Abstract: We examined whether experience of opaque or transparent screens modulates the use of distal or proximal cues for spatial orientation by domestic chicks. Chicks were housed in isolation with a ball suspended in the middle of the cage to serve as an imprinting stimulus. At 8 days of age chicks were re-housed in larger cages in pairs, and from 10-12 days of age either two opaque screens or two transparent screens were placed either side of the imprinting stimulus. Chicks could lose sight of the imprinting stimulus or their cage mate in cages with opaque screens, but were unable to do so in cages with transparent screens. At 13 and 14 days of age, chicks were trained to find the imprinting stimulus behind one of two screens. For half the chicks the screens were identical (both white) and for the remainder they were distinctly different (one was white and the other was covered in a coloured pattern). After training, unrewarded probe tests were presented to chicks using both eyes (BIN), the left eye (LES) or right eye (RES) with the apparatus rotated by 1800 so that proximal and distal cues indicated opposite sides of the apparatus. Generally, chicks chose the screen on the distal side more often and with a shorter latency in probe tests with identical screens than in probe tests with different screens. In probe tests with different screens proximal cues were used more frequently, although LES chicks chose the distal side more than the proximal side, whereas RES chicks chose the proximal side more than the distal side. In BIN probe tests with different screens, chicks reared in cages with opaque screens were quicker in making a choice, and tended to choose the screen on the distal side more often than chicks reared in cages with transparent screens. They also failed to chose a screen less often than the latter. Additionally, chicks reared in cages with opaque screens failed to make a choice less often than chicks reared in cages with transparent screens in monprobe tests with identical screens. The results suggest that chicks using the LES are less easily disrupted by conflicting proximal cues than chicks using the RES, and that experience of visual barriers from 10-12 days of age improves the chicks ability to attend to distal cues.

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Experience-induced modulation of the use of spatial information in the domestic chick

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ABSTRACT

We examined whether experience of opaque or transparent screens modulates the use of distal or proximal cues for spatial orientation by domestic chicks. Chicks were housed in isolation with a ball suspended in the middle of the cage to serve as an imprinting stimulus. At 8 days of age chicks were re-housed in larger cages in pairs, and from 10-12 days of age either two opaque screens or two transparent screens were placed either side of the imprinting stimulus. Chicks could lose sight of the imprinting stimulus or their cage mate in cages with opaque screens, but were unable to do so in cages with transparent screens. At 13 and 14 days of age, chicks were trained to find the imprinting stimulus behind one of two screens. For half the chicks the screens were identical (both white) and for the remainder they were distinctly different (one was white and the other was covered in a coloured pattern). After training, unrewarded probe tests were presented to chicks using both eyes (BIN), the left eye (LES) or right eye (RES) with the apparatus rotated by 180^{0} so that proximal and distal cues indicated opposite sides of the apparatus. Generally, chicks chose the screen on the distal side more often and with a shorter latency in probe tests with identical screens than in probe tests with different screens. In probe tests with different screens proximal cues were used more frequently, although LES chicks chose the distal side more than the proximal side, whereas RES chicks chose the proximal side more than the distal side. In BIN probe tests with different screens, chicks reared in cages with opaque screens were quicker in making a choice, and tended to choose the screen on the distal side more often than chicks reared in cages with transparent screens. They also failed to chose a screen less often than the latter. Additionally, chicks reared in cages with opaque screens failed to make a choice less

often than chicks reared in cages with transparent screens in monocular probe tests

with identical screens. The results suggest that chicks using the LES are less easily

disrupted by conflicting proximal cues than chicks using the RES, and that experience

of visual barriers from 10-12 days of age improves the chick's ability to attend to

distal cues.

Changes to the environment early in life, such as the addition of nesting material and other objects to cages, have been found to improve spatial memory in rats (Pham et al. 1999) and mice (Williams et al. 2001). Providing domestic chicks with the opportunity to move around visual barriers between days 8 and 12 has been found to improve spatial memory, as assessed by visual displacement and detour tests, and compared to chicks provided with similar sized transparent barriers (Freire et al. 2004). Visual barriers allow chickens to move out-of-sight of conspecifics, an action that shows a peak at around 11 days of age in semi-natural environments (Vallortigara et al. 1997) and appears to be one of the factors that contributes to the apparent sensitive period for effective spatial learning in chickens around this age (Rashid & Andrew 1989; Andrew 1991; Dharmaretnam & Andrew 1994). Presently, it therefore seems likely that environment-induced modulation of spatial cognition could be a reasonably common phenomenon in vertebrates.

As in mammals, the right hemisphere in birds appears to play a key role in the processing of spatial information (Vallortigara & Regolin 2002). In birds, hemispheric specialisation can be shown by monocular testing, since the complete decussation at the optic chiasma allows information from each eye to be processed largely by the contralateral hemisphere (Rogers 1995). Rashid & Andrew (1989) trained domestic chicks to find food in one corner of a square tray with each wall painted differently and then examined the search patterns when the chicks were presented with the tray rotated by 180^0, so that proximal (intra-tray) cues indicated one corner and distal (extra-tray) cues indicated the opposite corner. Using monocular testing, they showed that at certain ages chicks using the left eye system (LES, and so the right hemisphere) made more use of distal (extra-apparatus) cues than chicks using the right eye system (RES). With domestic chicks trained to find food in the centre of an

arena, Tommasi & Vallortigara (2001) found that, when they were tested in a larger arena, chicks using the LES searched in the centre, whereas chicks using the RES searched on the basis of absolute distance from the walls. Similarly, Marsh tits using monocular retrieval of cached food items use relative spatial position when using the LES but only local cues when using the RES (Clayton & Krebs 1994). Hence, monocular testing of birds in spatial tasks has proved to be a useful technique for identifying the role of each hemisphere in spatial processing.

