

## RESEARCH ARTICLE

# Hierarchical processing of feature, egocentric and relational information for spatial orientation in domestic chicks

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## ABSTRACT

Animals can use different types of information for navigation. Domestic chicks (*Gallus gallus*) prefer to use local features as a beacon over spatial relational information. However, the role of egocentric navigation strategies is less understood. Here, we tested domestic chicks' egocentric and allocentric orientation abilities in a large circular arena. In experiment 1, we investigated whether domestic chicks possess a side bias during viewpoint-dependent egocentric orientation, revealing facilitation for targets on the chicks' left side. Experiment 2 showed that local features are preferred over viewpoint-dependent egocentric information when the two conflict. Lastly, in experiment 3, we found that in a situation where there is a choice between egocentric and allocentric spatial relational information provided by free-standing objects, chicks preferentially rely on egocentric information. We conclude that chicks orient according to a hierarchy of cues, in which the use of the visual appearance of an object is the dominant strategy, followed by viewpoint-dependent egocentric information and finally by spatial relational information.

**KEY WORDS:** Allocentric, Egocentric, *Gallus gallus*, Left-right asymmetry, Spatial orientation

## INTRODUCTION

Spatial orientation is essential for the survival of animals, allowing them to retrieve the location of food, home, mates, potential predators, etc. Spatial reference systems used for navigation have been classified into 'egocentric' and 'allocentric' (O'Keefe and Nadel, 1978; Paillard, 1991; Burgess, 2006). Egocentric (or self-centred) navigation involves information supplied by bodily cues originating from kinaesthetic inputs and motor commands and does not require visual or any other external information. This mechanism allows the navigator to distinguish left from right, up from down, and front from back in reference to its body axis. The kinaesthetic and motor output information also allows tracking of the distance and velocity of active movement in each direction. In contrast, allocentric (or world-centred) navigation is based on external (e.g. visual) information encoded independently from the body (Klatzky, 1998; Paillard, 1991; Allen, 2004; Burgess et al., 2004). An allocentric navigator can, for instance, orient based on different visual cues from the surrounding environment. This mechanism can analyse how visual cues are positioned in relation to


each other; local landmarks can be used as beacons, and visual scenes can be analysed through a snapshot-based strategy (Judd and Collett, 1998; Dawkins and Woodington, 2000). A defining feature of allocentric mechanisms is that they allow the animal to re-orient. After being disoriented (e.g. being spun around in different directions while deprived of visual stimulation), an animal can no longer find a goal location by relying exclusively on egocentric information and must re-orient based on external cues (for reviews, see Wang and Spelke, 2002; Burgess, 2006; Vallortigara, 2009). When visual information is integrated with egocentric information, we can speak of viewpoint-dependent egocentric orientation (Vallortigara and Zanforlin, 1986; Vallortigara et al., 1996; Nakajima et al., 2003; for a review, see Morandi-Raikova and Mayer, 2022).

Birds are extremely popular animal models for investigating spatial cognition because of their highly developed visual system, and also because of some species' homing and migratory behaviour (Egevang et al., 2010; Gagliardo, 2013; Mayer et al., 2013; Bingman, 2018; Mouritsen, 2018). Although the domestic chicken (*Gallus gallus*) is a non-migratory ground-living species, it has been used since the mid-1980s to study spatial orientation (for reviews, see Vallortigara et al., 2009; Tommasi et al., 2012; Morandi-Raikova and Mayer, 2022). Chicks can locate a goal using the visual appearance of an object as a beacon (Vallortigara, 1996; Chiandetti et al., 2005; Morandi-Raikova et al., 2020), using distance information (Vallortigara and Zanforlin, 1986; Chiesa et al., 2006), view-matching strategies ('snapshot memory') (Dawkins and Woodington, 2000; Pecchia and Vallortigara, 2010; but see Lee et al., 2012), the information provided by the geometrical shape of an enclosure (Vallortigara et al., 1990; Chiandetti et al., 2007, 2015; Mayer et al., 2016, 2018), as well as spatial relational information (Rashid and Andrew, 1989; Tommasi and Vallortigara, 2000; Morandi-Raikova et al., 2020). Finally, chicks can use viewpoint-dependent egocentric orientation (Vallortigara and Zanforlin, 1986; Vallortigara et al., 1996; Nakajima et al., 2003).

The ability to discriminate left from right is crucial for egocentric spatial orientation. Spontaneous side bias, i.e. facilitation for responses directed to the left versus to the right side of space, can impact egocentric orientation. Thus, studying side bias is crucial to fully understand how animals integrate egocentric and allocentric navigation mechanisms to orient in their environment. Domestic chicks have shown spontaneous side bias in a variety of tasks. For instance, in an adaptation of the classical cancellation tasks used in human studies, pigeons and chickens showed a left-side bias, preferentially foraging for grains on the left side of their body (Diekamp et al., 2005). Similar evidence of a leftward spatial bias was also obtained in a line-bisection task (Regolin, 2006) and ordinal numerical tasks (Rugani et al., 2010). More complex spatial biases have been reported in cardinal numerical cognition tasks. In these tests, chicks associate smaller numerosities with the left space and bigger numerosities with the right space (Rugani et al., 2014;

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Received 20 July 2023; Accepted 29 January 2024

2015; for a review, see Rugani and Rosa-Salva, 2021). These effects have a striking resemblance to the side biases found in humans in similar tasks (e.g. numerical tasks: Dehaene et al., 1993; Fischer and Shaki, 2014; cancellation tasks: Uttl and Pilketon-Taylor, 2001; Bottini and Toraldo, 2003; line bisection tasks: Bowers and Heilman, 1980; Jewell and McCourt, 2000). These asymmetries have often been ascribed to a right hemispheric superiority in controlling spatial attentional resources or, for the cardinal numerical tasks, to a differential role of the two sides of the brain in the perceived valence of changes in magnitude (Vallortigara, 2018).

Despite the ample evidence of behavioural and brain asymmetries in domestic chicks (for reviews, see: Vallortigara and Rogers, 2005; Rogers et al., 2013), the presence of side bias during egocentric orientation tasks has not been directly investigated yet. Indirect evidence, however, suggests the presence of an advantage for targets located in the left hemispace during viewpoint-dependent egocentric orientation. Chicks trained to peck at a central target had better performance if the non-rewarded stimulus was placed on the right rather than on the left (Vallortigara et al., 1988). However, this effect, interpreted as an advantage of the left-eye system for detecting stimuli at the periphery of the visual field, was limited to male chicks. Likewise, when chicks detour a centrally placed obstacle to reach a goal, they do it mostly from the left side (especially males; Vallortigara et al., 1999). This aligns with what we recently observed in a test of chicks' ability to orient by allocentric information (Morandi-Raikova et al., 2020). In that study, male chicks were trained to orient by local feature cues or spatial relational information. The animals' tracks consistently showed a leftward bias, regardless of the cue used to solve the spatial orientation task. Contradicting results have been reported too (Vallortigara et al., 1996). Chicks trained to discriminate between two identical white feeders showed learning facilitation when the rewarded feeder was located on the right side, suggesting the presence of an egocentric rightward bias. However, during this task, chicks tended to employ the left eye system to inspect the goal object. Moreover, when task-irrelevant colour cues were incorporated into the task, increasing its complexity, chicks showed a potential left-side bias (re-learning facilitation when the rewarded stimulus was on the left) (Vallortigara et al., 1996). Based on this set of studies, we hypothesised that a leftward bias should characterise chicks' egocentric viewpoint-dependent orientation.

