interactions among subjects allowed  
70 sophisticated social behaviour to develop almost normally. We must understand how  
71 the predispositions interact with the post-natal learning, more specifically, how the  
72 innately predisposed preference controls the way by which post-natal experiences leads  
73 to the adaptive development of social behaviours.

74       Filial imprinting gives us a unique opportunity to analyse the initial phases of the  
75 behavioural development. Since the pioneering observation by Spalding (1873),  
76 imprinting has long been studied in precocial birds like geese (Lorenz, 1937), ducklings  
77 (Hess, 1958) and domestic chicks (Horn, 1985, 1998, 2004). It has been shown that  
78 these animals would learn to form a social attachment even if the first-seen object was a  
79 nonbiological artefact (also see reviews by Sluckin 1964; Bolhuis and Honey 1998,  
80 Bolhuis 1999; Matsushima et al., 2003). Under experimental settings, chicks exposed to  
81 a moving artefact (e.g., a blue box rotating along its axis; Horn, 2004) learn to follow  
82 similar objects based on the memorized colour (blue) and/or the shape (box). Recent  
83 studies revealed further that the newly-hatched chicks and ducklings memorise physical,  
84 geometrical and conceptual aspects of the object that these animals have been exposed  
85 to (colour cues for Maekawa et al. 2006; topological features for Wood & Wood 2015;

86 3-dimensional geometry for Versace et al. 2016; relational concept for Martinho &  
87 Kacelnik 2016; abstract multimodal patterns for Versace et al. 2017b), indicating that  
88 the imprinting comprises a complex set of perceptive and cognitive developments.

89 Despite the arguments of Lorenz (1937), who stressed the irreversible nature of  
90 imprinting, the preference does change after imprinting. Even if initially imprinted to an  
91 artefact, chicks gradually develop a predisposed preference for a stuffed hen that has  
92 natural visual configurations, particularly those of the neck and the head region (Bolhuis  
93 et al., 1985; Johnson et al., 1985; Johnson and Horn, 1988; also see Rosa-Salva et al.  
94 2010 for the innate preference for face-like configuration). Chicks may depend on the  
95 memorised visual cues as the first step, but they gradually reveal a predisposed  
96 preference for more natural objects. It is noticeable that the predisposed preference  
97 develops after non-specific experiences such as being placed in a running wheel, if  
98 given during a sensitive period within 36 hours post-hatch (Johnson et al., 1989). A  
99 similar transient time window has been shown for predisposed preference for moving  
100 objects of changing speed (Versace et al. 2019). A series of behavioural, localized  
101 brain-lesion and pharmacological experiments clearly showed that the predispositions  
102 have neural substrates and behavioural processes distinct from those responsible for the  
103 memory formation (Bolhuis and Honey 1998). However, it remains unclear how the  
104 memory formation interacts with the predispositions through the course of filial  
105 imprinting.

106 Biological motion (BM) is another factor of the imprinting stimulus for which  
107 predisposition has been observed. Johansson (1973) reported that simple animations  
108 composed of relatively few light points created a vivid and immediate percept of  
109 locomotion for human perceivers, if these points were appropriately placed to represent  
110 major parts of the body. In a manner similar to the configurational visual  
111 predispositions described above, the BM preference appears in visually inexperienced  
112 naïve chicks if they are primed by a certain amount of motor activity (Vallortigara et al.,  
113 2005). The predisposed BM preference thus precedes the imprinting memory formation,  
114 so are the predisposed preference for a rotating stuffed hen described above (Johnson et  
115 al., 1985; Johnson and Horn, 1988). The idea of the BM predisposition gained further  
116 evidence from a distinct gravity bias where chicks do not show tendency to align with  
117 an upside-down inverted version of the BM animation of a walking chicken

118 (Vallortigara and Regolin, 2006). Furthermore, the BM preference was significantly  
119 strengthened after nonspecific visual experience, where newly-hatched chicks were  
120 exposed to different kinds of motion pictures, not necessarily BM animations (Miura &  
121 Matsushima, 2012). In addition, BM preference was not induced in 4-day-old chicks if  
122 chicks had been raised in darkness, suggesting a transient “time window” like other  
123 forms of predisposition described above. If the emergence of the BM predisposition  
124 preceded the imprinting memory formation, we would expect these two processes to  
125 interact. Accordingly, BM point-light animations proved to be among the most potent  
126 imprinting stimuli, and those chicks with a higher BM preference showed a higher  
127 imprinting score (Miura & Matsushima, 2016).

128 The induction of BM preference is also closely linked with activation of a hormone  
129 that controls the sensitive period of imprinting (triiodo-thyronine  $T_3$ ; Yamaguchi et al.,  
130 2012; Miura et al., 2018). Imprinting enhances expression of the converting enzyme  
131 (*Dio2*) that leads to a higher content of  $T_3$  in the telencephalon, which then primes the  
132 memory system of imprinting so that even aged (4 to 8 days old) chicks will become  
133 imprintable (Yamaguchi et al., 2012). In other words, imprinting makes subsequent  
134 imprinting possible through feedback activation by thyroid hormone. Even if the first-  
135 seen moving object was an artefact, the induced BM preference would cooperate with  
136 the  $T_3$ -based priming of the memory system, making chicks form lasting social  
137 attachment selectively to those natural BM objects such as a mother hen (Takemura et  
138 al., 2018).

139 This scenario fits well with idea of the two-mechanism device underlying face  
140 processing in primate infants (Morton and Johnson, 1991). Two-to-four-month-old  
141 human babies show an innate preference for face-like visual patterns (Mauer and  
142 Barrera, 1981; also see Mondloch et al., 1999). Even new-borns are equipped with a  
143 visual processing machinery for face-like visual patterns (Sugita, 2008 in rhesus  
144 monkeys; Buiatti et al., 2019 in human babies; Reid et al., 2017 in human foetus) in  
145 accord with the idea of the *Conspec* process (Morton and Johnson, 1991). The *Conspec*  
146 process guides the initial preference, making infants pay more attention to face-like  
147 patterns, but it subsequently becomes un-observable in isolation and replaced by the  
148 later *Conlern* process, which makes babies learn the visual characteristics of specific  
149 human faces. The *Conspec-Conlern* dual processes may also function for the perception

150 of BM because the preference for BM appears very early in 2-day-old babies (Simion et  
151 al., 2008). The induction of the BM preference could act as a *Conspic* process based on  
152 core knowledge (Spelke, 2000; Spelke and Kinzler, 2007), which lead neonates (human  
153 babies and chicks) to form adaptive social attachments. For comprehensive reviews, see  
154 Vallortigara (2012), Rosa-Salva et al. (2015) and Di Giorgio et al. (2017).

155       Regarding the function of BM preference in imprinting, however, this scenario has  
156 some caveats because of several reasons. First, in most studies so far, conclusions were  
157 drawn from comparisons among groups of chicks trained by different visual stimuli, but  
158 each chick was trained by a single stimulus. This may not be appropriate, because  
159 hatchlings in nature are exposed to many moving objects, and subsequently learn to  
160 follow one, most preferably the mother hen. We must analyse behavioural consequences  
161 after training by simultaneously presented multiple visual stimuli. Furthermore, when  
162 we compare groups of chicks, each imprinted to one stimulus, different imprinting  
163 scores among groups could be due to different level of arousal or attention, rather than  
164 different predisposition of learning. On the other hand, as simultaneous display made  
165 chicks attend to the two visual stimuli equally, different preference scores would  
166 specifically represent a different degree of learning.

167       Second, chicks were often tested shortly after imprinting. The BM preference  
168 quickly arises in the early post-hatch period (Miura and Matsushima, 2012), but it may  
169 not last long enough to maintain subsequent imprinting selectively to biological objects.  
170 Third, components of the imprinting memory are not yet fully specified. Colour and  
171 shape cues have been identified at the level of neuronal representations in responsible  
172 telencephalic regions (IMM: Horn, 1998; Horn, 2004; visual Wulst: Maekawa et al.  
173 2006 for the colour), but the motion cue has not been specified as a critical sub-  
174 modality component of the memory; the responsible brain regions involved in the  
175 motion cues are also not identified.

176       In the present study, we examined the following two hypotheses. First, we  
177 hypothesized that (1) *the BM pattern would enhance the memorised preference for the*  
178 *associated colour*. Alternatively, chicks could form a learned attachment not solely on  
179 the colour cue, but on the association of motion and colour. Chicks might have a biased  
180 preference for a specific motion-colour association. Second, (2) *the induced BM*  
181 *preference would last long enough to control subsequent imprinting*. A stronger

182 imprinting to BM animation could thus emerge when chicks had been pre-induced for a  
183 BM preference. Alternatively, the induced BM preference rapidly decays and does not  
184 control the subsequent imprinting.

185 Experiments-1 to -4 were designed to examine the hypothesis (1), and experiments-  
186 5 and -6 the hypothesis (2). Experimental procedures, predictions, results and  
187 conclusions will be separately described in each section.

188

## 189 **GENERAL METHODS**

### 190 *Compliance with Ethical Standard*

191 Experiments were conducted under the guidelines and approval of the Committee  
192 on Animal Experiments of Hokkaido University (approval number 18-0093). The  
193 guidelines are based on the national regulations for animal welfare in Japan (Law for  
194 Humane Treatment and Management of Animals; after partial amendment No. 68,  
195 2005). After the experiments, the chicks were euthanised by carbon dioxide.

### 196 *Subjects*

197 Domestic white Leghorn chicks (*Gallus gallus domesticus*, egg-laying strain called  
198 as “Julia”) were used. Training started at 15–24 h post-hatch, and it was denoted as  
199 Day-1. Fertilised eggs supplied from a local hatchery (Iwamura Co., Hokkaido, Japan)  
200 were incubated in the laboratory by using type P-008B incubators (Showa Furanki Co.,  
201 Saitama, Japan) with its temperature controlled at 37.7°C and the humidity at ~80%.  
202 The inside of the incubator was kept in complete darkness until hatch. Chicks were  
203 individually hatched in small boxes separated by black plastic walls, so that they could  
204 interact acoustically but not visually. To avoid post-hatch visual experiences, hatchlings  
205 were individually housed in boxes placed in another incubator of the same type, kept in  
206 darkness until the experiment. Chicks were sexed just before the experiment.

207 In each batch of hatchlings, subjects were pseudo-randomly assigned to groups,  
208 until the number of accepted subjects reached n=10 in each group. Male and females  
209 were pseudo-randomly assigned to groups in a balanced manner (1:1) in experiment-3  
210 to -6, but not in experiment-1 and -2. In one group of experiment-1, the number of  
211 chicks was n=9 by our mistake but we did not add another subject. In experiment-5, the  
212 number of chicks was n=11 in one group and we added one more subject in the other  
213 group. When chicks did not run at all during both two training sessions on Day-1, these



214 chicks were discarded and not tested. If, on the other hand, the chick run and hit the  
215 sensor once or more during the training sessions, it was accepted and tested. A total of  
216 183 chicks were used, and the present paper is based on 171 accepted chicks (79 males  
217 and 92 females); see supplementary\_data\_file\_#3 for the sex and the number of subjects  
218 in experiments-1 to -6.

### 219 ***Apparatus and General procedures***

220 We used an I-shaped maze (9 cm × 70 cm) equipped with a 50-cm-long treadmill  
221 consisting of a rubber belt at the centre and an LCD monitor at each end (Fig. 1). The  
222 apparatus was the same as the one we used in a previous study (Miura et al., 2018).  
223 During imprinting, an infrared sensor and a transparent Plexiglass partition were placed  
224 at a point 10 cm from one monitor, and the other monitor was occluded by an opaque  
225 partition. When chicks ran and hit the sensor, the rubber belt of the treadmill moved for  
226 a short period of 0.3 s, drawing the chick backward by about 30 cm at a time. The  
227 treadmill motions were digitally counted, and the number of approaches was recorded  
228 for each trial.

229 At tests, the partitions were removed, and the treadmill was turned off. The subject  
230 chick was enclosed in a start box placed at the centre of the treadmill for about 10 s and  
231 was then allowed to freely exit and choose between the two arms. We recorded the total  
232 stay time near each monitor for 5 min, starting from the point when the chick exited the  
233 starting box. Each test was repeated twice with inter-test intervals of 1 min, after the  
234 sides of test animations were switched. The behaviour of the subject chick was  
235 monitored through a CCD camera (250 kilo pixels) placed on the ceiling, and the videos  
236 were stored for offline analysis in a video recorder (DCR-SR60, Sony, Japan). The  
237 apparatus was placed in a sound-proof wooden box, and the inside of the box was  
238 illuminated by infrared LED lamps.

239 Two sessions of training (each for 1 hour) were repeated with a 1-hour break  
240 interval, during which chicks were individually housed in boxes in a dark incubator. For  
241 testing the colour preference, chicks were presented with full-screen plates (background  
242 colour test, *i.e.*, overall screen was in yellow or red) twice for 5 min each. We measured  
243 the total period in which the subject chick stayed on either arm of the test maze,  
244 indicated by dashed lines in Fig. 1. The stay time on one side was subtracted by that on  
245 the other side, and the difference provided the preference score of +600 s (most

246 preferred) to -600 s (least preferred). Further details of the procedures will be described  
247 separately for each experiment.

#### 248 ***Point-light animations and video clips displayed on LCD monitors***

249 Animations and video clips are summarized in Supplementary\_document\_#1. Four  
250 types of point-light animations (each composed of 13 identical light points) were used  
251 after combining different motions (Walking or Linear) and colours (red or yellow),  
252 namely Wp (red), Wp (yellow), Lp (red), and Lp (yellow). Here, W and L abbreviate  
253 Walking and Linear motion, respectively. The suffix p indicates that the animation was  
254 composed of light points. In experiment-5, in order to test BM preference, we used two  
255 animations Wp (white) and Lp (white) that were composed of white light points. In  
256 experiment-2 and -3, two animations were simultaneously presented in combinations;  
257 Wp (red) and Lp (yellow), Wp (yellow) and Lp (red) in experiment-2; Wp (red) and Wp  
258 (yellow), Lp (red) and Lp (yellow) in experiment-3. The walking directions were  
259 alternately switched from one scene to the next.

