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**An experimental investigation of
the spatial-numerical association
in newborn domestic chicks
(*Gallus gallus*)**

Supervisor

Professor Rosa Rugani

Candidate: Chiara Brutti

Student ID number: 1223469

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ABSTRACT

The mental number line (MNL), a horizontally oriented disposition of numbers, has been shown to be the basis from which humans map numbers onto space. This disposition, characterized by a left-to-right directionality, sees the placement of smaller numbers on the left and large numbers on the right side of space, also facilitating response times. This spatial-numerical association (SNA) had initially been considered a by-product of culture, but subsequent research conducted with human newborns and non-human animals has challenged this hypothesis. Previous studies by Rugani et al. (2015; 2020) demonstrated that chicks, too, by means of numerical magnitudes, show this left-to-right number-space mapping. Therefore, based on the formerly mentioned studies, this thesis further investigated the spatial-numerical association of response codes (SNARC)-like effect in a sample of 3-day-old lateralized domestic chicks (*Gallus gallus*). After undergoing a shaping and training procedure, during which they had been exposed to a fixed numerosity (5 red squares), chicks took part in three different tests: 2vs2, or small number test; 8vs8 or large number test; and 5vs5, the control test. In these tests, chicks were exposed to two panels showing the same number of red squares (two, eight, or five, depending on the condition) and had to complete a total of five trials per test by circumnavigating one of the two panels. The expected and hypothesized results involved the presence of a leftwards bias for small numerosities and a rightwards bias for large numerosities, which would support the proposal of an innate origin of the SNA.

1. INTRODUCTION

1.1 Number-space association: an overview

Numbers and numerical processing play a considerable role in people's lives; their use ranges from the more theoretical, as when carrying out mathematical operations, to the more practical, as when sorting out real-life objects in space.

When thinking about numbers, humans have the tendency to associate them with space, underlying the fundamental role that number knowledge and processing have in our daily life. This spontaneous spatial-numerical association was first demonstrated by Galton (1880), who, by asking human adults to draw the way they visualized numbers, discovered that thinking and reasoning about numbers comported their arrangement in visuospatial representations and forms, which could vary depending on the person. These orderings, however, were typically organized along a horizontally displayed continuum labeled mental number line (MNL), whose disposition is characterized by a left-to-right orientation, suggesting the placement of smaller numerosities on the left and larger numerosities on the right.

The first empirical support for this phenomenon comes from Dehaene et al. (1993), who conducted a series of seminal studies confirming the existence of the MNL, pointing at how numerical magnitude exerted its effect on subjects' response times (RTs). Subjects were able to respond faster when small numerosities were located on the left side of space and when large numerosities were located on the right side of space: this was termed the SNARC (spatial-numerical association of response codes) effect, resistant to handedness and hemispheric influences on RTs. Interestingly, this phenomenon seemed to be more closely dependent on relative, rather than absolute, magnitude; depending on the numerical interval considered, the same number could lead to opposite responses. For example, number 5 would be associated with the right side of space for intervals going from 1 to 5 due to it representing a larger magnitude, while in an interval such as 1-9, in which it is considered smaller, it would be associated with the left side of space (Dehaene et al., 1993).

The SNARC, however, was not immune to the direction of writing, being especially evident in people exposed to a right-to-left writing system, suggesting that it may have

simply been a by-product of a person's culture. This has led to the hypothesis that the SNARC effect might be leaning more toward the nurture rather than the nature side of the debate, thus denoting a substantial cultural role over a weaker biological one.

Further evidence supporting this conclusion came from research on populations exposed to non-Western influences on formal education; Zebian (2005), for example, demonstrated the existence of a reverse SNARC effect in a study involving the participation of Arab subjects, whose writing system is right-to-left oriented.

A more thorough study was conducted by Shaki et al. (2009), who compared how adults from three different groups represented numbers in space: Canadians, Palestinians, and Israelis. While the first group read both words and numbers in a left-to-right orientation, the second showed a right-to-left reading style, and the third read numbers from left to right but words from right to left. As expected by the researchers, Canadians and Palestinians showed the SNARC and reversed SNARC effect, respectively, but the most exciting finding stemmed from the Israeli group, who, due to their contrasting reading habits, showed no reliable SNARC.

Adding to these studies, Bächtold et al. (1998) demonstrated how experimental contextual manipulation could affect the position of numbers along a left-to-right oriented MNL.

They instructed participants to conceive numbers differently according to their condition: numbers were perceived as distances along a ruler in one condition and as hours on a clock face in a second one. The different formats and ways of conceiving numbers elicited a left-to-right SNARC effect in subjects pertaining to the ruler group, while those in the clock group showed an inverted SNARC effect. These findings can be explained by observing how the placement of the same numbers in these two representations is the exact opposite: while, for example, numbers from 1-5 are located on the left side of the ruler (more closely representing the MNL), the same pattern is reversed in the clock condition, with the numbers appearing on the right side of space.

From all these findings, cultural habits, contextual variables, and experience's seminal roles in determining how numbers are mapped and oriented in space become very clear, almost shadowing the potential biological origins.

1.2 Experimental evidence in human newborns

Despite the previously mentioned findings, the debate regarding the emergence of number-space mapping did not cease; on the contrary, it became even more heated due to new evidence inferring a biological role in the origin of this phenomenon. Over the years, a significant number of studies have highlighted a potential phylogenetic origin of the spatial-numerical association (SNA) previously observed only in adults. By demonstrating the existence of pre-linguistic and innate precursors of SNA, these studies have proven that culture and experience are not the only elements involved in the linkage of numbers and space.

To investigate the origin of the spatial-numerical association (SNA), de Hevia et al. (2014) conducted a study with seven-month-old preverbal infants who had not been previously exposed to formal education (including both mathematical and reading/writing skills) and who lacked symbolic knowledge. Following the initial habituation phase, in which infants got accustomed to increasing and decreasing left-to-right oriented numerical sequences, subjects underwent the testing phases: results indicated that infants looked longer at new increasing sequences but not at decreasing ones. Interestingly, no bias was reported for either condition when subjects were habituated to right-to-left oriented sequences.

Similar findings resulted from experiments employing slightly older subjects: eight-month-old infants. Through the use of an eye-tracker to record their subjects' eye movements, Bulf et al. (2016) demonstrated how eight-month-old infants were able to orient their spatial attention differently when presented with different numerosities: when presented with a small number (e.g., two dots), infants directed their attention towards the left side of space, while when presented with a large number (e.g., nine dots), they focused on the opposite side of space, (i.e., the right side).

