

Research report

# Effects of light stimulation of embryos on the use of position-specific and object-specific cues in binocular and monocular domestic chicks (*Gallus gallus*)

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Received 19 January 2005; received in revised form 23 March 2005; accepted 29 March 2005

Available online 31 May 2005

## Abstract

Chicks hatched from eggs incubated in the dark (D-chicks) or from eggs exposed to light during the last 3 days before hatching (L-chicks) were trained on day 4 to peck at small cones for food reinforcement. The cones had different patterns (checked or striped) and were located in different positions (either on the left or on the right of a rectangular arena) so as both object-specific (pattern) and position-specific cues could be used to discriminate cones that contained or that did not contain food. After learning, the position of the cones was reversed so that object- and position-specific cues provided contradictory information. No effect of light incubation was observed in binocular chicks that chose cones on the basis of object-specific cues. Monocular D-chicks also tended to approach and peck the cones with the correct pattern in the wrong position, whereas monocular L-chicks did not show any clear choice. Initial choices for one side or other of the arena were mostly determined by the first side visible through the non-occluded eye in D-chicks, particularly when using their left eye. These results suggest that light exposure of the embryo makes neural mechanisms that do not receive direct visual input (i.e., those of the occluded side) more available to be used in assessment of novelty.

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**Keywords:** Light exposure; Embryo; Brain lateralisation; Asymmetry; Spatial cognition; Domestic chick

## 1. Introduction

The domestic chick is used widely as a model system for research on cerebral lateralisation of functions [2,19–21, 34,36,40]. This species possesses features such as a nearly complete decussation of the fibres at the optic chiasma, relatively poor interhemispheric connections compared to mammals (but see [6]) and independent scanning by the two eyes [2]. This makes it possible to study the separate functions of the two hemispheres using the simple technique of the tempo-

rary occlusion of one eye, which largely confines processing of visual information to the hemisphere contralateral to the stimulated eye.

Using monocular tests it has been proven that the chick's brain is lateralised for control of a range of visual responses. The right eye/left hemisphere system (RES) attends to categorisation of visual stimuli and it seems to be responsible for the visual control of a considered response (see for reviews [21,38]). The RES superiority in visual discrimination learning has been demonstrated in other avian species besides chicks [12], namely in pigeons [10], zebra finches [1] and quails [28]. The left eye/right hemisphere system (LES) seems to be more involved in certain aspects of spatial cognition [14,39,26,27], in the assessment of novelty [2] and in establishing identity with a past

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experience by attending to the details of individual stimuli [29,31,32].

Some of these behavioural asymmetries have been associated with neuroanatomical asymmetries caused by the exposure of the right eye of the embryo to light during the later stages of development before hatching. The chick embryo is oriented in the egg so that only the right eye receives light stimulation passing through the shell and the membranes, whereas the left eye is covered by the rest of the body [18]. This asymmetric light stimulation in embryo promotes asymmetric development of the visual projections that originate from the left side of the thalamus (fed by the right eye) and continue to the visual Wulst [23]. This has been revealed by injecting fluorescent tracers in the Wulst and then looking for ipsi- and contra-lateral labelled cell bodies in the thalamus. The exposure of the right eye to light leads to an increased number of visual projections from the left side of the thalamus (which receives inputs from the right eye) to the right Wulst region of the forebrain compared to the equivalent and opposite projection from the right side of the thalamus to the left visual Wulst [23]. Intriguingly, in the pigeon a similar asymmetry has been found, but in the tectofugal rather than in the thalamofugal pathway [9]. The exposure of the right eye to light leads to an increased number of visual projections from the right tectum to the contralateral rotundus. It could be that the difference in the pathway involved has something to do with the different developmental pattern of the two species, the pigeon being an altricial species the chicks being a precocial one [7].

Embryos incubated in completely dark conditions do not develop any asymmetry in the visual pathways [23]. Moreover, withdrawing the embryo's head from the egg 2 days before hatching, occluding the right eye with a patch and allowing the left eye to be stimulated by light, reverses the pattern of asymmetry, with an increased number of visual projections from the right thalamus to the contralateral Wulst (summarised in [7]).

