We tested the hypothesis that experience of moving out-of-sight of an imprinting stimulus during a period of right hemispheric dominance (11 days of age), but not during a period of left hemispheric dominance (8 days of age), biases attention to distal spatial cues. Chicks were trained to locate a hidden imprinting object behind one of two differently marked screens placed at either end of a rectangular arena, and then presented with five unrewarded probe tests with the arena rotated by 180°. Chicks that had experience of going behind opaque screens on day 11 chose the screen in the same direction as during training (i.e. using distal cues) significantly more often than chicks given experience with opaque screens on day 8 or chicks provided with two transparent screens on either of these two days (P=0.016). We conclude that the similarities between behaviour patterns of chicks in the laboratory and in semi-natural environments suggest that moving out-of-sight of the mother at 11 days of age is an ecologically important behavioural pattern that requires dominance by the right hemisphere to shift the chick's response to distal spatial information for locating a hidden goal.
Experience during a period of right hemisphere dominance alters attention to spatial information in the domestic chick.

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ABSTRACT

We tested the hypothesis that experience of moving out-of-sight of an imprinting stimulus during a period of right hemispheric dominance (11 days of age), but not during a period of left hemispheric dominance (8 days of age), biases attention to distal spatial cues. Chicks were trained to locate a hidden imprinting object behind one of two differently marked screens placed at either end of a rectangular arena, and then presented with five unrewarded probe tests with the arena rotated by 180°. Chicks that had experience of going behind opaque screens on day 11 chose the screen in the same direction as during training (i.e. using distal cues) significantly more often than chicks given experience with opaque screens on day 8 or chicks provided with two transparent screens on either of these two days (P=0.016). We conclude that the similarities between behaviour patterns of chicks in the laboratory and in semi-natural environments suggest that moving out-of-sight of the mother at 11 days of age is an ecologically important behavioural pattern that requires dominance by the right hemisphere to shift the chick’s response to distal spatial information for locating a hidden goal.
The domestic chick, with its accomplished spatial memory, known lateralization of brain and behaviour and neuronal and behavioural plasticity is a useful model for studying the development of brain and behaviour (e.g. Rogers 1995). Domestic chicks are able to detour around a barrier to obtain a goal (Regolin et al. 1995) and can use landmark, geometric (Tommasi & Vallortigara 2000) and magnetic (Freire et al. 2005) cues to locate a hidden goal. In the domestic chick, the hippocampus is involved in spatial memory formation (Nakajima et al. 2003) and processing of geometric spatial features occurs in the right hippocampus (Tommasi et al. 2003), as has been found in other vertebrates (see Vallortigara et al. 2004, for a review). The right hippocampus of chicks has also been found to have longer dendrites and more synapses than the left hippocampus (Freire & Cheng 2004), further supporting a functional similarity between the avian and mammalian hippocampus (Colombo & Broadbent 2000).

Studies involving monocular tests of spatial memory in chicks suggest that the right hemisphere is primarily concerned with processing and responding to non-local spatial information (distal cues), whereas the left hemisphere is concerned with local cues (Rashid & Andrew 1989; Tommasi & Vallortigara 2004; Freire & Rogers 2005). For example, Tommasi & Vallortigara (2004) trained chicks to locate food hidden in the centre of an enclosure next to a landmark (a red cylinder) and then tested the chicks monocularly with the landmark displaced to reveal that chicks using the left eye searched in the centre of the apparatus (thereby using geometric spatial information) and chicks using the right eye searched near the displaced landmark. Since input from the left eye is processed primarily in the right hemisphere (Rogers 1995), this result indicated use of the right hemisphere controlling responses to geometric spatial cues. Further support for this deduction was demonstrated by
lesioning of the hippocampus (Tommasi et al. 2003); lesions in the right hemisphere removed the response to geometric cues, whereas lesions to the left hemisphere, or a sham procedure, did not. In another test, chicks were tested in a delayed-response task with local and positional cues (i.e. relative left and right position) in conflict and those chicks using the left eye preferentially used positional information, whereas chicks using the right eye showed no preference for local or positional information, as expected (Regolin et al. 2005). When these cues were not in conflict, the chicks tested using either the left or right eye were able to use both local and positional cues, suggesting that both local and positional spatial information is available to both hemispheres. Hence, the hemispheric differences seem to be at the level of decision making rather than there being specialized availability of perceptual information.