These results, however, have been argued to be determined by the interaction of few-month-old infants with the adults around them as well as their environment. In fact, Patro et al. (2016) illustrated how quickly the number-space link could be generated in preschool children through the use of spatially-oriented visuomotor activities. In this study, 3- and 4-year-old children were trained with a non-numerical spatial movement task in which they had to move a frog across a pond on a touch screen; following this first phase, they were exposed to a numerosity comparison task. Results indicated a SNARC-

like effect when children were trained to move the frog in a left-to-right fashion, while a reversed SNARC-like effect was observed when they had to perform right-to-left movements.

Nonetheless, subsequent research conducted by de Hevia et al. (2017) provided evidence of an SNA existing in infants as young as 3-day-old, a fact that heavily reduced the possibility of caregiver interactions influencing the development of the number-space link. Researchers demonstrated that neonates, too, are able to associate small quantities with the left and large quantities with the right side of space, a result reached by testing newborns' responses to either small or large auditory quantities paired with geometrical shapes on either side of a screen. Such findings, therefore, are seminal in this field, indicating that it is as early as the start of postnatal life that the spatial-numerical association can first emerge.

More recent evidence comes from Di Giorgio et al. (2019), who provided evidence that SNA in newborns is relative rather than absolute. After habituation to a numerical value (a 12-item group), neonates showed a spontaneous association of a smaller value (a 4-item group) with the left side of space and a larger value (a 36-item group) with the right side of space. Excitingly, when habituated to a larger number (36) or a smaller number (4), the previous value (12) was then associated with the left side in the former case and with the right side in the latter.

Evidence from research on human newborns and children, therefore, points to a biological predisposition to the association between numbers and space, preceding the interaction with cultural and environmental influences and thus ruling out the primary role of formal education in determining SNA directionality.

1.3 Experimental evidence in animals

After carrying out experiments with human newborns, the seminal role of biology became apparent, but the prominence of culture and environmental variables was still considered to be affecting the development of the SNA.

One method to truly explore the biological and innate origin of the SNA is to work with subjects who have had little to no experience with numbers and space and who do not have developed symbolic thought or linguistic abilities; for this reason, comparative

studies with animals have been carried out. Using animal subjects, in fact, allows for ruling out linguistic and cultural variables, factors that were thought to determine the number-space link, thus permitting researchers to focus exclusively on the inborn predisposition that determines the spatial-numerical association.

Over the years, studies demonstrated that some animals, too, are able to dispose and orient numbers in space in a left-to-right fashion, findings that prompted researchers to hypothesize the existence of a common ancestor from which both animals and humans inherited this ability (Brugger, 2015; Vallortigara, 2018).

Rugani et al. (2010), for example, conducted a study with two different bird species: adult Clark's nutcrackers (*Nucifraga columbiana*) and domestic chicks (*Gallus gallus*). After being presented at training with a sequence of identical objects, subjects had to pinpoint one based on its ordinal position, where they tended to show a bias toward the left side of space; this apparent leftward predisposition prompted the researchers to hypothesize a similarity with the human MNL.

Similar results have been reported by Drucker & Brannon (2014), whose study revealed that rhesus macaques (*Macaca mulatta*) could identify an element in an array of identical items based on ordinality, showing a leftward bias.

Further animals involved in SNA research have been apes, particularly gorillas and orangutans. Gazes et al. (2017), whose aim was discovering whether the spatial-numerical association represented a seminal property of the cognition of primates, demonstrated that apes do have the ability to associate magnitudes with space. Yet, how this association is oriented (left-to-right or right-to-left) seems to depend on the individual considered.

To explain these distinctions, researchers theorized that individual experiences play a huge role in differentiating SNA orientation across subjects; in fact, while the spatial organization of information seems to be fundamental in cognition, it is culture and individual experiences (e.g., caregiving) that determine the way these representations are disposed (Gazes et al., 2017). All of these studies, therefore, provide indisputable evidence of an evolutionary origin of the mapping of numbers in space.

1.4 Ruling out culture and experience: the employment of naïve animals

To overcome the problems linked with cultural and personal experiences, the best solution would be to work with naïve animals, specifically few-day-old subjects. This subject choice allows researchers to work with an animal that has not yet been exposed to the external world, thus ensuring that any effect observed will result from innate mechanisms rather than experientially acquired ones.

Rugani et al. (2015a) conducted seminal research in this regard by working with newborn animals: 3-day-old domestic chicks (*Gallus gallus*). Following a training phase involving familiarization with a target number, chicks proved their ability to associate numbers with space spontaneously: when presented with smaller numerosities (compared to the target number), chicks associated them with the left side of space, while when exposed to larger numerosities, the association involved the right side of space.

The employment of few-day-old chicks was an excellent strategy to prevent the influence of experience, given the highly controlled laboratory conditions and the lack of contact with the external environment; thanks to these circumstances, it would have been easier to discern any innate ability in SNA development.

Interest in SNA testing increased following this influential study, with more researchers working to discover SNA's origin (Rugani and de Hevia, 2017); concurrently, the employment of non-linguistic subjects stimulated subsequent research, given its potential to exclude educational and cultural influences.

With this renewed interest, the paradigm developed by Rugani et al. (2015a) was implemented in various studies conducted with different species; the results, however, were not consistent, depending on whether the paradigm was adjusted or not to the specific experimental conditions. An example is provided by Triki and Bshary's (2018) research on the existence of the MNL in fish; working with cleaner fish (*Labroides dimidiatus*), they employed the previously mentioned paradigm, which, however, had been developed for chicks. This lack of adaptation thus led to a failure to find evidence favoring the MNL in their sample. Conversely, when the paradigm was regulated to match the experimental subjects and conditions while still maintaining the critical idea and features, research yielded positive results, as in the case of the studies on human newborns carried out by de Hevia et al. (2017) and Di Giorgio et al. (2019).

Once the findings of the research conducted by Rugani et al. (2015a) were published, an alternative explanation was put forward by Mangalam and Karve (2015); in their comment, they argued that individual biases (either leftward or rightward) could strongly impact on chicks' performance in the experiment, further considering the enhancement of the biases throughout the testing phases. In response to this, however, Rugani et al. (2015b) demonstrated that the methodological and bias-related concerns were unsubstantiated, thus ensuring no confounding with the reported results.