Asymmetrical light exposure of embryos has been shown to affect some forms of behavioural lateralisation after hatching. For example, performance in the pebble floor task, in which chicks are required to categorise grains of food as distinct from pebbles, is impaired only by glutamate-treatment of the left (and not the right) visual Wulst in L-chicks; this asymmetry is absent in D-chicks [6,7]. Lateralisation of attack responses shows the same dependency on light exposure of the eggs [16,18]. In this case, the response is higher in chicks using their left eye than it is in chicks using their right eye, provided that the eggs have been exposed to light [24]. In chicks hatched from eggs incubated in the dark, the levels of attack are the same when they use the left or right eye.

There are other forms of lateralisation in chicks that, however, do not depend on light exposure of the embryo, and these include imprinting and social recognition [37,3,8], response to olfactory versus visual cues [22,33] and lateralisation of auditory responses [4].

Lateralisation of spatial cognition has yet not been investigated for possible effects of asymmetric light stimulation in embryo. This is in spite of the fact that spatial cognition is probably among the best studied lateralised functions in the chick (see for a review [35]). A well-established fact, that seems to hold also for other species of birds (see e.g., for pigeons [11]), is that the two hemispheres of the chick's brain tend to attend differently to object-specific local cues and to position-specific, large scale, global cues. For instance, Tommasi and Vallortigara [26] trained chicks to find food hidden below sawdust on the floor by ground-scratching in the centre of a closed uniform arena: the centre was indicated by a conspicuous landmark. After learning, the landmark was relocated to a novel position and chicks were tested binocularly or with only one eye in use. A striking asymmetry appeared: binocular chicks and chicks using only their left eye searched at the centre (ignoring the landmark), whereas chicks using only their right eye searched at the corner (ignoring purely spatial information). Clayton and Krebs [5] tested the memory of food-storing and non-food-storing birds for feeders that had a trial-unique location in an experimental room as well as a trial-unique colour pattern. When, after a short retention interval, birds were given dissociation tests in which the correct feeder changed its position and a different feeder was placed at the original location, all birds searched by preference using position-based cues when tested with only their left eye and using feeder-specific cues when tested with only their right eye. More recently, using a working memory rather than a reference memory test, Regolin et al. [15] found that both object- and position-specific information is available to the two cerebral hemispheres in working memory; however, when a conflict between cues arises, the right hemisphere preferentially attends to position-specific cues, whereas the left hemisphere tends to attend to object-specific cues (and see also [25,30,38]).

The aim of this paper was to investigate whether asymmetric light stimulation of the embryo may affect relative reliance on object-specific and position-specific cues in chicks hatched from eggs exposed to light in the last days before hatching or maintained in darkness.

## 2. Materials and methods

### 2.1. Subjects and rearing conditions

The subjects were 458 Hybro *Gallus gallus* chicks (a local variety derived from the White Leghorn breed). The fertilized eggs came from two local commercial hatcheries (Avicola Berlanda Edio and C. Snc, Carmignano di Brenta, Padua, Italy and Agricola Berica, Montegalda, Vicenza, Italy) and were delivered to our laboratory weekly when the eggs were at day 14 of incubation. Thereafter, and until day 18, the eggs were incubated in the dark in the laboratory in an automatically turning incubator FIEM snc, MG 100H (45 cm × 58 cm × 43 cm), under controlled temperature (37.7 °C) and humidity (about 50–60%) conditions. On day 18 of incubation, the eggs were separated into two incubators

(60 cm × 65 cm × 66 cm), with a capacity of about 100 eggs, under the same temperature conditions and at a lower humidity level until hatching (which occurs on day 21). Half of the eggs ( $N = 117$ ) were kept in complete darkness also from day 18 of incubation onwards (D-chicks); the other half ( $N = 112$ ) were exposed to light from day 18 of incubation (L-chicks). A 25 W incandescent light bulb provided about 250 Lux within of the incubator. After hatching, the chicks were housed in pairs in metal home cages (22 cm wide, 30 cm large and 40 cm high) illuminated continuously by 30 W fluorescent lamps (placed 45 cm above the cages) and kept in standard rearing conditions (28–31 °C temperature and 68% humidity) with food and water available ad libitum. Chicks were reared in 229 pairs homogeneous with respect of gender: 122 pairs of males and 107 pairs of females (in this strain of chicks the sex can be determined soon after hatching by the arrangement of the wing feathers).

