Title

Hatching with Numbers: Pre-natal Light Exposure Affects Number Sense and the Mental Number Line in young domestic chicks

Short Title

Prenatal stimulation enhances numerical cognition

Authors

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Abstract

Humans order numerosity along a left-to-right Mental Number Line (MNL), traditionally considered culturally rooted. Yet, some species at birth show spatial-numerical associations, suggesting neural origins. Various accounts link MNL to brain lateralization, but lack evidence.

We investigated brain lateralization effects on numerical spatialization in 100 newborn chicks. In-ovo light exposure yielded strongly-lateralized brains in half the chicks and weakly-lateralized in the other half. Chicks learned to select the 4th item in a sagittal array. At test, the array was rotated 90°, with left and right 4th items correct. Strongly-lateralized chicks outperformed weakly-lateralized ones when ordinal and spatial cues were reliable (experiment 1), but not with unreliable spatial cues (experiment 2). Moreover, only strongly-lateralized chicks showed left-to-right directionality, suggesting the right hemisphere's key role in integrating spatial and numerical cues. We demonstrate that brain lateralization is fundamental for developing a left-to-right oriented MNL.

Keywords

Number Sense, Brain lateralization, Prenatal stimulation, Mental Number Line, Spatial-numerical association, Comparative cognition

Introduction

Humans share with other animals basic numerical capacities[1–5], this evolutionarily ancient number sense serves as a building block for our unique mathematical abilities[6]. A distinctive feature of the human numerical system is the Mental Number Line, MNL: an association of small numbers with the left and large ones with the right[7]. Traditionally, this has been considered a by-product of culture, primarily linked to reading and writing direction[8,9]. However, evidence of numerical spatialization in young infants[10–14], newborns[15–18] and non-humans[19–25] has challenged the predominant role of culture in determining the left-to-right orientation of the MNL and indicates that nervous systems across various species, despite their different levels of complexity, are prewired in how they relate numbers to space.

The first evidence of numerical spatialization in non-human subjects was observed in day-old domestic chicks[20,21] and adult Clark's nutcrackers[20]. Birds learned to identify a target item, e.g., the 4th, in a sagittally oriented array of identical items. They were then tested with the array rotated by 90°, thus laying on a fronto-parallel plane with the items oriented left-to-right. Animals selected the 4th left item more often than the 4th right one, suggesting that number is intrinsically represented from left to right[21]. Using this paradigm, the same left-to-right proto-counting tendency has been observed also in adult rhesus monkeys[19], and preschoolers[11].

Behavioral observations supporting the Mental Number Line have increased [11,13,14,16–20,23–26]; but see for null results[27,28]. Concurrently, specific neurons responding to numerosities have been identified in human[29–31], monkey[32], crow[33], and chick[34] brains. Nevertheless, the biological mechanisms and neural underpinnings of MNL are still largely unknown.

The right hemisphere is specialized for analyzing spatial information[35,36] and for processing numerical information[30,37]. This functional overlap[38,39] has been proposed as the basis for the spatial-numerical association that prompts animals to start counting from left to right. This will be referred to as the right-hemisphere dominance model[23,40]. Two other explicatory models have been put forward: the emotional-valence[4] and the Brain Asymmetric Frequency Tuning, BAFT[41].

The emotional-valence model is grounded in evidence demonstrating that the right hemisphere processes negative emotions and the left hemisphere handles positive ones[4,42]. This model posits that small numerosities are associated with a negative valence, and activate the right hemisphere that directs movement toward the left; large numerosities would be associated with a positive valence, activating the left hemisphere that leads movement toward the right[4].

BAFT links spatial frequency processing to higher cognitive functions. The right hemisphere specializes in processing low spatial frequencies, while the left hemisphere high frequencies[43]. Because smaller numerosities correspond to low frequencies, and large numerosities to high frequencies, such hemispheric specialization may explain MNL[41].

The core information (spatial, emotional, or spatial frequency) central to each model, and the consequent hemispheric specialization involved in processing such information changes. All models agree on hemispheric specialization underlying MNL, despite this, no research has explored whether brain lateralization affects number spatialization. Direct manipulation of brain lateralization is essential to determine if and how it affects spatial numerical association. The origin of the Mental Number Line could be clarified if specific predictions could be made based on the different core information (spatial, emotional, or spatial frequency) targeted by each of the three models (for predictions, see Results).

In the present study, the level of hemispheric lateralization was manipulated to assess the effect on number spatialization. Monocular occlusion was used to disentangle the role of the two cerebral hemispheres.

Lateralization levels in domestic chicks can be easily manipulated by controlling exposure to light in the final period of incubation, between embryonic days 18 and 21[44,45]. During development, the embryo rotates such that the right eye faces outward toward the translucent

eggshell and any available environmental light. In contrast, the left eye is oriented toward the body, receiving little to no light. The chick embryo's visual system, specifically the thalamofugal pathway, undergoes differentiation in-ovo. Egg exposure to light leads to an asymmetrical stimulation of the two eyes such that there is an increase in forebrain projections from the left side of the thalamus (fed by the light-stimulated right eye) compared with the right side[46]. Such asymmetries extend to the strength of visual projections from the thalamus to the visual Wulst (a laminated bulge in the dorsal telencephalon, functionally analogous to mammalian visual cortex[44,47]). Specifically, the right visual Wulst receives more bilateral information from the two eyes than the left one[48]. Evidence shows that as little as two-hour light exposure prior to hatching is sufficient to induce these brain asymmetries[49,50]. As a consequence of light-enhanced hemispheric differentiation, light-incubated chicks, Li-chicks, are strongly lateralized, as demonstrated by cognitive and behavioral biases[51]. For the present study, we expect that Li-chicks will exhibit a clearer left-to-right oriented numerical spatialization compared to dark-incubated chicks, Di-chicks, whose brain lateralization is mainly prevented[45].

Additionally, higher levels of lateralization enhance cognitive performance[51]. Strongly lateralized chicks perform better in dual tasks requiring simultaneous foraging and predator monitoring[52], in discrimination and categorization tasks such as distinguishing pebbles from food grains[50], and in transitive inference tasks where they learn hierarchical stimulus relationships (A > B > C > D > E) that then apply to novel pairings (i.e., AE and BD)[53]. While the influence of lateralization on numerical cognition is still unknown, based on previous findings, we hypothesize that numerical performance would be enhanced in Li-chicks compared to Di-chicks. This study aims to clarify whether light prenatal stimulation affect later number sense.

To investigate hemispheric dominance, we used temporary monocular occlusion by applying a removable eye patch to cover one eye of the chicks. Due to the complete decussation of fibers at the optic chiasm[54] and lack of a structure homologous to the corpus callosum (even though other smaller tracts allow interhemispheric communication[55,56]), information received by each eye is mainly elaborated by the contralateral hemisphere[46,57]. Restriction of the visual input by monocular occlusion will allow us to disentangle how the two hemispheres elaborate ordinal information, and to test whether one hemisphere is dominant.

Results

Here, we tested 100 male domestic chicks (*Gallus gallus*) of the Aviagen ROSS 308 line (experiment 1, n = 48; experiment 2, n = 52). Dark-incubated chicks (Di-chicks; n=24 in experiment 1, n =26 in experiment 2) were obtained from eggs incubated in darkness, while Light-incubated chicks (Li-chicks; n =24 in experiment 1, n =26 in experiment 2) were obtained from eggs exposed to light (LED 4.8W lightbulb) from day 18 to 21 of incubation.

All chicks were trained to peck for food reward at the 4th item in an array of 10 identical, equally-spaced, and sagittally aligned items (plastic caps); Fig. 1A. Each chick underwent four tests. In the first test, the array was oriented as during training, i.e., sagittally with respect to the chick's starting point; Sagittal test, Fig. 1A. This test was run in Binocular condition of vision.

Subsequently, each chick underwent tests in which the array was rotated by 90° so as to lay fronto-parallel with respect to the chick's starting position; Fronto-Parallel tests; Fig. 1B. Fronto-Parallel tests were administered to the chick in three conditions of vision: Binocular, Fig. 1C, Monocular-Left (right eye patched and left eye in use; Fig. 1D), and Monocular-Right (left eye patched and right eye in use; Fig. 1E).

The Fronto-Parallel test presented the array as oriented from left to right. This orientation produces two possible correct options, both equidistant from the subject: the 4th left and the 4th right item; Fig. 1B. The critical difference between experiment 1 and 2 was that, during testing, spatial information was available in experiment 1, but was unreliable in experiment 2. Specifically, in experiment 1, the inter-item distance and the total length of the array was constant

and identical to the training array, thus chicks could identify the items on the basis of either ordinal or spatial cues. In experiment 2, the inter-item distance was uniform within each trial, but varied systematically between trials (1.44 cm, 2.55 cm, 3.11 cm, and 3.66 cm resulting in total array lengths of respectively 43.0, 53.0, 58.0, and 63.0 cm) thus the spatial information was unreliable.

