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***Title: Hemispheric specialization in spatial vs. ordinal  
processing in the day-old domestic chick (*Gallus gallus*)***

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***Running Head: When number conflicts space.***

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1 **ABSTRACT**

2 Different species show an intriguing similarity in representing numerosness in  
3 space starting from left to right. This bias has been attributed to a right  
4 hemisphere dominance in processing spatial information. Here, to disentangle  
5 the role of each hemisphere in dealing with spatial vs. ordinal-numerical  
6 information, we tested domestic chicks during monocular vs. binocular vision. In  
7 the avian brain, the contralateral hemisphere mainly elaborates the visual input  
8 to each eye. Four-day-old chicks learnt to peck at the 4<sup>th</sup> element in a sagittal  
9 series of 10 identical elements. At test, chicks faced a left-to-right-oriented  
10 series, where inter-element distance was manipulated, so that the 3<sup>rd</sup> element  
11 was where the 4<sup>th</sup> had been at training; this compelled chicks to use either  
12 spatial or ordinal cue. Chicks tested binocularly selected both the 4<sup>th</sup> left and (to  
13 a lesser extent) right elements. Chicks tested monocularly chose equally the 3<sup>rd</sup>  
14 and the 4<sup>th</sup> elements on the seeing side. Interhemispheric cooperation resulted  
15 in the use of ordinal-numerical information; whilst, each single hemisphere  
16 could rely on spatial or ordinal-numerical cue. Both hemispheres can process  
17 spatial and ordinal-numerical information, but their interaction results in the  
18 supremacy of processing ordinal-numerical cue.

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21 **KEYWORDS:** Number cognition; Asymmetry; Hemispheres; Lateralization;  
22 Mental Number Line; Spatial-Numerical Association, Domestic chick.

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## 1 INTRODUCTION

2 A peculiar characteristic of numbers is their spatial representation along a left-  
3 right oriented continuum<sup>1</sup>. Healthy participants are faster at processing small  
4 numbers when responses are executed in the left side of space, and large  
5 numbers when responses are executed in the right side of space (spatial-  
6 numerical association of response codes, SNARC effect<sup>2</sup>). For a long time, the  
7 orientation of the spatial numerical association (SNA) has been attributed to  
8 cultural factors, such as reading and writing habits<sup>3</sup>. Israeli participants (who  
9 read words from right to left and numbers from left to right) showed no SNA<sup>3</sup>,  
10 while Palestinians, who read both words and numbers from right to left, show a  
11 reversed SNA<sup>4</sup>. A growing number of studies in pre-verbal children<sup>5-9</sup> and non-  
12 human species<sup>10-17</sup> support the hypothesis that SNA may be present soon after  
13 birth and exist before experience or cultural influence, but see<sup>18,19</sup>. Different  
14 species (domestic chicks<sup>10-12</sup>, adult Clark's nutcrackers<sup>11</sup>, and adult Rhesus  
15 macaques<sup>20</sup>) showed an intriguing similarity in representing growing ordinal-  
16 numerical information as oriented from left to right. Animals were trained to  
17 select a target element (e.g. the 4<sup>th</sup> one), in a sagittally-oriented series of  
18 identical and fixed elements. They were then tested with a series, identical to  
19 the first one but frontal, i.e., rotated by 90°; hereafter we refer to this kind of task  
20 as spatial-and-ordinal task. At test, even if the correct alternatives were two –  
21 the left and the right target- animals chose the left one. This suggests that a  
22 disposition to associate the numerical magnitudes from left to right may  
23 originate from a neuro-biological precursor<sup>21,22</sup>. Interestingly enough, if the use  
24 of the spatial cues -the distance of the target element from the beginning or

1 from the end of the series- is prevented, chicks choose both the left and the  
2 right target. When the distance between the elements was changed on each  
3 trial, both during training and during fronto-parallel testing, chicks correctly  
4 identified the target (the 4<sup>th</sup> element), but they did not show any left bias. The  
5 left bias seems to be related to the possibility of using spatial and ordinal  
6 information both during learning and recall. Bilateral responses occur also when  
7 chicks learnt to identify the target on spatial and ordinal cues (spatial-and  
8 ordinal training) but at test they could either use the spatial or the ordinal one  
9 (spatial vs. ordinal test). Taken together this evidence indicates that the left-to-  
10 right bias in this test occurs solely when both spatial and ordinal information  
11 were available during learning as well as recall <sup>12</sup>. An intra-hemispheric coupling  
12 of ordinal cues, which is bilaterally represented, and of spatial cues, unilaterally  
13 (right) represented, has been proposed to explain differential attentional  
14 allocation toward the left and right visual hemifields.