## 2.2. Procedure

The procedure consisted, for all pairs of chicks, of a training phase, in which chicks were required to learn to find food located in small cones on the floor of an arena (for details of the original procedure see [13]), followed by test trials within the same arena in which orienting cues to the food were experimentally manipulated. The test situation and the apparatus are shown in Fig. 1. The apparatus consisted in a white plastic rectangular arena (50 cm long, 30 cm wide and 10 cm high). The floor of the arena was uniformly covered by a grey waterproof sheet of paper. The arena had low edges from which the animals could observe the outside environment and spot the more relevant landmarks in the room to be possibly used as cues when orienting. The apparatus was placed on the ground of the experimental room, exactly in its centre. The room was 2 m long, 5 m wide and 2.5 m high and provided some large visual cues, such as a blue door, a tripod and a video camera, a white table with a television set and a video recorder on it, and a red chair for the experimenter. The experimenter stood by the arena throughout the testing trials in order to check the animals' behaviour which could also simultaneously be monitored through the video image recorded by the video camera and at the

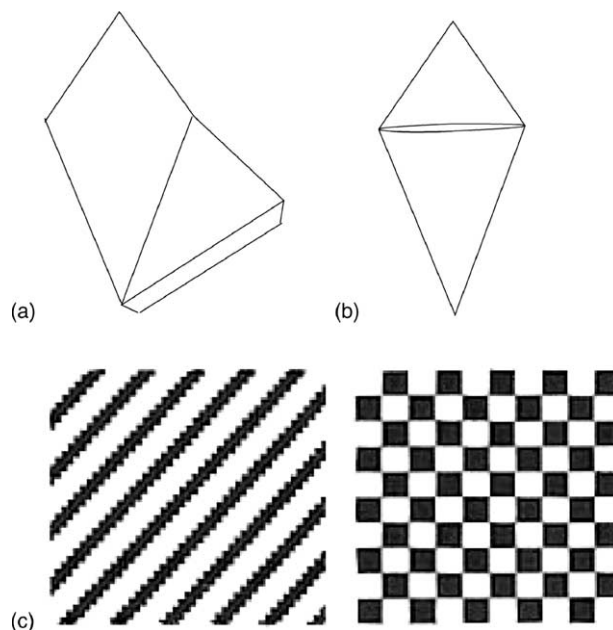


Fig. 2. The matrix of the stimuli employed for the learning trials and the test (a). The 3D stimulus folded to contain some grains of food (b). (c) The two different kind of textures: the white and black striped texture (positive cone) and the white and black checked texture.

same time projected on the connected television screen. The experimenter's position varied systematically between the pairs, but never changed between the various trials for the same pair. Also the arena was randomly rotated by 180° between testing of each pair in order to exclude the possible effect of cues not experimentally controlled.

Small cones (3 cm high with an open base of 1 cm in diameter) were used both for training and testing (Fig. 2a). The conical shape was obtained by accurately folding a little piece of waterproof material (3 cm high and 2.5 cm wide as shown in Fig. 2b) and the space inside of each cone could be used either to contain some grains of food to be used as reinforcement (reinforced cones) or could be left



Fig. 1. The apparatus used for training and testing, with the two chicks and the position of the two sets of cones.

empty (not reinforced cones). There were a total of eight cones, half of them were white and black striped, the other half were white and black checked (Fig. 2c). The two types of cones were comparable for amount of black and white surface. In preliminary trials, some birds were trained on either type of cone to exclude any preference for one of the two textures. Since neither learning differences (time to reach learning criterion) nor any kind of preferences (number of spontaneous pecks) for one particular type of cone were detected, we decided to arbitrarily choose one type of texture (the striped cones) and to use it as the positive (reinforced) stimulus throughout the experiment.

In the late evening of day 3 all chicks were food deprived. All the pairs underwent training and, thereafter, testing on day 4, starting in the early morning. For training, each pair of chicks was brought in turn in a little cardboard box to the experimental room nearby the rearing room, in which temperature and humidity were controlled (respectively, 25 °C and 70%); the room was illuminated with four neon bulbs of 58 W each. Chicks were placed in the same apparatus that would be later used for the testing, and trained to learn to find some food previously hidden by the experimenter inside the reinforced cones. The eight cones were positioned carefully in the arena so that all of the cones with the same pattern were located together on the same, longer side, of the arena, at a regular distance from one another. During the training, only the four positive cones (i.e., the striped ones) were reinforced (i.e., they contained some grains of chick crumbs); the checked cones, in contrast, were never reinforced (i.e., they never contained any food).