Additionally, lesioning of the hippocampus in birds impairs spatial memory but has little effect on other cognitive tasks (Hampton & Shettleworth 1996; Fremouw et al. 1997), and spatial memory in lesioned zebra finches can be improved by transplantation of embryonic tissue into the hippocampus (Patel et al. 1997). Recent work on domestic chicks showed that dendrite length and linear spine density were greater in the right compared to the left hippocampus (Freire & Cheng 2004), and lesioning of the right hippocampus, but not the left hippocampus or a sham operation, impaired relocation of hidden food using relative positional information (Tommasi et al. 2003). Thus the chicken brain is lateralized in its processing of spatial information - the right hippocampus processes distal and geometric cues but the left hippocampus does not.

Evidence that experience during development may modulate the above lateralization in spatial cognition and hippocampal function has, however, proved to be elusive. Freire & Cheng (2004) found no evidence that rearing with visual barriers, which are known to improve spatial memory, influenced asymmetry in the length of dendrites and linear spine density in the multipolar projection neurons of the hippocampus of domestic chicks. In contrast, exposing rats to a novel environment

early in life (which is known to improve spatial memory; Tang 2001) produced a long-lasting right-shift in hippocampal volume (Verstynen et al. 2001).

It is known that lateralization in brain structure and certain visual functions in the chicken are susceptible to environmental modulation at certain ages, since even a brief exposure to light during in the later part of incubation leads to asymmetry in the visual projections (Koshiba et al. 2003). As chickens have several shifts in hemispheric dominance in the first few weeks of life (summarized in Rogers 1995), it may be that different types of experiences influence the development of brain and behaviour during periods when a particular hemisphere is most active. If so, for reasons presented above we would predict that experience with spatial cues at around days 10 and 11 of age might have an effect on the right hemisphere and hence spatial ability using the LES later in life.

In the following experiment we used a variation of the rotated floor test (RFT, Rashid & Andrew, 1989) to test the hypothesis that experience of visual barriers from days 10-12 modulates the chick's use of distal and proximal cues. One modification required the chicks to locate an imprinting stimulus hidden in one of two locations at opposite ends of a rectangular apparatus. Testing involved rotating the apparatus by 180^0 so that proximal (intra-apparatus) and distal (extra-apparatus) cues now indicated opposite sides, and thus provided a means of assessing the choice made by the chick. Experience prior to testing was manipulated by experience of visual barriers or transparent barriers between 10 and 12 days of age. By using monocular testing in addition to binocular, we examined whether this manipulation of experience could generate the known asymmetry in performance in the RFT (Rashid & Andrew 1989). We predicted that chicks reared with visual barriers would pay more attention to distal cues than proximal cues in the RFT, when tested using the left eye.

METHODS

The subjects were 64 broiler chicks (Kootingal Hatchery, Kootingal, NSW) obtained

as fertile eggs at 18 days of incubation and hatched in two replicates of 32. Chicks

were reared in isolation from about 2 h after hatching to 7 days of age in grey sheet

metal cages (25 x 25 x 30 cm high). A yellow tennis ball was suspended by string 10

cm above the floor in the centre of the cage to provide an imprinting stimulus (broiler

chicks have previously been found to imprint on tennis balls, Freire & Nicol 1999).

Temperature was maintained at 35^0C for the first week after hatching and lighting

from halogen strips was provided on an LD 12:12 h cycle. The floor of the cage was

lined with white paper and sprinkled with chick starter crumbs that were periodically

tapped with a round dowel (as recommended in Laboratory birds: refinements in

husbandry and procedures 2001) in order to encourage pecking and eating which can

be a concern in isolation housed chicks. It should be noted that no chicks died in the

first week and they showed no signs of distress. Water was available *ad libitum* from

a clear Perspex petri dish for the first 3 days and for the remainder of the time from a

bird drinker placed outside the cage with the cup on the floor. In the first few days

chicks were sexed by inspection of the wing feathers, as advised by the hatchery.

When the chicks were 8 days old, we paired them (one male and one female as

far as possible), marked the back feathers of one chick with a black marker pen and

placed each pair into a cardboard box (50 x 50 x 60 cm high). Pairing at this age was

undertaken to uphold the welfare of the chicks by allowing them to express social

behaviour, as well as allowing chicks to observe a social companion disappear from

sight. Chicks were observed regularly during this time to ensure that fighting did not

break out or that welfare was compromised: fighting was not observed nor were there

any other signs that pairing at this age caused distress. There was no indication that the marking placed on the back of one of the chicks attracted pecking or otherwise compromised welfare in any way. A yellow tennis ball was suspended by a string in the centre of the pen, 10 cm above the floor. The floor was covered with wood shavings and food (starter crumbs) and water (from an externally placed drinker) were available *ad libitum*. From 8 days onwards, the temperature was 30^0C and the lighting schedule remained unchanged. At lights-on on day 10, we added two screens (20 x 20 x 30 cm high) to each box centrally 10cm either side of the imprinting stimulus. Opaque screens made of wood (0.5cm thick) and painted grey were added to eight boxes (Treatment O). Transparent screens made from 0.3-cm thick acrylic sheet were added to the other eight boxes (Treatment T). All the screens were removed just prior to lights-off on day 12 after hatching.

