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## **Influence of pre- and post-hatching experience on spatial behaviour of domestic chicks**

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## **Abstract**

We tested the hypothesis that domestic chicks incubated in the light and provided with experience of moving out of sight of each other (occlusion experience) during rearing would have better spatial cognitive abilities than control chicks. Ninety-six chicks were incubated in the light or dark and half the chicks had the opportunity to move behind opaque barriers from 10-12 days of age and the remainder we provided with identically-sized transparent screens as a control. Chicks were tested in a detour test, a rotated floor test and their dispersal was observed in a large pen. Chicks reared with opaque screens used distal cues significantly more when searching for their companion in a rotated floor test ( $P=0.042$ ) and light incubated chicks tended to make a choice in less time than dark incubated chicks in this test ( $P=0.089$ ). There were no significant differences in the time taken for the chicks to solve the detour test depending on the chicks' occlusion experience or incubation environment, possible because the ability of chicks to use the sounds from the social goal masked any differences in orientation ability. Incubation and occlusion treatments were not found to have a significant effect on dispersal. We conclude that although minor manipulation of the incubation and rearing environment affect the use of spatial information and ease of processing of conflicting cues in chicks, it is unclear whether these changes in behavioural phenotype translate to actual advantages in movement, dispersal and welfare of chickens in loose-housed commercial systems.

## **Introduction**

Chick embryos are positioned in the egg so that the right eye is directed towards the eggshell and the left eye directed towards the chick's body. If the egg is exposed to light only the right eye receives light leading to asymmetry in the thalamofugal visual projections to the forebrain (Deng and Rogers, 1997). Chicks which are exposed to light during incubation are more strongly lateralized for processing visual information than are dark-incubated ones (Rogers, 1996). The advantage of being lateralized is considered to be due to the two hemispheres being able to process different information (Vallortigara et al 2004) and thus the brain can be used more efficiently. Recently, light incubated chicks, and therefore more visually lateralized chicks, were shown to be better at directing pecks at food items scattered on a pebble floor whilst simultaneously being vigilant for a model overhead predator than dark incubated chicks (Dharmaretnam and Rogers, 2005; Rogers et al., 2004), supporting the above hypothesis.

Spatial navigation and orientation is among the best studied lateralized functions and evidence that the two sides of the brain contribute differently to spatial orientation has been shown in mammals and birds (Colombo and Broadbent, 2000). The right hemisphere is dominant for processing spatial information in chicks (Rashid and Andrew, 1989; Vallortigara and Regolin, 2002; Tommasi et al., 2003), as well as in humans (Witelson, 1976). Recent evidence suggests that in a working memory task

chicks could use both hemispheres for object and position specific information, but when there was a conflict between different cues the right hemisphere attended to position specific cues and the left hemisphere attended to object specific cues (Regolin et al., 2005). In accordance with these findings it has been found that chicks using their right hemisphere tend to pay more attention to distal cues whereas chicks using their left hemisphere relate more on proximal cues or search in random (Freire and Rogers, 2005; Rashid and Andrew, 1989).

One interesting aspect of lateralization is that changes in chick's behaviour coincide with changes in hemisphere dominance. This has been shown in broods reared in semi natural conditions which began to move independent of the mother hen and going out of sight from her on day 10 (Vallortigara et al 1997). At this age, there is also a shift towards control of the left eye system (Rogers and Elrich, 1983; Workman and Andrew, 1989). In the laboratory, providing chicks with the ability to move out of sight of an imprinting stimulus, by using opaque screens, has been found to lead to improved orientation in a detour test and better retrieval of a visually displaced goal (Freire et al 2004). The timing in behavioural changes and hemispheric dominance has recently been shown to lead to an improvement in the ability of chicks to use non-local (i.e. distal) spatial information, since the same experience at ages when the right hemisphere is not dominant does not lead to the improvement in the use of distal spatial cues (Freire and Rogers, 2007).