260 These animations were made from a video clip of a walking chicken as in our  
261 previous study (Miura and Matsushima, 2016). In experiment-4, the original video clip  
262 (in black and white) was used after changing the colour (in red or yellow), either the  
263 whole body or its parts (head or tail).

264 Animations and video clips were displayed on black background at a speed of 30  
265 frames/s. We made editing by Adobe Premiere (Elements 7) and the colour was set  
266 either to red (R: 255, G: 0, B: 0) or yellow (R: 255, G: 255, B: 0). These stimuli were  
267 displayed on the LCD monitors (size 10.4", 800 × 600 pixels, Logitech LCM-T102AS,  
268 Japan; flash rate: 56–75 Hz, brightness: 230 cd/m<sup>2</sup>, pitch size: 0.264 × 0.264 mm) using  
269 free viewer software (A-player, version 6.0) on a Windows PC. The width of the  
270 presentation was set at 9 cm on the monitor.

#### 271 ***Statistical analysis***

272 In each group, binary choice data were examined for the difference from the chance  
273 level by using one-sample *t*-tests without adjusting p-value by Bonferroni correction.  
274 Prior to comparisons among groups, we applied Levene's test to assess the inequality of  
275 variances in each experiment. When Levene's test failed to detect a significant  
276 difference in variance among groups, we applied parametric methods: two-sample *t*-  
277 tests, one-way or two-way ANOVA with or without repeated measures. When a

278 significant difference was detected by ANOVA, if necessary, we made post-hoc  
279 multiple comparisons by using Tukey's multiple comparison of means, or Dunnett's test  
280 depending on the design of the experiment. When parametric tests were not applicable,  
281 non-parametric methods were used instead; Wilcoxon rank sum test (i.e., Mann-  
282 Whitney's U-test), Wilcoxon's signed rank test, or Steel-Dwass's multiple comparisons  
283 depending on the design.

284 To examine the correlation between two behavioural scores obtained from a group  
285 of chicks, we used Pearson's product-moment correlation coefficient. Because the  
286 number of individuals in a group was small (n=9-11 chicks), we did not adopt the  
287 method of converting the computed correlation coefficients to z-values to compare.  
288 Instead, to compare the degree of correlation between two groups, we constructed  
289 generalized linear models (GLMs) and evaluated them in terms of AIC (Akaike  
290 Information Criteria).

291 We used R (version 3.4.1) and EZR on R commander (version 1.36). The  
292 significance level was set at  $p = 0.05$ . When comparisons were repeated in one  
293 experiment, the significance was judged after Bonferroni correction of the p-value.  
294 Results of these statistical calculations (number of males and females in each group,  
295 results of one-sample t-test and Levene's test of variance equality, and constructions of  
296 generalized linear models (GLMs) are summarized in Supplementary\_document\_#2.  
297 Behavioural data are shown in Supplementary\_data\_file\_#3.

298

## 299 **RESULTS**

### 300 **Experiment-1: Comparisons of imprinting using single point-light animations**

#### 301 *Procedures and Predictions*

302 We examined if BM pattern would enhance the learning of associated colour. Four  
303 groups of chicks were examined in experiment-1. In training, chicks were exposed to  
304 one type of point-light animation composed of different motions (Walking or Linear in  
305 light points, abbreviated as Wp or Lp) and colours (red or yellow) displayed on the  
306 training screen (left LCD monitor) (Fig. 1). Notice that the Wp possessed the BM  
307 feature, whereas the Lp did not. At 0.5 hour after the end of training, the trained chicks  
308 received binary choice tests using full-screen presentation of colours (red or yellow) on  
309 the two testing screens (left and right LCD monitors), both without any shape or motion

310 cues. If the hypothesis (1) was correct, chicks would consistently form an attachment to  
311 the colour that was associated with Wp but not Lp.

312 Chicks were further tested for their preference for the familiar point-light animation  
313 over the unfamiliar one, which was made of different motion and different colour. For  
314 example, if trained by Wp (red), the unfamiliar animation was Lp (yellow). If the  
315 alternative of the hypothesis (1) was correct, chicks would consistently form an  
316 attachment to the Wp point-light animation over the Lp.

### 317 ***Results and Discussion***

318 As shown in the left column of Fig. 2, the colour test scores (as the yellow-side stay  
319 time subtracted by the red-side) were neutral or positive (indicative of yellow  
320 preference) in all 4 groups. Results of t-test against chance level (i.e., preference score =  
321 0) are shown by asterisks (or ns) on the box-whisker plots in this and the following  
322 figures; the statistical data are summarized in supplementary\_document\_#2. Two-way  
323 ANOVA revealed a significant main effect of motion (Wp or Lp;  $F=6.4483$ ,  $df=1$ ,  
324  $p=0.0157$ ), but no main effect of colour (red or yellow;  $F=0.7657$ ,  $df=1$ ,  $p=0.3875$ ) nor  
325 interaction ( $F=3.754$ ,  $df=1$ ,  $p=0.0607$ ) appeared. This result does not support the  
326 hypothesis (1), which predicted that the colour test scores (i.e., yellow preference over  
327 red) would be negative in Wp (red) but positive in Wp (yellow), and a significant effect  
328 should have emerged on the interaction.

329 The right column of Fig. 2 shows the memory test scores (as the familiar-side stay  
330 time subtracted by the unfamiliar-side). Two-way ANOVA revealed a significant main  
331 effect of motion (Wp or Lp;  $F=17.5842$ ,  $df=1$ ,  $p=0.0001$ ), but no main effect of colour  
332 (red or yellow;  $F=2.7794$ ,  $df=1$ ,  $p=0.1044$ ) nor interaction ( $F=1.9304$ ,  $df=1$ ,  $p=0.1734$ )  
333 appeared. This result supports the alternative to the hypothesis (1), that chicks form  
334 learned preference for the BM motion associated with the colour. In the following  
335 experiment-2 and -3, we further examined this possibility by simultaneous presentation  
336 training.

337

### 338 **Experiment-2 and -3: Comparisons of imprinting using simultaneously presented** 339 **two point-light animations**

#### 340 ***Procedures and Predictions***

341 We examined if a BM pattern would enhance the learning in an unbiased manner.  
342 Two groups of chicks were examined in each experiment. In training, chicks were  
343 exposed to two animations simultaneously displayed on the training screen. These  
344 animations were composed of different motion-colour associations. Different preference  
345 scores in test should represent different levels of learning, rather than different levels of  
346 attention or arousal.

347 In experiment-2, group-1 was trained by Lp (yellow) and Wp (red), and group-2 by  
348 Wp (yellow) and Lp (red) (Fig.3). If the hypothesis (1) was correct, chicks would  
349 consistently prefer the colour associated with Wp, namely red in group-1 and yellow in  
350 group-2 in the colour test. In experiment-3, group-1 was trained by Wp (yellow) and  
351 Wp (red), and group-2 was trained by Lp (yellow) and Lp (red) (Fig. 4). If the  
352 hypothesis (1) was correct, chicks would not show a preference at test.

353 Chicks were further tested for their preference between the two animations used in  
354 training. Notice that chicks were familiarized to both animations. If the alternative of  
355 the hypothesis (1) was correct, chicks would consistently prefer the Wp animation in  
356 experiment-2 irrespectively of the associated colour, but no such preference would  
357 emerge in experiment-3. On the other hand, if the corollary of the alternative was  
358 correct, chicks would show a biased preference for a specific association of motion and  
359 colour, consistently in both experiment-2 and -3.

### 360 ***Results and Discussion***

361 Results of experiment-2 are shown in Fig. 3. As in the left column, the colour test  
362 scores were positive (indicative of yellow preference) in both groups, and a two-sample  
363 *t*-test failed to reveal a significant difference between them ( $df=18$ ,  $t=0.375$ ,  $p=0.7120$ ).  
364 Again, the data do not support the hypothesis (1), which predicted that scores would be  
365 negative in group-1 and positive in group-2 with a significant difference between them.  
366 The right column of Fig. 3 shows the memory test scores (the yellow-side stay time  
367 subtracted by the red-side). A two-sample *t*-test revealed a significant difference  
368 between the groups ( $t=4.4013$ ,  $df=18$ ,  $p=0.0003$ ); chicks preferred Wp if associated with  
369 red, but not with yellow.

370 Results of experiment-3 are shown in Fig. 4. The colour test scores (left column)  
371 were positive (indicative of yellow preference), and a two-sample *t*-test failed to reveal  
372 a significant difference between them ( $df=18$ ,  $t=0.0322$ ,  $p=0.9746$ ). The hypothesis (1)

373 was not supported. The memory test scores (right column) indicated red preference in  
374 group-1, and a two-sample t-test revealed a significant difference between the groups  
375 ( $df=18$ ,  $t=2.6286$ ,  $p=0.0170$ ). Taking experiment-2 and -3 together, chicks formed a  
376 preference for Wp (biological motion pattern) if associated with red, despite their  
377 consistent yellow preference in the colour tests.

378

#### 379 **Experiment-4: Comparisons of imprinting using simultaneously presented realistic** 380 **chicken videos in different colours**

##### 381 *Procedures and Predictions*

382 Biased preference for the walking light-points in red (Wp (red)) emerged  
383 consistently in experiment-2 and -3. We examined if a similar preference bias could  
384 occur when more naturalistic videos of real chicken were used. Four groups of chicks  
385 were examined in experiment-4 (Fig. 5). In training, chicks were exposed to a pair of  
386 videos simultaneously displayed on the training screen. These pairs were of the same  
387 motion type (Walking motion or Linear movement of a still image, denoted as W or L,  
388 respectively), but different in colours (red or yellow) as in experiment-3. In 3 pairs of  
389 the Walking videos, the whole body, the head or the tail was coloured. In 1 pair of the  
390 Linear videos, only the head was coloured.

391 If the hypothesis (1) was correct, colour preference would not emerge.  
392 Alternatively, if a biased preference for a specific motion-colour association occurred,  
393 chicks would prefer the W-x (red) video over the W-x (yellow) in all of the 3 groups (x  
394 = whole, head, or tail), but no such bias would appear for the L-head (red) video over  
395 the L-head (yellow). The coloured portion of the body could also be critical, as  
396 suggested by the configurational predisposition to the head-neck regions of the jungle  
397 fowl (Johnson and Horn 1988). Furthermore, the head motion had a bigger change in  
398 speed than the tail. Considering the predisposed preference for the changing speed  
399 (Rosa-Salva et al. 2016), W-head in red could be a stronger imprinting stimulus than W-  
400 head in yellow, whereas less or no preference could emerge in the group trained by W-  
401 tail.

##### 402 *Results and Discussion*

403 As shown in the left column of Fig. 6, the colour test scores were neutral or positive  
404 (indicative of yellow preference) in 4 groups. A one-way ANOVA revealed no  
405 significance ( $F=0.4976$ ,  $df=3$ ,  $p=0.6863$ ); the hypothesis (1) was not supported.

406 The memory test scores (right column; the yellow-side stay time subtracted by the  
407 red) revealed differences among groups. As parametric test was not applicable, Steel-  
408 Dwass multiple comparisons were adopted for every pair out of the 4 groups. We  
409 detected significant difference between group-1 vs each of groups-2, -3, -4; group-1 vs -  
410 2 ( $t=3.1039$ ,  $p=0.0103$ ), group-1 vs -3 ( $t=3.6339$ ,  $p=0.0015$ ), group-1 vs -4 ( $t=3.6339$ ,  
411  $p=0.0015$ ). Although the difference was not significant in the other 3 pairwise  
412 comparisons (group-2 vs -3:  $t=2.1166$ ,  $p=0.1477$ ; group-2 vs -4:  $t=1.8898$ ,  $p=0.2323$ ;  
413 group-3 vs -4  $t=0.1890$ ,  $p=0.9976$ ), “*red head*” in biological motion (group-2) tended to  
414 show higher scores than that in non-biological linear motion (group-3), but “*red tail*”  
415 did not (group-4). Chicks thus showed a clear bias toward “*biological motion in red*”.

416 The present result contrasts with those obtained so far by using artefact (coloured  
417 toys) as training and tests (Izawa et al. 2001; Yamaguchi et al. 2012, 2018), where  
418 comparable scores were obtained irrespectively of whether chicks were trained by a red  
419 toy or a yellow alternative. Although further systematic surveys are necessary, it is  
420 possible to assume that the red bias appears only when associated with visual stimuli  
421 that bears BM. The predisposed colour bias to red, if any, could strongly depend on the  
422 way the colour is presented.

423

## 424 **Experiment-5: BM preference induced by non-BM visual stimulus**

### 425 ***Procedures and Predictions***

426 To methodologically validate the following experiment-6, we examined the time  
427 course of the BM preference induction. Two groups of chicks were compared in  
428 experiment-5. On Day-1, chicks were trained by a video of a moving toy (group-1) or  
429 stationary toy (group-2) both coloured in red (Fig.7). The trained chicks were tested  
430 twice, first at 0.5 hour (Day-1) and second at ~24 hour (Day-2) after the end of training.  
431 Each test consisted of a BM preference test and a memory test. The BM preference was  
432 tested with Wp (white) vs Lp (white), and the imprinting memory was tested with a  
433 moving red toy (familiar) vs a moving yellow toy (unfamiliar).

434 If the hypothesis (2) was correct, group-1 chicks would consistently show a BM  
435 preference in both Day-1 and Day-2, whereas group-2 chicks would not show a BM  
436 preference. As argued in the Introduction, the BM preference might increase or decrease  
437 on Day-2, but we would anyway find a difference between the two groups. In the  
438 memory tests, group-1 chicks would show higher scores than group-2 on Day-1, but the  
439 difference between the two groups could be smaller on Day-2, if the imprinting memory  
440 of the artefact decayed. Furthermore, if the BM preference was linked with the  
441 imprinting memory formation at the individual level, we would find a significant  
442 correlation between the two scores in group-1 chicks.