At 13 and 14 days of age the chicks were trained and tested in a rotated floor test. The apparatus (Figure 1) consisted of a rectangular pen (160 x 65 x 60 cm) made from cardboard sheeting and with wood shavings on the floor. Parallel and 30 cm from the shorter sides were two screens (20 x 20 x30 cm high) made from wood. Half the chicks were trained and tested with both screens covered in white plastic paper, making them visually similar (these will subsequently be referred to as two identical screens). It should be noted that we made the apparatus as uniform as possible, and hence in tests with identical screens there may not have been any obvious proximal cues discernable to the chicks. The remainder of the chicks were trained and tested with one screen covered in white plastic paper and the other in a floral plastic wallpaper (mainly yellow, red and green on a white background), making them visually dissimilar (different).

All treatments and procedures were approved by the University of New England's Animal Ethics Committee (AEC03/90). After the tests were completed, the chicks were given away to a local grower of broiler chickens where they were kept until they reached slaughter weight.

Training Phase

Each chick was trained to locate the imprinting stimulus (yellow ball) behind one of the screens in the testing apparatus. The type of screen and its relative position to the chick (i.e. on the left or right) was balanced (as far as possible) for rearing treatment and sex. Training involved placing a chick in the centre of the apparatus next to the ball. The ball was moved slowly behind one screen and the chick usually followed it. If a chick did not follow the ball, it was gently pushed with a board until it was next to the ball. After the chick had remained with the ball for 15 s, it was placed in a circular start cage (20 cm diameter, 30 cm high, made from wire) in the centre of the apparatus. The ball was placed next to the start cage, and when the chick was oriented towards it, the ball was moved away from the chick until it was behind a screen. The chick was then released and allowed to search for the ball. After the chick approached to within 5 cm of the ball for 15 s, or after 2 minutes, it was picked-up (this was termed a displacement trial). The chick was then replaced in the start cage and received another displacement trial. Once the chick had completed 3 displacement trials, it was placed back in the home pen for between 5 – 15 min.

The chick was then placed into the apparatus again and received two consecutive displacement trials. If a chick failed to approach to within 5 cm of the ball within 2 minutes of release in both trials, it was deemed to not be imprinted, and was removed from the experiment (8 chicks were not imprinted). After the second

displacement trial, the chick was placed in the start cage again for 15 s. With the ball already behind the screen, the chick was released and allowed to search for the ball (this was termed a relocation trial; Figs 1a and 1b). After the chick had spent 15 s within 5 cm of the ball, or after 1 minute, it was returned to the home pen for 5-15 min. Chicks that did not approach the imprinting stimulus in the required time were given a latency score of 1 min.

The chick then received another relocation trial. If a chick failed to approach the ball within 1 minute, it was placed in the start cage and received a displacement trial, after which it was returned to the home pen for 5-15 min. The sequence of relocation trial followed by displacement trial continued until the chick reached the criterion of approaching to within 5 cm of the ball within 15s of release. Once criterion was reached the chick was returned to the home pen and the probe testing phase was begun.

Probe Testing Phase

The testing phase consisted of unrewarded probe tests (i.e. no imprinting stimuli were placed in the apparatus) with the apparatus rotated by 180^0 (Figs 1c and 1d) interspersed with rewarded relocation trials, and if necessary displacement trials, as described above. Each chick received three probe tests- one binocular, one using the left eye (LES) and one using the right eye (RES) - determined according to a semi-random sequence. We carried out monocular testing by temporarily placing a conical piece of tape (2 x 2.5 cm) over one eye between 10-15 min before testing. The cone was attached to the feathers around the eye and did not press on the eye. Chicks initially attempted to remove the cone, but after about 7 min such activities were not observed and chicks showed normal behaviour in the home pens. We carefully

removed the patches immediately after testing. The probe tests ended when a chick moved behind one screen, or after 3 min. Chicks that did not approach the imprinting stimulus in the required time were given a latency score of 3 min.

Following a probe test, the chick was returned to the home pen for about 10 minutes. It was then returned to the start cage and presented with a relocation trial as described above (i.e. the apparatus and the ball was as in the training phase). When a chick approached to within 5 cm of the ball within 15 s of release, it was returned to the home pen. If it failed to approach the ball within 1 minute it was placed in the start cage again and presented with a displacement trial, and then returned to the home pen. This procedure was repeated until the chick approached to within 5 cm of the ball within 15 s of release (criterion) and could be presented with another probe test.

Behaviour in the relocation trials and probe tests was recorded by an overhead video camera (Kobi DSP colour CCD) and VCR (JVC, J695). We used a blind procedure to record the side of the first screen that the chick walked behind and the latency to walk behind a screen by allocating each chick that was displayed on the video recordings a random number from 1 to 64. Corresponding rearing treatment and sex were not disclosed until completion of the behavioural observations, though the type of screens used could be seen on the recordings.

Statistical Analysis

Four chicks from Treatment O (three males and one female) and four chicks from Treatment T (two males and two females) were deemed not to be imprinted on the tennis ball and were removed from the experiment. Thus 28 chicks from each rearing treatment were tested with either identical (N=14) or different (N=14) screens.

