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The role of brain lateralization on ordinal tasks: An investigation on day-old domestic chicks (*Gallus gallus*)

Il ruolo della lateralizzazione cerebrale in compiti ordinali: Una ricerca sul pulcino di pollo domestico (*Gallus gallus*)

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Abstract

Numerical abilities are a crucial aspect of cognition involved in many of the everyday choices. Studies in this field have grown rapidly, and recent research with both human and non-human animals demonstrated that there is an association between the representation of numbers and space (spatial numerical association, SNA), that relates small numbers with the left and large numbers with the right, as in the human mental number line (MNL). The effects of this phenomenon can be inferred from the discovery made by Dehaene and colleagues in 1993, whom noticed faster reaction time in subjects performing a *parity judgement task*, when they had to respond to smaller numbers on the left and to greater numbers on the right. This effect was labelled at that time: "Spatial Numerical Association Of Response Code (SNARC effect). Recent studies investigated the association between the SNA and the hemispheric lateralization in the domestic chick (*Gallus gallus*). Chicks represent a great model for this type of research due to their precocious cognitive abilities. This model offers the remarkable possibility of manipulation of the lateralization through light exposure during the last days of incubation. Previous collected data show that the degree of hemispheric lateralization affects ordinal-spatial-numerical performances. In particular, a lower lateralization degree prevents the left bias and does not affect overall numerical abilities even though higher lateralized chicks perform better. These findings are in line with the hypothesis that numerical information is processed bilaterally by both hemispheres, while spatial information is preferentially processed by the right hemisphere. The aim of this study was to test how pre-hatching light stimulation affects chicks' performance in a purely ordinal task. The spatial cue in this study is absent, as spatial information is made uninformative through the constant variation (at each trial) of the inter-element distance, hence allowing only the numerical information processing. We expected a better performance by strongly lateralized chicks and no bias in both experimental groups. All birds (strong and weak lateralized chicks) underwent four ordinal tests: one in which target elements were sagittally-oriented and three in which the elements were fronto-parallel aligned with respect to the birds' starting position. For the front-parallel disposition, besides a binocular condition, a monocular occlusion technique was used to

disentangle each hemisphere processing for both sides. Interestingly, we found that there is no longer a difference between hatch conditions, suggesting that spatial information might be determinant for the observation of the left bias.

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Introduction

The most exciting aspect of science is the unceasing birth of new questions for every fresh answer that you receive. Among the classic questions, there are the ones concerning the origin of the mind and the nature of our behaviour, about the ‘’how’’ and ‘’why’’ we (as animals, hence considering all other species) do what we do. Studying the origins of the mind necessarily implies going back to its evolutionary roots, and to analyse all the minds which developed together with and before ours, in all the different forms through which they have manifested, seeking for points of contact and linkages. For this reason, a comparative approach represents a particularly valuable resource in this field. Particularly, the study of the mind, the brain, and consequently the study of cognition and behaviour can not be exempt from this approach. The study of comparative psychology and cognition boasts an ever-increasing amount of findings, thanks to the continuous and growing interest in this subject. As a matter of fact, in recent years important strides and advances have been achieved in the specific field of numerical cognition, about its characteristics, its neural and biological foundations and the spectrum of cerebral mechanisms involved.

1.1 The number question

Without numbers, and without being able to estimate and to rapidly assess quantities, the human species would not have been capable of achieving any of the goals that it has reached (so far) (Gersten and Chard, 1999; Jordan *et al.,* 2010; Louange and Bana, 2010). Starting from the most basic evolutionary needs, such as discriminating the larger quantity of food (Krebs, 1974) or determining how many predators are in the surroundings (McComb *et al.,* 1994), until getting to count money for paying the groceries, choosing the shortest queue for the cinema and even sending spacecrafts on Mars. All of this happens because numbers are everywhere. We need them for identifying objects, ranking, and quantifying them. Numbers are intertwined with all other physical variables (Nieder, 2016) and, if mastered, could represent a useful tool to enhance our everyday problem solving. To date, it has been well-documented that numbers are part of our biological system and our most ancient origins. Initially associated with the concept of language and culture, the dealing-with-number was mainly attributed to humans. There has been an important debate about the evolutionary roots, or even more trivially, its presence or not, of this sort of ‘’number processor’’ into the brain. After many years of research it is possible to state that in human and non-human species there is the possibility and the ability to encode, process and understand (with all the due individual and species-specific differences) numerical information (Piazza and Eger, 2016). This knowledge comes firstly from studies with human adults, both the ones that had a formal education (Barth *et al.,* 2003) as well as innumerate (Pica *et al.,* 2004); allowing the detaching of numerical competences from the formal study of numbers or mathematics. In addition, there has been a notably mole of research conducted by comparative and developmental psychologists, which have proved that, interestingly, already in the first stages of development there's a certain level of numerical cognition in nonverbal subjects (non-human animals and pre-verbal infants). Thanks to this body of work it is possible to state that dealing with numerousness in a non-symbolic and nonverbal manner is a universal shared ability (Dehaene, 1997). Given that research show that a predisposition towards the ability to precocious deal and process with information of numerical nature in quite distant species, such as mammalians, birds, fishes, vertebrates and invertebrates (Bisazza *et al.,* 2014; Brannon and Terrace, 1998; Butterworth, 2002; Dacke and Srinivasan, 2008; Howard *et al*., 2018; Rugani *et al*., 2022; Rumbaugh *et al*., 1987; Scarf *et al.,* 2011; Tornick *et al*., 2015), it has been hypothesised that this representational system encoding numerosity could share common ontogenetic and phylogenetic traits.

Additionally, thank to observations on crows dealing with numerosities (Ditz and Nieder, 2016) two commonalities with humans' magnitude and numbers processing have been found, suggesting a further prove that this rudimentary numerical system, other than being universal, has also a shared evolutionary heritage. This shared finding is the presence of both the “Numerical Distance Effect” and the “Number Size Effect” (both consequences of Weber's law) (Dehaene *et al*., 1998). The former refers to the facilitation in discriminating between two numbers that increases as the difference between the magnitudes of those two numbers grows. This has been observed in humans, monkeys, and birds (D'Amato *et al.,* 1990; Dehaene *et al*., 1998; Rilling and Mcdiarmid, 1965). The latter refers to the finding that discriminating between two numbers is impaired as numerical size increases, found in rats (Mechner, 1958).

This evidence permits the theorization of a shared possession of a domain-specific and biologically determined representation of number, kind of a “Number sense” (for the first time labelled in such a way by Tobias Dantzig in 1954, and subsequently echoed by Stanislas Dehaene) (Dantzig, 1954; Dehaene 1997). Dehaene defines the number sense as a system organised on numerical proximity and characterised by a fuzzier representation for increasingly larger numbers (Dehaene *et al.*; 1997).

More recently, this form of numerical intuition has also been defined as a system that allows number representation in an *approximate manner,* hence labelled “Approximate Number System” (ANS) (Dehaene *et al.*, 2011; Feingenson *et al.*, 2004).

An interesting and essential question therefore arises, that is: where can this type of representation be placed in the brain? Although more research is needed, a further and recent contribution to support the idea of this innate core system, the number sense, was obtained thanks to a neurophysiological study, in which numerically naive monkeys have been tested by recording single-cell activity (Viswanathan and Nieder, 2013).This research revealed the existence of neurons that are spontaneously tuned to preferred numerosities, and specifically, these numerosity-selective neurons were present in the primate’s brain posterior parietal cortex (PPC) and the lateral prefrontal cortex (lPFC). An akin kind of “Number neurons” (neurons responsive to specific numbers) were also found for the first time in crows’ nidopallium caudolaterale (NCL) (Ditz and Nieder, 2015) and more recently also in caudal nidopallium of young chicks (Kobylkov *et al.,* 2022). NCL is considered to be the avian brain area analogue to primates’ prefrontal cortex (PFC) (Nieder, 2016) and even though they are not homologous structures (Güntürkün and Bugnyar, 2016; Preuss and Wise, 2022) the same code for number is shown by both brains’ structure. Thus, it is possible to posit that such number neurons could be the result of convergent evolution and represent a neurobiological substrate for an intuitive sense of number and so an inborn feature of the brain.

1.1.1 The association between number and space

If the first question was whether a numbers sense existed or not, now that it is established, there is the need to trace a more specific investigation and analysis of the nature of this outcome. Just as it needed a succession of discoveries over time for the establishment of the idea that the existence of an innate ability to deal with numbers is universal, culture-independent and not only a human uniqueness, a similar path was faced by Galton's idea in 1880.

Galton's pioneering observations in 1880 arose from interviews he conducted on some patients, who declared to perceive and imagine numbers as automatically dyed with a specific colour or described them as consistently moving within space following their head's movement ,or conversely, staying still and fixed in the same location in this 'mental space' despite eye movements (Galton, 1880b). Such testimonies paved the way for the postulation of the theory by Galton himself, who labelled this abstract and analogical representation of numbers as a "Mental Number Line" (MNL) oriented from left to right. From that moment on, a succession of innumerable experimental paradigms sought and tried to confirm the existence of this link between numbers and space. One of the most famous experimental designs in favour of the existence of the MNL and its orientation from left to right, are two studies conducted by Stanislas Dehaene and colleagues in 1990 and then in 1993. In 1990, while the researchers asked the participants (human adults) to classify some numbers displayed on a screen as "smaller" or "bigger" than number 65, responses showed a "distance effect", in which answers were much faster as participants had to classify numbers increasingly farther to number 65. Moreover, an additional and serendipitous finding was observed when responses had to be given pushing a button (on the left for small numbers and on the right for big numbers): the smaller the number, the faster the left-hand response, while the larger the number, the faster the right-hand response (Dehaene *et al.*, 1990). In 1993 similar outcomes were observed while Dehaene and colleagues were subministrating a *Parity Judgement Task*, in which subjects are asked to judge whether two digits are even or odd (Dehaene *et al.*, 1993). This kind of spatial-association effect between side and numbers was at time defined as "Spatial numerical Association of Response Codes": "*As a tribute to Lewis Carroll, I called this finding the SNARC effect*" (Dehaene, 1997, p.81) and was hypothesised to arise as a consequence of the automatic activation of the mental number line after numbers' onset on the screen. The MNL and the SNARC effect are not the only phenomena from which it is possible to state an association between number and space (SNA). A close link between these two domains can also be inferred from lesion studies. Human patients, after a brain damage to the right hemisphere, exhibit a hemispatial neglect towards the left hemispace. When asked to bisect a numerical interval, they misplace the midpoint, resembling a similar error-pattern to the bisection of physical lines (Zorzi *et al.*, 2002). This outcome could show that the MNL might not be just a metaphor.

Presumably due to the coincidence of the left-right orientation of both the SNARC effect and the writing/reading western rules (Shaki *et al.*, 2008, 2009), and due to the *always-keep-in-mind* percentage of anthropocentrism that haunts our attitude and scientific progress, for many years the idea concerning the integration of number and space has been considered to be only a by-product of formal instruction, language or symbolic knowledge. Over the years, however, a huge number of demonstrations of the spontaneous association between numbers and space have been observed in all stages of human developmental phases. In children tested in a manual bisection paradigm (De Hevia and Spelke, 2009; Hoffmann *et al.*, 2013), a systematic bias in the localization of the midpoint of a horizontal line occurred, showing the same non-symbolic numerical and abstract organisation as adults do. Similar outcomes were observed from pre-schoolers (McCrink *at al.*, 2014; Patro and Haman, 2012), infants (Bulf *et al.*, 2016; Hevia *et al.*, 2014a) and even newborns (Di Giorgio *at al.*, 2019; de Hevia *et al.*, 2014b, 2017), proving that the presence of a SNA comes prior to the onset of any mathematical or formal education, even though variations (reverse SNARC effect) within different cultures (Cooperrider *et al.*, 2017; Göbel *et al.*, 2011; Zebian, 2005) or individual differences, still allow the posit that culture, context and experiences could shape and modulate it.

A step closer to a more biologically-oriented view, that made this debate even more intriguing, happened when Regolin trained a group of few-days-old chicks to perform an adaptation of the line bisection task, looking for factors that could underpin the Pseudoneglect (defined as the tendency of healthy individuals to allocate more attention to the left side of perceptual space than on the right side (Bowers and Heilman, 1980)) and Hemineglect phenomena (defined as the reduced awareness of stimuli on one side of space after cerebral lesion (Parton *et al.,* 2004)) documented in humans. While investigating these factors something interesting was observed. Chicks exhibit a sided-tendency in their mistake pattern (Regolin, 2006), showing a similar performance to the humans' one, when tested for the diagnosis of visuospatial attention deficit. Similar outcomes resulted also from Diekamp and colleagues' research on chicks and pigeons (Diekamp et al., 2005). Indeed, tested in an adaptation of the cancellation task, both groups of birds displayed the same leftward bias observed in humans. Likewise, very recent evidence from blue jays (*Cyanocitta cristata*) and pigeons (*Columba livia*) lead to similar conclusions about the tendency to organise the numbers spatially (even though here it was not observed a preferential sided tendency) (Lazareva *et al*., 2020).

Probably the most convincing demonstration of the existence of a spatial-numerical association in non-human animals results from a study on the domestic chick (*Gallus gallus*), conducted by Rugani and colleagues in 2015. In this study newborn chicks were trained to reach a food reward behind a panel depicting a target number of elements (randomly displayed). At testing instead, the chicks faced two panels depicting the same number of elements (but distinct from the target shown at training). By varying the magnitude of the numbers depicted, the chicks responded following the association ''small numbers-left space'' and ''large number-right space'', hence: when the panels depicted a number smaller than the target, they searched for food behind the left panel; *vice versa* the chicks circumnavigated the right panel when the number was larger than the target. Moreover, three different control experiments ((1) shape, size, colour of element; (2) overall area and occupancy; (3) perimeter, density and occupancy) were applied to prevent the non-numerical cues' influence. Lastly, an additional finding of this study, that was only been observed in humans before is the ''relativity'' of the SNA shown by the chicks. In fact, the association of a specific number was not absolute and fixed with one side, on the contrary, it was flexible and varied depending on the target number that the chicks had experienced at training (Rugani *et al*., 2015c).

