

Article

Lateralized Declarative-Like Memory for Conditional Spatial Information in Domestic Chicks (*Gallus gallus*)

Maria Loconsole ^{1,*}, Elena Mascalzoni ^{1,†}, Jonathan Niall Daisley ¹, Massimo De Agrò ^{1,2},
Giorgio Vallortigara ³ and Lucia Regolin ¹

¹ Department of General Psychology, University of Padua, 35131 Padua, Italy; elena.mascalzoni@gmail.com (E.M.); jndaisley@gmail.com (J.N.D.); massimo.de-agro@ur.de (M.D.A.); lucia.regolin@unipd.it (L.R.)

² Animal Comparative Economics Laboratory, Department of Zoology and Evolutionary Biology, University of Regensburg, 93053 Regensburg, Germany

³ Centre for Mind/Brain Sciences, University of Trento, 38068 Trento, Italy; giorgio.vallortigara@unitn.it

* Correspondence: maria.loconsole@phd.unipd.it

† M.L. and E.M. equally contributed to the paper.

Abstract: Declarative memory is an explicit, long-term memory system, used in generalization and categorization processes and to make inferences and to predict probable outcomes in novel situations. Animals have been proven to possess a similar declarative-like memory system. Here, we investigated declarative-like memory representations in young chicks, assessing the roles of the two hemispheres in memory recollection. Chicks were exposed for three consecutive days to two different arenas (blue/yellow), where they were presented with two panels, each depicting a different stimulus (cross/square). Only one of the two stimuli was rewarded, i.e., it hid a food reward. The position (left/right) of the rewarded stimulus remained constant within the same arena, but it differed between the two arenas (e.g., reward always on the left in the blue context and on the right in the yellow one). At test, both panels depicted the rewarded stimulus, thus chicks had to remember food position depending on the previously experienced contextual rule. Both binocular and right-eye monocularly-tested chicks correctly located the reward, whereas left-eye monocularly-tested chicks performed at the chance level. We showed that declarative-like memory of integrated information is available at early stages of development, and it is associated with a left hemisphere dominance.

Keywords: hemispheric dominance; brain lateralization; conditional-spatial memory; declarative-like memory; long-term memory system; domestic chick; *Gallus gallus*



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1. Introduction

One of the most intriguing, and at the same time debated, topics in animal cognition is how animals represent their surroundings and how these representations are created and stored in the brain [1–3]. Human studies have demonstrated that explicit—semantic—knowledge about the world is encoded within a dedicated memory storage system (i.e., declarative memory), the content of which can be consciously retrieved and described in propositional-symbolic-forms [4–6]. This knowledge can be used to make inferences, to generalize rules, to categorize new stimuli and events and, more generally, to predict possible outcomes in novel situations [4,7]. The presence of a similar declarative-like memory system in animals has been well attested in several models: e.g., in the chimpanzee [8], the rhesus monkey [9], the rat [10,11], the jay [12,13] and the domestic chicken [14,15]. However, little is known about how soon this mechanism is available during the individuals' development, and how information is represented and eventually stored in the memory system [16–18]. In their 2001 study, Cozzutti and Vallortigara demonstrated the presence of declarative-like memory in 5-day old domestic chicks, as evidence of the precocious development of this system. The authors exposed young chicks

to two feeders, located in two different spatial positions and containing two different food types. Chicks were then satiated with one of the two food types (devaluation phase). At test, subjects preferentially approached the feeder containing the non-devaluated food, thus showing the ability to remember the existence of the two different types of food and their different spatial locations. In addition, the authors found an effect of lateralization: binocular-tested chicks and right eye monocularly-tested chicks succeeded in the task, inspecting the location where the non-devaluated food had been experienced during the exposition phase. Chicks tested monocularly with their left eye only in use inspected both locations at the chance level. Domestic chicks have a virtually complete optic chiasm decussation, with each eye projecting exclusively to the contralateral hemisphere [19,20] and, given the lack of a corpus callosum and a reduced inter-hemispheric cross-over, this allows the study of brain lateralization by means of temporary monocular occlusion using eye-patching [21].