One technique that reveals the processing of local and non-local spatial information is the rotated floor test, in which chicks are trained to locate a goal hidden on one side of the apparatus, usually marked with distinguishing local features, and are then tested with the apparatus rotated by 180°, so that extra-apparatus (distal) cues are in conflict with intra-apparatus (local) cues (Rashid & Andrew 1989). The kind of distal spatial information that the chicks use in this latter test is largely undetermined, but is thought to include features of the ceiling, lighting direction, and possibly olfactory (Rashid & Andrew 1989; Freire & Rogers 2005) or magnetic cues (Freire et al. 2005). By presenting local and distal spatial information in conflict, the rotated floor test has consistently revealed a difference in response for chicks using the right or left eye, indicating that the right hemisphere preferentially responds to distal cues and the left hemisphere to local cues (Rashid & Andrew 1989; Freire & Rogers 2005).

Although it has been known for some time that the domestic chick shows sharply timed periods of bias in dominance of either hemisphere during development
(Andrew 1991, 2002), the role of these shifts in dominance on the above lateralized spatial responses is largely undetermined. Of particular relevance to spatial memory is the shift in dominance to the right hemisphere on day 11 (Rogers & Ehlrich 1983), a period when chicks in semi-natural conditions actively move out-of-sight of the mother hen (Workman & Andrew 1989). Chicks in the laboratory also show a peak in moving out-of-sight of an imprinting stimulus on day 11, and providing chicks with the opportunity to experience being out-of-sight of an imprinting stimulus by moving behind a screen from days 10-12 improves performance in a detour and a visual displacement test (Freire et al. 2004) and increases dendrite length and linear spine density in the hippocampus (Freire & Cheng 2004), indicating that experience at this time affects both behaviour and brain development. Hence particular experiences during this sensitive period shape spatial memory, and mark it as one of the known periods during development when it may be important that one or the other hemisphere is dominant for the appropriate, age-related control of behaviour.

The function of these shifts in hemispheric dominance is largely unknown, and has been suggested to act to constrain behaviour or to allow each hemisphere to learn particular responses (Andrew 2002). It is unclear whether the effectiveness of the experience of moving out-of-sight of the imprinting stimulus is restricted to day 11 or can occur at other ages or, perhaps more relevantly, during periods of dominance of the left hemisphere. The left hemisphere is dominant on day 8, as revealed by asymmetrical sensitivity to the action of cycloheximide (Rogers & Ehlrich 1983). We used a rotated floor test to test the hypothesis that experience of moving out-of-sight of the imprinting stimulus during a period of right hemispheric dominance (day 11), but not during a period of left hemispheric dominance (day 8), shifts attention to distal spatial information.
MATERIALS AND METHOD

The subjects were 24 layer chicks exposed to light throughout incubation (Nulkaba Hatchery, Cessnock, NSW) and obtained as day-old chicks. A yellow tennis ball was suspended by string 10 cm above the floor in the centre of the cage to provide an imprinting stimulus. Chicks were reared in isolation from arrival until 7 days of age in grey sheet metal cages (25 x 25 x 30 cm high). Temperature was maintained at 35°C 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 in order to encourage eating; chicks were also observed regularly throughout this time to ensure that they found the food and water. Brief isolation at this age did not cause distress and chicks were housed in pairs after the imprinting period to allow them to exhibit social behaviour. Water was available *ad libitum* from a 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- primary and secondary wing feathers are the same length in females but different lengths in males.

When the chicks were 7 days old, we paired them (one male and one female as far as possible) and placed each pair into a home pen (50 x 50 x 60 cm high). Pairing at this age was undertaken to uphold the welfare of the chicks by proving access to litter and allowing them to express social behaviour. Chicks were observed regularly during this time to ensure that aggression fighting did not occur or that welfare was compromised: aggression was not observed nor were there any other signs that pairing at this age caused distress. 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*.

At lights-on on day 8, we added two screens (20 x 20 x 30 cm high) to each box centrally and 10cm either side of the imprinting stimulus, and these were removed just prior to lights-on on day 9. Similarly, we added two screens to each box on day 11, and removed these just prior to lights-on on day 12. Some pairs of chicks were provided with opaque screens made of wood (0.5cm thick) and painted grey and others with transparent screens made from 0.3-cm thick acrylic sheet. There were three treatments as shown in Table 1.

At 13-15 days of age the chicks were trained and tested in a rotated floor test (Figure 1). The apparatus 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. One of these screens was covered in white plastic paper and the other in a floral-patterned plastic paper (mainly yellow, red and green on a white background), making them visually dissimilar. It should be noted that we made the inside of the apparatus as uniform as possible. The apparatus was in a laboratory next to a white wall which could be seen by the chick, though no other walls could be seen from the apparatus. The ceiling was also white and one halogen strip light could also be seen from the apparatus.

