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Is spatial-numerical association driven by numerical magnitude or ordinal position? Evidence from day-old domestic chicks

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Index

Abstract

People tend to associate small numerosity with the left side space and large one with the right side. Such spatial-numerical association (SNA) is not only present in adults and infants, but also in many non-human species, including domestic chicks (*Gallus gallus*). Previous research demonstrated that chicks associate different ordinal positions and numerosity with space. Specifically, when chicks are trained to peck at target stimulus in a specific ordinal position, they tended to count from the left. Moreover, similar to human, they associate small numerosity with the left side and large numerosity with the right side of space. However, it remains unknown how numerical magnitude and ordinal position interact and contribute to the SNA effect. To address the question, we trained chicks using either an ascending (2-5-8) or a descending (8-5-2) numerical sequence. At test, two panels with stimuli composed of red squares randomly distributed on a white background. During the test phase, two panels with identical stimuli (2 vs.2, 5 vs.5, 8 vs.8) were placed laterally and symmetrically in front of the chicks. Their choices revealed that the ascending group exhibited a general preference to associate numerical stimuli with the left space, while the descending group reported SNA driven by numerical magnitude. Notably, from the analysis of the first two stimuli, SNA driven by numerical magnitude emerged also in the ascending group. In conclusion, both numerical magnitude and ordinal position influence the SNA effect. Crucially, different numerical sequence orders modulated the strength of SNA.

1. Introduction

1.1 Spatial-numerical association

Numbers are embedded in every corner and moment of our daily lives. They are not only the language through which humans measure the world, but also a means of capturing the underlying relationships between all things. Therefore, understanding how humans perceive and process numbers has become a significant topic in the field of psychology. An increasing number of researchers have shown that numerical cognition is closely associated with spatial representation (Toomarian & Hubbard, 2020. In other words, humans process abstract numerical concepts in a concrete way, by mapping them onto spatial structures.

More specifically, people tend to associate smaller numbers with the left side and larger numbers with the right side. This phenomenon, first revealed by Dehaene, Bossini and Giraux (1993), is known as Spatial-Numerical Association Response Codes (SNARC). It suggests a spatial representation of numbers along a left-to-right mental number line (review in Toomarian & Hubbard, 2018).

However, how is this mental number line formed? Is it acquired through cultural environments, or is it determined by innate neurological structures? Which variables play a pivotal role in the emergence of spatial-numerical associations (SNA)? To address these questions, various experiments have been conducted, not only in humans, including adults and pre-verbal infants, but also in non-human species such as vertebrates (review in Rugani & de Hevia, 2017) and invertebrates (Giurfa et al., 2022). Through cross-species, cross-cultural and developmental studies, the influence of cultural and biological factors on SNA have become increasingly evident.

First, I will introduce the distinct meanings of numbers--namely, nominal, ordinal and cardinal. In particular, the latter two concepts are fundamentally important for this article. Next, I will present key findings from experiments involving both humans and non-human animals, which demonstrate the pervasive presence of SNA across species, and highlight its potentially evolutionary significance for survival. Finally, I will motivate and present the research I have conducted.

1.2.1 Symbolic and non-symbolic numbers

In daily life we can encounter *symbolic numbers* everywhere, on our passports, in train stations, or while navigating online. Symbolic numbers refer to numbers that are represented through culturally defined symbols, such as *Indo-Arabic* digits (e.g., "2") or written number words (e.g., "two"). Different from *non-symbolic number* representations, such as arrays of dots or sequences of sounds, symbolic numbers are culturally constructed and vary across writing systems and languages. Therefore, their understanding requires individual learning and cultural transmission. In contrast, non-symbolic representation relies on innate or early developing cognitive systems (Feigenson et al., 2004).

Although the processing of these distinct numerical representations shares a common neural basis in the inferior parietal regions (Dehaene et al., 2003), they also activate different brain areas (Holloway et al., 2009). Holloway and colleagues (2009) found that the elaboration of non-symbolic numbers requires greater visual processing to individuate stimuli and involves the activation of the right posterior superior parietal lobe. Instead, symbolic number processing involves the left angular gyrus and the left superior temporal gyrus.

In addition, it is important to distinguish non-symbolic number processing from continuous magnitude processing. Non-symbolic numbers refer to discrete quantities, while continuous magnitudes indicate analog properties, such as the length of a line, the size of an object or the duration of time. In 2003, Walsh proposed a common cognitive system for processing diverse magnitudes. However, some studies demonstrated that, though there is a shared mechanism, the patterns of neural activation are not entirely overlapping (e.g., Pinel et al., 2004).

1.2.2 Meanings of number

Numbers carry different meanings. For instance, consider the sentence: "The boy with the registration number 22 finished in 22nd place in the exam, which had 22 candidates in total." In this case, the registration number has a *nominal meaning,* that is used as a label to identify a specific person or object. Other examples include randomly generated one-time passwords, identity card codes or telephone numbers. These numbers are arbitrary and do not imply magnitude or order. In contrast, "22nd place" represents an ordinal position within a sequence. Ordinal numbers, which are

used for ranking do not support any calculation. Finally, the last use of the number in the sentence has a *cardinal meaning*, referring to the total quantity of candidates.

The processing of different types of numerical meaning may involve distinct cognitive systems. Delazer and Butterworth (1997) reported a patient with a left frontal infarct who exhibited deficits in cardinal number processing but relatively preserved ordinal processing. The patient was able to count, write and produce number sequences but had difficulties in answering the question such as "which of two presented numerals is larger". In contrast, a patient with lesions described by Turconi and Seron (2002), with lesions in the left posterior parietal cortex and the right parieto- occipital junction, showed the opposite behavioral pattern. The patient was unable to determine the ordinal relationships among numbers and letters but had little impairment in comparing numbers. This double-dissociation provides compelling evidence that the neural systems underlying cardinal and ordinal number processing are anatomically distinct.

1.3 SNARC effect on adults

The seminal study conducted by Dehaene et al. (1993) demonstrated the tendency of human adults to associate small numbers with the left side and large numbers with the right side. The experiment required participants to classify Indo-Arabic digits as odd or even by pressing one of two lateralized keyboard buttons. Even if numerical magnitude was not task-relevant, small numbers were more quickly classified with the left-side button, whereas large numbers speeded up responses with the right-side button.

From the study of Dehaene and his colleagues (1993), it is found that:

1. *Relativity* plays a key role in spatial-numerical associations effect. The same number (e.g., 4) is responded faster with the left-hand button when belonging to the numerical range from 4 to 9; conversely, it is responded faster with the right-hand button when belonging to the numerical range from 0 to 4. This suggests that the spatial-numerical mapping is influenced by the number's relative position within a given numerical range.

2. *Independence from handedness*. More specifically, the SNARC effect is not linked to the responding hand, but rather to the external space.

3. Influences of *cultural factors*, such as the direction of writing and reading. For instance, Iranian

participants, who write digits from right to left, do not exhibit the SNARC effect but demonstrated a reversed pattern compared to French participants. Notably, the amount of time spent in Western cultures with left-to-right reading direction habits can influence both the strength and direction of the SNARC effect.

4. *Automaticity of SNA*. The presence of SNARC effect, even though the tasks do not require magnitude assessment, implies that SNA is automatically activated during number processing.

According to a meta-analysis conducted by Wood et al. (2008), the strength of the SNARC effect is determined by the degree of magnitude activation (also consider Shaki & Fischer, 2024). Moreover, the SNARC effect tends to be more pronounced in elder individuals (Wood et al., 2008). Two possible interpretations have been proposed to explain these findings. One suggests that the effect strengthens with age due to increasing cultural exposure to SNA. Alternatively, the stronger effect in elder adults may be related to reduced inhibitory control, which makes automatic associations harder to suppress (Wood et al., 2008).

Moreover, spatial-numerical mapping is not limited to symbolic numbers, the SNARC-like effects have also been observed in magnitude judgment and parity judgment tasks, both in children and adults, using non-symbolic numerical stimuli such as dot arrays (Zhou et al., 2016; Adriano et al., 2022; Zhang et al., 2024). Therefore, SNA is a cognitive phenomenon commonly observed in human numerical processing. It does not rely on language or symbolic representation but may be rooted in our more fundamental system for perceiving quantity.