#### 443 ***Results and Discussion***

444 If induced on Day-1, the BM preference lasted until Day-2 (Fig. 8(a) (b)). A two-  
445 way ANOVA with a repeated measure revealed a significant main effect of the group  
446 factor ( $F=55.3711$ ,  $df=1$ ,  $p<0.0001$ ), but not of the Day factor ( $F=2.2272$ ,  $df=1$ ,  
447  $p=0.1660$ ) nor the interaction ( $F=0.0769$ ,  $df=1$ ,  $p=0.7876$ ). This result supports the first  
448 part of the hypothesis (2) that the induced BM preference lasts for at least ~24 hours  
449 with no signs of increase or decrease.

450 On the other hand, the memory scores decayed on Day-2 when compared with Day-  
451 1 (Fig. 8(c) (d)). A two-way ANOVA with a repeated measure revealed a significant  
452 main effect of the group factor ( $F=59.9201$ ,  $df=1$ ,  $p<0.0001$ ) and the Day factor  
453 ( $F=19.5100$ ,  $df=1$ ,  $p=0.0010$ ) without significant interaction ( $F=1.9398$ ,  $df=1$ ,  
454  $p=0.1941$ ).

455 Between the memory score and the BM preference, a significant correlation was  
456 not detected in any of the 4 plots (Fig. 8 (e) (f)); group (1)/Day-1 ( $t=-0.82946$ ,  $df=9$ ,  
457  $p=0.4283$ ); group (1)/Day-2 ( $t=1.0856$ ,  $df=9$ ,  $p=0.3059$ ); group (2)/Day-1( $t=2.0232$ ,  
458  $df=9$ ,  $p=0.07374$ ); group-2/Day-2 ( $t=0.6356$ ,  $df=9$ ,  $p=0.5409$ ), after Bonferroni  
459 correction of p-value ( $=0.05/4$ , namely  $p=0.0125$ ). At the individual level, the BM  
460 preference is not supposed to predict the memory score.

461 Taken together, the lasting nature of the induced BM preference validated the  
462 procedure of the experiment-6. Imprinting memory of the red toy was also not  
463 predictable by the individual BM preference.

464



465 **Experiment 6: Effects of pre-induction of the BM preference on subsequent**  
466 **imprinting**

467 *Procedures and Predictions*

468 To know if a pre-induced BM preference makes chicks specifically learn the  
469 coloured animation associated with BM, 3 groups of chicks were examined in  
470 experiment-6. Considering the lasting BM preference (experiment-5), we tested whether  
471 those chicks with pre-induced BM preference on Day-1 could show enhanced scores in  
472 imprinting on Day-2 (Fig. 9). For the Day-2 training, we presented chicks with  
473 simultaneously displayed Wp (yellow) and Lp (red), which gave rise to no significant  
474 preference in group-2 of experiment-2 (memory test in Fig. 3).

475 On Day-1, group-1 and -2 were trained by a moving toy (red), whereas group-3 was  
476 trained by a stationary toy (red). Chicks were tested for BM preference at 0.5 hour after  
477 the end of training. On Day-2, group-1 and -3 chicks received imprinting training, but  
478 group-2 chicks were not trained but kept in darkness. Chicks were subsequently tested  
479 for recent memory and old memory at 0.5 hour after the training.

480 If the hypothesis (2) was correct, group-1 chicks would show higher scores in the  
481 recent memory test (on Day-2) than group-3 chicks. However, since the recent memory  
482 test is accomplished by a binary choice between a BM stimulus (Wp (yellow)) and a  
483 non-BM stimulus (Lp (red)), the observed preference could represent the pre-induced  
484 and lasting BM preference. Group-2 was thus added as a control to group-1. We  
485 predicted that group-1 would show higher scores in the recent memory test than group-  
486 2. Or the recent memory scores of group-2 would have a high level of correlation with  
487 the individual BM scores compared with group-1.

488 In addition, we compared the old memory scores in Day-2 among the 3 groups. If  
489 newly-formed imprinting memory (on Day-2) interfered with the retention of old  
490 memory (on Day-1), group-2 could have higher scores than group-1.

491 *Results and Discussion*

492 BM preference scores on Day-1 were compared between group-1 and -2, and  
493 independently between group-1 and -3 (Fig. 10(a)). Pairwise multiple comparisons  
494 (Dunnett's test) showed a marginal but significant difference between group-1 and -3  
495 ( $t=2.357$ ,  $p=0.0476$ ). On the other hand, no significant difference occurred between

496 group-1 and -2 ( $t=0.821$ ,  $p=0.6285$ ), allowing us to compare the Day-2 data between  
497 these two.

498 Recent memory scores on Day-2 were compared between group-1 and -2, and  
499 between group-1 and -3. Pairwise multiple comparisons (Dunnnett's test) showed a  
500 significant difference between group-1 and -3 ( $t=3.231$ ,  $p=0.0061$ ). Group-1 chicks  
501 tended to show a higher score than group-2, but the difference was not significant  
502 ( $t=1.692$ ,  $p=0.1774$ ).

503 We made a post-hoc comparison between group-1 of experiment-6 and group-2 of  
504 experiment-2. A two-sample t-test revealed a significant difference ( $t=2.892$ ,  $p=0.0097$ ).  
505 Both groups were trained by simultaneous presentation of Wp (yellow) and Lp (red),  
506 and the difference was whether BM preference was pre-induced or not. BM preference  
507 was therefore strong enough to override the bias, making chicks prefer Wp (yellow) to  
508 Lp (red).

509 Correlation between the BM preference and the recent memory score was  
510 calculated for group-1 (c), -2 (d) and -3 (e) after Bonferroni correction of p-value for the  
511 repetition ( $p=0.05/3$ , namely  $p=0.0166$ ). Pearson's product-moment coefficient was  
512 significant in group-2 ( $t=3.711$ ,  $df=8$ ,  $p=0.0059$ ), but not in group-1 ( $t=0.7233$ ,  $df=8$ ,  
513  $p=0.1286$ ) and group-3 ( $t=0.4030$ ,  $df=8$ ,  $p=0.1410$ ).

514 To obtain reliable estimates of the contributing factors, we constructed 2 sets of  
515 generalized linear models (GLMs) for the recent memory scores, one set for the merged  
516 data of group-1 and -2, and another set for group-1 and -3. For each set, we constructed  
517 8 models with all possible combinations of two variables including their interaction,  
518 namely the BM preference score and the training (group-1 and -2: Day-2 training or not;  
519 group-1 and -3: Day-1 training by moving or stationary toy). Details are shown in  
520 Supplementary\_document\_#2.

521 In the set (group-1 and -2), the best model ( $AIC=268.76$ ) was composed of BM  
522 score ( $p=0.0408$ ), group ( $p=0.0311$ ) and the interaction ( $p=0.1104$ ) as explanatory  
523 variables. The second-best model ( $AIC=270.05$ ) did not include the interaction, and  
524 both coefficients of BM score ( $p=0.1670$ ) and group ( $p=0.1350$ ) were less significant.  
525 The AIC of the null model was 271.71. The Day-2 training thus contributed to the  
526 recent memory of group-2 in a manner stronger than that of group-1.

527 In the set (group-1 and -3), the best model (AIC=276.32) was composed only of  
528 group ( $p=0.0080$ ) as explanatory variable, and the second-best model (AIC=277.97)  
529 was composed of BM score ( $p=0.0096$ ) and group ( $p=0.030$ ). The AIC of the null  
530 model was 282.33. The effect of the Day-1 training was reconfirmed.

531 The old memory test on Day-2 failed to reveal a significant difference between  
532 group-1 and -2 after pairwise multiple comparisons (Dunnett's test;  $t=0.3930$ ,  
533  $p=0.8941$ ). The assumed interference of the old memory by the recent memory  
534 formation thus gained no experimental support.

535

## 536 **GENERAL DISCUSSION**

537 *Induced BM preference functions as a Conspec process, which guides the subsequent*  
538 *imprinting toward selective memorisation of the BM stimulus despite the associated*  
539 *colour*

540 Contrary to our initial prediction, the full-screen colour test of experiment-1 to -3  
541 failed to show a consistent learned preference for the colour associated with BM point-  
542 light animation. On the other hand, chicks can show a preference for the imprinting  
543 stimuli, when tested by the motion pictures in its associated colour, at least for the BM  
544 imprinting stimuli. In experiment-1 (Fig.2), group-1 and -2 preferred Wp (BM),  
545 whereas group-3 and -4 did not prefer Lp (non-BM). In experiment-2 (Fig.3), when  
546 simultaneously trained by Wp (red) and Lp (yellow) (group-1), chicks preferred the  
547 former Wp (red). However, when the motion-colour association was swapped (group-  
548 2), chicks did not show a preference between Wp (yellow) and Lp (red). Wp is therefore  
549 not powerful if shown in yellow. Accordingly, in experiment-3 (Fig.4), group-1  
550 preferred Wp (red) to Wp (yellow), but group-2 did not show a preference between Lp  
551 (red) and Lp (yellow). The first hypothesis (1) is thus rejected, and we conclude a  
552 corollary of the alternative idea that chicks form a biased preference for the BM in red.

553 Results of experiment-4 (right column, Fig.6) confirmed this conclusion, as a strong  
554 preference bias appeared for the red chicken walking over the yellow (group-1).  
555 Furthermore, the red head tended to be more powerful than the yellow head (group-2),  
556 but the preference disappeared in the linear motion (group-3) and the tail in colour  
557 (group-4). Our interpretation is that chicks have a predisposed preference for a walking  
558 chicken with a red crest on the head, or a red face ("*biological motion in red*"). We must

559 also note that the head movement was jerkier than the tail. The preference for the  
560 walking head can be accounted for in terms of the predisposed preference for objects of  
561 changing speeds as a visual cue of animacy (Rosa-Salva et al. 2016). Conversely, the  
562 motionless colour plate test (left column, Fig.6) almost consistently revealed a yellow  
563 preference in experiment-1 to -4. We are presently unable to explain why yellow was  
564 preferred, but this bias could simply represent an innate preference for a brighter screen.

565 In order to understand the functional role played by the early induction of BM  
566 preference, the “*biological motion in red*” bias was confounding because BM stimuli  
567 did not necessarily caused a successful imprinting as in the case of group-2 of  
568 experiment-2 (Fig. 3, memory test). In experiment-6, we therefore tried to separate the  
569 Day-1 process (induction of the BM preference) and the Day-2 process (learning based  
570 on the BM preference). The results (Fig. 10) showed that the induced BM preference  
571 made chicks override the “*biological motion in red*”, and chicks formed a learned  
572 preference for Wp (yellow), guiding the subsequent imprinting toward selective  
573 memorisation of the BM stimulus despite the associated colour.

574 Instead, the different results of experiment-2 (group-2) and -6 (group-1) might be  
575 attributed to the other factors, for example, the different ages of the memory test (post-  
576 hatch 15~24 h in experiment-2, and 39~48 h in experiment-6). We might assume that  
577 the aged chicks showed an unconditional preference bias to Wp (yellow). However,  
578 such a bias did not occur in group-2 of experiment-6 (no training on Day-2; central data  
579 of Fig. 10(b)), and the preference bias was predicted by the induced BM preference  
580 (Fig. 10(d)).

581 Experiment-5 was designed to methodologically validate experiment-6. As a pre-  
582 requisite, we examined if the induced BM preference on Day-1 could survive until Day-  
583 2, and it did as we had predicted. On the other hand, a decay in the memory test scores  
584 (Fig. 8(c) and (d)) occurred. It cannot be ascribed to the repeated testing on Day-1 and -  
585 2, because similarly low scores appeared in the old memory test of experiment-6, in  
586 which chicks were tested only once (Fig. 10(f)).

587 The main results are summarised in Fig. 11(a). If the BM preference was not pre-  
588 induced, the “*biological motion in red*” predisposition predominates. If pre-induced, this  
589 predisposition was overridden and a preference for Wp (yellow) could be developed  
590 through exposure to the combined imprinting stimuli in which BM was associated with

591 yellow. We may argue that a stronger *Conspec* mechanism (*i.e.*, the BM preference)  
592 leads to a clearer *Conlern* mechanism (*i.e.*, the preference for BM in yellow). However,  
593 at the individual level, we found no significant correlations between the BM preference  
594 (*Conspec*) and the recent memory scores (*Conlern*) in experiment-6 (Fig. 10(c)). The  
595 link between these two processes might not be direct nor strong.

596 It remains to be asked if other forms of motion preference could contribute beside  
597 the BM preference studied here. It has been reported that the visually naïve chicks show  
598 preference for a simple ball in proactive motion (self-propelled causal agency:  
599 Mascialzoni et al., 2010, Rosa-Salva et al., 2016; also see Lorenzi et al., 2017 for the  
600 involved brain areas). At present it remains unanswered as to if these forms of motion  
601 preference could share common neuro-cognitive bases.

602 As a take-home-message, we can summarise our findings by saying that imprinting  
603 is composed of dynamical processes in which predispositions and memory formation  
604 interact to develop adaptive social attachments, as opposed to the widely accepted view  
605 that imprinting is an irreversible memorization of the first-seen object that the chicks  
606 happened to encounter during a brief sensitive period after hatching.

607

#### 608 ***Breakdown of imprinting and possible hormonal mechanisms linking Conspec with*** 609 ***Conlern processes in chicks***

610 The present study revealed the somewhat complicated nature of imprinting. The  
611 breakdown list of the recognition memory is summarised in Fig. 11(b). As Lorenz  
612 (1937) pointed out, “imprinting has a number of features which distinguish it  
613 fundamentally from a learning process” (as cited by Sluckin, 1973), and it holds true in  
614 many senses. Not only is imprinting free of reinforcement process (as well as being  
615 independent of the basal ganglia system for reward-value updating; Izawa et al., 2001) ,  
616 the recognition memory is formed based on several different sensory sub-modalities  
617 such as colours, shapes, motions and configurations.

