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The impact of ascending and descending numerical sequences on scanning direction in day-old domestic chicks (*Gallus gallus*)

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ABSTRACT

Among the fundamental characteristics that evolved biologically in humans and animals are brain asymmetries. In vertebrates, the preferential engagement of the right hemisphere orients visuo-spatial attention to the left space, thus leading to a left-to-right visual scan. This evolutionary predisposition has been proposed as a basis for the organisation of numerical information along a left-to-right Mental Number Line. Indeed, in humans, visuo-spatial attention is bidirectionally linked to number processing: small numbers are associated with the left, large numbers with the right. We investigated whether exposure to different numerical sequences could influence the biological left-to-right scanning of quantity in young animals.

We trained 40 domestic chicks (*Gallus gallus*) to circumnavigate three sagittal panels displaying 2, 5, or 8 elements. Chicks were assigned into two Groups: 20 chicks underwent the ascending sequence (2-5-8) or descending sequence (8-5-2) order. At test, both groups were exposed to the same panels positioned fronto-parallel, in both Test sequence 2-5-8 and Test sequence 8-5-2 arrangements (from left to right).

A mixed ANOVA revealed a main effect of the Group. The ascending group showed a significant preference for scanning the triplets from left to right, regardless of numerosity disposition. Conversely, the descending group revealed no directional preference.

These findings demonstrate, for the first time, a modulation of the biological left-to-right scanning direction in young chicks as an effect of numerical sequences, mirroring the results observed in humans.

1.INTRODUCTION

1.1 Visuo-spatial attention

Attention is a fundamental cognitive process that plays a crucial role in human perception, enabling individuals to effectively navigate and orient themselves within the environment. Indeed, it helps them to focus on relevant stimuli while filtering out irrelevant information, organising sensory information and efficiently processing and responding to the stimuli in the environment. The perceptive system has five principal functions: it 1) determines which stimuli to give attention to, 2) localizes the stimuli, 3) recognises them, 4) extract their key features, and 5) maintains their visual consistency (Nolen-Hoeksema et al., 2017).

Selective attention

Perception starts with selective attention, which refers to the process of focusing attention to some objects, while ignoring others. With regards to visual attention, eye movements are a key indicator of visual exploration, which describes an active scanning of the environment. Specifically, visual attention consists of the pattern of fixations, defined as brief pauses of the eyes in a location, and saccades, described as rapid eye movement.

Notably, attention does not necessarily require eye movements. Indeed, it is possible to focus the attention on something without looking directly at it (Yu et al., 2022).

Attention is linked to memory, we usually do not clearly remember information that did not receive our focus (Treisman, A. M., 1969).

Spatial localization and object recognition

In order to localize information we need to distinguish objects between one another and from the background. Seminal studies in this field have been inspired by the Gestalt approach. Gestalt psychologists emphasize the role of our perception in what we see, specifically it is important to perceive objects as an integrated whole. For example, regarding discriminations of objects, there are images that can be interpreted in two ways and one perception does not eliminate the second one. In a similar vein, the way we organise objects affects how we perceive them: a set of dots may appear differently depending on the structure we recognise. When locating an object we are also influenced by their distance and movement patterns.

Subsequently, to recognise the object, it is essential to properly integrate multiple visual features. This integrative process is simplified by the initial processing of the global aspects of the visual scene, which serves as a contextual cue for interpreting elements. The foundation of this mechanism was firstly proposed by Anne Treisman (Treisman, 1986) through the *Feature Integration Theory*: during the preattentive stage primary attributes are perceived, like shape and color, and then in the attentive stage, selective attention is used to put together the different elements.

This theoretical framework aligns with the division of visual elaboration into early and later stages. In the initial phase, the perceptive system relies on retinal information, while in the latter this data is compared with categories already stored in visual memory. To further investigate these mechanisms, numerous studies (e.g. Hubel & Weasel, 1959) have been conducted on animal models, focusing on recording the activity of a single cell in the visual cortex. These studies aim to understand whether specific cells respond differently to varying stimuli attributes, such as orientation and spatial location within the visual field.

Feature extraction

Abstraction represents another fundamental cognitive function that allows individuals to transform raw sensory input from sense organs into abstract categories. This process is advantageous, as abstract representations are more easily stored in memory and are more efficient to compare.

Perceptual constancy

Finally, to properly live and interact with the external environment, perception must be stable throughout changes in sensory input. This function is essential for maintaining a coherent and continuous experience of the world. Mechanisms such as size, color and shape constancy allow the brain to interpret varying stimuli as consistent and familiar, thereby making perception a reliable way for interacting with a complex environment (e.g. Kraft & Brainard, 1999 for color constancy).

1.1.1 *Left bias and pseudoneglect in human adults, infants and non-human animals*

The neural basis of visuo-spatial attention is underpinned by a functional specialization of the right hemisphere (Thiebaut de Schotten et al., 2011). Evidence comes from research on patients with neglect, a neuropsychological condition resulting from a damage to one of the hemispheres (mostly the right one), characterized by a failure to attend to stimuli on the contralesional side (mostly the left). In support for the special role of the right hemisphere, neglect usually occurs after right hemisphere damage (Bisiach & Luzzatti 1978; Mesulam, 1981; Stone et al., 1991). Instead, patients with left hemisphere damage do not report neglect symptoms in visuo-spatial tasks, such as clock drawing and line bisection (Beis et al., 2004).

Additional evidence of right-hemispheric dominance for visuo-spatial processing comes from the phenomenon of *pseudoneglect*: it is a subtle leftward spatial bias observed in healthy individuals in attentional tasks (Jewell & McCourt, 2000; Nava et al., 2022). The most commonly used assessment for pseudoneglect is the visual line bisection task, which requires participants to bisect lines on paper.