1.4 SNA effect on preverbal infants

The tendency to associate numbers with spatial orientations is not limited to adults with mature symbolic numeral systems. It has also been observed in preverbal infants and newborns (Bulf et al., 2016; de Hevia et al., 2017; Di Giorgio et al., 2019), suggesting early developing or even innate origins of SNA. Increasing research focuses on whether a mental number line exists in infants, aiming to explore the potential biological foundations of SNA.

De Hevia et al. (2014) tested 7-month-old infants to investigate their capacity to discriminate numerical sequences. Increasing or decreasing sequences consisted of three numerical stimuli presented successively on the screen, oriented either from right to left or from left to right. They

found that infants looked significantly longer at novel, increasing sequences presented in a left-to-right orientation, regardless of the orientation shown during the habituation phase. However, such preference vanished when sequences were presented from right to left, implying that a left-to-right congruence with the mental number line may facilitate the processing of ordinal information, even at a very early age.

Furthermore, a study conducted by Bulf et al. (2016) employed a Posner-like paradigm and an eye-tracking system to record infants'gaze behavior when exposed to a central visual cue and a lateral visual target. The visual cues were either numerical (e.g., 2 or 9 dots) or non-numerical (e.g., an X-shaped figure).In congruent trials, a small numerosity cue (e.g., 2 dots) preceded a left-side target, and a large numerosity cue (e.g., 9 dots) preceded a right-side target. In incongruent trials, the reverse arrangement was used. Results showed that infants exhibited significantly faster gaze orientation in congruent trials than in incongruent trials. This suggests the presence of an automatically activated SNA on preverbal infants, with small number associated with the left side of space and large number with the right.

However, these experiments testing infants, preventing researchers from ruling out the possibility that early exposure to environmental factors and interactions with caregivers influenced the development of a mental number line. To address this limitation, Di Giorgio and colleagues (2019) conducted two experiments with newborns, aiming to assess whether the mental number line characterizes an innate biological structure and depends on absolute value or relative magnitude.

In their experiments, the stimuli consisted of static two-dimensional images presented bilaterally on either side of a central fixation point. Each image displayed a well-defined number of black square elements within a white square area. During the habituation phase, newborns were shown two identical stimuli, each depicting 12 elements. No significant difference was observed in the newborns' looking times toward the left or right side. However, in the test trials, when the stimuli contained smaller numerosity (e.g., 4 vs. 4), newborns looked significantly longer to the left stimuli, while for larger numerosity (e.g., 32 vs. 32), they looked significantly longer to the right stimulus.

To rule out the influence of continuous magnitudes, the overall area of black square for all stimuli was controlled in the second experiment. In the first condition, neonates were habituated to a stimulus containing 4 elements and then tested with a stimulus containing 12 elements. At test, neonates looked significantly longer to the right-side stimuli. In the second condition, neonates were habituated to the number 32 and again tested with number 12. This time, they showed a

preference for the left-side stimuli. These results are consistent with those from the first experiment and inform on the relative nature of SNA and its independence from continuous variables early in life.

So far, the evidence suggests that SNA is rooted in a biological predisposition that emerges prior to cultural and environmental influences. During ontogeny, the directionality of reading and writing plays a secondary role in shaping the orientation of the SNA (e.g., Dehaene et al., 1993; Toomarian & Hubbard, 2018).

To further explore the universality and evolutionary origins of the SNA, researchers have turned to studies involving non-human animal species. These investigations aim to determine whether the mapping of numerical magnitude onto spatial representations is a uniquely human phenomenon or a more general cognitive tendency shared across species. However, caution is urged in interpreting findings from animal models. Species from distinct evolutionary lineages may independently evolve similar cognitive traits (Emery & Clayton, 2004), and differences in brain structure and lateralization must be taken into account when drawing parallels to human cognition.

1.5 SNA in non-human animals

A growing body of research has demonstrated that vertebrates possess the ability to process numerical ordinality. For instance, primates such as chimpanzee (*Pan troglodyte*) (Biro & Matsuzawa, 2001), macaques (*Macaca mulatta*) (Washburn & Rumbaugh, 1991) and ring-tailed lemurs (*Lemur catta*) (Merritt et al., 2011) can be trained to make ordinal judgement. Other species, such as the rufous hummingbird (*Selasphorus rufus*) are able to identify a specific ordinal spatial position (Vámos et al., 2020), while rodents like the Norway rat (*Rattus nervegicus*) (Suzuki & Kobayashi, 2000) and the zebrafish (*Danio rerio*) (Potrich et al., 2019) have been shown to use ordinal information to select target stimuli.

Over time, research has also revealed that some animals are capable of associating numbers with space in a left-to-right orientation, exhibiting a form of SNA analogous to the human mental number line. These findings raise the possibility that both humans and non-human animals may inherit this spatial-numerical mapping from a common evolutionary ancestor, suggesting that the origins of the SNA could be deeply rooted in the vertebrate lineage.

For example, a study by Drucker and Brannon (2014) investigated the SNARC-like effect in Rhesus monkeys (*Macaca mulatta*). The monkeys were first trained to select the fourth position from the bottom in a vertical array of five elements. When the array was rotated 90 degrees into a horizontal line, monkeys preferred to select the fourth position from the left, rather than from the right. This leftward bias suggests a SNARC-like spatial mapping of ordinality. However, as with the previous study, it is difficult to rule out alternative explanations. For instance, the effect might be due to hemispheric specialization in spatial attention--a phenomenon known as pseudoneglect, where monkeys (and humans) exhibit a natural bias towards the left side of space. Thus, these results cannot definitively establish the presence of an innate mental number line in non-human primates.

Another study demonstrated that a SNARC-like effect was present in chimpanzees (*Pan troglodytes*), our closest evolutionary relatives (Adachi, 2014). In this study, a group of adult chimpanzees was trained to touch the numerosity stimuli on the screen. At test, only two numerals (1 and 9) were displayed. Chimpanzees responded significantly faster when "1" was located on the left side and "9" on the right side, compared to the reverse configuration.

Remarkably, SNA has been observed not only in vertebrates, but also invertebrates. Giurfa et al. (2022) demonstrated that even honey bees are able to extract numerical information from visual stimuli and associate numbers with space. Similarly to humans and primates, bees associate relatively smaller numerosity with the left side and larger numerosity with the right side. This suggests that SNA is evolutionarily conserved across nervous systems, irrespective of their neural complexity.

1.6 Previous research on chicks

Additionally, there here have been many studies on domestic chicks.

A seminal study (Rugani et al., 2010) has been conducted to examine the ordinal capacity of adult nutcrackers (*Nucifraga columbiana*) and few-day-old domestic chicks (*Gallus gallus*). During the training phase, birds learnt to peck at a bottle cup at a specific ordinal position (e.g., the fourth or sixth) among sixteen identical, equally spaced and sagitally aligned bottle cups. At test, the bottle cups were rotated by 90 degrees, becoming parallel to the birds' central starting position. The birds preferentially selected the correct element starting from the left end rather than the right one of the

series (see Fig.1A).

Moreover, chicks have been shown to associate with space also numerical magnitude and to do so in a relative way (Rugani et al., 2015). A group of day-old chicks was familiarized with panel depicting a certain number of elements (e.g. 5). At test, two panels depicting identical target (2 or 8) were positioned in symmetric way, each in one side with respect to the starting area where the chick was released. As a result, chicks demonstrated a significant preference for the left panel in the Small Number Test and the right panel in the Large number Test. Importantly, in a new group of chicks, which was familiarized with target "20" at training, leftward bias persisted for the Small Number Test "8" and rightward bias for the Large Number Test "32". Furthermore, the other variables, such as shape, color, size of each element, overall area, overall perimeter and density, have been shown to have no influence on chicks' performance. The presence of SNA relative and independent from non-numerical cues in non-human species suggests that SNA is biologically determined, possibly imposed by brain asymmetry and only later modulated by cultural experience (Rugani & de Hevia, 2017).

In line with the findings of Rugani et al. (2015), a subsequent study by Rugani et al. (2020) provided a consistent conclusion that SNA originates from pre-linguistic precursors. In this study, 3-day-old domestic chicks were presented with stimuli depicting 5 elements within a white square area. During the test phase, two identical stimuli, each displaying a well-defined number of elements, were positioned bilaterally and symmetrically with respect to the chick's starting point. In the control tests (5 vs. 5), no lateral preference was observed, suggesting the absence of individual side bias. In contrast, in the experimental conditions, chicks exhibited a significant leftward bias in the Small Number Test (2 vs. 2) and a rightward bias in the Large Number Test (8 vs. 8) (see Fig.1). The study indicates that it is the numerical magnitude, rather than random individual preference, that drives the spatial-numerical mapping in newborn chicks.