Each pair of chicks underwent five learning trials: the first lasted 2 min, the other four lasted 1 min only each. From one trial to the next chicks were placed back into the closed cardboard box to wait about 2 min for the apparatus to be ready for the next trial (the arena was cleaned any grains of food, the cones were filled with some new grains and correctly positioned in the arena). At any new training trial, as well as during testing, the starting position of each chick (i.e., at which end of the arena it was placed) was fully balanced across individuals.

All of the pairs of chicks learned quite rapidly to search for the hidden food within the striped cones, only requiring one or two trials in order to do so and no pair was discarded due to poor learning (i.e., three or four incorrect trials). After the first or second trial all chicks consistently pecked spontaneously only at the reinforced cones (ignoring the other type of cones).

After the learning trials, chicks were placed back into their home cage and randomly assigned to one of three viewing conditions. The monocular condition involved closure of the right eye for LE group (left eye-in-use) and the left eye for RE group (right eye-in-use). A total of 56 pairs were tested in RE condition, 56 pairs in LE condition and 117 in binocular condition (Binocular, BIN). The patching procedure is minimally disturbing for the animal, requiring handling for only a few seconds (the same time of handling was applied to the chicks of the binocular condition without applying any removable eye patch); it consists of gently placing a special removable tape onto one eye (the tape is cup-shaped and does not prevent the normal movements of the chick's eye lid). Following patching, all chicks were allowed 30 min in their own home cages to become accustomed to the new monocular condition.

For the test, chicks were brought again into the experimental room and gently placed in the arena each at a different end. The room conditions and the main landmarks (including the ex-

perimenter's position) were identical to those experienced during the learning trials. For the test (lasting 90 s), the position of the cones was reversed but not the position of the whole arena, which was the same in the training and test trials for any given pair of chicks.

The white and black striped cones (reinforced) were positioned along the side of the arena where the checked cones had been placed during training. For all of the measurements, "O" (object-specific cues) indicates that the first choice made by the chick was for the reinforced pattern of the cones (but now in the incorrect position) and "P" (position-specific cues) indicates the choice for the position of the cones that had been reinforced during the learning trials (but now of the incorrect pattern).

The test was video recorded and video recordings were subsequently scored: the arena was divided into two lateral sectors (12 cm wide) containing the cones, and a central sector (6 cm wide); in this way the experimenter could watch from above the animals while moving and categorise the choice for a side when a chick moved towards a particular type of cone (a choice was considered to have been made when the chick's head and most of its body had entered a given side sector).

Every measurement was recorded for each of the two chicks in a pair, but was then combined in order to classify the couples: "OO" and "PP" labelled those pairs in which both chicks expressed the same choice (i.e., "PP" = both chose the position of the cones, "OO" both went for the reinforced pattern of the cones); "PO" was the label indicating a non-homogeneous choice by the two chicks in a pair, i.e., the chicks each entered a different compartment or pecked at the two different types of cones.

### 2.3. Data analyses

The behavioural indices observed were:

- First side of entrance choice and first peck choice, labelled OO when the two members of the same couple expressed the same choice in the direction of the reinforced pattern but in the wrong spatial position, PP when they chose the cones in the correct spatial position (the same of the learning trials, but of different pattern) and PO when the two chicks expressed a different choice from each other.
- Latency of first side of entrance and latency to first peck calculated for each pair as the mean (expressed in seconds) of the two chicks' latencies. "Latency" is the time elapsed from placement in the arena of the two chicks and the entrance of each chick in one of the two lateral sectors (by one particular type of cone), or the first peck assigned. The mean of each pair was considered as a single data point because the behaviour of the two members of a pair cannot be considered as independent, as the members of a same pair might influence one another's behaviour.
- Time spent close to each of the two type of stimuli, expressed as mean of the times scored by the individual members of the pair.
- Total number of pecks to both types of cones expressed as mean pecks assigned by each pair of chicks.

For the ANOVA, the between-subject factors were: sex (male versus female), eye in use (LE versus RE versus BIN) and hatching (dark versus light).

Data for choice were analysed by chi-square tests; data for latencies, time spent in each sector and number of pecks were analysed by analysis of variance (ANOVA).