Traditionally, behavioural lateralisation in chicks was thought to emerge as a result of the exposure of developing embryos to light (Rogers, 1982). Chick embryos are asymmetrically oriented inside the egg, with the left eye covered by the body and the right eye facing the eggshell (Kuo, 1932). If eggs are exposed to light during a critical period, the right eye system is selectively stimulated by the light passing through the eggshell. However, it has since been shown that some forms of lateralisation do not depend on light exposure (Mascetti and Vallortigara, 2001; Deng and Rogers, 2002; Rogers et al., 2004, 2013). This poses the question of whether other recurring side biases can also emerge in dark-incubated animals.

The first aim of the present study was, thus, to systematically investigate whether dark-incubated domestic chicks possess a side bias during viewpoint-dependent egocentric orientation, using a simple and highly controlled task. The second aim of this study was to test whether egocentric information would be preferred over spatial relational information or that provided by local features. While chicks can orient in relation to free-standing objects, they prefer to follow local object features rather than spatial relational information when the two are put in conflict (Morandi-Raikova et al., 2020; see Ferreira et al., 2022, for similar evidence in adult chickens). However, the

preferential use of egocentric versus different kinds of allocentric information has not been systematically assessed yet.

We performed three experiments: in the first experiment, we assessed whether chicks preferred to approach the feeder on the left or right side of their body, testing for side bias in egocentric orientation. In the second experiment, we studied whether chicks preferentially relied on egocentric information provided by their body axis or on the feeder's features when the two were in conflict. Lastly, in the third experiment, we investigated their preference for using egocentric or allocentric spatial relational information provided by free-standing objects.

## MATERIALS AND METHODS

### Ethical statement

All experiments were carried out in accordance with the ethical guidelines current to European and Italian laws. All experiments and experimental procedures were licensed by the Ethical Committee for animal welfare of the University of Trento (Organismo Preposto al Benessere Animale) and by the Ministero della Salute, Dipartimento Alimenti, Nutrizione e Sanità Pubblica Veterinaria (permit number: 560/2018-PR).

### Subjects

Overall, 40 (20 male, 20 female) domestic chicks, *Gallus gallus* (Linnaeus 1758), of the Aviagen ROSS 308 strain, from nine different batches, were used to complete the three experiments. For each experiment, chicks from at least two different batches were used. Fertilised eggs were obtained from a commercial hatchery (CRESCENTI Società Agricola S.r.l., Allevamento Trepola, cod. Allevamento127BS105/2). During incubation and hatching, eggs and chicks were maintained in complete darkness, with a stable temperature of 37.7°C and a constant humidity (40% for incubation and 60% for the hatching stage). On the hatching day, all birds were separated and individually housed in metal cages (28 cm wide×32 cm high×40 cm deep) in the animal house. At this time, the sex of the birds was determined based on the sexual dimorphism of the wing feathers present in this strain. All chicks were kept in the same room, with a constant room temperature of 30–32°C and naturalistic variations in light conditions, providing 14 h of light (day) and 10 h of darkness (night). In the home cages, food and water were available *ad libitum*. Individual housing was implemented from the first day of life to facilitate chicks' habituation to visual isolation from conspecifics (each chick must be trained and tested alone to avoid social cues that could influence the positional choice; see below). Rearing in single cages strongly reduces the stress and freezing responses shown by the animals when they are brought to the experimental arena for single testing. However, except during the training and test sessions, chicks were in constant acoustical contact with familiar conspecifics located in nearby cages, an important form of social enrichment in this species. Moreover, as part of our refinement protocols, to minimise any discomfort due to this temporary acoustical separation, chicks were gradually but extensively habituated to being alone in the test arena and to all aspects of the training procedures before even beginning the training proper (see below). After the completion of the experiments, which took a maximum of 10 days (starting with the 4th day after hatching), chicks were transferred to group housing conditions in larger enclosures and later donated to local farmers.

### Experimental apparatus

Custom-made cages were used to transport the animals to the experimental room and to release them into the experimental arena

without manipulating them. These transportation cages (26 cm wide×32 cm high×40 cm deep) were made of black polypropylene (Poliplak). Each was illuminated by a 16 W LED lamp placed on the ceiling. One of the shorter walls contained a sliding door that allowed the animals to be released into the experimental arena without touching them.

The experimental apparatus (Fig. 1A) consisted of a large circular arena (270 cm diameter) that was surrounded by black polypropylene sheets (30 cm high). The arena floor was uniformly covered by rubberised fabric and carefully cleaned after each trial. The arena's walls contained four release sites (12 cm wide×14 cm high) at the four cardinal points of the arena. Each release site was connected to a corridor (12 cm wide×14 cm high×25 cm long), placed on the outer wall of the arena, to which the transportable cages with the chicks were attached. The experimental arena was surrounded by a large circular black tent (275 cm diameter, 220 cm high), to prevent chicks from seeing outside the arena. Homogeneous illumination of the experimental arena was provided by four LED lamps (64 W) hanging on the tent's ceiling, 180 cm above the arena floor. A fisheye wide-angle lens camera (ELP USB camera, 8-megapixel lens of 180 deg) was positioned between the four LED lamps. The camera was connected to a computer outside the arena, enabling the experimenter to record the videos and score the animal's behaviour online during training and testing. The computer screen was the only light source present in the experimental room, outside the tent.

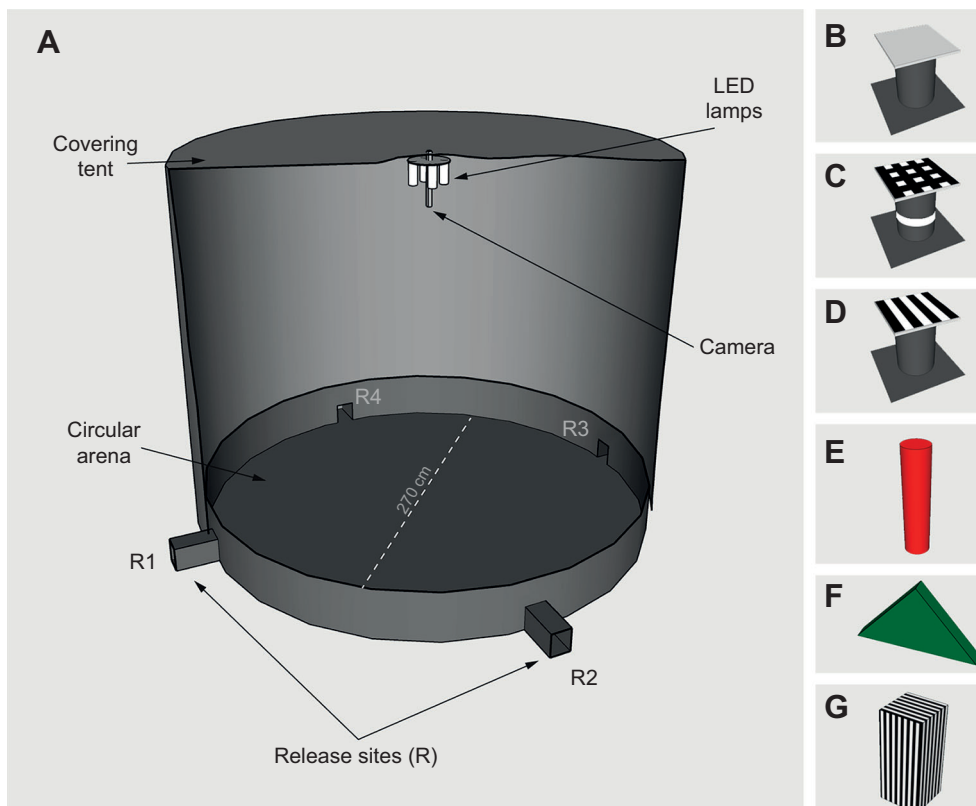
### Habituation training

At the beginning of each training day, chicks were transported to a room adjacent to the experimental room and individually placed into the transportation cages (both rooms were kept at a constant internal temperature of 28°C and allowed acoustical contact with