Today, it is becoming more common in many parts of the world to keep laying hens in loose-house (i.e. large floor groups) commercial systems (Tauson 2005). These systems can be rather large and complex and put demands on the birds' ability to move around and find resources such as a range, perches, food and water. One concern is that commercial rearing, which can be in small, barren cages fails to provide chicks with the necessary opportunities to develop the spatial cognitive abilities to move in large loose-house systems later in life.

The hypothesis being tested was that a more lateralized chick with experience of occlusion at 11 days of age would have better spatial cognitive abilities than non-lateralized, barren-reared, chicks. To investigate this we examined chicks incubated in the light or dark, and gave half these chicks the opportunity to move out of sight at day 10-12 and the remainder we provided with identically-sized transparent screens as a control. Chicks were tested in a detour test, a rotated floor test and their dispersal was observed in a large pen.

## **Materials and methods**

### *Animals and treatment*

Ninety six White Leghorn x Australorp domestic chicks (Barter and Sons, Luddenham, NSW) in five batches were used. Chicks had been exposed to both natural and fluorescent light in the incubator (Li treatment) or incubated in the dark from 17 days of incubation till hatching (Da treatment). Six groups of eight chicks per treatment were used. All chicks were marked individually with a coloured leg band and painted with a whiteboard marker on the back with one of four different patterns (+, -, · and •) in either black or red.

### Housing (day 2-9 and day 13-15)

From arrival until day 9 the chicks were housed in cardboard boxes 40 x 40 x 60 cm (length x width x height) with wood-shavings on the floor and ad libitum access to water and food. The water was provided in bird drinkers and the food was starter crumbs provided in a glass bowl (12 cm wide and 5 cm high) placed in a corner of the box. Room temperature was kept at  $28 \pm 4^\circ$  C. The light schedule was 16:8 light:dark cycle with lights on from 8.00h, until 24.00h.

Prior to lights-off on day 12, the chicks were returned to groups of eight in a cardboard box, as during days 2-9.

### Occlusion observations and housing (day 10-12)

On the morning of day 10 the chicks were paired and each pair was placed in a new 40 x 40 x 60 cm box with two screens 20 x 30 cm (width x height) placed parallel to each other 20 cm apart. The food bowl was placed in the middle of the box between the screens and a drinker was placed on one side. Half of the chicks had transparent screens, made of 3 mm Perspex, and the other half had opaque wooden screens to provide experience of occlusion, provided in a 2x2 factorial design. Scan sampling every 5 minutes for 2h (10.00am -12.00pm) were carried out every day from day 10 until day 12 to record whether the chicks were on different sides of the screens and thus, if having opaque screens, being out of sight of each other.

### Dispersal pens, housing and observations (day 16-17)

In the morning on day 16 the chicks were moved to the dispersal pens. These pens measured 1.5 x 1.5 m and the sides were 0.6 m high and made of wire-mesh. The pens contained one drinker and one food bowl. The floor was covered with sheets of cardboard with a grid pattern made by sticky tape. Each square in the grid was 30 x 30 cm and in total there were 25 squares per pen. Wood shavings were scattered on the floor. The walls of the pen facing another pen with chicks were covered by up to 30 cm so the chicks could not see the chicks from the other groups unless they went up on the perch. The squares were numbered from 1-5 and A-E thus giving each square its own code with a number and letter combination.

Approximately 30 minutes after four chicks had been released into the pens the first observations were carried out. Two pens were observed at the same time and scans were made at one minute intervals to record the square/squares that the chicks were located in. Each observation period continued for 20 minutes and was repeated 6 times per day over two consecutive days.

### *Behaviour tests*

### Detour test (day 13)

A detour test was carried out on all chicks on day 13. The arena was 156 cm long, 50 cm wide and 60 cm high with solid walls and wood shavings covering the floor. In one end of the arena there was a companion cage of wire-mesh measuring 25 x 25 x 25 cm (width x length x height). The detour screen was U-shaped 34 cm long, 23 cm wide and 32 cm high with the short side made of wire mesh (bottom of the U) facing the companion cage and the two flanking sides made of cardboard.