### 3. Results

#### 3.1. First side of entrance and first peck choice

Results for first side of entrance and first peck for binocular chicks are shown in Table 1. There was no significant heterogeneity between L- and D-chicks for either first side of entrance ( $\chi^2_{(2)} = 0.299$ , n.s.) and first peck choice ( $\chi^2_{(2)} = 0.488$ , n.s.). Also, no significant heterogeneity associated with sex was apparent for either first side of entrance (D-chicks:  $\chi^2_{(2)} = 3.293$ , n.s.; L-chicks:  $\chi^2_{(2)} = 0.393$ , n.s.) and first peck choice (D-chicks:  $\chi^2_{(2)} = 2.112$ , n.s.; L-chicks:  $\chi^2_{(2)} = 1.637$ , n.s.).

Lumping together data for the two sexes and the two light-exposure conditions, it appeared that binocular chicks showed preferential choice for object characteristics, either when all three categories of choice were considered (first side of entrance:  $\chi^2_{(2)} = 16.615$ ;  $p = 0.0002$ ; first peck choice:  $\chi^2_{(2)} = 22.741$ ;  $p = 0.0001$ ) or when only homogeneous choices were considered (first side of entrance:  $\chi^2_{(1)} = 3.375$ ;  $p = 0.066$ ; first peck choice:  $\chi^2_{(1)} = 19.000$ ;  $p = 0.0001$ ). Thus, analyses of first choices (entrance and peck) in binocular chicks revealed that the animals seemed to choose on the basis of the specific characteristics rather than on the basis of the position of the cones; no differences between the two hatching conditions were apparent.

Table 1

First side of entrance and first peck choices for the Binocular, LE and RE conditions

	First side of entrance			First peck		
	OO	PO	PP	OO	PO	PP
<b>BIN</b>						
F dark	16	3	13	12	9	6
M dark	15	7	7	17	7	3
Total dark	31	10	20	29	16	9
F light	16	6	10	16	9	5
M light	10	5	9	12	3	5
Total light	26	11	19	28	12	10
<b>LE</b>						
F dark	1	11	1	5	0	2
M dark	3	9	2	5	2	0
Total dark	4	20	3	10	2	2
F light	9	4	3	3	1	3
M light	2	7	4	4	3	3
Total light	11	11	7	7	4	6
<b>RE</b>						
F dark	1	11	1	5	0	2
M dark	3	9	2	5	2	0
Total dark	4	20	3	10	2	2
F light	9	4	3	3	1	3
M light	2	7	4	4	3	3
Total light	11	11	7	7	4	6

F, females; M, males.

The results for monocular chicks are shown in Table 1. Overall, first side of entrance in the monocular condition revealed no differences between D- and L-chicks (three categories:  $\chi^2_{(2)} = 5.234$ , n.s.; two categories (OO and PP only):  $\chi^2_{(2)} = 0.030$ , n.s.). When considering separately the two eye conditions, however, a clear difference emerged. A significant difference between the two hatching conditions was observed in left-eyed chicks ( $\chi^2_{(2)} = 7.418$ ,  $p = 0.025$ ), but not in right-eyed chicks ( $\chi^2_{(2)} = 0.476$ , n.s.). The effect was due to a higher value of heterogeneous choice among chicks of the same pair (i.e., PO choices, with one animal choosing position and the other choosing the pattern of the cones) in left-eyed chicks coming from eggs maintained in darkness. Among left-eyed D-chicks it seemed that most animals simply tended to enter the side facing the eye not covered by the eye-patch (binomial one-tailed 0.0748), without being affected by the characteristics of the cones (when considering only the homogeneous groups, OO and PP, no differences were observed,  $\chi^2_{(1)} = 0.033$ , n.s.).

First peck choice in the overall monocular group revealed a striking difference between incubation conditions: D-chicks pecked mostly at the cones with the correct pattern, ignoring position (three categories of choice:  $\chi^2_{(2)} = 13.852$ ,  $p = 0.017$ ; two categories (OO and PP only):  $\chi^2_{(1)} = 12.848$ ,  $p = 0.005$ ); L-chicks, in contrast, did not show any clear choice ( $\chi^2_{(2)} = 1.841$ , n.s.). Thus, D-chicks preferred at first to peck at the cones with the correct pattern largely ignoring their position. In contrast, no clear choice appeared in L-chicks.