15 Nevertheless, the neural correlates of this left-to-right oriented numerical  
16 association remain largely unknown. In humans, the posterior parietal cortex  
17 has been indicated as the brain region dedicated to respond to numbers<sup>23-25</sup>.  
18 Moreover, in humans, populations of neurons tuned to small numbers have  
19 been described in the parietal cortex<sup>26</sup>. These neurons are organized  
20 topographically, forming a map in the brain. Such a neural organization might  
21 determine the organization of magnitudes along the MNL<sup>22</sup>. Neurons tuned on  
22 numerosness are located in the intra-parietal cortices of the macaque brain<sup>27</sup>,  
23 and in a brain association area (*nidopallium caudolaterale*, NCL) of the avian  
24 brain<sup>28</sup>. Up to now the topographical organization of “number-neurons” in

1 animals has not been defined. Since non-verbal numerical cognition is shared  
2 by many animal species<sup>22</sup>, it is plausible that a similar map of number-neurons  
3 would be a common characteristic of the architecture of animal neural systems.  
4 In performing the original version of the “spatial-and-ordinal” task, specifically  
5 designed to investigate left vs. right processing of spatial and ordinal  
6 information, both hemispheres of the avian brain store, retain and proficiently  
7 retrieve both numerical as well as spatial information<sup>16</sup>. Spatial asymmetries in  
8 the “spatial-and-ordinal” task may be accounted for by a model that assumes  
9 differential encoding, processing and integration by the two hemispheres for  
10 spatial and numerical information.

11 A first attempt to study the hemispheric correlates of left-right bias in a serial  
12 ordinal task, has been done using the technique of temporary monocular  
13 occlusion. Due to the conformation of the avian brain, visual input to each eye is  
14 mainly elaborated by the contralateral hemisphere. Two main features of the  
15 bird brain make this possible: i) the visual fibres decussate nearly completely at  
16 the optic chiasm<sup>29,30</sup>; ii) the absence of a structure homologous to the corpus  
17 callosum (even though smaller tracts exist which allow inter hemispheric  
18 communication<sup>31-34</sup>). Thus, each eye mainly projects toward the visual system  
19 of the contralateral hemisphere<sup>35</sup>. By temporarily restricting the visual input to a  
20 single eye (by simply patching of the other eye), it is possible to determine the  
21 role of the contralateral hemisphere<sup>35</sup>. Chicks learnt to find food reinforcement  
22 into the 4<sup>th</sup> element, in a sagittally-oriented series of identical and fixed  
23 elements. At a subsequent fronto-parallel test, the series was maintained  
24 identical but it was rotated by 90°. The test was conducted in three different

1 conditions of vision: binocular, right monocular and left monocular. At fronto-  
2 parallel test, right monocular chicks pecked at the 4<sup>th</sup> right position, left  
3 monocular and binocular chicks pecked at the 4<sup>th</sup> left position<sup>16</sup>. This indicates  
4 that both hemispheres process, maintain and correctly use ordinal-numerical  
5 information. The similarity in the left-oriented responses showed by the left  
6 monocular and binocular chicks suggests that the left bias is linked to a right  
7 hemisphere dominance, which allocates attention toward the left hemispace<sup>36</sup>.  
8 But did the hemispheres use different strategies to solve the task? To approach  
9 this question, in the present study we used a spatial-and-ordinal training, which  
10 allows learning of both spatial and ordinal information, and a “spatial vs. ordinal”  
11 test, in which we created a conflict between spatial and ordinal cues<sup>12</sup>. We  
12 trained chicks to identify the 4<sup>th</sup> element in a series of 10 identical elements.  
13 During training the elements were in fixed positions, thus birds could identify the  
14 4<sup>th</sup> both on the basis of (i) its ordinal information: the 4<sup>th</sup> position in the  
15 sequence; (ii) its spatial information: the element that is located at a given  
16 distance from the beginning of the series. At test inter-elements distances were  
17 increased so that the 3<sup>rd</sup> element was at the distance from the beginning of the  
18 series in which the 4<sup>th</sup> element had been experienced during training. By  
19 integrating this paradigm with the use of temporary monocular occlusion, we  
20 studied how the two hemispheres encode, process and integrate spatial vs.  
21 ordinal information. We expected that chicks would be able to solve the task,  
22 and select the 4<sup>th</sup> element, but that the leftward bias would be lost if based on  
23 spatial cues.

24

## 1 MATERIAL AND METHODS

2

### 3 Subjects

4 Subjects were 13 male domestic chicks (*Gallus gallus*). We weekly obtained  
5 hours-old chicks from a local commercial hatchery (Agricola Berica,  
6 Montegalda, Vicenza, Italy). Immediately after their arrival, they were caged, in  
7 groups of three, in standard metal cages (28.0 x 40.0 x 32.0 cm width, depth  
8 and height, respectively) at controlled temperature (28–31 C) and humidity  
9 (68%). Food (chick starter) and water were available *ad libitum*. Three times a  
10 day we also fed them some mealworms (*Tenebrio molitor larvae*), to familiarize  
11 them with this food, that was used as reinforcement during training.

12 Chicks were reared in these conditions from Monday morning (11.00 am) to  
13 Wednesday afternoon (5.00 pm), when they were singly caged. On Thursday  
14 (10.00 am) the food jars were removed, while water was left available. A few  
15 hours later (1.00 pm) birds underwent pre-training. Pre-training began when  
16 they were 4 days old, due to their yolk sac reserves, chicks are little motivated  
17 to peck for food reward before day 4 post-hatching. Two hours after the pre-  
18 training was over, each chick underwent training. Once this was over, they were  
19 singly caged overnight with food and water available *ad libitum*.