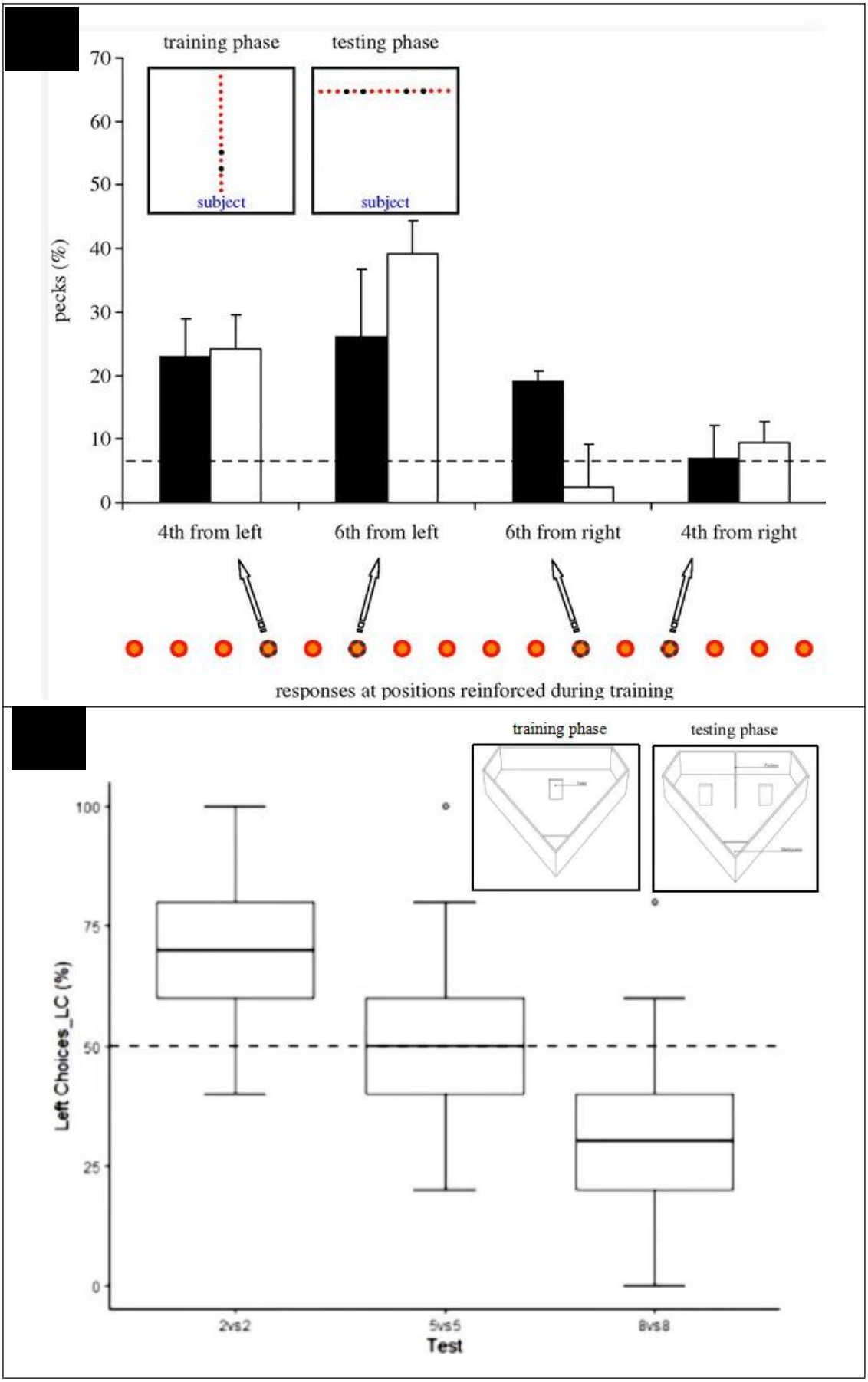

Fig.1 Experimental apparatus and results in Rugani et al., 2010 (A) and Rugani et al., 2020 (B).

1.7 The present study

1.7.1 Objectives

This study aims to further investigate the roles of ordinal position and numerical magnitude in shaping SNA in 3-day-old domestic chicks.

The existence of SNA resembling the human mental number line has already been demonstrated in domestic chicks (Rugani et al., 2007; 2010; 2015; 2020; 2025). However, it remains unclear whether this association is more strongly influenced by the ordinal position of a numerical sequence or by numerical magnitude. This study aims to address exactly this research gap. Specifically, we trained day-old chicks with either ascending or descending numerical sequences. At test, we examined their spatial preference for the specific numerosity.

1.7.2 Why we choose newborn chicks as subjects

First of all, to eliminate the influence of prior experience on performance, chicks (*Gallus gallus*) serve as ideal subjects. Their motor and cognitive capacity develops rapidly, allowing them to interact with external environment and participate in experimental tasks almost immediately after hatching. Even at just a few days old, chicks are capable of independently navigating their surroundings, pecking, and foraging for food. A series of studies employing diverse experimental paradigms have demonstrated the excellent performance of chicks in numerical tasks (Rugani et al., 2007; 2010; 2015; 2020; 2025), confirming their suitability as model organisms and providing important references for the present research.

Secondly, chicks few days after hatching can discriminate between different numerosity, perform rudimentary arithmetic operations, and utilize ordinal information to identify a target element within a sequence of identical stimuli (Rugani, 2018). Remarkably, chicks are able to distinguish large numerosities when other irrelevant non-numerical variables are controlled (Rugani et al., 2015). These findings demonstrate numerical abilities that are of central interest in the present study.

Thirdly, chicks have been shown to retain information for up to 60 seconds, even when the time allowed for initial perception is brief (Vallortigara et al., 1998), indicating an early stage working

memory that is fundamental to maintain mental representation of numerical sequences.
Lastly, different from primates, chicks are much easier to raise and manage, providing a more economical and efficient means of data collection.

1.7.3 Novelty of experimental design

The experimental parading is built on the studies of Rugani et al. (2015; 2020). While previous experiments employed only a single training stimulus, in the present study the training stimuli consisted of a sequence of three non-symbolic numerical stimuli. Specifically, chicks were divided into two groups: The ascending group was trained with a numerical sequence organized in an ascending order (2-5-8); the descending group was trained with the same numerical sequence but organized in a descending order (8-5-2). At training, the numerical sequences were positioned sagitally with respect to the chick's starting point. At Test, each chick underwent three tests: Small Number Test, Large Number Test and Control Number Test. This study was designed to: (1) assess SNA after training with a sequence and not a single numerical stimulus, thus extending the previous findings (Rugani et al., 2015; 2020), (2) understand the interaction between biological predispositions and experiential influences on SNA, and (3) weigh the role of ordinal position (first vs. last stimulus) and numerical magnitude (small vs. large) in determining SNA.

1.7.4 Hypotheses

The first hypothesis reckons on replicating the left-to-right SNA observed in previous studies using a single numerical reference (Rugani et al., 2015; 2020). If chicks are able to retain all three stimuli in working memory during the habituation phase, we expect to find stronger spatial biases for the first and third stimuli in the sequence, namely the smallest (2) and largest (8) numerosity. Thus, we might find a leftward bias for "2", a rightward bias for "8", and no significant bias for "5", which may serve as a central or reference value within the sequence.
Conversely, if chicks only maintain the first two stimuli in working memory, spatial biases are expected primarily for those two stimuli. For instance, in the ascending sequence group (2-5-8), if chicks retain the entire numerical sequence in working memory, we may observe significant

differences in lateral preference for the numerosity "2" and "5" compared to "8".

Second, we explore whether spatial biases are driven by absolute numerical magnitude or by ordinal position within a learned sequence. If SNAs are governed by numerical magnitude, we expect consistent lateral preferences: small numerosity (e.g., 2) were associated with leftward biases and large numerosity (e.g., 8) rightward biases, independent of the training sequence. In contrast, if SNAs are shaped primarily by ordinal position, we predict that chicks will show a leftward bias for the first item in the training sequence regardless of its numerical value. That is, we expect a leftward preference for "2" in the ascending group (2–5–8) and for "8" in the descending group (8–5–2), and a rightward preference for “8” in the ascending group (2-5-8) and for “2” in the descending group (8-5-2).