There were three possible outcomes to the probe tests: the chick (1) walked behind the screen on the same side as the imprinting stimulus was located during relocation trials (indicating a preference to use distal, extra-apparatus cues), (2) walked behind the opposite side (indicating a preference to use proximal, intra-apparatus) cues or (3) the chick failed to walk behind a screen within 3 minutes (fail). In some cases tabulation of the outcome resulted in many cells with expected counts of less than 5 so the data were grouped in two meaningful ways for analysis using a chi-squared test (Sokal & Rohlf 1981). First, the effect of screen type on the outcome of the probe tests was examined by combining the two rearing treatments. Second, the effect of rearing treatment on outcome was examined by combining screen type. Additionally, the number of chicks choosing the screen indicated by the proximal cues or failing to chose a screen were combined if required.

As the sex of the chicks had no effect on the number of trials required to reach criterion during training (ANOVA: $F_{1,48}= 0$, NS), the mean latency of training trials (ANOVA: $F_{1,48}= 0.1$, NS) or on the outcome or latency to chose a screen in the probe tests (ANOVA: $F_{1,48}= 0.35$, NS), sex was removed from the analysis. Hence, response latencies in the probe tests were analysed using an ANOVA, with rearing treatment (Treatment O or T) and screen type (identical or different) as the between subject factors, and eye system (BIN, RES or LES) as the within subject factor, followed by F-tests when appropriate.

RESULTS

Relocation Trials

Chicks required between 1 and 4 relocation trials to reach criterion during the training phase. After the first probe trial, chicks again took between 1 and 4 relocation trials to reach criterion. After the second probe trial, they took between 1 and 3 relocation trials to reach criterion. The number of trials decreased as the experiment progressed, so that fewer trials were required to reach criterion at the end of testing compared to during the training phase (Fig. 2: ANOVA: $F_{2,104}$=10.2, $P<0.0001$), with no significant interactions (phase*treatment interaction, ANOVA: $F_{2,104}$= 1.6, NS; phase*screen interaction, ANOVA: $F_{2,104}$=0.8, NS). The number of trials required to reach criterion was not significantly influenced by the type of screen used (identical or different screens: ANOVA: $F_{1,52}$=0.05, NS), rearing treatment (ANOVA: $F_{1,52}$=0.15, NS) or their interaction (rearing * screen interaction, ANOVA: $F_{1,52}$=1.3, NS). Similarly, the mean latency to approach the imprinting stimulus during the relocation trials decreased as the experiment progressed (Fig. 3: ANOVA: $F_{2,104}$=5.9, $P<0.01$), with no significant interactions (phase*treatment interaction, ANOVA: $F_{2,104}$= 1.0, NS; phase*screen interaction, ANOVA: $F_{2,104}$=1.9, NS). Again, the mean latency to approach the imprinting stimulus was not significantly influenced by the type of screen used (ANOVA: $F_{1,52}$=0, NS), rearing treatment (ANOVA: $F_{1,52}$= 0.04, NS) or their interaction (rearing * screen interaction, ANOVA: $F_{1,52}$=0.8, NS).

Probe Tests

In general, chicks oriented on the basis of distal cues more and failed to make a choice less in probe tests with identical screens than in probe tests with different

screens in BIN (Fig. 4; Chi-squared test: χ^2_1=5.8, P<0.01), LES (Fig. 5; Chi-squared test: χ^2_1=3.6, P=0.057) and RES tests (Fig. 5; Chi-squared test: χ^2_2=10.5, P<0.01). The latency to make a choice was shorter in probe tests with identical screens than in probe tests with different screens (pooled means 74.9±7.4 s and 105.6±7.7 s respectively; ANOVA: $F_{1,52}$= 11.08, P<0.01), suggesting that the use of different screens in the probe tests impaired performance. No significant interaction were found between the type of screen used in probe tests and other factors (screen*eye system, ANOVA: $F_{2,104}$=0.48, NS; screen*treatment, ANOVA: $F_{1,52}$=1.59, NS; screen*eye system*treatment, ANOVA: $F_{2,104}$=0.54, NS). The data were further analysed to determine the effect of rearing treatment on outcome and latency to make a choice in BIN, LES and RES probe tests.

Binocular probe tests

In probe tests with identical screens, Treatment O and T chicks showed a preference to respond to distal cues (Fig. 4, Binomial test: P<0.0001 and P<0.05 respectively). In probe tests with two different screens, however, Treatment O chicks chose the screens indicated by the distal cues more than Treatment T chicks which tended to chose at random or failed to chose a screen (Fig. 4; Chi-squared test: χ^2_1=3.6, P=0.058). Additionally, there was a significant interaction between eye system and rearing treatment in response latency (Fig. 6; ANOVA: $F_{2,104}$= 6.1, P<0.01), indicating that Treatment O chicks were faster at making a choice than Treatment T chicks (Fig. 6; F test: $F_{1,149}$=14.3, P<0.001).

Monocular probe tests

In general, chicks failed to chose a screen more often in monocular probe tests (50) than when tested binocularly (3). Treatment O chicks failed 22 times and Treatment T chicks failed 28 times, with five chicks from Treatment O and ten chicks from Treatment T failing in probe tests using either the LES or RES. Overall, chicks chose the side indicated by distal cues in monocular probe tests with identical screens (Fig. 5a; 28/10, Binomial test: P<0.01). In probe tests with different screens, however, LES chicks still chose the distal side more often than the proximal side, whereas RES chicks chose the proximal side more often than the distal side (Fig. 5b, Chi-squared test: χ^2_1=4.2, P<0.05).