These experimental manipulations allow us to test and compare the three models proposed to explain the origin of the Mental Number Line.

i) According to the right-hemisphere dominance model[23,40], which highlights the role of the right hemisphere in processing spatial and numerical cues, a left bias is expected whenever

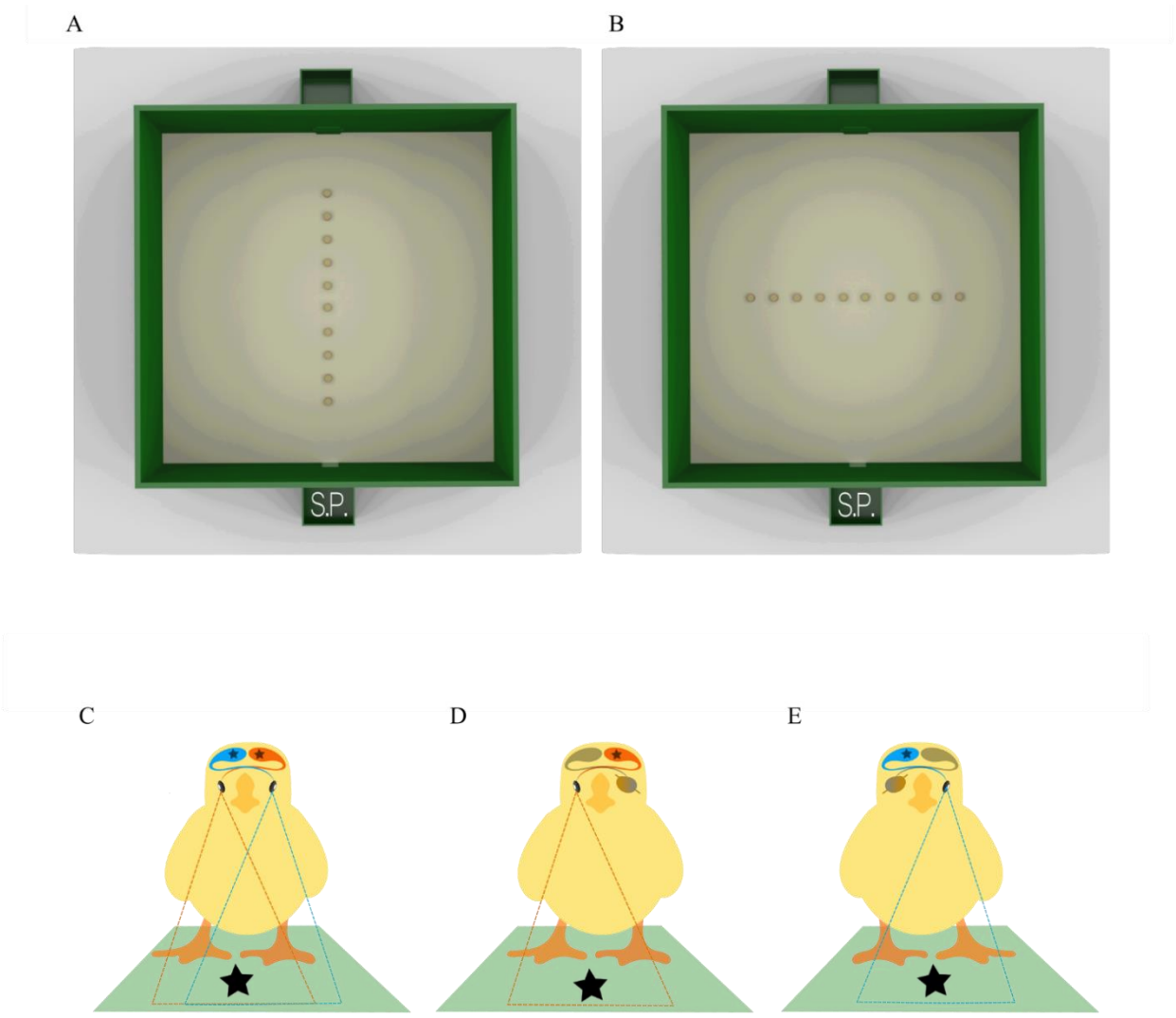

Fig. 1. Illustration of the experimental apparatus and of the three conditions of vision. (**A**) Array configuration used at training and Sagittal test. (**B**) Array configuration used for the Fronto-Parallel tests. S.P. indicates the chick's starting position. (**C**) Binocular condition of vision: both eyes, thus both hemispheres (left in red and right in blue), in use. (**D**) Right monocular condition with the right eye and the left hemisphere in use, in red. (**E**) Left monocular condition with the left eye and the right hemisphere in use, in blue. In monocular conditions, the eye not receiving light and its contralateral hemisphere are depicted in gray.

both eyes and hemispheres are in use, particularly in individuals with greater interhemispheric

differences, i.e., Li-chicks. Moreover, the left bias should be more pronounced when only the right hemisphere processes information, as in the monocular-left eye condition.

ii) Following the emotional-valence model[4], which suggests that the left hemisphere processes positive emotions, considering that food itself is associated with positive emotions, a right bias might be expected in bilateral processing during food search. This bias should be more pronounced in Li-chicks due to their enhanced hemispheric differentiation.

iii) As for the BAFT model[41], the left/right symmetry in item disposition within the array would lead to an absence of bias with no difference between strong and weak lateralized animals.

In each trial, chicks were allowed a single peck. We recorded the selected item to calculate the percentage of responses at each position and averaged them separately for each group and test. We analyzed the group percentage for choosing the 4th item above chance (10%), using Bonferroni correction for multiple comparisons (data and significant results are reported in Table 1; Additional analyses on the selection of each item are reported in **Error! Reference source not found.** and **Error! Reference source not found.**). To assess side bias in the Fronto-Parallel tests, we compared correct choices on the left (4L) *versus* the right (4R) using a paired *t* test, with Cohen's *d* as the effect size, and Bonferroni correction, see Table 1. Moreover, we tested whether brain lateralization influenced accuracy by comparing the percentage of correct choices (i.e., the selection of the 4th item in the Sagittal test; the 4L or 4R items in the Fronto-Parallel tests) between Li-chicks and Di-chicks using a two-sample *t* test, with Cohen's *d* as the effect size, and Bonferroni correction. We conducted both frequentist and Bayesian statistics to ensure reliable interpretations of our results.

Results in the tests allowing utilization of reliable ordinal and spatial cues (experiment 1)

Sagittal test conducted under binocular vision condition.

In the Sagittal test (Fig. 2A), Li-chicks selected the 2nd and 4th items above chance; Di-chicks selected the 1st, 2nd and 4th items above chance (Table 1 and **Error! Reference source not found.**). Moreover, Li-chicks showed higher accuracy in selecting the 4th item than Di-chicks (Li-chicks: n = 24, mean = 36.38, SE = 1.666; Di-chicks: n = 24, mean = 28.44, SE = 1.913; t(45.1) = 3.132, P = 0.003, d = 0.904; BF = 12.588); Fig. 2A.

Fronto-Parallel transfer test conducted under binocular vision condition.

In the Fronto-Parallel Binocular test Fig. 2B), Li-chicks successfully transferred what was learned to the rotated series: they selected only the 4th left item above chance (Table 1). Li-chicks showed a left bias, pecking the 4th left item more than the 4th right one (n = 24, t(23) = 4.791, P < 0.001, d = 1.635; BF = 337.124). Di-chicks pecked both 4th left and 4th right items above chance (Table 1) without a difference (n = 24, t(23) = 0.218, P = 1.000 d = 0.076; BF = 0.219). Remarkably, only Li-chicks showed a left bias, indicating a tendency to proto-count from left to right.

Comparing accuracy between the two groups, Li-chicks selected the 4th left item more than Di-chicks did (Li-chicks: n = 24, mean = 28.85, SE = 2.572; Di-chicks: n = 24, mean = 18.96, SE = 2.149 ; t(44.6) = 2.952, P = 0.010 , d = 0.852; BF = 8.456); while Li-chicks choose the 4th right item less than Di-chicks (Li-chicks: n = 24, mean = 11.89, SE = 1.532; Di-chicks: n = 24, mean = 19.79, SE = 2.321; t(39.8) = -2.840, P = 0.014 , d = 0.820; BF = 6.657).

Fronto-Parallel transfer test conducted under left-monocular vision condition.

In the Fronto-Parallel Monocular-Left test (Fig. 2C), Li-chicks were able to transfer learning to a differently oriented series, correctly selecting the 4th left item above chance, even if they also pecked at the 1st left item (Table 1; **Error! Reference source not found.**). Moreover, they selected the 4th left more than the 4th right item (n = 24, t(23) = 6.056, P < 0.001, d = 2.088; BF =

5598.452,). Di-chicks failed: they only selected the 1st and 2nd left items above chance (**Error! Reference source not found.**).

As for the difference between the two groups, Li-chicks selected the 4th left item more than Di-chicks (Li-chicks, n = 24, mean = 22.59, SE = 1.783; Di-chicks, n = 24, mean = 12.78, SE = 1.696; t(45.9) = 3.988, P < 0.001, d = 1.151; BF = 104.830).