618 We must notice that we do not know whether the pre-induction of BM preference  
619 could improve the BM perception *per se*, as would be expected as a *Conspec* process.  
620 Through a consequent *Conlern* process, chicks would have an improved discrimination  
621 of objects by the motion cues. Naïve chicks do not discriminate between the mother hen  
622 and a possible predator like polecat (see Johnson and Horn 1988; Vallortigara et al.

623 2005), but chicks could do so after the BM induction. In human adults, capability to  
624 discriminate different motions by point-light animations is enhanced by acquiring the  
625 novel motor patterns that correspond to the animation without specific visual experience  
626 (Casile and Giese 2006). Similar post-imprinting development through behavioural  
627 execution might be assumed in chicks.

628 Induction of the BM preference is correlatively (Takemura et al., 2018) as well as  
629 causally (Miura et al., 2018) linked with increased gene expression of *Dio2*, which  
630 codes the type-2 deiodinase, an enzyme responsible for the conversion of thyroid  
631 hormone (T<sub>4</sub>) to its active form (T<sub>3</sub>) in the brain. The influx of T<sub>3</sub> would acutely  
632 activate the ongoing imprinting, and chronically prime the memory system so that the  
633 chicks remain imprintable for considerable days or a week afterwards (Yamaguchi et  
634 al., 2012). Cellular/molecular bases of the acute (activational) and chronic  
635 (organisational) effects of T<sub>3</sub> are yet largely unknown (see Aoki et al., 2015;  
636 Yamaguchi et al., 2018), but the neuro-hormonal actions would link the predisposed  
637 BM preference (*Conspec* mechanism) with the further memorisation of the BM object  
638 in colour (*Conlern* mechanism). As important topics in future studies,  
639 neuropharmacological manipulations could be designed to disconnect these functional  
640 links.

641

642 ***Imprinting gains resilience or developmental stability through functional links***  
643 ***between the memory formation and the social predispositions***

644 The present study makes us assume a resilient development of social cognition, as  
645 reported in the study of socially deprived rhesus monkeys (Harlow and Suomi, 1971).  
646 Even if the first-seen object after hatching was not the mother hen, chicks would soon  
647 develop a set of predispositions toward a “*biological motion in red*”. This would act  
648 together with the prolongation of the sensitive period of learning (memory priming)  
649 through T<sub>3</sub>. The pure colour preference of the initial imprinting object rapidly fades  
650 (even before the completion of the 2-hour training; Takemura et al., 2018) and is  
651 replaced by the formation of specific motion-colour memory, leading to adaptive social  
652 attachment.

653 In this context, recent findings from the chick model of autism spectrum disorder  
654 (ASD) is worthy of attention (Nishigori et al. 2013; Sgadò et al. 2018; Lorenzi et al.

655 2019). Embryonic exposure to valproic acid consistently impaired development of  
656 various types of social behaviours, whereas imprinting memory formation (using  
657 artificial objects or video images) remained intact. As the machineries underlying the  
658 social development, several neural nuclei (septum, hypothalamic preoptic area and  
659 amygdaloid nucleus) have been suggested (Mayer et al. 2017a, b; Lorenzi et al. 2017).  
660 Most of these candidate nuclei receive direct projections from the limbic part of  
661 arcopallium, and localized lesion to this part is shown to impair social facilitation of  
662 foraging behaviour (Xin et al. 2017). Furthermore, neurons in the arcopallium receive  
663 excitatory synaptic inputs from intermediate medial mesopallium (IMM; Csillag 1999),  
664 which has been known as the hub for imprinting memory formation (Horn 1998, 2004).  
665 The embryonic valproic acid treatment could selectively interfere with the development  
666 of the social neural network, while sparing the sensory learning mechanisms in IMM  
667 and IMHA (Aoki et al. 2015) intact. The link between the BM preference induction and  
668 the attachment formation found in this study could be broken down in these ASD model  
669 chicks.

670 It is surprising that chicks retain these adaptive neurocognitive processes for  
671 imprinting even after artificial breeding for thousands of generations under  
672 domestication. A recent report clearly shows that the preference is maintained in  
673 genetically isolated lines for a considerable period over 18 years (Versace et al., 2017a).  
674 Furthermore, chicks are equipped with faculties responsible for numerosity  
675 comprehension (Rugani et al., 2009, 2013, 2015), probability inference (Santolin et al.,  
676 2016), categorisation based on conceptual relationship (Martinho and Kacelnik, 2016;  
677 Versace et al. 2017b; also see Hogue et al. 1996 for the transitivity inference in female  
678 hens, and Daisley et al. 2010 for chicks) and spatial geometry (Pecchia and Vallortigara,  
679 2010; Pecchia and Vallortigara, 2012; Lee et al., 2012). Intensive domestication could  
680 have weakened or erased these “cognitive” capabilities that are not explicitly beneficial  
681 in the animal husbandry.

682 The idea of modular organizations may assume that these distinct processes could  
683 independently evolve to meet different ecological needs. What if, on the contrary, these  
684 cognitive processes are mutually linked, as suggested by Versace et al. (2018),  
685 particularly during the early *Conspec* process? The physiologically linked relationships  
686 are unbreakable and make these apparently distinct processes act altogether, allowing

687 some of the cognitive phenotype to survive even in the absence of any favourable  
688 positive selection. Accordingly, exogenous application of T<sub>3</sub> also facilitated the learning  
689 reinforced by delayed rewarded, as it did the secondary imprinting (Yamaguchi et al.,  
690 2012), suggesting that the underlying cognitive flexibility is tightly associated with the  
691 early processes of imprinting. Further analytical surveys would reveal critical  
692 developmental linkages shared by these distinct domains of intelligent behaviours.

693

#### 694 **Supplementary materials**

695 Supplementary\_document\_#1; list of point-light animations and videos

696 Supplementary\_document\_#2; statistical calculations

697 Supplementary\_data\_#3; behavioural data (excel file)

698

#### 699 **References**

- 700 Aoki N, Yamaguchi S, Kitajima T, Takehara A, Katagiri-Nakagawa S, Matsui R,  
701 Watanabe D, Matsushima T, Homma KJ (2015) Critical role of the neural pathway  
702 from the intermediate medial mesopallium to the intermediate hyperpallium apicale  
703 in filial imprinting of domestic chicks (*Gallus gallus domesticus*). *Neuroscience*  
704 308:115-124. doi:10.1016/j.neuroscience.2015.09.014
- 705 Barki A, Volpato GL (1998) Early social environment and the fighting behaviour of  
706 young *Oreochromis niloticus* (Pisces, Cichlidae). *Behaviour* 135:913-929.  
707 <https://www.jstor.org/stable/4535568>
- 708 Bolhuis JJ (1999) Early learning and the development of filial preferences in the chick.  
709 *Behavioural Brain Research* 98:245-252. doi:10.1016/S0166-4328(98)00090-4
- 710 Bolhuis JJ, Honey RC (1998) Imprinting, learning and development: from behaviour to  
711 brain and back. *Trends in Neuroscience* 21:306-311. doi:10.1016/S0166-  
712 2236(98)01258-2
- 713 Bolhuis JJ, Johnson MH, Horn G (1985) Effects of early experience on the development  
714 of filial preferences in the domestic chick. *Developmental Psychobiology* 18:299-  
715 308. doi:10.1002/dev.420180403
- 716 Buiatti M, Di Giorgio E, Piazza M, Menna G, Taddei F, Baldo E, Vallortigara G (2019)  
717 *Proceedings of the National Academy of Sciences USA* 116:4625-4630.  
718 doi:10.1073/pnas.1812419116



719 Carlson M, Earls F (1995) Psychological and neuroendocrinological sequelae of early  
720 social deprivation in institutionalized children in Romania. *Annals of the New York*  
721 *Academy of Sciences* 807:419-428. doi:10.1111/j.1749-6632.1997.tb51936.x

722 Casile A, Giese MA (2006) Nonvisual motor training influences biological motion  
723 perception. *Current Biology* 16: 69-74. doi:10.1016/j.cub.2005.10.071

724 Csillag A (1999) Striato-telencephalic and striato-tegmental circuits: relevance to  
725 learning in domestic chicks. *Behavioural Brain Research* 98:227-236.  
726 [https://doi.org/10.1016/S0166-4328\(98\)00088-6](https://doi.org/10.1016/S0166-4328(98)00088-6)

727 Daisley JN, Vallortigara G, Regolin L (2010) Logic in an asymmetrical (social) brain:  
728 Transitive inference in the young domestic chick. *Social Neuroscience* 5:309-319.  
729 doi:10.1080/17470910903529795

730 Di Giorgio E, Loveland JL, Mayer U, Rosa-Salva O, Versace E, Vallortigara G (2017)  
731 Filial responses as predisposed and learned preferences: early attachment in chicks  
732 and babies. *Behavioural Brain Research* 325:90-104. doi:10.1016/j.bbr.2016.09.018

733 Harlow HF, Dodsworth RO, Harlow MK (1965) Total social isolation in monkeys.  
734 *Proceedings of the National Academy of Sciences USA* 54:90-97.  
735 doi:10.1073/pnas.54.1.90

736 Harlow HF, Suomi SJ (1971) Social recovery by isolation-reared monkeys. *Proceedings*  
737 *of the National Academy of Sciences USA* 68:1534-1538.  
738 doi:10.1073/pnas.68.7.1534

739 Hess EH (1958) "Imprinting" in animals. *Scientific American*, 198:81-90.

740 Hogue ME, Beaugrand JP, Laguë PC (1996) Coherent use of information by hens  
741 observing their former dominant defeating or being defeated by a stranger.  
742 *Behavioural Processes* 38:241-252. doi:10.1016/S0376-6357(96)00035-6

743 Horn G (1985) *Memory, Imprinting and the Brain, An Inquiry into Mechanisms*. Oxford  
744 University Press, Oxford, U.K.

745 Horn G (1998) Visual imprinting and the neural mechanisms of recognition memory.  
746 *Trends in Neurosciences* 21:300-305. doi:10.1016/S0166-2236(97)01219-8

747 Horn G (2004) Pathways of the past: the imprint of memory. *Nature Review*  
748 *Neuroscience* 5:108-120. doi:10.1038/nrn1324

749 Ichihashi T, Ichikawa Y, Matsushima T (2004) A non-social and isolate rearing  
750 condition induces an irreversible shift toward continued fights in the male fighting  
751 fish (*Betta splendens*). *Zoological Science* 21:723-729. doi:10.2108/zsj.21.723  
752 Izawa EI, Yanagihara S, Atsumi T, Matsushima T (2001) The role of basal ganglia in  
753 reinforcement learning and imprinting in domestic chicks. *NeuroReport* 12:1743-  
754 1747. doi:10.1097/00001756-200106130-00045  
755 Johansson G (1973) Visual perception of biological motion and a model for its analysis.  
756 *Perception & Psychophysics* 14:201-211. doi:10.3758/BF03212378  
757 Johnson MH, Bolhuis JJ, Horn G (1985) Interaction between acquired preferences and  
758 developing predispositions during imprinting. *Animal Behaviour* 33:1000-1006.  
759 doi:10.1016/S0003-3472(85)80034-8  
760 Johnson MH, Horn G (1988) Development of filial preferences in dark-reared chicks.  
761 *Animal Behaviour* 36:675-683. doi:10.1016/S0003-3472(88)80150-7  
762 Johnson MH, Davies DC, Horn G (1989) A sensitive period for the development of a  
763 predisposition in dark-reared chicks. *Animal Behaviour* 37:1044-1046.  
764 Lee SA, Spelke ES, Vallortigara G (2012) Chicks, like children, spontaneously reorient  
765 by three-dimensional environmental geometry, not by image matching. *Biology*  
766 *Letters* 8:492-494. doi:10.1098/rsbl.2012.0067  
767 Lorenz K (1937). *The Companion in the Bird's World*. *The Auk* 54:245-273.  
768 doi:10.2307/4078077  
769 Lorenzi E, Mayer U, Rosa-Salva O, Vallortigara G (2017). Dynamic features of animate  
770 motion activate septal and preoptic areas in visually naïve chicks (*Gallus gallus*).  
771 *Neuroscience* 354:54-68. doi:10.1016/j.neuroscience.2017.04.022  
772 Lorenzi E, Pross A, Rosa-Salva O, Versace E, Sgadò P, Vallortigara G (2019)  
773 Embryonic exposure to valproic acid affects social predispositions for dynamic  
774 cues of animate motion in newly-hatched chicks. *Frontiers in Physiology* 10:501.  
775 <https://doi.org/10.3389/fphys.2019.00501>  
776 Maekawa F, Komine O, Sato K, Kanamatsu T, Uchiyama M, Tanaka K, & Ohki-  
777 Hamazaki H (2006) Imprinting modulates processing of visual information in the  
778 visual wulst of chicks. *BMC Neuroscience* 7:75. doi:10.1186/1471-2202-7-75

779 Makinodan M, Rosen KM, Ito S, Corfas G (2012) A critical period for social  
780 experience-dependent oligodendrocyte maturation and myelination. *Science*  
781 337:1357-1360. doi:10.1126/science.1220845

782 Martinho A III, Kacelnik A (2016) Ducklings imprint on the relational concept of  
783 “same or different”. *Science* 353:286-288. doi:10.1126/science.aaf4247

784 Mascalonzi E, Regolin L, Vallortigara G (2010) Innate sensitivity for self-propelled  
785 causal agency in newly hatched chicks. *Proceedings of the National Academy of*  
786 *Sciences USA* 107:4483-4485. doi:10.1073/pnas.0908792107

787 Matsushima T, Izawa EI, Aoki N, Yanagihara S (2003) The mind through chick eyes:  
788 memory, cognition and anticipation. *Zoological Science* 20: 395-408.  
789 doi:10.2108/zsj.20.395

