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Secondary imprinting in the domestic chick: Binocular and lateralized monocular performance

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Newly-hatched chicks were reared with a coloured imprinting object on day 1 of life (primary imprinting) and then with an object of a different colour (secondary imprinting) on day 2. They were then tested on day 3 for preferences between the primary and the secondary imprinting object in binocular and in monocular conditions. The main results were that (1) left-eyed chicks usually showed clearer choice than right-eyed chicks; (2) there were colour preferences that appeared to affect choice differently in left- and right-eyed chicks; (3) eye asymmetries were in general more pronounced in males than in females. Experiments using composite stimuli (that contained simultaneously the colours of both the primary and the secondary imprinting objects) and experiments in which retention of memories for the primary and secondary imprinting objects were tested against the preference for novel objects showed that the eye asymmetries cannot be explained neither by hemispheric differences in response to novelty nor by different rates of forgetting of primary and secondary imprinting objects in the two hemispheres. It is argued that properties of single-units responses in neural structures involved in imprinting in the left and right hemisphere can account for these behavioural results.

The domestic chick (*Gallus gallus*) has proved to be an ideal model for studies of imprinting (for recent reviews see Bolhuis & Honey, 1998; Horn, 1998). One unexpected observation that has arisen from such studies concerns the differential role played by the left and right brain hemispheres in imprinting. When newly hatched chicks are exposed to a visually conspicuous object, they approach it, learn its characteristics, and form a social attachment to it. (In the natural environment, the object is usually the hen, but in the laboratory imprinting can be easily obtained using a variety of simple artificial objects.) The intermediate part of the hyperstriatum ventrale (IMHV), an associative forebrain structure, is part of a memory system that encodes a representation of the imprinting object (Horn, 1990). The right and the left IMHV appear, however, to have different roles in memory of the imprinting object. Evidence from lesion and electrophysiological studies suggest that both the right and the left IMHV act as long-term stores (Nicol, Brown, & Horn, 1995), but the right IMHV is also crucial in establishing another store, outside the IMHV region, and referred to as S' (McCabe,

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1991). The right IMHV passes information out to S' over a period of several hours (Horn, 1990). It has been also suggested that the right IMHV may add to the depth of processing by contributing contextual information and thus enriching simple representations initially stored in the left IMHV during the course of memory formation (Horn & Johnson, 1989).

This might be consistent with behavioural evidence from monocular tests showing that the right eye only responds to large changes in the visual appearance of an imprinting stimulus, whereas the left eye responds to more subtle changes in at least some visual characteristics of the imprinting object (Deng & Rogers, in press; Vallortigara, 1992; Vallortigara & Andrew, 1991, 1994a, 1994b). Monocular tests take advantage from the virtually complete decussation of nerve fibres at the optic chiasm that, combined with the natural occurrence of independent scanning by the two eyes (Andrew, 1991), makes it possible to investigate the roles played by the neural system fed by the left and right eye (largely confined to, respectively, the contralateral right and left hemisphere) using a non invasive procedure.

Although Lorenz (1935) claimed that imprinting was irreversible (see also Bateson, 1966) this view has been challenged by several studies that demonstrated that imprinting preferences can be reversed, using both artificial imprinting stimuli (Bolhuis & Trooster, 1988; Cherfas & Scott, 1981; Hoffman, Ratner & Eiserer, 1972; Salzen & Meyer, 1967; 1968) and live hens (Kent, 1987). A weaker form of Lorenz's claim suggests that, although the animal may direct its filial responses towards a novel stimulus, information about the original stimulus is not forgotten. A number of studies provided results consistent with this interpretation (Bateson, 1981; Bolhuis & Bateson, 1990; Cherfas & Scott, 1981; Cook, 1993). However, no research has yet investigated the problem of how the left and right hemispheres manage the task of encoding and storing information concerning a second imprinting stimulus, presented after imprinting on the first stimulus has taken place. Several different hypotheses can be suggested. A first possibility is that each hemisphere takes charge of storing and encoding the characteristics of only one imprinting stimulus. This seems to be very unlikely, however, for, although the avian brain shows a considerable degree of hemispheric segregation, transfer of information between the hemispheres does occur to some extent (see, e.g., Deng & Rogers, 1997; 1998a, 1998b). A more interesting possibility is that each hemisphere would form slightly different records of the primary and secondary imprinting objects. Evidence from free-choice experiments with chicks wearing eye patches (Deng & Rogers, in press; Vallortigara, 1992; Vallortigara & Andrew, 1991; 1994) and single-unit recordings from left and right IMHV (Nicol, Brown, & Horn, 1995) suggests that the right hemisphere tends to attend to subtle details of imprinting stimuli, making the finest discriminations between different, but similar, versions of imprinting stimuli. The left hemisphere, in contrast, tends to attend to general properties of visual stimuli, making a broad categorization of imprinting objects (Andrew, 1991; Vallortigara & Andrew, 1994a, 1994b). It has been suggested that these different encoding strategies may serve the different functions of recognizing individuals (right hemisphere) and responding rapidly to all members of the general category of social partners (Vallortigara, 1992; Vallortigara & Andrew, 1994).

Other hypotheses can be also considered. For instance, differences in response to novelty between the two hemispheres have been reported (for review see Andrew, 1991), as well as differences in the time course of memory formation (e.g., Andrew, 1997). They can both affect storing and encoding of memories of successively presented imprinting objects.

Secondary imprinting thus offers interesting possibilities to explore the nature of hemispheric specialization in the chick. In this paper we started by studying how information obtained from the two imprinting stimuli (primary and secondary) was stored and combined in the two hemispheres, and tried to put under experimental scrutiny the above mentioned hypotheses.

Experiment 1

In Experiment 1 chicks were reared on day 1 with an imprinting object of a certain colour. Then on day 2 the imprinting object was swapped with an identical object of a different colour. On day 3 chicks were tested for preferences for the primary or secondary imprinting object in binocular or monocular conditions.