Having excluded all other variables (such as a lack of motivation or a failure in remembering the two different food types), the authors explained the drop in performance of left-eyed chicks as either an overall failure in the recollection of declarative-like memory or a failure in the integration of information. More precisely, under this condition the consequences of devaluation (i.e., satiation of food A) and the knowledge about each feeder content (i.e., food A is in container X, food B is in container Y) might not have been integrated, as they were originally encoded separately (the devaluation phase was separated from, and subsequent to, the exposure phase). Consequently, chicks might have failed in using the integrated representation to guide their behavior (i.e., go to container Y to get the non-devaluated food B). In their discussion, Cozzutti and Vallortigara pointed out the impossibility of their study to disentangle between these two explanations: on the one hand, there could be a left hemispheric specialization for retrieving declarative-like information; alternatively, chicks could have failed at test because they were unable to integrate two different memory representations in retrospect. In this latter case, poor performance of left-eyed chicks could have been due to an impairment of the right hemisphere in integrating information, rather than to a true lateralization for declarative-like memory. This would also be in line with previous evidence on pigeons that showed absence of lateralization in a simple color discrimination task, but a left-hemispheric dominance in the subsequent rule reversal, when a more demanding cognitive processing was required [22].

The aim of the present study was to better clarify the role of hemispheric specialization in declarative-like memory, and the nature of the stored representation. We designed a new paradigm, in which chicks were directly exposed to the conditional rule (i.e., if X go A and if Y go B). This way chicks experienced at once all the required information and could form an integrated memory already before the test. In this case, a lateralization effect as that originally found in Cozzutti and Vallortigara (2001) could only be ascribed to a left hemispheric specialization for declarative like-memory. *Vice-versa*, success in the task of both monocularly tested groups would suggest that the previously described lateralization effect [18] was due to an information integration defect rather than to declarative-like memory *per se*.

2. Materials and Methods

2.1. Subjects and Rearing Conditions

Two-hundred and nine (107 males and 102 females) chicks (*Gallus gallus domesticus*) were used in the present study, supplied by a local hatchery (Agricola Berica, Montegalda, Vicenza, Italy) as fertilized eggs. Eggs were incubated in the laboratory at a controlled temperature (37.5 °C) and humidity (55–66%). Three days before hatching, eggs were placed at a lower humidity and exposed to light; this procedure is known to positively affect neural and behavioral development in chicks [23,24]. In particular, light exposure of embryos seems to affect the development of hemispheric specialization (although some lateralization effects have also been reported in chicks hatched in darkness) [21,25].

On the day of hatching subjects were feather-sexed and singly housed in standard metal cages (28 cm × 32 cm × 40 cm), constantly lit by fluorescent lamps (36 W) placed 15 cm over the top of the cage (45 cm from the cage floor). A red plastic ball was hung in the centre of the cage at about 5 cm from the floor as a form of social enrichment. Chick starter food was scattered under the ball, while water was provided ad libitum in a transparent jar positioned by one of the sidewalls. The rearing room was maintained at a controlled temperature (30 °C) and humidity (68%).

Each chick was hatched in the laboratory and tested in the following days. By the end of their first week of life chicks were donated to local farmers. A minimum of 4 to a maximum of 10 chicks were tested weekly, depending on several technical factors (e.g., hatching rate, experimental schedule, chicks' motivation at test, etc.). In total, the study lasted about a year.

All experiments were conducted in accordance with the ethical guidelines indicated by the European and Italian laws.

2.2. Experimental Setup

We exposed chicks to two environmental contexts (either a blue or a yellow arena) in which they had to circumvent a panel depicting a geometrical shape (either a square or a cross) to find a food reward. The position (left/right) of the rewarded panel changed according to the context (e.g., always on the left in the blue arena and always on the right in the yellow one). At test, chicks were placed in one of the two arenas and presented on both sides with the rewarded stimulus. To correctly locate food, birds had to rely on an integrated memory for the spatial position of the rewarded stimulus in the two different contexts. Chicks were tested either in binocular or monocular (right-eye-in-use or left-eye-in-use) condition of vision, to assess any lateralization effect.