#### 3.2. Total number of pecks

Results for total number of pecks at the position of the cones or pattern are shown in Fig. 3. The analysis of

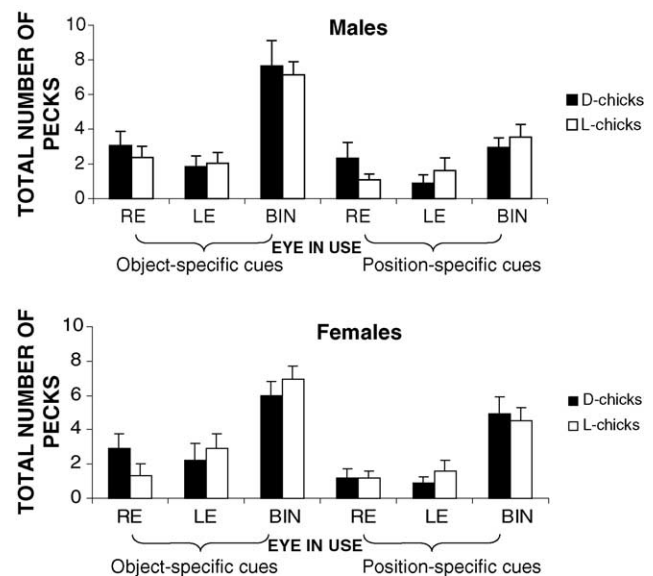


Fig. 3. Total number of pecks (group means  $\pm$  S.E. are shown).

variance with sex (male versus female), eye in use (LE versus RE versus BIN) and hatching (dark versus light) as between-subject factors and choice (pecks on pattern versus pecks on position), revealed the significant main effects of eye ( $F_{(2,217)} = 53.366$ ,  $p = 0.0001$ ) and of choice ( $F_{(1,217)} = 40.406$ ,  $p = 0.0001$ ).

An analysis restricted to the two monocular conditions revealed, however, a significant eye  $\times$  hatching interaction ( $F_{(1,104)} = 4.574$ ,  $p = 0.0348$ ). Separate analyses for the LE and RE conditions revealed a significant effect of choice for LE chicks ( $F_{(1,52)} = 5.558$ ,  $p = 0.0222$ ) and for RE chicks ( $F_{(1,52)} = 12.748$ ,  $p = 0.0008$ ) but no other main effects nor interactions (LE sex:  $F_{(1,52)} = 0.002$ ,  $p = 0.9669$ ; hatching:  $F_{(1,52)} = 1.863$ ,  $p = 0.1781$ ; sex  $\times$  hatching:  $F_{(1,52)} = 0.114$ ,  $p = 0.7367$ . RE sex:  $F_{(1,52)} = 3.007$ ,  $p = 0.0888$ ; hatching:  $F_{(1,52)} = 1.477$ ,  $p = 0.2297$ ; sex  $\times$  hatching:  $F_{(1,52)} = 1.167$ ,  $p = 0.2851$ ). As can be seen from Fig. 3, it seems therefore that, irrespective of whether pecks were directed at the pattern of the cones or position, in RE-chicks there was more pecking by D-chicks, whereas in LE-chicks there was more pecking by L-chicks.

### 3.3. Latencies of first side of entrance and to first peck

Here the analyses were restricted to the homogeneous (PP and OO) pairs only, to avoid lumping together in a single data point the pair latencies in cases of heterogeneous choice (i.e., PO choices).

Results for latencies of first side of entrance are shown in Fig. 4. The ANOVA revealed significant main effects of sex ( $F_{(1,136)} = 3.977$ ,  $p = 0.0481$ ) and eye in use ( $F_{(2,136)} = 13.805$ ,  $p = 0.0001$ ) as well as of the sex  $\times$  eye interaction ( $F_{(2,136)} = 3.603$ ,  $p = 0.0299$ ). There were no other significant effects (hatching:  $F_{(1,136)} = 0.002$ ,  $p = 0.9656$ ; sex  $\times$  hatching:  $F_{(1,136)} = 0.975$ ,