Method

Subjects. Subjects were 60 male and 87 female domestic chicks (*Gallus gallus L*) of the Hybro strain (a local hybrid variety derived from the White Leghorn strain). Chicks were collected soon after hatching in a commercial incubator (Incubatoio Alba, Ponte di Castegnero, Vicenza, Italy) and transported in closed cardboard boxes to the laboratory. They were reared singly in metal cages (24 x 35 x 50 cm) with food and water ad libitum. Temperature (31-33°C) and humidity (68%) were controlled in the animal house. In the center of the cage a roundish ball (4 x 3 x 3 cm), that served as the imprinting object, was freely suspended by a thread at about the height of the chick's head.

Apparatus and Procedure. The apparatus (see Figure 1), which was placed in a separate room, consisted of a rectangular white box (72 x 20 x 30 cm) at the opposite ends of which the two imprinting stimuli were located, each behind a transparent glass. The time spent in the two compartments (each 14.7 cm in lenght) closest to one or the other imprinting stimulus (Figure 1) was measured. An electronic device connected with a computer allowed the experimenter to record, by simply pressing separate buttons, the time spent in each of the two compartments (in 1-min bins). The direction of the chick's head at the starting point (in the central compartment) as well as the position of the two imprinting stimuli were balanced across animals. Data were collected by four different experimenters and research assistants who were unaware of the exposure conditions chicks received as well as of each other's results.

The test comprised 6 min overall. The percentage of time spent close to the primary imprinting object was recorded as [(Time close to the primary imprinting object)/(Time spent close to the primary + Time spent close to the secondary imprinting object)] x 100.

Data were analyzed by analysis of variance. Significant departures from chance levels (50%) in times spent close to the primary imprinting object were estimated by one-sample, two-

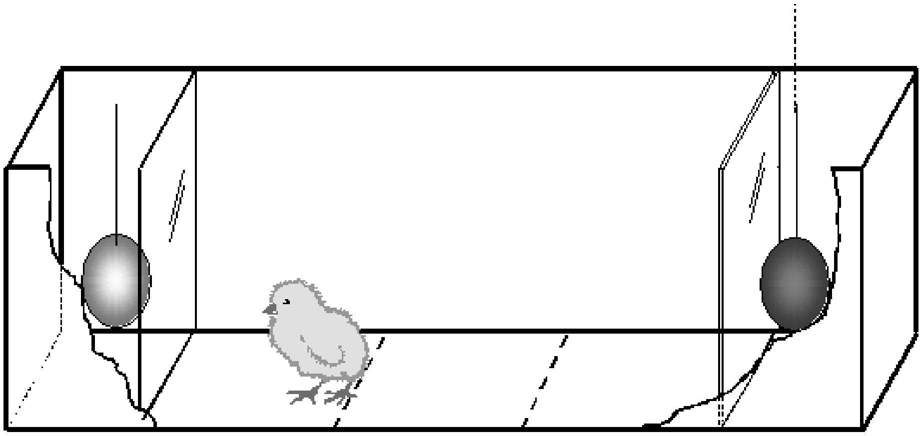


Figure 1. Schematic representation of the test apparatus. The dotted lines indicated the two sectors closest to one or other of the two testing objects.

Table 1
Design of Experiment 1.

Sex	Day 1	Day 2	*n =* re	le	bin	Day 3: Test
Males	Orange	Pink	10	10	10	Orange vs Pink
Males	Pink	Orange	11	10	9	Pink vs Orange
Females	Orange	Pink	15	17	15	Orange vs Pink
Females	Pink	Orange	13	14	13	Pink vs Orange

Note. re: right eye. le: left eye. bin: binocular.

tailed t-tests (see Vallortigara & Andrew, 1991, for details of analysis of free-choice tests in imprinting experiments).

Chicks were tested in binocular or monocular conditions. Monocular testing was carried out by means of eye patches made of special, removable, masking tape, positioned on the chicks 20 min before testing to habituate them to the new condition (binocular chicks were not eye-patched but received an equivalent amount of handling and habituation time as monocular chicks).

The design of this experiment is described in Table 1. Thirty male chicks were reared on day 1 with an orange imprinting object (primary imprinting) and on day 2 with an identical object but pink (secondary imprinting). They were then tested some in binocular conditions (*n* = 10), some with only their left eye in use (*n* = 10) and some with only their right eye in use (*n* = 10). Thirty male chicks were reared on day 1 with a pink imprinting object and on day 2 with an orange imprinting object. They were then tested some in binocular conditions (*n* = 9), some with only their left eye in use (*n* = 10) and some with only their right eye in use (n = 11).

Forty-seven female chicks were reared on day 1 with an orange imprinting object and on day 2 with an identical object but pink. They were then tested some in binocular conditions (n = 15), some with only their left eye in use (*n* = 17) and some with only their right eye in use (*n* = 15). Forty female chicks were reared on day 1 with a pink imprinting object and on day 2 with an orange imprinting object. They were then tested some in binocular conditions (*n* = 13), some with only their left eye in use (*n* = 14) and some with only their right eye in use (*n* = 13).