Overall, this evidence indicates that the disposition to associate numbers onto space with a left-to-right orientation, has a phylogenetic origin unrelated to culture, and that the SNA could be a widespread cognitive strategy.

1.2 The domestic chick: a model and a central thread

The advantage and the importance of comparative studies, lies in the fact that animals represent a perfect model to prove nativistic foundations of behaviours observed in humans, due to the lack of any culture or language-specific biases. Moreover, selecting very young subjects allows the control over previous experiences influences. An example of such a suited model in the comparative literature is the Domestic chick (*Gallus gallus*), one among the most precocial species, showing very sophisticated behaviour, a wide range of cognitive abilities and a fully developed motor system at a very early age (soon after hatching). That of young chicks embodying a perfect model, is something quite known in this field. Spalding already back in 1873 said: ‘’*chickens, for example, run about, pick up crumbs, and follow the call of their mother immediately on leaving the shell. For putting this matter to the test of experiment, chickens, therefore, are most suitable and convenient subjects*.” (Spalding, 1873, p.2). Also, as cited by Rose, Hans Krebs remarked that “*God had provided just the right organism with which to study any particular biological problem*” (Rose, 2000, p.1).

Studies on new-born chicks allow a strict control over “past” experiences, considering also the ones *in ov*o*,* and indeed supply an additional empirical evidence for the nature-nurture issues on the origin of knowledge (Vallortigara *et al*., 2010). Moreover, the chicks also have a soft unossified skull, facilitating cerebral injections without the need to resort to any implantation (Rose, 2000) and develop as embryos outside mothers' body (into the egg), embodying an highly malleable experimental model (Daisley *et al*., 2009). A discovery that makes this species an even more worthwhile reference-model for psychological, perceptual, cognitive and neurobiological investigation, is the one made by Rogers and Anson in 1979. Through injection of cycloheximide the two researchers observed for the first time some asymmetries in the chicks' brain (Rogers and Anson, 1979). Since that moment the domestic chick (as other Avian species) due to the brain asymmetries shown, the lack of *corpus callosum* and lateral eye's placement, became a milestone in the study of lateralization (*see more in chapter 1.3*) that is, a key animal model to investigate the mechanisms underlying the processing of information (for example spatial and numerical ones) by both hemispheres such as their interactions.

Between all vertebrates, chickens have always been considered as not so valuable, suffice it to think of the common sayings ''chicken brain'' or ''birdbrain''. Despite this unduly labelling, the chicks proved to possess remarkable cognitive abilities, such as a distinct spatial orientation and a variety of numerical competences, for example: young chicks proved to master the capacity to discriminate between different numerosities (Rugani *et al.*, 2008), succeed in proto-arithmetic task (Rugani *et al.*, 2009), find the center of a squared-shaped arena (Tommasi and Vallortigara, 200). As already mentioned in the previous section, early studies emphasised how the chicks also show a leftward bias, similar to the one shown by humans, while performing some attentional/numerical tasks (Regolin, 2006). There is a specific ability which comprises both numerical competences and the capacity to represent elements disposed onto space, following an ascending directional mapping: the ordinal ability.

1.2.1 Ordinal abilities

It was pointed out that there are several examples of non-symbolic numerical skills throughout the animal world. Nevertheless, there is a particular aspect of number abilities that has not been explored yet, which is ordinality. Ordinality is related to ranking an element into a sequence (Wiese, 2003). Just as much as a rapid estimate of numerosity, and the ability to quickly assess a numerical information, might have been advantageous for wild animals' survival (Lourenco and Aulet, 2023), accordingly, a spontaneous ordinal judgement (for example in establishing a rank in an hierarchical society as happens with baboons (Bergman, 2003)) could have had its ecological value. Ordinal abilities can be investigated in a non-symbolic way, through simply training and then testing animals to identify a target element into a sequence of identical other elements based exclusively on its ordinal position. Evidence relating ordinal abilities has been widely documented between several non-human species, such as brown capuchin (Judge *et al.*, 2005), rats (Davisand Bradford 1986), chimpanzees (Boysen *et al.*, 1993), rhesus monkeys (Drucker and Brannon, 2014) and bees (Giurfa *et al.*, 2022).

There is a link behind the sentence: ''*the ordered representation of the numbers onto space*'', which could describe either the definition of the Mental Number Line and also the capacity of dealing with numerical-ordinal information, since in this case too, numbers are mapped onto space. Consequently, there is space to hypothesise that the SNA could be revealed through ordinality assessing, due to the common intrinsic relationship existing between number and space. Investigating such ability in non-human species may represent a good chance and an ideal condition to shed light on the biological foundation on this left-to-right bias. A series of experiment conducted by Rugani and colleagues (*see next section*) on the domestic chick (*Gallus gallus*), aimed to explore the SNA in spatial-ordinal tasks, and allowed the achievement of a wide amount of evidence indicating that this kind of association in not only a human prerequisite.

1.2.2 Young Domestic Chicks (*Gallus gallus*) and ordinal abilities

This section concerns itself with a review on the ordinal studies conducted using the Domestic Chick as a model, in order to shed light on the predisposition to "count from left to right" beyond the human species. In 2007, the seminal work by Rugani, Regolin and Vallortigara led to the first evidence suggesting a predisposition to count from left to right in chicks in a very early stage of development (five-days-old). In this study, the chicks were trained to peck at a target element to receive food reinforcement. Three groups were formed, and for each of them a different target was different: it could be the third, the fourth or the sixth element in a series of ten identical containers. All containers were equidistant, fixed, and aligned sagittally with respect to the starting position. All groups of chicks successfully acquired the discrimination, pecking above chance exclusively the target element; this result proved that the chicks could process serial-ordinal information. However, due to the fixed position of the elements, the absolute distances could have been used as spatial information on which their performances could have been based. In order to exclude alternative explanations, a new group was trained to identify the fourth element in a series of ten, as it was done for the training in the first experiment, but now, at test the series was rotated by 90°. In this condition the series was now fronto-parallel with respect to the chick's position and the possible correct responses were two: the fourth element starting from the left or from the right end of the series. At this moment that an asymmetry arose: even though both correct responses were equally reinforced, the chicks showed a significant preference for the fourth element from the left of the series rather than the one from the right. For the first time a non-human species showed the tendency to map the numerical number line from left to right (Rugani, Regolin and Vallortigara, 2007). A possible explanation for this bias was given attributing to the right hemisphere (processing information coming from the left eye) a stronger activation, hence the dominance on the left hemisphere and consequently on the chicks' behaviour (Andrew, 1990), as it was already been thought when this bias was observed in chicks (Diekamp, 2005; Regolin, 2006) resembling somehow the human phenomenon of ''pseudoneglect'' shown while performing visuospatial tasks (Vingiano, 1991). This lateral bias appears to be a robust phenomenon, indeed it has been replicated in subsequent studies.

In 2010, two different species of bird were investigated: young domestic chicks and adult Clark's nutcrackers (Rugani, *et al.*, 2010), following a very similar experimental design as the previous experiment, with a only difference: the series for this study was longer (16 elements). The procedure was left unchanged: the elements were fixed and equidistant at both training and test. Again, the preference appeared for targets located in the left hemispace. It has been posited that, in both studies, since the elements were fixed, the overall length of the series and all other absolute distances (from the starting point, from the two ends, walking times) were all available and could constitute a notable amount of cues for the performance of the chicks. If this is the case, the left bias would hence become the manifestation of the right-specialisation for a preferential processing of spatial information (Rashid and Andrew, 1989; Regolin *et al.*, 2005; Tommasi and Vallortigara, 2001).

Therefore, the aim of subsequent research has been ensuring that the information processed by the chicks in this type of task was related only to ordinal-numerical information, and unravel the spatial cues' role. By keeping the same paradigm but manipulating the inter-element distance at each trial, (during both training and testing), the use of spatial information was disrupted. The chicks in this condition successfully pecked at the fourth element above chance but, interestingly, did not show any left hemispace bias. This outcome shows that chicks preferentially rely on numerical information rather than spatial information (Rugani *et al.*, 2011). Further confirms were obtained in an additional control applied during this study, in which a new group of chicks was trained with the elements kept at a fixed distance at training, but then, at testing, the group was divided into two halves: one faced a frontoparallel series with an enlarged distances (so that the previous position occupied by the fourth element was now the location of the third element), and the other group faced a series reduced in length (so that the fifth element occupied the position previously occupied by the fourth element). Even in this case, both the fourth element from the left and the fourth element from the right were pecked equally and above chance. Another control experiment was conducted to rule out the possibility that any "novelty effect" could have influenced the performance, so the colour of the element was changed, but in this condition a left bias was observed, hence excluding this alternative explanation (Rugani *et al.*, 2011). Overall, these findings seem to indicate that even when the spatial information is not used by the chicks to solve the task, its availability has a crucial role for the emergence of the left sided bias. Hence, both hemispheres could be equally involved in representing purely ordinal-numerical information while the spatial one could be represented unilaterally, in the right hemisphere.

To disentangle the role of each hemisphere in this intrahemispheric coupling of spatial and ordinal cues, Rugani and colleagues in 2016 attempted for the first time to use a temporary monocular occlusion technique on the chicks' eye. The chicks were performing the same serial ordinal tasks originally designed to investigate the left bias in previous studies. Due to the almost complete decussation of birds' optic chiasm (Ocklenburg and Güntürkün, 2012; Weidner *et al.*, 1985) and taking advantage of the paucity of a strong structure allowing inter-hemispheric communication in avian brains (studies on pigeons found small tracts that could work nearly as the *corpus callosum* in humans (Robert and Cuénod, 1969; Zeier and Karten 1973)) reducing the visual input to one eye only, permits to obtain information of the single contralateral hemispheres' processing (Rogers *et al.*, 2013). Three different vision conditions characterised this study: (1) Binocular Frontoparallel (2) Left Monocular Frontoparallel (left eye in use, right hemisphere processing) and (3) Right Monocular Frontoparallel (right eye in use, left hemisphere processing). Results show that the chicks perform successfully at test in all conditions of vision, pecking the target element above chance. Interestingly, chicks in monocular conditions only pecked on the correct choice in the same hemifield of the unobstructed eye. This outcome shows that, firstly, both hemisphere can correctly and independently represent and use the numerical information acquired during binocular training and also that, due to fact that only one end of the series (homolateral to the unpatched eye) was clearly visible, the chicks used it as a benchmark to “start to count” (Rugani *et al*., 2016). Furthermore, the fact that the chicks also pecked at the first element above chance only under the two monocular conditions confirms the hypothesis of considering the end of the series as the “starting-counting point”. In fact, it has been observed before (Davis and Bradford, 1986) that animals have the tendency to seek food in the closest possible location if facing a series of identical elements. Additionally, the saliency of the first element has consequences on the chicks’ behaviour only under the monocular condition, whenever only one hemisphere is processing. As a matter of a fact, at the binocular condition responses to the first element could efficiently be suppressed, resulting in the exclusive preference for the fourth element on the left side. Recurrence of the left-sided bias was expected from previous results (Rugani *et al*., 2007, 2010, 2011) since the availability of both numerical and spatial information. The similarity between performances of the chicks under binocular condition and left monocular condition, suggests that when both hemispheres are processing information completing a task, and the spatial information is accessible, chicks’ behaviour is driven by the right hemisphere, in charge of spatial information processing (Rashid and Andrew, 1989; Regolin *et al*., 2005; Tommasi and Vallortigara, 2001). Once proved that both hemispheres of the chicks’ brain are capable of succeed in spatial-ordinal task, the following study (Rugani and Regolin, 2020) aimed to answer the question: ‘’do hemispheres use different strategies to sort out the task?’’. The method utilised to solve this query was, using the same experimental design and paradigm as in the previous study, the creation of a conflict between spatial and numerical cues at test, after a training session in which both information were kept available. Therefore, after the chicks underwent the training and sagittal test with the element fixed and placed identically, they faced the frontoparallel tests (Binocular; Left Monocular; Right Monocular) in which the distances between the elements were increased so that the actual third element was in the position previously occupied by the fourth element. The results are consistent with the literature, showing a successful performance for the identification of the fourth element both at the sagittal and in the binocular test, proving that the chicks (ignoring the third at the frontoparallel task) process preferentially ordinal information rather than spatial information. A left bias was observed under binocular vision. For what concerns both monocular vision conditions, a balanced resort to both spatial and numerical information was observed. The chicks indeed chose above chance equally the fourth element and the third element respectively on the homolateral side of the unobstructed eye. This reliance either on spatial than numerical information implies that both hemispheres can represent and use independently both kinds of cues acquired previously. The bilateral representation of the numerical information observed in this study is consistent with the literature (Bugden *et al.*, 2019; Piazza and Eger, 2016).

Lastly, it is possible to posit that the SNA could be ascribable to a model assuming a differential (1) encoding (2) processing and (3) integration by each hemisphere for numerical and spatial information.

Given that, all species evolved into an environment in which numerical cues usually are intertwined and need to be constantly encoded with other information, it is possible hypothesising that different brains could be equipped with a system capable of processing all combinations of them. A new issue arises, though, when brain asymmetries have been proposed as representing the basis of the SNA (de Hevia *et al.*, 2012). Nowadays, thanks to more than a century of research, it is possible to state that brain asymmetries are widespread all over species (*see more in next section*). Brain asymmetry implies hemispheric specialisation and lateralized abilities. Since lateralization in non-human-species was discovered in the Domestic chicken (*Gallus gallus*) (Rogers and Anson; 1979), this species represented one of the most studied animal models in this field. As mentioned above, the latest findings in spatial-ordinal investigation by Rugani and colleagues assumed the SNA observed in young chicks could be linked to a right hemisphere dominance, hence positing that cerebral lateralization could play a role in this association. A subsequent study conducted at our Laboratory (unpublished), investigated the effects of lateralization on ordinal performances. The performance of two groups of chicks (differing in lateralization conditions) was compared, following the same experimental design used for previous studies on this topic. Owing that in this species the asymmetries are triggered mainly by an environmental factor (light stimulation during the last three days of incubation) (Rogers and Anson; 1979) one group was hatched in light condition ("strong lateralized group": *Li-chicks*) while the other was incubated in dark condition (*Di-chicks)*. Moreover, the monocular-occlusion technique was implemented. The hypotheses were (1) an overall better performance from the lateralized chicks compared to non-lateralized chicks, and due to the lack of hemisphere specialisation in non-lateralized chicks (hence no right-hemisphere dominance for spatial information) (2) the prevention of left bias in the completing of the ordinal task was expected. The outcomes of this experiment are consistent with previous studies in the literature and with the hypothesis. Both groups mastered the identification of the target element (even though strong lateralized chicks showed more accuracy), and moreover, in the frontoparallel binocular test, strong-lateralized chicks displayed the left bias while non-lateralized chicks did not. In the frontoparallel monocular tests, both groups show a preference for the homolateral side of the unpatched eye (Rugani *et al*., 2016, 2020) showing also a fallacy in the inhibition to peck the first element (Rugani *et al*., 2011, 2016, 2020).