20 On Friday, in the early morning (7.00 am) chicks were food deprived and then  
21 re-trained (8.00 am). Testing took place for each chick immediately after the re-  
22 training. At the end of the behavioural observations, all chicks were caged in  
23 social groups of five birds, with food and water available *ad libitum*, and a few  
24 hours later they were all (i.e. those used in this as well as in all other

1 experiments described) donated to local farmers. All procedures employed were  
2 evaluated and approved by the Committee for Animal Welfare of the University  
3 of Padova.

## 6 **Apparatus**

7 All the experimental phases (pre-training, training, re-training, sagittal test and  
8 fronto-parallel tests) took place in an experimental room located near to the  
9 rearing room. During experimental phases temperature and humidity were kept  
10 respectively at 25° C and 70%. The room was lit by four 58 W lamps, placed on  
11 the ceiling, 148 cm above the experimental apparatus. The apparatus consisted  
12 in a plastic square-shaped arena (80 x 80 x 40 cm), which floor was covered  
13 with wood-shaving. The apparatus was connected with a starting box (7 x 11 x  
14 11.5 cm) located outside of the arena itself. A slit (7 x 11 cm) connected the  
15 arena with the starting box. The slit was normally closed by an opaque plastic  
16 partition (8 x 12 cm); the partition was lifted for a few seconds at the beginning  
17 of each trial to let the chicks enter the arena. Ten identical elements (plastic  
18 bottle tops, 3.2 cm in diameter and 0.8 cm height) were aligned along the  
19 midline of the arena's floor. The elements were spaced 2.5 cm from one  
20 another, for an overall length of 54.5 cm. The sagittal series was symmetrically  
21 placed in the centre of the apparatus, thus the beginning of the series was 13  
22 cm apart from the slit and 39.6 cm from the side walls (**Fig.1a**). Each element  
23 was filled with wood-shaving. All the elements contained a mealworm, but only  
24 in the 4<sup>th</sup> element it was reachable by the chicks. At the beginning of each trial,

1 all elements looked identical. In each experimental phases, the apparatus was  
2 randomly rotated in the experimental room, from trial to trial, to avoid any  
3 possible use of external cues.

4

#### 5 **Pre-training**

6 On Day 4, chicks singly underwent a pre-training. The bird was firstly placed, for  
7 about 3 seconds, in the starting box and then, by sliding the partition, it was  
8 allowed to enter the arena. It could then spend a couple of minutes in the arena,  
9 walking wherever it wanted to be acquainted with the novel environment. As  
10 soon as the bird stopped to emit distress calls, a pre-training procedure started.  
11 Initially a mealworm was visibly positioned (not covered by wood-shaving) into  
12 the niche of the 4<sup>th</sup> element. Thereafter, the mealworm was progressively buried  
13 in the wood shaving, up to be completely hidden into it. Only chicks' pecking  
14 responses to the target element were reinforced. Once the mealworm was  
15 completely hidden, the learning criterion of this experimental phases was three  
16 consecutive correct trials.

17

#### 18 **Training**

19 Training begun two hours after the pre-training was over. During training, all  
20 elements were filled with wood-shaving, so that at the beginning of each trial,  
21 they looked identical. Only the 4<sup>th</sup> element hid a piece of mealworm. At the  
22 beginning of each trials, the chick was placed in the starting box for a few  
23 seconds. The removable partition was lift from above thus the chick could enter  
24 the arena and walk towards the series of elements and peck at one of them. A

1 trial was considered over when the chick pecked at one element. Only one peck  
2 on each trial was allowed. A trial was considered correct if the chick pecked at  
3 the 4<sup>th</sup> elements. A trial was considered null and thus terminated after 180  
4 seconds in the absence of a peck at whichever element. The learning criterion  
5 for this experimental phase was -at least- eight correct responses across 20  
6 valid trials<sup>15,24</sup>. All the chicks reached it and advanced to the subsequent  
7 experimental phase.

8

### 9 **Re-training**

10 Before the beginning of each of the following tests, chicks underwent a re-  
11 training. The experimental procedure was identical to that used in the training  
12 phase. The re-training criterion was fixed at three consecutive correct  
13 responses. All chicks passed this phase in 5–10 minutes. A few minutes after  
14 the completion of the re-training, chicks underwent a test.

15

### 16 **Sagittal test**

17 Two hours after training each chick underwent a re-training. Immediately  
18 thereafter, chicks underwent the sagittal test. This consisted of 20 consecutive  
19 trials. The experimental apparatus and the series was exactly identical to those  
20 used during training. During each trial, the chick was allowed one peck. Only  
21 correct responses could be reinforced: the food reinforcement was available  
22 only in some pre-established trials (trial number 4, 5, 7, 10, 13, 14, 16 and 19),  
23 and chicks could gain the food only by correctly choosing in those trials<sup>16,37,38</sup>  
24 this reward schedule was applied also for the fronto-parallel test. This rewarding

1 schedule is needed to prevent extinction of responses over multiple unrewarded  
2 test trials<sup>12,16</sup>. All other trials were unrewarded. If no response occurred within  
3 60 seconds, the trial was terminated. At the end of each trial, the chick was  
4 gently placed back at the starting box and after approximately 5 seconds it was  
5 given a new trial.