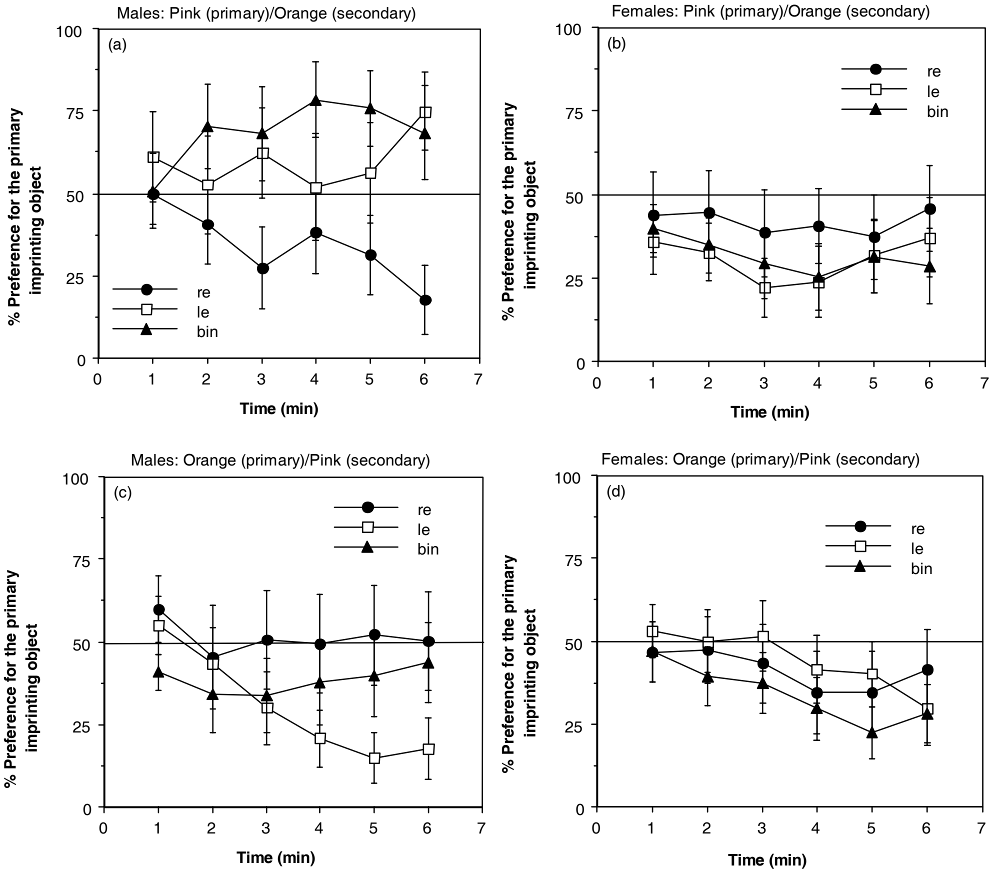


Figure 2. Preferences for the primary imprinting objects in male and female chicks reared with either a pink- or an orange-coloured stimulus as the primary imprinting object and then tested for preferences between the the same two stimuli (means with SEM are shown). Stimuli used for primary and secondary imprinting are indicated above each graph. bin= binocular chicks; re= chicks using only their right eye; le= chicks using only their left eye.

Results and Discussion

Choices are shown in Figure 2. Data have been analyzed with Sex, Eye, and Stimulus Colour of the primary and secondary imprinting stimulus (pink/orange *vs.* orange/pink) as between-subject factors, and Time of Testing (1-6 min) as a within-subject factor.

The analysis revealed a significant main effect of time, $F(5, 675) = 3.37$, $p < 0.01$, and a significant Time x Eye x Stimulus interaction $F(10, 675) = 1.88$, $p < 0.05$. The main effect of sex was close to significance, $F(1, 135) = 3.24$, $p < 0.08$, with males showing a stronger tendency to choose the object of primary imprinting than females (means ±SEM: males: 46.51 ±2.14, females: 37.46 ±1.72). There were no other statistically significant effects.

An analysis limited to the two monocular conditions revealed a significant main effect of time, $F(5, 460) = 4.03$, $p < 0.02$, and a significant Sex x Eye x Stimulus, $F(1, 92) = 4.73$, $p < 0.05$, and Time x Eye x Stimulus, $F(5, 460) = 2.95$, $p < 0.02$, interactions.

No significant effects were apparent in females. In males, there was a significant Eye x Stimulus, $F(1, 36) = 6.81$, $p < 0.02$ and a significant Time x Eye x Stimulus interaction, $F(5, 180) = 3.12$, $p < 0.02$. In the orange (primary)/pink (secondary) condition, there was no significant main effect associated to the eye in use, and the interaction Time x Eye only approached significance, $F(5, 90) = 1.995$, $p < 0.09$. However, left-eyed chicks tend to choose the secondary imprinting object (mean with SEM: 30.28 ±4.15; $t(59) = 4.75$, $p < 0.001$), whereas right-eyed chicks chose at random (mean ±SEM: 51.27 ±5.64; $t < 1$). In the pink (primary)/orange (secondary) condition, on the other hand, there was a significant main effect of eye, $F(1, 18) = 5.07$, $p < 0.05$ with left-eyed chicks choosing the primary imprinting object (mean ±SEM: 65.04 ±5.79; $t(53) = 2.59$, $p < 0.02$) and right-eyed chicks choosing the secondary imprinting object (mean ±SEM: 43.14 ±4.69; $t(65) = 3.37$, $p < 0.01$). The Time x Eye interaction was not significant.

These results showed that there are different patterns of choice between the two eyes and that the asymmetries were present only in males. However, asymmetries in the pattern of choices in males are complex. The more striking effect is a change in the direction of choice in left-eyed chicks as a function of colour. In the orange/pink condition, left-eyed chicks chose the secondary imprinting object (the pink one); in the pink/orange condition, left-eyed chicks chose the primary imprinting object (again the pink one). Thus, apparently, left-eyed chicks have a strong preference for the pink-coloured object. Right-eyed chicks showed a trend for an opposite pattern of results, with a preference for the orange-coloured object, but results are not so clear-cut: they showed in fact no choice in the orange/pink condition and a choice for the secondary imprinting object (orange) in the pink/orange condition. The analysis of variance did not show any effect of rearing conditions in right-eyed chicks (stimulus: $F(1, 19) = 1.30$, n.s.; Time x Stimulus: $F < 1$, n.s.). Nonetheless, right-eyed chicks did not show any clear choice when reared in the orange(primary)/pink (secondary) condition (mean ±SEM: 51.27 ±5.64), but showed a clear preference for the secondary imprinting object (orange) when reared in the condition pink (primary)/orange (secondary) (mean ±SEM = 34.14 ±4.70; $t(65) = -3.38$, $p < 0.01$).

Experiment 2

In order to clarify the complex pattern of results obtained in Experiment 1, we developed a procedure that would minimize the effects of colour preferences. In Experiment 2 chicks were imprinted, on day 1, on an object of a certain colour and then, on day 2, on an object of a different colour. At test, chicks had the possibility to choose between a composite object obtained by assembling the two original colours and either the primary or the secondary imprinted object. In this way, both colours (that of primary imprinting and that of secondary imprinting) were simultaneously presented at test (though in different amounts).