The outcome of these works suggests that language is not indispensable for accounting for SNA and that this association could be a common characteristic shared by a core system between all species (Rugani *et al*., 2015a; Vallortigara, 2012). However, the origin of the SNA still remain to be totally determined.

1.3 Brain Lateralization

As mentioned above, a typical human tendency is to attribute to himself the uniqueness of some characteristics. This anthropocentric attitude has its roots also in the topic of lateralization. Starting from the assumption that, for a very long time the whole scientific community had settled on the idea that *symmetrical* was the adjective corresponding to ''normality''(or for better saying to a ''non-abnormality'' condition), *asymmetrical* as a consequence, implied something pathological, and not related to healthy subjects. It was thanks to Broca's insight (Broca, 1865), and with some delay, Wernicke's contribution (Wernicke, 1874), that the thoughts about humans' cerebral asymmetry changed, and research on brain lateralization had its onset.

A brain is considered to be lateralized if one hemisphere (or any other brain region) differs from the other in structure and/or in the performing of a different set of functions (Bisazza *et al*., 1998). It took decades for the first research on non-human species to start to be of interest for the scientific community. Among the reasons that slowed the study of asymmetries in other species, one is definitely the strong belief that brain asymmetries depended on language and handedness, which are uniquely human features. Indeed, the first studies on animals were mainly focused on seeking this kind of human-like asymmetries. The outcome resulting from the research in non-linguistic species, seeking for similar results of human language asymmetry, was scarce or not very significant. The same happened whenever, for investigating lateral preferences in animals' movements reaching for food, the benchmark was human handedness. Itani and colleagues in 1963 studied 394 monkeys reaching movements, with the underwhelming results of a third of them showing a left-handed preference, a third a right-handed preference and a third with no preferential side at all (Itani *et al*, 1963). Only towards the end of the 1970s this field of research saw a rebirth, surprisingly, thanks to two discoveries on bird models.

Nottebohm in 1977 published the results of a unilateral transection of the canary's *nervus glossopharyngeus pars tracheosyringealis* (equivalent to the human larynx). Transecting this nerve on the right side implied the loss of 10% of the birds' syllables, while an equal transection of the left side rendered the animal almost mute (Nottebohm, 1977). In 1979 Lesley Rogers after injecting an antibiotic that inhibits ribosomal protein synthesis (cycloheximide) into the left or right forebrain of a two-days-old domestic chick (*Gallus gallus*) observed that only when the injection was given to the left hemisphere chicks showed elevated attack and copulations scores. Additionally, the chick could not be able anymore to complete a visual discrimination task (grains vs. pebbles) which was, indeed, correctly performed by the chicks that received the treatment on the right hemisphere. This result suggests that the left hemisphere was the one in charge of behaviour control. In the following years Rogers conducted a wide number of studies on this species leading to fascinating discoveries, such as the ontogenetic causes establishing the hemispheric specialization as well as the neural pathways involved (Rogers.,1982; Rogers and Sink, 1988). After this first study onwards, some researchers found that lateralization could be easily investigated simply by testing animals monocularly (Mench and Andrew, 1986). This was feasible because birds can use their eyes independently to scan the environment (Wallman and Pettigrew, 1985). This method became the standard practice to investigate lateralized behaviour. In particular, owing to the fact that in species with laterally positioned eyes, hence with a reduced overlap of the visual fields, information coming from one eye goes to the contralateral side of the brain (retinal fibres in the avian brain entirely decussate at the optic chiasm, sending contralateral visual inputs to the brain (Butler and Hodos, 2005), applying an eye-patch while testing the subjects and then comparing the performance divided for the single eye, permits to reveal lateralized differences. Moreover, following this method, it is possible to disentangle the hemisphere in charge of a specific behavioural control since it coincides with the "seeing" eye (Mench and Andrew, 1986; Vallortigara and Andrew, 1991).

In the vein of these studies, other researchers tested chicks to unravel the individual specialization of each hemisphere. Andrew in 1991 observed that information is processed in very different ways between the two halves of the chicken's brain. It was found that the left hemisphere is specialised in the categorization of stimuli (i.e. food, other chicks), focused attention, sustained responding, and landmark cues, while the right one attends more specifically details (Andrew, 1991) including spatial stimuli (Rashid and Andre, 1989), react to novelties or unexpected stimuli, geometric cues, social cognition, escape responses, predators detection (Vallortigara and Andrew, 1991; *see* Rogers, 2013 for a review). Knowing that the hemispheres of the brain give different contribution in completing cognitive challenges and display differences, both functional and structural, between left and right (Daisley *et al.*, 2009) chicks currently embody an established animal model for the study of the neurobiological and cognitive foundations of left/right differences (Güntürkün *et al.*, 2020).

1.3.1 The avian brain and visual system

Over the past twenty years, the view of avian as model species has changed. Thanks to more advanced techniques, it has been possible to conduct specific and very accurate inspections of the avian brain, obtaining new and interesting results. If we compare the brain of a raven (*Corvus corax*) and the brain of a rhesus macaque (*Macaca mulatta*) we would notice that the primate's brain weighs more than six times the birds' brain (Rose, 2022). This could be explained by the differences in the sizes of the animals, but if we compare the number of neurons, the ratio halves, with crows showing a higher number of neurons. This is remarkable because a higher density in neurons (in a smaller brain) implies neurons connected with shorter distances, hence increased processing speed (*ibidem*).

Before the 20th century, the avian brain was thought to be mostly a hypertrophied striatum because when investigating birds' telencephalon, no layering of cells was found (expecting a division similar to the mammals' telencephalon: an outer region with six layers of cell called neocortex and an inner region called basal ganglia), if not only a region apparently similar to mammals' basal ganglia (Reiner, 2005). It is known now that the avian telencephalon is not organised in laminar architecture as mammals' cortex (Karten and Shimizu, 1989), it is in fact pallilal (pallium in mammals corresponds to hippocampus, cortex and parts of the amygdala), with a nuclear organisation. Even though this distinction reflects a very evident difference in the anatomical look, since birds do not show any separation between white and gray matter as mammalians do, recent studies, found some similarities allowing the assumption that the avian pallium and the mammalian pallium could be homologous (Rose, 2022). The avian pallium is composed by the Wulst (also labelled hyperpallium) and the dorsal ventricular ridge (DVR). The DVR is divided then into mesopallium, nidopallium and arcopallium (*ibidem*). A particular section of the posterior pallium: the *nidopallium caudolaterale* (NCL) has been found to be the counterpart of the prefrontal cortex (PFC) of the mammalians, and its associated with neural activity regarding categories, numerosity, reward, and working memory (Waldmann and Güntürkün, 1993). Thinking of the evolutionary distances between this species, this is a remarkable finding.

Another difference between birds and mammalians that is particularly evident is the absence of the *corpus callosum* in the avian brain. This lack could play a role in the shaping of lateralization, since the presence of this commissure in mammalians' brain is the key factor that allows one hemisphere the inhibition of its homologue (Denenberg, 1981). However, other smaller commissures proved to fulfil similar functions in birds. In the midbrain of the domestic chick (*Gallus gallus*) there are the Tectal and the Posterior commissures in the Thalamus, and also an anterior commissure, which seem to be involved in the suppression of lateralization (Parsons and Rogers, 1993). Going back to similarities between the avian and mammalian species, it was observed that in both visual systems, the two main projections are the thalamofugal (lemnothalamic) and the tectofugal (collotalamic) (Butler and Hodos, 2005). In addition, only the birds also possess a centrifugal system. In birds, the tectofugal pathways from the retina goes to the optic tectum and to the nucleus rutundus (thalamus), reaching the entopallium (telencephalic recipient) while the thalamofugal pathway runs from the retina to the visual Wulst of the telencephalon (hyperpallum) through the nucleus opticus principalis thalami (OPT) (Bischof and Watanabe, 1997) (see Figure 1.1).

Thanks to the study of Boxer and Stanford, a first report on asymmetries in the thalamofugal visual projection in chicks was obtained in 1985. Researchers using a detection label (horseradish peroxidase) found more contralateral projections from the left side of the thalamus to the right hyperpallial region of the forebrain than vice versa (Boxer and Stanford, 1985). These results were confirmed four years later, by Andrew and Rogers, using injections of dye as a flow tracer (Andrew and Rogers, 1989). Thanks to this technique, in another study it was also found that brain lateralization is stronger in male chicks rather than in females (Rajendra and Rogers, 1993).

Subsequently, Rogers and Bolden found corresponding results, but this time using chicks that were incubated in different light conditions. Their outcome shows the same asymmetry in the thalamofugal visual projection ascending from the thalamus to the visual hyperpallium, as previous studies, in chicks incubated in light condition, but when the same investigation was conducted on a group of chicks incubated or reared in dark condition, this asymmetry was absent (Rogers and Bolde, 1991). No asymmetries, instead, were found in the tectofugal pathway in following studies on the chicks (Rogers and Deng, 1999; Rogers and Sink, 1988).

Overall, it appears that in domestic chicks, the right hemisphere receives (more) visual inputs from both eyes through the thalamofugal system, while the left hemisphere, through the same pathway, receives visual input only from the right eye. These differences are thought to be in line with the hemispheric differences showed in cognition tasks (Rogers, 1996), and that this lateralization is linked to the influence of light during incubation (see next section) (Rogers and Deng 1999).

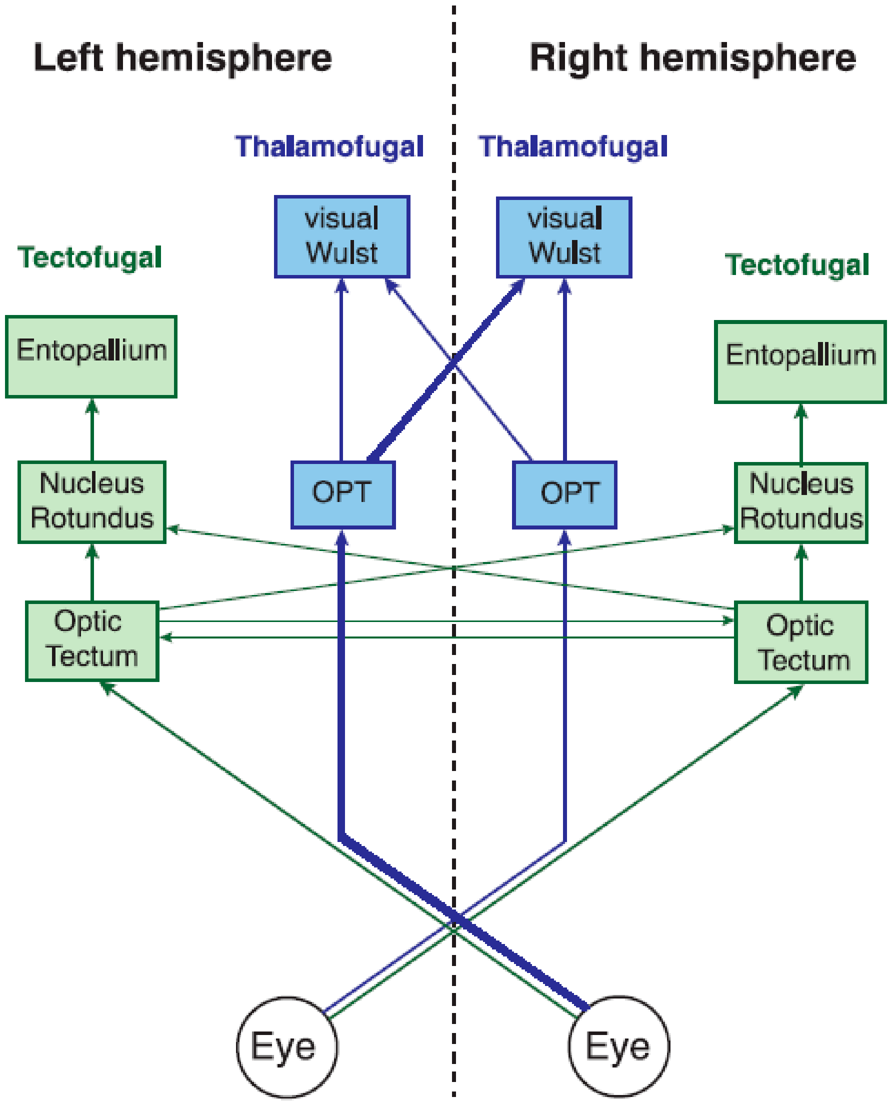

Figure 1.1 *Graphic representation of the organisation of both thalamofugal pathway (represented in blue) and tectofugal pathway (represented in green), composing the visual systems in the avian brain. OPT= nucleus opticus principalis thalami. The width of the lines has been marked as to indicate the ''number'' of projections, to enlighten the lateralization that occurs after light stimulation in the chicks' thalamofugal pathway. In chicks hatched in light conditions, there is an increment of projections from the left OPT to the right Wulst, that receives information from both eyes compared to the left hemisphere. No asymmetries were found in the tectofugal pathway in chicks. (Picture adapted from Morandi-Raikova et al., 2021).*

1.3.2 How and why lateralization emerges

One of the most important findings in the study of lateralization was the discovery made by Rogers concerning how lateralization emerges in the domestic chick (*Gallus gallus*). In 1982 indeed, the researchers found that if the chicks' eggs are exposed to light during the last three days of incubation there are crucial effects on the development of the visual pathways. This happens because the embryo, during the last few days before hatching, is turned in such a way that its left eye is occluded by its own body, while the right eye remains next to the membrane of the yolk sac. As a consequence, light passing through the egg's shell can reach only the right eyelid (which is transparent) and therefore reach the retina (Rogers, 1982). Even a small amount of light stimulation (two hours) during the 19th, the 20th or the 21h day of incubation, has been observed to be sufficient to establish a lateralization for some behaviours, like visual-category learning or control of copulation and attack (*ibidem*). Any light stimulation prior to the sensitive period of the last three days of incubation does not have any effect on lateralization (Zappia and Rogers, 1983). Moreover, during the sensitive period for light stimulation, the chicks' tectofugal pathway has already completed the development, while the thalamofugal pathway is still developing, this could be the reason why it is the latter that shows the asymmetries (Deng and Rogers, 1997). Almost every chick hatched in light condition shows this pattern of lateralization because the posture inside the egg while the embryo develops is encoded in the genes. Additionally, whenever manipulating the chicks before hatching so that the left eye is the one exposed to light, new-borns show a reversed asymmetry (Rogers, 1990). Chicks incubated in absence of light stimulation, i.e. in dark conditions, are not lateralized, more fearful and form weaker social hierarchies (Rogers, 2008). The discovery of the role of light exposure for the development of lateralization in domestic chick embryos revolutionised this research field. A huge number of findings followed and confirmed these first results. Nowadays, it is well established that lateralization is ubiquitous in the animal kingdom. Several animal species showed some hemispheric specialisations, like invertebrates (Fresnelli *et al*., 2012), primates (Petersen, 1984), octopuses (Frasnelli *et al*., 2019), rats (Denenberg, 1981; Glick, 1973), fishes, reptiles and amphibians (Bisazza *et al.,* 1998), and as a consequence, a question arise: Why do hemispheres develop different functions?