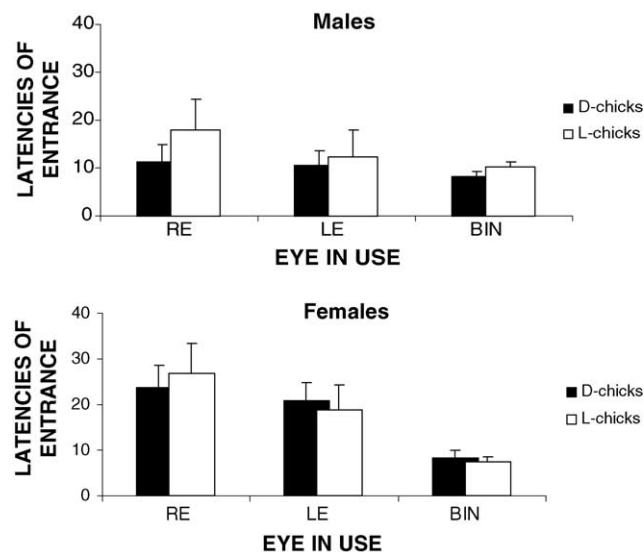


Fig. 4. Latencies of first side of entrance (group means  $\pm$  S.E. are shown).

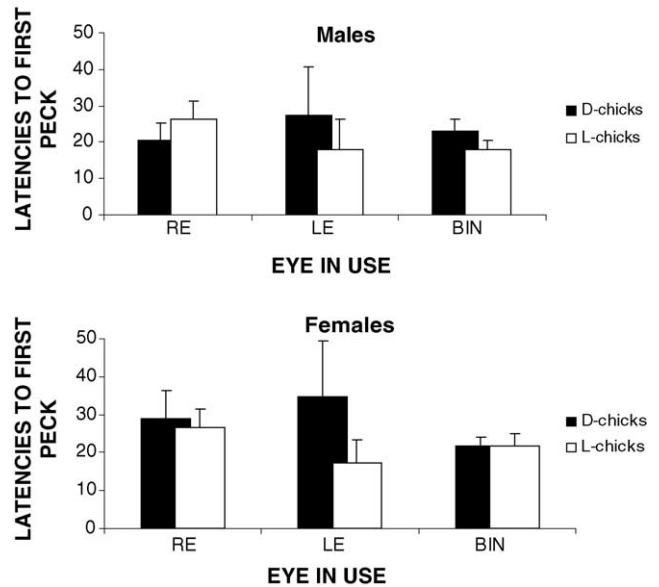


Fig. 5. Latencies to first peck (group means  $\pm$  S.E. are shown).

$p = 0.3251$ ; eye  $\times$  hatching:  $F_{(2,136)} = 0.353$ ,  $p = 0.7031$ ; sex  $\times$  eye  $\times$  hatching:  $F_{(2,136)} = 0.760$ ,  $p = 0.4696$ ).

Overall, females showed longer latencies of first side of entrance than males. However, an analysis restricted to the monocular groups revealed a significant main effect of sex ( $F_{(1,44)} = 4.195$ ,  $p = 0.0465$ ), without any sex  $\times$  eye ( $F_{(1,44)} = 0.075$ ,  $p = 0.7855$ ) interaction, thus revealing that sex differences were apparent only in the monocular conditions but not in the binocular condition. This was likely due to a floor effect, because binocular chicks had shorter latencies than monocular chicks. Post hoc analyses (Fisher Protected LSD) revealed that latencies of BIN were shorter than those of both RES ( $p = 0.0001$ ) and LES ( $p = 0.0066$ ), whereas latencies of LES tended to be shorter than those of RES ( $p = 0.0916$ ).

Latencies to first peck are shown in Fig. 5. The ANOVA did not reveal any statistically significant effect (sex:  $F_{(1,136)} = 0.490$ ,  $p = 0.4852$ ; eye in use:  $F_{(2,136)} = 1.984$ ,  $p = 0.1415$ ; hatching:  $F_{(1,136)} = 0.532$ ,  $p = 0.4670$ ; sex  $\times$  eye:  $F_{(2,136)} = 1.809$ ,  $p = 0.1677$ ; sex  $\times$  hatching:  $F_{(1,136)} = 1.860$ ,  $p = 0.1749$ ; eye  $\times$  hatching:  $F_{(2,136)} = 0.324$ ,  $p = 0.7237$ ; sex  $\times$  eye  $\times$  hatching:  $F_{(2,136)} = 1.151$ ,  $p = 0.3192$ ).

## 4. General discussion

First choices (entrance and peck) showed that choices of binocular chicks were mainly based on object-specific cues (the characteristics of the cones) rather than on position-specific cue (the location of the cones). Thus, it seems that in this task object-specific cues are dominant. No differences were observed between L- and D-chicks in the binocular condition.