790 Maurer D, Barrera M (1981) Infants' perception of natural and distorted arrangements o  
791 f a schematic face. *Child Development* 47:523-527.  
792 <https://www.jstor.org/stable/1129230>

793 Mayer U, Rosa-Salva O, Vallortigara G (2017a) First exposure to an alive conspecific  
794 activates septal and amygdaloid nuclei in visually-naïve domestic chicks (*Gallus*  
795 *gallus*). *Behavioural Brain Research* 317: 71-81. doi:10.1016/j.bbr.2016.09.031

796 Mayer U, Rosa-Salva O, Morbioli F, Vallortigara G (2017b) The motion of a living  
797 conspecific activates septal and preoptic areas in naive domestic chicks (*Gallus*  
798 *gallus*). *European Journal of Neuroscience* 45:423-432. doi:10.1111/ejn.13484

799 Miura M, Matsushima T (2012) Preference for biological motion in domestic chicks:  
800 sex-dependent effect of early visual experience. *Animal Cognition* 15:871-879.  
801 doi:10.1007/s10071-012-0514-x

802 Miura M, Matsushima T (2016) Biological motion facilitates filial imprinting. *Animal*  
803 *Behaviour*, 116:171-180. doi:10.1016/j.anbehav.2016.03.025

804 Miura M, Aoki N, Yamaguchi S, Homma KJ, Matsushima T (2018) Thyroid hormone  
805 sensitizes the imprinting-associated induction of biological motion preference in  
806 domestic chicks. *Frontiers in Physiology*, 9:1740. doi:10.3389/fphys.2018.01740

807 Mondloch CJ, Lewis TL, Budreau DR, Maurer D, Dannemiller JL, Stephens BR,  
808 Kleiner-Gathercoal KA (1999) Face perception during early infancy. *Psychological*  
809 *Science*, 10:419-422. doi:10.1111/1467-9280.00179

810 Morton J, Johnson MH (1991) CONSPEC and CONLERN: a two-process theory of  
811 infant face recognition. *Psychological Review* 98:164-181.  
812 <https://philpapers.org/archive/MORCAC-15.pdf>

813 Moulson MC, Shutts K, Fox NA, Zeahah CH, Spelke ES, Nelson CA (2015) Effects of  
814 early institutionalization on the development of emotion processing: a case for  
815 *relative* sparing? *Developmental Science* 18:298-313. doi:10.1111/desc.12217

816 Naumova OY, Rychkov SY, Kornilov SA, Odintsova VV, Anikina VO, Solodunova  
817 MY, Arintcina IA, Zhukova MA, Ovchinnikova IV, Burenkova OV, Zhukova OV,  
818 Muhamedrahimov RJ, Grigorenko EL (2019) Effects of early social deprivation on  
819 epigenetic statuses and adaptive behavior of young children: a study based on a  
820 cohort of institutionalized infants and toddlers. *Plos One* 14:e0214285.  
821 doi:10.1371/journal.pone.0214285

822 Nishigori H, Kagami K, Takahashi A, Tezuka Y, Sanbe A, Nishigori H (2013) Impaired  
823 social behavior in chicks exposed to sodium valproate during the last week of  
824 embryogenesis. *Psychopharmacology* 227:393-402. doi:10.1007/s00213-013-2979-  
825 y

826 Pecchia T, Vallortigara G (2010) View-based strategy for reorientation by geometry.  
827 *Journal of Experimental Biology* 213:2987-2996. doi:10.1242/jeb.043315

828 Pecchia T, Vallortigara G (2012) Spatial reorientation by geometry with freestanding  
829 objects and extended surfaces: a unifying view. *Proceedings of the Royal Society B*  
830 279:2228-2236. doi:10.1098/rspb.2011.2522

831 Reid VM, Dunn K, Young RJ, Amu J, Donovan T, Reissland N (2017) The human fetus  
832 preferentially engages with face-like visual stimuli. *Current Biology* 27:1825-1828.  
833 doi:10.1016/j.cub.2017.05.044

834 Rosa-Salva O, Regolin L, Vallortigara G (2010) Faces are special for newly hatched  
835 chicks: evidence for inborn domain-specific mechanisms underlying spontaneous  
836 preferences for face-like stimuli. *Developmental Science* 13:565–577.  
837 doi:10.1111/j.1467-7687.2009.00914.x

838 Rosa-Salva O, Mayer U, Vallortigara G (2015) Roots of a social brain: developmental  
839 models of emerging animacy-detection mechanisms. *Neuroscience and*  
840 *Biobehavioral Reviews* 50:150-1168. doi:10.1016/j.neubiorev.2014.12.015

841 Rosa-Salva O, Grassi M, Lorenzi E, Regolin L, Vallortigara G (2016) Spontaneous  
842 preference for visual cues of animacy in naïve domestic chicks: the case of speed  
843 changes. *Cognition* 157:49-60. doi.org/10.1016/j.cognition.2016.08.014

844 Rugani R, Fontanari L, Simoni E, Regolin L, Vallortigara G (2009) Arithmetic in  
845 newborn chicks. *Proceedings of the Royal Society B* 276:2451-2460.  
846 doi:10.1098/rspb.2009.0044

847 Rugani R, Cavazzana A, Vallortigara G, Regolin L (2013) One, two, three, four, or is  
848 there something more? Numerical discrimination in day-old domestic chicks.  
849 *Animal Cognition* 16:557-564. doi:10.1007/s10071-012-0593-8

850 Rugani R, Vallortigara G, Priftis K, Regolin L (2015) Number-space mapping in the  
851 newborn chick resembles humans' mental number line. *Science* 347:534-536.  
852 doi:10.1126/science.aaa1379

853 Santolin C, Rosa-Salva O, Vallortigara G, Regolin L (2016) Unsupervised statistical  
854 learning in newly hatched chicks. *Current Biology* 26:1218-1220.  
855 doi.:10.1016/j.cub.2016.10.011

856 Sgadò P, Rosa-Salva O, Versace E, Vallortigara G (2018) Embryonic exposure to  
857 valproic acid impairs social predispositions of newly-hatched chicks. *Scientific*  
858 *Reports* 8:5919. https://doi.org/10.1038/s41598-018-24202-8

859 Simion F, Regolin L, Bulf H (2008) A predisposition for biological motion in the  
860 newborn baby. *Proceedings of the National Academy of Sciences USA* 105:809-  
861 813. doi:10.1073/pnas.0707021105

862 Sluckin W (1964) *Imprinting and early learning*. Page 26 (Chapter 2, "Approach and  
863 Following Responses"; Section 2, "Moving objects"). Spottiswoode, Ballantyne &  
864 Co. Ltd., London & Colchester, U.K.

865 Spalding D (1873) Instinct with original observations on young animals. *Macmillan's*  
866 *Magazine* 27:282-293. Reprinted in *British Journal of Animal Behaviour* 2:2-11 in  
867 1954.

868 Spelke ES (2000) Core knowledge. *The American Psychologist* 55:1233-1243.  
869 doi:10.1037/0003-066X.55.11.1233

870 Spelke ES, Kinzler KD (2007) Core knowledge. *Developmental Science* 10:89-96.  
871 doi:10.1111/j.1467-7687.2007.00569.x

872 Sugita Y (2008) Face perception in monkeys reared with no exposure to faces.  
873 Proceedings of the National Academy of Sciences USA 105:394-398.  
874 doi:10.1073/pnas.0706079105

875 Takemura Y, Yamaguchi S, Aoki N, Miura M, Homma KJ, Matsushima T (2018) Gene  
876 expression of Dio2 (thyroid hormone converting enzyme) in telencephalon is linked  
877 with predisposed biological motion preference in domestic chicks. Behavioural  
878 Brain Research 349:25-30. doi:10.1016/j.bbr.2018.04.039

879 Tooker CP, Miller RJ (1980) The ontogeny of agonistic behaviour in the blue gourami,  
880 *Trichogaster trichopterus* (Pisces, Anabantoidei). Animal Behaviour 28:973-988.  
881 doi:10.1016/S0003-3472(80)80086-8

882 Tóth M, Halász J, Mikics É, Barsy B, Haller J (2008) Early social deprivation induces  
883 disturbed social communication and violent aggression in adulthood. Behavioral  
884 Neuroscience 122:849-854. doi:10.1037/0735-7044.122.4.849

885 Vallortigara G, Regolin L, Marconato F (2005) Visually inexperienced chicks exhibit  
886 spontaneous preference for biological motion patterns. PLoS Biology 3:1312-1316.  
887 doi:10.1371/journal.pbio.0030208

888 Vallortigara G, Regolin L (2006) Gravity bias in the interpretation of biological motion  
889 by inexperienced chicks. Current Biology 16:279-280.  
890 doi:10.1016/j.cub.2006.03.052

891 Vallortigara G (2012) Core knowledge of object, number, and geometry: a comparative  
892 and neural approach. Cognitive Neuropsychology 29:213-236.  
893 <https://doi.org/10.1080/02643294.2012.654772>.

894 Versace R, Schill J, Nencini AM, Vallortigara G (2016) Naïve chicks prefer hollow  
895 objects. Plos One 11:e0166425. doi:10.1371/journal.pone.0166425

896 Versace E, Fracasso I, Baldan G, Della Zotte A, Vallortigara G (2017a) Newborn chicks  
897 show inherited variability in early social predispositions for hen-like stimuli.  
898 Scientific Reports 7:40296. doi:10.1038/srep40296

899 Versace E, Spierings MJ, Caffini M, ten Cate C., Vallortigara G (2017b) Spontaneous  
900 generalization of abstract multimodal patterns in young domestic chicks. Animal  
901 Cognition 20: 521-529. doi: 10.1007/s10071-017-1079-5

902 Versace E, Martinho-Truswel A, Kacelnik A, Vallortigara G (2018) Priors in animal  
903 and artificial intelligence: Where does learning begin? Trends in Cognitive  
904 Sciences 22:963-965. doi:10.1016/j.tics.2018.07.005

905 Versace E, Ragusa M, Vallortigara G (2019). A transient time window for early  
906 predispositions in newborn chicks. bioRxiv 623439. doi:10.1101/623439

907 Wood SMW, Wood JN (2015) A chicken model for studying the emergence of  
908 invariant object recognition. Frontiers in Neural Circuits 9:7.  
909 doi:10.3389/fncir.2015.00007

910 Xin Q, Ogura Y, Uno L, Matsushima T (2017) Selective contribution of the  
911 telencephalic arcopallium to the social facilitation of foraging efforts in the  
912 domestic chick. European Journal of Neuroscience 45:365-380.  
913 doi:10.1111/ejn.13475

914 Yamaguchi S, Aoki N, Kitajima T, Iikubo E, Katagiri S, Matsushima T, Homma KJ  
915 (2012) Thyroid hormone determines the start of the sensitive period of imprinting  
916 and primes later learning. Nature Communications 3:1081.  
917 doi:10.1038/ncomms2088

918 Yamaguchi S, Aoki N, Matsushima T, Homma KJ (2018) Wnt-2b in the intermediate  
919 hyperpallium apicale of the telencephalon is critical for the thyroid hormone-  
920 mediated opening of the sensitive period for filial imprinting in domestic chicks  
921 (*Gallus gallus domesticus*). Hormones and Behavior 102:120-128.  
922 doi:10.1016/j.yhbeh.2018.05.011

923

924 **Figure legends**

925 **Fig. 1** Experiment-1, -2 and -3, apparatus and procedures. In training, the stimulus was  
926 presented on an LCD monitor on one side, and the other side was occluded. When the  
927 chick approached the monitor, an infra-red sensor detected it and triggered the treadmill  
928 to withdraw the chick backward. At tests, the treadmill was turned off and chicks  
929 walked between the two monitors that displayed different visual stimuli. The stay time  
930 spent in each arm was measured during two sessions (300 sec each) with counter-  
931 balanced side of presentation, and difference of the stay time represented the preference  
932 score. Colour tests were immediately followed by memory tests. See text and following  
933 figures for the abbreviations of visual stimuli such as Wp (red) and Lp (yellow).

934

935 **Fig. 2** Experiment-1, results. Four groups of chicks (n=9 or 10) were compared. In  
936 training, chicks were exposed to one of 4 types of animations with different motion  
937 patterns (Walking (W) for BM and Linear (L) for non-BM) and colours (red and  
938 yellow). The suffix (p) denotes that the animation was composed of light points on dark  
939 background screen. In this and the following figures (Fig. 3, 4, 6), results of the colour  
940 test (left column) and the memory test (left column) are shown in box-whisker plots  
941 (median with upper/lower quadrants and maximum/minimum range), and dots indicate  
942 individuals. Asterisks placed on the boxes indicate the significant difference against the  
943 chance level revealed by one-sample t-test; \*\*\* (p<0.001), \*\* (p<0.01), \* (p<0.05), ns  
944 (not significant). For the inter-group comparisons, see text and  
945 Supplementary\_document\_#2.

946

947 **Fig. 3** Experiment-2, results. Two groups of chicks (each n=10) were compared. In  
948 training, chicks were exposed simultaneously to two of the 4 types of animations used  
949 in experiment-1; Wp (red) and Lp (yellow) in group-1, Wp (yellow) and Lp (red) in  
950 group-2.

951

952 **Fig. 4** Experiment-3, results. Two groups of chicks (each n=10) were compared. In  
953 training, chicks were exposed simultaneously to two of the 4 types of animations in a  
954 manner different from experiment-2; Wp (red) and Wp (yellow) in group-1, Lp (yellow)  
955 and Lp (red) in group-2.

956

957 **Fig. 5** Experiment-4, procedures. Walking (W) represents a BM motion, and Linear (L)  
958 a non-BM motion. Suffixes (whole, head and tail) denote that the whole body, the head  
959 or the tail was coloured. For example, W-whole (red) indicates a Walking chicken video  
960 with its whole body coloured in red. Test procedure is the same as experiment-1 to -3.  
961 Note that the point-light animations used in experiment-1 to -3 were composed based on  
962 the video clip of walking chicken used in experiment-4.