Method

Subjects were 164 male and 164 female "Hybro" chicks. Rearing conditions were the same as in the previous experiment. The apparatus was the same used in the previous experiment. The composite stimulus used during test was a ball identical to that used in the previous experiment, with one half (upper half) of a colour and the other half (lower half) of a different colour (upper and lower versions were counterbalanced). The design of this experiment is described in Table 2. Other aspects of the procedure were as described in Experiment 1.

Table 2
Design of Experiment 2.

			n =			
Sex	Day 1	Day 2	re	le	bin	Day 3: Test
Males	Orange	Pink	13	11	13	Orange vs Composite
Males	Pink	Orange	14	14	15	Pink vs Composite
Males	Orange	Pink	11	13	15	Pink vs Composite
Males	Pink	Orange	15	15	15	Orange vs Composite
Females	Orange	Pink	15	14	15	Orange vs Composite
Females	Pink	Orange	15	14	15	Pink vs Composite
Females	Orange	Pink	15	13	13	Pink vs Composite
Females	Pink	Orange	13	9	13	Orange vs Composite

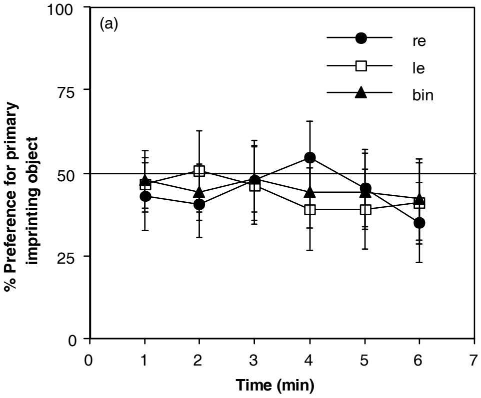
Note. re: right eye. le: left eye. bin: binocular. The composite object was half orange and half pink.

Results and Discussion

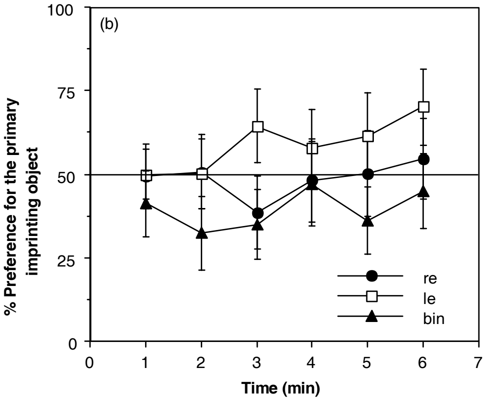
Time spent near the imprinting object (either primary or secondary) is shown in Figure 3. The data have been analyzed with a mixed factorial design with eye (bin, re, le), rearing condition (orange primary/pink secondary, pink primary/ orange secondary), test conditions (primary *vs.* composite, secondary *vs.* composite), and sex (males, females) as between-subject factors, and time (1-6 min) as a within-subject factor.

The analysis revealed significant Eye x Test, $F(2, 304) = 4.07$, $p < 0.02$, Rearing x Test, $F(1, 304) = 4.81$, $p < 0.03$, Sex x Rearing x Test, $F(1, 304) = 4.05$, $p < 0.05$ and Sex x Eye x Rearing x Test, $F(2, 304) = 3.05$, $p < 0.05$, interactions. There were no other statistically significant effects. An analysis limited to the two monocular conditions revealed however only a significant Eye x Test effect, $F(1, 198) = 7.56$, $p < 0.01$, and Sex x Eye x Test, $F(1, 198) = 3.57$, $p < 0.05$. The Sex x Eye x Rearing x Test interac-

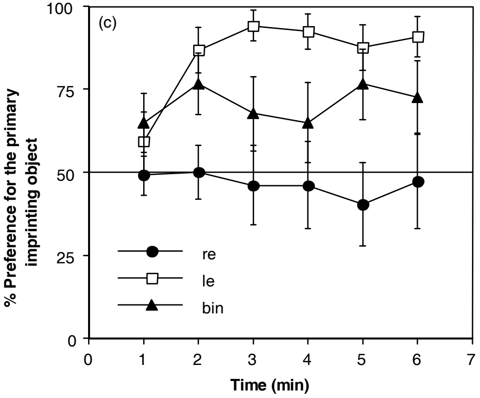
Males:
Rearing: Pink (primary)/Orange (secondary)
Test: Pink vs. Composite



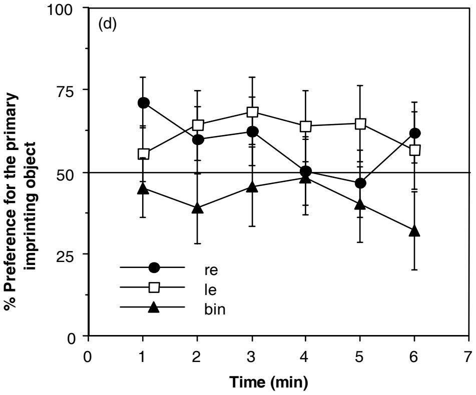
Females:
Rearing: Pink (primary)/Orange (secondary)
Test: Pink vs. Composite



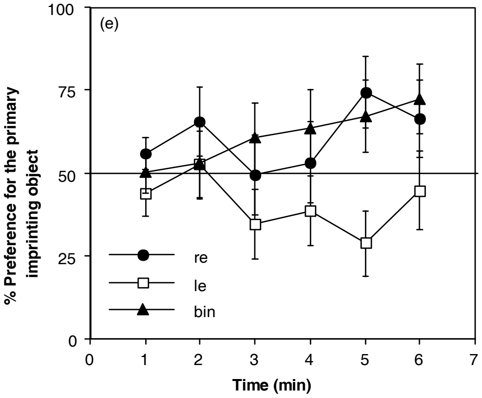
Males:
Rearing: Orange (primary)/Pink (secondary)
Test: Orange vs. Composite