II. Materials and methods

2.1 Subjects

Subjects were 53 male domestic chicks (*Gallus gallus*) of the Aviagen ROSS 308 line. Fertilized eggs were obtained from a local commercial hatchery (Società Agricola La Pellegrina Spa, San Pietro in Gù, Padova, Italy). After the incubation, the chicks were housed in standard metal cages (40 cm x 25 cm x 35 cm). Each cage contained two or three chicks of the same gender. The rearing room, illuminated by fluorescent lamps (36W) located 45 cm above each cage, was constantly monitored for temperature (28-31°C) and humidity (68%). Food and water in transparent glass jars (5cm in diameter, 5 cm high), were placed in a symmetric way. For example, in a cage with two chicks, each chick was provided with its own water jar, positioned at two corners on one side of the cage, with a food jar placed centrally between them.

2.2 Apparatus

In the experimental room, temperature was controlled (about 25°C). Artificial light was provided by four 58-W lamps placed on the ceiling (194 cm above the floor of the experimental apparatus).
The experimental apparatus was located in the experimental room. A boat-shaped arena was constructed using green plastic panels. The apparatus was divided into three areas using two transparent removable partitions (30 cm x 40 cm; see Fig.1A). The external wall comprised a 40 cm high plastic panel, the floor a white plastic sheet covered by wood chips. A "starting area" was limited by a transparent removable glass (28 cm x 28 cm) positioned at 10 cm from the main vertex of the arena. The transparent glass was used to confine subjects for five seconds before the beginning of each training and test trial. This was essential to let the subject process the stimuli. During the test phase, only the first area was used for behavioral observations. The first transparent partition was replaced by a green plastic panel bisected by an opaque partition (see Fig.2B). Two panels, one on the left side and one on the other side, were positioned 15 cm apart and 40 cm away from the main vertex of the arena. The panels were symmetrically oriented towards the transparent

glass of the starting area.

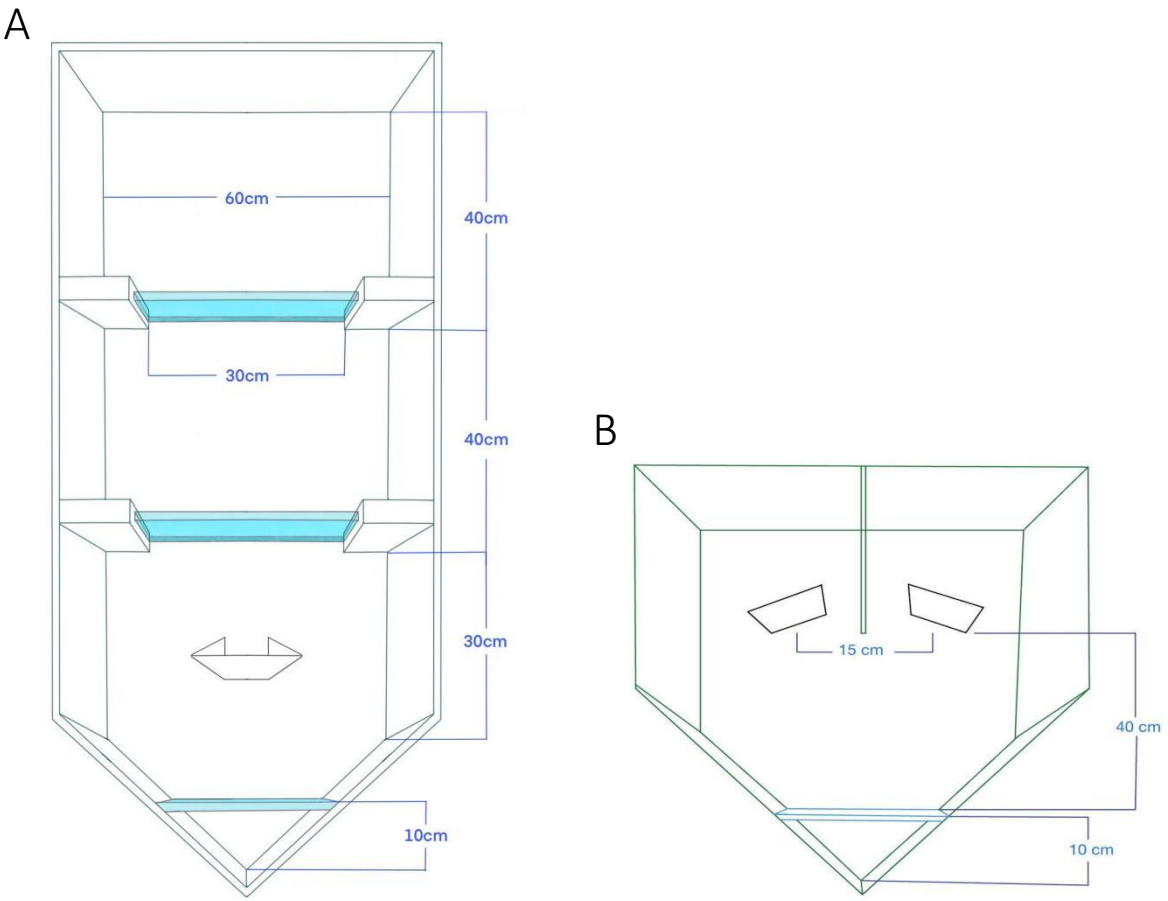

Fig.2 Schematic representation of the experimental apparatus used at training (A) and test (B).

2.3 Stimuli

Training and test stimuli consisted of three different numerosity (2, 5, 8) of red squares (0.8 cm x 0.8 cm) distributed on a white rectangular board (11 cm x 9 cm; see Fig. 3). The stimuli were positioned on three sagittally aligned panels in ascending (Fig.3A) or descending sequence throughout training. The placement of squares on the board for each stimulus was randomly assigned, ensuring that the stimuli associated with each numerosity in the training phases varied from one another. For each stimulus, two identical copies were printed and placed on the front and back of a panel. The panels (15 cm x 12.5 cm) were placed in the center of each area, with a bottle

cup affixed to the rear bottom. All panels were provided with 3 cm sides bent back to prevent the chicks from spotting behind the panel (where the mealworm was hidden in the bottle cup during training) before circumnavigating it. During the preparation of stimuli, chicks were placed in an opaque white box (20 cm x 40 cm x 30 cm) with an opaque cover to prevent them for seeing experimenters.

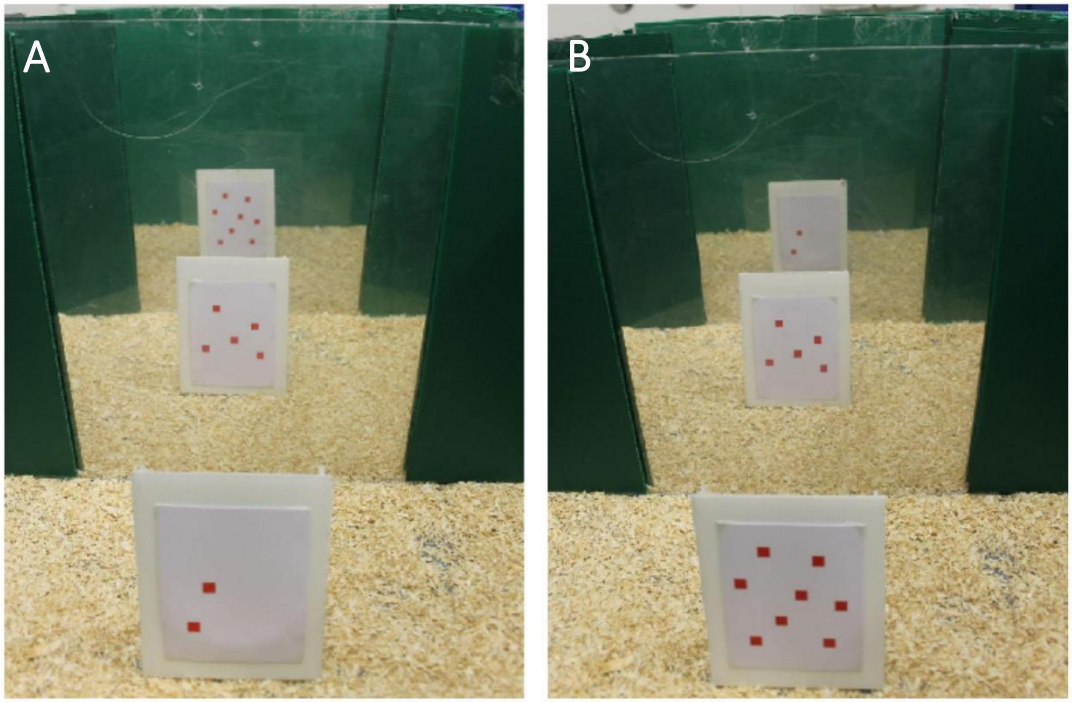

Fig.3 The order of the numeric stimuli in ascending group (A) and descending group (B).