963



964 **Fig. 6** Experiment-4, results. Biased imprintability of the red animation over the yellow,  
965 when chicks were trained by a walking motion pattern, and when the whole or the head  
966 region was coloured. Four groups of chicks (each n = 10) were compared.

967

968 **Fig. 7** Experiment-5, procedures. Chicks were trained either with Moving toy or  
969 Stationary toy (both in red) and tested twice at 0.5 h (Day-1) and at ~24 h (Day-2). The  
970 first test was for the BM preference (Wp (while) vs. Lp (white)), and second test for the  
971 memory (familiar red Toy vs. unfamiliar yellow Toy).

972

973 **Fig. 8** Experiment-5, results. Data obtained from 2 groups of chicks (group-1 and -2)  
974 with different training on Day-1 (Moving toy and Stationary toy, respectively). The  
975 Day-1 (open circles) and Day-2 (filled circles) test scores are connected by dashed lines  
976 in (a) to (d). In contrast to the stable BM preference (a, b), the memory test revealed  
977 decaying scores (c, d) in both groups. Memory scores (y-axis) plotted against individual  
978 BM preference scores (x-axis) failed to reveal significant correlations in all 4 sets of  
979 data (e, f).

980

981 **Fig. 9** Experiment-6, procedures. On Day-1, chicks were trained with a moving toy  
982 (red) in group-1 and -2, and a stationary toy (red) in group-3. These chicks were tested  
983 for the BM preference at 0.5 hour after the end of training. On Day-2, group-1 and -3  
984 chicks were further trained by simultaneous presentation of Lp (red) and Wp (yellow)  
985 animations; group-2 chicks were not trained and kept in darkness. The 3 groups of  
986 chicks were then tested for the recent memory with point-light animations, and for the  
987 old memory with toys.

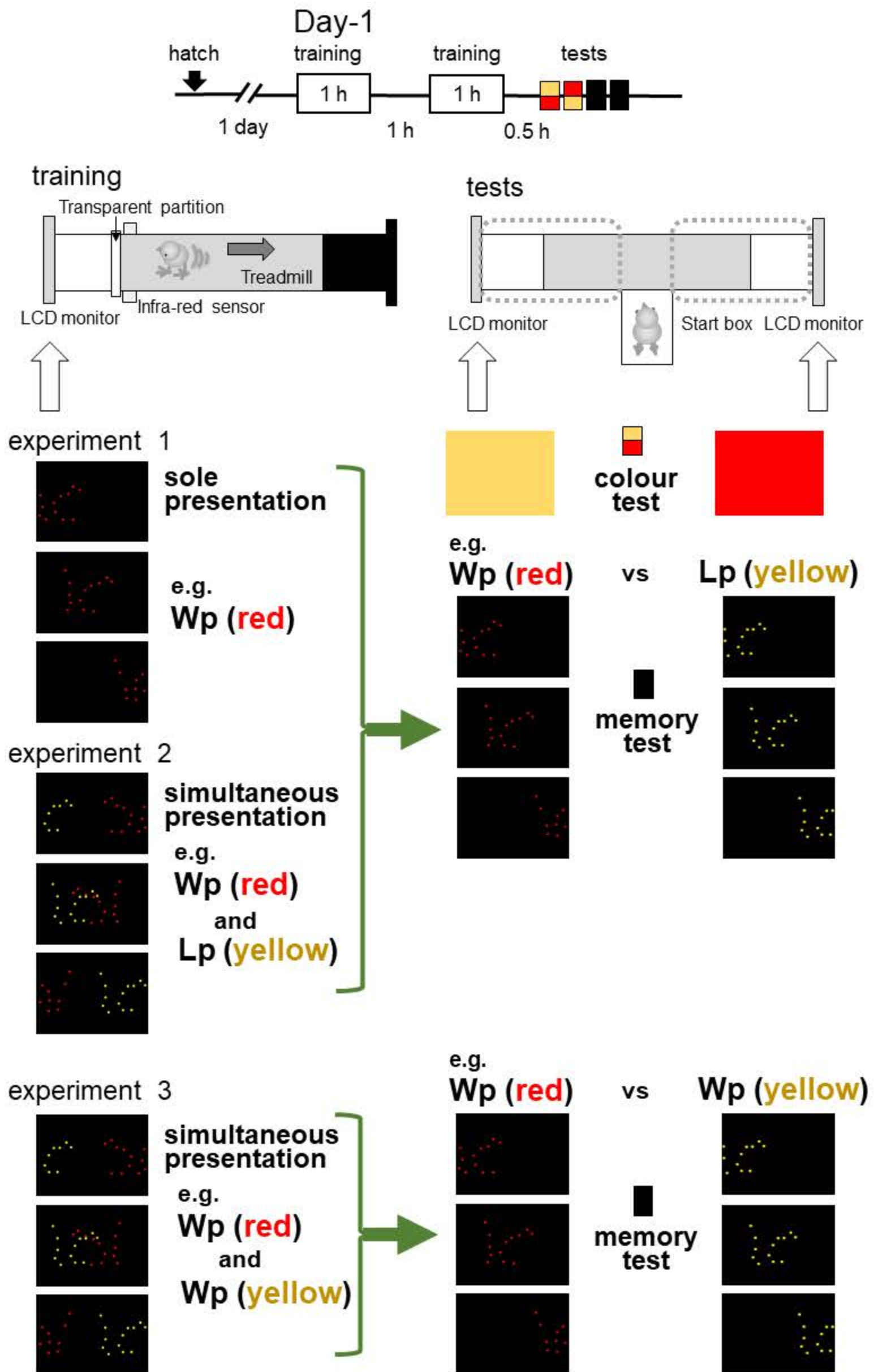
988

989 **Fig. 10** Experiment-5, results. Test scores obtained from 3 groups of chicks (group-1, -  
990 2, and -3) with different training on Day-1 and Day-2 are shown; (a) BM preference on  
991 Day-1, (b) recent memory test and (f) old memory test on Day-2. In (c) to (e), the recent  
992 memory test score (as y-axis) was plotted against the individual BM preference score  
993 (as x-axis); a significant positive correlation appeared in group-2 (d) but not in -1 (c)  
994 and -3 (e) after Bonferroni correction of p-values.

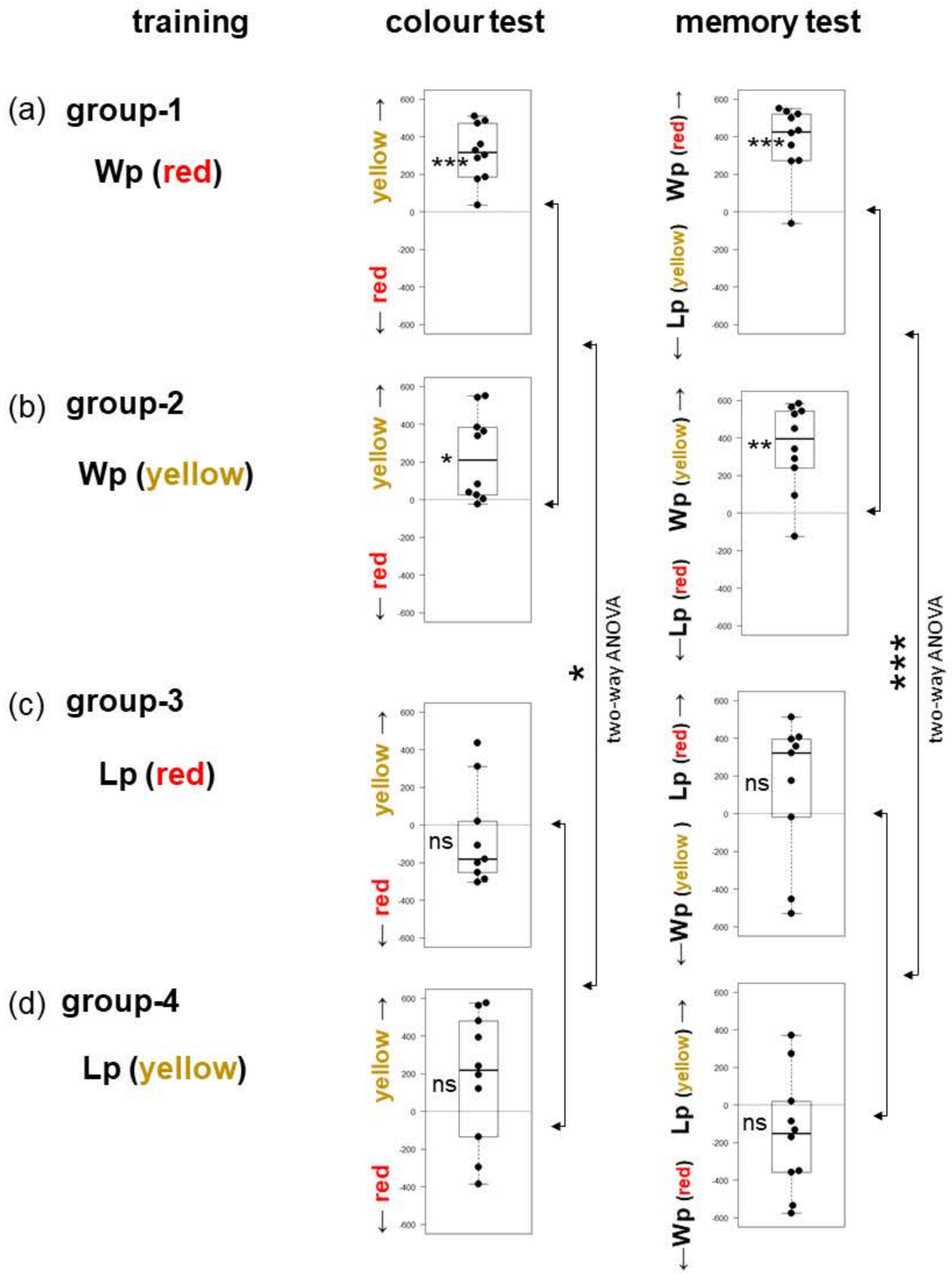
995

996 **Fig. 11** Graphical summary. (a) Results are compared between experiment-2 and -6. In  
997 experiment-2, naïve chicks trained with Wp (yellow) and Lp (red) showed no  
998 preference (group-2). In experiment-6, if the BM preference was pre-induced, chicks  
999 showed a preference for Wp (yellow) after the same training (group-1). If not pre-  
1000 induced, no preference emerged (group-3). (b) Possible scenario upon breakdown of the  
1001 recognition memory of imprinting.

# EXPERIMENT 1, 2, 3



EXPERIMENT 1



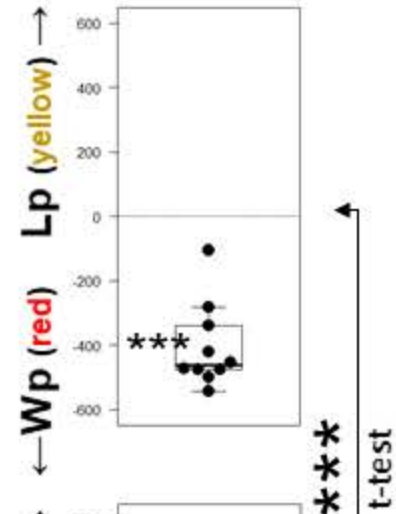
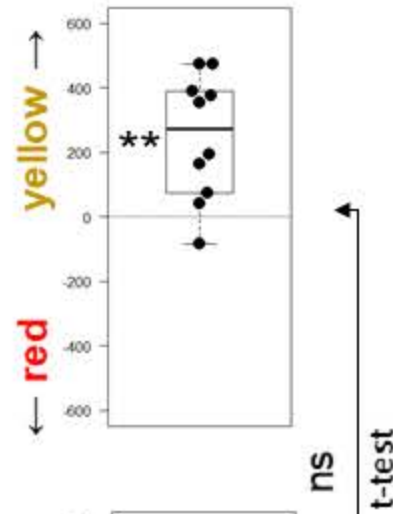
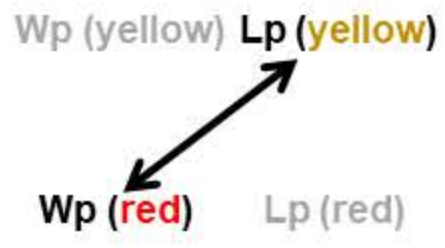
EXPERIMENT 2

training

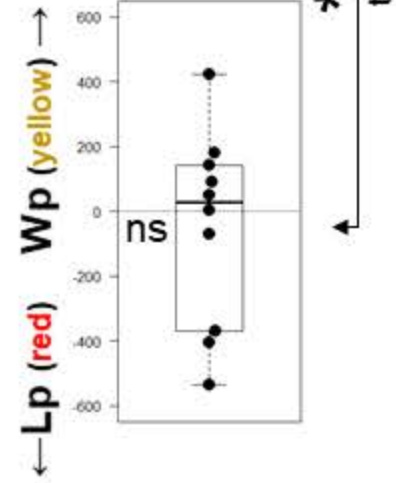
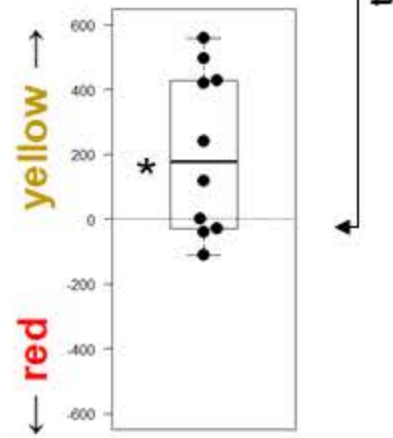
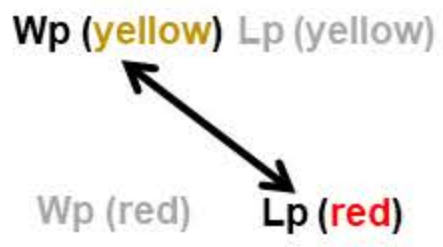
colour test