During rearing, chicks were exposed to two contexts: these were arenas identical to the metal rearing cage (28 cm × 32 cm × 40 cm), except one had all walls and floor lined with blue paper, the other was lined with yellow paper. In each case, two white panels (11 × 4 cm) were present: one with a black cross depicted on it and the other with a black square. The symbols were approximately of equal size (2 cm × 1.5 cm). The panels were positioned at adjacent corners of the colored contexts, each of them obscuring a food jar (not visible to the chick). Only one of the jars was filled with food (chick crumb), such that only one of the two presented shapes would become associated with the reward (S+). The S+ position changed according to the color of the arena (e.g., S+ was on the left in the blue arena and on the right in the yellow one, Figure 1). The S+ (i.e., either the square or the cross) and its position (left/right) were counterbalanced across subjects.

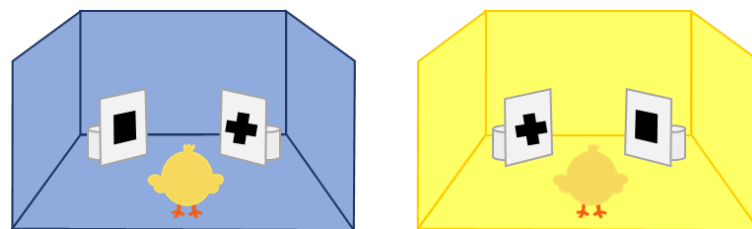


Figure 1. The two contexts (blue and yellow arenas) used for the exposure phase. Only one of the screens hid a jar containing food. Food would be consistently located depending on the color of the context (e.g., always behind the stimulus depicting a square, thus in the figure on the left in the blue arena and on the right in the yellow one). Jars looked identical and were not visible from the chick's starting point (shown in the picture).

2.3. Exposure Phase

The exposure phase lasted 3 days, from day 2 to day 4 of life. It took place in an experimental room adjacent to the rearing room, but acoustically isolated. The room was kept at a controlled temperature of 27–28 °C.

Each chick underwent the exposure phase individually. Each chick was left free to explore one of the two arenas (context 1) for two hours and was then moved to the other arena (context 2) for a further two hours, for a total of 4 h of exposure per day for the first 2 days (day 2 and day 3 post-hatch). On day 3 (day 4 post-hatch), the chick was exposed for only one hour to each context, for a total of 2 h. Each bird always experienced the S+ in the same spatial position in one context (e.g., S+ on the left in the blue arena) and on the opposite side in the other (e.g., S+ on the right in the yellow arena). With this procedure each chick associated only one stimulus with the reward, with its position varying depending on context color. For the remaining time chicks were placed back in their standard rearing cage with the red ball, and with food and water available ad libitum.

2.4. Eye-Patching

Before the test, chicks were randomly assigned to one of three experimental conditions: binocular condition of vision (BIN; $n = 71$, 37 males), where chicks were tested with both eyes uncovered; left-eye monocular condition (LE, $n = 71$; 38 males) in which the chick's right eye was covered and chicks could only use their left eye; right-eye monocular condition (RE; $n = 67$, 32 males) in which the chick's left eye was covered and chicks could only use their right eye.

Thirty minutes before testing, the eye of LE and RE chicks was temporarily occluded with a removable eye-patch. The eye-patch was handmade by the experimenter for each chick. It consisted of removable, disposable, paper tape, shaped like a cone so that, once applied on the chick's eye, it would occlude vision from that eye, without preventing normal blinking. The eye-patching procedure takes about 20 s and is harmless for the chick. At the end of the testing phase, the tape is gently removed with no impact on the chick's eyesight. Eye-patching is a well-established non-invasive procedure that allows the study of hemispheric specialization in birds without the need of any invasive procedure [21,26–28]. As in most avian species, chicks' optic chiasm has a virtually complete decussation, thus by occluding one eye it is possible to restrict input almost exclusively from the contralateral eye to the brain areas ipsilateral to the patched eye [18–20]. BIN chicks were handled for a similar amount of time to simulate the eye-patching procedure.