Pseudoneglect in adults

A comprehensive review based on 73 studies (Jewell & McCourt, 2000), has revealed that the systematic leftward error in line bisection is modulated by ontogenetic and learning-related aspects: 1)age, younger people exhibit more leftward errors, instead, older ones shows more rightward errors; 2)task modality, midsagittal-pointing tasks and tactile bisection tasks elicit the left bias, while kinesthetic tasks tend to produce rightward errors; 3)sex, males have a slightly larger bias than females; 4)hand used, although both hands generally exhibit a leftward tendency, there is a relative directional bias in the hand that is actually used; 5)scanning direction, participants tend to error in the direction where scanning begins. Notably, no significant differences emerge with respect to handedness/laterality effects. Indeed, both right and left handed individuals exhibit a consistent leftward bias.

Pseudoneglect in infants

Recent findings suggest that pseudoneglect is not exclusive to later development stages, but rather emerges in infancy, indicating that right-hemispheric dominance for visuo-spatial attention may be present from the first months of life. Nava et al. (2022) provided the first evidence of pseudoneglect in 4 and 5-month-old infants with a spontaneous leftward gaze bias when viewing horizontal lines, regardless of the presence of a central cue. This bias suggests early asymmetries in visuo-spatial attention. Remarkably, when presented with vertical lines, preverbal infants exhibited a rightward bias, thus indicating a role played by stimulus orientation.

Overall, the evidence of pseudoneglect in humans reveal the influence of both biological and ontogenetic actors in shaping attentional biases, such as right hemispheric control and spatial scanning.

Pseudoneglect in animals

Given the observed pseudoneglect in human children, exploring similar spatial biases in non-human species could provide valuable insights into the evolutionary origins of lateralized attention.

Remarkably, a similar leftward bias as the pseudoneglect in humans was reported by Diekamp et al. (2005) in chicks (*Gallus gallus*) and pigeons (*Columba livia*). These animals were tested in a cancellation task and showed a consistent bias on the left hemispace. Moreover, the same results were found by Regolin (2006) in an adapted line bisection task, in which chicks had to peck in the center of a line.

Later, Chiandetti (2011) explored the bias in the same cancellation task as Diekamp et al. (2005), but in two groups of domestic chicks (*Gallus gallus*): one lateralized and one which was not. Indeed, during the last three days of incubation the right eye is exposed to light, as the chicken embryo is positioned in the egg with the left eye shielded (Rogers, 1990). Performance on tasks that required chicks to search for and collect food grains within a designated area in front of them revealed a leftward bias especially on light incubated chicks. This supports the role of the right hemisphere in shifting spatial attention.

This overview on visuo-spatial attention in humans and animals indicates 1) the presence of a biological left-side bias in scanning visual scenes, and 2) the fundamental role of the right hemisphere in guiding attention during the exploration of the external environment.

1.2 Visuo-spatial processes and numerical cognition

Importantly, the leftward bias in attention also influences the mapping of numerical information onto space (e.g. Rugani et al., 2007; 2025). Indeed, visuo-spatial attention and number processing seems to share neural substrates, which involve frontoparietal regions, in particular the intraparietal sulcus in the posterior parietal cortex (Toomarian & Hubbard, 2018; see also Hubbard et al., 2005 and Hubbard et al., 2009, Göbel et al., 2006). The following section explores the intersection between visuo-spatial processing and numerical cognition, examining how spatial biases influence numerical representations.

Visuo-spatial attention is closely linked to the way we picture numbers in our mind. Historically, Galton was the first one that investigated how different people visualised numbers in their mind's eye: all the representations were different from one another but they suggested the idea of a linear space when visualising increasing numbers (Galton, 1880).

The connection between numbers and space has been experimentally revealed by the discovery of the *Spatial-Numerical Association of Response Codes* (SNARC) effect, describing association of: small numbers with the left side of space, and large numbers with the right side (Dehaene et al., 1993). The SNARC effect has been widely replicated using a variety of tasks (review in Toomarian & Hubbard, 2018; meta-analysis in Wood et al., 2008). The SNARC effect has been considered as indicative of a spatial representation of numerical magnitude along a horizontal *Mental Number Line* (MNL). The MNL is typically oriented in a left-to-right scanning direction with smaller numbers on the left (Toomarian & Hubbard, 2018).

Is the MNL rooted in biology or shaped by experience?

1.2.1 *Left-to-right scanning and counting direction in human adults, children, infants, newborns and non-human animals*

Fischer (2024) proposed a hierarchical model of cognition known as GES: Grounding, Embodiment and Situatedness.
Grounding refers to phylogenetic and innate components, for example hemispheric lateralization. *Embodiment* corresponds to the sensorimotor experiences related to learning history. *Situatedness* refers to context-dependent (e.g. Fischer et al., 2010) or body position (Winter & Matlock, 2013).
Below, I briefly apply the GES framework to research on human adults, children, infants, newborns, and non-humans animals.

In adults

Grounding. In adults, the first empirical evidence of the SNARC effect comes from the above-mentioned seminal study by Dehaene et al. (1993), which demonstrated that numerical magnitude influenced participants' response times: participants were faster at classifying small numbers with the left side response key and large numbers on the right side response key. Interestingly, the effect was not driven by the absolute magnitude of the numbers, but rather by the numerical range employed in that task. Moreover, the effect was found to be independent from handedness. Importantly the same *Spatial-Numerical Association* (SNA) has been found in indigenous groups (Dehaene et al., 2008; Eccher et al., 2025), suggesting a potential innate, biologically determined MNL.

Importantly, SNA is mediated by visuo-spatial attention. In a line bisection task, visual lines made up (Fischer, 2001) of or flanked by (Porru et al., 2025) small numbers (e.g., 11111; 1___1) lead to an underestimation of the mid-point. Instead, when larger numbers were presented (e.g. 9), the midpoint was misperceived toward the right hemispace. Moreover, Porru et al. (2025) demonstrated that symbolic and non-symbolic numbers have different effects on flanked line bisection tasks: specifically, the asymmetric non-symbolic condition resulted in a stronger leftward bias. These findings suggest that the format in which numerical information is presented also contributes to the modulation of spatial representations.