6

### 7 **Fronto-parallel tests**

8 In the morning of the fifth day, each chick underwent a re-training and then at a  
9 fronto-parallel test. During fronto-parallel tests, we used a series of 10 elements  
10 (**Fig.1b**); the elements were fronto-parallel oriented with respect to the slit (that  
11 allowed the chick to enter the arena) and approximately 35.0 cm away from it.  
12 Thus, the new test series was rotated by 90° when compared with the training  
13 series. The distance between elements was enlarged (i.e. 5.35 cm), with  
14 respect to the inter-element distance which was used during training, but it was  
15 kept constant throughout the 20 testing trials. We specifically selected this  
16 distance, to create a conflict between the spatial and the ordinal information: the  
17 3<sup>rd</sup> element of this new series was located at the same absolute distance, from  
18 the end of the series, in which was the 4<sup>th</sup> element in the previous phases (20.3  
19 cm from the beginning of the series). The overall length of the series was 73.15  
20 cm and the ends of the series were at 3.4 cm from the arena's lateral walls.

21 The fronto-parallel test was conducted in three different conditions of vision:  
22 binocular, left monocular and right monocular. The interval between two  
23 consecutive tests was of two hours, and before each test chicks underwent re-  
24 training. All chicks firstly underwent the binocular test, then they underwent the

1 two monocular tests, in balanced order. Even if the order of the monocular tests  
2 was shown not to have an effect chicks' performance in a "space and number"  
3 ordinal task<sup>16</sup>, a group of chicks (n= 6) underwent the left-monocular fronto-  
4 parallel test as second test, another group (n=7) underwent the right-monocular  
5 fronto-parallel test as second test.

6 Twenty minutes before the beginning of a monocular test, a temporary eye  
7 patch -made of special, removable, paper tape- was gently applied so as to  
8 occlude vision from one eye (without preventing normal blinking). This period  
9 allowed subjects to get acquainted to the new condition of vision before the  
10 testing time. Before the binocular test, chicks received an equal amount of  
11 handling and acquainting time, but in this case the eye-patch was not applied).

12

### 13 **RESULTS**

14 For each test, we scored the pecks emitted by each chick to any of the 10  
15 elements and we computed the percentages of correct responses [(number of  
16 pecks to a given element/20) x 100] and averaged separately. We planned to  
17 use parametric paired t-tests to compare: i) the pecks emitted at the target  
18 elements (4<sup>th</sup> from left and 4<sup>th</sup> from right), ii) the pecks emitted in the correct  
19 ordinal position (to the 4<sup>th</sup> element), iii) the pecks emitted in the correct spatial  
20 location (to the 3<sup>rd</sup> element). Thus for this last analysis, the planned  
21 comparisons were: i) the 4<sup>th</sup> left element vs. the 3<sup>rd</sup> left element, ii) the 4<sup>th</sup> right  
22 element vs. the 3<sup>rd</sup> right element.

23 For each test, before each pairwise analysis we run a test to check of normality  
24 (Shapiro-Wilk). Since the Shapiro-Wilk test indicated that data were not

1 normally distributed, we used the non-parametric equivalent: the One-sample  
2 Wilcoxon Test to analyze departures from chance level (10%) and the  
3 Wilcoxon's signed-rank test to analyze differences among the 3<sup>rd</sup> and the 4<sup>th</sup>  
4 elements. We reported the effect size as the rank-biserial correlation ( $r_B$ ). We  
5 conducted all analyses using Jasp 0.11.1. Sample size was calculated using the  
6 formula for quantitative variables:  $n=(2\sigma^2)/(\mu_1-\mu_2)^2 \times f(\alpha,\beta)$ ; with the following  
7 values:  $\alpha=0.05$ ;  $\beta=0.80$ ; average=30%; standard deviation=18%. In this  
8 formula:  $\sigma$  is the variance;  $\mu_1$  and  $\mu_2$  are the means of the two groups;  $f(\alpha,\beta)$  is  
9 a function of type I error ( $\alpha$ ) and type of II error ( $\beta$ ). The sample size was  
10 evaluated according to the principle of Reduction in animal research and  
11 approved by the University Committee for animal welfare.

12

### 13 ***Sagittal test:***

14 Chicks pecked at the 4<sup>th</sup> element above chance (N=13, Mean=53.846,  
15 ES=2.542; Median=55.000; V=91.000,  $p=0.002$ ,  $r_B=1.000$ ); also the 3<sup>rd</sup> element:  
16 was pecked above chance (N=13, Mean=19.231, ES=3.041, Median=20.000;  
17 V=59.500,  $p=0.020$ ,  $r_B=0.526$ ) (see **Fig. 2a**). However, chicks chose more often  
18 the 4<sup>th</sup> rather than the 3<sup>rd</sup> element ( $W=91.000$ ,  $p=0.002$ ,  $r_B=1.000$ ).

19 Since the first rewarded trial in all tests was the 4<sup>th</sup> trial, we restricted the  
20 analysis to the first four trials, to exclude any effect of learning during testing. It  
21 appeared that from the very first trials chicks selected the 4<sup>th</sup> element above  
22 chance (N=13, Mean=63.462, ES=3.598; Median=75.000; V=91.000,  $p=0.001$ ,  
23  $r_B=1.000$ ); the 3<sup>rd</sup> element was not selected above chance (N=13,  
24 Mean=15.385, ES=5.325, Median=0.000; V=63.000,  $p=0.020$ ,  $r_B=5.000$ ) (see

1 **Fig. 3a).** Chicks chose more often the 4<sup>th</sup> rather than the 3<sup>rd</sup> element  
2 (W=66.000, p=0.003, r<sub>B</sub>=-1.000).