These results suggest that whenever the right hemisphere is processing the information, light exposure affects the left bias and numerical performance. This evidence, on one side, supports the relevance of the right hemisphere in directing SNA directionality[23,58]. On the other side, it shows how significant experiences that stimulate brain development, although limited to a few hours of exposure to moderate ambient light, can boost cognitive performance.

Fronto-Parallel transfer test conducted under right-monocular vision condition.

In the Fronto-parallel Monocular-Right test (Fig. 2D), Li-chicks succeeded correctly in selecting the 4th right item, which was pecked more than the 4th left item (n = 24, t(23) = -6.151, P < 0.001, d = 1.844; BF = 6887.511), even if they also selected the 1st left item above chance (Table 1 and **Error! Reference source not found.**). Di-chicks failed: they did not select the 4th left nor right item above chance; the only item selected above chance was the 1st right one (**Error! Reference source not found.**).

As for the accuracy differences between the two groups, Li-chicks selected the 4th right item more than Di-chicks (Li-chicks: n = 24, mean = 21.48, SE = 2.191; Di-chicks: n = 24, mean = 13.42, SE = 1.792; t(44.3) = 2.850, P = 0.013, d = 0.823; BF = 6.799).

Again, only Li-chicks succeeded while Di-chicks failed, corroborating evidence on the importance of light stimulation in favoring the development of both hemispheres[48] and boosting cognitive performance.

Overall, these data showed that prenatal light experience can stimulate brain development and hemispheric specialization, which emphasizes the Spatial Numerical Association and enhances performance in a spatial/numerical task. This provides novel evidence of the role of brain lateralization in determining SNA and in boosting proto-numerical counting.

Results in the tests allowing utilization of reliable ordinal and unreliable spatial cues (experiment 2)

Sagittal test conducted under binocular vision condition.

In the Sagittal test (Fig. 3A), both groups succeeded: Li-chicks exclusively selected the correct 4th item above chance (Table 1), while Di-chicks selected the 4th item, but also mistakenly pecked the 2nd item (Table 1 and **Error! Reference source not found.**). Yet, accuracy in pecking the 4th item did not differ in the two groups (Li-chicks: n = 26, mean = 32.27, SE = 2.203; Di-chicks: n = 26, mean = 31.49, SE = 3.008; t(45.8) = 0.211, P = 1.000, d = 0.059; BF = 0.283).

Fronto-Parallel transfer test conducted under binocular vision condition.

In the Fronto-Parallel Binocular test (Fig. 3B), Li-chicks and Di-chicks selected both 4th left and right items above chance (Table 1). For each group, no differences emerged in selecting the 4th left and 4th right items, indicating a lack of side bias in both groups (Li-chicks: n = 26, t(25) = 1.910, P = 0.135, d = 0.624; BF = 0.997; Di-chicks: n = 26, t(25) = -0.218, P = 1.000, d = 0.098; BF = 0.219). Li-chicks did not differ from Di-chicks in responses on the 4th left (Li-chicks: n = 26, mean = 21.77, SE = 1.939; Di-chicks: n = 26, mean = 18.73, SE = 1.813; t(49.8) = 1.147, P = 0.513, d = 0.318; BF = 0.478) or 4th right item (Li-chicks: n = 26, mean = 15.80, SE = 1.812; Di-chicks: n = 26, mean = 19.67, SE = 1.966; t(49.7) = -1.446, P = 0.309, d = 0.401; BF = 0.655). This confirms that whenever the spatial information is unavailable at the test, the left bias

disappears[59], highlighting the role of the right hemisphere in processing spatial information and determining the left-to-right orientation of the Spatial-Numerical Association.

Fronto-Parallel transfer test conducted under left-monocular vision condition.

In the Fronto-Parallel Left-Monocular test (Fig. 3C), Li-and Di-chicks failed: Li-chicks selected the 1st left item (**Error! Reference source not found.**); Di-chicks selected the 1st and the 2nd left item above chance (**Error! Reference source not found.**). As for the accuracy, the two groups equally selected the 4th left (Li-chicks: n = 26, mean = 15.01, SE = 1.832; Di-chicks: n = 26, mean = 14.84, SE = 1.937; t(49.8) = 0.066, P = 1.000, d = 0.018; BF = 0.279) and the 4th right items (Li-chicks: n = 26, mean = 7.94, SE = 1.193; Di-chicks: n = 26, mean = 8.33, SE = 1.356; t(49.2) = -0.218 P = 1.000, d = 0.060; BF = 0.284).

Whenever spatial information is unavailable, the right hemisphere fails to transfer tasks. This indicates that independent of the hemisphere's development, unilateral right hemispheric processing is insufficient in dealing with an ordinal task; thus, ordinality does not appear to be lateralized to the right hemisphere.

Fronto-Parallel transfer test conducted under right-monocular vision condition.

In the Fronto-Parallel Monocular-Right test (Fig. 3D), only Li-chicks succeeded and selected the 4th right item; even if they also pecked the 1st and the 2nd right items (Table 1 and **Error! Reference source not found.**), moreover they selected the 4th right more than the 4th left item (n = 26, t(25) = -4.946, P < 0.001, d = - 1.447; BF = 573.520). Di-chicks failed to select the 4th left or right item; instead they selected only the 1st right item above chance (**Error! Reference source not found.**). As for the difference in accuracy between the two groups, Li-chicks did not select the 4th right item more than Di-chicks (Li-chicks: n = 26, mean = 17.98, SE = 1.889; Di-chicks: n = 26, mean = 12.46, SE = 1.815; t(49.9) = 2.110, P = 0.080, d = 0.585; BF = 1.678). This suggests that lateralization influences numerical cognition even in the absence of spatial information, and that the left hemisphere plays a significant role in processing ordinal information.

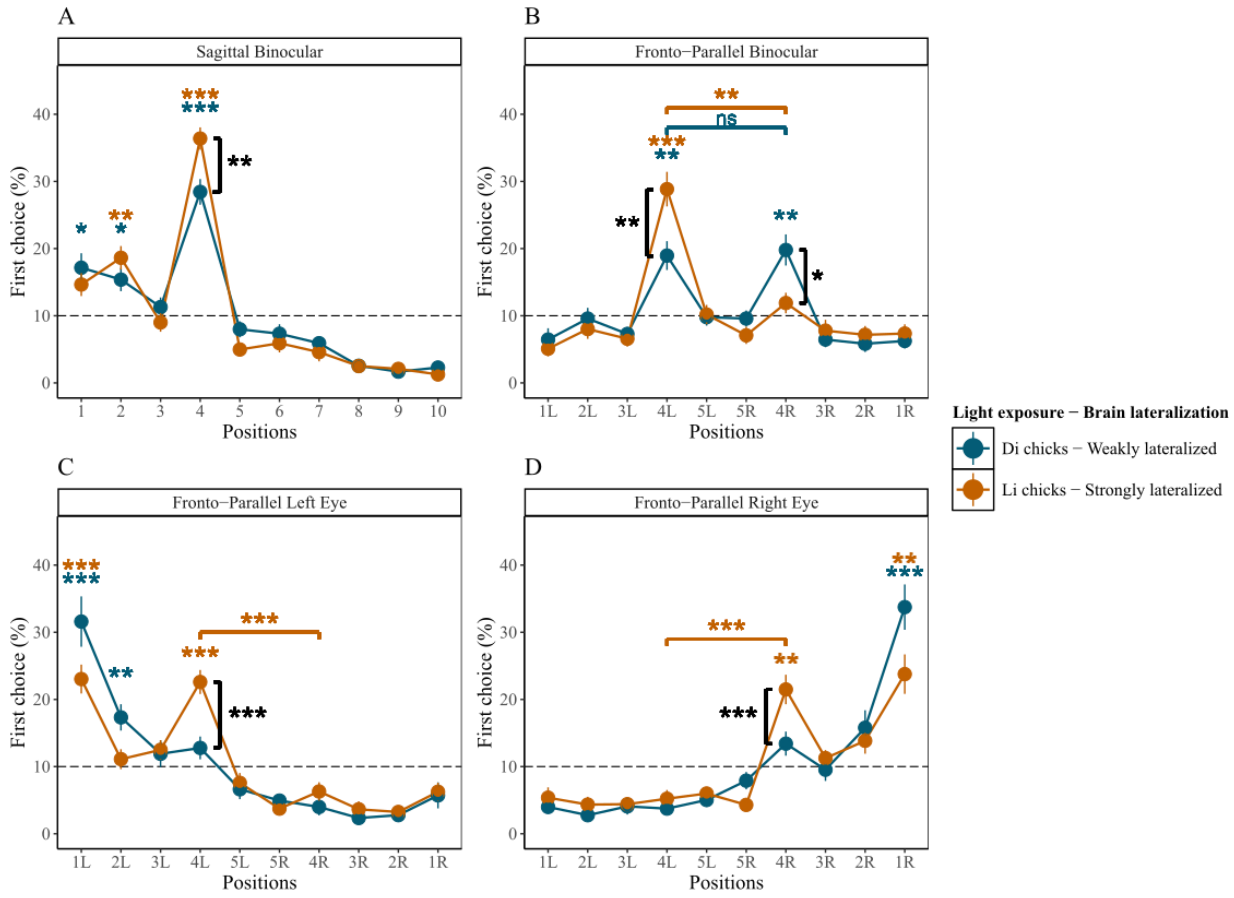

Fig. 2. Results of experiment 1. The average percentage of chicks' choices (y-axis) as a function of item positions (x-axis), light exposure modulating brain lateralization, and tests. Error bars indicate standard errors. The gray dashed line indicates chance level (10%). (**A**) Results of the Sagittal test. (**B**) Results of the Fronto-Parallel Binocular test. (**C**) Results of the Fronto-Parallel Monocular Left test. (**D**) Results of Fronto-Parallel Monocular Right test. ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$.