Clearcut evidence of the crucial involvement of visuo-spatial attention in number processing has been provided by Fischer et al. (2003) who showed that small Indo-Arabic digits speeded up the detection of left sides targets, conversely, large Indo-Arabic digits speeded up the detection of right sides targets (but see failed replication in Colling et al., 2020). Later, Ranzini et al. (2015) found that overt attentional shifts influenced number processing. This effect was observed exclusively when numerical magnitude was relevant to the task at hand.

SNA and its relation to neglect, as mentioned in the introduction, provide further evidence for the grounded, spatial representation of numerical cognition, mediated by visuo-spatial attention. Patients with neglect likely have impaired access to the MNL (Zorzi et al., 2002). Interestingly, rehabilitation techniques for neglect have been shown to improve performance on visual tasks related to numerical processing (Umiltà et al., 2009). Another condition linked to the MNL is Gerstmann's syndrome, which involves the left angular gyrus. This syndrome is characterized by a cluster of symptoms including acalculia (difficulty performing arithmetic), agraphia (writing impairment), finger agnosia (inability to distinguish fingers), and left-right disorientation (Ardila, 2020). The association between these spatial and numerical deficits further supports the concept of a spatially grounded representation of numbers.

Embodiment. The seminal study by Dehaene et al. (1993), also provides evidence for cultural influences on the MNL. Their study included both French and Iranian participants, with the latter group showing no SNA, likely due to the differing reading habits (from right to left). Notably, the longer Iranian subjects had lived in France, the more their performances resembled that of European ones, suggesting an experience-driven influence on spatial-numerical mapping.

Zebian (2005) conducted a study on individuals from Arabic cultures, where the reading direction is oriented from right to left. This research revealed a reversed SNARC effect, where participants associated small numbers with the right side of space and large numbers with the left side.

This cultural dimension was further explored by Shaki and Fischer (2009), who tested three groups of adult subjects: Canadians, who read words and numbers from left to right; Palestinians, right to left for both; Israelis, right to left for words, left to right for numbers.

The results revealed clear group differences. Canadians showed a typical SNARC effect, Palestinians exhibited a reversed SNARC, and Israelis displayed no consistent spatial bias. These findings indicate that culture-determined directional experiences such as reading and counting direction habits, play a significant role in shaping SNA.

Recently, Shaki and Fischer (2024) reported a critical influence of counting direction tasks within the same population. In left-to-right counters, the central view of small digits speeded up the detection of left sided target, while the presentation of large digits speeded up the detection of right sided target. Instead, right-to-left counters reported the opposite pattern.

Situatedness. However, is a spatial representation of numbers solely determined by brain architecture and individual direction habits?

Fischer et al. (2010) addressed this question by showing that the SNARC effect can shift rapidly based on task and context. In their study, Western and Israeli participants read cooking instructions in which quantities were arranged in a congruent or incongruent manner with their habitual reading direction. This brief exposure was sufficient to weaken the SNARC effect in Western subjects and reversed it in Israelis subjects in the incongruent condition. The findings suggest that even short-term, context-dependent experiences can modulate spatial-numerical mapping.

A similar conclusion was reached by Shaki and Fischer (2008) in a study involving bilingual Russian-Hebrew speakers who read texts aligned either from left to right or from right to left. The strength of the SNARC varied depending on the orientation of the text, further supporting the influence of situational factors.

Further evidence comes from an oral number generation task where participants were instructed to produce random numbers while looking either to the left or to the right along a horizontal line: notably, larger numbers tended to be generated when looking to the right (Winter & Matlock, 2013).

In conclusion, evidence from adult participants indicates that the SNARC effect and the MNL are shaped by all three levels of the GES framework: grounding, embodiment, and situatedness.

In children, infants and newborns

Having reviewed the evidence for the SNARC effect and the MNL in adults, it is essential to consider how SNA develops in preverbal infants. Investigating this developmental stage provides insights into the origins of this association and allows a deeper exploration of its biological nature and ontogenetic modulation.

Grounding. A seminal study on 8-month-old infants by de Hevia & Spelke (2010) investigates whether human infants possess an innate association between number and spatial extent. Through a series of experiments, the researchers found that mapping between number and space appears to emerge independently of formal instruction or symbolic knowledge. These findings support the hypothesis that humans possess an early-developing, possibly innate, number-space mapping system, which may serve for later mathematical reasoning.

Further research by de Hevia et al. (2014) found out that preverbal 7-month-old infants, still lacking symbolic knowledge, exhibit a preference for increasing left-to-right numerical sequences.

In the study, three experiments were conducted. In experiment 1, infants habituated to both increasing and decreasing left-to-right sequences showed a significant preference for the increasing one. Experiment 2 followed the same procedure, but with right-to-left sequences. In this case, participants exhibited no preference, suggesting the absence of a fixed spatial orientation in their number-space mapping. Experiment 3 replicated Experiment 1 but without an habituation phase: infants who were first shown the increasing sequence displayed a preference for it, whereas those who were first exposed to the decreasing sequence showed no preference.

Evidence of early crucial involvement of visuo-spatial attention in number processing comes from Bulf et al. (2016), who provided the first demonstration of an oriented SNA in preverbal infants aged 8 to 9 months using non-symbolic numerical stimuli. Their findings suggest that the associations are automatic: infants were faster at locating objects on the left when cued by small numbers and on the right when cued by larger numbers.

More recently, Di Giorgio et al. (2019) investigated if SNA is evident immediately after birth and whether it depends on the relative or absolute magnitude. Hour-old newborns were habituated to a specific numerical quantity and then tested. Newborns showed a spontaneous association of small numbers on the left and larger on the right. Importantly, this mapping was relative rather than absolute: when they were habituated with 4, they associated 12 to the right space, while those who habituated with 36 associated 12 to the left. These findings indicate that SNA is innate and based on relative numerical quantity.