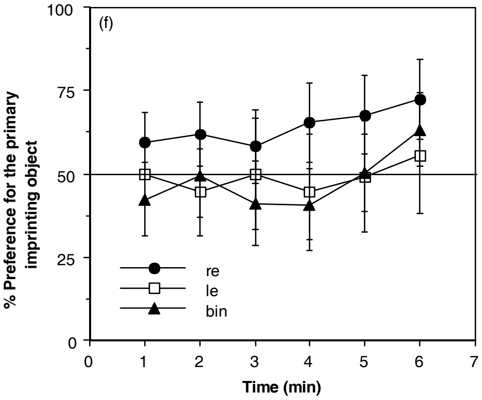
Females:
Rearing: Orange (primary)/Pink (secondary)
Test: Orange vs. Composite



Males:
Rearing: Pink (primary)/Orange (secondary)
Test: Orange vs. Composite



Females:
Rearing: Pink (primary)/Orange (secondary)
Test: Orange vs. Composite



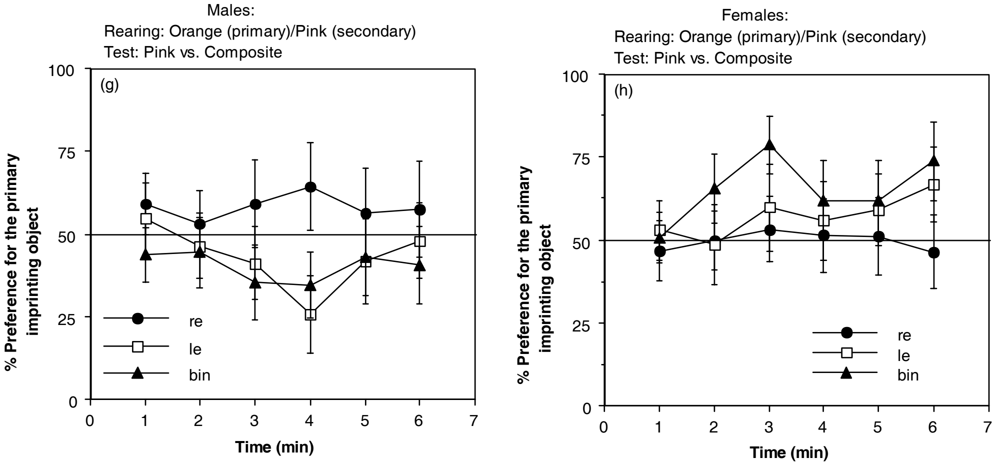


Figure 3. Preferences for the primary imprinting objects in male and female chicks reared with either a pink- or an orange-coloured stimulus as the primary imprinting object and then tested for preferences between the primary or the secondary imprinting object and a composite object obtained by assembling both coloured-stimuli together (means with SEM are shown). Stimuli used for primary and secondary imprinting and stimuli used at test are indicated above each graph. bin= binocular chicks; re= chicks using only their right eye; le= chicks using only their left eye.

tion was only close to significance, $F(1, 198) = 3.37, p < 0.07$.

An analysis limited to monocular females did not reveal any statistically significant effects. An analysis limited to the monocular males revealed a significant Eye x Testing conditions interaction, $F(1, 98) = 8.46$, $p < 0.005$. In males tested in the condition secondary *vs.* composite there was only a significant main effect of eye, $F(1, 50) = 4.25$, $p < 0.05$. Right-eyed chicks chose the secondary imprinting object, $t(155) = 3.00$, $p < 0.01$, whereas left-eyed chicks chose the composite object, $t(167) = -2.77$, $p < 0.01$. In males tested in the condition primary *vs.* composite there was a significant main effect of eye, $F(1, 48) = 4.21$, $p < 0.05$, and also significant Eye x Rearing, $F(1, 48) = 4.51$, $p < 0.05$, and Time x Eye x Rearing, $F(5, 240) = 2.69$, $p < 0.05$, interactions. When the orange-coloured object was the one used during primary imprinting (and the pink-coloured the one used during secondary imprinting), left-eyed chicks clearly chose the primary imprinting object, $t(65) = 11.70$, $p < 0.001$, whereas right-eyed chicks did not show any clear choice; when the pink-coloured object was the one used during primary imprinting, no significant choices appeared in either eye conditions.

Several possible explanations for the complex pattern of choices of left- and right-eyed chicks may be considered. A first possibility is related to the transfer of memories from left to right IMHV and then to S' documented by lesion studies (see introduction). It might be that when chicks are tested on day 3, memories of the primary imprinting object are well established in both hemispheres, whereas the most recent ones (i.e., those of the secondary imprinting object) are still being established. This, however, is in contrast with data showing clear choice (though opposite in direction) between secondary imprinting object and composite object in both right- and left-eyed chicks. Actually, the only monocular condition in which there was no significant choice was the primary *vs.* composite condition of right-eyed chicks (see Figure 3). Moreover, transfer from left to right IMHV to S' seems to require about 6 h and therefore memory traces in the two hemispheres should be completed in our testing conditions in which each imprinted object remained with the chicks for about 24 h.

A different hypothesis may be required to account for interest in slight novelty by left-eyed chicks. This is well documented in social recognition, at least in male chicks (Vallortigara & Andrew, 1991). Obviously chicks are presented with both colours and therefore they are familiar with both of them. However, it could be reasonably assumed that the relative degree of novelty of a stimulus would depend on how long ago was its last presentation. Consider the primary *vs.* composite condition. In the last 24 h chicks were reared with, say, a pink coloured ball. Then they were presented with a red ball (primary) and a pink/red composite. It is not unreasonable to assume that the completely red-coloured ball represents a degree of change (relatively) higher than the pink/red (which has some of the most recent coloration). Thus, one would predict left-eyed chicks would explore the red ball (the primary imprinting ball). Right-eyed chicks, on the other hand, would probably approach the pink colour because it is more familiar (more recently seen) but, on the other hand, the composite ball does present some novelty because of the simultaneous presence of two colours and the change in the overall pattern of coloration. So perhaps no clear choice should be predicted. Now consider the secondary *vs.* composite condition. In this case the slight novelty is present in the composite ball (i.e., the colour of the primary imprinting ball which has not been seen for 24 h). Thus left-eyed chicks would be expected to explore the composite and right-eyed chicks to choose clearly the more familiar secondary imprinting object. This is obviously a *post hoc* reconstruction, but it fits the data well.