An additional concern was expressed by Núñez and Fias (2017), who identified novelty as the factor responsible for chick's spatial-numerical association; instead of magnitude, it may have been the exposure to novel numerosities than the training one that prompted chicks to respond in a SNARC-like manner. As a consequence, in their publication Núñez and Fias (2017) stressed the need to test chicks with a previously observed magnitude in order to exclude the role of novelty, which was fulfilled by means of the control test (5 vs. 5), presenting the numerosity previously introduced in the training.

2. OBJECTIVES

Utilizing the previously mentioned, influential paradigm devised and employed by Rugani et al. (2015a) and starting from their most recent research in the study of SNA in newborn chicks (Rugani et al., 2020), this study aimed at further analyzing the role played by numerical magnitude in determining a SNARC-like effect, as opposed to individual spatial biases in a sample of 3-day-old domestic chicks (*Gallus gallus*).

The difference between the present study and past literature (i.e., Rugani et al., 2015a; Rugani et al., 2020) is that the subject sample was solely comprised of light-hatched laboratory-born chicks, who came to the laboratory as eggs and hatched there; previous studies, on the other hand, also employed subjects who came from local hatcheries as few-hour-old chicks.

In this study, each chick began by undergoing shaping and training: during these procedures, they learned how to circumnavigate a single centrally located panel presenting a target number (5 red squares) in order to obtain a food reward.

After habituation, chicks took part in three consecutive 5-trial tests: the small number test (2 vs. 2), the large number test (8 vs. 8), and the control test (5 vs. 5). During these, chicks were presented with two identical panels, both depicting the same numerosity, which would vary according to the testing phase being undertaken: they could present either 2, 8 or 5 red squares, whose order was randomly arranged. To prevent Order from being the cause of any observed SNA, the sample ($n = 11$) was divided into two groups: one started with the small number test ($N = 5$; 2 vs. 2, 8 vs. 8, 5 vs. 5), while the other started with the large number test ($N = 6$; 8 vs. 8, 2 vs. 2, 5 vs. 5).

The experimental hypothesis expected chicks to show a leftward bias in the 2 vs. 2 or small number test, when chicks were presented with numerosities smaller than a target number (5; introduced during training) and a rightward bias in the 8 vs. 8 or large number test, when presented instead with larger numerosities. Furthermore, the employment of a control test (i.e., 5 vs. 5), showing the same number with which chicks familiarized during training, allowed for a neutral comparison with the other two tests, given that it was presumed that chicks in this test would not show biases of any sort. This latter procedure represented a great asset in the present research (as well as in the previously mentioned ones), as it allowed the detection of any individual spatial biases subjects could have;

moreover, by excluding the element of novelty (as a consequence of presenting chicks with a number they were acquainted with), the experiment also followed the advice provided by Núñez and Fias (2017), as mentioned in the above section.

Finally, in the scoring process, apart from focusing on the panel choice of each subject, the side of circumnavigation was also taken into account, as it holds great value in determining the lateralization in the processing of numerical magnitude.

Previous research conducted by Daisley et al. (2009) has highlighted the lateralization in chicks' visual system; according to the particular situation and task they were presented with, chicks were able to independently use one or the other eye, therefore involving the most specialized hemisphere. This lateralization, moreover, appears to be especially evident in light-hatched chicks compared to their dark-hatched counterparts (Daisley et al., 2009). Taking this into account and considering that looking behind the panel while circumnavigating implicates the use of a single eye, the eye in use would indicate which hemisphere is preferred for processing. Therefore, going behind the panel from the left (inspecting with the right eye) would underline a left hemisphere bias, while going behind the panel from the right (inspecting with the left eye) would indicate a right hemisphere bias in processing.

3. MATERIALS AND METHODS

3.1 Subjects

The experimental sample comprised a total of 11 male domestic chicks (*Gallus gallus*), Broiler Ross 308, provided by a local commercial hatchery (Società Agricola La Pellegrina Spa, Borgoricco, Padua, Italy). The choice of male chicks was made accordingly to evidence illustrating male responsiveness and motivation to food reinforcements (Vallortigara et al., 1990), extensively used in this study.

The subjects hatched in the Comparative Cognition (COMP COG) Laboratory of the Department of General Psychology (DPG) of the University of Padua (Via Venezia, 8), where the experiment would also take place.

Upon their arrival, eggs were immediately placed in the incubator, whose temperature and humidity were consistently monitored and maintained at 37-38°C and 50%, respectively; they could be either 7-day-old eggs, which would hatch in two weeks, or 14-day-old eggs, hatching the subsequent week. At 18 days of incubation (three days prior to the hatching), the eggs were moved from the dark incubator to the one in which they would hatch; here, the temperature was kept between 37-38°C and the humidity between 65-70%. Maintaining constant temperature and humidity is also essential to aid the chicks' breaking of the eggshell: these conditions, in fact, provide an adequate climate that renders the eggshell softer, thus facilitating hatching.

The chicks involved in the experiment were light-incubated and therefore lateralized; this was achieved through the exposure of the eggs to a light source during the last three days of embryonic development. Eggs were usually moved from the dark incubator to the one equipped with the light source on Thursday, thus allowing the exposure to light to last until Monday when chicks would be born; all of the chicks, therefore, were light-hatched and lateralized.

Once hatched, chicks were immediately placed in standard metal cages (28 cm × 32 cm × 40 cm) provided with food and water; these were located at the corners of the cages in transparent glass jars (5 cm in diameter, 5 cm high) and available *ad libitum*. In addition to the chicken feed, chicks were also given two or three mealworms (*Tenebrio molitor* larvae) once per day, as these would later be used as reinforcement during training.

In the rearing room, temperature and humidity were kept under constant control to create a suitable environment for the subjects: the former was maintained within the 28-31° range, while the latter was maintained at 68%. Fluorescent lamps (36 W), positioned 45 cm above each cage's floor, provided lighting.

The rearing conditions remained stable from the day of hatching/arrival (11 a.m.) to the morning of Day 2 (8 a.m.), at which point the chicks were deprived of food, thus ensuring active participation in the subsequent training and testing phases (involving mealworms). Shaping began a couple of hours later (10 a.m.); during this procedure, chicks had the opportunity to get accustomed to the new environment and learn how to circumnavigate a plastic panel located at the center of the experimental apparatus. Once shaping was concluded, each chick was placed back in its cage, where it would rest for an hour before undertaking the first training phase. The experiment comprised a total of three tests, undertaken by each chick, in which different numerosities were presented: two, eight, or five red squares. In order to move on to each testing phase, chicks had to complete a round of training; upon each test conclusion, chicks would be put back in the rearing cages to rest for one hour.