Since lateralization is so widespread, it is possible to posit that it plays a role for animals’ fitness. Different studies have shown that having a lateralized brain could be advantageous; for example, lateralized pigeons find more grains among pebbles (Güntürkün *et al.*, 2000), and chimps which exhibit and hand-preference catch more termites (Mcgrew and Marchant, 1999). Could lateralization be beneficial because selecting preferentially one limb/side increases speed and accuracy? It has also been hypothesised that lateralization is advantageous because it enhances the capacity to perform two tasks simultaneously, reducing parallel and redundant processing by both hemispheres (Güntürkün *et al.*, 2020). A study that supports this last hypothesis was that conducted by Rogers and colleagues in 2004. The researchers tested two groups of chicks, one composed by chicks incubated in light conditions and the other composed by chicks incubated in dark conditions. Both were tested in a dual task: pecking for grains among pebbles on the ground while being vigilant for a model predator moving over the cage. Aware that, light exposure produces a left-eye (right-hemisphere processing) advantage in chicks in detecting an overhead predator, and that discriminating food involves functions of the left visual wulst, the expectations were that lateralized chicks could use both hemispheres at the same time and perform better than dark incubated chicks. The results confirm the hypothesis, showing a worse performance by dark incubated chicks that were less attentive and vigilant and also more affected by the presence of the predator than light incubated chicks (Rogers *et al.*, 2004). A similar outcome was found in a study on goldbellies (*Girandinus falcatus*): when the predator was present, lateralized fishes were able to catch food two times faster than non-lateralized fishes. Moreover, the former used one eye for each task, whereas the latter switched between tasks using both eyes simultaneously (Dadda and Bisazza, 2006).

These results show that being lateralized has benefits, hence lateralization might have evolved because, exploiting the possibility of carrying out a different task with each hemisphere, individuals enhanced their survival.

However, still little is known about lateralization, its phylogenesis and ontogenesis, and further research needs to be conducted.

2

Objectives

Years of research in comparative numerical cognition field provided a strong background literature that allowed to attest chicks' ability in ordinal tasks. Namely, chicks have been proven to be able to identify a certain target location based on its position into a sagittally oriented series of other possible and identical locations associated. Moreover, a parallel has been serendipitously observed between humans (Jewell and McCourt, 2000; Regolin, 2006) and chicks' behaviour: the presence of a leftward bias while performing a generalization of the ordinal task (Rugani *et al.*, 2007, 2010, 2011, 2016, but see more in *Chapter 1.2.2*) .

Why do chicks show this tendency? Why, among two correct choices, is the preferred one the one on the left?

An hypothesis is that this bias might be linked to the association between numbers and space (SNA) shown both in adults and new-born humans, as well as in other animal species (see *Chapter 1.1.1*), according to which numbers are abstractly displayed along a "Mental Number Line" oriented from left to right. To investigate this link between numbers and space, Rugani and colleagues (2011) ran a series of ordinal experiments. During both training and test sessions, to explore the supposed role of spatial cues in affecting numerical information, the sources of spatial information were manipulated and/or disrupted. In fact, a lack of bias was observed by applying this kind of control, although the chicks continued to succeed in. More generally, results proved that the left-hemi-space bias is prevented each time the spatial information is made unavailable, suggesting both that this tendency has inherently a spatial nature and that numbers provide a natural and relevant cue. This left-to-right oriented bias has later been defined as a manifestation of a right hemisphere dominance in processing spatial information, that implies a selective allocation of attention towards the left hemi-field. Therefore, in 2016, Rugani and colleagues tested this possible explanation taking advantage of the almost complete decussation of optic chiasm fibres (Ocklenburg and Güntürkün, 2012; Weidner *et al.*, 1985) and the reduced interhemispheric communication characterizing avian brains (see *Chapter 1.3.1*) by restricting the visual input of a single eye (through the application of a temporary patch on the chick's eye). This monocular occlusion allowed the researcher to disentangle the role and processing of each hemisphere separately. The chicks tested under monocular left vision behaved like the chicks tested under binocular conditions, showing the same leftward bias, while both hemispheres were able to proficiently process and retrieve spatial and numerical information. Rugani and Regolin a few years later (2020) tested chicks in the same condition but added, at test, a mismatch between spatial and numerical information. The researcher expected the loss of the left bias while maintaining the correct completion of the task. In fact, a bilateral activation of the hemisphere was observed whenever a mismatch between the two sources of information was presented, resulting in a balanced propensity to start to "count" either from right to left and form left to right. This allows inferring that the leftward bias takes place solely when the chicks can rely on both spatial and numerical cues. Hence, in each case where there is an availability of the spatial information. Since 1989, this kind of information has been known to be processed in the forebrain by the right hemisphere (Rashid and Andrew, 1989). This hemisphere seems to take control of the chick's behaviour in these cases, leading to a preferential attentional allocation towards the left. Looking back at 1979, a similar expression for this "*sided-preference*" was observed by Rogers and Anson, who noticed a bias in chickens' responses to stimuli seen by the left eye. This preference seems to correlate with an asymmetry in the organization of the chicks' visual projections (Boxer and Standford, 1985). According to the authors' observations, the thalamofugal projections from the thalamus to the hyper-striatal region of birds' forebrain show some asymmetries than the tectofugal projections, and seem to guide the behaviour. This has been hypothesised to be a consequence of embryo head orientation during the development into the egg. It was discovered indeed, in 1997, that embryo's left eye is occluded by his body during the last days of incubation, while the right one remains oriented outwards, hence receiving light entering through the shell (Rogers, 1997). This exposure to light during the last days of incubation, seem to cause lateralization in male chicks (*ibidem*).

Could this light-dependent lateralization cause the left bias showed by chicks in ordinal performances?

At our Laboratory, in 2022, the effects of lateralization caused by light stimulation have been investigated through the comparison of ordinal performances between two groups of male chicks. One group hatched in light condition (hence a "strong lateralized group", *Li-chicks*) while the other was incubated in absence of light, i.e., in dark condition (hence with a lower degree of lateralization, *Di-chicks)*. At testing, moreover, the monocular-occlusion technique was implemented beside to binocular condition, expecting a better performance from the lateralized chicks and the loss of left bias in non-lateralized chicks. The results of this (unpublished) experiment are consistent with all previous studies in the literature and lay the foundations for the current study. In fact, Di-chicks correctly identified the correct target element, irrespective of the side. Li-chicks conversely, neglected the right correct response. However, it should be noted that the chicks could rely both on spatial and numerical information in this experiment. Therefore, it cannot be said on which one of the two sources of information the left-bias could depend on. Since up to now (1) the left-bias has been observed in ordinal tasks whenever both numerical and spatial information were available, and (2) when in other studies a conflict between them was created, there was never the control over the lateralization condition, the need to address those gaps was the rationale of this study. In order to demonstrate which one between numerosity or spatial information is the crucial parameter that drives chicks behaviour, it is fundamental to exclude spatial potentially confounding variables in the experimental design.

The purpose of the present research was to examine which factors could play a role into the spatial-numerical association shown in young domestic chicks (*Gallus gallus*) in the execution of ordinal tasks. Our research objectives indeed were to determine how brain lateralization affects chicks' performance and whether the absence of spatial information influences the processing of uniquely numerical and ordinal information. The relationship between brain lateralization and the accuracy in the accomplishing of the task has been assessed, taking advantage of the hemispherical asymmetry manipulation that can be carried out modulating the quantity of light that chicks in embryo receives in the last three days before hatching. Two groups differing in hatch conditions (Li-chicks and Di-chicks) were formed. Meanwhile, the numerical information impact on the ordinal task execution has been determined preventing the access to any kind of spatial cues while testing, thus allowing the chicks to respond solely on the basis of numerical cues. Moreover, to investigate the effect of embryonic light exposure on each hemisphere singly, both binocular and monocular behavioural responses have been observed.

This present work is part of a line of previous studies that have examined the spatial-numerical association abilities of chicks. These studies were carried out at the Department of General Psychology (DPG) of the University of Padua.

3

Methods and materials

All experimental procedures comply with national and European directives regarding the use of animals for research and have been approved by the Committee on Animal Welfare of the University of Padua, by the Ethics Committee of the University of Padua for Animal Experimentation and the Ministry of Health of the Italian Republic (Prot. N.9245, 17/01/2019).

3.1 Subjects

For this experiment 34 male broiler domestic chicks (*Gallus gallus)* of the Ross 308 line (Aviagen) were used, provided by a local hatchery (Incubatoio Società Agricola LA PELLEGRINA S.P.A., San Pietro in Giù, PD, and Italy). Males were selected over females, as we know from previous studies that the former seem to perform better in food-reinforced attention tests (Vallortigara *et al.*, 1990) and have a higher asymmetry of the thalamofugal visual projections (Rajendra and Rogers, 1993). The local hatchery delivered every three weeks, on Monday, both eggs and chicks. The chicks were only a few hours old, while the eggs could be on the 7th or 14th day of incubation. Chicks were sexed and caged, whereas egg were left finishing the hatching process into a FIEM incubator MG 70/100 cm (45cm long x 58cm deep x 43cm high) at a controlled temperature and humidity (37.5°C; 70-75%) at the Comparative Cognition Laboratory (Dept. Of General Psychology, University of Padova). This study comprised two experimental groups: strong-lateralized chicks and weak-lateralized chicks, thus a part of the eggs was incubated in light condition while the remaining in dark condition. Already born chicks and the ones hatched from eggs incubated in light condition composed the group with strong lateralization degree (Li-chicks) since light stimulation induces the typical cerebral asymmetry observed in chicks (Rogers, 1982) (*see Chapter 1.3*), whereas the weak-lateralized group was constituted by dark incubated chicks (Di-chicks).

3.2 Breeding Conditions

A few hours after hatching, all of the subjects were housed in metal cages inside a rearing room (temperature maintained between 29/31° and humidity level of 60%).

After being sexed by checking their wings' feathering, male chicks were caged in groups of 2 or 3 per cage. To allow individual identification, the chicks were marked with water soluble colours. All cages were standardised in size (28cm long x 32cm deep x 40cm high) and adjacent to one another. Each of them had an opening at the top to allow air circulation and permit a proper light condition, provided by neon lights (36W) placed above the cages (45cm from the ground, 15cm from the top of the cages) which followed an artificial light-dark cycle resembling the natural day-night sequence (light was on from 7.00am to 7.00pm; from 10.00pm to 12.00pm and again from 3.00am to 5.00am). The floor was covered by an absorbent paper substrate, whereon chicks could freely walk and peck. Food (grit) and water were provided *ad libitum* in transparent glass jars placed at the corners of the cages. Since the chicks were only a few hours old, to show them ''how to drink'' for the first time from the jars, the tip of their beak was gently dipped to touch the water. A similar procedure was followed with grains that were partially spread on the floor so that chicks could eat them following their natural tendency to peck small objects on the ground. Newborn chicks indeed, even within a few hours of hatching, are predisposed to peck at small objects (Murphy et. al, 2014) and develop very soon an efficient feeding behaviour on their own, receiving little help in feeding from parents in an ecological environment (Dawkins, 1968). Moreover, the chicks were taught how to approach with "dynamic" food, i.e. fed with mealworms (*Tenebrio molitor larvae*), to make them accustomed and become familiar with what would have been used as food reinforcement for the experimental procedure over the next few days. On Mondays, the chicks were also weighed for the first time, in order to compare their weight as newborns with a second measurement, which took place on Tuesdays, to ensure that they were eating enough and growing healthy. Similar conditions were maintained on the second day, after the second session and cage cleaning; they were fed mealworms and left with water and food without restriction overnight. The only difference was that on Tuesdays the chicks were fed mealworms presented inside a stimuli element (see Chapter *3.3.1*). This procedure allowed the chicks to get used to the stimuli and avoid scared or aggressive reactions towards the elements during training or testing sessions (Rugani and Regolin; 2020; 2021). On Wednesday, the experimental procedure began.