2.4 Procedure

Every morning starting from the third day from hatching, chicks were food deprived two hours before behavioral observations to reinforce the mealworm-following behaviors of the chicks.

The experimental procedure consisted of 3 phases: shaping, training and test phases.

Shaping, the first phase of the procedure (see Tabel 1), began in the third day and lasted for about 30 minutes. After shaping, chicks were moved to their cages for 1-hour rest until the training phase began. *Training* phase consisted of 5 trainings, each made up of 7 trials. Before each training, a criterion had to be reached. The criterion was defined as the successful completion of three consecutive trials.

In the fourth day, chicks underwent Training 2. After a 1-hour rest, Training 3 preceded Test 1. After another hour of rest, Training 4 preceded Test 2. In the fifth day, chicks underwent Training 5 and immediately afterwards the Control test.

After daily experiments, chicks were placed in the cages with food and water available ad libitum. At the end of the procedure, chicks were donated to local farmers.

Table 1. Weekly schedule of the experiments.

Day 3	**Day 4**	**Day 5**
Removal of food jars (2 hours before shaping)	Removal of food jars (2 hours before training)	Removal of food jars (2 hours before training)
Shaping (30 minutes)	Criterion (3 trials) + Training 2 (7 trials)	Criterion (3 trials) + Training 5 (7 trials)
1-hour rest	1-hour rest	Control test (5 trials)
Criterion (3 trials) + Training 1 (7 trials)	Criterion (3 trials)+ Training 3 (7 trials)	
	Test 1(5 trials)	
	1-hour rest	
	Criterion (3 trials) + Training 4 (7 trials)	
	Test 2 (5 trials)	

Shaping

At the beginning of the shaping phase, a single white panel without any stimulus was placed at the center of the first area, and the first partition was replaced by a green plastic panel to facilitate the chick's navigation without causing distraction or confusion regarding the expansive space. The chick was moved to the center of the area and permitted to explore the unfamiliar surroundings alone. After 2 minutes of familiarization, two pieces of mealworm were offered to the chick to establish a positive association with the arena.

Then the chick was delimited behind a transparent glass in the starting area, watching a piece of mealworm progressively positioned next to the panel. Once released, the chick ran to the mealworm. Five trials were repeated and each time the mealworm was placed closer to the bottle cup behind the panel. After reaching the criterion of three consecutive successful attempts of finding the mealworm behind the panel, the stimuli were positioned on the panels, and the green plastic partition was

removed.

The chick was moved to the starting area, delimited by a transparent glass. A piece of mealworm was moved gradually behind the panel and positioned on the bottle cup. After the chick discovered and consumed the mealworm, another mealworm was showed to the chick behind the first transparent partition and moved slowly behind the second panel. The partition was elevated. The chick had to traverse beneath the partition and proceed behind the panel to access the mealworm. The same operation was repeated for the third area. Once the chick has passed a partition, a green plastic panel was placed behind the partition, to prevent the chick from seeing the previous stimuli. If the chick was distracted and remained in the same area, a stick was used to assist the chick in locating the mealworm in the bottle cup by gently tapping the rear of the panel. After reaching the criterion, namely three consecutive successful trials, the chick was moved to the cage for one one-hour break.

Training

Chicks were divided into two groups, each subjected to the same procedure but with a different numerical sequence.

After the break, reaching the criterion determined the beginning of the training. Three pieces of mealworm were positioned in the bottle cups of each panel. The chick was moved to the starting area, delimited for 5 seconds and released from the transparent glass. Once it found the mealworm, the partition to the next area was elevated. After the chick passed through, the partition returned to the initial position and a green plastic panel was placed behind of the partition. Once passed the criterion (three consecutive successful trials), the chick entered the training phase made up of 7 trials.

What differentiate training phase from criteria is that the chick had to wait behind each transparent partition. The time of waiting progressively increased until the chick could remain in front of the partition for 5 seconds before advancing to the subsequent stimulus. Except for Training 1, in Training 2, 3, 4 and 5, the chick always had to wait for 5 seconds prior to the removal of the partitions. The objective of this phase was to direct the chick's attention to the stimuli and to memorize the numerical sequence. After each trial, the chick was moved to an opaque box while the experimenter positioned mealworm pieces behind the panels. The trial was considered incomplete if the chick remained in the initial area for over 1 minute or the total duration was longer than 2

minutes. Overall, depending on the chick's behavior, the training phase lasted between 10 and 20 minutes.

In the transition from the criterion to Training 1, the use of the stick was progressively diminished, hence limiting the possible development of a chick's dependence on the stick. During Training 2, 3, 4 and 5, the stick was no longer utilized for guidance. Furthermore, in these training phases, the food reward was provided in all but one trial to prevent automatic adherence the trajectory and neglect of the stimuli.

Test

There were two experimental tests (Small number test,Large number test) and one control test (Middle number test). Stimuli depicted 2 red squares (0.6 cm x 0.6 cm) in the Small number test, 5 red squares in the Middle number test, and 8 red squares in the Large number test. Two identical test stimuli, different from the training stimuli in the spatial arrangement of the elements, were bilaterally placed, one on the left and one on the right side with respect to the chicks starting position. The experimental tests (Small number test, Large number test) preceded the control test (Middle number test). The order of the experimental tests was counterbalanced across participants and the chicks were randomly assigned to one of them. The control test was always administered at the end. In all the test phases, food reward (i.e., mealworm) was not provided.

Each test was preceded by three trials to reach the criterion and 7 trials of training. Each test consisted of five trials.

Before test trials, the chick was placed in the apparatus without panels and was allowed to navigate independently. This practice was useful to make the chick familiarize with the novel environment used at test (see Fig. 1B). Then the chick was moved to the opaque box until the stimuli were arranged. At the onset of test trials, the chick was restricted for 5 seconds to the starting area, behind the transparent partition, from which it could see both panels on either side. Then the partition was removed and the chick was released to the arena. Once the head or at least 3/4 of the chick's body had entered the area behind one of the two panels, the lateral choice was registered and the trial ended. Only the lateral panel first selected by the chick was considered. The chick was moved to an opaque box outside the apparatus for 15 seconds until the new trial began. When the chick did not go behind any panel for over 1 minute or hit the panel, the trial was considered invalid. After 3 consecutive invalid trials, the test was suspended for 20-30 minutes and restarted following 3 trials

to reach the criterion. Once 5 valid choices were made, the chick was moved to the cage.

The chicks' behavior was observed by a monitor of a video camera not to disturb the chicks by direct observation. All trials were video-recorded to be later scored offline by a second experimenter who was unaware of the purposes of the study.

III Results

Data analysis

The dependent variable that we measured was chicks' lateral choices.

To quantify this behavior, we used two specific indices that reflect the directionality and strength of spatial preferences:

- **LC percentage of Left choice (LC)**, calculated for each chick and each test using the following formula: LC(%) = (Number of Left Choices / Total Number of Choices) × 100. It ranged from 0, indicating that the left panel was never selected, to 100%, meaning that the left panel was consistently chosen in each trial.

- **Relative index**. In addition to analyzing the means choice values for each group, we also examined the relative differences in chicks' performance across test conditions. We hypothesized that the SNA effect would be more pronounced when comparing the LC in response to the largest versus the smallest numerosity. To capture this effect, we computed a Relative index using the formula: LC (Small number test) - LC (Large number test).

Furthermore, based on the assumption that chicks encoded the first two stimuli in the training sequence more effectively, we calculated two additional indices for each group to reflect their relative spatial preference: LC (Medium number test) - LC (Small number test) for ascending group, and LC (Medium number test) - LC (Large number test) for descending group. Notably, the numerosity "5" presented in the medium position, served as the common reference stimulus for both groups. This relative indices with first two panels allowed us to explore whether the ordinal position of numerical stimuli influenced the strength and direction of chicks' SNA.