memory test

(a) group-1



(b) group-2



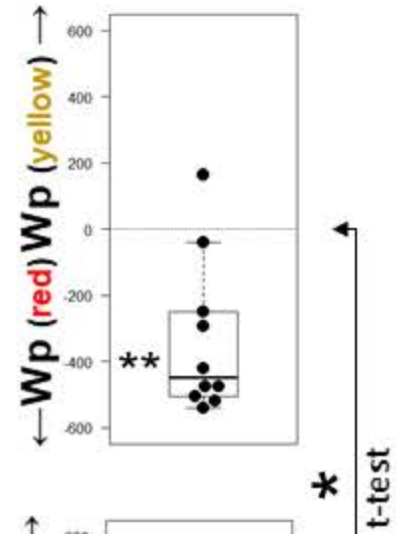
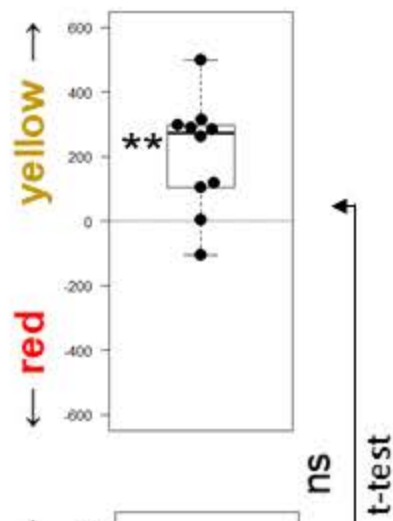
EXPERIMENT 3

training

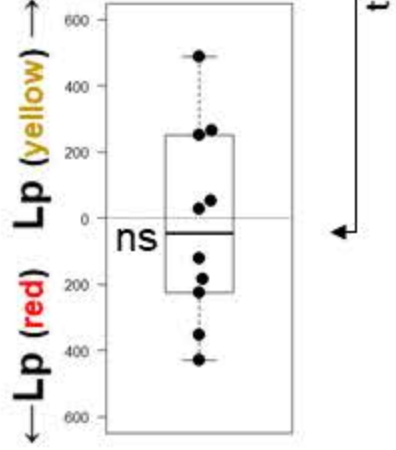
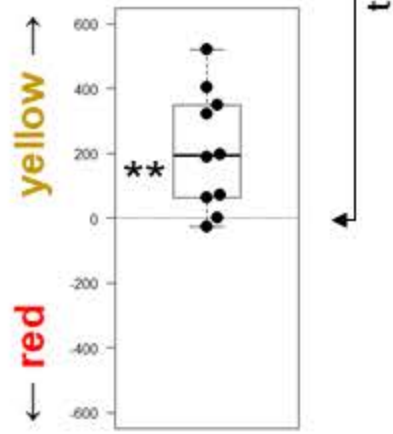
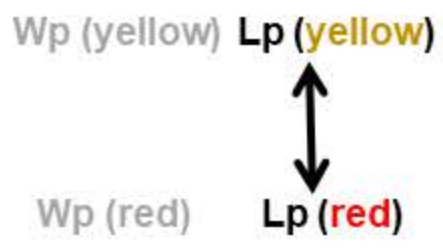
colour test

memory test

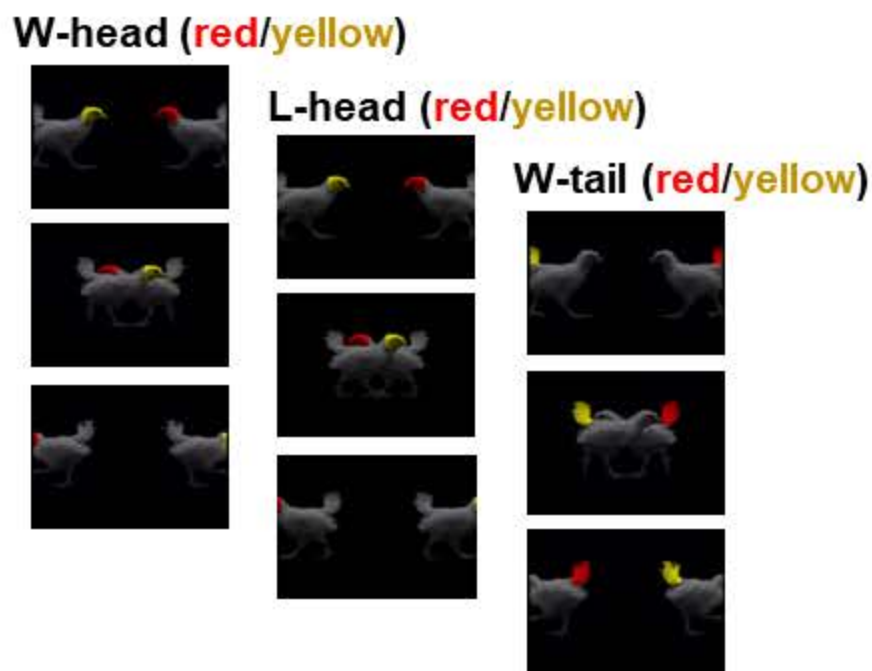
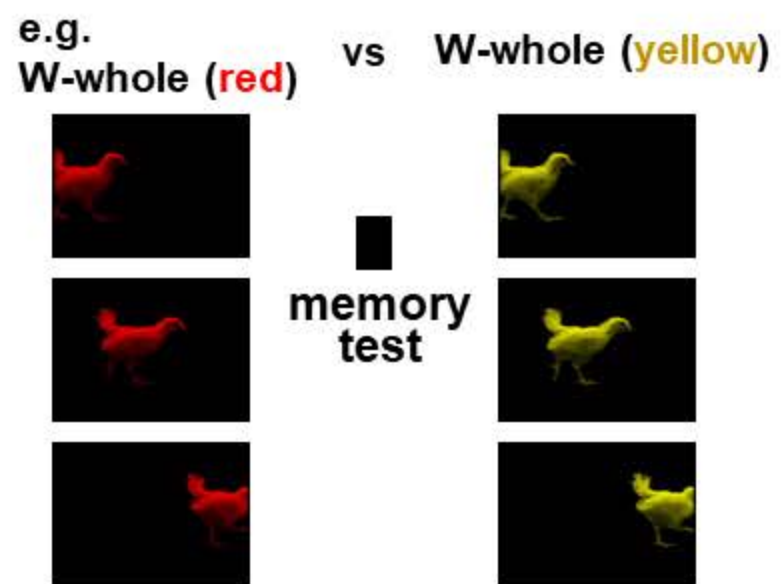
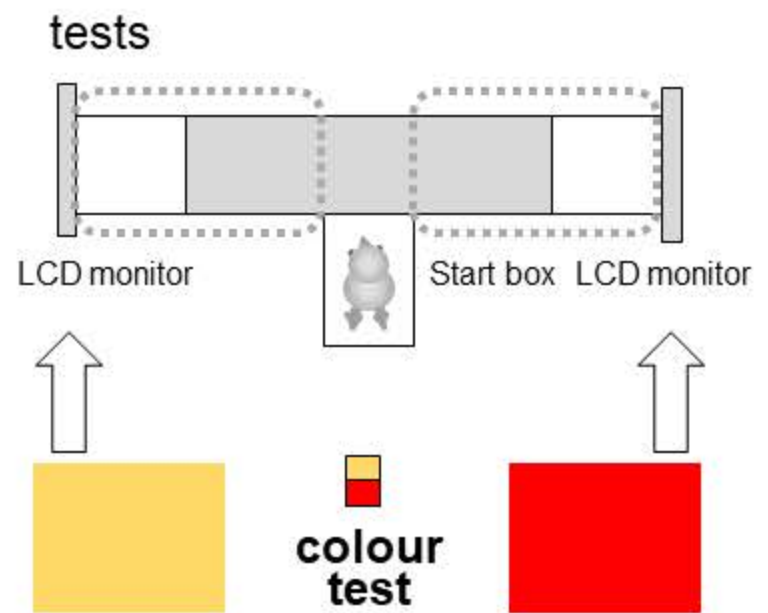
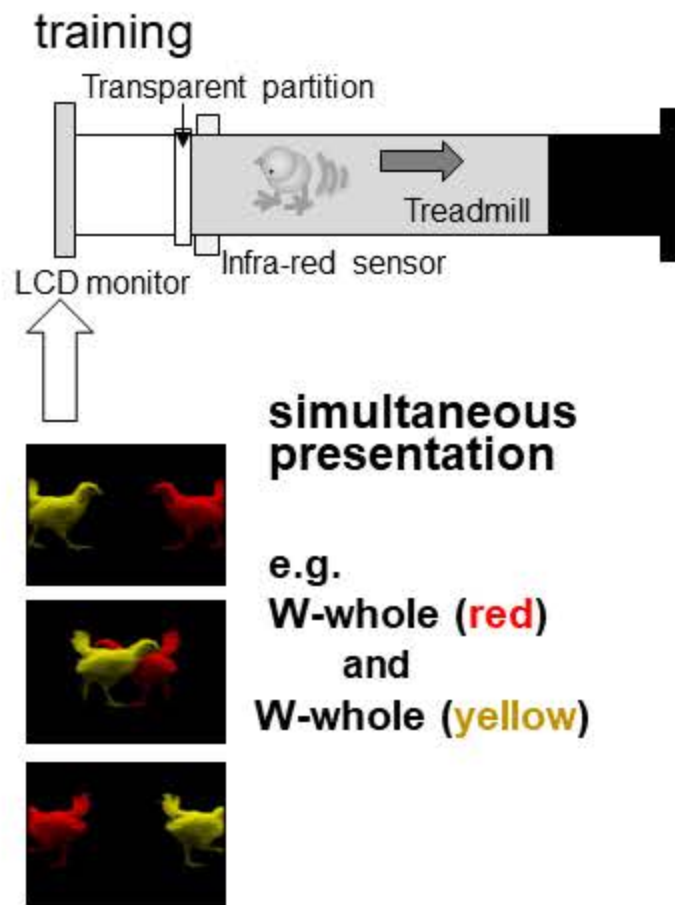
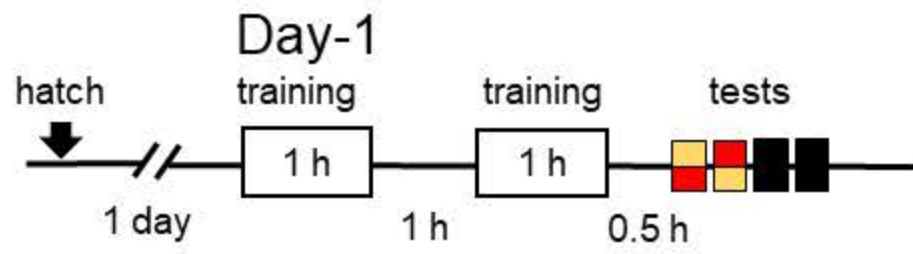
(a) group-1