3.3 Experimental Setup

3.3.1 Arena & Stimuli

For this study, the apparatus consisted of a square-shaped (100cm long x 100cm deep x 400cm high) green polypropylene (Poliplak®) arena, which was used both for training and for testing phases (Fig.3.1). The apparatus was placed on an elevated surface allowing the experimenter an easy manipulation of the stimuli and moreover to avoid any experimenter cueing. Indeed, it is known in comparative studies that inadvertent cuing is a crucial factor to be kept under control (a striking example of this kind of methodological error while assessing animal cognition is *“The Clever Hans phenomenon*”, demonstrated by Pfungst (Pfungst, 1907)). Panels were 40cm high so that the experimenter could be hidden from chicks’ view while test took place. On the external part of two of the four panels composing the arena’s perimeter were located two specular boxes (15cm long x 15cm deep x 10cm high), one of which constituted the starting point. Chicks were placed in the “starting box” at the beginning of the training/test session and between each trial. The boxes and the arena were connected by a door which chicks could pass through only when the experimenter lifted a cardboard partition made out of the same arena’s material and colour. The area’s floor was covered with pine wood shavings. As stimuli, ten identical red plastic bottle tops were used (diameter: 3.0cm; height: 0.9cm). Only one (the fourth of the series) was then taught as target stimulus. The stimuli were placed along a line that ranged in length between four measures (40cm; 50cm; 55cm; 60cm) which were randomly changed throughout the experimental procedure at each trial. Distance between caps varied depending on the total line’s length, with a minimum distance of 2,8cm and a maximum of 5cm. When elements were sagittally-aligned relative to chick’s starting point, only the first cap had a fixed position which worked as a benchmark for the other caps, at 28cm distance from the entrance door and 50cm for the side panels. When the series was fronto-parallel-oriented the reference point for the distribution of the caps was the median of the total line length and the centre of the arena for the whole series placement. Before chicks’ entrance in the apparatus all the caps were filled with wood shaving (same used for covering the floor) so that each of them looked identical. When, during the procedure, the presence of food-reinforcement for the on-going trial was required, the mealworm was carefully hidden underneath wood shavings to maintain the same appearance between target cap and the other caps.

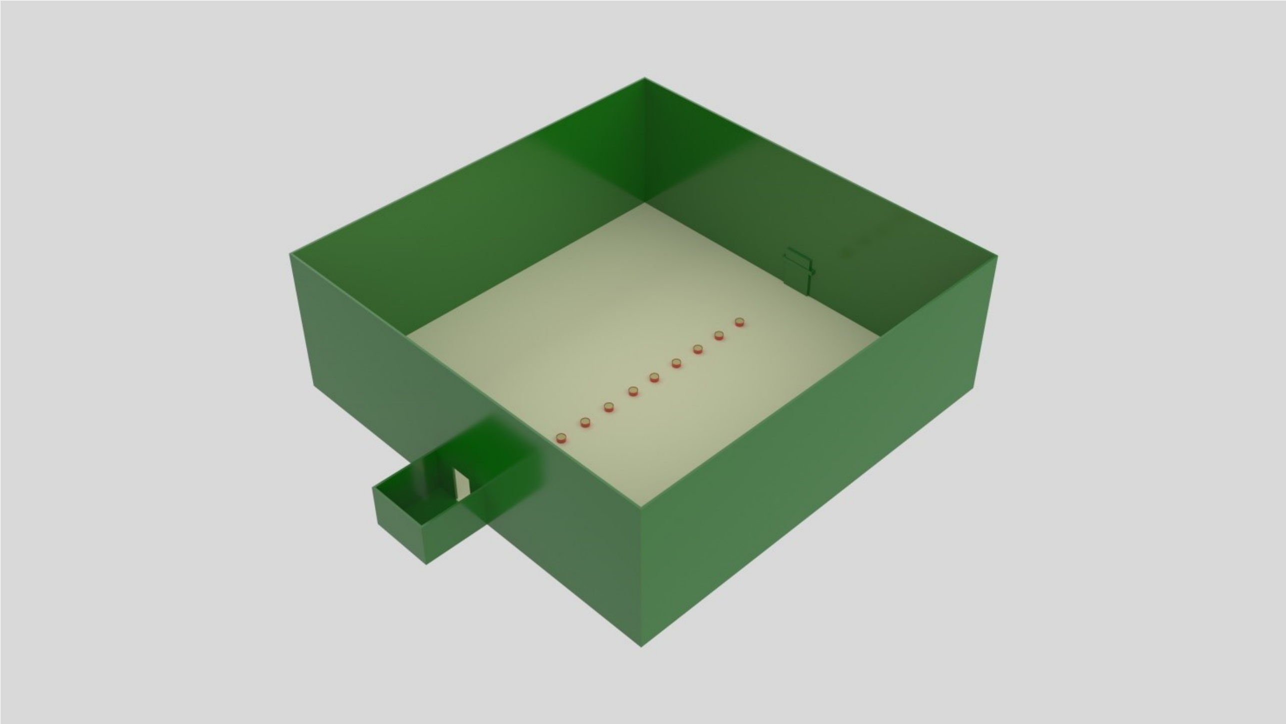

Figure 3.1 *Graphic representation of the experimental apparatus with a sagittally-oriented series of stimulus.*

3.4 Procedure

Data were collected through the observation and scoring of the chicks' behaviour while solving ordinal tasks. The experimental procedure was divided into a shaping/training session and four different testing sessions. Both phases took place in the same room, separated but close to the rearing room. The chicks were moved individually to the experimental room into a transportation cage, which consisted in a semi-transparent and opaque box (30.0cm long x 42.0cm deep x 21.0cm high) covered on the bottom with an absorbent paper layer. The experimental room was kept at fixed temperature and humidity levels (28°; 70%). Training phase took place when the chicks were three-days old, always happening on Wednesdays, since all of the them were born on Monday. Chicks then underwent testing phases on the fourth and fifth day of life. Before each experimental session, following previous paradigms used for testing chicks' ordinal performances (see Rugani *et al.*, 2007; 2016; Rugani and Regolin 2020; 2021), food jars were removed from the chicks' cages at least two hours prior to the session's beginning. Water instead was always available. This step is essential to avoid a sleepy or tired attitude (typically shown by chicks right after eating) while testing. Moreover it has been observed that the chicks were more motivated to look for food and consequently approached tasks with a sustained and active behaviour.

3.4.1 Shaping

At 8.00am on Wednesday the chicks were food deprived while still sharing the cage in couplets or triplets. At 10.00am the training session began, and one at the time the chicks were individually moved to the experimental room. Then an *habituation phase* started, during which the chick could freely walk around and inside the apparatus for a couple of minutes. Once acquainted with the environment, the subject was gently placed inside the starting box while stimuli were arranged and food reward was placed. When everything was set up, the experimenter lifted the sliding door thus allowing the chicks' passage. Chicks were trained to discriminate the fourth stimulus (cap) in a sagittally-oriented series of ten. For the very first trials a mealworm was placed upon the shavings filling the cap so as to be clearly visible for the chick as soon as entering the apparatus. In this way the chicks learned to express their choices by pecking the cap and getting food reward. A choice was considered valid if the peck was either inside or on caps' edge. Subjects had a maximum of 120 seconds from the moment they crossed the door to peck a cap, otherwise the trial was considered null. Whenever a cap was pecked, the trial ended and the chick was replaced into the starting box. Shaping consisted of two sessions of ten minutes, during each session distance between elements was fixed but around the fifth minute (mid-session) the whole apparatus was rotated into the experimental room to avoid external cues. During training every correct trial (peck on the fourth position) was food-reinforced, and mealworms were gradually hidden until a non-visible condition, forcing the chick to almost dig, pecking, into the cap to reach the recompense. After the shaping session n.1, the chicks were put back in the rearing room and underwent a 40 minute food-deprivation pause. From that moment on subjects were caged singularly. Individual caging is useful for avoiding separation stress during testing phases. Moreover, it prevents possible reciprocal influences and so a reduction of uncontrolled variables. After every chick underwent both shaping sessions a brief "qualitative" comment was pinned about each of them, in order to compare the first approach to the apparatus with the one of the following day. It could happen indeed, that some of them showed a disoriented or aggressive attitude, or a particular tendency to dig. The chicks that could not inhibit the attitude of digging, were too scared or impetuous also on the day after training, were discarded. After the second session the chicks were placed back into the rearing room with water and food *ad libitum* for the rest of the day and overnight.

3.4.2 Pre-training & Re-training

On Thursday, at 8.00am the chicks were food deprived, and at 10.00am singularly underwent a Pre-training session. This phase was considered over once the chicks reached a pre-established learning criterion of three consecutive correct trials (i.e. pecking the fourth cap) (Rugani *et al*., 2010; 2016; 2020; 2021). For the first preparation trials the mealworm was visible and then gradually hidden until totally covert. The correct trials were counted only when the mealworm was in a non-visible condition. This criterion was then repeated before each test session as a "Re-training" procedure. Depending on the chicks' characteristics, Re-training could last between 10 and 30 minutes. Chicks that took more than 35 minutes to pass the criterion were temporarily put back into the rearing room and let rest for a while, then re-trained. Chicks that never reached the criterion could not pass to the successive step. Re-training took place with the series oriented in a sagittal way, also before frontoparallel test.

3.4.3 Training

On Thursday, around 11.00am right after passing the Pre-training criterion, the chicks underwent a Training session. Subjects performed a 20-trials ordinal task in which the objective was to peck the target element (cap on the fourth position of a sagittally-oriented series of ten). Only correct pecks were reinforced. Food reinforcement was slightly reduced in dimensions (mealworms were cut in smaller pieces) to avoid early satiety and a motivation decrease since the high number of trials that the chicks would have faced by day. Maximum time allowed until considering the trial as null was 120 seconds. From training on, each peck was scored. The passing criterion was 8 correct pecks out of 20 trials (Rugani *et al.*; 2011). Chicks that did not reach the criterion in the first attempt could repeat the training again twice, otherwise were discarded. Chicks that reached at least 8 trials out of 20, furthered to the next experimental stage after 30 minutes. 34 chicks (18 Di-chicks; 16 Li-chicks) underwent the testing phase.

3.5 Testing Sessions

For this work, the testing phase was composed of four different tests. Each chick first underwent the Sagittal test and then the Frontoparallel test in three vision conditions (*Binocular Fronto-parallel; Right Monocular Fronto-parallel; Left Monocular Fronto-parallel*). So, after chicks were trained and learnt to peck the fourth position of a series (sagittally-oriented) of identical elements, were then required to generalise the correct response to an identical series but rotated by 90°(*Fronto-parallel* condition). Both groups (Light-hatched chicks and Dark-hatched chicks) underwent all of the experimental conditions so as to investigate the differences between different lateralization conditions. The same apparatus used for the Shaping and the Training sessions was also used for all the testing phases. Each test consisted of twenty trials, but unlike previous phases, the chicks could receive food reward (if pecking the correct position) only in some pre-established trials (*N.4, 5, 7, 10, 13, 14, 19*) to prevent a motivational drop in case of consecutive unrewarded trials (Rugani *et al.*, 2015b; 2016; Rugani and Regolin 2020; 2021). For the testing phases, a systematic manipulation of the position into the series was implemented in each trial (Rugani *et al.*, 2011. The distances varied in overall length of the array, so that even keeping the same elements, the spacings were of different width. These four measures were arbitrarily alternated for the twenty trials following four combinations (A, B, C, D). Each chick was randomly assigned a combination.

Modifying the array's length and distance between the target and the ends of the series (ratio between distances) means preventing the possibility of considering other cues, such as spatial information, in order to allow chicks to identify the target element only by its ordinal position.

3.5.1 Sagittal Test

On the morning of day 4 (Thursday), after two hours of food deprivation (8.00am-10.00am) the chicks entered the testing phase. A test session could take place only after the Re-training criterion (3 consecutive correct pecks) had been passed. Reaching the criterion could take from 10 to 20 minutes. The sagittal test, beginning immediately after it, lasted between 30 and 45 minutes, depending on the attitude (pecking a cap right after exiting the door/walking around the arena) and the time needed to arrange the array following the pre-established measure sequence throughout the 20 trials. Each trial lasted a maximum of 60 seconds, and in the case of no-peck within the minute, the trial was considered as null. For this test, the series was sagittally aligned with respect to the entering door (Fig. 3.2). The first element of the series was placed at a fixed distance of 28.0cm from the starting point and the whole sequence followed the median line of the arena's floor (50.0cm from both side panels). The position of the last cap, i.e. the end of the line, varied according to the series' total length: 32.0cm from the panel with the 40.0cm disposition; 22.0cm with the 50.0cm disposition; 17.0cm with the 55.0cm disposition; 12.0cm with the 60.0cm disposition. Respectively, distances between elements were: 2.8cm; 3.8cm; 4.4cm; 5.0cm. For each trial, the procedure of manipulation and, when needed, the placement of food-reward, took around 1 minute, during which the chick waited in the starting box behind the sliding partition for the new trial to start. Additionally, two supplementary procedures were implemented to prevent any external cue's influence. First, a random exchange of position was done between caps in order to avoid that any specific physical detail (a stain, a smell, shavings' shape) could catch chick's attention and drive its choice. Lastly, the apparatus was randomly rotated twice during the test to prevent any possibility of external spatial reference.