Generally, rearing treatment had no effect on the number of chicks that chose the distal side when tested monocularly. In probe tests with identical screens, however, Treatment O chicks failed to chose a screen less often than Treatment T chicks (Fig. 5a, Chi-squared test: χ^2_2=7.3, P<0.05). Rearing treatment was not found to influence response latency in probe tests using the LES (F test: $F_{1,149}$=2.4, NS) or RES (F test: $F_{1,149}$=0.3, NS). However, Treatment T chicks responded faster when using the LES than the RES (Fig. 6; F test: $F_{1,104}$=5.0, P<0.05) whereas Treatment T chicks showed no asymmetry in response latency (F test: $F_{1,104}$=0.02, NS).

DISCUSSION

In summary, chicks chose the screen on the distal side (i.e. the same side as during training) more often and with a shorter latency in probe tests with identical screens than in probe tests with different screens. In probe tests with different screens, however, LES chicks chose the distal side more often than the proximal side, whereas

RES chicks chose the proximal side more often than the distal side. In BIN probe tests with different screens, Treatment O chicks were quicker in making a choice than Treatment T chicks and they tended to choose the screen on the distal side more often than Treatment T chicks. Treatment O chicks also failed to make a choice less often than Treatment T chicks in monocular probe tests with identical screens.

We found no gender effects on performance in the rotated probe tests, suggesting similar spatial cognitive abilities in male and female chicks. Likewise, Tommasi & Vallortigara (2004) found no difference in male and female chicks' use of proximal cues for locating hidden food, though males responded more strongly to non-spatial changes to the proximal cues (i.e. their removal or replacement). Additionally, no evidence was found that the rearing treatment influenced the ability to walk around a screen in the probe tests. First, Treatment O and T chicks did not differ in either the number of training trials or in the latency to move around a screen during training. Second, no evidence was found that rearing treatment influenced performance in BIN probe tests with identical screens, or in monocular probe tests with different screens. Thus the results suggest that Treatment O and T chicks were equally motivated to search for the imprinting stimulus.

Our findings that chicks generally chose the screen on the distal side in probe tests with two identical screens supports previous work indicating that chicks readily use distal cues in the RFT (Rashid & Andrew 1989; Regolin et al. in press). Generally, fewer chicks showed a preference for the distal side in probe tests with two different screens, suggesting that the relatively obvious proximal cues presented a conflict situation. Indeed, the slower latency to choose a side and the greater number of failures in probe tests with two different screens than with two identical screens supports this idea. Our findings extend earlier work indicating that chicks are able to

orient accurately and quickly using distal cues by showing that the presence of obvious proximal cues that present conflicting information may interfere with orientation based on distal cues.

Furthermore, although chicks responded strongly to distal cues in BIN probe tests with identical screens, there was a tendency for Treatment O chicks to use distal cues more often than Treatment T chicks in BIN probe tests with different screens (P=0.058), suggesting that in a conflict situation Treatment O chicks were still able to attend to distal cues but that Treatment T were more likely to switch to proximal cues or fail to chose. Indeed, the finding that Treatment O chicks were faster at choosing a screen in BIN probe tests than were Treatment T chicks supports the suggestion that Treatment T chicks were in greater conflict than Treatment O chicks. Generally, chicks were more likely to fail in monocular than in binocular probe tests indicating that covering one eye significantly impaired performance in probe tests. Interestingly, Treatment O chicks failed to make a choice less often than Treatment T chicks in monocular probe tests with identical screens. It is unlikely that the latter finding indicates a motivational difference in search behaviour between Treatment O and T chicks, since no other evidence for such differences either in training or in other probe tests was found. Instead, it may be that Treatment O chicks were better able to detect and use subtle proximal cues than Treatment T chicks when orientation was impaired by covering one eye, though this remains to be determined. Combined, the above findings indicate that rearing with opaque screens improved the chick's response to distal cues by apparently reducing the effect of interference from conflicting proximal cues.

Providing chickens with experience of opaque screens for 5 days, between 8-12 days of age, has been found to improve search in a visual displacement test and a

detour test, suggesting that there is an effect of experience on the chick's ability to orient (Freire et al. 2004). It appears that day 11 may be a crucial period since chickens in the laboratory spend more time out-of-sight of an imprinting stimulus on this day than in the days before or after (Freire et al. 2004) and similarly chickens in semi-extensive housing systems actively move out-of-sight of the mother at 11 days of age (Vallortigara et al. 1997), a time when the right hemisphere assumes dominant control of visual behaviour (Rogers & Ehrlich 1983).

Although it appears that moving out-of-sight shapes the development of spatial memory, the precise experiences that shape this remain to be determined. It does not appear that the locomotory changes associated with moving around objects is important, since the transparent screens used in the present study and by Freire et al. (2004) would be expected to allow similar locomotory patterns. Chicks reared in pens without screens and periodically placed out-of-view of an imprinting stimulus by the experimenter showed no improvement in spatial memory relative to chicks similarly handled but without being placed out-of-sight of the imprinting stimulus, suggesting that the chick's active behaviour is central to the developmental process (Freire et al. 2004). One possibility is that, by moving out-of-sight of prominent visual cues such as the imprinting stimulus or the other chick, the chicks are actively facilitating their ability to learn to use distal spatial cues.