2.5. Test

After the three days of exposure (from day 2 to day 4), chicks were tested (on day 5). Testing took place in the same experimental room used for the exposure phase. For the testing session, only one of the two exposure arenas was used for each chick. In addition, at test, both panels showed the S+ image but no food reward was present, i.e., both jars behind panels showing the rewarded symbol (either square or cross) were empty (Figure 2). Water was always provided ad libitum in a glass jar on the cage wall opposite to the side where the two panels were located.

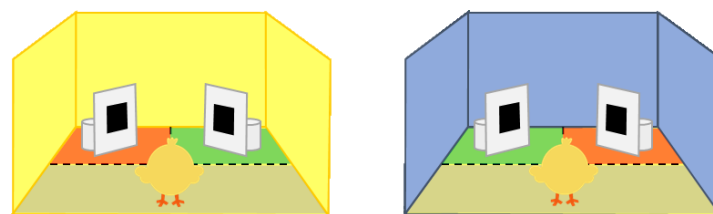


Figure 2. The testing apparatus. In this example the S+ corresponded to the square. The dotted lines delineate the three choice areas (colored for illustrative purposes) within the apparatus. The red and the green areas indicate the wrong-choice area and the correct-choice area, respectively. The front area (where the chick is positioned in the picture) corresponds to the non-choice area. As for the exposure phase, the jars looked identical and were not visible from the chick's starting point (shown in the picture). At test both jars were empty.

Subjects were placed in the testing arena and left for a total of six minutes, in line with previous studies on chicks that adopted a similar methodology [29,30] to explore their environment. The floor surface was divided virtually into three areas: a non-choice area (which included the chick's starting point) and two areas each comprising one of the panels (left or right). If the chick remained within the non-choice area for the whole duration of the test, its behavior was scored as absence of choice and the subject was not used in further data analysis. We recorded the total number of seconds in which chicks remained with their whole body within each choice area. We considered the preference for a panel as "correct" when it was consistent with the position of the S+ experienced by that chick in the same color context during the exposure phase.

2.6. Data Analysis

All data were analyzed in R 4.0.2 [31]. We measured the overall time spent by the subjects within each choice area, and used it as our dependent variable. We subsequently performed multiple nested linear mixed effect models with a Gaussian error structure, using the package lme4 [32] having subjects as the random intercept effect. Dependent variables were: stimulus value (correct or wrong choice area); condition (BIN, LE, and RE); position of the stimulus (left or right); color of the arena (blue or yellow); stimulus shape (cross or square); subject sex (male or female) and the interaction of all these factors. The individual subject was included in the model as the random effect. We then ran an Akaike information criterion (AIC) based model selection to choose the best fitting model, and thus the best predictors.

Subsequently, we ran a post-hoc analysis with Bonferroni correction on the selected model (R package emmeans [33]) to determine the direction of effect of all the predictors.

We also recorded, which of the two choice areas was first entered by the chicks. This was coded as binomial data, i.e., 1 for approaching the correct area and 0 for the incorrect area. We ran a generalized mixed effect model based on the binomial curve with the resulting model including the interaction between the BIN/RE/LE systems and the S+ position at exposure. A post-hoc multiple comparison analysis was carried out on the model output. Raw data collected during the experiment are available in Supplementary S3.

3. Results

The model that resulted with the lowest AIC value (4825.8), and the one that was consequently employed in the subsequent analysis was the one containing the predictors: stimulus value, condition, position, color and their interactions. Consistent with previous literature on similar tasks [18], the predictor "sex" did not appear in our final model, suggesting that there were no differences between males and females. We found no differences in terms of the overall time spent close to either stimulus in the three experimental conditions (ANOVA, Sum. Sq. = 10,330; Mean Sq. = 5165; $F = 0.912$; $p = 0.403$, suggesting that the experimental intervention did not affect the chicks' general levels of activity. The effects of the single predictors are available in Supplementary S1.