Embodiment. Cultural influences on SNA have also been documented in early development. Shaki et al. (2012) replicated a study by Shaki and Fischer (2009) with three-year-old children from cultures with different reading directions (British, Palestinians and Israelis). The children already exhibited directional biases in counting, which aligned with the findings in adults and with the dominant reading direction of their respective cultures. In British and Palestinian children the counting bias strengthened with increasing reading experience. In contrast, Israeli children, who are exposed to opposite directions in reading and counting, exhibited a counting bias from right to left but tended to weaken with age.

This pattern suggests that spatial biases emerge prior to formal reading instruction, likely due to observational learning such as watching caregivers read, or turn pages, or shift gaze direction. Over time, these early tendencies are further shaped through education.

In conclusion, the evidence from children, infants and newborns underlines the importance of both grounding and ontogenetic factors in the development of SNA and the role of visuo-spatial attention in number processing.

In animals

Interestingly, similar SNA observed in human infants and newborns (Bulf et al., 2016; Di Giorgio et al., 2019) has also been observed in non-human animals, suggesting that these mechanisms may not be uniquely human. Comparative studies on animals are particularly valuable as they give the possibility to investigate the innate origin of SNA without the confounding of cultural influence (like observational learning from caregivers in infants).

Grounding. A seminal study into spatial biases in numerical cognition was conducted by Rugani et al. (2007) on domestic chicks (*Gallus gallus*). In this experiment day-old chicks identified a target based on its ordinal position within a series of ten identical ones. Notably, this performance was consistent even when controlling the position and distance from the starting point. Furthermore, when the sequence was rotated by 90°, chicks made the correct choice starting more often from the left side.

This leftward bias in spatial-numerical mapping was further investigated by Rugani et al. (2010), who tested both nutcrackers (*Nucifraga columbiana*) and newly hatched domestic chicks (*Gallus gallus*). Both species showed a consistent leftward bias: they preferentially made the correct choice from left rather than right. These findings suggest similarities with the MNL observed in humans.

Building on these studies, Drucker and Brunner (2014) replicated the paradigm of Rugani et al. (2007) on Rhesus monkeys (*Macaca mulatta*), reporting analogous results. The monkey exhibited a SNARC-like effect, further supporting the cross-species presence of MNL. The same results of scanning direction preference from left to right were recently found in the same species by Rugani et al. (2024).

More direct evidence of a left-to-right oriented MNL in animals was provided by Rugani et al. (2015). In this study 3-day-old domestic chicks (*Gallus gallus*) were trained with the number 5 and subsequently tested with a smaller (2) or larger numerosity (8). A preference for associating a smaller numerosity with the left side and a larger numerosity with the right side emerged. Interestingly, when trained with the number 20, chicks associated 8 elements with the left, demonstrating that chicks' spatial-numerical mapping is based on the relative rather than absolute numerical magnitude.

Recently, Rugani et al. (2025) revealed that the left-to-right MNL can be found only in lateralized chicks.

In conclusion, animal research supports 1) the innate origin of a left-to-right MNL, and 2) the critical involvement of visuo-spatial attention and brain lateralization in number processing. However, the influence of ascending or descending numerical sequences on spatial preferences in newborn animals has never been explored.

2.OBJECTIVES

In my thesis work I aimed to investigate whether exposure to different numerical sequences (ascending or descending) have an impact on spatial behaviour in newborn animals, specifically in domestic chicks.

2.1 Chicks as animal model

There are several reasons for selecting this bird species.
First, although cross-species comparisons must be carefully made, chicks exhibit a left-to-right MNL that closely resembles the one observed in humans (see Introduction). Furthermore, studies suggest similar brain structures between chicks and mammals (Vallortigara, 2018; Matsushima et al., 2003).
Second, chicks lack a structure homologous to the corpus callosum (Ehrlich et al., 1988); consequently, their visual pathways fully cross at the optic chiasm, resulting in each hemisphere receiving input exclusively from the contralateral eye (Ocklenburg & Güntürkün, 2012). These neural features contribute to pronounced brain asymmetries. In fact, chicks can be strongly or weakly lateralized (Rogers, 1990; Vallortigara & Rogers, 2020; Rugani et al., 2025), making them a valuable model for studying the impact of lateralization on behaviour. Additionally, lateralization seems to make chicks use both spatial and object cues (Chiandetti et al., 2005).
Finally, chicks provide an excellent model for nature versus nurture investigations: 1) as a non-linguistic and non-symbolic species, they are free from cultural or language-based influences; 2) being precocial, they are born with already advanced visual and motor abilities, enabling immediate post hatching testing.

3.MATERIALS AND METHODS

All the experimental procedures regarding the use of animals have been approved by the Animal Welfare Committee of the University of Padua, the University of Padua's Ethical Committee for Animal Experimentation. and the Ministry of Health of the Italian Republic.

3.1 Subjects

The study sample consisted of 40 male domestic chicks (*Gallus gallus*) of the Aviagen ROSS 308 line. They were bought from a commercial hatchery (Società Agricola La Pellegrina Spa, San Pietro in Gù, Padova, Italy).

According to G*Power (repeated measures, interaction analysis) at least 36 chicks (18 per group) are required to reach a medium effect size (f = .5) taken from previous studies (Rugani et al., 2007; 2015; 2020), 80% power and 5% significance.

Only male individuals were tested as they are more responsive to food reinforcement, compared to females who are more responsive to social stimuli (Vallortigara et al., 1990, Regolin et al., 2005b).

3.2 Breeding conditions

The subjects hatched in the Comparative Cognition Laboratory of the Department of General Psychology (DPG) of the University of Padua. At the moment of their arrival, eggs were placed in the incubator, whose temperature and humidity were maintained at around 37°C and 55%, respectively.

Three days prior to hatching, the eggs were placed in the hatching machine, whose temperature and humidity were also controlled and preserved. There, eggs were exposed to a light source in order to lateralize them.