Once all tests had been completed, the chicks were caged in social groups of two subjects and provided with food and water available *ad libitum*. Given the conclusion of all behavioral observations, chicks were donated to local farmers at the end of the week.

3.2 Apparatus

The entire experimental process was conducted in a dedicated room near the rearing one. To guarantee the safety of the subjects, the experimental room's temperature and humidity were kept under strict control and maintained at, respectively, 25°C and 70% throughout all experimental phases. The room was further equipped with four lamps (58 W) placed on the ceiling, 194 cm above the base of the apparatus, which provided uniform lighting conditions.

The same experimental apparatus was used consistently across all training and testing phases to avoid confounding results (**Figure 1a, 1b**); it consisted of a diamond-shaped arena constructed with white plastic panels and a white plastic sheet for the floor and outer walls of 20 cm blocking external distractions. To prevent the chick from

immediately entering the arena, a starting area was delimited by a transparent glass removable partition ($10\text{ cm} \times 20\text{ cm}$) located about 10 cm from the central vertex of the apparatus. Before the beginning of each trial, the chick was positioned behind the partition for five seconds, after which it was released. During this time, however, despite being confined, the bird could still visually inspect the arena, given the see-through nature of the material used. Each chick was allowed one choice per trial: once a panel was circumnavigated, the chick was moved to an adjacent opaque box ($20\text{ cm} \times 40\text{ cm} \times 40\text{ cm}$) to prevent it from seeing the changing of the stimuli and the cleaning of the experimental setup.

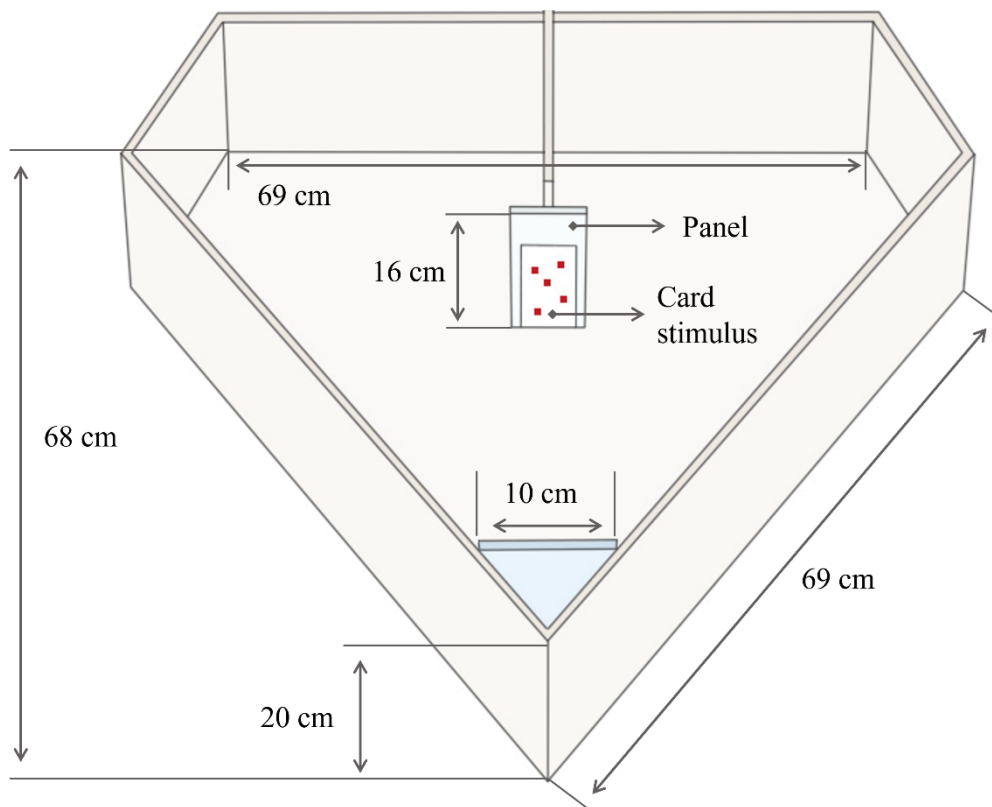


Figure 1a

Schematic representation of the experimental apparatus during the training phase.

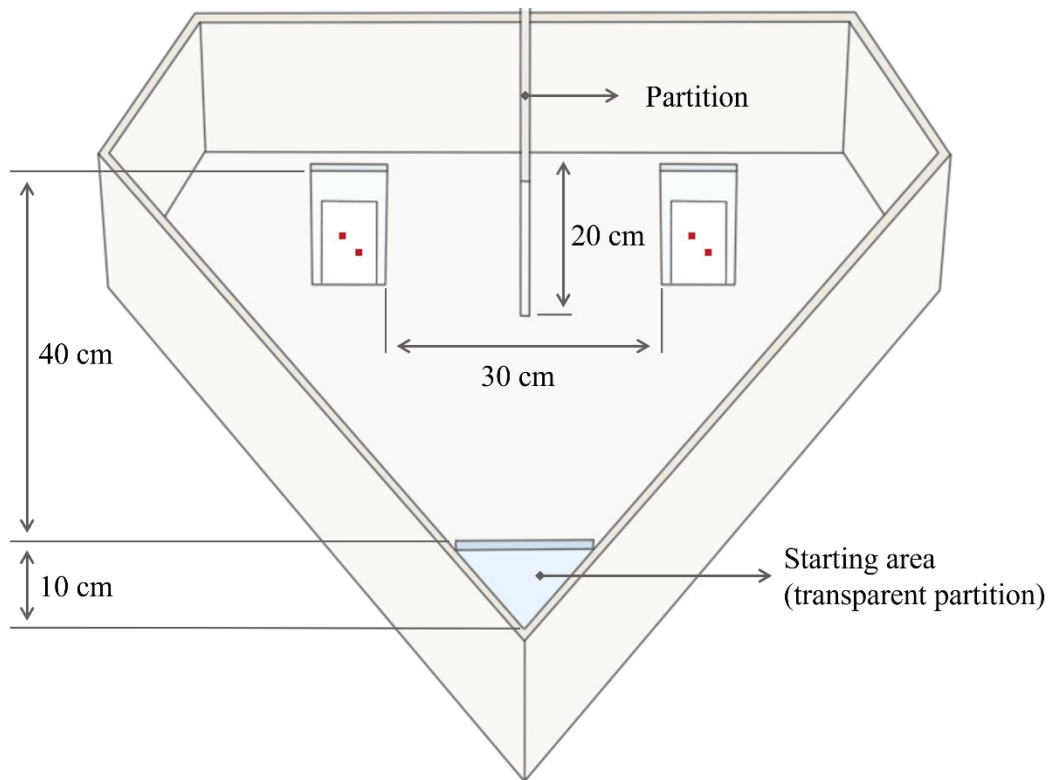


Figure 1b

Schematic representation of the experimental apparatus during one of the testing phases (2 vs. 2).