All the test conducted in the experiment were filmed using a video camera placed in front of and above the apparatus to allow a clear view of the chicks' movements. The videos then underwent an offline scoring process with the aim of checking the chicks' choices (whether they circumnavigated the left or the right panel).

As for the independent variables, we considered:

- **Group** as a between-subject variable. Chicks were randomly assigned to one of two training groups: ascending group (2-5-8) or descending group (8-5-2);

- **Test** as a within-subject variable. Each chick underwent two experimental tests and one control test, each involving different numerical magnitudes: Small number test (2 vs 2), Medium number

test (5 vs. 5), and Large number test (8 vs. 8). Order of Test as a between-subject variable.

- **Test order** as a between-subject variable. To control possible order effects, the order of two experimental tests was counterbalanced. Half of the chicks underwent the Small number test first followed by the Large number test (SL order), while the other half received the reverse order (LS order).

Fifty male three-day-old domestic chicks that made at least three valid choices in each of the three test conditions were included in the final analysis. Three chicks in the descending group did not meet this criterion and were excluded. This resulted in a final sample of 50 chicks: 26 chicks in the ascending group and 24 in the descending group. In the ascending group, 13 chicks were assigned to the SL order and 13 to the LS order; in the descending group, 12 chicks were tested following the SL order and 12 following the LS order.

To determine whether the chicks' behavior was due to numerical magnitude or ordinal position of the stimuli, the data of experiment were analyzed as follow:

(i) A mixed Analysis of Variance (ANOVA) was conducted involving the following variables: Group (ascending vs. descending) × Test (2, 5, 8) × Test Order (SL vs. LS);

(ii) One-sample t-tests were carried out to control whether chicks in the ascending or descending group showed a significant lateral preference for each numerosity. Specifically, the goal was to determine whether the LC percentages in each group, for each test conditions significantly differed from chance level (i.e., 50%);

(iii) Within each group, a paired-sample t-test was used to compare LC percentage across the three test conditions. This analysis aimed to verify whether a leftward bias was reported in response to small, medium and large numerosity;

(iv) Independent-samples t-tests were applied to compare performance between the two groups on each test.

In addition,

(v) Independent-samples t-tests were conducted on the relative indices to determine whether there were significant differences in chicks' responses between two groups;

(vi) Independent sample t-tests conducted on the relative indices with first two panels were carried out to assess whether significant differences existed between the groups.

The following hypotheses guided the data analysis:

(A) if a significant Group by Numerical Magnitude interaction is observed, it would imply that:

- ordinal position and not numerical magnitude contributes to modulating the spatial mapping of numerical information. Specifically, we hypothesized that the stimuli encountered first by the chicks would be associated with left side, whereas those encountered later would be associated with the right side. For instance, in the ascending group, chicks might show a leftward preference for numerosity "2" and a rightward preference for numerosity "8". Conversely, in the descending group, a leftward preference might emerge for numerosity "8" and rightward for numerosity "2". To sum up, the directionality of lateral choices for the stimuli in the same ordinal position would be identical for both groups; or

- both ordinal position and numerical magnitude contributed to determine SNA. For example, numerical magnitude determined the direction of SNA effect, while the sequential order modulated its strength, either enhancing it or attenuating it.

(B) If a main effect of Numerical Magnitude emerges, it would suggest that numerosity plays a dominant role in spatial-numerical mapping. Following Rugani et al.'s (2020) study, it was predicted that the LC would be affected by the Test magnitude (2 vs. 2, 8 vs. 8, and 5 vs. 5); more specifically, we expected the Small number test to have the highest LC percentage, the Medium test to remain at the chance level, and the Large number test to result in the lowest percentage LC(2 vs. 2) > LC(5 vs. 5) > LC(8 vs. 8) ;

(C) If a main effect of Group is found, it would indicate that the numerical sequence (ascending or descending) influences chicks' spatial preferences regardless of the numerosity used at test.

Results

First, a Group by Numerical Magnitude interaction was found on the LC percentages ($F(2) = 4.117$; $p = 0.019$, $\eta^2 = 0.043$). Neither Group ($p = .57$) nor Numerical Magnitude ($p = .28$) did not reach significance. All other interactions were not statistically significant ($p > 0.05$).

Secondly, in the ascending group, only the LC percentage in the Large number test was significantly above chance (mean = 62.051, SD = 25.770, $t(25) = 0.025$, $p = 0.025$, Cohen's d = 0.468). Although the percentages in the Small number test, and Medium number test, were slightly above chance, they did not reach statistical significance (Small number test: mean = 58.141, SD = 24.560, $t(25) = 1.690$, $p = 0.103$, Cohen's d = 0.331; Medium number test: mean = 58, SD = 0.28.084, $t(25) = 1.059$, $p = 0.300$, Cohen's d = 0.208).

In the descending group, a significant leftward bias was found in the Medium number test (mean = 64.722, SD = 31.051, t(23) = 2.323, p = 0.029, Cohen's d = 0.474). The LC in the Small number test was slightly larger than chance level but not statistically significant (mean = 58.750, SD = 29.827, t(23) = 1.437, p = 0.164, Cohen's d = 0.293). In contrast, in the Large number test a trend indicating a rightward bias was observed, though it also did not reach significance (mean = 43.264, SD = 26.055, t(23) = -1.267, p = 0.218, Cohen's d = -0.259; see Fig. 4).

Thirdly, no significant differences emerged from the pairwise comparisons among the three tests conditions in the ascending group (p > 0.1). In contrast, in the descending group, the LC percentage in the Large number test was significantly different from both the Small number test (t(23) = 2.449, p = 0.022, Cohen's d = 0.500) and the Medium number test (t(23) = 3.140, p = 0.005, Cohen's d = 0.641).

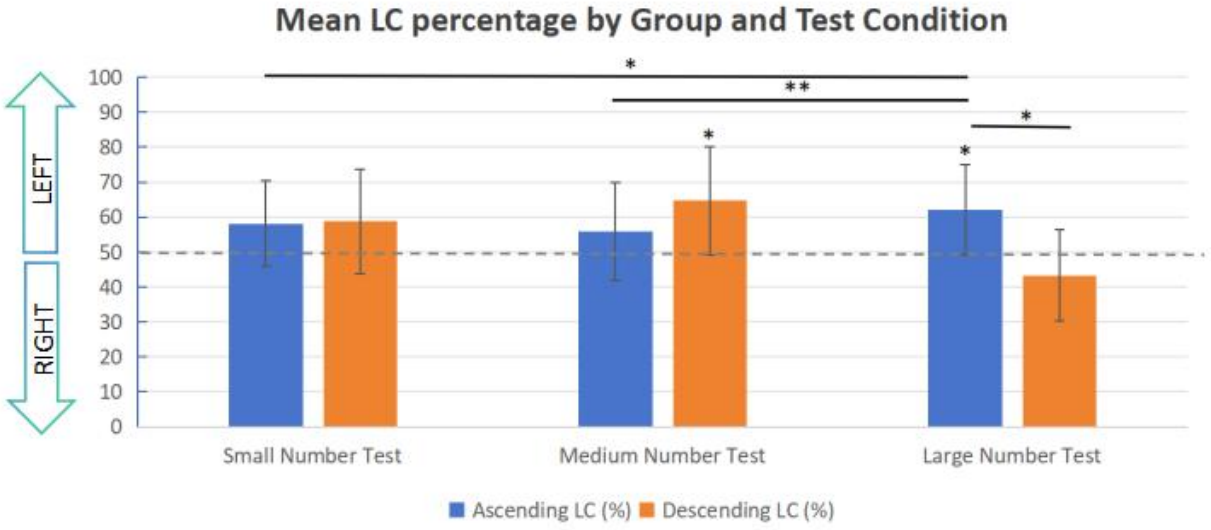

Fig.4 Mean percentage of Left Choices (LC) in the three test conditions for the ascending and descending groups. The central line indicates the chance level. Error bars indicate standard deviation. Asterisks denote statistically significant differences.

Subsequently, only in Large number test the LC percentage differed significantly between the two groups, with the descending group showing a significantly lower LC than the ascending group (t(48) = 2.562, p = 0.014, Cohen's d = 0.725)).