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 position relative to the chick (i.e. on the left or the right) was balanced between treatments. 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 pushed gently with a small 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 the screen. The chick was
then released and allowed to search for the ball (this was termed a visual displacement
trial). After the chick approached to within 5 cm of the ball for 15 s, or after 2
minutes, it was picked-up. 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. 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; Fig. 1a & 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, and
presented with a visual displacement trial before being returned to their home pen.

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 20s of release on two consecutive trials.
(criterion reached in 3.8±0.3 trials). 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 five unrewarded probe tests (i.e. no imprinting stimuli were placed in the apparatus) with the apparatus rotated by 180° (Fig. 1c & 1d) interspersed with rewarded relocation trials (so as to prevent extinction) and, if necessary, displacement trials as described above. 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 present in the same place and behind the same screen as in the training phase). When a chick approached to within 5 cm of the ball within 20 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 20 s of release and could be presented with another probe test.

Behaviour in the relocation trials and probe tests was observed by an overhead video camera (Kobi DSP colour CCD) displayed on a monitor. We recorded the side of the first screen that the chick walked behind and the latency to walk behind a screen.

All treatments and procedures were approved by the University of New England’s Animal Ethics Committee (AEC05/98). Chicks were observed at least six times a day to ensure welfare. After the experiment, the chicks were group housed and observed to ensure that they displayed appropriate social behaviour (they did not
appear to show any abnormal effect due to the early isolation or imprinting), before being given away to local poultry rearers.

Statistical Analysis

Since all chicks walked behind a screen within 3 minutes of release in probe tests, there were two possible outcomes to each test: 1) the chick walked behind the same screen as it had during training trials (i.e. response to local, intra-apparatus cues), or 2) the chick walked behind the screen in the same direction as the correct screen during training trials (i.e. response to distal, extra-apparatus cues). The proportion of responses to distal cues was arcsine transformed, as recommended for proportional data ($p' = \text{asin}(\sqrt{p}) \times 52.298$; Sokal & Rohlf 1994), and analyzed using a General Linear Model with treatment and sex as factors (since homogeneity of variance assumptions were met: Levene’s test $L=0.49$, $P=0.62$).

RESULTS

There were no signs of distress following the addition of the screens on days 8 and 11, with chicks moving freely around the screens to reach food and water. Following the five visual displacement training trials on days 13 and 14, chicks located the hidden imprinting stimulus readily in relocation trials, reaching criterion in between 2-7 trials with a mean latency of 2.5-50.8s. No difference was found between treatments in the number of trials required to reach criterion (ANOVA: $F_{2,18}=2.1$, $P=0.15$) or in the mean latency to locate the imprinting stimulus (ANOVA: $F_{2,18}=2.4$, $P=0.12$).

In between probe tests, however, control chicks required more relocation trials ($7.5 \pm 0.5$ trials) prior to presentation of the next test than T8 chicks ($5.1 \pm 0.3$ trials; $F$
test: $F_{1,21}=14.7$, $P=0.001$) and T11 chicks (6.1±0.4 trials; F test: $F_{1,21}=4.9$, $P=0.04$: ANOVA: $F_{2,18}=7.7$, $P=0.004$). On average, control chicks also took longer to locate the imprinting stimulus (27.6±1.8s) than T8 chicks (17.4±2.8s; F test, $F_{1,21}=10.9$, $P=0.004$) and T11 chicks (21.2±1.5s; F test, $F_{1,21}=4.3$, $P=0.05$: ANOVA: $F_{2,18}=5.8$, $P=0.01$) in the relocation trials between the tests. No significant difference was found between T8 and T11 in either the number of relocation trials prior to presentation of the next test (F test: $F_{1,21}=2.6$, $P=0.12$) and the latency to locate the ball in relocation trials (F test: $F_{1,21}=1.5$, $P=0.24$). No significant effects of sex were found on the number of trials or latency, both before reaching criterion (trials: sex effect: ANOVA: $F_{1,18}=1.0$, $P=0.34$: sex/treatment interaction: ANOVA: $F_{1,18}=1.8$, $P=0.20$) or between probe tests (trials: sex effect: ANOVA: $F_{1,18}=0.9$, $P=0.35$: sex/treatment interaction: ANOVA: $F_{1,18}=0.2$, $P=0.79$).