Another possibility might be a different rate of forgetting by the two hemispheres. Left-eyed chicks showed a clear choice in both testing conditions. Right-eyed chicks, in contrast, showed a clear choice between the secondary imprinted object and the composite, but they did not show any clear choice between the primary and the composite. Could it be that right-eyed chicks remembered only the most recent (i.e., secondary) imprinting object? If so, similar effects should occur even when chicks were presented at test with a completely novel object rather than a composite of the (already familiar) primary and secondary imprinting objects. The following experiment was designed to test this hypothesis.

Experiment 3

In this experiment chicks underwent a rearing procedure similar to that described in the previous experiments. They were first reared on day 1 with an object of a certain colour (orange or pink) and on day 2 with an object of a different colour (pink or orange). At test, a novel-coloured (blue) imprinting object was used, together with either the primary or the secondary imprinting object.

Method

Subjects were 116 male and 118 female Hybro chicks. Rearing conditions were the same as in the previous experiments. The apparatus was the same as in the previous experiments. The design of this experiment is described in Table 3. Other aspects of the procedure were as described in Experiment 1.

Table 3
Design of Experiment 3.

			n =			
Sex	Day 1	Day 2	re	le	bin	Day 3: Test
Males	Orange	Pink	9	9	10	Orange vs Blue
Males	Pink	Orange	9	9	9	Pink vs Blue
Males	Orange	Pink	11	9	11	Pink vs Blue
Males	Pink	Orange	10	9	11	Orange vs Blue
Females	Orange	Pink	10	9	10	Orange vs Blue
Females	Pink	Orange	10	11	10	Pink vs Blue
Females	Orange	Pink	10	9	10	Pink vs Blue
Females	Pink	Orange	10	9	10	Orange vs Blue

Note. re: right eye. le: left eye. bin: binocular.

Results and Discussion

The percentage of time spent close to the primary imprinting object is shown in Figure 4. Data have been analyzed with a mixed factorial design with sex, eye, rearing (pink [primary] / orange [secondary], orange [primary] / pink[secondary]), and test (pink/orange vs. blue; orange/pink vs. blue) as between-subject factors, and time (1-6 min) as a within-subject factor. The analysis revealed a significant main effect of sex, $F(1, 210) = 5.03$, $p < 0.03$, with females showing a stronger preference for the primary or secondary imprinting object with respect to the novel blue object than males, and a significant main effect of time, $F(5, 1050) = 15.63$, $p < 0.001$. There were no other statistically significant effects.

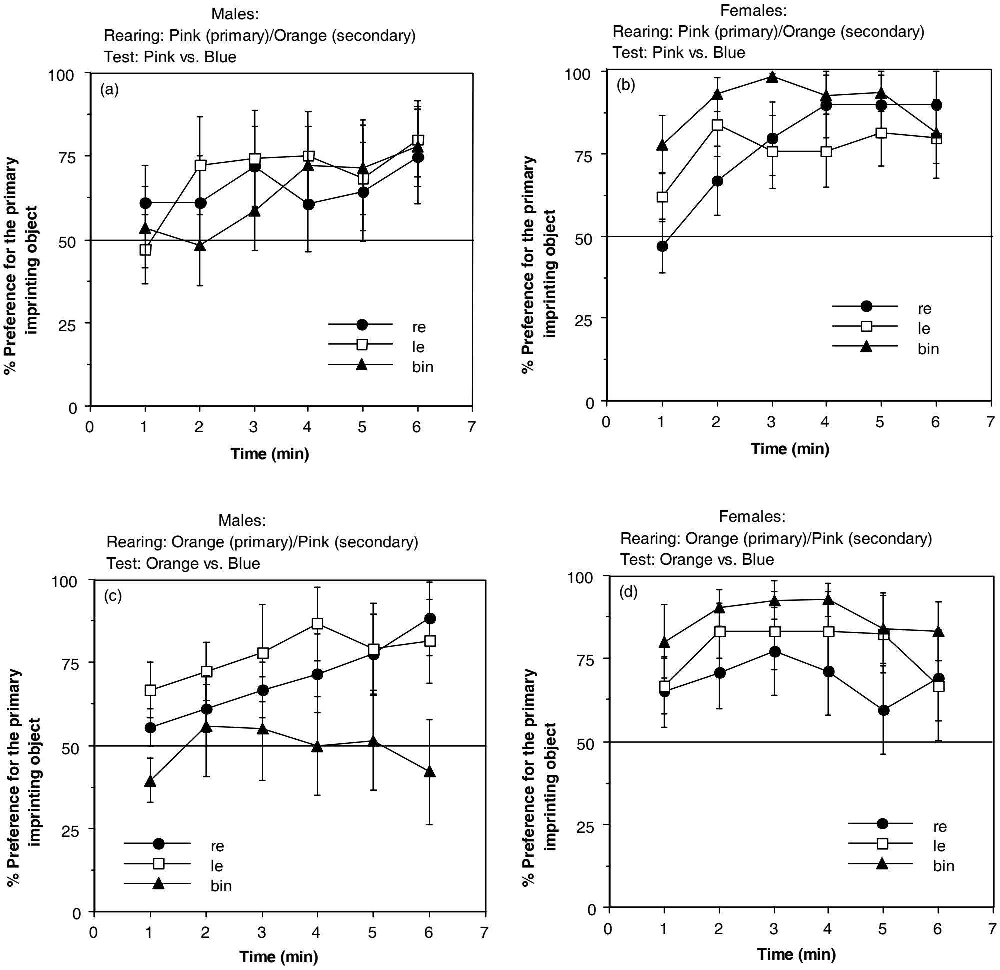


Figure 4. Preferences for the primary imprinting objects in male and female chicks reared with either a pink- or an orange-coloured stimulus as the primary imprinting object and then tested for preferences between the primary or the secondary imprinting object and a novel blue object (means with SEM are shown). Stimuli used for primary and secondary imprinting and stimuli used at test are indicated above each graph.