After hatching (usually on Monday), chicks were placed in groups of two or three of the same sex in a metal cage (40 cm x 25 cm x 35 cm). Each cage contained one glass jar (5 cm in diameter, 5 cm high) of water per chick and one glass of food. Water and food containers were positioned symmetrically in the cage. The first two days, chicks were also given three worms (*Tenebrio molitor* larvae), as these would later be used as a reinforcement during the experimental procedure.

The rearing room was kept at a temperature of 28-31°C, in order to maintain an appropriate environment, and was illuminated by fluorescent lamps (36W) set at 45 cm above cages.

3.3 Apparatus and stimuli

As the rearing room, also the experimental room was controlled in terms of temperature and humidity. To ensure the room was uniformly illuminated, the ceiling had four 58-W lamps that provided artificial lighting (placed 194 cm above the floor of the experimental apparatus).
Training and tests took place in a boat-shaped experimental apparatus, made up of green plastic panels. The apparatus was divided into three areas by two transparent removable barriers (30 x 40 cm) and the floor was covered by wood chips. (see Figure 1)
The transparent partitions were covered by a green plastic panel during the shaping and training phases to prevent the chick from being distracted from the previous stimulus.

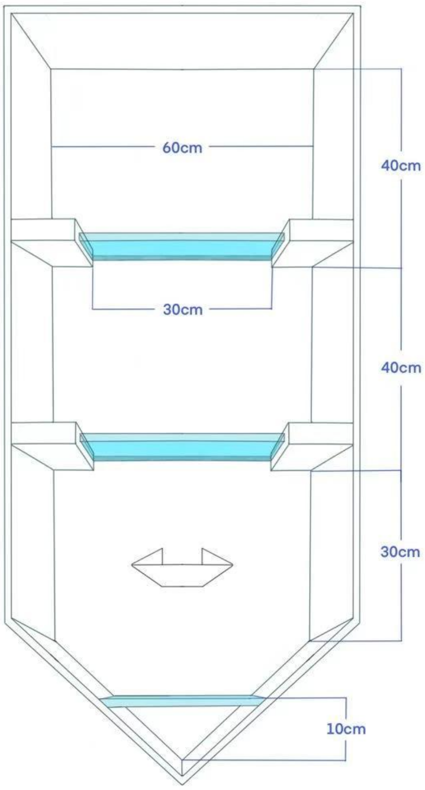

Figure 1: Schematic representation of the apparatus used at training.

The first area of the boat-shaped arena was used for the test (see Figure 2). It was separated from the others by a green plastic panel (49.5 cm x 100. 5 cm) placed in front of the first removable panel.

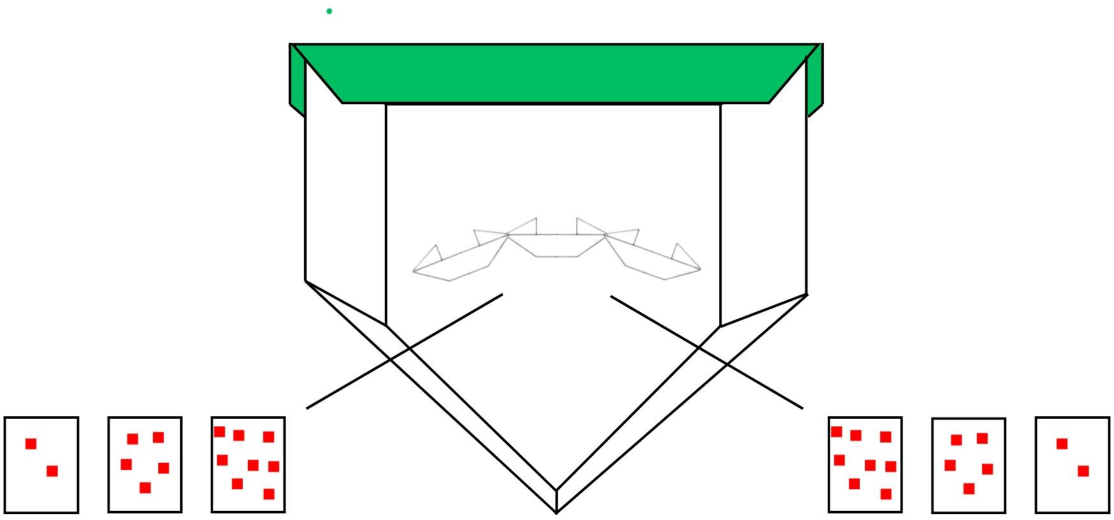

Figure 2: Schematic representation of the apparatus used at test with stimuli displayed as 2-5-8 (from left to right) or 8-5-2 (from left to right).

The stimuli consisted of three different types of static 2D images, attached to identical rectangular boards (11 cm x 9 cm). Each image depicted 2, 5, or 8 red squares (0.8 cm x 0.8 cm).

The boards were attached to a white plastic panel (15 cm x 12.5 cm), with a bottle cup on the back placed on the bottom, where a piece of a mealworm was put. In addition, a 3 cm side bent was added on both sides to prevent the chicks from seeing the food reinforcement. (see Figure 3)

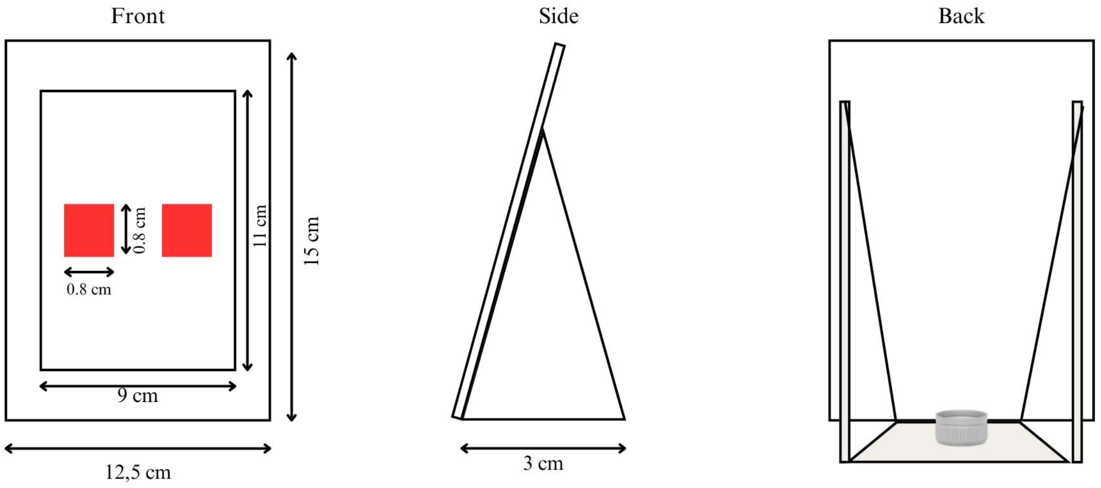

Figure 3: Schematic representation of a panel from the front, side and back.