3

4

5 ***Fronto-parallel tests:***

6 *Binocular test:*

7 Chicks selectively chose both elements in the 4<sup>th</sup> position above chance (4<sup>th</sup> left  
8 element: Mean=41.538, ES=3.978, Median=45.000; V=78.000, p=0.001,  
9 r<sub>B</sub>=0.714; 4<sup>th</sup> right element: Mean=16.538, ES=3.222, Median=15.000;  
10 V=62.000, p=0.037, r<sub>B</sub>=0.590) (see **Fig. 2b**). They did not peck on the elements  
11 in the 3<sup>rd</sup> position above chance (3<sup>rd</sup> left element: Mean=5.769, ES=2.029,  
12 Median=5.000; V=15.000, p=0.957, r<sub>B</sub>=0.071; 3<sup>rd</sup> right element: Mean=3.462,  
13 ES=1.042, Median=5.000; V=0.000, p=0.999, r<sub>B</sub>=-1.000).

14 The chicks pecked more often at the 4<sup>th</sup> left than the 4<sup>th</sup> right element  
15 (W=72.500, p=0.010, r<sub>B</sub>=0.859). They selected more often the 4<sup>th</sup> elements than  
16 the 3<sup>rd</sup> element both on the left and on the right side (left: W=91.000, p=0.002,  
17 r<sub>B</sub>=1.000; right: W=55.000, p=0.006, r<sub>B</sub>=1.000).

18 For what concerns the first four trials, chicks selectively chose the 4<sup>th</sup> elements  
19 above chance (4<sup>th</sup> left element: Mean=44.231, ES=6.426, Median=50.000;  
20 V=90.000, p<0.001, r<sub>B</sub>=1.308; 4<sup>th</sup> right element: Mean=21.154, ES=6.231,  
21 Median=25.000; V=70.000, p=0.044, r<sub>B</sub>=4.000) (see **Fig. 3b**). No difference was  
22 there between choice of the 4<sup>th</sup> left and the 4<sup>th</sup> right element (W=54.000,  
23 p=0.064, r<sub>B</sub>=0.636).

1 *Left monocular test:* Chicks selectively chose the 4<sup>th</sup> left element above chance  
2 (4<sup>th</sup> left element: Mean=31.923, ES=4.441, Median=30.000; V=76.500, p=0.002,  
3  $r_B=0.681$ ), but not the 4<sup>th</sup> right element (Mean=5.385, ES=1.647, Median=5.000;  
4 V=5.000, p=0.992,  $r_B=-0.643$ ) (see **Fig. 2c**). Also the 3<sup>rd</sup> left element was  
5 pecked above chance (Mean=22.308, ES=2.164, Median=25.000; V=76.500,  
6 p=0.002,  $r_B=0.681$ ), but not the 3<sup>rd</sup> right element (Mean=2.692, ES=1.342,  
7 Median=2.000; V=2.000, p=0.0999,  $r_B=-0.600$ ).

8 Chicks pecked more often at the 4<sup>th</sup> left element than at the 4<sup>th</sup> right element  
9 (W=78.000, p=0.002,  $r_B=1.000$ ). They pecked at the 4<sup>th</sup> just as much as the 3<sup>rd</sup>  
10 elements, both on the left and on the right sides (left: W=52.500, p=0.089,  
11  $r_B=0.591$ ; right: W=14.000, p=0.090,  $r_B=0.867$ ).

12 For what concerns the first four trials, chicks selectively chose the 3<sup>rd</sup> and the  
13 4<sup>th</sup> left elements above chance (3<sup>rd</sup> left element: Mean=23.077, ES=5.979,  
14 Median=25.000; V=76.000, p=0.017,  $r_B=3.222$ ; 4<sup>th</sup> left element: Mean=32.692,  
15 ES=7.692, Median=25.000; V=81.000, p=0.007,  $r_B=2.600$ ) (see **Fig. 3c**). No  
16 difference was there between choice of the 3<sup>rd</sup> and the 4<sup>th</sup> left element  
17 (W=22.000, p=0.193,  $r_B=0.571$ ).

18 *Right monocular:* The chicks chose both the 4<sup>th</sup> right element and the 3<sup>rd</sup> right  
19 element above chance (4<sup>th</sup> right element: Mean=25.000, ES=3.397,  
20 Median=25.000; V=76.000, p=0.002,  $r_B=0.670$ ; 3<sup>rd</sup> right element: Mean=29.625,  
21 ES=3.645, Median=30.000; V=85.500, p=0.001,  $r_B=0.967$ ); but not the 4<sup>th</sup> left  
22 element (Mean=5.000, ES=1.179, Median=0.000; V=9.000, p=0.993,  $r_B=-1.000$ )  
23 and the 3<sup>rd</sup> left element (Mean=0.769, ES=0.521, Median=0.000; V=0.000,  
24 p=1.000,  $r_B=-0.143$ ) (see **Fig. 2d**).