The full results of the post-hoc analysis is reported in Supplementary S2. Crucially, for the time spent near the two stimuli (correct or wrong choice area), we found an overall preference for the correct area over the incorrect one for both BIN (post-hoc analysis, estimated difference between the averages (est.) = 47.182; SE = 12.6; $p = 0.004$) and RE chicks (post-hoc analysis, est. = 57.05; SE = 13; $p = 0.0003$), but not for the LE chicks (post-hoc analysis, est. = -2.11; SE = 12.6; $p = 1$); refer Figure 3.

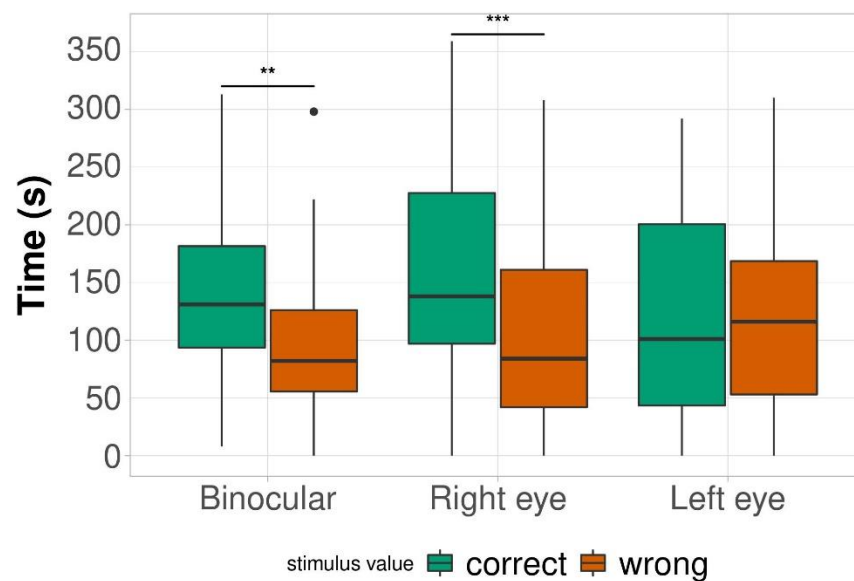


Figure 3. Average time (sec.) spent at test in the correct area (green) and in the incorrect area (red) in each condition (BIN, RE and LE). Whereas binocular and right eye chicks significantly spent longer time in the correct area, Left eye chicks behaved at the chance level. Asterisks indicate statistically significant contrasts: ** $p < 0.001$; *** $p < 0.0001$.

Since we found four different factors in the final model (Figure 4), post-hoc analysis for the full interaction has a low power, given the fact that they are based on only a few subjects each (Supplementary S2). For this reason, most of the effects should be interpreted with caution. However, it is interesting to note that LE chicks had a significant preference for the incorrect area when positioned on the right in the blue arena (post-hoc analysis, est. = -104.56 ; SE = 25.1 ; $p = 0.0007$).