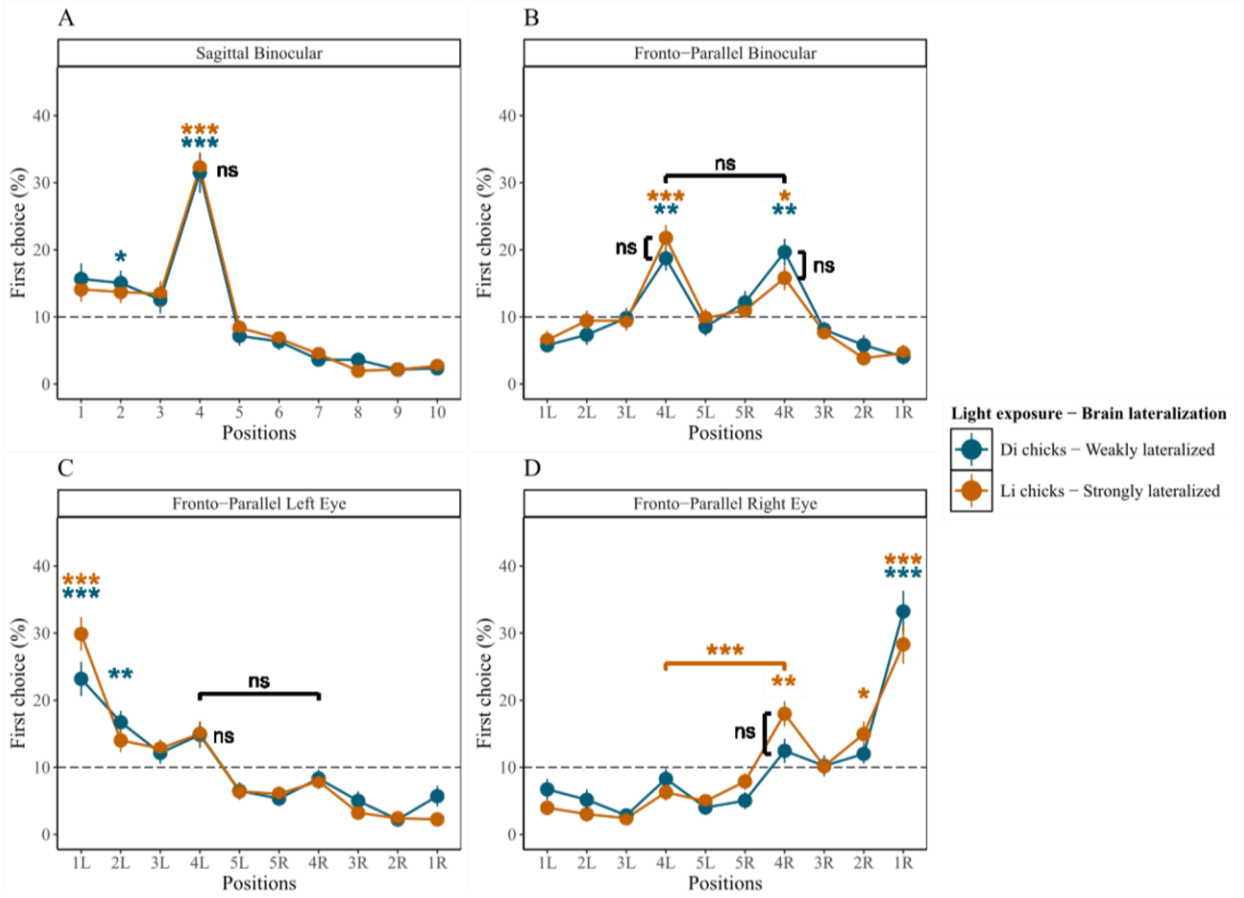

Fig. 3. Results of experiment 2. The average percentage of chicks' choices (y-axis) as a function of item positions (x-axis), light exposure modulating brain lateralization, and tests. Error bars indicate standard errors. The gray dashed line indicates chance level (10%). (**A**) Results of the Sagittal test. (**B**) Results of the Fronto-Parallel Binocular test. (**C**) Results of the Fronto-Parallel Monocular Left test. (**D**) Results of Fronto-Parallel Monocular Right test. ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$

Discussion

General

Our main findings are that prenatal exposure that can modulate brain lateralization in domestic chicks impacts the left-to-right oriented numerical spatialization and numerical performance.

In experiment 1, which allowed chicks to reliably use both ordinal and spatial cues in identifying the 4th item, chicks exhibited different behaviors despite identical learning experiences and tasks, either showing or not showing a left bias, depending on their prenatal light exposure. In the Fronto-Parallel Binocular test, when both eyes and hemispheres processed the information, chicks hatched from light-incubated eggs, Li-chicks, selected only the 4th left item; while chicks hatched from dark-incubated eggs, Di-chicks, equally selected the 4th left and the 4th right item. Only Li-chicks (i.e., more strongly lateralized) demonstrated left-to-right proto-counting, indicating that brain lateralization influences Mental Number Line directionality. When the two hemispheres engaged in differential processing, as observed in Li-chicks, a unidirectional left-to-right oriented numerical spatialization emerged. Conversely, when prenatal stimulation did not enhance hemispheric specialization, resulting in more homogeneous hemispheric processing, animals (Di-chicks) showed no directional bias. These pioneering findings corroborate all models positing hemispheric specialization as the neural basis for spatial numerical association[4,23,40,41], while establishing lateralization essential prerequisite for numerical spatialization. However, pre-

hatching light stimulation did not affect chicks' performance when spatial information was unavailable (experiment 2). This finding substantiates the relevance of spatial information and highlights that its integration with numerical processing within shared cortical regions is fundamental to the neurobiological underpinning of number spatialization. This integration is coherent with the fact that in the chick's brain the right hemisphere is dominant in processing spatial information[35,36], but can also process numerical information[37,59] (consistently with primates and human literature[30]). The present data align with previous research that used monocular occlusion to disentangle the engagement of the two hemispheres with spatial or ordinal cues[37]. In prior research, day-old chicks learned to select the 4th item in an array of 10 identical sagittal-aligned items maintained in fixed positions, so that both spatial and ordinal cues were available during learning. At test, chicks faced a left-to-right-oriented series where the inter-item distance was manipulated so that the 3rd item was at the same distance from the beginning of the series as the 4th item had been at training. This forced chicks to choose either spatial or ordinal cues. Chicks tested binocularly selected both the 4th left and right items above chance expectation, confirming that a coherent use of numerical and spatial information is essential in limiting birds' responses toward the left[59]. Chicks tested monocularly chose the 3rd and 4th items on the seeing side, suggesting that birds relied on spatial or ordinal cues to a similar extent in different trials and that each hemisphere can process both cues[37].

Here, in monocular conditions, Li-chicks succeeded in Fronto-Parallel tests. Even if they directed their pecks to the visible side [40,60], they selected the 4th item above chance expectation. This was the case in experiment 1 when ordinal and spatial cues were available at test. Nevertheless, in experiment 2, when spatial information was available at training but unreliable at test, chicks succeeded in the right-eye/left-hemisphere, but not in the left-eye/right-hemisphere condition. This corroborates the hypothesis of the right hemisphere specificity in the analysis of spatial cues and suggests that the left hemisphere is more specialized in processing ordinal information. The left-to-right spatialization of numerosity appears to be based on preferential processing by the right hemisphere when spatial information is available and hemispheric specialization is favored by environmental stimulation.