The stimuli were placed on top of either one or two panels. Each panel (16 cm × 8 cm) had a white plastic structure with bent back sides of about 3 cm, which hid the mealworm available during the training phase, thus encouraging the chick to circumnavigate it to find the reward (**Figure 2a, b, c**). To keep the arena clean and prevent the chicks from seeing the larvae simply by nearing the panel borders, the mealworms were contained in white plastic bottle lids (3 cm in diameter, 1 cm high), which were also present during the testing phases, despite being empty.

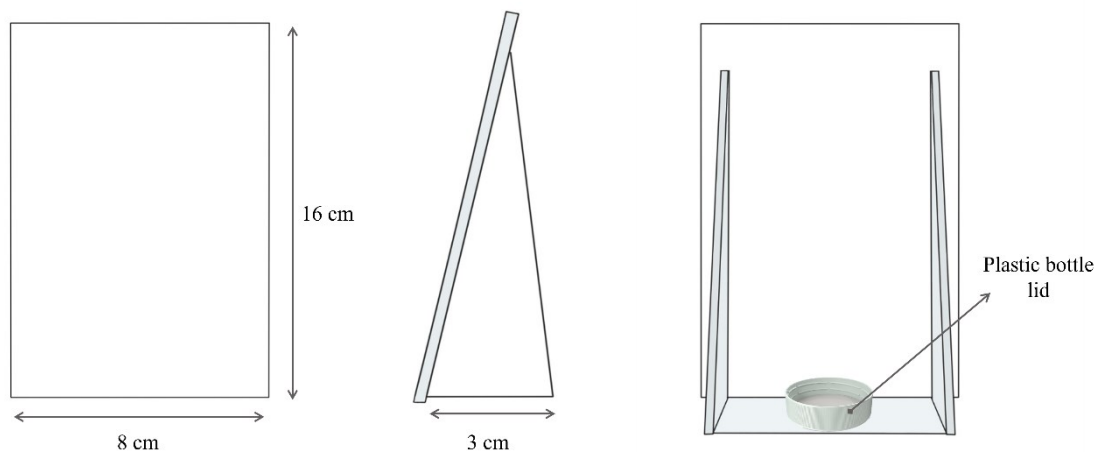


Figure 2a, b, c

Schematic representation of the panel: front (a), side (b) and back (c) view.

According to the experimental phase, the panels' disposition changed: during training, the chick was presented with a single central panel facing the starting area and located 30 cm from it; during testing, instead, two identical panels were placed symmetrically 30 cm apart, resulting on the left and right side with respect to the apparatus' central vertex. A white plastic partition was used to divide the far side of the apparatus into two symmetrical zones during testing; this was to prevent the chick from running in between the panels and looking for the reinforcement, which, however, was absent during this phase. Given the subsequent scoring process, the addition of the partition aided the scorers in their task.

3.3 Stimuli

The stimuli employed in the experiment were static 2D images involving a number of small red squares printed on white rectangular cards (11.5 cm × 9 cm). Depending on whether the chick was undertaking a training or a testing trial, a stimulus or a pair of stimuli were positioned on the panel(s).

The training involved the use of stimuli depicting five red squares (1 cm × 1 cm). Each training phase consisted of 20 trials; given the possibility of the chicks learning how to identify the stimuli based on the spatial disposition of the red squares, every new trial

involved the use of a different stimulus. For this reason, a total of 20 5-red-squared cards were used, for which the distance of the elements was randomly determined, varying from 0.3 cm to 3.8 cm; moreover, the distance of the squares from the external borders of the card was also randomly determined, ranging from 0,8 cm to 9,2 cm.

Depending on the specific test, the stimuli employed presented either 2, 5, or 8 identical red squares, with the same parameters previously mentioned for the training stimuli regarding the distance between the elements themselves and the distance from the card borders.

Each test was made up of 5 trials, and due to the same spatial disposition learning issue described above, five different stimuli with distinct element arrangements were used for each of the three tests (2 vs. 2, 5 vs. 5, 8 vs. 8). Since the test phases required the use of two panels, a copy was printed for every test stimulus.

3.4 Shaping and training

The experimental procedure began on the morning of Day 3, the testing day, when each chick underwent shaping; this first step aimed at letting the subject get acquainted with the novel experimental apparatus and feeding in it.

To encourage the chick to approach the panel, a single one was positioned at the center of the arena, and a mealworm was placed between it and the starting area.

Once placed in the starting area, the chick was free to move around and explore the novel environment for two minutes, given the absence of the transparent partition.

At this point, the chick's response was slowly and progressively shaped by placing pieces of mealworms closer and closer to the panel throughout five subsequent trials, such that while in the first shaping trial the mealworm was placed closer to the starting area, in the fifth (last) trial it was just below the panel. This first phase was concluded once the chick could confidently and quickly approach the panel to eat the mealworm.

Subsequently, chicks learned to circumnavigate the panel to search for food. At this point, the transparent partition was introduced, confining the chick within the starting area. From behind the partition, the chick saw a plastic, despite very realistic, mealworm attached to a transparent thread, being progressively dragged from the front of the panel towards the back, where a real mealworm had previously been located. Once the chick

was released in the arena, it could search for food, placed in a small white bottle cap behind the panel. Therefore, at the end of shaping, the chick moved from the starting area and circumnavigated the panel to find the food reinforcement confidently.

Once familiarization was over, each chick underwent training. As previously mentioned, on each of the twenty training trials, the stimulus positioned on the panel changed; however, the number of red squares was always five. Following the positioning of the stimulus, the chick was placed in the starting area, where it was confined for five seconds to let it observe the number of elements presented and associate that particular number with the presence of the reinforcement. When released, the chick was given one minute to circumnavigate the panel to collect the reward; in case it exceeded the time limit, the trial was considered null, and the chick was removed from the arena and placed in the opaque white box near the apparatus. Had the first null trial been followed by two subsequent null ones, the training would be over, and the chick would have been placed back in the cage to let it rest.