Probe Tests

In general, chicks were slower in making a choice of screen in probe tests (52.2±5.1s) than in training trials (22.1±1.5s), partly because they were observed to walk from one screen to the other screen more often in probe tests than in training trials. Chicks provided with opaque screens on day 11 (T11) chose the screen in the correct distal position significantly more often than T8 chicks or control chicks (Fig. 2; ANOVA: $F_{2,18}=5.2$, $P=0.016$; pairwise comparisons of T11 and T8 chicks, $F_{1,18}=10.6$, $P=0.004$; pairwise comparison of T11 and control chicks, $F_{1,18}=5.6$, $P=0.029$). Chicks provided with opaque screens on day 8 (T8) did not differ from control chicks in their choice of screen ($F_{1,18}=0.8$, $P=0.38$). No significant effect on the choice of screen was found for sex (ANOVA: $F_{2,18}=0.6$, $P=0.46$) or the sex and treatment interaction (ANOVA: $F_{2,18}=0.5$, $P=0.61$). A significant interaction was
found between the latency to chose a screen and sex (ANOVA: $F_{2,18}=4.4$, $P=0.028$); females had longer response latency than males in the control condition, but not in the other conditions (i.e. T8 and T11; Fig. 3).

DISCUSSION

In summary, chicks provided with opaque screens on day 11 (T11) chose the screen on the distal side (i.e. in the same direction as during training) more often than chicks provided with opaque screens on day 8 (T8) and chicks provided only with transparent screens (control). There were no differences between treatments in the early stages of training but control chicks needed more relocation trials between tests and on average took longer than T8 and T11 chicks in these trials. Latency to make a choice was longer in tests than in relocation trials. This result and the observation of chicks walking from one screen to the other repeatedly during tests both suggest that chicks were in conflict as to which cues to respond to during the tests.

The present study extends the findings of Freire & Rogers (2005) in various respects. First, these findings show that only one day of experience of opaque screens is sufficient to create a preference to use distal spatial cues for locating a hidden object (N.B., Freire & Rogers (2005) provided 3 days of experience). This is important because behavioural patterns in semi-natural conditions that are likely to provide visual experience similar to the addition of opaque screens, namely moving out-of-view of the mother (Workman & Andrew 1989) and moving ahead of the mother (Vallortigara et al. 1997), peak for just one day. In particular, the latter behavioural pattern (moving ahead of the mother) was observed on day 11 but not on day 10 and not on day 12 in female chicks (male chicks showed some of this
behavioural pattern on day 12, but this was substantially lower than on day 11; Vallortigara et al. 1997). Previously, providing opaque screens from days 8-12 has been found to improve the performance of chicks in detour and visual displacement tests and increase dendrite length and spine density in the hippocampus (see introduction). Our current results suggest that the use of spatial information, and possibly the hippocampus, is shaped by experience of visual screens on day 11 only, and similarities between behaviour in the laboratory and in a semi-natural environment suggest that this may be an ecological valid phenomenon.

Second, the present findings show that the timing of experience of opaque screens is important in shaping the use of spatial information, suggesting a link between hemispheric dominance and development of behaviour. In the present study, visual experience when the right hemisphere is known to be dominant, but not when the left hemisphere is known to be dominant, produced a clear preference for distal spatial information. It should be stressed, however, that we were not able to completely discount the possibility that our findings arose from maturation processes independent of shifts in hemispheric dominance. Nonetheless, our results show that hemispheric dominance per se (i.e. regardless of which hemisphere is dominant) is not sufficient to shape the use of spatial information, since visual experience on day 8, when the left hemisphere is known to be dominant, did not increase the use of distal spatial cues.

The findings presented here support the hypothesis that shifts in hemisphere dominance function to allow each hemisphere to learn and develop particular responses (Andrew 2002). As shown previously, passive observation of an imprinting stimulus being out-of-sight on day 11 is not sufficient to generate an improvement in performance in detour and visual displacement tests (Freire et al. 2004), suggesting
that active behaviour of the chick is important in shaping the use of spatial information. It seems likely that chicks are predisposed to move out-of-sight of the mother on day 11, since this behaviour at this age is seen in very different environments (Workman & Andrew 1989; Freire et al. 2004). Domestic chicks show a predisposition to approach stimuli with characteristics of the mother (Bateson, 1979) and with biological motion patterns (Vallortigara et al., 2005) at a younger age, and appear to do so using neural processes separate from those used during imprinting itself (Bolhuis & Honey 1998). Thus two separate processes could be involved in shaping the use of distal spatial information on day 11: 1) a predisposition to actively move out-of-sight of the mother on day 11 which requires right hemisphere control of behaviour and 2) a resultant shift to use distal spatial information, which relies on the right hippocampus (Freire & Cheng 2004). We propose that the combination of right hemisphere control and moving out of sight is necessary for shaping the use of distal information, and that if either does not occur, there will be no effect.