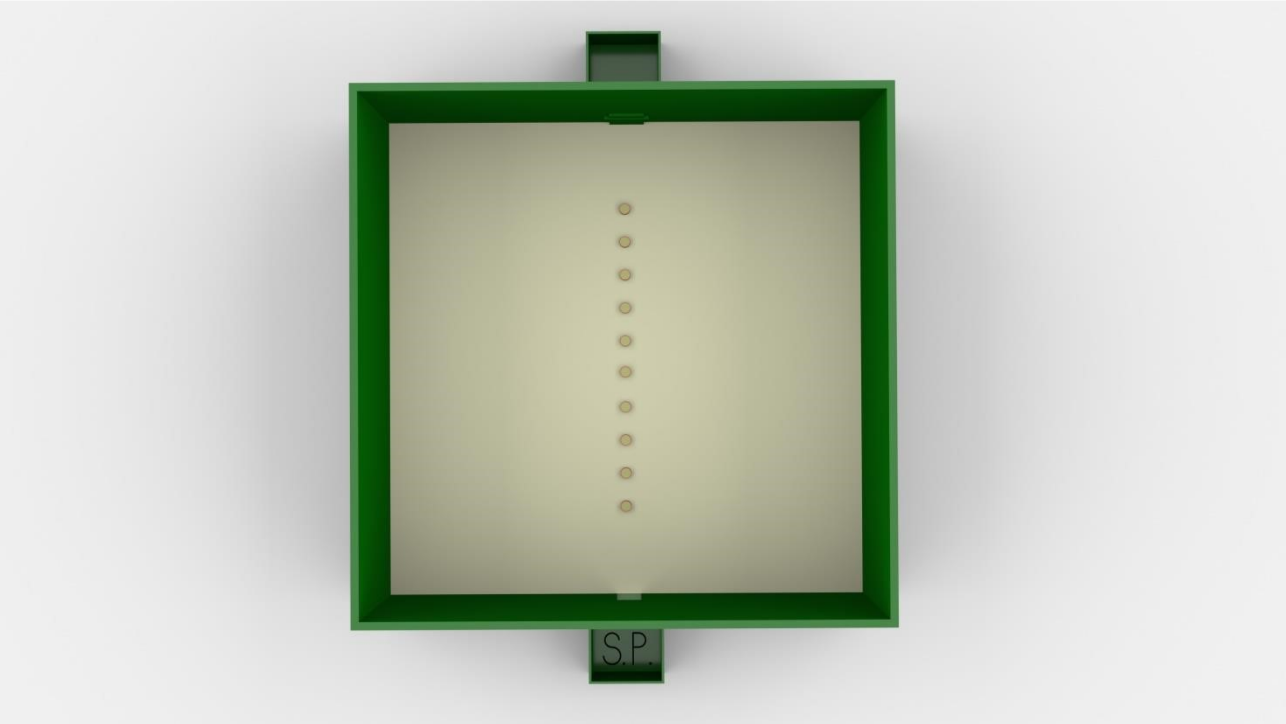

Figure 3.2 Graphic *Representation of the Arena with the series of target elements sagittally-oriented relative to the starting point (S.P.).*

3.5.2 Frontoparallel Tests

Between each test, the chicks were placed back in the cages in the rearing room for a one-hour break in a food-deprivation condition. Hence around 12:00am (Thursday) the chicks underwent the re-training and then started with the first Frontoparallel condition: the Binocular one. This test's procedure (Number of trials; Rewarded trials, Time-frame; Number of elements, Distances and measures manipulation) is identical to the sagittal one. The only variation is the rotation by 90° of the series (Fig. 3.3), which implies two possible correct responses: the fourth cap from the left (4L) and the fourth cap from the right (4R). Series' distance from the starting point coincides with the arena's centre (50,00cm). The position of the ends (the left and the right first cap) varied according to the length of the series, with a minimum of 20,00cm when the series was arranged at an overall length of 60,00cm and a maximum distance from the side panels of 30,00cm when the series was arranged at 40,00cm. After this test session, the chicks were put back in the rearing room and left resting until the next day. Food and water were available *ad libitum.* At 8.00am of day 5 (Friday) food jars were removed from the cages and after two hours wait, the chicks underwent the Re-training procedure. A monocular vision condition was provided for the last two Frontoparallel test designed for this study: Left Monocular (left eye in use- right hemisphere processing) and Right Monocular (right eye in use- left hemisphere processing). Although it has been previously observed that the order of the monocular test has no effect on the performances (Rugani *et al.*, 2016; Rugani and Regolin, 2020), chicks were randomly assigned the starting test when assigning the measures-combination. Monocular vision was made possible thanks to a temporary and non-invasive patching technique. The eye patch (specific and removable soft paper tape) was applied 10/15 minutes before beginning the test session so that the chicks could get acquainted with the new condition. Normal blinking was kept unaltered and a new patch was promptly provided in case chicks showed an itching reaction. Once the chick was calm and ready, the two Monocular tests were carried out following the same procedure as for the Binocular condition. As mentioned before, there was one hour (in food-deprivation condition) break in between and always a Re-retraining session before starting.

During testing, if any interference occurred (food reinforcement accidentally becomes visible, the chicks moves one stimulus digging or walking on it, the chicks gets scared or distracted by a big noise coming from the outside, eye patch falls) the trial was considered null, so the subject was put back into the starting box for a few seconds and the trial was re-started.

The position of the element pecked by the chick was scored after each trial. In addition to direct observation and hand-scoring, test trials were also scored by video-recordings taken with a digital camera (Sony HDR-CX240E) placed above the arena. The recordings were then scored by a blind observer.

When all the test phases were over, the chicks were put back into cages in triplets with water and food and in late afternoon were donated to local farmers.

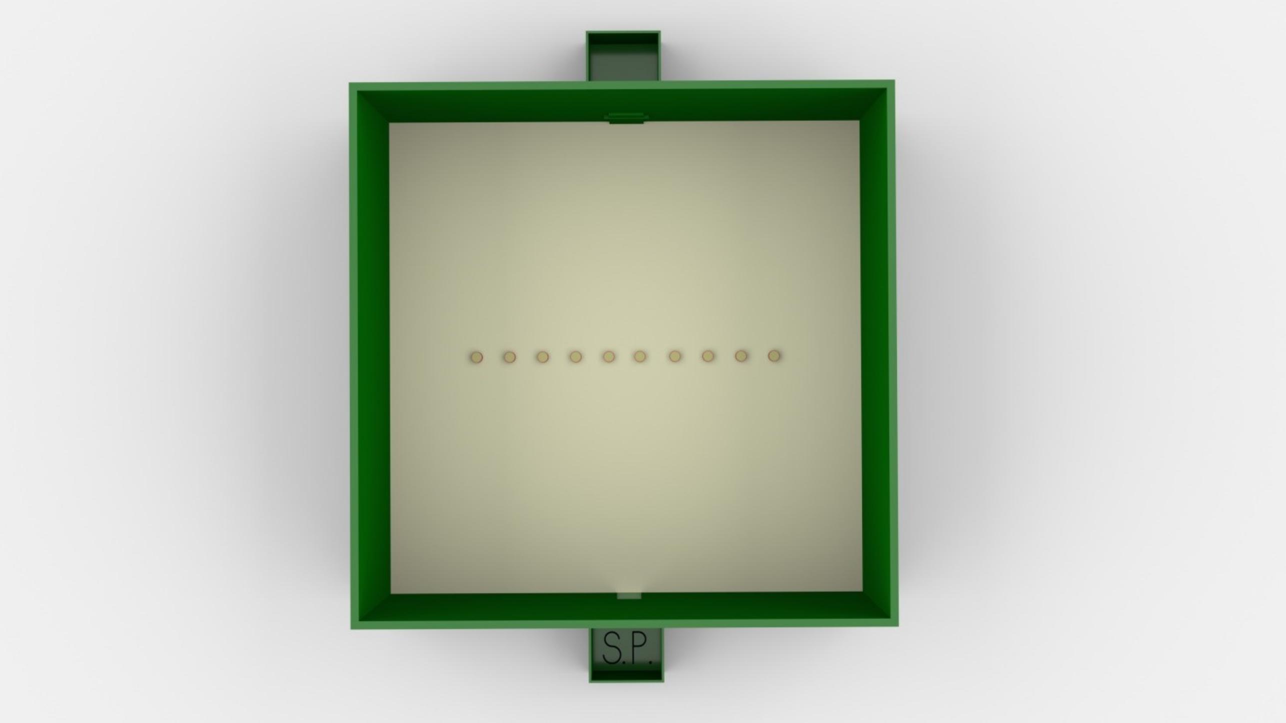

Figure 3.3 Graphic *Representation of the Arena with the series of target elements following a Frontoparallel orientation relative to the starting point (S.P.).*

4

Results

To assess the significance of departures from the chance level (10%), a one-sample Wilcoxon test was performed. For each trial of the four tests, the first peck towards any of the ten elements was scored and then through the formula: "*number of pecks to a given element/20 x 100",* the percentage was computed for each element separately. The analyses have been conducted using R 4.2.0. The effect size has been referred to as the rank-biserial correlation (r). For this experimental design, multiple regression (ANOVA Type III) has been conducted to predict the value of the percentage of correct choices (fourth cap) made by the chicks using the hatch condition and/or the choice side as factors and also to predict their interaction terms. Pairwise comparisons for interactions were adjusted using the Bonferroni correction. For the graphs and plots, the package GGplot2 3.4.1 was used.

4.1 Sagittal

4.1.1 Chance Level

Chicks hatched in light condition (*Li-chicks*) pecked at the fourth element above chance (n=16, mean=27.857, SE=8.526,p<0.001, r=0.883), also the first element (n=16, mean=15.972, SE=9.544, p=0.011, r=0.594) and the second element were pecked above chance (n=16, mean=14.078, SE=9.151,p=0.037 r=0.525). However, both the first and the second position were chosen less than the fourth position.

Chicks hatched in dark condition (*Di-chicks*) pecked at the fourth element above chance (n=18, mean=31.132, SE=16.625, p<0.001, r=0.852), also the first element (n=18, mean=16.368, SE=12.603, p=0.028, r=0.501) and the second element (n=18, mean=16.065, SE=10.257, p=0.008, r=0.585) were pecked above chance. However, the fourth element was chosen more than the third one, and the second element less than the third one.

All other elements were not selected above chance by both Li-chicks and Di-chicks. (*see Table. 4.1, in Appendix)*

4.1.2 Linear Regression

Linear regression was conducted on the percentage of correct choices (fourth element) as a function of the hatch condition (light, dark).

It was not found any significant effect of the hatch condition (estimate=-3.275, t=-0.709, p=0.484): Light incubated chicks (Li-chicks) have similar performances to dark incubated chicks (Di-chicks) as shown in *Figure 4.1.*

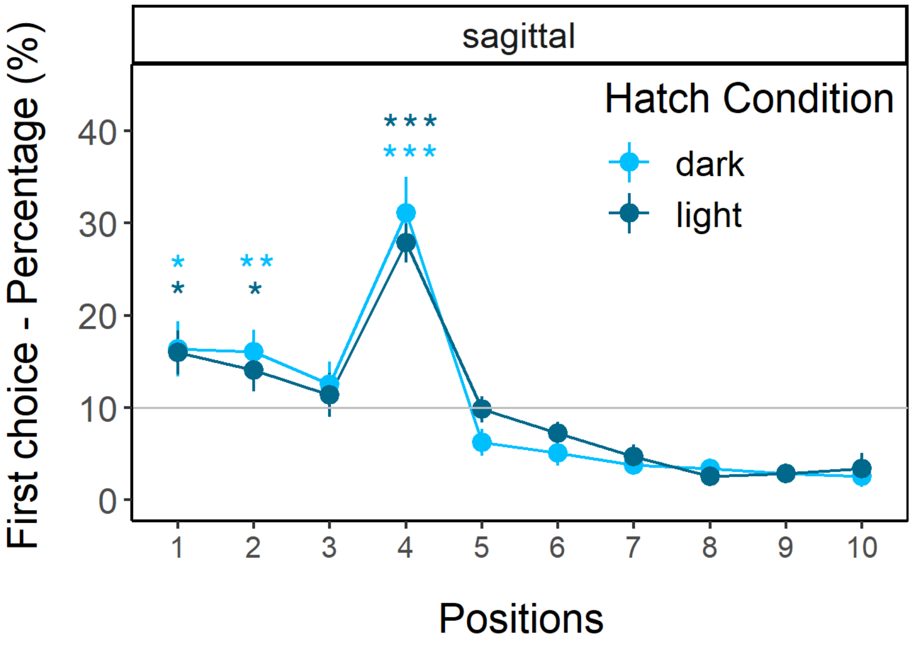

Figure 4.1. ***Sagittal test: Results.*** *The graphic represents the mean percentage of the subjects' choices (emitted pecks) to each element in the Sagittal Test. On the horizontal axis: element positions. On the ordinate axis: the percentage of each choice. The grey line represents the chance level (10%; Y=10). Asterisks correspond to the p-value levels: p<0.05 (*); p<0.01 (**); p<0.001 (***). Both Light and Dark chicks chose the fourth, the first and the second element above chance. No other significant effects were observed.*

4.2 Binocular Frontoparallel

4.2.1 Chance Level

The chicks hatched in light condition (Li-chicks) pecked at the fourth element above chance on both sides, respectively on the left side (4L) (n=16, mean=20.378, SE=11.899,p=0.033, r=0.735) and on the right side (4R) (n=16, mean=17.549, SE=9.116, p=0.003, r=0.73).

The chicks hatched in dark condition (Di-chicks) pecked at the fourth element above chance both on the left side: 4L (n=18, mean=31.132, SE=16.625, p<0.001, r=0.852) than on the right side: 4R (n=18, mean=16.368, SE=12.603, p=0.028, r=0.501). The chick also pecked at the fifth element (n=18, mean=16.065, SE=10.257, p=0.008, r=0.585) above chance.

All other elements were not selected above chance by both Li-chicks and Di-chicks (*see Table 4.1.*).

4.2.2 Multiple Linear Regression

A Multiple Linear regression was conducted on (1) the percentage of correct choices (fourth cap on the left side (4R); fourth cap on the right side (4R)) as a function of hatch condition (light, dark), (2) side of the choice (4L, 4R) and (3) the interaction between hatch condition and choices.

No main effect of hatch condition was observed (estimate=4.998, t=1.446, p=0.153): the overall performance of Li-chicks was similar to Di-chicks'one.

It was not found any significant effect between choices (estimate=4.693, t=1.399, P=0.167): The fourth right element was chosen just as much as the fourth element from the left.

The interaction between the two variables (hatch condition; choices) was not significant (estimate=-7.522, t=-1.538, p=0.129): the levels of one variable do not influence the other and *vice versa* (*see Figure 4.2*) .