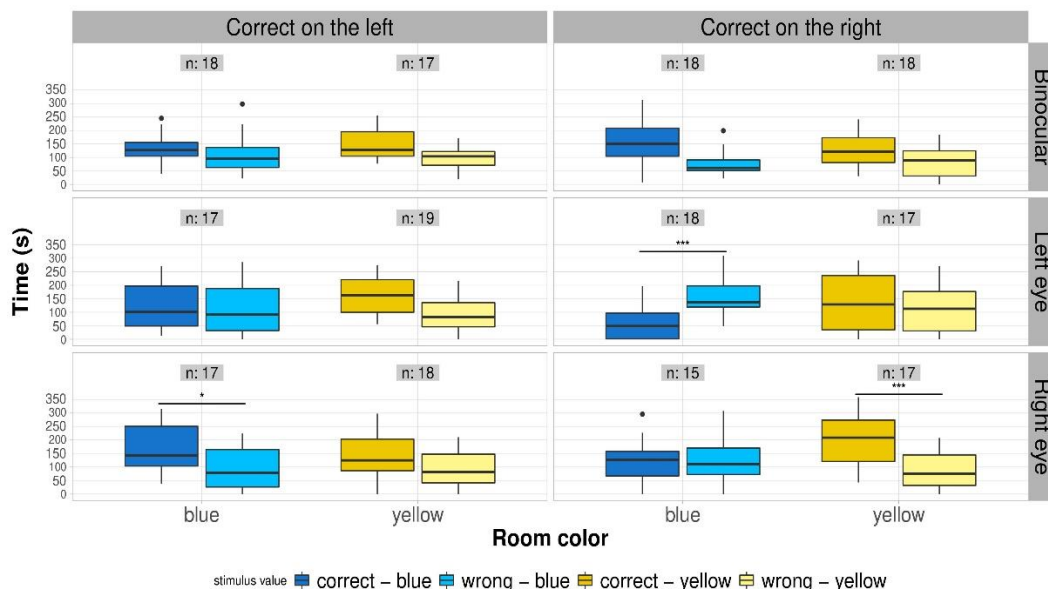


Figure 4. Time (sec.) spent in the correct choice area (dark blue and dark yellow) or incorrect choice area (light blue and light yellow) for each experimental condition separately illustrated for correct position (left/right) and color of the arena (blue/yellow). Dark blue indicates time spent in the correct choice area in the blue context; light blue indicates time spent in the incorrect choice area in the blue context. Dark yellow indicates time within the correct area in the yellow arena; light yellow indicates time spent within the incorrect area in the yellow arena. Asterisks indicate statistically significant contrasts: * $p < 0.05$; *** $p < 0.0001$.

Regarding which of the two stimuli was approached first, we found no preference in any of the three groups (exact binomial test. Binocular: $x = 33$, $n = 71$, $\text{prob.} = 0.465$, $p\text{-value} = 0.635$; left eye: $x = 36$, $n = 71$, $\text{prob.} = 0.507$, $p\text{-value} = 1$; right eye: $x = 40$, $n = 67$, $\text{prob.} = 0.597$, $p\text{-value} = 0.142$).

4. Discussion

Overall, the data presented in this study suggest that chicks are able to acquire and integrate information from the environment and to successfully retrieve it to solve a food-searching task, according to a spatial-conditional rule.

This finding is consistent with previous literature about declarative-like memory in animals (see Introduction) and, since chicks spontaneously encoded additional contextual information (i.e., panel position and color of the arena), it suggests a predisposition to form explicit integrated memories from experience even in the absence of formal training. This is not surprising, considering that domestic chicks are a precocial species, and are able to autonomously interact with the surrounding environment immediately after hatching [34]. This ability may have a different developmental trajectory in altricial species. Studies on human infants suggest an early development of declarative memory, which has been described in 6-month-old infants [35], however, at this age the memory system could be immature as the information encoded cannot be flexibly manipulated [36]. Further studies on different animal models are needed to better explore this possibility.

Our results further support the presence of long-term memory of past events in young chicks. Chicks successfully processed the additional contextual information about the characteristics of the context and the spatial location of the rewarded stimulus and integrated it so that they were subsequently able to remember the corresponding conditional rule (e.g., when the context is blue go left, when the context is yellow go right). Chicks had already been proven capable of acquiring relational rules [37] and integrated information [38], and to generalize information about the characteristics of a certain stimulus [39]. However, in all these cases chicks were tested following specific training [37,39] or in a paradigm that required the working memory system only, being all the required information already available in the testing environment [38]. Adult pigeons even failed or required 6-months extensive training to acquire (binocularly) a similar spatial conditional rule (i.e., geometric shape and up/down spatial position [40]). Conversely, in our study, we showed how chicks could exhibit similar cognitive capabilities (i.e., acquiring a conditional rule and integrating different types of information) even in the absence of dedicated training. They were also able to retain the information in a long-term memory system, rather than immediately use it in the present, meaning that they can create and store integrated representations of their experience.