The greater delay in choosing a screen in probe tests than in training trials, and the walking from one screen to the other screen observed here suggests that chicks perceived the experimentally-induced conflict in local and distal information. Similarly, Regolin et al. (2005) showed that domestic chicks could use local spatial information from two differently marked screens as well as positional spatial information. It is unlikely that the delay in choosing a screen in probe tests compared to training trials arose from the chicks considering the situation novel (i.e. the screens were not where they were observed previously), since Regolin et al. (2005) showed that novelty per se, provided by substitution of one screen with a novel screen, completely abolished the response to positional information. In both the T11 chicks in the present study and binocular tested chicks in the study of Regolin et al. (2005),
response to non-local information (i.e. other than the marking of the screen) was around 70%.

Female control chicks in the present study took more time to make a choice than male control chicks, suggesting that the former chicks may have been in a greater state of conflict than the latter chicks. Since control chicks responded more strongly to local cues than to distal cues, these results may indicate that that females were relatively more responsive to distal than to local cues than males (though it should be noted that there were no sex differences in choice of screen). This latter finding supports those of Tommasi & Vallortigara (2004), who reported that female chicks relied less on local information than male chicks in a task in which the local cue was a landmark and the non-local cue the geometric shape of the apparatus. In contrast, Vallortigara (1996) found that females learnt a discrimination task using local cues more rapidly than males, whereas males were better at learning using positional (i.e. relative left and right) cues. Notwithstanding the difficulties in drawing comparisons from three different tests, there were also differences in age, which may be important: Tommasi & Vallortigara (2004) tested chicks at 16 days of age, whereas Vallortigara (1996) tested them at 9 days of age. Nonetheless, there appear to be sex differences in the use of spatial information and the results presented here suggest that experience of moving out-of-sight of an imprinting stimulus at day 8 or day 11 removes this sex difference. Since chicks move out-of-sight of the mother in semi-natural conditions (Workman & Andrew 1989; Vallortigara et al. 1997), the observed sex difference in chicks denied the opportunity to move out-of-sight may be apparent only after specific laboratory rearing.

In conclusion, similarities between the behaviour patterns of chicks in the laboratory and in semi-natural environments suggest that moving out-of-sight on day
11 is an ecologically important behavioural predisposition that shifts the chick’s response to distal spatial information for locating a hidden goal. This bias in the use of spatial information appears to require dominance of the right hemisphere at the time of the experience, supporting the theory that shifts in hemispheric dominance allow each hemisphere to learn and develop particular responses.

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REFERENCES


Table 1. Type of screens added on days 8 and 11 to create three treatments

Figure 1. Rotated floor testing apparatus. The two types of screens are marked by dashed and solid lines. The arrangement at the start and at the point of release of the chick in relocation trials (a and b respectively) is shown, as is the arrangement during probe tests (c and d respectively) with the apparatus rotated by 180°.

Figure 2. Percentage of choices (mean±SEM) of the screen in the correct distal position in the rotated floor test for the three rearing treatments. Chicks provided with opaque screens on day 11 (T11) made significantly more choices of the correct distal position than chicks provided with opaque screens on day 8 (T8) or control chicks provided with transparent screens.

Figure 3. Latency to make a choice in the probe tests by male and female chicks from each rearing treatment. Female control chicks (white bars) took more time to make a choice than male chicks (dark bars), suggesting that the former may have experienced more conflict in the tests than the latter. Means ±SEM are presented.
Table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 8 screens</th>
<th>Day 11 screens</th>
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<tbody>
<tr>
<td>T8</td>
<td>Opaque</td>
<td>Transparent</td>
</tr>
<tr>
<td>T11</td>
<td>Transparent</td>
<td>Opaque</td>
</tr>
<tr>
<td>Control</td>
<td>Transparent</td>
<td>Transparent</td>
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Note that the screens were removed before lights-on on the next day. There were 8 chicks per treatment, 4 of each sex for each treatment except for T8 which had 3 females and 5 males.
Figure 1

a) Start
   Ball
   Start cage

b) Release

Screen

c) Start

d) Release
Figure 3

Latency to choose a screen (s)

T8  T11  Control

Treatment