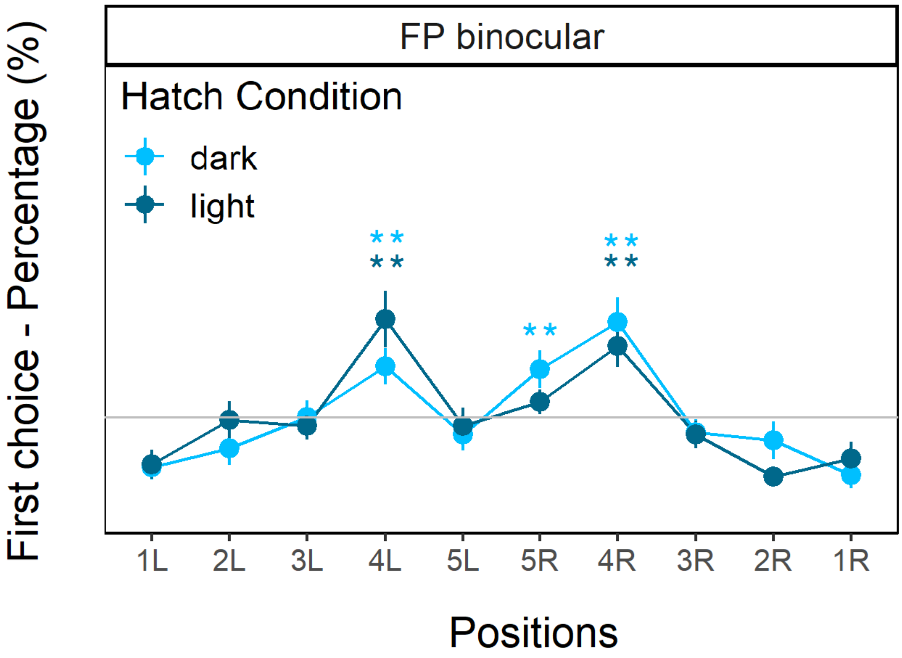

Figure 4.2. ***Frontoparallel Binocular test: Results.*** *The graphic represents the mean percentage of the subjects' choices (emitted pecks) to each element in the Frontoparallel Binocular Test. On the horizontal axis: element positions. On the ordinate axis: the percentage of each choice. The grey line represents the chance level (10%; Y=10). Asterisks correspond to the p-value levels: p<0.05 (*); p<0.01 (**); p<0.001 (***). Both Light and Dark chicks chose the fourth element from both sides (4R and 4L) above chance. Dark chicks also pecked the fifth element from the right side (5R) above chance. No other significant effects were observed.*

4.3 Left Monocular Frontoparallel

4.3.1 Chance Level

Chicks hatched in light condition (*Li-chicks*) pecked at the fourth element above chance on the left side: 4L (n=16, mean=18.877, SE=7.350,p<0.001, r=0.78) but not at the fourth on the right side (4R). Furthermore, the first element on the left was pecked above chance: 1L (n=16, mean=29.787, SE=9.593, p<0.001, r=0.882).

Chicks hatched in dark condition (*Di-chicks*) pecked at the fourth element above chance on the left side: 4L (n=18, mean=15.118, SE=9.730, p=0.021, r=0.51) but not on the right side (4R). First position on the left side: 1L (n=18, mean=26.718, SE=12.720, p=<0.001, r=0.846) second position on the left side: 2L (n=18, mean=18.982, SE=6.916, p<0.001, r=0.827) and third position on the left side 3L were also chosen above chance (n=18, mean=15.385, SE=6.963, p=0.004, r=0.698).

All other elements were not selected above chance by both Li-chicks and Di-chicks (*see Table 4.1*).

4.3.2 Multiple Linear Regression

The only main effect that has been found in the Left Monocular Frontoparallel test (left eye in use; right hemisphere) is in the choice preference (estimate=-9.545, t=-4.051, p<0.001): there is a significant preference for the element in the fourth position on the left side (4L) from both groups. While for the hatch condition (estimate=3.759, t=1.547, p=0.127) and for the interaction (estimate=-2.024, t=-0.589, p=0.558) no main effects were observed, consequently Li-chicks and Di-chicks had similar performances both oriented towards the left hemi-field (*see Figure 4.3*).

Since no differences between conditions have been observed, the overall average of the accuracy in the choices has been examined for the Left Monocular Frontoparallel Test for both the correct element on the left side (4L) (n=34, mean=16.887, sd=8.773, se=1.504) and on the right side (4R) (n=34; mean=6.390, sd=4.935, se=0.846). All others values reported in the *Table 4.2*

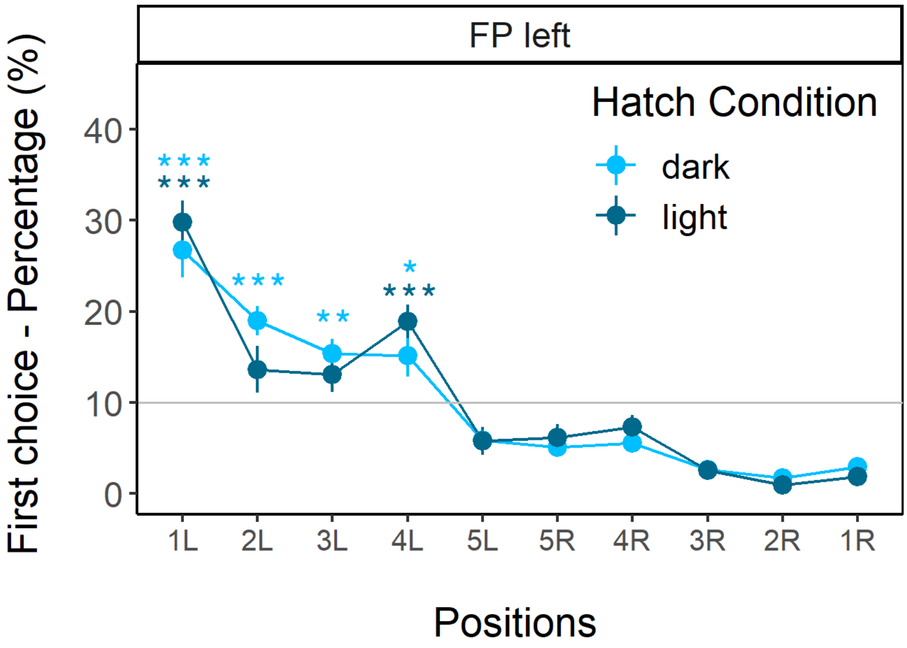

Figure 4.3. ***Frontoparallel Monocular Left test: Results.*** *The graphic represents the mean percentage of the subjects' choices (emitted pecks) to each element in the Frontoparallel Binocular Test. On the horizontal axis: element positions. On the ordinate axis: the percentage of each choice. The grey line represents the chance level (10%; Y=10). Asterisks correspond to the p-value levels: p<0.05 (*); p<0.01 (**); p<0.001 (***). Both Light and Dark chicks chose the fourth and the first element from left (4L;1L) above chance. Dark chicks also pecked the second and the third element from the left side (2L; 3L) above chance. No other significant effects were observed.*

4.4 Right Monocular Frontoparallel

4.4.1 Chance Level

Chicks hatched in light condition (Li-chicks) pecked at the fourth element above chance on the right side: 4R (n=16, mean=18.449, SE=10.268,p=0.002, r=0.807) but not at the fourth on the left side (4L). Also the first element on the right side was pecked above chance: 1R (n=16, mean=28.100, SE=13.743, p<0.001, r=0.829) so as the second element on the right side: 2R (n=16, mean=170256, SE=10.870, p=0.005, r=0.642). All other elements were not chosen above chance.

Chicks hatched in dark condition (Di-chicks) only pecked above chance at the first position on the right side: 1R (n=18, mean=33.865, SE=14.873, p=<0.001, r=0.846) all others element were not selected above chance (*see Table 4.1*).

4.4.2 Multiple Linear Regression

For the Right Monocular Frontoparallel condition any hatch condition effect (estimate=-3.701, t=-1.305, p=0.196) nor choice effect (estimate=4.519, t=1.643, p=0.105) were found. The interaction between the two variables instead resulted as significant (estimate=9.767, t=2.436, p<0.05). Indeed a post hoc analysis was conducted to investigate each factor and determinate the direction of the predictors.
The results showed that, in terms of choice, a significant effect resulted for the right-side correct response (4R) (estimate=-6.07, t.ratio=-2.140, p<0.05): the fourth cap from the right side was the significant choice, pecked more by Li-chicks than Di-chicks. Left-side correct choice (4L) did not show any main effect (estimate=3.70, t.ratio=1.305, p=0.196): Fourth cap from the left was not significantly chosen, even though it was pecked more by Di-chicks than Li-chicks.
In terms of hatch condition, a significant effect resulted for the Light Condition (estimate=-14.29, t.ratio=-4.897, p<0.0001): Li-chicks showed a strong preference for the fourth cap from the right (4R), meanwhile no significant effect was observed for the Dark condition (estimate=-4.52, t.ratio=-1.643, p=0.105): Di-chicks did not show any

significant difference in the side choice even thought 4R was chosen more than 4L. (*See Figure 4.4)*

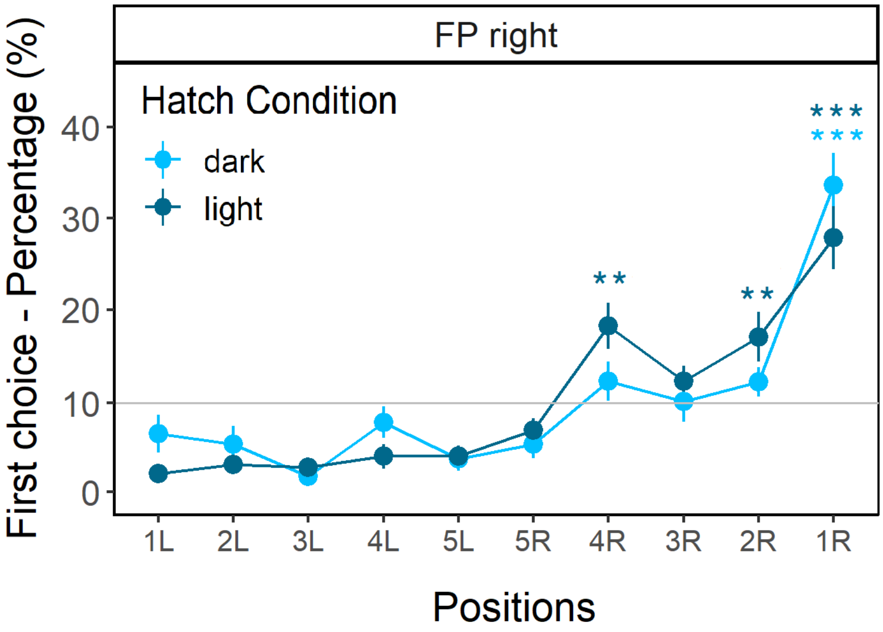

Figure 4.4. ***Frontoparallel Monocular Right test: Results.*** *The graphic represents the mean percentage of the subjects' choices (emitted pecks) to each element in the Frontoparallel Monocular Right Test. On the horizontal axis: element positions. On the ordinate axis: the percentage of each choice. The grey line represents the chance level (10%; Y=10). Asterisks correspond to the p-value levels: p<0.05 (*); p<0.01 (**); p<0.001 (***). Both Light and Dark chicks chose the first element from right (1R) above chance. Light chicks also pecked the second and the fourth element from the right side (2R; 4R) above chance. No other significant effects were observed except a strong preference for the right side from the Light chicks.*

5 Discussion

Through this experiments it was investigated whether and how embryonic light exposure could affect ordinal performances in newborns of domestic chicks (*Gallus gallus*) and how spatial information affects numerical information processing in both hemispheres of the chicks' brain.

At the sagittal test it was observed that the ordinal-numerical strategy for the identification of the target element is encoded, learned and it is used proficiently in a spatial arrangement of the elements that is similar to the training one, in which the chicks were binocularly trained to identify the fourth element in a series of ten, sagittally oriented. .These findings are consistent with previous studies (Rugani *et al.,* 2007, 2010, 2011, 2016; Rugani and Regolin 2020). Both groups though, beside the target element, pecked above chance also the first and the second element of the series. This outcome (pecking consistently at the first elements) has been observed also in the frontoparallel monocular conditions (but not in the binocular frontoparallel condition) by both groups. Such a tendency has already been observed in previous research involving chicks (Rugani *et al*., 2011, 2016; Rugani and Regolin, 2020), rats (Davis and Bradford, 1986; Suzuki and Kobayashi, 2000) and fish (Potrich *et al*., 2019). Possible explanations for this "attractivity" phenomenon are both (1) the saliency of the first element of a series of other identical elements and (2) the tendency of animals to search for food in the closest possible location (Davis and Bradford, 1986; Rugani *et al*., 2011, 2016). Furthermore, for the monocular condition, due to the possibility to see only half of the overall visual field, chicks' attention could be even more easily attracted by the perceptual salience of the first element of the series and consequently, relying uniquely on the processing of a single hemisphere, it is more difficult to inhibit. Beside the target, the first and the second element, the chicks incubated in light condition did not peck any other elements. For what concerns the not-lateralized group, beside the target and the first element, which were pecked in all of the four test conditions, also the third element (in the Frontoparallel Monocular Left condition) and the fifth element (in the Frontoparallel Binocular condition) were pecked above chance. This interesting outcome is consistent with observation of previous studies on ordinal tasks. Davis and Bradford in 1986 observed that rats, once identified the target element, anchored to the closest end of the series to “start to count” using a *working up* strategy (Davis and Bradford, 198), hence making more mistakes related to the element immediately preceding the target (this would explain the pecks on the third element). In 2000, Suzuki and Kobayashi extended David and Bradford’s task and noticed that rats could use also a *working down* strategy, anchoring to the last element of the series for identifying for example the sixth element (Suzuki and Kobayashi, 2000); this phenomenon was observed in previous study with chicks as well (Rugani *et al.,* 2007; Rugani and Regolin, 2020) and shows that this trend could occur in both directions, explaining then why in this study chicks might have mistaken the fifth element in binocular condition.

The tendency documented in previous studies to start to ‘‘count’’ from left to right (Rugani *et al.,* 2007, 2010, 2016) seems to be specific for the conditions in which an integrated use of both numerical and spatial information is permitted. Whenever this is not the case (Rugani *et al.,* 2011; Rugani and Regolin 2020) chicks choose equally both target elements (either from left than from right), proving that the bias is specifically related to the spatial information processing. In this study, by varying the distances between the elements of the series, this possibility was prevented. As expected from the literature, no side-bias was observed. The chicks performed the binocular task proficiently and without significant differences between hatch conditions, hence showing no dependency on eggs’ light exposure (the significant effects are illustrated in *Figures 4.1; 4.2; 4.3; 4.4*).