The training phase would end after 20 consecutive trials in which the chick successfully circumnavigated the panel; this stage saw the employment of a procedure used in previous studies within this field (Rugani et al., 2013; Rugani et al., 2014).

Rugani et al. (2013; 2014) have demonstrated that chicks are able to identify a panel by means of the number depicted on it: this was achieved after a few trials during which the chick found the food reinforcement behind the panel, thus establishing an association between the number depicted on it and the presence of the reward.

However, since no mealworms would be present during the testing phases, chicks had to get accustomed to working despite this lack of food. This was achieved by employing an intermittent pattern of food reinforcement: among the 20 trials, trials number 15, 16, 18, and 19 were devoid of mealworms; trials number 17 and 20 each presented a big one; whereas, in the remaining ones, a small piece of food was made available. Thanks to this strategy, chicks succeeded in circumnavigating the panel during training, with the same success being carried over to the testing phases, preventing performance extinction.

The usual amount of time required for each training phase was between 10 to 20 minutes, assuming the chick's cooperation; those chicks who showed little interest or motivation in following or eating the mealworms were initially put back in the rearing cage to rest,

and if the same behavior was further observed in subsequent attempts, the chicks would be discarded from the study.

Once a chick had completed the first training phase, it immediately undertook Test 1 to preserve the recognition of the 5-red squared panel; after finishing the first test and resting for an hour, each subject underwent a second training just before moving on to Test 2, and the same procedure was implemented for Test 3.

3.5 Test

The testing phase comprised three tests, each consisting of five trials: the 2 vs. 2 or “small number test”, the 5 vs. 5 or “control test”, and the 8 vs. 8 or “big number test”.

Chicks were randomly assigned to one of two groups varying in the ordering of the tests: either a 2 vs. 2, 8 vs. 8, 5 vs. 5 (N = 5) group or an 8 vs. 8, 2 vs. 2, 5 vs. 5 (N = 6) group. The arena was equipped with two symmetrically located panels, one on the left and one on the right, and two identical stimuli placed on each; during this phase, however, the chicks were not reinforced, meaning that they would not find any mealworms once behind the panel.

At the start of each trial, the chick was confined within the starting area by means of the transparent partition, where it would stay for about five seconds; from here, it was able to visually inspect the stimuli before being released. Once the partition was lifted, the chick could walk around the arena, and as soon as a panel was circumnavigated, the trial would end. Each subject was allowed only one choice, which would be scored; to be considered a valid choice, the chick had to enter the area behind one of the panels with its head and at least $\frac{3}{4}$ of its body, therefore beyond the bent sides of the panel.

In the inter-trial period, the chick was removed from the apparatus and placed in the opaque box adjacent to it to allow the changing of the stimuli and, possibly, the cleaning of the arena; in general, the chick spent around 15 seconds in the box before being repositioned in the starting area at the beginning of a new trial.

To prevent any spatial learning due to cues provided by the specific position of panels or stimuli, the panels were interchanged, and the stimuli were substituted on each new trial. Once the preparation of the arena had been completed, a procedure that needed to be very quick, the chick was repositioned in the starting area, and a new trial began.

If the chick did not choose between the two panels within the allocated time limit of one minute, the trial was promptly repeated with the stimuli turned upside-down; this procedure continued until each chick completed the three experimental testing sessions, each comprising five valid trials.

In order to avoid disturbing the chicks, the subjects' behavior was observed from the camera's screen, thus avoiding direct observation; also crucial was maintaining a quiet environment devoid of external distractions. All the tests' trials were video-recorded and scored offline.

The number of trials in which each subject chose and circumnavigated the left panel was computed for each test, and the percentages of left choices were obtained by calculating: $(\text{number of left choices}/5) \times 100$.

4. RESULTS

All the tests 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 right panel), as well as the direction of circumnavigation. The percentage of Left Choices (LC), or the percentage of trials in which the left panel was circumnavigated, was calculated for each chick and each test: it ranged from 0, meaning that the left panel hadn't been chosen at all, to 100, meaning that the left panel was consistently chosen in each trial.

Following Rugani et al.'s (2020) study, it was predicted that the LC would be affected by the Test variable (2 vs. 2, 8 vs. 8, and 5 vs. 5); more specifically, the expected order restriction saw the small number test as that with the highest LC percentage, the control test at chance level, and the large number test with the lowest percentage ($LC(2 \text{ vs. } 2) > LC(5 \text{ vs. } 5) > LC(8 \text{ vs. } 8)$).

In order to determine whether the chicks' observed behavior was due to an individual bias or numerical magnitude, a Small Number Bias (SNB) and a Large Number Bias (LNB) were calculated for each chick: the SNB was calculated by subtracting the LC obtained from the control test from those of the small number test ($LC(5 \text{ vs. } 5) - LC(2 \text{ vs. } 2)$), while the LNB was obtained by subtracting the LC of the control test from those of the large number test ($LC(5 \text{ vs. } 5) - LC(8 \text{ vs. } 8)$). The expected results were an $SNB < 0$, highlighting a leftward bias in response to small numerosities and an $LNB > 0$, highlighting a rightward bias in response to large numerosities.

4.1 Data analysis

The data collected were subjected to Bayesian analyses in R (version 4.2.1), employing the Bayes Factor package; the interpretation of the Bayes Factor (BF) was carried out according to Lee and Wagenmakers' classification (2014).

Concerning frequentist analyses, JASP 0.16.1.0 was utilized.

4.2 Results

Firstly, the effect of Order and Test variables on the percentage of Left-sided Choices (LC; **Figure 3**) was considered. The Bayesian ANOVA (BfANOVA) analysis, against the ‘Intercept only’ model, generated moderate evidence supporting a Test effect (BF = 6.973), (repeated measures ANOVA: $F(2,18) = 6.674$; $p = 0.007$, $\eta^2 = 0.278$); but no effect (anecdotal) of Order (BF = 0.687), (repeated measures ANOVA: $F(1,18) = 0.805$; $p = 0.463$, $\eta^2 = 0.034$).