The group of four chicks from the same box was placed in the companion cage in the test arena and after one minute of adaptation one chick was taken out and placed behind the detour screen. The time taken to reach the companions and the number of orientation errors (leaving detour cage but returning back in again) were recorded. All chicks in the group were tested in succession. Detour tests lasting more than 10 minutes were aborted and these chicks were returned to the companion cage.

### Rotated Floor test (day 14-15)

Eight randomly selected chicks from each treatment were used in the Rotated Floor test. Each chick was tested five times. The test arena was 156 cm long, 50 cm wide and 60 cm high with solid walls and wood shavings covered the floor. Two screens were placed parallel to the short side of the arena opposite each other and 50 cm from the middle of the arena. One screen was white and one screen had a coloured pattern (yellow, red, green and blue). Training involved placing a chick in a start cage in the middle of the arena with a companion chick in a small wire cage behind one of the screens. The chick was then released and if it approached the companion chick, it was allowed to remain there for 30s, before being returned to the middle of the arena. If the chick did not manage to reach the companion chicks within 3 minutes, it was gently pushed behind the screen where the other chick was and given approx 30s with its companion. This procedure was repeated until the chick reached the criteria of approaching the companion chick twice in succession, and without going behind the other screen, within one minute.

After reaching the training criterion, chicks were tested with the apparatus rotated by 180 degrees. The chick was again placed in the middle of the arena in the start cage but for tests there was no companion chick (i.e. test trials were unrewarded). After five seconds the start cage was removed and the time taken for the chick to make a choice- measured as going behind one of the screens was recorded. If the chick chose the same screen as the companion had been hidden behind that was scored as a choice using proximal cues (i.e. the colour of the screen). If the chick chose the same direction as during training, it was scored as a choice using distal cues (i.e. cues outside the apparatus).

After testing the chick was put back into its home box. The chick then had to complete a successful training trial before its next test. Each chick was tested five times.

### *Statistical analysis*

We excluded the chicks that did not solve the detour test. The duration taken to solve the test was not normally distributed, even after several transformations. We have performed a GLM on the log transformed detour time with incubation and occlusion treatments as fixed factors, as this transformation yielded that closest to a normal distribution.

The number of squares that chicks used in total per scan ranging from 1-4 were noted and also the mean distance between all the chicks in the group. Each

chick was given a score depending on the distance to its nearest neighbour and the sum of all the chicks scores in the group were then divided by the number of the chicks in the group. If two chicks were in the same square the distance between them were scored as 0 and if a chick were in a square next to another chick they each got score 1, having one square between one chick and the one closest to it gave a score of two and so forth up until four (which was the maximum distance for an individual chick). The maximum average for the whole group was 4 (4 squares x 4 chicks / 4chicks) if there were one chick in each corner of the pen, and the minimum for the whole group zero if they were all located in the same square.

## Results

### *Occlusion experience*

A significant incubation treatment and occlusion treatment interaction was found ( $F_{1,38}=4.13$ ,  $P=0.049$ ), suggesting that Da chicks spent more time out of view of each other than Li chicks, but Li chicks spent more time on different sides of transparent screens than Da chicks (Figure 1).

### *Detour test*

Seventy chicks managed to solve the detour test in  $92.0\pm 13.3$ sec, and 8 chicks did not manage to solve the detour test within 10 min. There were no significant difference between the time it took for the chicks to solve the detour test depending on the chicks occlusion experience (ANOVA:  $F_{1,99}=1.23$ ,  $P=0.256$ ) or incubation environment ( $F_{1,99}=1.69$ ,  $P=0.196$ ), nor were there any interaction between these two factors ( $F_{1,99}=0.24$ ,  $P=0.625$ ; Table 1).

Li chicks detoured around the right screen on  $x\pm\text{sem}\%$  of tests, and Da chicks on  $y\%$  of tests (Chi-squared test,  $\chi^2 = x$ ,  $P=0.80$ ). Chicks provided with opaque screens detoured around the right screen on  $x\pm\text{sem}\%$  of tests, and chicks reared with transparent screens on  $y\%$  of tests (Chi-squared test,  $\chi^2 = x$ ,  $P=0.38$ ; Table 1).