Also in both monocular conditions of vision light-incubated chicks performed proficiently and generalized at the target element located in their unobstructed hemifield. This shows that the bilateral representation of numerical processing acquired during binocular training can be represented and use independently by each hemisphere, as already observed in previous studies (Bugden *et al*., 2019; Piazza and Eger, 2016; Rugani *et al*., 2016; Rugani and Regolin, 2020).

An important consideration that has to be done is that, through an analysis on the overall possible choices (i.e. considering all of the positions) it was observed, at a group level, a pecking pattern much more spread out specifically by the chicks incubated in dark-condition than the light-incubated chicks (all results are summarised in *Table 4.1*). This implies that, even though chicks hatched from eggs incubated in the dark were able to rely on numerical cues, they pecked at other elements beside the target (the fifth element, as above-mentioned) more than the other group.

These data confirm previous results, showing that chicks hatched from eggs incubated in light condition successfully memorize the numerical information and learn to solve the task, pecking at the target element above chance level in all of the conditions with more accuracy. Almost similar findings emerge from non-lateralized chicks', which still show the completion of the task under left monocular condition, although an increased range of uncertainty in the spread of the response accuracy, underperforming uniquely under the right monocular condition. In fact, when facing the right frontoparallel monocular test, even if marginally so, the fourth element is not pecked above chance while most of the choices converge at the first element of the series. Summarising: both groups exhibit similar outcomes, showing no significant differences between hatch conditions, a part of the right monocular condition for dark-incubated chicks.

In a study conducted by Chiandetti in 2005, light-incubated chicks and dark-incubated chicks were trained to peck at small cones for food reinforcement under binocular and monocular conditions. Due to the observation of similar behaviour between both groups under binocular condition, but differentiating under the monocular one, it was hypothesised that the asymmetric light stimulation could play a role more on the interhemispheric communication rather than in the allocation of separate functions into each hemisphere (Chiandetti *et al.*, 2005). Although this hypothesis was made speculatively, looking at the outcome obtained in the present study, it is intriguing to suppose that an analogous hypothesis could also suit this case too. In fact, if the light stimulation strengthened the connection between the hemispheres, it would be explained why although both groups of chicks showed to learn the task effectively and in the same way at the binocular test, they indeed exhibit differences under monocular condition. As a matter of a fact, chicks with stronger cerebral connections due to the pre-hatching light simulation, after encoding numerical information in both hemispheres, perform accurately and commit less mistakes at monocular tasks. Chicks incubated in the absence of light, on the other hand, given the lower strength in the interhemispheric connections, would have no problem in performing the task in a binocular vision condition but would show less accuracy in monocular tasks (as observed in this research).

Altogether, this study proved that both chicks' hemispheres independently encode, process and successfully use numerical information to solve ordinal tasks. Moreover, the present results corroborate previous observations about the prevention of the left bias when removing the spatial information.

Although these outcomes are statistically significant and in line with all previous studies, it is necessary to specify that the hypotheses presented in this study are still of a speculative nature. It is also important to highlight a limit to the evidential values of the reported results. For this study 34 domestic chicks (*Gallus gallus*) were tested. Through the conduction of a power analysis, the sample size needed to reach the medium effect (α=0.05, power= 0.8, f=0.25) turned out to be 48 subjects. The number of subjects tested for this research did not reach the number required due to the spread of the avian influenza (HPAI) (fowl plague), which comported restriction in the availability of eggs. Reaching the required sample size could imply different outcomes and possibly lead to new interesting implications. Further investigation is therefore necessary.

6 Conclusions

Up to now, in the field of comparative numerical cognition, and more specifically, concerning the SNA in domestic chicks (*Gallus gallus*) with different degrees of lateralization, there was been no study that had investigated how numerical–ordinal information is processed by the left and right hemispheres singularly. This study proved that both chicks' hemispheres independently encode, process and successfully use numerical information to solve ordinal tasks, irrespective of the hatch condition. Moreover, the present results corroborate the previous literature on the SNA in young chicks, which stated the presence of a leftward bias whenever both spatial and ordinal information were present while performing ordinal tasks. In this study was observed, indeed, a prevention of the left bias by means of the removal of spatial information.

Overall, this research might represent a step closer to the unravelling of the origin of SNA, the ontogenesis of numerical knowledge and of the lateralization phenomenon; a goal that nevertheless has yet to be reached, making future studies to directly address these issues still necessary.

This work was carried out as part of a larger research project led by Rosa Rugani (Lucia Regolin and Yujia Zhang).

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Appendix

Table 4.1

	Test	Hatch_cond	Choice	Mean	sd	n	p	p.sig	r
1	FP binocular	dark	1L	4,751461988	5,580577061	18	0,998699052	ns	-0,769
2	FP binocular	dark	1R	3,903508772	6,079996023	18	0,99861745	ns	-0,742
3	FP binocular	dark	2L	6,710526316	7,487020979	18	0,982147506	ns	-0,553
4	FP binocular	dark	2R	7,543859649	8,488506928	18	0,955761284	ns	-0,446
5	FP binocular	dark	3L	10,04385965	7,26668488	18	0,468493666	ns	0,0262
6	FP binocular	dark	3R	8,377192982	5,995659991	18	0,878595454	ns	-0,305
7	FP binocular	dark	4L	15,38011696	8,027380136	18	0,007034133	**	0,601
8	FP binocular	dark	4R	20,07309942	10,88656218	18	0,001870783	**	0,783
9	FP binocular	dark	5L	8,143274853	6,987309093	18	0,844597712	ns	-0,254
10	FP binocular	dark	5R	15,07309942	8,559621128	18	0,007908247	**	0,707
11	FP binocular	light	1L	5,016447368	6,055658092	16	0,993454615	ns	-0,655
12	FP binocular	light	1R	5,625	7,041543391	16	0,984102117	ns	-0,585
13	FP binocular	light	2L	9,703947368	7,835965452	16	0,554094425	ns	-0,0273
14	FP binocular	light	2R	3,75	4,281744193	16	0,999352597	ns	-0,918
15	FP binocular	light	3L	9,095394737	5,843423867	16	0,835195197	ns	-0,28
16	FP binocular	light	3R	8,174342105	5,803387387	16	0,911760449	ns	-0,411
17	FP binocular	light	4L	20,37828947	11,89907107	16	0,003302143	**	0,735
18	FP binocular	light	4R	17,54934211	9,116420884	16	0,003515647	**	0,73
19	FP binocular	light	5L	9,095394737	7,581486386	16	0,703042087	ns	-0,138
20	FP binocular	light	5R	11,61184211	5,111747555	16	0,088987353	ns	0,419
21	FP left	dark	1L	26,71874403	12,72048219	18	0,000176576	***	0,846
22	FP left	dark	1R	2,946718649	4,681871702	18	0,999761643	ns	-0,818
23	FP left	dark	2L	18,9829148	6,916045561	18	0,000239797	***	0,827
24	FP left	dark	2R	1,743014945	3,175937114	18	0,999946613	ns	-0,907
25	FP left	dark	3L	15,38537247	6,96319756	18	0,00499211	**	0,698
26	FP left	dark	3R	2,605588044	3,755445789	18	0,999899379	ns	-0,872
27	FP left	dark	4L	15,11896571	9,730997556	18	0,021813666	*	0,51
28	FP left	dark	4R	5,57399763	4,7077338	18	0,997657968	ns	-0,723
29	FP left	dark	5L	5,842888812	6,123919601	18	0,994930674	ns	-0,618
30	FP left	dark	5R	5,081794901	4,282381506	18	0,99950339	ns	-0,842
31	FP left	light	1L	29,7871517	9,593060483	16	0,000230322	***	0,882
32	FP left	light	1R	1,891447368	4,045241325	16	0,999817411	ns	-0,885

33	FP left	light	2L	13,64647833	10,17843465	16	0,089021639	ns	0,369
34	FP left	light	2R	0,953947368	2,051675441	16	0,999921623	ns	-0,938
35	FP left	light	3L	13,07662539	7,633538015	16	0,059781779	ns	0,441
36	FP left	light	3R	2,532894737	4,104897645	16	0,999676625	ns	-0,845
37	FP left	light	4L	18,87770898	7,350262942	16	0,000989861	***	0,78
38	FP left	light	4R	7,308436533	5,174274903	16	0,961259158	ns	-0,462
39	FP left	light	5L	5,762383901	6,187272485	16	0,97829156	ns	-0,549
40	FP left	light	5R	6,162925696	5,8138779	16	0,977821289	ns	-0,511
41	FP right	dark	1L	6,629471964	8,798668924	18	0,981182456	ns	-0,497
42	FP right	dark	1R	33,8658363	14,83770147	18	0,000268583	***	0,846
43	FP right	dark	2L	5,489121087	8,396872156	18	0,987709283	ns	-0,538
44	FP right	dark	2R	12,31428926	6,963854922	18	0,060883439	ns	0,381
45	FP right	dark	3L	1,973684211	3,561935987	18	0,999927305	ns	-0,914
46	FP right	dark	3R	10,12727898	9,341559271	18	0,763745479	ns	-0,178
47	FP right	dark	4L	7,864039865	7,30852592	18	0,936707937	ns	-0,364
48	FP right	dark	4R	12,38320816	9,194610226	18	0,146252742	ns	0,279
49	FP right	dark	5L	3,837719298	5,444673732	18	0,999423285	ns	-0,805
50	FP right	dark	5R	5,515350877	6,707423932	18	0,995828031	ns	-0,633
51	FP right	light	1L	2,236842105	3,194235343	16	0,999814829	ns	-0,911
52	FP right	light	1R	28,1004902	13,74366706	16	0,0007284	***	0,829
53	FP right	light	2L	3,266038872	3,786709554	16	0,999714784	ns	-0,883
54	FP right	light	2R	17,25608445	10,87030438	16	0,005540961	**	0,642
55	FP right	light	3L	2,933436533	2,67985823	16	0,999833332	ns	-0,89
56	FP right	light	3R	12,40679825	6,610254457	16	0,068290002	ns	0,487
57	FP right	light	4L	4,163011696	5,357747323	16	0,997725186	ns	-0,726
58	FP right	light	4R	18,4494324	10,26868397	16	0,00203158	**	0,807
59	FP right	light	5L	4,144736842	4,689332897	16	0,998340764	ns	-0,775
60	FP right	light	5R	7,043128655	5,207523534	16	0,962881669	ns	-0,44
61	sagittal	dark	1	16,36852102	12,60357266	18	0,028241383	*	0,501
62	sagittal	dark	10	2,529239766	4,669402001	18	0,999868297	ns	-0,878
63	sagittal	dark	2	16,06587694	10,25701177	18	0,008484247	**	0,585
64	sagittal	dark	3	12,50499825	10,63069988	18	0,202800465	ns	0,214
65	sagittal	dark	4	31,13260359	16,62588691	18	0,000351222	***	0,852
66	sagittal	dark	5	6,245938921	6,188007268	18	0,980740883	ns	-0,51
67	sagittal	dark	6	5,103963613	5,855466641	18	0,993209054	ns	-0,61
68	sagittal	dark	7	3,80454341	4,580856462	18	0,999522106	ns	-0,82
69	sagittal	dark	8	3,40805718	4,272352881	18	0,999766047	ns	-0,896

70	sagittal	dark	9	2,83625731	4,0743813	18	0,999749708	ns	-0,816
71	sagittal	light	1	15,97232972	9,54473853	16	0,011641834	*	0,594
72	sagittal	light	10	3,4375	6,511208285	16	0,996324733	ns	-0,684
73	sagittal	light	2	14,07894737	9,151439548	16	0,037182101	*	0,525
74	sagittal	light	3	11,42027864	9,505011203	16	0,376401749	ns	0,109
75	sagittal	light	4	27,85797214	8,526456451	16	0,000346425	***	0,883
76	sagittal	light	5	9,830688854	5,644658448	16	0,579574408	ns	0,883
77	sagittal	light	6	7,259094427	4,790209993	16	0,96527515	ns	0,883
78	sagittal	light	7	4,6875	5,31311271	16	0,996661126	ns	0,883
79	sagittal	light	8	2,571594427	2,664499212	16	0,99984963	ns	0,883
80	sagittal	light	9	2,884094427	4,116753547	16	0,999650554	ns	0,883

Tab. 4.1. *Results of choices made by both groups, divided for the Hatch condition (Light/Dark chicks)in each test ordered alphabetically: Binocular Frontoparallel Test (FP binocular); Left Monocular Frontoparallel test (FP left); Right Monocular Frontoparallel test (FP Right); Sagittal test (sagittal). Highlighted, the rows corresponding to significant effects (choices above chance level (10%)).Choices in Sagittal test are labelled with the number corresponding to the position; Choices in the Fronto parallel tests are labelled with the number corresponding to the position and the letter that refers to the side (Left/Right)*

Table 4.2

	Test	**Choice**	**Mean**	**sd**	**n**	**se**
1	FP binocular	4L	17,732198	10,196387	34	1,748666
2	FP binocular	4R	18,885449	10,023279	34	1,718978
3	FP left	4L	16,887786	8,772959	34	1,50455
4	FP left	4R	6,390204	4,935479	34	0,846428
5	FP right	4L	6,12238	6,639314	34	1,138633
6	FP right	4R	15,237902	10,046245	34	1,722917
7	sagittal	4	29,591601	13,349022	34	2,289338

Tab. 4.2 *Results of choices made by all of the chicks (34) in each test, ordered alphabetically: Binocular Frontoparallel Test (FP binocular); Left Monocular Frontoparallel test (FP left); Right Monocular Frontoparallel test (FP Right); Sagittal test (sagittal), not considering the different Hatch Conditions. Choices in Sagittal test are labelled with the number corresponding to the position; Choices in the Frontoparallel tests are labelled with the number corresponding to the position and the letter that refers to the side (Left/Right)*