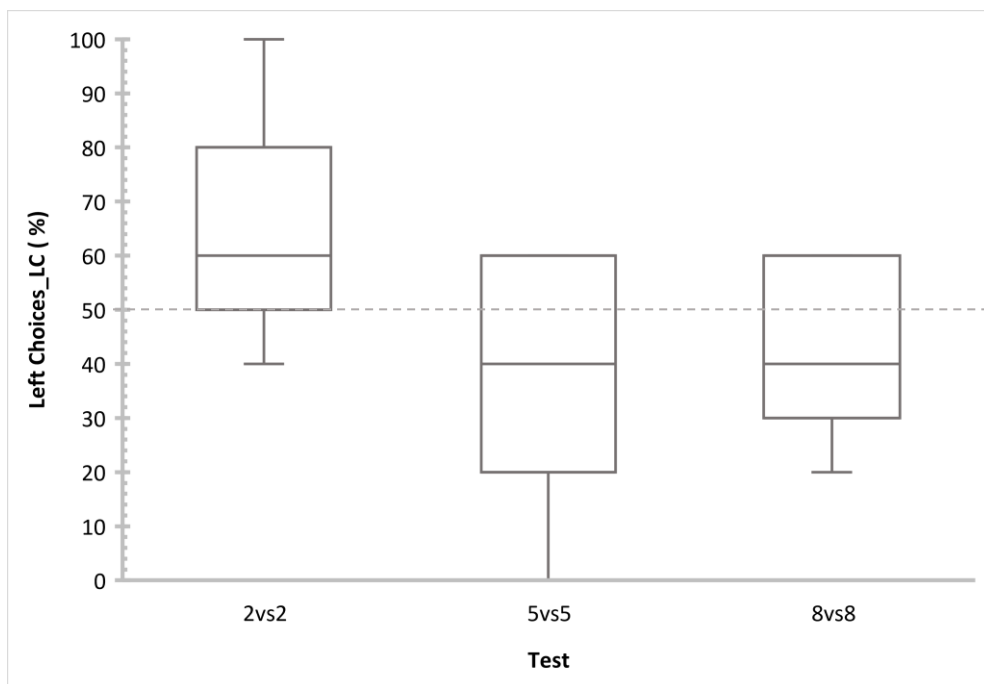


Figure 3

The figure represents the Left Choices [LC] as a function of numerical magnitudes; depicted here is each test's LCs (means, SE, 1Q, and 3Q). A dotted line represents the chance level (50%). The Bayesian and the frequentist one-sample t-test vs. chance level are reported below. When facing smaller magnitudes chicks took more left-sided choices (two: BF = 1.883; $p = 0.044$, Cohen's $d = 0.695$), and slightly more right-sided choices when facing larger magnitudes (eight: BF = 0.537; $p = 0.255$, Cohen's $d = -0.364$) than the one experienced during training (five); in the 5 vs. 5 test they showed a right bias (BF = 1.549; $p = 0.057$, Cohen's $d = -0.649$).

Secondly, to test the equality constraints of the present model, the unconstrained model, indicated by $LC_2 \text{ vs. } 2 \neq LC_8 \text{ vs. } 8 \neq LC_5 \text{ vs. } 5$, was compared to every possible

constrained model (e.g., LC_8 vs. 8 ≠ LC_2 vs. 2 = LC_5 vs. 5). The unconstrained model was preferred to the majority of the possible constrained models by a factor ranging from 2 to 17, with the exception of one model (LC_5 vs. 5 = LC_8 vs. 8 ≠ LC_2 vs. 2), with a factor of 0.527. In the former case, the results pointed to anecdotal to strong evidence favoring a distinct performance in the three tests, while in the latter, it supported with anecdotal evidence the similarity in performance in the 5 vs. 5 and 8 vs. 8 tests.

The results of frequentist analyses showed that: LC_2 vs. 2 was moderately larger than chance (50%): mean = 63.636, SD = 19.633, $t(10) = 2.304$, $p = 0.044$, Cohen's $d = 0.695$; LC_8 vs. 8 was slightly smaller than chance: mean = 43.636, SD = 17.477, $t(10) = -1.208$, $p = 0.255$, Cohen's $d = -0.364$; while the LC_5 vs. 5 was significantly lower than chance: mean = 34.545, SD = 23.817, $t(10) = -2.152$, $p = 0.057$, Cohen's $d = -0.649$.

Concerning the Number Bias, the first step saw the computation of the Small Number Bias (SNB) and the Large Number Bias (LNB), followed by the comparison of each Number Bias with the null = 0. T-test Bayes factor analysis yielded a moderate evidence favoring the Number Bias for SNB (BF = 9.851; One sample t-test: $t(10) = -3.525$; $p = 0.005$, Cohen's $d = -1.063$), and an anecdotal evidence opposed to the Number Bias for LNB (BF = 0.517; One sample t-test: $t(10) = -1.166$; $p = 0.271$, Cohen's $d = -0.351$).

Regarding the side of circumnavigation, evidence for each numerical magnitude appeared to be only anecdotal: 2 vs. 2 (BF = 1.593; $X^2 = 3.320$; $p = 0.068$, Phi = 0.246); 5 vs. 5 (BF = 0.364; $X^2 = 0.146$; $p = 0.703$, Phi = 0.051); 8 vs. 8 (BF = 1.743; $X^2 = 3.541$; $p = 0.060$, Phi = 0.254). Results are presented below in Table 1.

Table 1

Data relative to the side of circumnavigation in all test conditions for each panel.

Test	Side	Left panel		Right panel		BF	X ²	P	Phi
		Left	Right	Left	Right				
2 vs. 2	Count	26	9	10	10	1.593	3.320	0.068	0.246
	%	74.286	25.714	50.000	50.000				
5 vs. 5	Count	10	9	17	19	0.364	0.146	0.703	0.051
	%	52.632	47.368	47.222	52.778				
8 vs. 8	Count	19	5	17	14	1.743	3.541	0.060	0.254
	%	79.167	20.833	54.839	45.161				

5. DISCUSSION

The present study follows and is based on previous research conducted by Rugani et al. (2015a; 2020), with the aim of discovering the origin of spatial-numerical association (SNA) in newborn domestic chicks (*Gallus gallus*); the paradigm, procedures, and analyses employed here are, therefore, the same.

The main objective of this research was to investigate the existence of a SNARC-like effect in 3-day-old chicks and the role of numerical magnitude, as opposed to individual spatial biases, in determining this association. The employment of young, naïve subjects would allow the exclusion of variables that could potentially impact their performance (e.g., experience) while focusing solely on the biological mechanisms underlying the spatial-numerical association.