(b) group-2

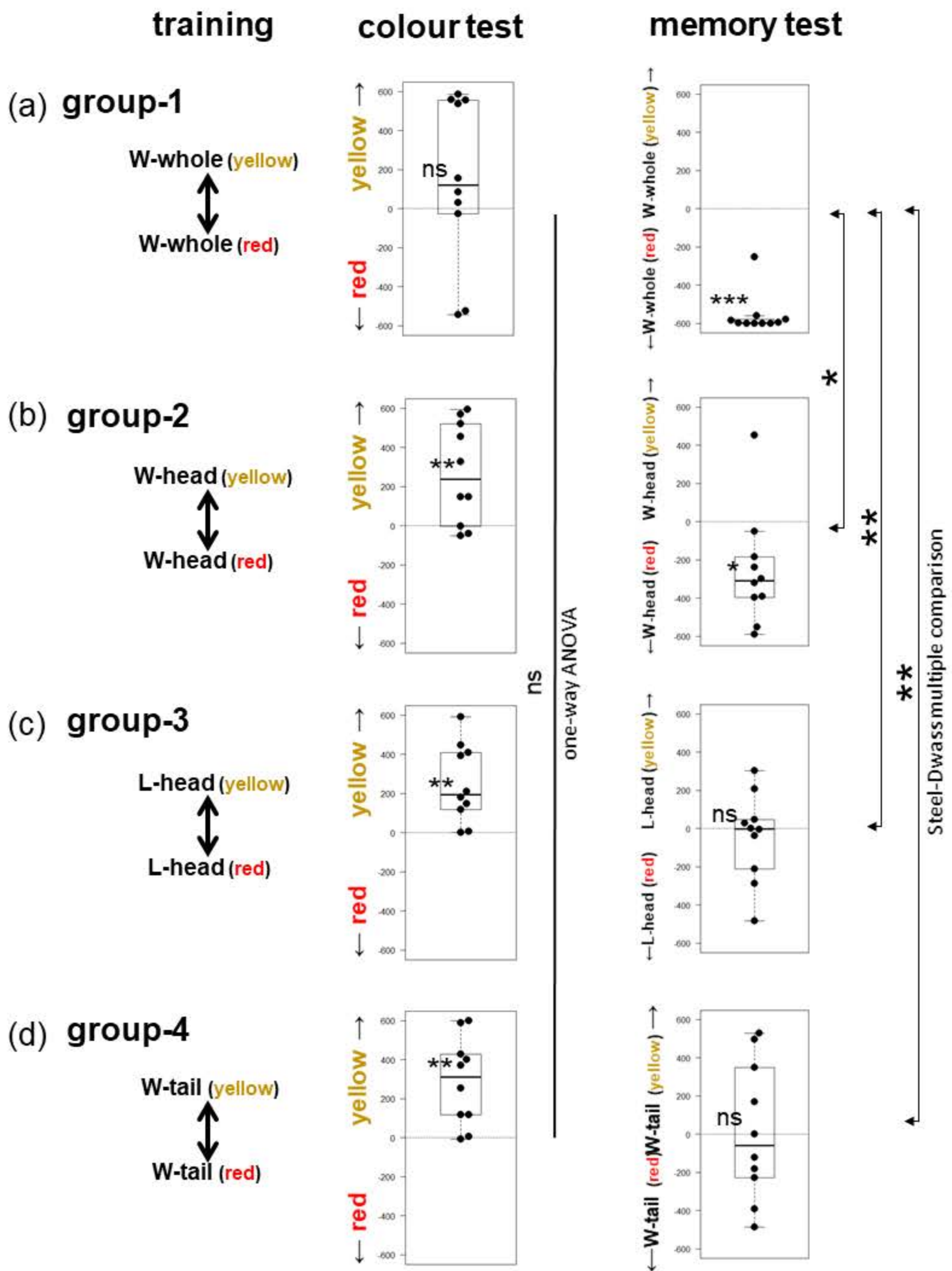


# EXPERIMENT 4



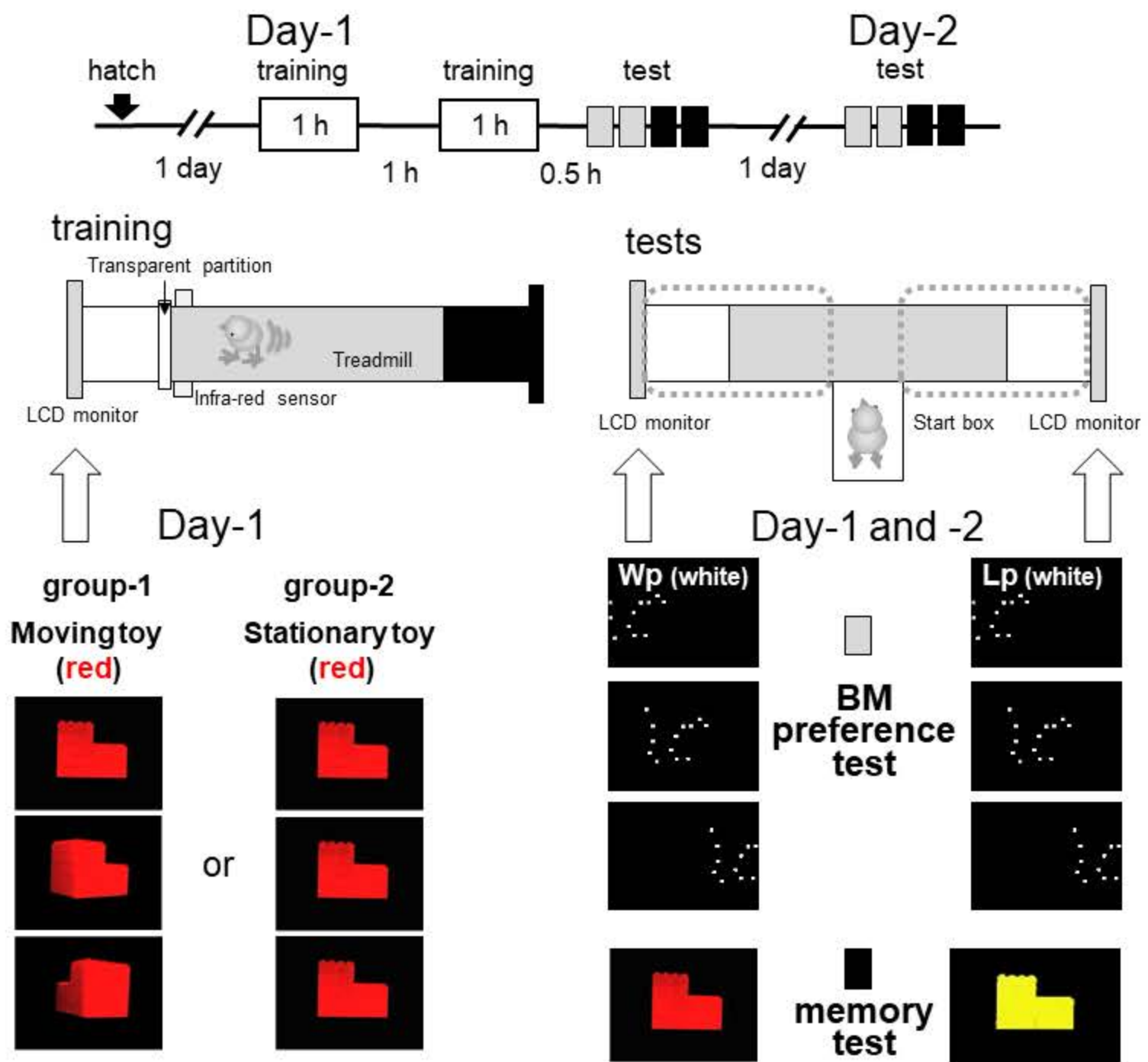


# EXPERIMENT 4



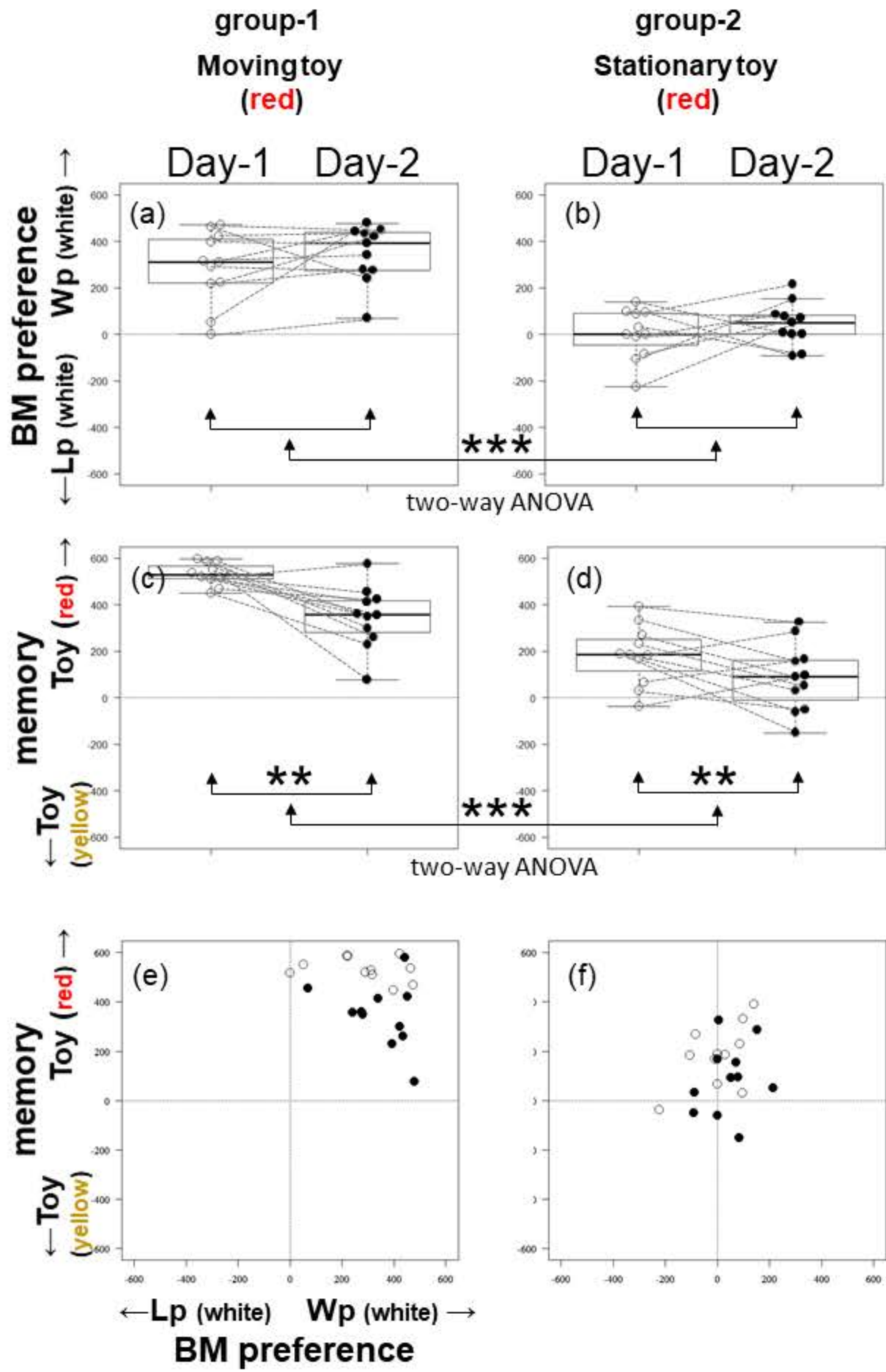


# EXPERIMENT 5

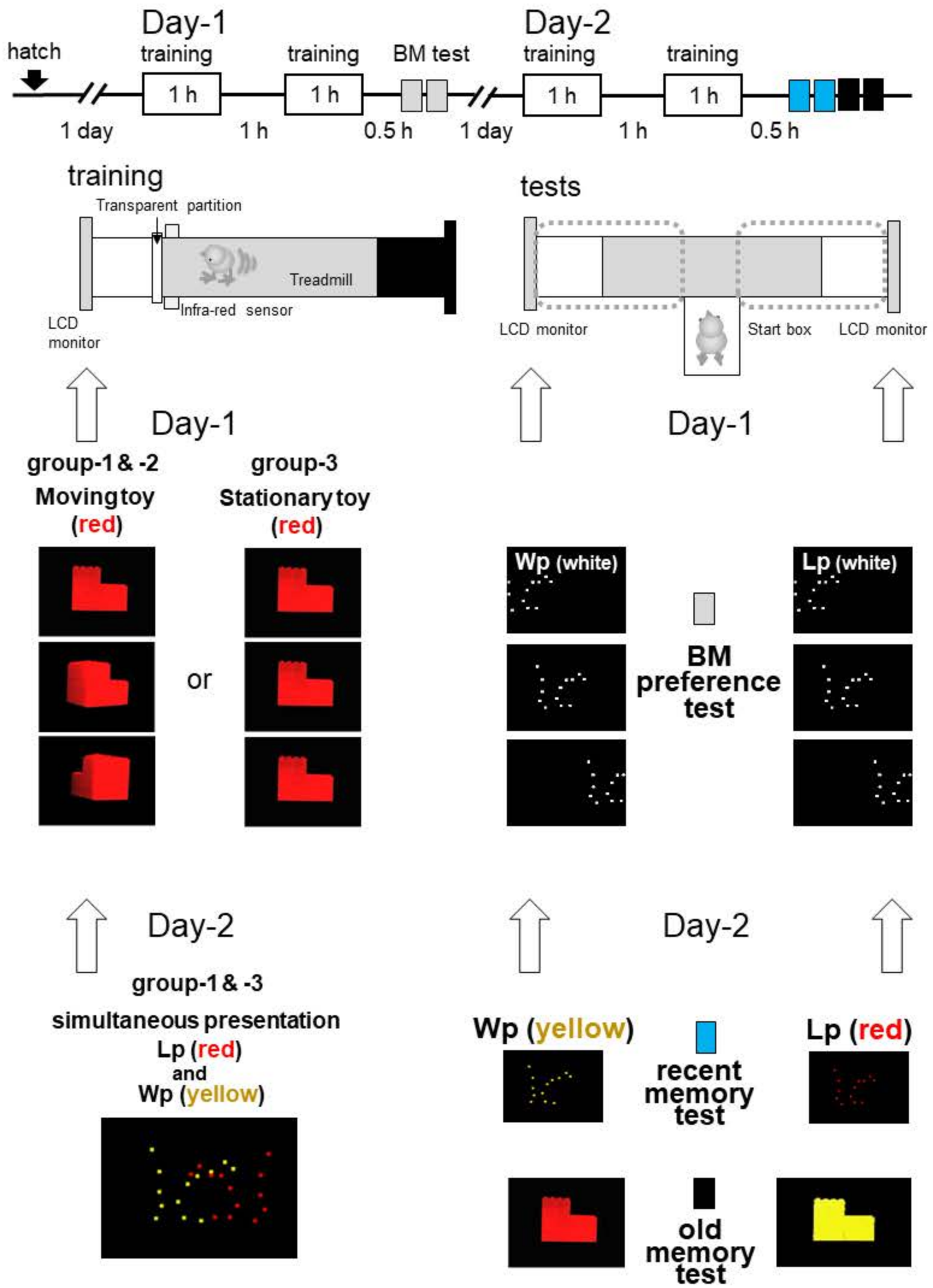


# EXPERIMENT 5

ns



# EXPERIMENT 6

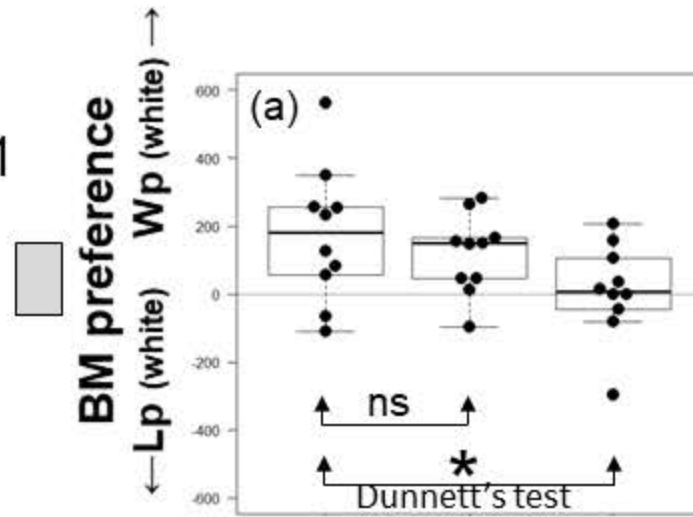


# EXPERIMENT 6

training	group-1	group-2	group-3
Day-1	Movingtoy (red)	Movingtoy (red)	Stationarytoy (red)
Day-2	Lp (red) ↔Wp (yellow)	No training	Lp (red) ↔Wp (yellow)

## tests

Day-1



Day-2