In addition, Relative indices between two groups were significantly different (t(48) = -2.063, p = 0.045, Cohen's d = -0.584). Specifically, the relative index of the descending group (Mean = 15.186,

SD = 30.984) was significantly larger than the relative index of the ascending group (Mean = -3.910, SD = 35.147; see Fig.5).

Lastly, relative indices with first two stimuli of two group were also significantly different between the two groups (t(48) = -2.497, p = 0.016, Cohen's d = -0.707; ascending: Mean = -2.308, SD = 33.764; descending: Mean = 21.458, SD = 33.475).

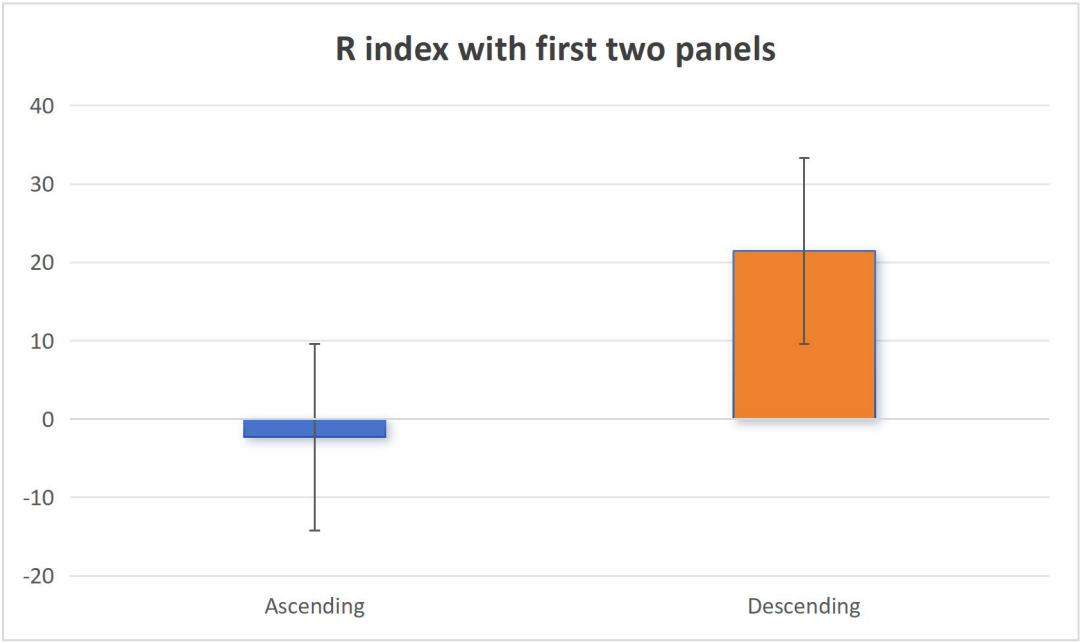

Fig.5 Relative indices with first two stimuli of the ascending group and the descending group.

IV Discussion

4.1 Interpretation of data

While the findings aligned with the initial hypothesis that both numerical sequence and magnitude contributed to determine SNA, the impact of the sequences on the two group was contrary to our expectations.

When examining the relative indices based on the first two stimuli, both groups showed the higher LC percentages for smaller numerosities compared to large ones, congruent with previous findings (Rugani et al., 2020). It indicates that SNA was present in the ascending group, albeit to a much weaker degree than in the descending group.This finding aligns with our hypotheses regarding magnitude-driven SNA, and chicks' memory capacity of up to two stimuli. This may further indicate that the SNA effect is stronger for stimuli that are retained more effectively in memory.

However, based on the results, the ascending group exhibited a general leftward spatial preference for all stimuli. In contrast, for the descending group, the leftward bias appeared to be modulated by numerical magnitude. Specifically, chicks tended to choose the left stimulus when presented with relatively smaller numerosity, and the right stimulus when depicting a larger numerosity.

In the ascending group, the leftward spatial attention may be enhanced. Supporting this interpretation, no significant differences were found across three test conditions. However, the reason why the leftward preference was most pronounced in response to the stimulus "8", as compared to "2" and "5", remains unclear. One plausible explanation involves the *numerosity adaptation effect*. In the study conducted by Burr and Ross (2007), participants were required to stare at a fixation point for 30 seconds while viewing two stimulus presented on the screen (see Fig.6). When subsequently presented with identical numerosities on both sides, participants tended to underestimate the numerosity on the side previously associated with fewer items. Moreover, they found that adapting to small numbers increased apparent numerosity, whereas adapting to large numbers decreased apparent numerosity.

Given that chicks underwent test immediately after training, and "8"was the final stimulus in the training sequence, a numerosity adaptation effect may have influenced their lateral choices.

Additionally, the unique visual structure of chicks should be considered. Their laterally placed eyes provide an extremely wide visual field of over 300 degrees, allowing them to potentially view two stimuli simultaneously, one with each eye. For example, if chicks viewed the “5” stimulus with its right eye and the “8” with its left, then the apparent numerosity of the right-side stimulus could have been decreased due to adaptation. As a result, chicks preferred the left stimulus that appeared to be larger (Rugani, 2017) and made more left choice. Furthermore, such effect can arise after only brief exposure, even as short as 1 second, though their persistence depends on the numbers of repeated exposures (Aagten-Murphy & David Burr, 2019).

However, there is no research on the numerosity adaptation effect in animals. It remains unclear whether such effect can persist for several minutes in chicks while the experimenters were preparing arena for testing. Even if the effect does exist, it is uncertain that chicks chose the left-side stimulus because it was perceived as a smaller numerosity (consistent with SNA), or, as previously suggested, because the left stimulus appeared more numerous, thereby inducing to a leftward choice. Another assumption is that chicks should focus more on a specific stimulus using the same eye to produce lateral numerosity adaptation effect. In conclusion, further investigation is needed to clarify these issues.

In the descending group, the numerosities “2” and “5” were associated with the left side, while “8” showed a slight rightward bias. This was further supported by the results of paired-sample t-tests, indicating that chicks were more likely to choose the right-side stimulus when presented with larger numberosity, compared to the small and medium ones.

Unexpectedly, the percentage of LC in Medium number test was the most pronounced in three test conditions. One plausible explanation is that chicks primarily memorized the first two stimuli.

Another possible interpretation is that the number “2”, as a subitizable quantity, elicited a different cognitive processing pathway compared to larger numerosities. Estimating is what occurs when the stimulus-number is greater than four, whereas subitizing is what occurs when the stimulus number is less than four. Non-symbolic numerical cognition is thought to rely on two distinct systems: object file system (OFS), which processes small numberosity, and analogue magnitude system (AMS), which elaborate larger numerosity. The processing of subitizing small sets up to 3-4 items is effortless, rapid and accurate (Revkin et al., 2008). Approximate representation has been found in various animals, such as horses (Uller and Lewis, 2009), amphibians (Uller et al., 2003), and fish (Agrillo et al., 2007), and chicks (Rugani et al., 2008).

However, the study of Liu et al. (2020) showed that subitizing is highly dependent on attentional resources. Given that the first two stimuli presented to the descending group were large numerosities requiring more effort, it is possible that spatial representations were more strongly activated to facilitate processing. Consequently, chicks may have less time and fewer attentional resources to process the ultimate stimulus, resulting in a weaker leftward preference for the number "2" compared to "5".

Explaining the difference in results between the two groups remains challenging. Independent-samples t-test results indicated that the primary divergence occurred in the Large number test, where rightward choices were significantly more frequent in the descending group, suggesting a stronger SNA. Moreover, the R index of the descending group was significantly larger than that of the ascending group. It indicates that chicks in the descending group tended to have more left choices in respond to the smallest numerosity compared to the largest one. In contrast, the ascending group showed a weaker spatial bias. The finding provides additional support for this conclusion.