conspecifics). In the morning, 3 h before training, chicks were food deprived to increase their motivation to perform the task, while water was always available *ad libitum*. During training, mealworms (*Tenebrio molitor* larvae), a highly palatable food for chicks, were used as a reward. At the end of each training day, chicks were taken back to their home cages in the animal house, where food and water were available *ad libitum* until the subsequent training day.

All chicks underwent an extensive habituation training that started on post-hatching day 5. This training aimed to familiarise the animals with the testing arena. During this phase, chicks were gradually trained to enter the experimental arena, open a feeder containing a mealworm by pecking at a grey plastic sheet (4×4 cm; Fig. 1B) placed on top of it and then return to the transportable cage whenever the light inside the arena was turned off. The habituation training lasted 3 days. Each day included eight sessions of six trials each – four in the morning and four in the afternoon. For each session, chicks were transported to the experimental room inside the transportation cage, which was attached to the corridor leading to one of the four release sites (the different release sites were alternated in random order between trials).

At the beginning of the habituation training, a few mealworms were administered directly inside the transportation cage. Once chicks became used to eating the mealworms, a mealworm was used to attract them into the illuminated arena, which they could explore for 2–5 min. Then, the light inside the arena was turned off, whereas the light in the transportation plastic cage was still on. Under such circumstances, most chicks spontaneously returned to their illuminated cages. If this did not occur, chicks were attracted towards the transportation cage with another mealworm. If the chicks still did not return to their illuminated transportation cages, they were further encouraged to escape in the direction of the transportation cage by the experimenter. To do so, the experimenter



**Fig. 1. The experimental setup.**

(A) Diagram of the large circular arena covered by a black tent. Chicks entered the arena through one of the four release sites (R1–R4). (B) Feeder with a grey lid used for the habituation training and for testing in experiments 1 and 3. (C) Feeder with a chequered pattern on the lid and a white stripe on the base, used as a feature cue in experiment 2. (D) Non-rewarded feeder with a striped lid used in experiment 2. (E–G) The three landmarks (a red cylinder, a green triangle and a striped box) providing spatial relational information in experiment 3.

produced a sound (e.g. hand clapping) while standing outside on the opposite side of the arena to the transportation cage (chicks tended to run away from the sound). In the subsequent session, each trial started by opening the sliding door and allowing chicks to run towards a feeder in front of the release site. During this session, one mealworm was placed on the feeder lid to encourage chicks to peck and remove it. Another mealworm was located inside the feeder and accessible only after removing the lid. Once all mealworms were found and eaten, the light inside the arena was turned off, and chicks returned to the transportation cages that were still illuminated. In the subsequent training sessions, the mealworm placed on the lid was removed, and in each trial the feeder was positioned further away from the release site (up to a distance of 70 cm; Fig. 2A). On the second and third days of habituation training, chickens entered through each of the four release sites (the feeder was always located 70 cm in front of the release site) twice a day. Each habituation trial was terminated when the chick had successfully eaten the mealworm, which always happened within 2 min (maximal duration of the trial). At the end of the 3 days of habituation training, all animals confidently entered the experimental arena, pecked on the feeder to obtain the reward and returned to the transportable cage once the light in the arena was turned off.

In experiment 1, chicks were tested for their side preference at the end of the habituation training. In experiments 2 and 3, chicks underwent further discrimination training before being tested in a conflict situation (in which different sources of spatial information conflicted with one another).

### Experiment 1: side bias

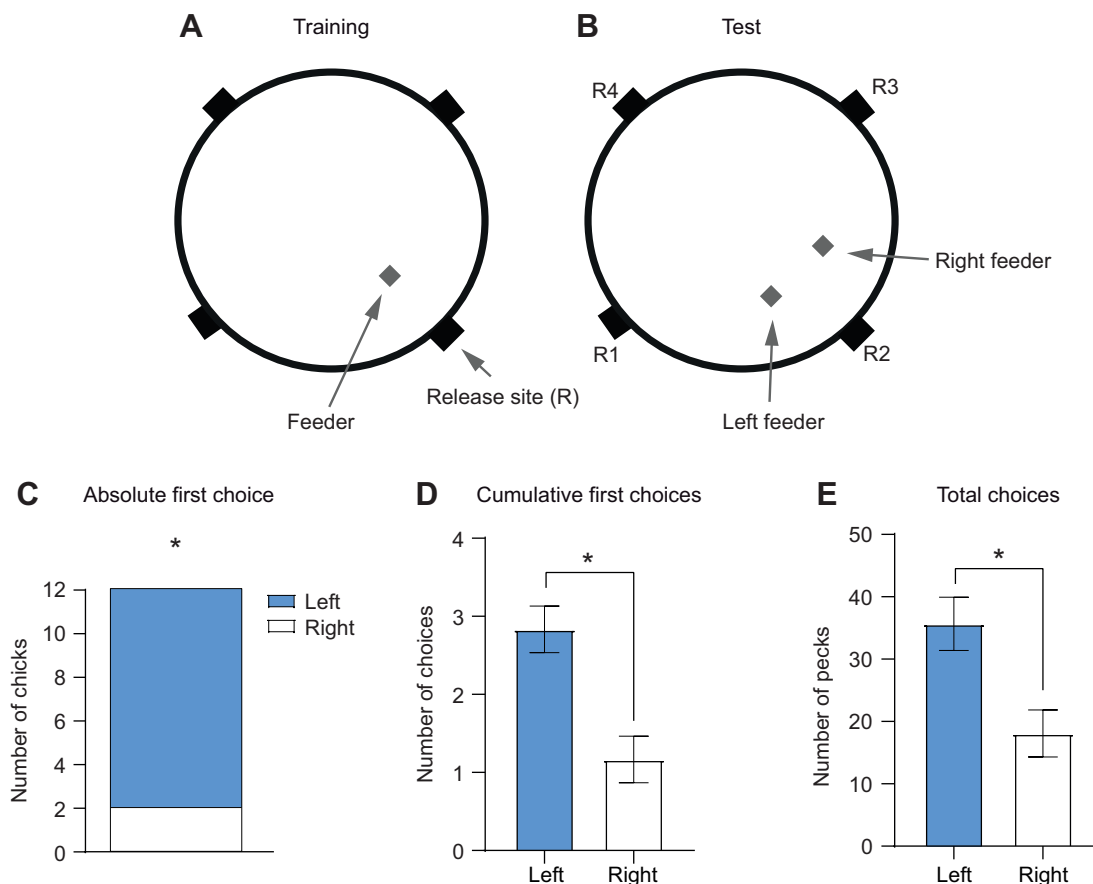
The first experiment investigated whether domestic chicks have a spontaneous side bias (i.e. an untrained preference to approach a feeder on their left or right side) during an egocentric orientation task.