1 Chicks pecked more often at the 4<sup>th</sup> right elements than at the 4<sup>th</sup> left element  
2 (W=76.000, p=0.004, r<sub>B</sub>=0.949). They identically selected the 4<sup>th</sup> right element  
3 and the 3<sup>rd</sup> right element (W=24.000, p=0.445, r<sub>B</sub>=-0.273).

4 In the first four trials, chicks selectively chose the 3<sup>rd</sup> and the 4<sup>th</sup> right elements  
5 above chance (3<sup>rd</sup> right element: Mean=19.231, ES=5.027, Median=25.000;  
6 V=76.000, p=0.016, r<sub>B</sub>=3.222; 4<sup>th</sup> right element: Mean=32.692, ES=7.153,  
7 Median=25.000; V=85.000, p=0.003, r<sub>B</sub>=2.091) (see **Fig. 3d**). No difference was  
8 there between the 3<sup>rd</sup> and the 4<sup>th</sup> right element (W=47.000, p=0.219, r<sub>B</sub>=0.424).

9  
10

11 **DISCUSSION**

12 The aim of this study was to disentangle the engagement of either hemisphere  
13 in dealing with the ordinal and spatial information in a serial ordinal task.  
14 Results of the sagittal test, performed in binocular condition of vision, indicate  
15 that chicks correctly memorized and used the integration of spatial and  
16 numerical cues to locate the target element. These findings are in line with  
17 previous studies<sup>10-12,16</sup> and sustain the idea that birds are precociously capable  
18 to use ordinal-numerical information to find a food source. Birds, in the sagittal  
19 test, chose the target element (the 4<sup>th</sup> one) over all the others. Whenever the  
20 analysis was restricted to the very first four trials, only the 4<sup>th</sup> element was  
21 selected above chance. If we consider the whole test, chicks pecked also at the  
22 3<sup>rd</sup> element above chance, but the 4<sup>th</sup> element was selected more than the 3<sup>rd</sup>. A  
23 possible explanation for this could be that, in identifying the target element,  
24 chicks anchored their evaluation on the closest end of the sequence (i.e.,

1 somewhat similarly to the “working up strategy” described by Suzuki and  
2 Kobayashi (2000)<sup>39</sup>. This possible interpretation is also supported by the  
3 outcome of a previous study. In such a study, different groups of day-old chicks  
4 learnt to identify the 3<sup>rd</sup>, the 4<sup>th</sup> or the 6<sup>th</sup> element in a sagittal series on ten  
5 identical elements. Whenever the 3<sup>rd</sup> (or the 4<sup>th</sup>) element were the target, chicks  
6 made more errors respectively on the 2<sup>nd</sup> (or on the 3<sup>rd</sup>) element; while chicks  
7 for whom the 6<sup>th</sup> elements was the target mistook more on the 7<sup>th</sup> element<sup>10</sup>.  
8 These results can indicate that birds would anchor to the 1<sup>st</sup> element, to identify  
9 the 3<sup>rd</sup> and the 4<sup>th</sup> element, hence making more mistakes on the element  
10 immediately preceding the target rather than on the element following the  
11 target. Similarly, the chicks may anchor to the last element (i.e., the 10<sup>th</sup> one)  
12 when identifying the 6<sup>th</sup> element (i.e., see the “working down strategy” described  
13 by Davis and Bradford (1986)<sup>40</sup>.  
14 Results from the binocular fronto-parallel test showed that chicks identified the  
15 target element, basing on serial ordering, and neglecting the elements at the  
16 correct distance (the 3<sup>rd</sup> elements). Both 4<sup>th</sup> elements were pecked above  
17 chance; considering the whole test, chicks pecked more often at the left than at  
18 the right target, but restricting the analysis to the first four trials, the 4<sup>th</sup> elements  
19 were equally selected (for similar findings see<sup>12</sup>). They were able to generalize  
20 what learnt to a new series, characterized by i) a different orientation with  
21 respect to the one experienced during training, ii) a different metric, in fact by  
22 increasing the inter-element distance also the overall length of the series  
23 increased. Interestingly enough they selected the numerical-ordinal target  
24 elements in a presence of a conflict between the numerically correct target (the

1 4<sup>th</sup> elements) and the spatially correct one (the 3<sup>rd</sup>, which was the one located at  
2 the correct distance at training).

3 To disentangle the engagement of each hemisphere in dealing with the “spatial  
4 vs. ordinal” test, we used the monocular occlusion technique. The domestic  
5 chick is a unique model to study hemispheric specialization. In binocular  
6 condition of vision, the information from the eyes reaches, throughout the  
7 tectofugal and thalamofugal pathways, the respective telencephalic areas: the  
8 Ecostriatum and the visual Wulst<sup>35</sup>. However, thanks to the decussation at the  
9 optic chiasm of both the tectofugal and thalamofugal pathways<sup>41</sup> and to an  
10 almost total lack of interhemispheric connections (see introduction), a simple  
11 temporary patch over one eye can be used to discern hemispheric  
12 specializations<sup>42</sup>. A common feature of the two visual pathways is their  
13 lateralization<sup>43</sup>, in chicks stimulus processing is usually carried out by the eye-  
14 system specialized for the given task<sup>44</sup>. If one hemisphere is dominant in  
15 processing spatial or ordinal information, we would expect an asymmetry in  
16 behaviour when the information is elaborated with the “specialized” eye-system.