Chicks successfully retrieved the conditional rule when they were tested in the binocular condition or in the monocular condition with their right eye in use, while they failed when tested monocularly with their left eye in use. This data complements the results of the original study from Cozzutti and Vallortigara (2001; see Introduction), deepening the nature of the stored declarative-like representation, and the role of the hemispheric specialization. Due to our experimental design, chicks were exposed to the conditional rule within one single event, therefore they could store it as a whole, already integrated, memory engram. If failure in the original study [18] had been due to a left hemispheric specialization for declarative-like memory, we would expect a similar impairment in monocularly tested LE chicks. On the other hand, if chicks' failure had depended on a defect in the integration of information acquired in two different moments, here we would have expected that both LE and RE chicks succeeded in the task. Our data support the first case scenario, suggesting that there is indeed a functional brain specialization of the left hemisphere in declarative-memory recollection.

This is consistent with literature that shows the presence of lateralization effects in the domestic chick and in other animal models [22,40–44]. Data from adult birds also support the idea of a lateralized system for storing task-contingency information, and for

its retrieval. Adult pigeons that learn a spatial conditional rule with both eyes in use fail in applying it when tested with the left eye only. Interestingly, reacquiring the rule in such monocular condition, requires an extremely long and extensive training, further suggesting the existence of a strong functional asymmetry [40].

The left hemisphere appears to be predominant in those tasks that require categorization of stimuli [45] (and thus declarative-like memory types of information), whereas the right hemisphere appears to take control in topographic spatial orientation [46] and in response to novelty [47]. Moreover, both right and left brain systems are engaged when encoding short term memory information about an imprinted object, though the left hemisphere is selectively responsible for the long term memory of the object in domestic chicks [48]. Such left hemisphere specialization for long-term memory storage could also play a key role in declarative-like memory tasks.

Additionally, human studies can offer some insights. Humans possess hemispheric specialization akin to domestic chicks [45,49]: humans too show higher right hemisphere activation in spatial and topographical tasks [50–52] and novelty processing [53] but higher left hemisphere activation in object categorization and familiarity recognition [54,55]. Moreover, the human brain is strongly left-lateralized for language production and comprehension [52,56]. A left lateralization similar to the one observed for human verbal language has also been found in deaf human subjects using American Sign Language to perform linguistic tasks [57,58], indicating a predominant role of the left hemisphere in semantic and/or symbolic representation regardless of a verbal linguistic aspect and this might also include the declarative memory system. In light of these evidences we can assume a general left hemisphere specialization for explicit knowledge about a certain category [4,45].

While the overall performance in the three experimental groups in the present study is consistent with data from the literature, and with our initial hypothesis, we found some unforeseen minor effects on chicks' performance. When S+ was on the right side in the blue arena LE chicks preferentially approached the stimulus in the incorrect choice area. This effect might be due to an attentional bias for the left hemisphere, which is also consistent with the trend for LE chicks to enter the correct area when S+ was on the left side in the yellow arena. However, it is not known why such a bias only emerged in these two scenarios. An alternative explanation might be in reference to the context colors: whereas chicks show a spontaneous preference for both the orange and the blue regions of the color spectrum [59,60] in some particular circumstances they can show an avoidance-like behavior to blue [61]. In the present study, we can hypothesize that there is some aversive reaction to the blue environment when it is being processed with the right hemisphere (LE chicks).

Interestingly, it has been shown that chicks show a lateralized behavior in an olfaction task when they are presented with a blue stimulus (i.e., a blue-colored bread crumb) but not when they are presented with a red stimulus [62]. Specifically, chicks that could only use their right nostril appeared to rely on both visual and olfactory information, while chicks that could use their left nostril only appeared to rely only on the visual characteristics of the stimulus. This suggests that the use of the left hemisphere might result in a better performance when the task requires integrated information, whereas the right hemisphere appears to preferentially recall single bits of information only (e.g., the color of the bread crumb).