Twelve chicks (6 males, 6 females) were used for this experiment. Chicks were directly tested at the end of the habituation training with one central feeder. During the test, two grey feeders, identical to the one used during habituation training, were placed in front of the release site (70 cm away from it and 20 cm apart from each other; Fig. 2B). The feeders were closed and contained no mealworms. Each test trial lasted for 1 min, and chicks were released into the arena from a different release site. The order in which the different release sites were employed was randomised between subjects. Chicks participated in four test sessions, composed of four trials each. In each test session, the first three trials were rewarded habituation trials with the central feeder to keep the animals motivated during the test, and the last one was an unrewarded test trial with two feeders. Only the behaviour from the four unrewarded test trials was analysed.

### Experiment 2: egocentric versus local feature

The second experiment investigated whether domestic chicks orient preferentially by egocentric information or feature cues when these two types of information conflict.

Twelve chicks (6 males, 6 females) were used for this experiment. After the end of the habituation training with one central feeder,



**Fig. 2. Design and results of experiment 1.** (A,B) Feeder placement during training (A) and testing (B). (C) Number of chicks ( $n=12$  total) showing an absolute first choice for the left or right feeder. (D) Cumulative first choices for the left and right feeders. (E) Total choices for the two feeders, expressed as the number of pecks/touches directed to each feeder during the whole test. The bar plots represent means  $\pm$  s.e.m. (\* $P<0.05$ ).



chicks started the discrimination training. The habituation feeder was replaced with two visually distinct feeders during this phase. One was a feeder covered by a chequered lid with a white stripe wrapped around its black base (Fig. 1C). The other was a feeder covered by a striped lid, whose base was uniformly black (Fig. 1D). Only the chequered feeder could be opened and contained a mealworm. Thus, the chicks' task was to learn that only pecking the chequered feeder would provide a reward.

During the discrimination training, chicks were divided into two groups ( $N=6$  each, 3 males, 3 females): (i) a group that was trained to peck at the baited chequered feeder always located on the left side of the release site and (ii) a group that was rewarded on the baited chequered feeder always located on the right side. This was done to assess whether the left–right placement of the feeder rewarded during the training would influence the chicks' performance. As the first experiment revealed a spontaneous leftward bias, we expected facilitation for chicks rewarded on a feeder placed on their left side. The discrimination training comprised eight sessions per day (four in the morning and four in the afternoon), with six trials per session. In each trial, the animals were released from a different site. The order in which the release sites were experienced was randomised between animals. Each trial ended when the chick found the mealworm. The training continued until chicks reached a learning criterion of 21 correct responses out of 24 trials during a single day (21/24 binominal test:  $P<0.001$ ). A response was defined as correct if the first choice of the animal was for the rewarded feeder (i.e. if the rewarded chequered feeder was the first one touched or pecked during the trial). At the end of the discrimination training, one group of chicks had learnt that the baited feeder was the chequered one always located on the left of the release site. In contrast, the other group had learnt that the reward could be found in the chequered feeder to the right of the release site.

Testing took place about 1 h after the end of the discrimination training. It was composed of one session with four non-rewarded trials, lasting 1 min each (none of the test feeders contained a mealworm, and none could be opened). During the test, too, the release site order was randomised between animals. To investigate whether domestic chicks preferentially orient by egocentric information or local features, during the test, the position of the two feeders was inverted to create a conflict between these two types of information (Fig. 3A,B). For the group of chicks that were used to find the baited chequered feeder on the left, at the test, this feeder was moved to the right of the release site, and vice versa for the other group. If the animals preferentially follow local features, they should approach the chequered feeder independently of its egocentrically defined position (left or right of the release site). However, suppose chicks preferentially follow egocentric information. In that case, they should approach the feeder on their left or right (consistent with the rewarded side at training) even though the chequered feeder is now on the other side.

### Experiment 3: egocentric versus spatial

This last experiment investigated whether domestic chicks orient preferentially using egocentric or allocentric spatial relational information provided by free-standing objects. As in the previous experiment, these two types of information were simultaneously available during the training and were put in conflict during the test.

Sixteen domestic chicks (8 males, 8 females) were used for this experiment. The day after the end of the habituation training, chicks started the discrimination training. During this phase, chicks had to learn the position of the baited feeder among four identical feeders with grey covers (Fig. 1B). Only the baited feeder could be opened

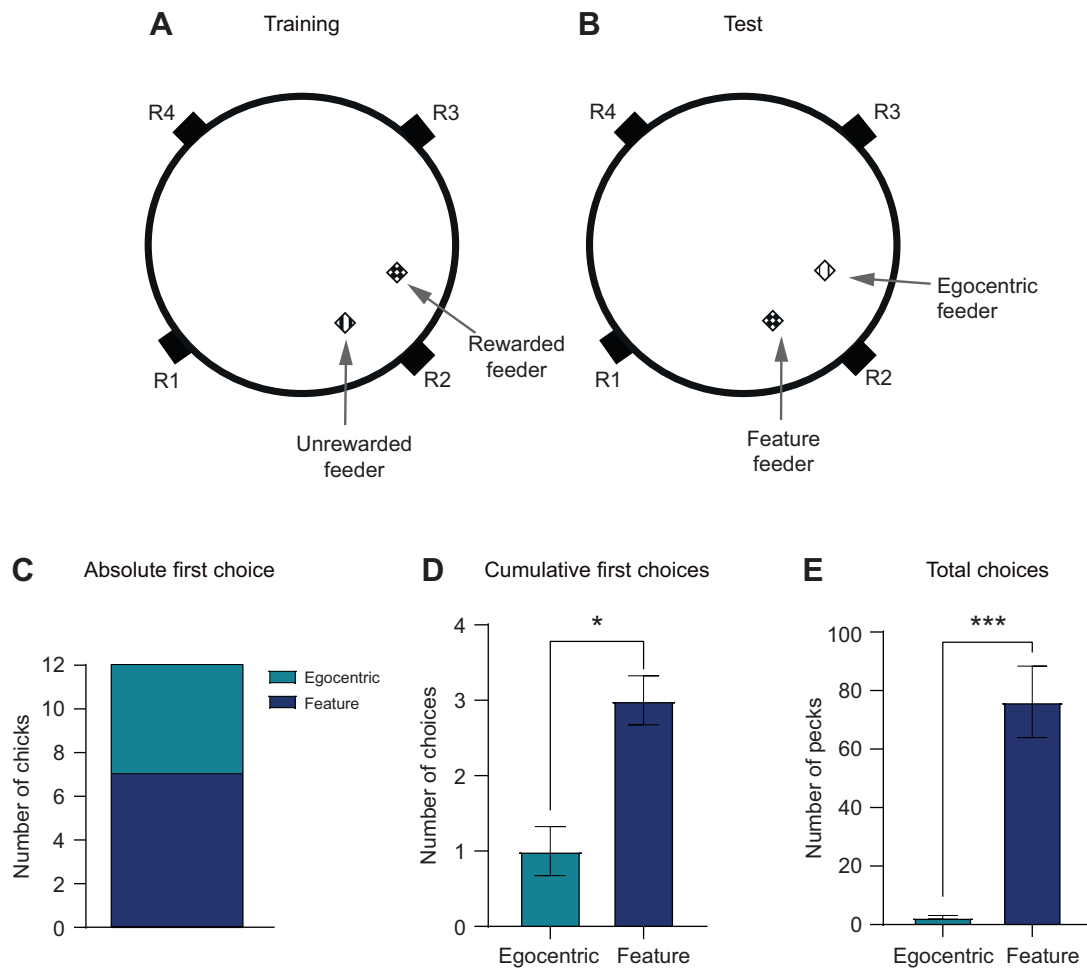
and contained a mealworm. During the discrimination training, three landmarks were placed inside the experimental arena (Fig. 1E–G). The landmarks were three objects of different shapes and colours: a red cylinder (6 cm diameter, 35 cm high), a green triangle (34 cm long×20 cm high) and a striped black-and-white box (20 cm high×9 cm wide). Each landmark was 90 cm distant from the centre of the arena and 30 cm from the walls. The red cylinder (L1) was placed 20 deg to the left of release site 1 (R1), the green triangle (L2) was placed 20 deg to the right of release site 3 (R3), while the striped box was placed 20 deg to the left of release site 4 (R4) (see Fig. 4A). We have shown that in this setup, chicks can orient using only the spatial relational information provided by these three landmarks (Morandi-Raikova et al., 2020).