Chicks using the LES based their orientation more on distal cues than proximal cues, whereas chicks using the RES based their orientation more on proximal cues than on distal cues in probe tests with different screens, supporting previous research showing that the processing of distal information involves the LES (Rashid & Andrew 1989; Tommasi & Vallortigara 2001; Regolin et al. in press) and presumably the right hippocampus (Tommasi et al. 2003). Perhaps surprisingly though, we found that

chicks using the RES and LES responded equally well to distal cues in probe tests with identical screens, indicating that the RES is able to attend to distal cues if there are no obvious proximal cues. Similarly, Vallortigara et al. (2004) found that chicks using the RES could orient using distal cues, though not as well as chicks using the LES. It is unlikely that the left hemisphere has some rudimentary ability to process distal information since lesions to the right hippocampus, but not the left hippocampus, disrupts response to geometric information (Tommasi et al. 2003). Instead, it may be that the left hemisphere is able to attend to distal cues in the absence of obvious proximal cues by engaging the right hemisphere through ipsilateral connections, or perhaps through connections to the right hemisphere such as the thalamofugal visual projections through the supraoptic decussation (Rogers 1995). It may be that, in normal functioning, the pathways allowing the left hemisphere to process distal information are largely redundant, but when there are no obvious proximal cues, the left hemisphere may be able to attend to distal cues. Although the above is speculative, it would suggest that the left hemisphere has not lost its ability to respond to distal cues but that instead its primary role is to respond to proximal cues.

In conclusion, response to distal cues in a rotated floor test was impaired by obvious proximal cues, indicating that relatively obvious proximal cues are necessary for conflict to arise. Previously reported advantages in the use of distal cues by the LES were confirmed, and indicated that chicks using the LES were less disrupted by conflicting proximal cues than chicks using the RES. Rearing with opaque screens improved the chicks ability to use distal cues and ignore conflicting proximal cues suggesting that the previously reported experience-induced improvement in spatial

memory (Freire et al. 2004) may arise because experience of visual barriers biases the chick's orientation mechanism towards using distal cues.

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Figure 1: Rotated floor testing apparatus showing the two screens and the arrangement at the start and at the point of release of the chick in relocation trials (a and b respectively) and probe tests (c and d respectively). Note that in probe tests the apparatus was rotated by 180^0. The figure shows training in one direction with different screens (distinguished by solid and dashed lines) for ease of explanation, although the direction of displacement of the ball and the screen behind which the ball was hidden was, as far as possible, balanced for rearing treatment and sex.

Figure 2: Mean number of relocation trials required to reach criterion during the training phase (Phase 1), after the first probe test (Phase 2) and after the second probe test (Phase 3) using identical and different screens, showing that the number of trials required to reach criterion decreased as the experiment progressed (P<0.0001).

Figure 3: Mean latency to approach the imprinting stimulus in relocation trials during the training phase (Phase 1), after the first probe trial (Phase 2) and after the second

probe trial (Phase 3) using identical and different screens, showing that the latency to approach the imprinting stimulus decreased as the experiment progressed (P<0.01).

Figure 4: Number of chicks choosing the screen indicated by the distal (black bars) or proximal (white bars) cues, or failing to make a choice (grey bars) in binocular probe tests. The majority of chicks chose the side indicated by distal cues in probe tests with identical screens, whereas in probe tests with different screens the majority of Treatment O chicks tended to continue to respond to distal cues, whereas Treatment T chicks tended to chose the proximal side or failed to chose a screen (P=0.058). N=14 per group.

Figure 5: Number of chicks choosing the screen indicated by the distal (black bars) or proximal (white bars) cues, or failing to make a choice (grey bars) when using the left eye (LES) or right eye (RES) in probe tests with (a) identical and (b) different screens. Fewer Treatment O chicks failed than Treatment T chicks: Treatment O chicks made more proximal choices in probe tests with identical screens (P<0.05). The majority of monocular chicks failed in probe tests with two different screens, but note the higher number of Treatment O chicks choosing distal cues when using their left eye, compared to all other groups.