To prevent the animal from learning the stimuli by the spatial collocation of the squares, every training and test involved stimuli arranged in a different spatial disposition on the rectangular board.

3.4 Experimental procedure

The experimental procedure was composed of a shaping phase, five training phases and two tests.

Shaping and Training 1 took place when chicks had 3 days (mostly on Wednesday). Training 2, Training 3 and Training 4, were conducted when chicks were 4-day old (mostly on Thursday). Training 5, Test 1 and Test 2 were administered to 5-day old chicks (mostly on Friday).

Chicks were deprived two hours before they underwent the first activity of each day, which is the shaping phase the third day and the training phase on the fourth and fifth day. Food deprivation makes them more active and motivated. Notably, while during training all trials but one or two were reinforced with food, during the test, no trial was reinforced.

Before the tests and between the trials of each test, chicks were placed in an opaque white box (20 cm x 40 cm x 30 cm) with a cover with the same characteristics.

Twenty chicks underwent shaping and training with ascendant stimuli (2-5-8, see Figure 4A) and twenty chicks underwent the same procedure with descendent stimuli (8-5-2, see Figure 4B).

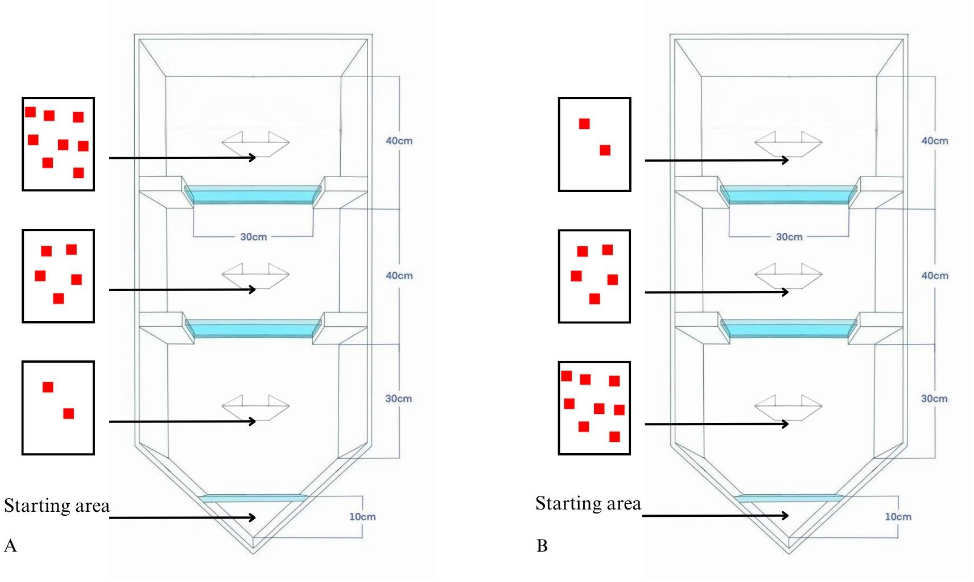

Figure 4: Schematic representation of the ascending (A) and descending (B) numerical sequence.

After Training 5, each chick underwent two Tests: in one test, the three panels were arranged in an ascending order (2-5-8, from left to right), in the other test, the same panels were arranged in a descending order (8-5-2, from left to right; see Figure 2). The order of the test was counterbalanced across subjects: 10 chicks from the ascending group underwent the test with the panels positioned in an ascending order (2-5-8, from left to right) first, 10 chicks from the ascending group underwent the test with the panels arranged a descending order (8-5-2, from left to right) first. The same applies to the chicks belonging to the descending group.

Below, I describe each phase (Shaping, Training, Test) in detail.

3.4.1 *Shaping and Training*

During shaping chicks were firstly trained to find the mealworm in the bottle cup behind the white panels, by slowly showing the bird the path. Secondly, the chick learned to walk along the full arena independently with the stimuli placed sagittally, one per area, in the center of it.

Each training and test phase only began after reaching a criterion. The criterion was defined as the successful completion of three subsequent trials (i.e., finding reward after the three sagittally displaced panels without the help of the experimenter). The criterion was introduced to: 1) focus the chick on the main task, and 2) assess its motivation.

Each training consisted of seven trials, where the chick had to go behind each panel and eat a piece of mealworm placed in the bottle cup, from the first stimulus to the third one. The experimenter recorded the time.

Each trial started with the chick placed behind a glass panel (27.5 x 28.3 cm) in the starting area. After five seconds, the glass partition was removed to let the bird move freely within the first part of the arena. After eating the first mealworm, the chick had to show interest in going to the next area by looking or pecking the second transparent partition for around four to five seconds. Once the bird had moved to the new area, the transparent partition was covered with a green plastic panel. The same procedure was repeated to access the final area, and the trial ended once the chick had eaten the last mealworm.

3.4.2 *Test phase*

The test consisted of three panels placed in the center of the first area (see Figure 2), with the chicks being tested with the panels arranged both in an ascending (2-5-8 from left to right) and descending sequence (8-5-2 from right to left).