### *Rotated Floor test*

Chicks reared with opaque screens used distal cues significantly more when searching for their companion in the Rotated Floor test (ANOVA:  $F_{1,28}=4.56$ ;  $P=0.042$ ) than chicks reared with transparent screens. But there was no significant effect of incubation experience ( $F_{1,28}=0.13$ ;  $P=0.723$ ) or a significant interaction between occlusion and incubation treatments ( $F_{1,28}=0.12$ ;  $P=0.733$ ). The non occlusion experienced chicks did not use proximal cues more than distal cues (T-test of mean=50%:  $t=-1.28$ ;  $P=0.22$ )

Li chicks tended to make a choice in less time than Da chicks ( $25.3\pm 3.4$ s and  $18.6\pm 1.9$ s respectively, ANOVA:  $F_{1,28}=3.11$ ,  $P=0.089$ ). There were no significant effects of occlusion experience ( $F_{1,28}=2.43$ ,  $P=0.130$ ) or interaction between occlusion experience and incubation treatment on latency to make a choice ( $F_{1,28}=0.39$ ,  $P=0.535$ ).

### *Dispersal observations*

Incubation and occlusion treatments were not found to have a significant effect on dispersal (ANOVA, incubation  $F=0.477$ ,  $P=0.500$ ; occlusion  $F=0.851$ ,  $P=0.371$ ; interaction, ??). Chicks initially stayed relative close to each other the first day but tended to disperse more on the second day (ANOVA,  $F=3.942$ ,  $P=0.066$ ). However, nearest neighbour distances were not significantly influenced by incubation treatment ( $F=1.564$ ;  $P=0.230$ ), occlusion treatment ( $F=0.529$ ;  $P=0.478$ ) or their interaction (??).

## Discussion

In summary, chicks provided with experience of wooden screens at 10-12 days of age used large-scale, distal, spatial information in a rotated floor test more than chicks provided with experience of transparent screens. Additionally, light incubated chicks had a shorter response latency than dark incubated chicks in the rotated floor test,

perhaps indicating an advantage in processing conflicting information. Incubating treatment and occlusion experience had little effect on behaviour in the detour test or the dispersal test, and several limitations of these tests are considered.

In this study, pairs of chicks were only rarely observed on different sides of the wooden screens, perhaps indicating a reluctance to be out-of-sight of each other. Nonetheless, even this small amount of experience of being out-of-sight was enough to account for a greater use of distal cues in the rotated floor test, as has been reported previously (Freire and Rogers 2007). The amount of occlusion experience necessary to produce this bias in the use of distal cues is not known, but it is likely that active, rather than passive, occlusion experience is critical to the change in use of distal cues (Freire et al 2004).

Additionally, Li chicks tended to make a choice faster in the rotated floor test than Da chicks, possibly indicating an advantage in the processing of conflicting spatial cues. Previously, Li chicks have been found to be better at dual tasks, that is, tasks involving the simultaneous processing of two types of information than Da chicks (Dharmaretnam and Rogers, 2005; Rogers et al., 2004). In the rotated floor test, proximal and distal spatial information are simultaneously presented in conflict. Studies involving monocular tests of spatial memory suggest that the right hemisphere is primarily concerned with processing and responding to distal information, whereas the left hemisphere is concerned with proximal cues (Rashid and Andrew, 1989; Tommasi and Vallortigara 2004; Freire and Rogers, 2005). Thus our findings suggest that in addition to the known advantage of visual lateralisation in dual tasks, visual lateralisation arising from Li incubation may also yield an advantage in processing conflicting spatial information.

The lack of difference between treatments in the detour test was perhaps not unexpected. Previously, only subtle differences in the number of orientation errors were found between occlusion-experienced and control chicks (Freire et al 2004) using an imprinting stimulus. In the present study, the use of companion chicks, which would call and respond to calls, is likely to have aided the test chick particularly when it was out-of-sight behind the detour screen.