Ecological Implications and Adaptive Variability

Experimental contexts allow for selective manipulation of the environment, enabling changes that are highly improbable in nature. In experiment 2, the item arrangement, that had been experienced as a stable context that provided coherent and reliable numerical and spatial information during training, was manipulated to eliminate spatial information at test. This manipulation resulted in the disappearance of left-to-right oriented directionality in the binocular condition and in a failure in the left-eye/right-hemisphere condition. We can speculate that in some naturalistic contexts, where reliable landmarks or beacons used for navigation remain stable, the coherent availability of spatial information and numerical information would likely maximize the engagement of the right hemisphere and trigger an imbalance favoring the left space. This left-biased space would serve as an anchor point from which to initiate environmental scanning, avoiding the delay presumably implied if there was not a hemisphere taking control of processing and guiding behavior[61]. Such an intrinsic, left-oriented bias might be advantageous for other ecological situations requiring number processing, such as quantifying conspecifics or food items. For example, when foraging, a consistent left-to-right scanning pattern could help animals to efficiently locate and quantify food sources without overlooking areas. The tendency to scan items from the preferred left side [62], might be evolved as an adaptive behavior to maximize fitness. This tendency could then have been assimilated by other cognitive processes that share neural substrates, including numerical cognition. Numerosities relevant for animals (such as the number of conspecifics, food items, or predators) are inherently distributed in space. The right hemisphere specialized in spatial processing incorporated some rudimentary forms of

enumeration. This resulted in a right hemispheric dominance for both space and number. This might explain the observed left-to-right bias in numerical cognition tasks. The absence of this bias in conditions where spatial cues are artificially eliminated, as in experiment 2, underscores the spatial nature of numerical processing of objects in the environment. Remarkably, the left-to-right directionality is not reported in weakly lateralized, Di-chicks, in both experiments, irrespective of spatial cues availability. It should be noted that in some situations like predator detection, even if a systematic approach to surveying the surroundings could be beneficial for prompt threats detection, it could also lead to more predictability. This could favor predators with a complementary approach directionality. From an evolutionary perspective, lateralization variability within a species can be viewed as an adaptive strategy. This variability may represent a form of evolutionary bet-hedging[63], where different degrees of lateralization confer different adaptive advantages in fluctuating ecological contexts. Bet-hedging strategies maintain population fitness by promoting phenotypic diversity, optimizing adaptation in unpredictable environments[64]. Lateralization variability might also contribute to the species' behavioral unpredictability, offering an advantage in predator-prey dynamics[65]. The persistence of both lateralized and non-lateralized individuals within a population may be an evolutionarily stable strategy[66], conferring differential advantages to different individuals, overall maintaining high fitness and making the overall population less predictable[67].

Monocular test outcomes support that light-induced lateralization enhances spatial-numerical performance

Our investigation produced a second major result and showed that prenatal exposure significantly affected performance. In the Sagittal test, Li-chicks outperformed Di-chicks, demonstrating that a higher degree of lateralization led to greater accuracy when both spatial and numerical cues were available (experiment 1).

The results of the monocular Fronto-Parallel tests further support the effect of embryonic stimulation in enhancing performance, as only Li-chicks succeeded in both the left and right monocular conditions. Remarkably, in the Fronto-Parallel test of experiment 1 allowing a coherent use of spatial and numerical information, Li-chicks tested in left-monocular condition of vision showed a bias alike to Li-chicks tested in binocular condition of vision. When a hemisphere is dominant for a function, behavior under its sole control often matches that observed in normal conditions of vision when both hemispheres are active[61]. In the present scenario, similarities between chicks tested in Binocular and Left Monocular conditions of vision suggest that when both hemispheres are processing the information, the availability of spatial cues triggers an overactivation of the right hemisphere resulting in a leftward bias[40].

Whenever processing was confined to a single hemisphere, either one, only strongly lateralized Li-chicks succeeded while weakly lateralized Di-chicks failed, corroborating evidence on the importance of light stimulation in favoring the development and specialization of both hemispheres and in boosting cognitive performance. This finding aligns with previous anatomical studies that demonstrated the presence of light-dependent lateralization in bilaterally responsive units of the right visual Wulst[48,68]. Nevertheless, in the specific case of Li-chicks tested with the left-eye/right-hemisphere in use, subjects failed the Fronto-Parallel test when the use of spatial cues was prevented (experiment 2). This highlights the reliance of the right hemisphere on spatial information.

Interpreting results through proposed models for the origin of the Mental Number Line

The present results reveal that brain lateralization influences performance in ordinal tasks involving both spatial and numerical cues, suggesting a joint contribution of hemispheric specialization and environmental stimulation to the spatial organization of numbers. These results allow us to reconsider the models proposed to explain the MNL.

1. The BAFT model would not predict any asymmetry as it refers number spatialization to differences in spatial frequencies[41], but in the present study the spatial distribution of the stimuli is symmetrical. The predictions based on this model fit with the results of the experiment 2, where chicks did not show any spatial bias in the Fronto-Parallel Binocular test. Yet the model fails in predicting the left bias found in the Fronto-Parallel Binocular and Left Monocular tests of experiment 1. While this model can still be valuable in explaining spatial-numerical association in other contexts, it fails to account for the left-to-right proto-counting observed in this experimental setting[11,19–21,40,59].

2. According to the emotional valence model chicks should exhibit a rightward bias in food-seeking behavior, since food rewards trigger positive emotions, particularly when lateralization is pronounced. The assumption that a larger numerosity can be associated with more positive emotion and consequent preferential processing by the left hemisphere would explain the right bias[4]. Conversely, the assumption that a smaller numerosity is associated with a negative emotion would activate the right hemisphere, driving animals toward the left[4]. Nevertheless, in the present study, chicks showed a left bias when they could rely on consistent spatial and numerical information (experiment 1) and no bias when spatial information was unreliable (experiment 2). Thus, the results obtained in the present study do not fit the emotional model.

3. The left bias meets the predictions based on the right-hemisphere dominance model, which links the origin of the Mental Number Line to the right hemisphere's specialization in visual/numerical processing —resulting in a predisposed left-to-right scanning tendency[23]. Remarkably, this model has been put forward to explain outcomes in both the previously-mentioned paradigms[23,24,40,58], thus explaining both the left-to-right oriented searching found in ordinal tasks, as well as the association of smaller numerosity with the left and larger numerosity with the right. Such association likely results from brain asymmetry driven by right-hemisphere dominance in visuospatial attention. Although in principle arbitrary, the left-to-right mapping direction during evolution may have been imposed by brain asymmetry: a common and ancient trait that occurs in a wide range of vertebrates[69,70] and invertebrates[71–74], which possibly optimizes simultaneous processing of different kinds of information[61]. In natural environments, relevant numerosity (e.g., predators, food items, conspecifics) are intrinsically linked to their spatial arrangements, and their enumeration might be facilitated whenever a clear scanning directionality is present. Consequently, numerical estimation may have evolved in conjunction with spatial processing biases, leading to a left-anchored, rightward-directed environmental scanning strategy. This hypothesis suggests that numerical cognition evolved incorporating spatial processing biases and reflecting the spatial nature of ecologically relevant numerosity.

It should be noted that BAFT and the emotional valence models have been elaborated to explain the performance in a different task[17,22–24]. This required animals first to learn to associate a food reward with a central numerosity, e.g., an array depicting 5 dots. At test, when presented with new but identical numerosity, placed one on the left and one on the right, animals chose the left option when the test numerosity was smaller than the one experienced during learning, e.g., 2, and the right option when the numerosity was larger, e.g., 8[17,22–24]. Such shift in the bias from left to right depending on numerosity cannot be simply associated with the lateralization of feeding responses typically guided by the left hemisphere in chicks[50,52,75]. Nevertheless, numerosities could correlate with spatial frequencies[41] or with emotional valence[4]. Remarkably, the right hemisphere dominance model provides a more comprehensive explanation. In fact, it accounts for the above mentioned task[17,23,24,58] as well as for the ordinal task[37,40].

Although all models[4,23,40,41] identify the key role of hemispheric specialization in determining numerical spatialization, the Right-Hemisphere Dominance model can explain animal behavior across multiple contexts, providing a more parsimonious and generalizable explanation of cognitive processes, and potentially revealing fundamental connections between seemingly distinct cognitive domains.

Materials and Methods

Subject

We tested 100 male domestic chicks (*Gallus gallus*) of the Aviagen ROSS 308 line (experiment 1, n = 48; experiment 2, n = 52). We chose male chicks because of their superior response to food reinforcement compared to females[36,76] and significantly greater degree of lateralization in the thalamofugal pathway[77]. The fertilized eggs were obtained weekly from two local hatcheries (Agricola Berica, Montegalda, Vicenza, Italy, or Società Agricola La Pellegrina Spa, San Pietro in Gù, Padova, Italy). Eggs on the seventh or fourteenth day of incubation were delivered to the lab and placed in a FIEM incubator MG 70/100 (45 × 58 × 43 cm) at a controlled temperature of 36-38 °C and 60% humidity. On the eighteenth day of incubation, eggs were moved to a hatching machine (60 × 32 × 40 cm) at controlled temperature and humidity (36-38 °C; 60%) until the 21st day of incubation (hatching day). Eggs were incubated under two conditions: in darkness to obtain Dark-incubated (weakly-lateralized) chicks (Di-chicks, n = 24 in experiment 1, n = 26 in experiment 2), and under light exposure using an LED 4.8W lightbulb to obtain Light-incubated (strongly-lateralized) chicks (Li-chicks, n = 24 in experiment 1, n = 26 in experiment 2). Only the chicks that completed all four tests were included in the final sample size. A few hours after hatching, chicks were feather-sexed and caged in pairs or triplets in standard metal cages (28 × 32 × 40 cm) with the floor covered with absorbent paper. The rearing room was maintained at a temperature of 28-31°C and humidity of about 60%. The cages were illuminated by neon lights (36 W) placed about 15 centimeters above each cage, with a standard 24-hour light-dark rearing cycle. Food (chick crumbles) and water were available in transparent glass jars (5 × 5 cm) *ad libitum*. Daily, chicks were familiarized and fed with some mealworms (*Tenebrio molitor larvae*) that were used as reinforcement during training. These rearing conditions were maintained until the initiation of the experimental protocol on Wednesday (8 a.m.) when food jars were removed from cages and chicks were isolated one per cage. Chicks underwent two hours of food deprivation before the start of each experimental session (shaping, training, and tests). Following the last testing session, chicks were rehoused in social groups with water and food *ad libitum*. On Friday afternoon, chicks were donated to local farmers.