17 In both monocular conditions of vision, the birds efficaciously performed at the  
18 fronto-parallel test, but in these cases they relied on both numerical and spatial  
19 cues. In fact, they identically chose the 3<sup>rd</sup> and the 4<sup>th</sup> element (either on the left  
20 or on the right) significantly above chance. This is true when we considered the  
21 whole 20 testing trials as well as when we limited the analyses to the very first  
22 four trials. In monocular conditions of vision birds mainly directed their  
23 responses on the elements located in their clear hemifield. Because of the eye  
24 patch, only one end of the series either left or right was clearly visible, and

1 chicks used this side as benchmark to start to “count”. Whenever their vision  
2 was limited to the left eye, they equally chose the 3<sup>rd</sup> and the 4<sup>th</sup> left elements;  
3 when they could see with their right eye, they equally chose the 3<sup>rd</sup> and the 4<sup>th</sup>  
4 right elements. Thus in different trials the animals relied either on spatial or  
5 ordinal cue. This shows that the spatial and ordinal information acquired during  
6 binocular training can be correctly represented, and independently used by  
7 each hemisphere, though with a different anchor end that identifies the starting  
8 point for “counting”. This anchoring has been reported also when both spatial  
9 and ordinal cues were available at fronto-parallel test<sup>16</sup>. The bilateral  
10 hemispheric representation of numerical processing found here is coherent with  
11 scientific literature<sup>45,46</sup>. Overall these findings enlarge our knowledge on the left-  
12 to-right oriented bias previously reported in birds<sup>11</sup> and non-human primates<sup>20</sup>.  
13 Here we show that both hemispheres encoded and used spatial and ordinal  
14 cues to find a food source. The left bias reported in previous studies seems  
15 therefore to be based on an integration of numerical and spatial cue by the right  
16 hemisphere.

17 Up to now there is no study that has investigated how numerical-ordinal  
18 information is elaborated and integrated by the two hemispheres. Future studies  
19 are needed to understand (i) how and where this integration of information  
20 occurs and (ii) if a same mechanism can be potentially extended to explain  
21 other SNA effect, like associations between numerosities and sides in space,  
22 which were described in infants<sup>7-9</sup> and also in chicks<sup>15</sup>. An insightful reflection  
23 could arise by comparing the behavioural responses in the fronto-parallel test  
24 performed in binocular conditions of vision with those of the monocular

1 conditions of vision. Whenever the birds could use both cerebral hemispheres,  
2 they selectively relied on numerical-ordinal cues, whereas when the suitable  
3 hemisphere was only one (either the left or the right one), they relied on both  
4 ordinal-numerical and on spatial cues. This might allow to speculate that the  
5 basis of the spatial numerical association (SNA) might be accounted for by a  
6 model that assumes differential encoding, processing and integration by the two  
7 hemispheres for spatial and numerical information. Up to now there is no  
8 sufficient evidence to individuate the origin of SNA. Nevertheless, our results  
9 seem to indicate that a valuable answer will be obtained once we will have  
10 better understood how and where numerosnesses are represented within the  
11 brain.

12

13

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23

24

1 **AUTHOR CONTRIBUTIONS**

2 R.R. and L.R. designed the study; R.R. analyzed the data; both authors  
3 interpreted the results; R.R. wrote the paper and created the figures; L.R.  
4 critically reviewed the paper.

5

6

7 **COMPETING INTERESTS**

8 All authors must include full disclosure statements at time of submission as a  
9 section at the end of the manuscript text.

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13 **Figure legends**

14 Fig. 1. Schematic illustration of the apparatus and the series of elements; “d”  
15 indicates the distance from the closest end of the series during training. All  
16 elements looked identical, but in the illustration, the target is grey. “S.B.”  
17 represent the starting box; the chick’s starting position. (a) The disposition of the  
18 series during training and during the sagittal test. (b) The disposition of the  
19 series during the fronto-parallel tests.

20 Fig. 2. The graphs represent the mean percentage  $\pm$  SE of choices for each  
21 element in the 20 testing trials; the dotted line represents chance level ( $y=10$ );  
22 asterisks indicate  $p<0.05$ ; the schematic chick heads indicate the condition of  
23 vision; the schematic illustration of the apparatus and of the series indicates the  
24 test. (a) Results of the binocular sagittal test. (b) Results of the binocular fronto-

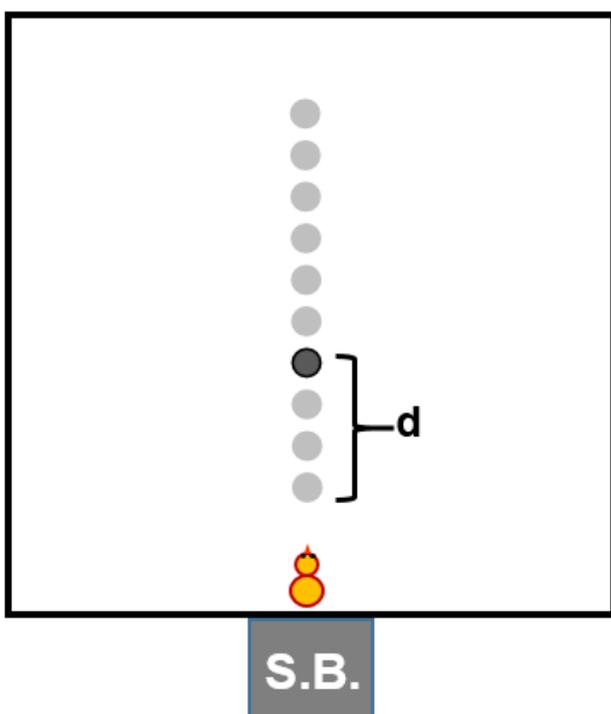
1 parallel test. (c) Results of the left monocular fronto-parallel test. (d) Results of  
2 the right monocular fronto-parallel test.