The experiment consisted of three trials per sequence. The chick was initially placed behind a glass panel for five to ten seconds, to let it observe the numbers. Afterwards, it was released and given one minute to circumnavigate the panels from the right or left side. Meanwhile, the experimenter kept track of the time it took to make the first choice from the chick release, and of the chick's first choice (left or right). A choice was defined as the chick positioning its heads and ¾ of its body behind one of the two panels.

If the chick did not choose within one minute or if it was exhibiting signs of agitation (e.g. throwing itself on the stimuli), the trial had to be re-made till a maximum of three consecutive null choices. After three null trials, the chick would have been placed back in the rearing room for thirty minutes to rest before trying the test again.

3.5 Experimental design

All and only the test procedure was recorded for two reasons: 1) to observe the chicks from the monitor connected to the video camera, without disturbing or affecting the chick's choice; 2) to ensure an offline scoring. My role was to help the experimenter to record the data and score them offline.

The dependent variable was the percentage of left choices, calculated with the Left preference index: (numbers of circumvention of the panels from left) / (number of total trials) x 100.

The independent variables were the Group (between-subject variable with two levels, ascending or descending), the Test sequence (within-subject variable with two levels, ascending or descending) and Test order (between-subject variable with two levels, first test with panels arranged as 2-5-8 or first test with panels arranged as 8-5-2).

We formulated three hypotheses:

Hypothesis 1. A main effect of the Group would indicate that the numerical sequence has an effect on spatial behaviour, regardless of the arrangements of the panels at test. Specifically, we expected the ascending group to always prefer to move from the left and the descending group to have no preference.

Hypothesis 2. A main effect of the Test sequence. Specifically, we predicted preference to approach the test sequence from number 2, especially when arranged from left to right as reported in human infants (de Hevia et al., 2014).

Hypothesis 3. A group by Test sequence interaction would indicate that the numerical sequence has an effect on spatial behaviour but depending on the Test sequence. Specifically, we expected chicks to circumnavigate from the first number seen at training (number 2 for the ascending group, number 8 for the descending group).

We did not expect any effect of Test order, as it was fully counterbalanced.

4.RESULTS

4.1 Data analysis

Data were first analysed with a mixed analysis of variance (ANOVA): Group (ascending vs. descending; between-subject factors) x Test sequence (2-5-8 vs. 8-5-2; within-subject factors) x Test order (2-5-8 first vs. 8-5-2 first; between-subject factors).
Then one sample, paired and independent samples post hoc t-test were conducted.

For all the analyses JASP 0.19.3 was utilized (JASP Team, 2024).

4.2 Results

The ANOVA revealed a significant effect of Group ($F(1, 38) = 7.810$; $p = 0.008$, $\eta2 = 0.143$), indicating that chicks' performance differed significantly between the two Groups.
All other effects and interactions did not reach the level of significance ($p>0.05$).

The ascending group reported preference for approaching the triplets of elements from left to right both in Test sequence 2-5-8 (Mean: 76; One sample t-test: $t(19)=4.465$; $p<0.001$, Cohen's d = 0.998) and in Test sequence 8-5-2 (Mean: 75; One sample t-test: $t(19)=3.135$; $p=0.005$, Cohen's d = 0.701). Instead, the descending group reported no spatial preference for approaching the triplets neither in Test sequence 2-5-8 (Mean: 43; One sample t-test: $t(19)=-0.867$; $p=0.397$) nor in Test sequence 8-5-2 (Mean: 53; One sample t-test: $t(19)=0.377$; $p=0.711$; see Figure 5).
Paired t-test confirmed that performance between the two Test sequences within groups was not significant ($p>0.05$). (see Figure 5)

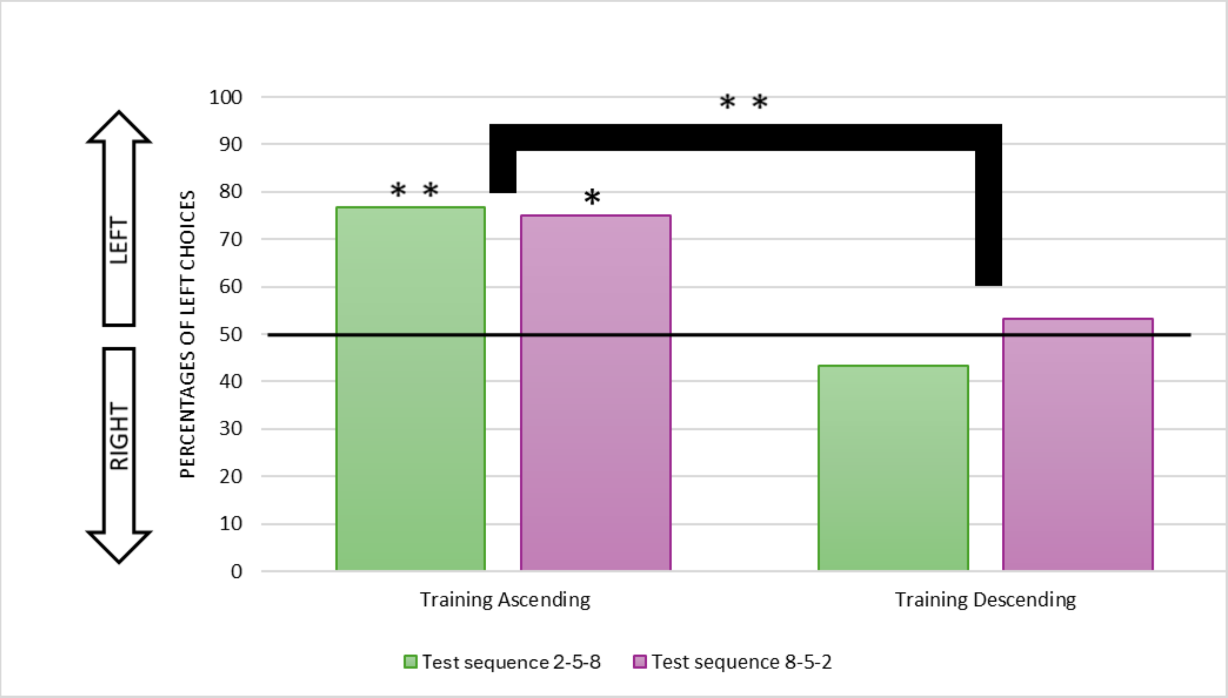

Figure 5: The graph displays the mean percentage of left choices of the Ascending and Descending group as a function of the Test sequence. The central line indicates the chance level.