Apparatus

The experimental apparatus was located in the experimental room, near the rearing room, and maintained at constant temperature and humidity (28 °C; 70%). The apparatus consisted of a square arena constructed from green polypropylene (100 × 100 × 40 cm) with the floor covered with wood shavings (Fig. 1). Inside the arena were ten identical red plastic caps (3 cm in diameter, 0.9 cm in height), each filled with wood shavings. To minimize the potential use of external cues, the entire setup was elevated and rotated randomly between trials or sessions [20,21]. Bottle caps were frequently shuffled to ensure that the choices made by the chicks did not depend on some unique characteristics of the caps[40,59,60]. The apparatus comprised two mirrored starting positions (15 × 15 × 10 cm boxes) outside opposing walls, one of which serves as a starting position (labeled as "S.P." in Fig. 1). The starting boxes were designed to allow consistent visual input of the inner apparatus to the chicks. Access to the arena was provided through an entrance door made of green polypropylene (10 × 17 cm), which could be lifted by a nylon thread.

Shaping

In both experiments, the shaping, pre-training, and training procedures were conducted in the same setting. Shaping started on Wednesday morning after 2 hours of food deprivation (8:00-10:00 am). This was essential in motivating foraging behavior during training and testing. During Shaping, the array made of 10 items was centrally aligned along the median sagittal line, thus sagittally oriented, with respect to either starting point (Fig. 1A), with the first cap positioned 28.5

cm from each entrance door. Each cap was positioned 1.44 cm apart from the subsequent one, with the overall array length being 43 cm. The array was situated 48.5 cm from either side wall.

The experimenter first introduced the chick into the arena for habituation, which lasted for about two minutes, allowing the bird to explore until it showed no signs of distress. Subsequently, the experimenter placed the chick into the starting point and the Shaping began. A piece of mealworm was placed and remained visible on the 4th cap to reinforce pecking behavior at that ordinal position. After the chick entered the arena and first pecked at any item, the trial was over, and the chick was immediately placed back into the starting box. If the chick did not peck at any item within 30 seconds, the experimenter used a metal stick to direct the chick to the fourth cap. After the chick had successfully pecked the 4th cap in 10 trials (whether consecutive or not), in the subsequent trials, the food was gradually covered with wood shavings until it was completely hidden. The shaping lasted for 10-15 minutes, followed by 30-40 minutes of rest back in the rearing cage with access to water but not to food.

Pre-Training

After the resting period, the Pre-Training began. From the Pre-Training all items looked identical as the food reward in the correct (4th) cap was completely buried in wood shavings. The chick had to complete three consecutive correct trials to reach the learning criterion and pass the re-training phase[37,40,59,60]. This usually took about 5-10 minutes. If the chick did not reach the learning criterion, the Pre-Training was repeated after 30 minutes of rest. If the chick again did not reach the criterion, it was excluded from the study.

Training

Immediately after completion of the Pre-Training, chicks underwent Training, comprising 20 trials, using the identical sequence employed in the Pre-Training phase. In each trial, only one choice was allowed, and the trial was terminated as soon as the chick pecked any item, with its choice being recorded. If the chick did not peck any item in 180 seconds, the trial was considered null and terminated. A choice was considered correct if the chick pecked at the 4th item. The learning criterion for passing the training phase was eight correct trials out of 20 trials[37,40,59,60]. If a subject did not achieve the learning criterion, after 40 minutes of rest it underwent another Pre-Training and, if this was successful the Training began. Each chick had three chances to pass the training criterion.

Re-Training

Re-Training was conducted prior to every test. The Re-Training procedure was the same as Pre-Training and ended with three consecutive correct trials. Test sessions started immediately after Re-Training was completed.

Test sessions

All chicks participated in all four tests: first the Sagittal Test, then the Fronto-Parallel Binocular Test. Thereafter, monocular tests were administered in counter-balanced order. The Fronto-Parallel Monocular Right Test was conducted prior to the Fronto-Parallel Monocular Left Test for n = 24 chicks in experiment 1 and n = 26 in experiment 2. The remaining chicks (n = 24 in experiment 1, n = 26 in experiment 2) underwent the Fronto-Parallel Monocular Tests in the reversed order.

Sagittal Test

The procedure for the Sagittal Test (Fig. 1A) was the same as the Training. The Sagittal Test consisted of 20 trials, and the time limit for each trial was 60 seconds. During Testing, food reinforcement was available only in pre-established trials to prevent the extinction of responses

over multiple unrewarded test trials (reinforced trials: 4, 5, 7, 10, 13, 14, 16 and 19[37,40,59,60]). Thereafter, subjects rested for at least 60 minutes before entering the Fronto-Parallel Tests.

In experiment 1 ordinal and spatial cues were available to identify the 4th correct item, in fact the array was arranged as during Training, with the length of the series being kept constant throughout the trials.

In experiment 2, to eliminate spatial cues to locate the 4th item, the inter-item distance varied between test trials (1.44 cm, 2.55 cm, 3.11 cm, and 3.66 cm), while remaining equally-spaced within each trial, resulting in total array lengths of 43.0, 53.0, 58.0, and 63.0 cm, respectively. The first cap was set at 28.5 cm from the starting position.

Fronto-parallel Tests

In both experiments, the Fronto-Parallel Test was conducted on each subject in three different conditions of vision (Fig. 1). The Binocular Fronto-Parallel Test was always administered first. Then half of the chicks underwent the Left Monocular Fronto-Parallel Test and finally the Right Monocular Fronto-Parallel Test, while the other half underwent the monocular tests in reverse order.

In the Monocular Fronto-Parallel Test, a temporary eye patch was carefully applied to restrict visual input to one of the chicks' eyes. The patch, made of removable paper tape, did not obstruct eyelid movements and enabled smooth removal post testing without harming the subject. Before the actual test, chicks were habituated to wear the eye patch for about 15 minutes, during which they were closely monitored; any signs of distress or excessive scratching prompted immediate intervention to ensure the animals' well-being[40,60,78].

In the three Fronto-Parallel Tests, the array was rotated 90°, fronto-parallel with respect to the Starting Point (Fig. 1B). Inter-item distances matched those in the Sagittal Test for each experiment. In this rotated array, both the 4th item from left (4L) and right (4R) were considered correct and rewarded during the pre-established trials (as described for the Sagittal Test). Each Fronto-Parallel Test comprised 20 trials, with two-hour rest periods between tests.

Statistical analyses

In each trial, chicks were allowed a single peck. We recorded the selected item to calculate the percentage of responses at each position as [(number of pecks to a given item ÷ total number valid trials) × 100] and averaged them separately for each group and test. We employed both frequentist and Bayesian statistical approaches, conducting corresponding Bayesian analyses for each frequentist test. We analyzed the group percentage for choosing each item above chance (10%), using Wilcoxon one-sample signed-rank tests with Bonferroni correction for multiple comparisons (data and significant results are reported in **Error! Reference source not found.** and **Error! Reference source not found.**), and one-sample Bayesian t-tests. To assess side bias in the Fronto-Parallel tests, we compared correct choices on the left (4L) *versus* the right (4R) using paired *t* tests, with Cohen's *d* as the effect size, and Bonferroni as the correction method; moreover, we conducted two-sample Bayesian *t* tests. Additionally, we tested whether brain lateralization influenced accuracy by comparing the percentage of correct choices (i.e., the selection of the 4th item in the Sagittal test, and of the 4L or 4R items in the Fronto-Parallel tests) between Li-chicks and Di-chicks using two-sample *t* tests, with Cohen's *d* as the effect size, and Bonferroni as the correction method, additionally we conducted two-sample Bayesian t-tests.

Bayesian factors were computed using BayesFactor package[79]. The analyses were conducted using R (version 4.3.1; R Core Team, 2022). We used the classification by Lee & Wagenmakers (2014) [80] to interpret BFs.

Table 1. Descriptive statistics. For each test in the two experiments, the accuracy of selecting the 4th item in the Sagittal test and the 4L or 4R items in the Fronto-Parallel (FP) tests is reported.