Thus, no effects involving the eye in use were apparent in choices between the imprinting and the novel object. Chicks showed a general avoidance of the novel object. There was a clear effect of sex, possibly due to stronger social affiliation tendencies in females than in males (Vallortigara, 1992).

Experiment 4

The results of Experiment 3 suggest that chicks prefer the primary or the secondary imprinting object to a completely unfamiliar coloured object irrespective of the eye in use. Apparently, both eye systems remember to a similar extent the primary and the secondary imprinting objects. The asymmetry revealed in Experiment 1 should therefore be in some ways specific of the two (already) familiar stimuli used during primary and secondary imprinting. To test this hypothesis the following experiments implemented a direct choice test between the two objects used during primary and secondary imprinting using colours other than pink (used in Experiment 1), in an attempt to eliminate or reduce colour preferences. Here we used blue and orange as colours (there is no evidence that blue is a strikingly preferred colour for imprinting; see e.g. Salzen, Williamson & Parker, 1979).

Method

Subjects were 42 male and 43 female Hybro chicks. Rearing conditions were the same as in the previous experiments. The apparatus was the same as in the previous experiments. The design of this experiment is described in Table 4. Other procedural details were the same as in Experiment 1.

Table 4
Design of Experiment 4.

			n =			
Sex	Day 1	Day 2	re	Le	bin	Day 3: Test
Males	Orange	Blue	8	6	6	Orange vs Blue
Males	Blue	Orange	8	7	7	Blue vs Orange
Females	Orange	Blue	6	7	8	Orange vs Blue
Females	Blue	Orange	6	8	8	Blue vs Orange

Note. re: right eye. le: left eye. bin: binocular.

Results and Discussion

The percentage preference for the primary imprinting object is shown in Figure 5. The analysis revealed a significant main effect of stimulus colour, $F(1, 73) = 7.50$, $p < 0.01$, with chicks reared in the condition orange (primary)/blue (secondary) showing a stronger preference for the secondary imprinting object (overall mean ±SEM: 17.33 ±2.10) than chicks reared in the condition blue (primary)/orange (secondary) (mean ±SEM: 34.03 ±2.39). There was also a significant Time x Eye x Stimulus interaction, $F(10, 365) = 1.88$, $p < 0.05$, which was also confirmed in an analysis restricted to the two monocular conditions, $F(5, 255) = 2.77$, $p < 0.02$. As can be seen in Figure 5, particularly in males, left-eyed chicks showed more clear choice than right-eyed chicks, though at different times in the two stimulus conditions: in the condition orange (primary)/blue (secondary) the eye asymmetry was present in the initial minutes of test, in the condition blue(primary)/orange (secondary) it was apparent in the

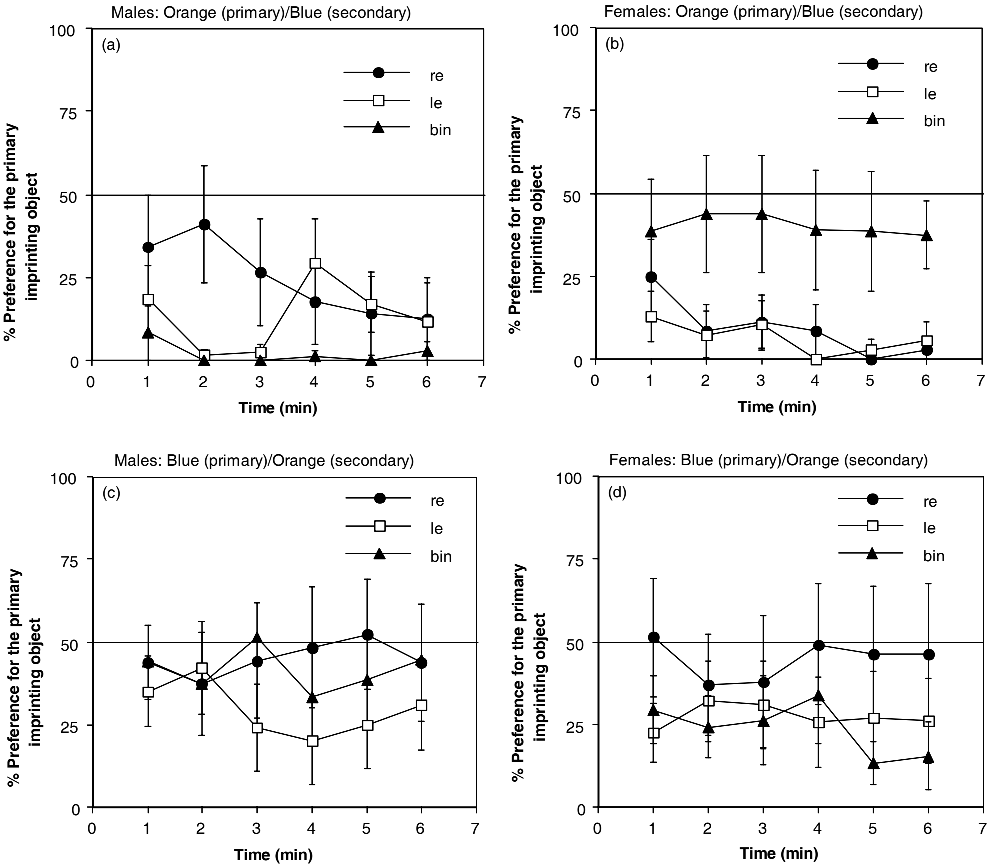


Figure 5. Preferences for the primary imprinting objects in male and female chicks reared with either a blue- or an orange-coloured stimulus as the primary imprinting object and then tested for preferences between the same two stimuli (means with SEM are shown). Stimuli used for primary and secondary imprinting are indicated above each graph. bin= binocular chicks; re= chicks using only their right eye; le= chicks using only their left eye.

final minutes of test. This was confirmed by statistical analyses confined to the first three and the last three minutes of test. In the condition orange (primary)/blue (secondary) there was a marginal effect of the eye in use during the first minutes, $F(1, 23) = 4.22$, $p < 0.06$, but not during the last minutes, $F < 1$, n.s. It is clear from inspection of Figure 5 that this was largely due to males, but the Sex x Eye did not turn to be statistically significant, $F(1, 23) = 1.19$, n.s. The reverse occurred in the condition blue (primary)orange (secondary) in which the effect of eye was observed in the last, $F(1, 28) = 4.21$, $p < 0.05$, but not in the initial minutes of test, $F < 1$, n.s.