During the discrimination training, the rewarded feeder was always positioned between the green triangle and the striped box (e.g. feeder D in Fig. 4A). In this phase, chicks always entered the arena from the same release site (for example, Fig. 4 illustrates the case of an animal entering from R4). In this case, chicks could identify the rewarded feeder D either in relation to the landmarks or based on egocentric information (e.g. the baited feeder is always to the left of the release site in Fig. 4A). The three other feeders were not rewarded at training: the feeder located on the opposite side of the release site (feeder A in Fig. 4A) and two feeders called control I and control II (feeders B and C in Fig. 4A).

For half of the animals ( $N=8$ , 4 males, 4 females), the baited feeder was always located to the left of the release site, while for the other half, the rewarded feeder was located to the right. Release sites were randomised between animals to control for the use of any other potential source of information. However, the landmarks were always rotated so that, regardless of the release site used to train a given animal, the allocentric relational information provided by the free-standing objects and the egocentric information were matched between animals within the same reward group, e.g. for an animal that was released from R1 and trained to peck at the feeder located to the left of the release site, the landmarks were rotated by 90 deg to the left compared with what is shown in Fig. 4A so that the correct feeder was located between the green triangle and the striped box.

The discrimination training comprised eight sessions per day (four in the morning and four in the afternoon), with six trials per session. Each trial ended when the chick found the mealworm. The training continued until chicks reached a learning criterion of 21 correct trials out of 24 (21/24 binominal test:  $P<0.001$ ).

Testing took place about 1 h after the end of the discrimination training and consisted of three conflict test trials of 1 min each. At the test, none of the feeders could be opened or contained a mealworm. In each test trial, the chick entered the arena from a different release site, chosen among the three release sites that the animal had not experienced at training. Note that egocentric and allocentric relational information conflict when chicks enter the arena from a different release site to that used at training. Thus, the feeder located on the previously rewarded side is no longer the one positioned between the green triangle and the striped box. For example, let us consider the case of an animal that, at training, entered the arena from R4 and found the baited feeder on the left side. During testing, when the animal entered the arena from R3, the feeder on its left side was now located between the green triangle on the right and the red cylinder on the left side of the release site. Thus, during the test trials, chicks that orient based on egocentric information should approach the feeder located to their left or their right (depending on the training group), regardless of its position in relation to the free-standing objects. In contrast, chicks that orient based on allocentric spatial relational information should choose the feeder between the green triangle and the striped box,



**Fig. 3. Design and results of experiment 2.** (A,B) An example of the placement of the two distinct feeders during training (A) and testing (B). (C) Number of chicks ( $n=12$  total) showing an absolute first choice for the egocentric or feature feeder. (D) Cumulative first choices for the egocentric and feature feeders. (E) Total choices for the two feeders, expressed as the number of pecks/touches directed to each feeder during the whole test. The bar plots represent means  $\pm$  s.e.m. (\* $P < 0.05$ , \*\*\* $P < 0.001$ ).

regardless of whether it is located to their left or right. The use of unfamiliar release sites during the test also prevented the use of view-matching strategies to locate the rewarded feeder during testing (see Dawkins and Woodington, 2000; Pecchia and Vallortigara, 2010). The order of entrance from the three new release sites was randomised between animals. During testing, ‘control I’ was defined as the first control feeder located clockwise after the ‘egocentric’ feeder and ‘control II’ as the second control feeder located clockwise after the egocentric feeder.

#### Data analysis

Behavioural data were scored offline. As preliminary analyses did not reveal any effect of sex or any interaction of sex with other factors (see Tables S1–S3 for the raw data of each experiment), data from males and females were pooled together in all further analyses. Likewise, preliminary analyses did not reveal any change across trials in chicks’ response times [scored as the time (s) needed to peck or touch a feeder for the first time], suggesting a similar level of motivation and impulsiveness during the test. We thus did not include this factor in further analyses.

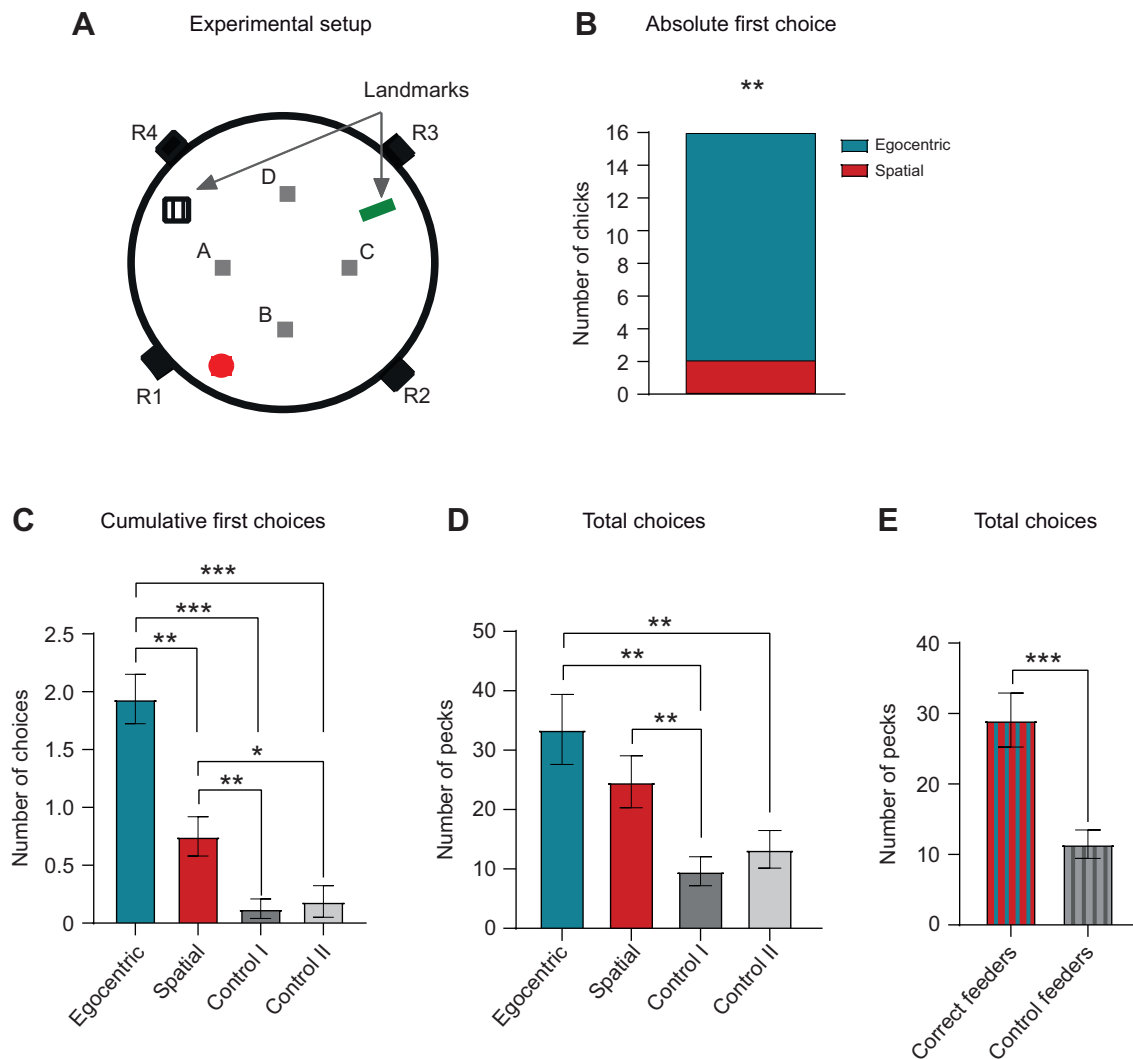
In experiments 2 and 3, the number of discrimination trials needed to reach the learning criterion (‘trials to criterion’) was recorded to assess chicks’ learning performance. An independent