Experiment 1: Ordinal and Spatial cue									
Test	**Hatch Condition**	**Choice**	**mean**	**SD**	**SE**	***n***	***r***	***P***	**BF**
Sagittal	**Di-chicks**	4	28.436	9.374	1.913	24	0.878	< 0.001	>10000
	Li-chicks	4	36.382	8.164	1.666	24	0.88	< 0.001	>10000
FP Binocular	**Di-chicks**	4L	18.958	10.527	2.149	24	0.727	0.005	169
		4R	19.792	11.371	2.321	24	0.724	0.003	188
	Li-chicks	4L	28.851	12.598	2.572	24	0.859	< 0.001	>10000
		4R	11.894	7.503	1.532	24	0.337	0.798	0.741
FP Monocular Left	**Di-chicks**	4L	12.780	8.309	1.696	24	0.376	0.532	1.287
		4R	3.990	6.154	1.256	24	-0.708	1.000	0.05
	Li-chicks	4L	22.595	8.736	1.783	24	0.876	< 0.001	>10000
		4R	6.297	6.747	1.377	24	-0.465	1.000	0.068
FP Monocular Right	**Di-chicks**	4L	3.746	3.668	0.749	24	-0.798	1.000	0.013
		4R	13.417	8.781	1.792	24	0.406	0.398	1.936
	Li-chicks	4L	5.219	6.356	1.297	24	-0.594	1.000	0.057
		4R	21.484	10.734	2.191	24	0.807	0.001	1843
Experiment 2: Ordinal cue only									
Test	**Hatch Condition**	**Choice**	**mean**	**SD**	**SE**	***n***	***r***	***P***	**BF**
Sagittal	**Di-chicks**	4	31.487	15.337	3.008	26	0.863	< 0.001	>10000
	Li-chicks	4	32.275	11.232	2.203	26	0.876	< 0.001	>10000
FP Binocular	**Di-chicks**	4L	18.725	9.246	1.813	26	0.74	0.001	836.246
		4R	19.666	10.024	1.966	26	0.786	0.002	1070.73
	Li-chicks	4L	21.771	9.888	1.939	26	0.819	< 0.001	>10000
		4R	15.800	9.239	1.812	26	0.603	0.024	21.919
FP Monocular Left	**Di-chicks**	4L	14.835	9.878	1.937	26	0.473	0.122	5.337
		4R	8.333	6.912	1.356	26	-0.316	1.000	0.102
	Li-chicks	4L	15.011	9.344	1.832	26	0.518	0.050	8.449
		4R	7.939	6.082	1.193	26	-0.302	1.000	0.084
FP Monocular Right	**Di-chicks**	4L	8.290	7.659	1.502	26	-0.269	1.000	0.102
		4R	12.457	9.255	1.815	26	0.29	0.850	0.839
	Li-chicks	4L	6.288	6.151	1.206	26	-0.515	1.000	0.06
		4R	17.984	9.634	1.889	26	0.78	0.001	214

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Supplementary Materials:
The PDF file includes:
Tables S1 and S2

References

1. Cordes, S. & Brannon, E. M. Crossing the divide: Infants discriminate small from large numerosities. *Developmental Psychology* **45**, 1583–1594 (2009).
2. Cordes, S., Gelman, R., Gallistel, C. R. & Whalen, J. Variability signatures distinguish verbal from nonverbal counting for both large and small numbers. *Psychonomic Bulletin & Review* **8**, 698–707 (2001).
3. Cantlon, J. F. & Brannon, E. M. Basic Math in Monkeys and College Students. *PLoS Biol* **5**, e328 (2007).
4. Vallortigara, G. Comparative cognition of number and space: the case of geometry and of the mental number line. *Phil. Trans. R. Soc. B* **373**, 20170120 (2018).
5. Rugani, R. Towards numerical cognition's origin: insights from day-old domestic chicks. *Phil. Trans. R. Soc. B* **373**, 20160509 (2018).
6. Dehaene, S. *The Number Sense: How the Mind Creates Mathematics*. (Oxford University Press, New York, 2011).
7. Galton, F. Visualised Numerals. *Nature* **21**, 252–256 (1880).
8. Zebian, S. Linkages between Number Concepts, Spatial Thinking, and Directionality of Writing: The SNARC Effect and the REVERSE SNARC Effect in English and Arabic Monoliterates, Biliterates, and Illiterate Arabic Speakers. *J Cogn Cult* **5**, 165–190 (2005).
9. Shaki, S., Fischer, M. H. & Petrusic, W. M. Reading habits for both words and numbers contribute to the SNARC effect. *Psychonomic Bulletin & Review* **16**, 328–331 (2009).
10. de Hevia, M. D. How the Human Mind Grounds Numerical Quantities on Space. *Child Dev Perspect* **15**, 44–50 (2021).
11. West, E. & McCrink, K. Eye Tracking Lateralized Spatial Associations in Early Childhood. *Journal of Cognition and Development* **22**, 678–694 (2021).
12. Rugani, R., Zhang, Y., Ahmed, N. & Brannon, E. Children perform better on left than right targets in an ordinal task. *Acta Psychologica* **226**, 103560 (2022).
13. de Hevia, M. D. & Spelke, E. S. Number-Space Mapping in Human Infants. *Psychol Sci* **21**, 653–660 (2010).
14. Bulf, H., de Hevia, M. D. & Macchi Cassia, V. Small on the left, large on the right: numbers orient visual attention onto space in preverbal infants. *Dev Sci* **19**, 394–401 (2016).

15. de Hevia, M. D., Izard, V., Coubart, A., Spelke, E. S. & Streri, A. Representations of space, time, and number in neonates. *Proc Natl Acad Sci USA* **111**, 4809–4813 (2014).
16. McCrink, K., Veggiotti, L. & Hevia, M. D. A left visual advantage for quantity processing in neonates. *Ann. N.Y. Acad. Sci.* **1477**, 71–78 (2020).
17. Di Giorgio, E. *et al.* A mental number line in human newborns. *Dev Sci* **22**, (2019).
18. de Hevia, M. D., Veggiotti, L., Streri, A. & Bonn, C. D. At Birth, Humans Associate "Few" with Left and "Many" with Right. *Current Biology* **27**, 3879-3884.e2 (2017).
19. Drucker, C. B. & Brannon, E. M. Rhesus monkeys (Macaca mulatta) map number onto space. *Cognition* **132**, 57–67 (2014).
20. Rugani, R., Kelly, D. M., Szelest, I., Regolin, L. & Vallortigara, G. Is it only humans that count from left to right? *Biol. Lett.* **6**, 290–292 (2010).
21. Rugani, R., Regolin, L. & Vallortigara, G. Rudimental numerical competence in 5-day-old domestic chicks (Gallus gallus): Identification of ordinal position. *Journal of Experimental Psychology: Animal Behavior Processes* **33**, 21–31 (2007).
22. Rugani, R., Vallortigara, G., Priftis, K. & Regolin, L. Numerical magnitude, rather than individual bias, explains spatial numerical association in newborn chicks. *eLife* **9**, e54662 (2020).
23. Rugani, R., Vallortigara, G., Priftis, K. & Regolin, L. Number-space mapping in the newborn chick resembles humans' mental number line. *Science* **347**, 534–536 (2015).
24. Giurfa, M., Marcout, C., Hilpert, P., Thevenot, C. & Rugani, R. An insect brain organizes numbers on a left-to-right mental number line. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2203584119 (2022).
25. Rugani, R., Platt, M. L., Zhang, Y. & Brannon, E. M. Magnitude shifts spatial attention from left to right in rhesus monkeys as in the human mental number line. *iScience* **27**, 108866 (2024).
26. Adachi, I. Spontaneous Spatial Mapping of Learned Sequence in Chimpanzees: Evidence for a SNARC-Like Effect. *PLoS ONE* **9**, e90373 (2014).
27. Triki, Z. & Bshary, R. Cleaner fish Labroides dimidiatus discriminate numbers but fail a mental number line test. *Anim Cogn* **21**, 99–107 (2018).
28. Beran, M. J., French, K., Smith, T. R. & Parrish, A. E. Limited evidence of number–space mapping in rhesus monkeys (Macaca mulatta) and capuchin monkeys (Sapajus apella). *Journal of Comparative Psychology* **133**, 281–293 (2019).
29. Piazza, M., Izard, V., Pinel, P., Le Bihan, D. & Dehaene, S. Tuning Curves for Approximate Numerosity in the Human Intraparietal Sulcus. *Neuron* **44**, 547–555 (2004).

30. Piazza, M. & Eger, E. Neural foundations and functional specificity of number representations. *Neuropsychologia* **83**, 257–273 (2016).

31. Pearson, J. M., Hayden, B. Y., Raghavachari, S. & Platt, M. L. Neurons in Posterior Cingulate Cortex Signal Exploratory Decisions in a Dynamic Multioption Choice Task. *Current Biology* **19**, 1532–1537 (2009).

32. Nieder, A. Representation of the Quantity of Visual Items in the Primate Prefrontal Cortex. *Science* **297**, 1708–1711 (2002).

33. Ditz, H. M. & Nieder, A. Neurons selective to the number of visual items in the corvid songbird endbrain. *Proc Natl Acad Sci USA* **112**, 7827–7832 (2015).