Figure 6: Mean latency to chose a screen in probe tests when the chicks were tested binocularly (black bars), and using the left (grey bars) and right (white bars) eyes for Treatment O and T chicks. Data for identical and different screens are pooled because they were not significantly different. * P<0.05; ***P<0.001).
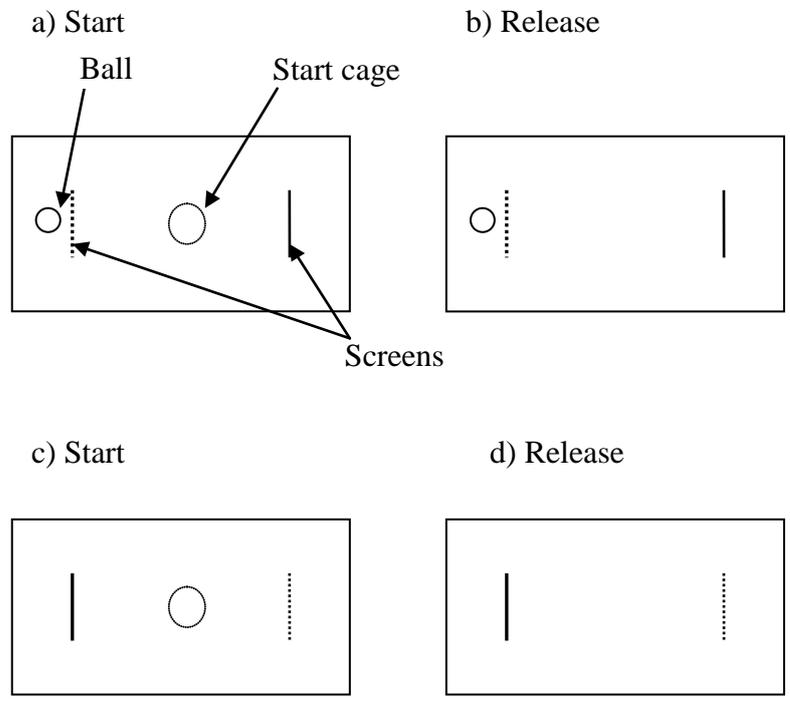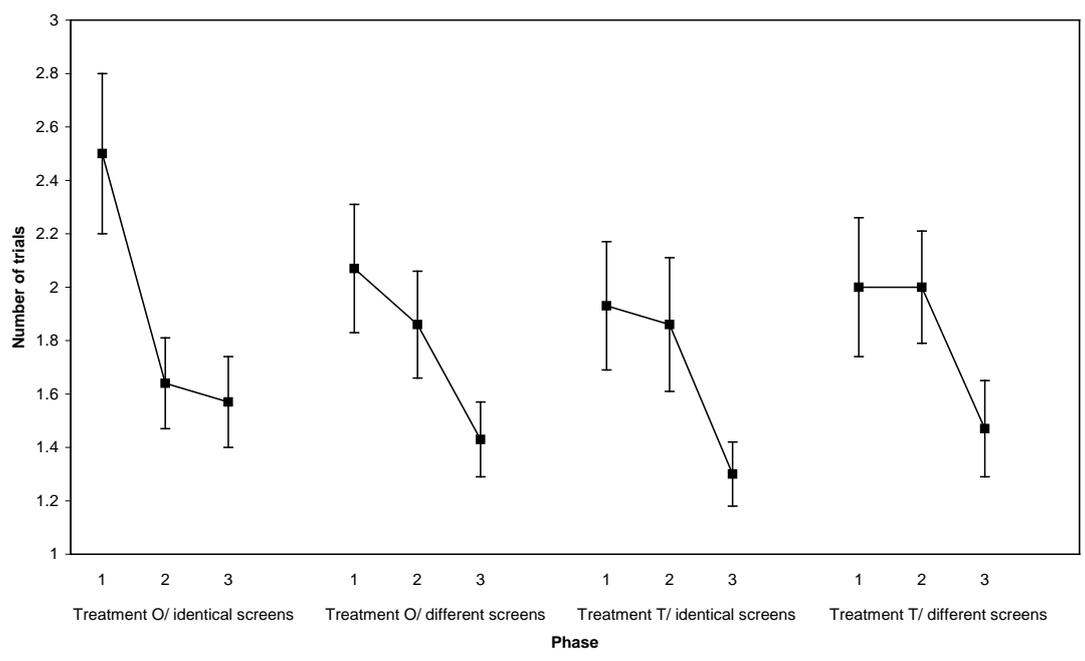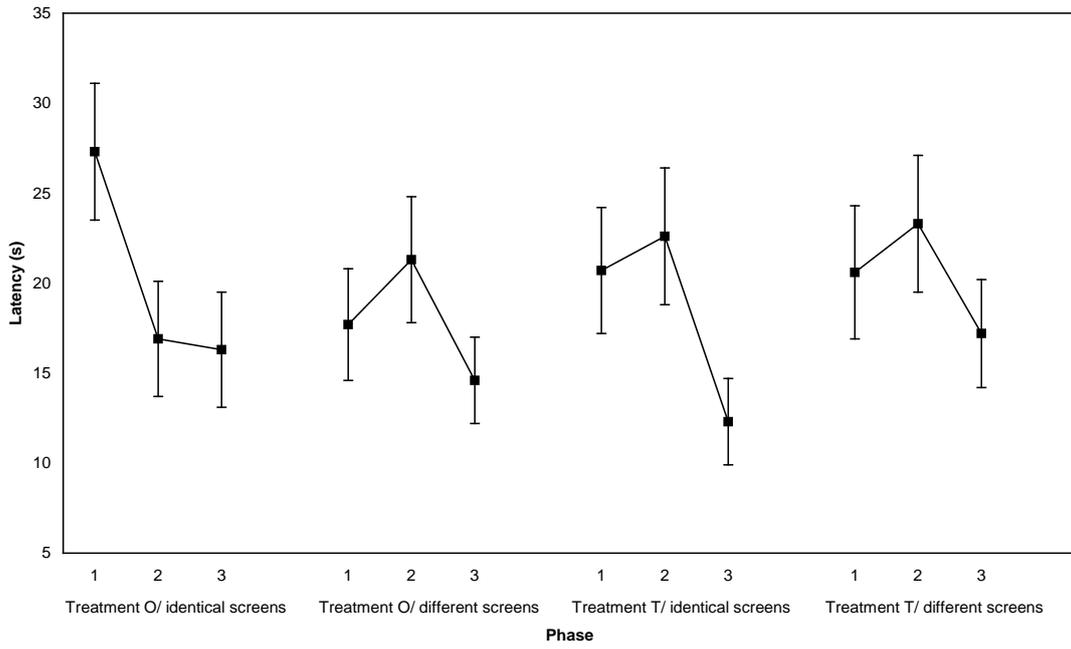
Figure 1