samples *t*-test analysed the number of trials to criterion to assess whether the left–right placement of the rewarded feeder influenced learning performance.

As for the test performance, three different behavioural measures were scored for each testing trial: absolute first choice, cumulative first choices and total choices.

For the absolute first choice, we recorded the first feeder the chick pecked or touched during the first test trial. The touching or pecking of feeders was visible on the video recordings and easily scorable. To test whether the absolute first choice revealed a preference for one feeder over the other, a chi-square goodness-of-fit test was run. Furthermore, for experiments 2 and 3, a chi-square goodness-of-fit test was performed on a  $2 \times 2$  contingency table to assess whether there was any effect on the rewarded training side (left versus right) on the frequency of absolute first choice for the two target test feeders (egocentric versus feature feeder in experiment 2, egocentric versus spatial feeder in experiment 3).

For the cumulative first choices, we recorded which feeder was the first to be pecked/touched in each trial. We then computed how many times each feeder was chosen first during the whole test. In experiments 1 and 2, the test consisted of four valid trials, whereas in experiment 3 the test consisted of three valid trials. Thus, the number of cumulative first choices assigned to each feeder could



**Fig. 4. Design and results of experiment 3.** (A) An example of the placement of feeders and landmarks for chicks entering from release site R4 at training. (B) Number of chicks showing an absolute first choice for the egocentric or spatial feeders ( $n=16$  total). (C) Cumulative first choices for the egocentric, spatial and the two control feeders. (D) Total choices for the four feeders, expressed as the number of pecks/touches directed to each feeder during the whole test. (E) Total choices for the correct and control feeders. The bar plots represent means $\pm$ s.e.m. (\* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ ).

range from 0 to 4 in experiments 1 and 2, or from 0 to 3 in experiment 3.

The total choices were computed as the sum of the overall number of pecks/touches the chick directed towards each feeder during the whole test duration. The number of choices scored in each trial was thus summed to obtain the total value for the whole test session. A repeated measures ANOVA was run on the cumulative first and total choices to assess whether the release site (within-subject factor, four levels: release site 1, 2, 3 and 4) and, for experiments 2 and 3 only, the rewarded training site as a between-subject factor (two levels: left and right) influenced this measure.

All statistical analyses were performed using SPSS (SPSS Statistics v23.0, IBM), while the graphs were created with GraphPad Prism 8.

## RESULTS

### Experiment 1: side bias

The absolute first choices (i.e. the first feeder each chick pecked or touched during the first test trial) were significantly more frequent for the feeder on the left than for that on the right of the release site

( $\chi^2_1=5.333$ ,  $P=0.021$ ). Indeed, 10 out of 12 animals showed an absolute first choice for the feeder on the left side of the release site (Fig. 2C). A preference for the feeder on the left side was also confirmed for the cumulative first choices. The feeder located on the left of the release site received overall more first pecks/approaches ( $2.83\pm0.3$ , mean $\pm$ s.e.m., rounded numbers) than the one located on the right ( $1.17\pm0.3$ ) (Fig. 2D). A repeated measures ANOVA with within-subject factors 'side' (two levels: left, right) and 'release site' (four levels: release site 1, release site 2, release site 3, release site 4) revealed a significant effect of side, but no main effect of release site nor any interaction between these two factors (all ANOVA results are reported in Table 1). The total choices confirmed this: chicks showed significantly more choices for the feeder on the left ( $35.7\pm4.3$ ) than for that on the right ( $18.08\pm3.8$ ) (Fig. 2E). The repeated measures ANOVA, performed on the total choices, revealed a significant effect of side, but no significant effect of release site and no interaction between these two factors (see Table 1).

In summary, regardless of the release site, most subjects preferred to approach the feeder on their left. This leftward bias was evident in all the dependent variables analysed.

**Table 1. ANOVA results of the cumulative first and total choices for each experiment**

Main effects and interactions	Cumulative first choices	Total choices
Experiment 1: side bias		
Feeder	<b><math>F_{1,3}=7.857, P=0.017</math></b>	<b><math>F_{1,11}=8.280, P=0.015</math></b>
Release site	$F_{3,33}=1.000, P=0.405$	$F_{3,33}=0.276, P=0.842$
Feeder×Release site	$F_{3,33}=1.320, P=0.284$	$F_{3,33}=0.202, P=0.895$
Experiment 2: egocentric vs local		
Feeder	<b><math>F_{1,10}=9.474, P=0.012</math></b>	<b><math>F_{1,10}=38.075, P&lt;0.001</math></b>
Release site	$F_{3,30}=0.978, P=0.333$	$F_{3,30}=0.199, P=0.896$
Rewarded training side	$F_{1,10}=0.000, P=1.000$	$F_{1,10}=1.866, P=0.202$
Feeder×Release site	$F_{3,30}=1.034, P=0.391$	$F_{3,30}=0.241, P=0.867$
Feeder×Rewarded training side	$F_{1,10}=1.053, P=0.329$	$F_{1,10}=1.650, P=0.228$
Release site×Rewarded training side	$F_{3,30}=2.857, P=0.122$	$F_{3,30}=0.454, P=0.717$
Feeder×Release site×Rewarded training side	$F_{3,30}=0.345, P=0.793$	$F_{3,30}=0.576, P=0.635$
Experiment 3: egocentric vs spatial		
Feeder	<b><math>F_{2,014,28.190}=21.805, P&lt;0.001</math></b>	<b><math>F_{3,42}=7.723, P&lt;0.001</math></b>
Release site	$F_{3,42}=0.113, P=0.952$	$F_{3,42}=0.524, P=0.668$
Rewarded training side	$F_{1,14}=0.000, P=1.000$	$F_{1,114}=0.731, P=0.407$
Feeder×Release site	$F_{4,402,61.628}=0.190, P=0.995$	$F_{4,33,60.58}=0.345, P=0.860$
Feeder×Rewarded training side	$F_{3,42}=1.529, P=0.221$	$F_{3,42}=2.903, P=0.065$
Release site×Rewarded training side	$F_{3,42}=0.339, P=0.797$	$F_{3,42}=0.242, P=0.866$
Feeder×Release site×Rewarded training side	$F_{4,402,61.628}=1.376, P=0.250$	$F_{9,126}=0.623, P=0.776$

Bold indicates significance.