In the present study, first choice was not predictive of general performance: in all cases, with chicks approaching both stimuli at chance level. It is possible that chicks initially reacted to the rewarded stimulus only. In fact, both panels at test depicted the rewarded stimulus: if this was the only information on which chicks were relying, it would have resulted in a random approach. However, as they did not find the food reward (importantly, at test no food was available to the subjects) they had to recall and subsequently rely on the declarative-like memory of their past experience. In doing so, chicks also retrieved the spatial conditional rule and could behave accordingly, i.e., when considering the entire test BIN and RE chicks succeeded in the task. Following this line of reasoning, we can

speculate that, even though chicks are able to create and to recall an integrated memory of additional contextual information, retrieving of those memories is not immediate, but only takes place when other, simpler (i.e., remembering just the shape of the rewarded stimulus) strategies demonstrated to be inefficient.

Overall, our results provide further evidence of declarative-like memory in domestic chicks together with its underlying mechanisms and along with a deeper analysis of the nature of the stored information. Chicks appeared to be capable of spontaneously encoding additional contextual information from different perceptual modalities, i.e., visual (the color of the context) and spatial/proprioceptive, i.e., the location of the rewarded stimulus in the arena. These were integrated into a long-term memory that could be recalled in order to answer a new environmental problem, in this specific case, when the initially acquired information was no longer reliable (i.e., when the rewarded stimulus was presented on both sides). Interestingly, the information that was initially learned binocularly, could be successfully retrieved, and used when chicks were tested with both eyes in use and with the right eye in use only, but not with the left eye. Given that birds have a virtually complete decussation of the optic nerve, and that the absence of a corpus callosum might restrict the interhemispheric transfer of information [40,63], our results suggest that long-term memories on conditional rules and integration of information are stored asymmetrically in the brain, with a left hemispheric specialization.

We believe that, given their precocious capability to extract additional non-necessary information and to integrate it into a dedicated long-term memory system, chicks constitute an optimal model for the investigation of the development of long-term memory systems in animals. Even though it is not possible to directly obtain information about animals' awareness of the self-experience, the capability to integrate multimodal information might be at the basis of an episodic-like memory. Further studies might deepen such possibility by implementing the present paradigm with the three episodic-like memory components of what–where–when [64]. Moreover, a deeper investigation of the lateralization of declarative-like memory is required. In fact, while we tested chicks that were exposed to light during egg incubation, it still unknown whether also dark-incubated chicks (thus, chicks with a less pronounced hemispheric specialization) would exhibit a similar behavior.

5. Conclusions

In the present study, 5-day old domestic chicks showed the capacity to spontaneously form integrated declarative-like memories of contextual, non-necessary information according to a conditional-spatial rule and to retrieve and use it to solve a food-searching task. This ability is strongly dependent on the left side of the brain, which is in accordance with the literature on brain lateralization in human and non-human species. Overall, these data show the presence of a precocious semantic non-verbal representation system in a non-human model.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/sym13050906/s1>, Supplementary results S1; Supplementary results S2; Raw data S3; Methods video S4.

Author Contributions: Conceptualization, E.M., J.N.D., G.V. and L.R.; methodology, E.M., J.N.D., G.V. and L.R.; validation, all authors; formal analysis, M.L. and M.D.A.; investigation, E.M. and J.N.D.; resources, L.R.; data curation, M.L., E.M., J.N.D. and M.D.A.; writing—original draft preparation, M.L.; writing—review and editing, all authors; visualization, M.L. and M.D.A.; supervision, G.V. and L.R.; project administration, L.R.; funding acquisition, L.R. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The experiments complied with all applicable national and European laws concerning the use of animals in research and were approved by the Italian National Ministry of Health (N.I.H.). All procedures employed in the experiments included in this study were examined and approved by the Ethical Committee of the University of Padova: Comitato Etico di Ateneo per la Sperimentazione Animale (C.E.A.S.A 07/11 I.C.S.), Prot. 09/2011.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data generated during this study are provided as supplementary material.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

BIN = binocular condition, chicks tested with both eyes in use; LE = left eye condition, chicks tested with the left eye in use, while the right eye is temporarily occluded with an eye-patch; RE = right eye condition, chicks tested with the right eye in use, while the left eye is patched.

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