Since the paired sample t-test reported no differences based on the Test sequence, a new variable "Bias" was computed by merging the choices of the two Test sequences. A one-tail independent t-test revealed a significant difference between the two Group (Mean ascending sequence: 75; Mean descending sequence: 48; t(38) = 2.857, *p* = 0.003, Cohen's d = 0.903), confirming the impact of the Group on the general directional bias.

5.DISCUSSION

The aim of my thesis was to understand for the first time the impact of numerical sequences on spatial behaviour in newborn animals.

My study tested 40 domestic chicks (*Gallus gallus*). Twenty chicks underwent a training involving an ascending numerical sequence, other twenty chicks underwent a training involving a descending numerical sequence. After training, all subjects were tested in two tests in which the same numerosity was organised as 2-5-8 or 8-5-2.

Firstly, the left bias is confirmed in this study, as the percentage of left choices is significant above chances, whereas right choices were not. This findings aligns with previous research in humans (Jewell and McCourt, 2000), newborns (Nava et al., 2022) and animals (Rugani et al., 2007; Rugani et al., 2010).

Secondly, the main effect of the Group suggests that exposure to numerical sequences plays a role in shaping the biological and innate left bias during visuo-spatial and numerical tasks. More precisely, the ascending Group may have reinforced this natural tendency, whereas the descending Group may have conflicted with it, resulting in a weaker leftward preference. It is plausible that when a numerical task is congruent with the innate bias, it is more easily learned and retained. Conversely, when the numerical task is incongruent with the innate bias, there may be an overlap between learned and biological processes. Notably, this effect has occurred in relation to numerical magnitude.

Third, no main effect of the Test sequence was found. This may be due either to the absence of a preference or to the fact that the training sequence was presented sagittally rather than horizontally, as is typically done for domestic chicks (Rugani et al., 2007; 2010; 2025) and preverbal infants (de Hevia et al., 2014).

Lastly, the absence of an interaction between variables suggests that the chicks may have interpreted the test panels as a new visuo-spatial context compared to the one experienced during training.

Overall, the findings indicate that the innate left-to-right preference to approach a visual scene in domestic chicks can be modulated by early experience with numerical sequences. Thus, there appears to be an interplay between innate cognitive biases and early learning. Spatial behaviour of domestic chicks is not fixed but can be modulated by exposure to numerical sequences. This study represents a first step toward understanding how experiences associated with numerosity can shape biologically determined spatial behaviour in animal models.

5.1 Limitations and Future directions

Despite the ability of the study to answer my research question, it is also important to indicate possible limitations.

Firstly, the test was included in a battery of tests made on the days before the one of our interest. Therefore, it would be interesting to assess the replicability of the results within a less demanding battery of tests, allowing also to test chicks on their third day of life. Indeed, in our experiment the test was conducted on their fifth day of life, by which time their sensorimotor development is already more advanced.

Secondly, so far studies on chicks' working memory have shown that they can hold in their mind up to two elements (Vallortigara et al., 1998; Regolin et al., 2005a), while our test involved three. Future research would be useful to explore whether chicks can retain more than two objects, which would provide stronger support for our findings.

Nevertheless, the most relevant aspect of the study was the directional preference.

Third, a control group would be necessary to establish a baseline against which our results can be compared. This would also enable us to clearly identify the effect of the Group. Indeed, our experimental procedure had never been used before, so chicks' behaviour in this context remains unclear.

Notably, ongoing experiments are replicating this procedure controlling for continuous variables, such as area and perimeter (e.g., Rugani et al., 2015; 2020), and with a different numerical range (8, 20, 32), to investigate if the results are dependent or independent from the specific stimuli used.

In conclusion, in future research it would be interesting to investigate if the left bias can be reversed as in humans (Zebian, 2005).

6.CONCLUSION

Visuo-spatial attention is a key perceptual feature in both humans and animals. Indeed, it has biases that can shape behaviour and understanding of the external world. One such bias is the left-hemispace bias, observed across species (Jewell & McCourt, 2000; Nava et al., 2022; Diekamp et al., 2005; Regolin, 2006; Chiandetti, 2011). This left side bias is critical also when processing numerical information, indeed visuo-spatial attention is closely linked to number processing (Fischer, 2001; Porru et al., 2025; de Hevia et al., 2014; Rugani et al., 2025). In humans, for instance, numbers are typically represented along a MNL oriented from left to right (e.g. Dehaene et al., 1993; de Hevia et al., 2014). While this orientation is partly innate, it is also subject to cultural and contextual influences, such as reading and counting habits (e.g. Shaki & Fischer, 2009) and task demands (e.g. Fischer et al., 2010).

Research on domestic chicks (*Gallus gallus*) has revealed striking similarities with humans in this regard. Rugani and colleagues (e.g. Rugani et al., 2007; 2010; 2015; 2020) demonstrated that these birds show a natural left-to-right bias and a form of left-to-right MNL. The present thesis reveals, for the first time, an impact of numerical sequences on the natural tendencies to explore and approach visual scenes from left to right.

These findings contribute to our understanding of the fundamental link between number and space, offering valuable insights for comparative, development, spatial and numerical cognition.

AUTHOR'S STATEMENT

This work was carried out as part of a larger research project led by Prof. Rosa Rugani, Dr. Arianna Felisatti and Matteo Macchinizzi. Chiara Gonzato contribution is restricted to a part of the project adapted to meet the requirement criteria for the bachelor mandatory internship.

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