The behavioral patterns observed in the ascending group were inconsistent with the results of previous findings (Rugani et al., 2020). This discrepancy may be attributed to the increased complexity of stimuli used in the current study. Differing from single item design employed in the previous study, the numerical sequence presented here imposed greater cognitive demands, thus chicks could adopt different strategies. For instance, in the ascending group, the numerical sequence began with a smaller numerosity (2 and 5). The neural system of subitizing, which may be lateralized (e.g., to the right hemisphere), could have been more strongly activated during the experiment. The elaboration of subitizing is rapid and accurate, involving cerebral region of lower hierarchy. To simplify the processing of numerical information, chicks may have generalized the association between these smaller numerosities and the left side to subsequent stimuli, resulting in an overall leftward bias. Whereas the first stimulus in the descending group involved a large numerosity that was likely more difficult for the chicks to process and retain. This may have necessitated the engagement of higher-order brain regions and activated numerical-spatial mapping mechanisms for quantity discrimination. A recent study on domestic chicks (Loconsole et al., 2023) also demonstrated that under high cognitive load in a proto-arithmetic task, numerical judgments were more accurate when the numerosity was congruent with spatial associations (i.e., small sets on the left and large sets on the right). These findings suggest that SNA may serve not only as a

spontaneous bias but also as a cognitive strategy that enhances performance in numerically demanding contexts.

In summary, the interaction between group and numerical magnitude played a crucial role in determining the strength and directionality of SNA. The ascending numerical sequence appeared to reinforce a general leftward bias weakening the SNA effect, whereas the descending sequence amplified it. However, the reason why the impact of the sequences on the two groups was contrary to the initial expectations remained unclear. Further investigation is necessary to assess the replicability of these findings and to test the falsifiability of the proposed explanations.

Our results demonstrates that both numerical magnitdue and learning experience play a critical role in modulating SNA, consistent with the findings from Gazes et al. (2017) in gorillas and orangutans, which showed that the orientation can be changed by training instruction.

This raises a broader question: why chicks, and many other species, including human, tend to associate small number with the left side and large number with the right side?

The *right-hemisphere dominance* model proposes that the functional overlap between spatial and numerical processing in the right hemisphere is at the basis of the origin of SNA (Rugani et al., 2010; 2015). According to this model, the right hemisphere's specialization in both spatial (Rashid & Andrew, 1989; Regolin et al., 2005; Tommasi & Vallortigara, 2001) and numerical information processing (Piazza & Eger, 2016; Rugani & Regolin, 2020) predisposes animals to start to “count” from left to right, which in turn explains the association of smaller numbers with the left and larger numbers with the right.

The *emotional-valence model* is grounded in neurobiological evidence on hemispheric lateralization of emotional processing, with the right hemisphere predominantly processing negative emotions and the left hemisphere processing positive ones (Davidson, 2004). This model posits that small numerosities, intrinsically associated with negative valence, activate the right hemisphere, consequently directing movements towards the left. Conversely, large numerosities, associated with positive valence, activate the left hemisphere and directs movements toward the right (Vallortigara, 2018).

The *Brain Asymmetric Frequency Tuning model* (BAFT) connects low-level spatial frequency processing with high cognitive functions, including numerical cognition. BAFT is based on the hemispheric specialization for spatial frequency, with the right hemisphere predominantly extracting low spatial frequencies and the left hemisphere preferentially selecting high spatial

frequencies (Gazzaniga, 2000; Kauffmann et al., 2014). BAFT extends this hemispheric specialization to numerical cognition, suggesting that smaller numerosities, which contain relatively lower spatial frequencies, are processed by the right hemisphere, with consequent attentional shift towards the left hemifield. On the contrary, larger numerosities, which contain relatively higher spatial frequencies, are processed by the left hemisphere, thus directing the attention towards the right hemifield (Felisatti et al., 2020).

4.2 Methodological limitations and suggestions

First of all, the choice of numerosity in the training sequences should be reconsidered, given that the processing of quantities below four may be automatic and therefore more easily ignored by chicks compared to the higher numerosities. An ongoing study using larger numerical sequence (8-20-32) is conducted to investigate whether these findings persist or change under different numerical range. Training numerosity should either all fall within or beyond the subitizing range to ensure consistency. Furthermore, overall area was not controlled. Although previous studies (e.g., Rugani et al., 2015) demonstrated that SNA is independent from continuous magnitude (e.g., area, density) and have no effect on chicks' performance, the stimuli used in the present study were more complex, and such confounds may have exerted an influence. Additional control experiments involving sequences of geometric figures are necessary to ruling out the effect of non-numerical variables and assess whether the space is used also to map the non-numerical stimuli.

A second limitation consists of the lack of time control during the training phases. The time each chick spent with each stimulus depended on its own behavior. Observations indicated that some chicks circumnavigated more around the first stimulus, others lingered at the second, and some quickly passed through all stimuli. As a result, individual differences in attention time to each stimulus may have affected learning.

Additionally, in some cases, chicks paused for 2-3 seconds in front of the transparent partition before moving around the panel. This behavior may have led to repeated exposure simultaneously to two stimuli, potentially influencing performance. An alternative arena design may include replacing the transparent partition with an opaque door. Instead of panels, red bottle cups could be used as stimuli, placed directly on the floor, with food rewards positioned in front of the door. This

set up may also help eliminate potential biases caused by chicks preferring the side they habitually circumnavigate first. Moreover, it better simulates an ecological context in which food resources are randomly distributed on the ground.

A third limitation is the exclusion of invalid trials from the analysis. The number of invalid responses may offer valuable insight into the chicks' emotional states. For example, a high number of null trials could suggest elevated anxiety, which activates the right hemisphere of the avian brain that controls fear and escape responses (Rogers, 2008), possibly resulting in increased left-side choices. It is worth examining the chicks' behaviors following a null trial: would chicks persist with their previous strategy or switch to a different one? Furthermore, including these invalid trials in the analysis might alter the observed proportion of left choices.

What's more, after a few trials during the test phase, chicks may realize that no food is available and begin checking the opposite panel. In such cases, their subsequent responses may shift from automatic association to strategic decision-making. For this reason, it may be informative to analyze LC percentages in only the first three trials and compare them to the overall statistics.

Moreover, in some invalid trials, chicks were observed jumping and kicking one of the two panels. Such behaviors may reflect anxiety-induced aggression. Therefore, it may be advisable to exclude these trials, as the activation of lateralized brain regions associated with negative emotions could interfere with the automatic processing of SNA.

4.3 Future research directions

The present study is the first to utilize spatial numerical sequences consisting of three stimuli to explore the impact of both numerical ordinality and cardinality on SNA in chicks. It also represents the first attempt to demonstrate how learning experiences with different sequences can shape chicks' behavior. These finding open new avenues for future research on how chicks perceive and process sequential numerical information.

Importantly, subsequent experiments should include additional controls to rule out potential confounds, such as continuous magnitude. A more refined experimental design could help isolate the specific contribution of duration in each area during training phases.

Further studies can focus on examining whether exposure to different numerical orders can modulate spatial attention. In addition to the sagittal spatial sequence used in the current paradigm,

future research could implement parallel or randomized sequences presented in different spatial layouts to assess whether similar SNA effects emerge under alternative conditions.

Another intriguing direction concerns the potential influence of numerosity adaptation in chicks. It remains to be seen whether the simultaneous perception of two distinct stimuli can affect their numerical discrimination abilities.

To sum up, future studies may explore how sequence learning shapes SNA, as well as its underlying cognitive and neural mechanisms.

V. Conclusion

The present study aims to investigate how numerical magnitude and ordinal position contribute to SNA. We used the LC percentages as an index to measure chicks' lateral preferences in two groups. As predicted by one of our hypotheses, numerical magnitude significantly interacted with ordinal position. Specifically, different numerical sequences modulated the strength of SNA. Unexpectedly, the SNA in the descending group was significantly more pronounced than in the ascending group, especially when focusing on chicks' lateral choices in response to the first two stimuli. Moreover, the ascending numerical sequence elicited a general leftward spatial attention, while descending sequence resulted in clear SNA effect.

Remarkably, from the analysis of the LC percentages for those stimuli, it was found that the SNA in the ascending group, though much weaker, followed the same direction as that in descending group. Such effect was absent in other stimulus pairs. It suggests that SNA may be more strongly expressed for numerosity that is more elaborated and better retained in memory. The finding could imply a plausible explanation of the behavioral differences between the two groups: in the descending group, the first two numerosities were larger and beyond the subitizing range, thereby requiring greater cognitive effort to process and store the numerical information. As a result, SNA may serve as cognitive strategy to facilitate the processing and thus become more strongly reinforced with respect to the ascending group. Therefore, the results suggest a potential positive correlation between cognitive load and the expression of SNA.

Given that this is the first study to employ training sequences consisting of three numerosity stimuli, further research is necessary to control for potential confounding variables, such as continuous magnitude and distinct mechanisms of numerosity processing.

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Author's statement

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