The reason for a lack of differences between treatments in the dispersal test could be due to no difference between chicks or the test not being sensitive enough to detect these differences. The arena used in this experiment was only 1.5 x 1.5 m, and this was perhaps not sufficiently large to reveal differences in dispersal due to differences in spatial abilities. It is yet to be determined whether the incubation and rearing effects in spatial tests presented here would translate to variations in dispersal and movement of chickens in adulthood in loose-housed commercial systems.

In conclusion, minor manipulation of the incubation and rearing environment affect the use of spatial information and ease of processing of conflict cues in chicks, indicating that these manipulations alter the behavioural phenotype of chickens. However, it is unclear whether these changes in behavioural phenotype will translate to actual advantages in movement, dispersal and welfare of chickens in loose-housed commercial systems.

## Acknowledgements

## References

Table 1. Mean time to solve detour test, mean number of orientation errors and the choice of direction (right or left) by chicks from each treatment.

Treatment	N	Mean $\pm$ se Detour time(s)	Mean number of orientation errors	Direction	
				N Right	N Left
Da, wooden screens.	16	64.6 $\pm$ 21.1	0.50 $\pm$ 0.33	7	9
Da, transparent screens	18	134.3 $\pm$ 38.5	1.28 $\pm$ 0.54	5	13
Li, wooden screens	17	75.6 $\pm$ 15.8	0.47 $\pm$ 0.21	10	7
Li, transparent screens	19	89.7 $\pm$ 23.0	0.74 $\pm$ 0.45	9	10

## Figure legends

Fig 1. Mean $\pm$ se number of observations of chicks on different sides of the opaque and transparent screens, showing a significant interaction of incubation and occlusion treatment (P=0.049).

Fig 2. Mean $\pm$ se percentage use of distal cues by chicks from different treatments when searching for the companion chick in the Rotated Floor Test. Chicks reared with opaque screens used distal cues more than chicks reared with transparent screens (P=0.04).

Fig 1

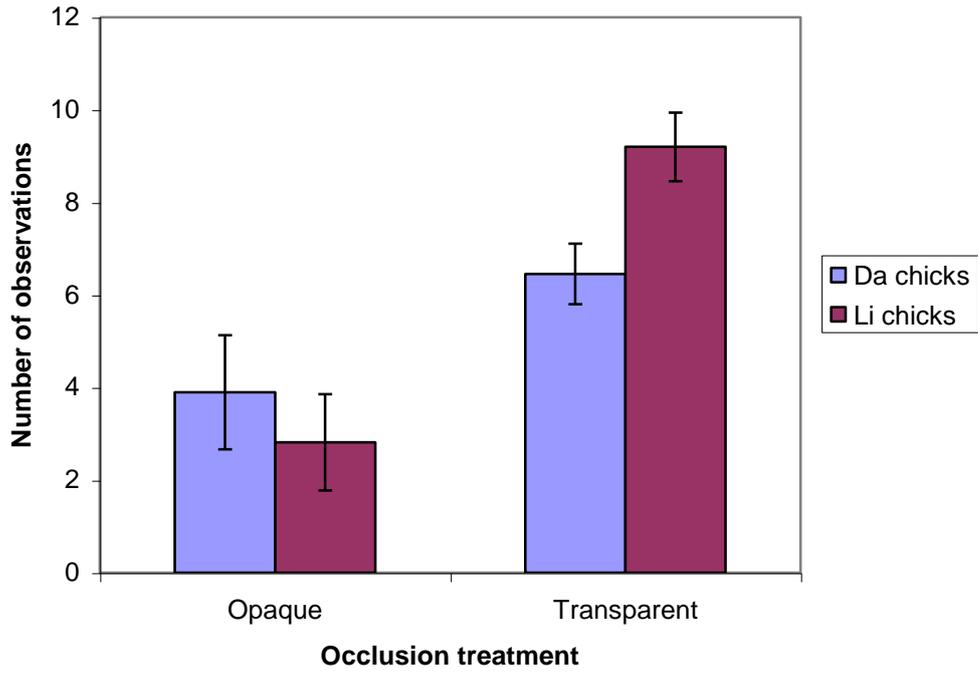


Fig 2