Results suggest that the two types of change in the colour of the imprinting object, from orange to blue and vice versa, are not experienced as equivalent by the chicks and, probably as a result of this, the eye asymmetry occurred at different times (in the initial or in the final minutes of test). With both types of changes, however, the asymmetry was similar: the left eye showed a clearest choice (preference for the secondary imprinting object in this case) than did the right eye.

General Discussion

This is the first study devoted to the investigation of how the left and right hemispheres of the chick's brain deal with the encoding and storing of primary and secondary imprinting memory information. These results clearly confirm previous evidence concerning the existence of secondary imprinting in the chick (see Bolhuis, 1991, for a review). In most cases, chicks came to prefer the second object to which they had been exposed over the first one. Furthermore, the results provide evidence that the two hemispheres may play different roles in the process of secondary imprinting. A simple explanation based on hemispheric differences in the rate of forgetting of the characteristics of the primary imprinting object was ruled out in Experiment 3, where the choice was between primary or secondary imprinting object and a novel object. Moreover, differences in the response to novelty in imprinting situations (e.g., Vallortigara & Andrew, 1991; 1994a, 1994b) cannot account adequately for all the results: in Experiment 2, choice of the secondary imprinting object was not due to the primary object being treated as novel and thus interesting because its properties had been forgotten.

Overall, left-eyed chicks showed clearer differentiation (i.e., clearer choice) between the primary and the secondary imprinting object than did right-eyed chicks. Although the hemispheric difference seems to be a matter of degree rather than kind, this result agrees with evidence indicating that more subtle forms of visual discrimination in social contexts, such as individual recognition, are associated with left eye use and thus right hemisphere involvement (Deng & Rogers, in press; Vallortigara, 1992; Vallortigara & Andrew, 1991; 1994a; Vallortigara, Cozzutti, Tommasi & Rogers, 2001). This sensitivity could be traced back to single-unit responses in the IMHV. Nicol, Brown, and Horn (1995) showed that, relative to dark-reared controls, imprinting leads to an increase in the proportion of neurons in the left IMHV that respond to the training stimulus, but has no effect on the proportion of neurons responding to the alternative stimulus (not used in imprinting). In the right IMHV a similar increase occurs, but this is associated also with a decrease in the proportion of neurons responding to the alternative stimulus. Thus, the signal-to-noise ratio at the cellular level would favour discrimination in the right over the left IMHV; the same was observed in the present experiments at the behavioural level. It is likely that these hemispheric differences make functional sense when considering the opposite requirements of between-category discrimination (e.g., establishing rapidly whether an individual is or is not a social partner) and within-category discrimination (e.g., establishing whether a member of the category of social partner is a familiar or a novel one, for the purposes of individual recognition; see Vallortigara, 1992).

One striking tendency in the present results was for females to show less difference between right and left eye choice than males. This was obvious in pink primary, orange secondary, with choice between secondary and novel blue. This sort of sex difference has been reported previously for bead experiments in chicks (Andrew, 1991). In these experiments, habituation curves for the evocation of pecking by a bead illuminated internally with violet light were established. The degree of transfer of habituation from an earlier experience with a bead differing in various features (colour, position, etc.) from the standard bead, to subsequent tests with the standard bead was then measured. In both sexes, left-eyed chicks ceased to show transfer of habituation at smaller differences than did right-eyed chicks (which is similar to some effects shown in the present experiments). In addition, left-eyed males showed the same degree of habituation after prior presentation of a red bead as after a violet bead, as though illuminated beads were treated as fully equivalent. Females did not transfer habituation in this way; red and violet beads were treated as largely different objects. There was also a second consistent sex difference: when binocular and monocular females were compared, the curves showing the course of habituation and the degree of transfer of habituation shown by binocular chicks were closely similar to those for either right- or left-eyed chicks. This was not true for males; instead, binocular curves differed markedly from both monocular curves, usually being substantially higher than in either right- and left-eyed chicks. This suggests that one of the eye systems/hemispheres is usually fully in control in binocular females, whereas in males there is an involvement of both eye systems/hemispheres.

A major difficulty in the interpretation of the present results is associated with a novel and unexpected finding, namely the presence of colour preferences which are differently modulated by use of the left or right eye. Colour preferences are well-documented in the behaviour of the domestic chicks, both for pecking (Hess, 1956) and for filial imprinting (Schaefer & Hess, 1959). However, this is the first evidence that these (presumably) innate preferences could vary depending on whether the chick is using its left or right eye. The pattern of results obtained in Experiment 1, for instance, cannot be explained in terms of differential novelty associated with a change from orange to pink and from pink to orange: left-eyed chicks exhibited a preference for the pink object (which was not shown by right-eyed chicks) irrespective of whether the pink object was the primary or the secondary imprinting object. The finding also raises the possibility that some preferences were not noticed in previous work because they are not normally expressed (perhaps because of inhibitory processes) in binocular chicks.

At this stage we can only speculate on the meaning and functional value of these eye asymmetries It is usually assumed that they depend entirely on differences in higher perceptual processing. However, recent demonstrations of asymmetries in the proportions of cone types in left and right eyes in starlings (Hart, Partridge, & Cuthill, 2000) raise the possibility that visual lateralization is generated peripherally as well as centrally. There is no evidence at present that such retinal asymmetries are present in chicks; but if that were the case, they would be clearly pertinent to understanding the present findings.It would be important to extend studies on asymmetry in primary and secondary imprinting to visual characteristics other than colour, such as shape, for which retinal processing is likely to assume less importance than central (tectal and telencephalic) processing.

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