a) Start

Ball   Start cage

Screens

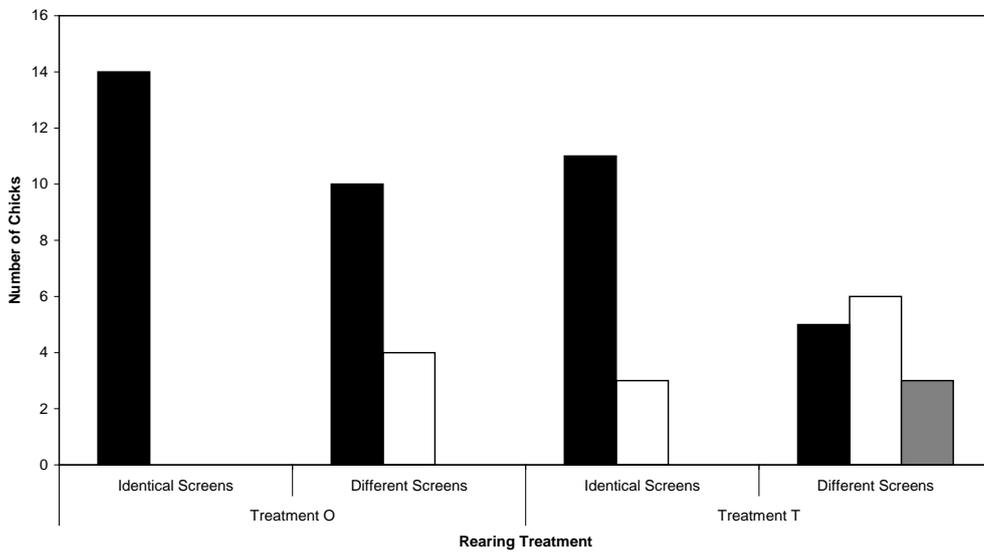b) Release

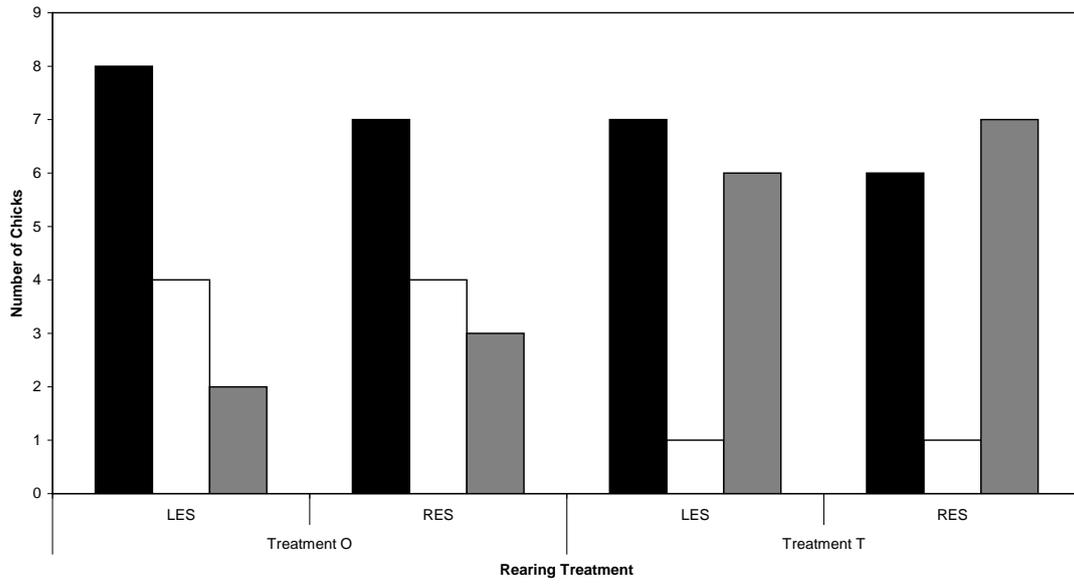c) Start

d) Release

Figure 2

Figure 3

Figure 4
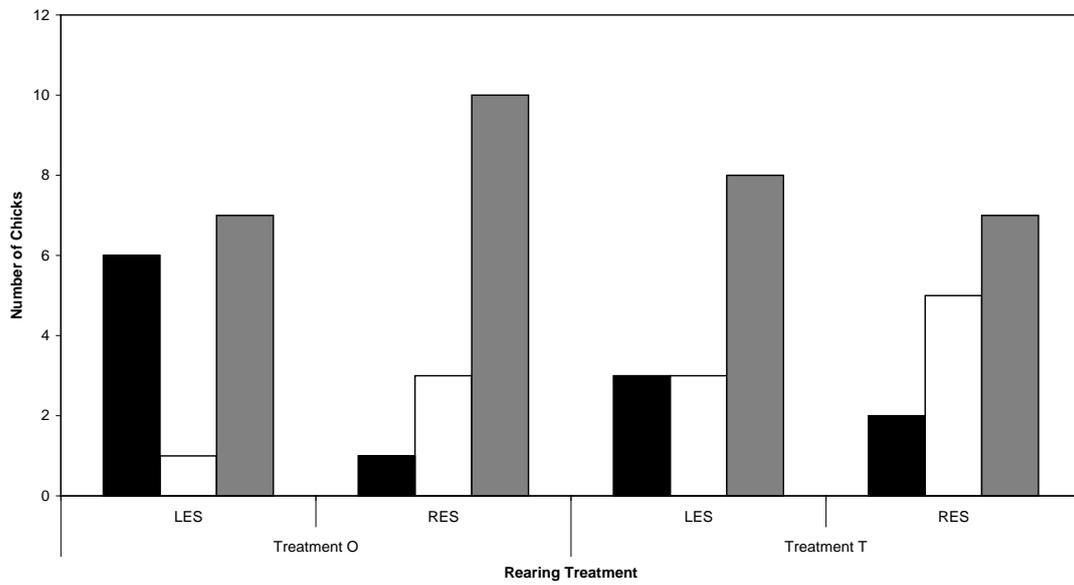
Figure 5a- identical screens

Figure 5b- different screens

Figure 6