### Experiment 2: egocentric versus local feature

On average, chicks reached the learning criterion in  $22.8 \pm 0.3$  trials. Chicks that were trained to find the baited (chequered) feeder on the left or the right of the release site needed a similar number of trials to reach the learning criterion, as revealed by an independent samples *t*-test ( $t_{10} = -0.243, P=0.813$ ; rewarded on the left:  $22.7 \pm 0.5$ ; rewarded on the right:  $22.8 \pm 0.5$ ).

The analysis of the absolute first choice performed during the first test trial did not reveal any significant preference for the chequered feeder (7 animals out of 12 went first to the chequered feeder;  $\chi^2_1=0.333, P=0.564$ ) (Fig. 3C). Also, no effect of the rewarded training side was observed when analysing the chicks' absolute first choice ( $\chi^2_1=0.343, P=0.558$ , obtained from a  $2 \times 2$  contingency table, rewarded training side: left versus right; absolute first choice: egocentric versus feature feeder).

However, when analysing the cumulative first choices, a significant preference for the chequered feeder did emerge, as shown by a mixed ANOVA, with 'feeder' (two levels: choices for the chequered feeder, choices for the egocentric feeder) and 'release site' (four levels: release site 1, release site 2, release site 3, release site 4) as within-subjects factors and 'rewarded training side' (two levels: left, right) as a between-subject factor. This analysis revealed a significant effect of the factor feeder, due to a significantly higher number of cumulative first choices for the chequered feeder ( $3.00 \pm 0.3$ ) than for the previously rewarded egocentric location ( $1.00 \pm 0.3$ ) (Fig. 3D). No other significant main effects or interactions emerged (see Table 1).

A preference for the chequered feeder also appeared in the total choices. Chicks expressed significantly more choices for the chequered feeder ( $76.2 \pm 12.2$ ) than for the one on the previously rewarded side ( $2.5 \pm 0.6$ ) (Fig. 3E). This was revealed by a mixed ANOVA performed on the total choices, where a significant effect of the factor feeder was found (no other significant effects or interactions emerged; see Table 1).

To summarise, two out of three dependent variables revealed that chicks relied on the feeder's visual features rather than on view-dependent egocentric information to find a goal location. The dissociation between the absolute first choices and the other two dependent variables suggests that some chicks could have learnt

both strategies and used egocentric information to direct their first choice. Nevertheless, when given the possibility to express multiple choices during the test, chicks preferred orienting by the appearance of the feeder. The fact that the release site did not influence the performance confirms that the animals' responses were not affected by any residual perceptual cue that might have been available in the arena.

### Experiment 3: egocentric versus spatial

On average, chicks needed  $57 \pm 10.5$  trials to reach the learning criterion. Chicks rewarded for choosing the feeder on the left needed only  $39 \pm 15$  trials to complete the training, while those rewarded for choosing the feeder on the right needed  $75 \pm 12.4$  trials to complete the training. This difference, however, was not significant according to an independent samples *t*-test ( $t_{14} = -1.852, P=0.085$ ).

As for the absolute first choice, significantly more chicks approached first the egocentrically correct feeder (chosen by 14 out of 16 chicks,  $\chi^2_1=9.00, P=0.003$ ) than the spatially correct feeder (chosen by the remaining 2 chicks; Fig. 4B). No effect of the rewarded training side was observed when analysing the chicks' absolute first choice ( $\chi^2_1=0.00, P=1.000$ , obtained from a  $2 \times 2$  contingency table, rewarded training side: left versus right; absolute first choice: egocentric versus feature feeder).

A repeated measures ANOVA was run on the first cumulative choices to investigate whether any of the four feeders (the two experimental feeders: egocentric and spatial, and the two control feeders: control I and control II) was preferred. The ANOVA had 'feeder' (four levels: egocentric, spatial, control I and control II) and 'release site' (four levels: release site 1, release site 2, release site 3, release site 4) as within-subject factors and rewarded training side (two levels: left, right) as a between-subject factor. This revealed a significant effect only of the factor feeder (no other significant effects or interactions were found; see Table 1). *Post hoc* comparisons (paired samples *t*-tests, reported in Table 2) showed that the number of cumulative first choices was significantly higher for the egocentrically correct feeder ( $1.94 \pm 0.2$ ) than for the spatially correct feeder ( $0.75 \pm 0.2$ ), control I ( $0.13 \pm 0.1$ ) or control II ( $0.19 \pm 0.1$ ). A significant preference was also found for the spatially correct feeder compared with the two control feeders. No



**Table 2. Paired t-test results of the cumulative first and total choices for experiment 3**

Post hoc comparisons	Cumulative first choices	Total choices
Egocentrically correct versus spatially correct	<b><math>t_{15}=3.335</math>, <math>P=0.005</math></b>	$t_{15}=1.256$ , $P=0.228$
Egocentrically correct versus control I	<b><math>t_{15}=6.926</math>, <math>P&lt;0.001</math></b>	<b><math>t_{15}=3.397</math>, <math>P=0.004</math></b>
Egocentrically correct versus control II	<b><math>t_{15}=5.916</math>, <math>P&lt;0.001</math></b>	<b><math>t_{15}=3.225</math>, <math>P=0.006</math></b>
Spatially correct versus control I	<b><math>t_{15}=3.478</math>, <math>P=0.003</math></b>	<b><math>t_{15}=3.648</math>, <math>P=0.002</math></b>
Spatially correct versus control II	<b><math>t_{15}=2.334</math>, <math>P=0.034</math></b>	$t_{15}=1.955$ , $P=0.069$
Control I versus control II	$t_{15}=-0.368$ , $P=0.718$	$t_{15}=-0.923$ , $P=0.371$

Bold indicates significance.

significant difference in the cumulative first choices appeared when comparing control I with control II (Fig. 4C).

Also, for the total choices, a similar ANOVA revealed a significant effect of the feeder (no other significant effects or interactions were found; Table 1). *Post hoc* analyses (reported in Table 2) revealed that significantly more total choices were directed to the egocentric feeder ( $33.5\pm5.9$ ) when compared with the control I ( $9.8\pm2.5$ ) and control II ( $13.3\pm3.2$ ) feeder. Moreover, the spatial feeder ( $24.7\pm4.4$ ) was chosen significantly more than the control I feeder (Fig. 4D). In contrast, no significant difference was observed between the egocentric and spatial feeders, between the spatial feeder and control II feeder or between the two control feeders. Lastly, the sum of the total choices performed for the two ‘correct’ feeders (egocentric and spatial) was significantly higher ( $t_{15}=4.088$ ,  $P<0.001$ ) than the sum of those directed to the two control feeders (control I and control II) (Fig. 4E).