34. Kobylkov, D., Mayer, U., Zanon, M. & Vallortigara, G. Number neurons in the nidopallium of young domestic chicks. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2201039119 (2022).

35. Rashid, N. & Andrew, R. J. Right hemisphere advantage for topographical orientation in the domestic chick. *Neuropsychologia* **27**, 937–948 (1989).

36. Regolin, L., Garzotto, B., Rugani, R., Pagni, P. & Vallortigara, G. Working memory in the chick: parallel and lateralized mechanisms for encoding of object- and position-specific information. *Behavioural Brain Research* **157**, 1–9 (2005).

37. Rugani, R. & Regolin, L. Hemispheric specialization in spatial versus ordinal processing in the day-old domestic chick (Gallus gallus). *Ann. N.Y. Acad. Sci.* **1477**, 34–43 (2020).

38. Harvey, B. M., Klein, B. P., Petridou, N. & Dumoulin, S. O. Topographic Representation of Numerosity in the Human Parietal Cortex. *Science* **341**, 1123–1126 (2013).

39. Zorzi, M., Priftis, K. & Umiltà, C. Neglect disrupts the mental number line. *Nature* **417**, 138–139 (2002).

40. Rugani, R., Vallortigara, G. & Regolin, L. Mapping number to space in the two hemispheres of the avian brain. *Neurobiology of Learning and Memory* **133**, 13–18 (2016).

41. Felisatti, A., Laubrock, J., Shaki, S. & Fischer, M. H. A biological foundation for spatial–numerical associations: the brain's asymmetric frequency tuning. *Annals of the New York Academy of Sciences* **1477**, 44–53 (2020).

42. Davidson, R. J. Well–being and affective style: neural substrates and biobehavioural correlates. *Phil. Trans. R. Soc. Lond. B* **359**, 1395–1411 (2004).

43. Gazzaniga, M. S. Cerebral specialization and interhemispheric communication: Does the corpus callosum enable the human condition? *Brain* **123**, 1293–1326 (2000).

44. Rogers, L. J. & Sink, H. S. Transient asymmetry in the projections of the rostral thalamus to the visual hyperstriatum of the chicken, and reversal of its direction by light exposure. *Exp Brain Res* **70**, (1988).

45. Rogers, L. J. & Bolden, S. W. Light-dependent development and asymmetry of visual projections. *Neuroscience Letters* **121**, 63–67 (1991).

46. Deng, C. & Rogers, L. J. Bilaterally projecting neurons in the two visual pathways of chicks. *Brain Research* **794**, 281–290 (1998).

47. Clark, W. J. & Colombo, M. The functional architecture, receptive field characteristics, and representation of objects in the visual network of the pigeon brain. *Progress in Neurobiology* **195**, 101781 (2020).

48. Costalunga, G., Kobylkov, D., Rosa-Salva, O., Vallortigara, G. & Mayer, U. Light-incubation effects on lateralisation of single unit responses in the visual Wulst of domestic chicks. *Brain Struct Funct* **227**, 497–513 (2022).

49. Rogers, L. J. Light experience and asymmetry of brain function in chickens. *Nature* **297**, 223–225 (1982).

50. Deng, C. & Rogers, L. J. Differential contributions of the two visual pathways to functional lateralization in chicks. *Behavioural Brain Research* **87**, 173–182 (1997).

51. Daisley, J. N., Mascalzoni, E., Rosa-Salva, O., Rugani, R. & Regolin, L. Lateralization of social cognition in the domestic chicken (*Gallus gallus*). *Phil. Trans. R. Soc. B* **364**, 965–981 (2009).

52. Dharmaretnam, M. & Rogers, L. J. Hemispheric specialization and dual processing in strongly versus weakly lateralized chicks. *Behavioural Brain Research* **162**, 62–70 (2005).

53. Daisley, J. N., Vallortigara, G. & Regolin, L. Logic in an asymmetrical (social) brain: Transitive inference in the young domestic chick. *Social Neuroscience* **5**, 309–319 (2010).

54. Weidner, C., Repérant, J., Miceli, D., Haby, M. & Rio, J. P. An anatomical study of ipsilateral retinal projections in the quail using radioautographic, horseradish peroxidase, fluorescence and degeneration techniques. *Brain Research* **340**, 99–108 (1985).

55. Theiss, M. P. H., Hellmann, B. & Güntürkün, O. The architecture of an inhibitory sidepath within the avian tectofugal system: *NeuroReport* **14**, 879–882 (2003).

56. Robert, F. & Cuénod, M., M. Electrophysiology of the intertectal commissures in the pigeon I. Analysis of the pathways. *Exp Brain Res* **9**, (1969).

57. Rogers, L. J. & Vallortigara, G. Brain and behavioural asymmetries in non-human species. *Laterality* **26**, v–vii (2021).

58. Rugani, R. & de Hevia, M.-D. Number-space associations without language: Evidence from preverbal human infants and non-human animal species. *Psychon Bull Rev* **24**, 352–369 (2017).

59. Rugani, R., Vallortigara, G., Vallini, B. & Regolin, L. Asymmetrical number-space mapping in the avian brain. *Neurobiology of Learning and Memory* **95**, 231–238 (2011).

60. Rugani, R. & Regolin, L. Approach direction and accuracy, but not response times, show spatial-numerical association in chicks. *PLoS ONE* **16**, e0257764 (2021).

61. Rogers, L. J., Vallortigara, G. & Andrew, R. J. *Divided Brains: The Biology and Behaviour of Brain Asymmetries*. (Cambridge University Press, 2013). doi:10.1017/CBO9780511793899.

62. Diekamp, B., Regolin, L., Güntürkün, O. & Vallortigara, G. A left-sided visuospatial bias in birds. *Current Biology* **15**, R372–R373 (2005).

63. Simons, A. M. Modes of response to environmental change and the elusive empirical evidence for bet hedging. *Proc. R. Soc. B.* **278**, 1601–1609 (2011).

64. Philippi, T. & Seger, J. Hedging one's evolutionary bets, revisited. *Trends in Ecology & Evolution* **4**, 41–44 (1989).

65. Güntürkün, O., Ströckens, F. & Ocklenburg, S. Brain Lateralization: A Comparative Perspective. *Physiological Reviews* **100**, 1019–1063 (2020).

66. Ghirlanda, S. & Vallortigara, G. The evolution of brain lateralization: a game-theoretical analysis of population structure. *Proc. R. Soc. Lond. B* **271**, 853–857 (2004).

67. Rogers, L. J. Brain Lateralization and Cognitive Capacity. *Animals* **11**, 1996 (2021).

68. Rogers, L. J. & Deng, C. Light experience and lateralization of the two visual pathways in the chick. *Behavioural Brain Research* **98**, 277–287 (1999).

69. Vallortigara, G. & Rogers, L. J. survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behav Brain Sci* **28**, 575–589 (2005).

70. *Comparative Vertebrate Lateralization*. (Cambridge University Press, 2002). doi:10.1017/CBO9780511546372.

71. Frasnelli, E., Vallortigara, G. & Rogers, L. J. Left–right asymmetries of behaviour and nervous system in invertebrates. *Neuroscience & Biobehavioral Reviews* **36**, 1273–1291 (2012).

72. Anfora, G. *et al.* Lateralization in the Invertebrate Brain: Left-Right Asymmetry of Olfaction in Bumble Bee, Bombus terrestris. *PLoS ONE* **6**, e18903 (2011).

73. Frasnelli, E. *et al.* The Bee as a Model to Investigate Brain and Behavioural Asymmetries. *Insects* **5**, 120–138 (2014).

74. Baracchi, D., Rigosi, E., De Brito Sanchez, G. & Giurfa, M. Lateralization of Sucrose Responsiveness and Non-associative Learning in Honeybees. *Front. Psychol.* **9**, 425 (2018).

75. Deng, C. & Rogers, L. J. Prehatching visual experience and lateralization in the visual Wulst of the chick. *Behavioural Brain Research* **134**, 375–385 (2002).

76. Vallortigara, G., Cailotto, M. & Zanforlin, M. Sex differences in social reinstatement motivation of the domestic chick (Gallus gallus) revealed by runway tests with social and nonsocial reinforcement. *Journal of Comparative Psychology* **104**, 361–367 (1990).

77. Rajendra, S. & Rogers, LesleyJ. Asymmetry is present in the thalamofugal visual projections of female chicks. *Exp Brain Res* **92**, (1993).

78. *Lateralized Brain Functions: Methods in Human and Non-Human Species*. vol. 122 (Springer New York, New York, NY, 2017).

79. Morey, R. D. & Rouder, J. N. BayesFactor: Computation of Bayes Factors for Common Designs. 0.9.12-4.7 https://doi.org/10.32614/CRAN.package.BayesFactor (2012).

80. Lee, M. D. & Wagenmakers, E.-J. *Bayesian Cognitive Modeling: A Practical Course*. (Cambridge University Press, 2014). doi:10.1017/CBO9781139087759.

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