Monocular tests revealed, however, some interesting differences between D- and L-chicks. D-chicks preferred to peck

first at the cones with the correct pattern, largely ignoring their position. They thus behaved mostly as binocular chicks did. In contrast, no clear choice was seen in L-chicks. This lack of choice was unlikely to be due to a difficulty in performing the discrimination. It seemed as if L-chicks were affected by cues that were instead ignored by D-chicks.

Another difference between D- and L-chicks was that, although no evidence for a difference between LE and RE chicks was apparent for pecking choice, D-chicks tended to use the eye not occluded for choice of first entrance and this was particularly striking for LE chicks. Assuming (see Section 1) that the left eye (right hemisphere) should be mainly attending to spatial cues, this suggests that D-chicks tend to neglect positional cues, which would also agree with evidence of choice for object-specific cues in first pecking by D-chicks. If this is correct, then lack of clear choice in L-chicks could be due to the fact that these chicks were more affected by positional cues than D-chicks, which might have resulted in an imbalance in choice between object- and position-specific cues in L-chicks.

Irrespective of whether pecks were directed at the cones' pattern or position, in RE chicks more pecking was scored by D-chicks, whereas in LE chicks more pecking was scored by L-chicks. There is evidence that the left hemisphere drives pecking to feed more than the right hemisphere [17]. Since RE D-chicks are exclusively dependent on the left hemisphere (see below) they would have a stronger tendency to peck. The LE chicks would need to access the left hemisphere, done better by the L- than the D-chicks.

In general, BIN chicks appeared to be more active than monocular chicks (they pecked more and made choices more rapidly). This was expected because the eye-patching procedure might produce some disturbance to the animals. However, there was an intriguing difference between RE and LE chicks in latencies of first choice: monocular chicks were in general the slower group to make a choice, but particularly when the right eye was in use. LE chicks were faster than RE chicks, but slower than binocular chicks. RE chicks, always slower than the other groups, spent most of the time close to the same type of cone: once they chose using a local or a global feature (the pattern or the position) they tended to remain in the same spatial position. It could be that LE chicks were similar in behaviour to BIN chicks because of a major right hemisphere involvement; the right hemisphere may be partly responsible for redirection of the first choice due to its more diffuse attention, whereas the left hemisphere would show more focussed attention (see [20,21]).

The most important result of the experiments seems to be that light-stimulation of the embryo makes it more likely that chicks use both types of information available. D-chicks seemed to orient mainly on the basis of the seeing eye, i.e., by the contralateral hemisphere. L-chicks in contrast seemed to be affected by both positional and object cues. Why, however, were no differences observed in binocular chicks? It could simply be a matter of the amount of difference perceived between stimuli: we have indeed preliminary data showing

that L- and D-BIN chicks do differ in their relative use of allocentric spatial cues and object-specific cues provided that the difference between the stimuli is large. Another, more intriguing possibility is that the effect of light stimulation is to make it possible for the non-stimulated hemisphere to take part in the control of overt behaviour. What is striking in the behaviour of eye-patched D-chicks is that their choice seems to reflect only the visual information available to the non-occluded side. This means that the hemisphere, which does not receive input takes no part in the decision on the course of action. In L-chicks, in contrast, choice seems to take into account positional cues even when only the right eye is in use (i.e., even when the left hemisphere, which is allegedly not specialised for attending to spatial cues, is receiving the main sensory input). This strongly suggests that in L-chicks, but not in D-chicks, the hemisphere which does not receive sensory input (because of the eye patching) is nonetheless able to affect the behaviour of the "seeing" hemisphere. This account of the data may explain why no effects of light and dark conditions can be observed in binocular chicks, as in binocular chicks both eyes receive direct sensory inputs.

Of course the hypothesis is still speculative and will require further research. It is also unclear, at present, how these effects of light stimulation of the embryo can be related to the known anatomical asymmetries in the thalamofugal pathway (see Section 1). But, if correct, the hypothesis suggests that the role of asymmetric light stimulation of embryos may have to do more with interhemispheric communication than with the allocation of separate functions into the left and right hemispheres.

## Acknowledgements

The experiments comply with the current Italian and European Community laws for the ethical treatment of animals. The research was supported by grants MIUR Cofin 2004, 2004070353\_002 "Intellat" and MIPAF "Benolat" to G.V.

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