In summary, in this experiment, regardless of the release site or rewarded side, chicks preferred to use egocentric rather than allocentric spatial relational information. However, this effect reached significance only for the absolute and cumulative first choices, but not for the total choices. This suggests that the first responses performed by the animals were mostly driven by egocentric information. Once the animals realised that their first choice did not reward them during that trial, they were able to utilise both types of information. In fact, the total choices of the animals were not casually distributed among all the feeders: chicks pecked significantly more on the two correct feeders than on the two control feeders, alternating between the two sources of information that were provided to them during training.

DISCUSSION

We tested domestic chicks’ orientation strategies. The first experiment revealed a leftward bias in a viewpoint-dependent egocentric orientation task. The second and third experiments showed that object features are more relevant for chicks than egocentric information and that egocentric information is more relevant than allocentric spatial relational information provided by free-standing objects.

The leftward bias of experiment 1 is in line with our predictions, based on the left eye system dominance for attentional allocation in chicks (e.g. Diekamp et al., 2005; Regolin, 2006; Rugani et al., 2010) and on indirect evidence of a leftward bias in egocentric orientation (Vallortigara et al., 1988, 1996, 1999). Moreover, in our previous study, using the same setup, we prevented the use of egocentric information (Morandi-Raikova et al., 2020); nevertheless, chicks’ tracks revealed a spontaneous tendency to explore the left

hemisphere on their way to the correct feeder. The current study is a direct confirmation of this tendency, demonstrating that viewpoint-dependent egocentric orientation is facilitated if the location of the goal is in the left hemisphere.

Remarkably, this side bias emerged in dark-incubated chicks. Often, potential lateralisation in egocentric (Vallortigara et al., 1988, 1996, 1999) or allocentric spatial orientation (Rashid and Andrew, 1989; Tommasi et al., 2003) has been reported for studies that did not specify whether the chicks were hatched from light-exposed or dark-incubated eggs. Those studies that did report the incubation conditions, however, paint a conflicting picture. For instance, Morandi-Raikova et al. (2020) report a potential leftward spatial bias in dark-incubated animals. In contrast, other studies have shown non-lateralised spatial processing in dark-incubated chicks (Mayer et al., 2016: orientation based on the geometrical shape of an enclosure) or found lateralised spatial processing in light-incubated chicks only (Chiandetti et al., 2005). In the face of this conflicting evidence, a particularly relevant finding of the current study is the direct demonstration that side bias in viewpoint-dependent egocentric orienting does not require in-egg light exposure to emerge. Overall, this is in line with the view that behavioural and brain lateralisation can be present in dark-incubated chicks (e.g. Mascetti and Vallortigara, 2001; Chiandetti et al., 2013; Chiandetti and Vallortigara, 2019; Lorenzi et al., 2019; Costalunga et al., 2022; Morandi-Raikova et al., 2021; Morandi-Raikova and Mayer, 2021).

In experiments 2 and 3, we further described the ‘hierarchy’ of cues preferentially used by domestic chicks for orientation in a well-controlled setup (a large, featureless circular arena with four identical entrances). Our previous study using the same setup found that chicks can orient using spatial relational information provided by free-standing objects. However, chicks prefer to orient using the features of the rewarded feeder as a beacon when these two sources of information conflict (Morandi-Raikova et al., 2020). In the current study, we tested the role of egocentric information in combination with object features and spatial cues. Overall we can conclude that, in our setup, domestic chicks prefer to orient by object features (used as a beacon) over egocentric viewpoint-dependent information. At the same time, egocentric information is preferred over spatial relational information provided by free-standing objects.

The hierarchy of cues chicks employ to orient in our setup is also reflected in another aspect of the results. Despite the strong leftward bias observed in experiment 1, in experiment 2, we did not observe any learning facilitation for the animals rewarded on the feeder placed to the left of the release site. This suggests that the dominant feature information prevents the expression of egocentric side bias. In contrast, in experiment 3, a non-significant trend suggested potential learning facilitation for chicks rewarded on the left during training. This would align with the fact that egocentric information is prioritised over spatial relational information. However, whereas in experiment 3, during testing the spatially correct feeder was chosen less frequently than the egocentrically correct feeder, the latter was chosen more than the control feeders. This indicates that, even in the presence of competing egocentric information, chicks still learnt spatial relational information.

Animals can use different reference systems for navigation, depending on the circumstances and ecology of the species (Paillard, 1991; Burgess, 2006). The preferential use of object features appears to be a stable trait of chickens, as it is also present in adult red junglefowl and another domesticated breed (Ferreira et al., 2022). Chickens are ground-living omnivore birds which forage

within a relatively small home range. This could have favoured attention to object features that allow the detection of small food items dispersed in their environment. Moreover, some important food sources for chickens (insects and small vertebrates) can move around. Therefore their precise location can change and is less informative than their appearance. This would explain why chickens follow object features even if these are displaced to a nearby location, as in experiment 2 and in Morandi-Raikova et al. (2020). Furthermore, navigation in a smaller-scale environment is probably less subject to disturbances from events causing disorientation (such as being carried away by the wind in unknown directions). This could promote less computationally heavy egocentric orientation strategies, compared with orientation by spatial relational information, which allows an animal to re-orient after losing its bearings.

The hierarchy between egocentric, featural and spatial relational allocentric cues varies among bird species. For instance, food-hoarding birds prefer spatial relational cues over object features (Brodbeck, 1994; Clayton and Krebs, 1994a,b; Herborn et al., 2011; Hurly et al., 2014; but see Hodgson and Healy, 2005; LaDage et al., 2009), unlike chicks. This could result from an ecological adaptation to the need to retrieve the hidden caches after a long delay in food-hoarding birds. Spatial relational cues provide more reliable information about an environment that changes over time (Brodbeck, 1994; Clayton and Krebs, 1994a,b; Brodbeck and Shettleworth, 1995).

In summary, in this study, we systematically investigated domestic chicks' use of viewpoint-dependent egocentric information, revealing an asymmetry favouring the left hemispace. We also describe for the first time the hierarchy of featural, egocentric and spatial relational cues used by domestic chicks for locating food resources, reflecting this species' foraging ecology.

#### Acknowledgements

This work was made possible through the use of the Animal Cognition and Neuroscience core facilities at the Center for Mind/Brain Sciences, University of Trento.

#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: A.M.-R., O.R.-S., U.M.; Methodology: A.M.-R., U.M.; Formal analysis: A.M.-R., A.S.; Investigation: A.M.-R., A.S.; Resources: G.V., U.M.; Data curation: A.M.-R., A.S.; Writing - original draft: A.M.-R., A.S.; Writing - review & editing: O.R.-S., G.V., U.M.; Visualization: A.M.-R.; Supervision: U.M.; Project administration: U.M.; Funding acquisition: G.V.

#### Funding

This project received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and the innovation programme (grant agreement no. 833504 SPANUMBRA) and Ministero dell'Università e della Ricerca Progetti di ricerca di Rilevante Interesse Nazionale (Prin) 2017 – Title: 'Number-space association: a comparative, developmental and neurobiological approach' (codice progetto 2017PSRHPZ) awarded to G.V. The funders had no role in study design, data collection, interpretation or the decision to submit the work for publication.

#### Data availability

The data from this study are available within the supplementary material (Tables S1, S2, S3).

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