From the data collected, it emerged that chicks showed a left bias in the small number test (2 vs. 2) and a right bias in the large number test (8 vs. 8; despite being slightly less strong), therefore demonstrating a SNARC-like effect; the surprising finding, however, was the presence of a robust right bias in the control test (5 vs. 5), which appeared to be even more prominent than that found in the 8 vs. 8 test. The existence of this bias goes against previous findings (Rugani et al., 2015a; Rugani et al., 2020), given that chicks did not respond impartially when presented with the same numerosity as that of training.

Overall, therefore, the results of this research do not seem to support the experimental hypothesis: despite showing a SNARC-like pattern in the small and large number tests, the presence of a bias in the control test, devised with the exact aim of detecting individual preferences in responding, questioned the strength of magnitude's role in determining SNA's directionality.

One reason that could explain the unusual responses may be a lack of motivation in concluding the entire testing section; it could be that, after completing the first two tests, when reaching the last (5 vs. 5), chicks may have simply been repeating a response that was 'reinforced'. Being placed into the opaque box near the apparatus while the stimuli were being changed may have served as reinforcement; consequently, the first panel circumnavigated might have been chosen for all or most subsequent trials purely because they lost motivation, and that was the only way to be removed from the apparatus. When considering the panel circumnavigated by each chick in the first trial of the 5 vs. 5 test,

the majority of subjects (7 out of 11) chose the right panel, which would seem to support this theory.

In light of these findings, the concerns brought about by Núñez and Fias (2017) become relevant; the performance of chicks in the control test, however, cannot be explained by exposure to novelty because the numerosity presented was identical to that of the training, undertaken immediately before the test. Thus, this confirms that the chicks' unusual responses in this test are due to a rightward bias.

It must be highlighted, however, that due to the reduced size of the sample, any result obtained cannot be generalized, as it would not be truthful to the real potential demonstrated by chicks in previous more thorough experiments (Rugani et al., 2015a; Rugani et al., 2020).

Throughout the testing phases, chicks appeared to be much more influenced by the numerosity presented at test: the obtained results yielded moderate evidence in favor of a Test effect (i.e., 2 vs. 2, 5 vs. 5, and 8 vs. 8) but no evidence in favor of the Order variable. This indicates that it was the magnitude presented in the different testing phases that impacted the chicks' performance and choices rather than the order in which they undertook these latter.

The computation of the Number Bias (i.e., SNB, LNB) and the subsequent analysis yielded moderate favorable evidence for the Small Number Bias but anecdotal evidence opposing the Large Number Bias. By subtracting the left choices of the large number test from those of the control test, the expected result was an $LNB > 0$ because it was presumed that chicks in the control test would choose impartially, thus with scores around the mean (50%) and that chicks in the large number test would choose mainly the right panel, thus obtaining a reduced LC percentage ($< 50\%$). However, given the detected right bias in the 5 vs. 5 test, results more closely resembled those of the small number test, with an $LNB < 0$. Regarding the side of circumnavigation, results did not point at any preferential response; this suggests that when circumnavigating the selected panel (either left or right), chicks used their eyes in an equal manner, therefore not showing any hemispheric bias.

While carrying out the experiment, an unexpected pattern of responses emerged in some subjects' performance. Despite ultimately including only laboratory-hatched chicks in the experiment, a small number of few-hour-old chicks ($n = 4$; obtained from local hatcheries) were also part of the original sample. Their performance, however, gave rise to unusual

results: they showed a reverse SNARC-like effect, characterized by a right bias for smaller magnitudes and a left bias for larger magnitudes, thus presenting the opposite pattern of what is usually expected. Unfortunately, this phenomenon could not be explored further due to time constraints, leading to the exclusion of the subjects from the sample.

These findings represent an exciting starting point for future research; an experimental procedure could be conducted involving two groups of chicks, one entirely composed of light-hatched, laboratory-born chicks and one entirely composed of few-hour-old chicks, hatching outside the laboratory. In this way, more light could be shed on this occurrence, potentially discovering whether this was just an isolated case or whether it represents a distinctive response pattern.

5.1 Limitations

It must be pointed out that this study has been subject to several limitations; hence any results obtained should be interpreted with caution.

Firstly, the small sample size ($n = 11$) does not allow the generalization of the obtained results to the bigger population, as the performance of a few chicks may not be close to the actual pattern of responses commonly identifiable in domestic chicks.

Secondly, the experiment was conducted during a protracted period that spanned both winter and summer, exposing subjects to two very different time intervals, temperature-wise. Despite the carefully controlled thermic conditions in the laboratory, in fact, chicks seemed to be affected by the temperature change, performing slightly better during winter compared to during summer.

Thirdly, the experiment suffered from months of inactivity as a consequence of the December-April avian flu outbreak in Northern Italy; this was the leading cause for the inability to reach the expected sample size.

As a result of these limitations, it becomes clear that the sample size employed is not the definitive one; the work carried out and discussed here is, therefore, solely functioning as a research exercise apt at completing the bachelor thesis project.

6. CONCLUSION

The present study was therefore not able to prove the experimental hypothesis in a sample of 3-day-old domestic chicks (*Gallus gallus*); due to the detection of a right bias in the control test (5 vs. 5), the study could not demonstrate the prominent role of magnitude in determining SNA, thus favoring the role of individual preferences. Nevertheless, despite the nonsignificant results, it should still be pointed out that a SNARC-like tendency was observed; chicks showed preferences in associating smaller numbers with the left and larger numbers with the right side of space.

Attention is, however, warranted on the numerous limitations encountered throughout the experimental procedure, which could have affected the results of the study; previous more thorough research conducted by Rugani et al. (2015a; 2020) is thus more appropriate in representing the pattern of SNA in domestic chicks.

The acquired results, however, could still provide an interesting starting point for future research despite failing to reach the expected conclusions. Given that few-hour-old chicks hatching outside the laboratory (ultimately excluded from the final sample) surprisingly presented a reversed SNARC-like pattern of responses, further insight into this phenomenon would allow an understanding of whether this represents an atypical response or a new avenue in SNA research.

AUTHORS' CONTRIBUTIONS

The work reported in this thesis is part of a larger research project led by Rosa Rugani. The contribution of the student (Chiara Brutti) is restricted to a part of such project adapted to meet the requirement criteria for the bachelor mandatory internship/thesis project.

Authors' contributions:

- **Rosa Rugani:** conceptualization, methodology, supervision, project administration, resources, original draft, revision, and modifications.
- **Chiara Brutti:** execution of the experiment, data collection.

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