3 Fig. 3. The graphs represent the mean percentage  $\pm$  SE of choices for each  
4 position in the first four trials; the dotted line represents chance level ( $y=10$ );  
5 asterisks indicate  $p<0.05$  (a) Results of the binocular sagittal test. (b) Results of  
6 the binocular fronto-parallel test. (c) Results of the left monocular fronto-parallel  
7 test. (d) Results of the right monocular fronto-parallel test.

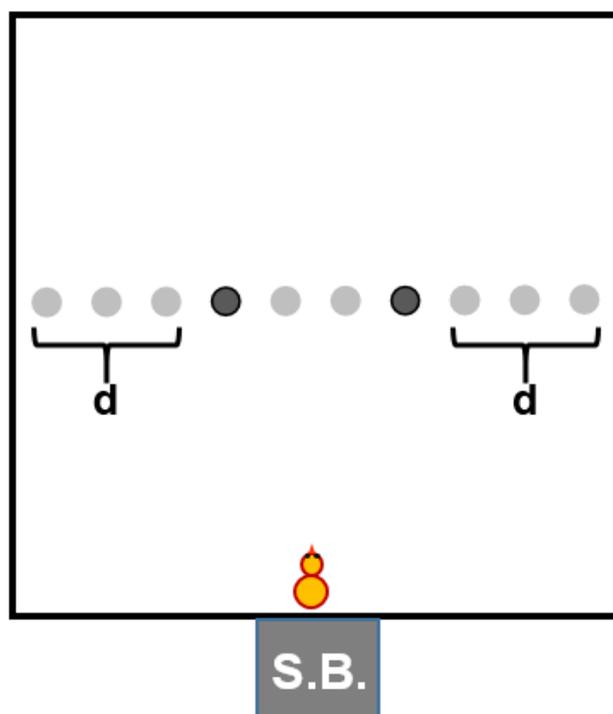
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9 **Figures**

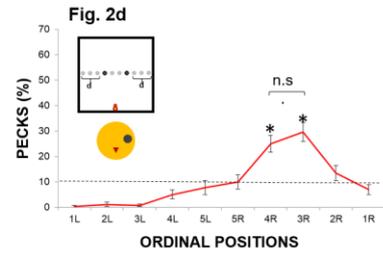
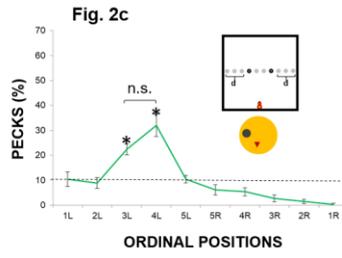
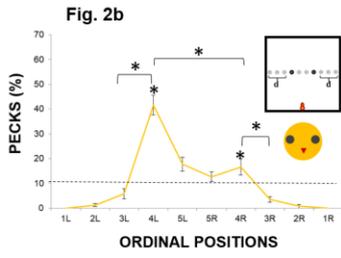
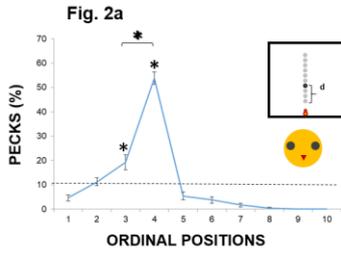
**Fig. 1a**



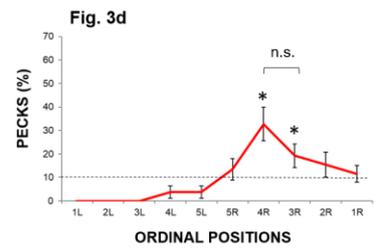
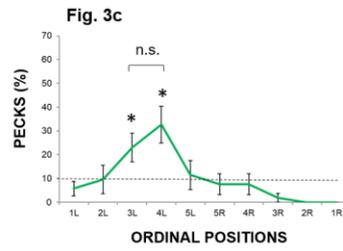
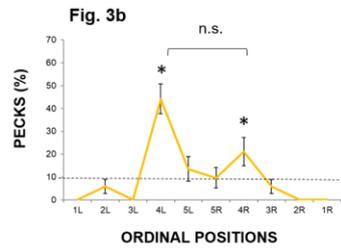
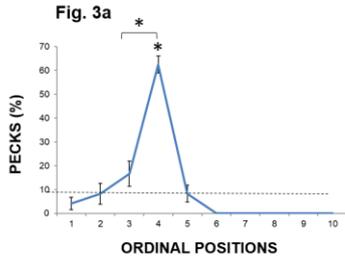
**Fig. 1b**



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